The Use of Skeletal Muscle to Amplify Action Potentials in Transected Peripheral Nerves

Yazan Al Ajam, FRCS (Plast)

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John Scales Centre for Biomedical Engineering
Institute of Orthopaedics and Musculoskeletal Science
University College London
Royal National Orthopaedic Hospital
Brockley Hill, Stanmore
Middlesex HA7 4LP
Abstract

Upper limb amputees suffer with problems associated with control and attachment of prostheses. Skin-surface electrodes placed over the stump, which detect myoelectric signals, are traditionally used to control hand movements. However, this method is unintuitive, the electrodes lift-off, and signal selectivity can be an issue.

One solution to these limitations is to implant electrodes directly on muscles. Another approach is to implant electrodes directly into the nerves that innervate the muscles. A significant challenge with both solutions is the reliable transmission of biosignals across the skin barrier.

In this thesis, I investigated the use of implantable muscle electrodes in an ovine model using myoelectrodes in combination with a bone-anchor, acting as a conduit for signal transmission. High-quality readings were obtained which were significantly better than skin-surface electrode readings. I further investigated the effect of electrode configurations to achieve the best signal quality.

For direct recording from nerves, I tested the effect of adsorbed endoneural basement membrane proteins on nerve regeneration in vivo using microchannel neural interfaces implanted in rat sciatic nerves. Muscle and nerve signal recordings were obtained and improvements in sciatic nerve function were observed.

Direct skeletal fixation of a prosthesis to the amputation stump using a bone-anchor has been proposed as a solution to skin problems associated with traditional socket-type prostheses. However, there remains a concern about the risk of infection between the implant and skin. Achieving a durable seal at this interface is therefore crucial, which formed the final part of the thesis. Bone-anchors were optimised for surface pore size and coatings to facilitate binding of human dermal fibroblasts to optimise skin-implant seal in an ovine model. Implants silanised with Arginine-Glycine-Aspartic Acid experienced significantly increased dermal tissue infiltration. This approach may therefore improve the soft tissue seal, and thus success of bone-anchored implants.

By addressing both the way prostheses are attached to the amputation stump, by way of direct skeletal fixation, as well as providing high fidelity biosignals for high-level intuitive prosthetic control, I aim to further the field of limb loss rehabilitation.
Impact Statement

Upper limb loss is a life-changing event for patients, significantly impacting their physical and psychological well-being. The mainstay of rehabilitation has been fitment with a prosthetic limb. Prostheses bring two challenges: firstly, how to attach the end-device to the amputation stump, and secondly how to control the prosthesis in an intuitive manner to allow the end user to perform upper limb functions closer to a native hand.

This thesis is divided into two parts. The first addresses recording muscle and nerve signals from implantable devices. The purpose of this section is to address the shortcomings of using surface electrodes to detect biosignals that are used to control a prosthetic limb. The second investigates surface coatings in bone anchors to improve the skin-implant seal. The work that has been done in this thesis has demonstrated that implantable muscle electrodes can be used reliably to transmit myoelectric signals across the skin barrier over a period of months, contributing to our understanding of signal recording and transmission across the skin barrier. Work on optimising electrode design has also contributed to finding the optimum configuration with regards to reducing cross talk. The significance of this part of the thesis is in its potential impact in translating this technology from animal *in vivo* studies to first in human trials.

While bone-anchored devices have solved the issue of attaching the prosthesis to the amputation stump by way of direct skeletal fixation, there remains the unresolved problem of a stable skin-implant seal. Lack of a stable seal has thus far been one of the main reasons to preclude the wide-spread adoption of bone-anchors in limb loss rehabilitation. In this part of the thesis, we showed that certain surface coatings can increase the adherence of dermal fibroblasts to the implant surface, which can potentially improve the skin-implant seal. The work presented in this thesis brings us closer to resolving this issue, by adding to the body of evidence on the effects of topography and surface coatings on binding of dermal fibroblasts to the implant. The impact of contributing to the knowledge in this area will further increase our understanding on how to optimise dermal attachment to the implant, and inform further research into the field.
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I dedicate this thesis to my loving wife and parents.
Statement of Originality

I, Yazan Al Ajam, confirm that the work presented in this thesis is my own. The conclusions I have drawn are based entirely on the outcomes of these experiments and the opinions expressed in this work are entirely my own. Where information has been derived from other sources, they have been duly referenced.
Publications

Work presented in this thesis has been published, presented or submitted as follows:


“A multi-channel multiplexed EMG recording system: realising variable electrode configurations”

Authors: Kylie de Jager, Michael Mentink, Henry T Lancashire, Yazan Al-Ajam, Stephen Taylor, Anne Vanhoestenberghe.

This work was also presented at the Biomedical Circuits and Systems Conference, Nara, Japan, 2019

Presenting Author: Kylie de Jager


“Optimising implanted epimysial electrode configuration for prosthetic control.”

Presenting Author: Yazan Al-Ajam.

PRS Global Open, 2019.

“Hard-wired Epimysial Recordings from Normal and Reinnervated Muscle Using a Bone-anchored Device.”

Authors: Henry T Lancashire, Yazan Al-Ajam, Robert P. Dowling, Catherine Pendegrass, and Gordon Blunn.


“Microchannel neural interface manufacture by stacking silicone and metal foil laminae.”

Authors: Henry Lancashire, Anne Vanhoestenberghe, Yazan Al Ajam, Catherine Pendegrass, Elliot Magee, Nick Donaldson, & Gordon Blunn.

10th World Biomaterials Congress, Montreal, Canada, 2016.

“Manufacture and in vivo evaluation of laminated microchannel neural interfaces: do endoneural basement membrane protein coatings enhance peripheral nerve regeneration through microchannels?”

Presenting Author: Henry Lancashire

“Myoelectric Signal Transmission from Implanted Epimysial Electrodes Using a Bone-Anchor as a Conduit.”

Presenting Author: Yazan Al-Ajam.


“Using bone-anchor as a hard-wired engineered conduit for myoelectric signal transmission from implanted muscle electrodes.”

Presenting Author: Yazan Al-Ajam.

Winner: Best Oral Presentation.

International TMR Symposium, Vienna, Austria, 2013

“The Use of a Bone-Anchored Device as a Hard-Wired Conduit for Transmitting EMG Signals from Implanted Muscle Electrodes.”

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Authors: Yazan Al-Ajam, Henry T Lancashire, Catherine Pendegrass, Norbert Kang, Robert P. Dowling, Stephen Taylor and Gordon Blunn.


“In vitro Neurite Outgrowth on Endoneural Basement Membrane Proteins.”

Presenting Author: Henry T Lancashire.

Runner Up: Oral Presentation Prize.

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“Basement Membrane Protein Coatings for In vitro Neural Regeneration for Nerve Electrodes.”

Presenting Author: Henry T Lancashire.

European Plastic Surgery Research Council Meeting, Hamburg, Germany, August 2013.

“Use of bone-anchored devices for intuitive muscular prosthetic control.”
Presenting Author: Yazan Al-Ajam


“The use of skeletal muscle to amplify action potentials in transacted peripheral nerves.”

Presenting Author: Yazan Al-Ajam.


“The Use of Skeletal Muscle to Amplify Action Potentials in Transected Peripheral Nerves.”

Presenting Author: Yazan Al-Ajam.


“Evaluation of a hard-wired epimysial electrode construct for multi-channel, high bandwidth prosthetic control.”

Presenting Author: Henry T Lancashire


“Bone-Anchored Devices for Neuromuscular Prosthetic Control.”

Poster Presentation

Presenting Author: Henry T Lancashire
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List of Abbreviations

AC Alternating Current
BION Bionic Neuron
CMAP Compound Motor Action Potential
CNS Central Nervous System
DC Direct Current
DoF Degrees of Freedom
ECM Extracellular Matrix
EMG Electromyogram / Electromyography
ENG Electroneurogram / Electroneurography
IED Interelectrode distance
FES Functional Electrical Stimulation
FINE Flat Interface Nerve Electrode
Fn Fibronectin
HDF Human Dermal Fibroblast
IMES Implantable Myo-Electric Sensor
IMS Industrial Methylated Spirits
ILP Integral Leg Prosthesis
ITAP Intraosseous Transcutaneous Amputation Prosthesis
LIFE Longitudinal Intra-Fascicular Electrode
MEA Microelectrode Array
MNI Microchannel Neural Interface

MUAP Motor Unit Action Potential

NMJ Neuromuscular Junction

OPL Osseointegration Prosthetic Limb

OPRA Osseointegration Prostheses for the Rehabilitation of Amputees

PBS Phosphate Buffered Saline

RGD Arginine-Glycine-Aspartic Acid

RPNI Regenerative Peripheral Nerve Interface

sECM Surface coating of neural extracellular matrix proteins: Collagen-IV, Laminin-2, -4, and Nidogen-1

sEMG Surface Electromyogram / Electromyography

SFI Sciatic Function Index

SNR Signal-to-Noise Ratio

THR Total Hip Replacement

TIME Transverse Intra-Fascicular Multichannel Electrode

TMR Targeted Muscle Reinnervation

TSR Targeted Sensory Reinnervation
Chapter 1 - Introduction

1.1 Aims and Overview

This thesis investigates the use of implantable electrodes for the control of upper limb prostheses in amputees. The primary focus is implantable epimysial electrodes, with additional research investigating implantable nerve electrodes and optimising the skin/implant seal for an intraosseous transcutaneous amputation prosthesis (ITAP). The overall aim is to provide a more selective, robust, reliable, and permanent alternative to the surface electrodes currently in use for control of myoelectric prosthetic limbs.

This thesis consists of 8 chapters:

Chapter 1 is an introduction into the current methods of rehabilitation with a prosthesis after upper limb loss. It outlines the problems faced by users of myoelectric devices and looks at potential solutions. It includes a description of how these devices function, including a review of both muscle and nerve biosignals. This chapter includes a review of the latest advances in prosthetic control, from both engineering and surgical perspectives. It also summarises the current work of other research groups to make the use of prosthetic limbs easier and more intuitive.

Chapter 2 focuses on the use of an animal model, including the cadaveric dissections performed to identify an appropriate muscle for testing implanted epimysial electrodes. Further, it outlines a proof of concept study of the use of the selected muscle (m. peroneus tertius), and another proof of concept study to test signal to noise ratios (SNR). The aim was to determine if m. peroneus tertius would be a suitable muscle model to investigate signal recording using a bipolar epimysial electrode for detection of electromyography (EMG) signals.

Chapter 3 investigates the use of an osseointegrated device for transmission of EMG signals in an ovine model. Signals from implanted epimysial electrodes were compared with surface electrode recordings. The aim was to determine whether an implanted electrode would record physiologic EMG signals with a higher SNR and less crosstalk than surface electrodes in a conscious animal during treadmill walking.
Chapter 4 presents the results of a study using *m. peroneus tertius* to investigate signal regeneration and EMG signal regeneration after targeted muscle reinnervation (TMR). The aim was to determine if neural regeneration and muscle activity would be observed following TMR with eventual restoration of high-quality EMG signals with a SNR comparable to native muscles.

Chapter 5 investigates the optimal electrode configuration for maximising SNR in epimysial electrodes. Electrode configurations (bipolar vs. monopolar vs. tripolar), and interelectrode distances were compared with regards to SNR in a 5-electrode array in an ovine model. Physiologic EMG signals were transmitted using an osseointegrated model as previously described. The aim was to find the optimal electrode configuration with the highest SNR while minimising crosstalk. The second aim was to investigate the effect of varying interelectrode distance on signal strength and SNR.

Chapter 6 investigates ENG signal transmission through a microchannel neural interface (MNI) with multiple protein coatings. The *in vivo* experiments were performed in a rat model to test the effects of adsorbed endoneural basement membrane proteins on sciatic nerve regeneration. The aim was to determine whether augmentation of MNI implants with protein coatings could improve nerve regeneration.

Chapter 7 investigates the effects of porosity and surface coatings on dermal and epidermal tissue integration with ITAP. The aims were to determine whether a porous flange would enhance soft tissue integration compared with commercially machined controls, and whether the addition of a silanised, Arginine-Glycine-Aspartic Acid (RGD) coating enhance this integration further in an *in vivo* ovine model.

Chapter 8 discusses the overall results of this thesis and explores areas for future work.
1.2 Upper limb amputation

Upper limb amputations result in significant functional impairment and psychological morbidity and are a major financial burden to both patients and health services. Upper limb amputations can be due to trauma, tumours, or vascular complications of disease. Between 2006-2007, 215 referrals were made to the UK Prosthetic Services for upper limb amputation [1]. This number increased due to injuries sustained by soldiers in overseas military campaigns; 54 traumatic or surgical amputations in British soldiers in Iraq alone in 2009 [2], and over 250 between 2006-2011 were reported [3].

The figure was higher for United States soldiers, with 77 upper limb amputations in the first 9 months of 2009 alone [4], and 1,407 in total during the Iraq and Afghanistan wars up to September 2010 [3]. This was due to both an increase in the use of improvised explosive devices, as well as improvements in survival, secondary to personal protective equipment shielding for the torso, and to the use of soldier-carried tourniquets to stem blood-flow following an amputation [3].

The number of civilian upper limb amputees is much higher. The European community has an estimated 94,000 upper limb amputees [5], more than twice that of the United States, which is known to have 41,000 people with an amputation of a hand or a complete arm [6] - an estimated prevalence of 1 in 6,100 of the general population. This translates to 1 million upper limb amputees globally, however, with recent increases in conflicts and natural disasters, the actual number is difficult to accurately estimate. Since the majority of traumatic amputations occur in young patients (16-54 years) [1], the financial and psychological morbidity is considerable. The number of people born with congenital upper limb deficiency is even higher, with an incidence of 4.1 per 10,000 live births [7], [8].

1.3 Allograft Reconstruction

The only alternatives to prosthetic restoration are autologous or allograft reconstruction. Autologous reconstruction is only appropriate for limited, distal loss of the upper limb (e.g., single digits). Allograft (the transplant of tissue from one individual to another individual of the same species) reconstruction can restore total loss of an entire upper limb. However, allograft upper limb transplantation surgery is complex and finding suitable donors is
difficult. Furthermore, the need for life-long immunosuppression and poor functional recovery of transplanted limbs for amputations above the elbow, are major obstacles which are likely to prevent more widespread adoption of this solution [9]. There will be a continuing, worldwide need for prosthetic solutions to restore some degree of upper limb function, which can allow individuals to return to their former activities of daily life.

1.4 Prostheses

Except for hand transplantation, prostheses have remained the mainstay for significant upper limb loss reconstruction. Prosthetic limbs pose two challenges to designers and end users: attachment of the prosthesis to the residual limb and control of the device.

1.4.1 Problems with Attachment

Despite advances in the materials used to construct prosthetic limbs and the new technologies to move and control them, there remain fundamental problems in the way prosthetic devices are attached to the residual limb for all prosthetic limb users. Currently, most upper limb prostheses are attached to the residual limb with a suction socket. The socket is made of a rigid (usually plastic) material, which fits over a silicon liner and is custom-fitted to the patient’s residual limb. The socket provides the rigid point of attachment for the artificial limb. Therefore, a well-fitting socket is critical for correct functioning of the prosthesis, and much time and effort is expended by the prosthetist (working closely with the wearer) to obtain an optimal fit. If the fitting is poor, the socket moves relative to the residual limb and the prosthesis also moves in an awkward, unpredictable or energy inefficient manner.

1.4.1.1 Socket/skin interface

Creating a tight-fitting socket ensures that the soft tissues of the residual limb are as rigid as possible. This ensures that there is better energy transfer between the end device, the socket, through the soft tissues and to the underlying bony skeleton of the residual limb. However, the better the fit, the more likely the patient is to develop a variety of cutaneous pathologies because of the constant contact between the liner/socket and the skin of the residual limb. These include; skin ulcers, blistering, intertrigo, contact dermatitis, epidermoid cysts, verrucous hyperplasia, pain and numbness [10]–[15]. Skin trauma caused by chaffing within the socket, excessive heat and moisture or sweating at the skin/socket interface can also
contribute to skin breakdown and infections since these cutaneous/soft tissue structures were never intended to carry a constant mechanical load like the underlying bony skeleton. Cutaneous problems are particularly frequent in patients with scarring or skin grafts on their residual limbs because these areas are fragile and prone to ulcerate. Scars and grafts are common in patients who have sustained traumatic amputations. Cutaneous complications can contribute to eventual abandonment of upper limb prosthetic devices in up to 80% of patients, at 2 years after issue [16].

1.4.1.2 Load transfers
The transfer of load from the residual limb, through the socket to the end device is non-physiologic compared with a load carried by the bony skeleton (e.g., by direct skeletal fixation of a prosthesis). The energy needed to move the prosthesis is also greater for a patient using a socket-fitted prosthesis. For example, a unilateral, transfemoral amputee using a standard, socket-fitted prosthesis needs at least 75% more energy to mobilise compared with an able-bodied individual [17]–[19]. This energy requirement is up to 25% lower in a patient who mobilises using a prosthesis secured directly to the skeleton. Despite not directly relating to upper limb amputees, these figures give an indication of the additional energy expenditure associated with prosthesis use. Moreover, the way in which the load is transferred for an upper limb amputee is restricted, either because the socket itself limits the range of movement of the joints proximal to the amputation (e.g., reduced shoulder movements for a transhumeral amputee) and/or because the load is carried by the skin and soft tissues in traction. In addition, the more proximal the amputation, the less suitable a socket attachment, and the greater the need for secure strapping of the prosthetic device to the torso to prevent it from moving around uncontrollably. Patients (especially women) complain that these straps are uncomfortable, unsightly, difficult to use and restrict the clothing they can wear, especially in hot weather (Figure 1.1).
Figure 1.1: Left transhumeral amputee donning his NHS-provided prosthesis, which is secured to the residual limb using a standard socket. The socket contains and restrains the shoulder joint limiting range of movement of this joint. The prosthesis socket is further secured using straps, which also allow the patient to flex the elbow using a cable-operated system when he shrugs his shoulders. The (absent) hand is operated by a myoelectric sensor placed over the *m. biceps* and *m. triceps* to control opening and closing.

Images courtesy of Mr Norbert Kang FRCS(Plast), permission received.

Amputees who experience painful complications due to neuromas or heterotopic ossification often find it impossible to use a standard socket-fitted prosthesis [20]. In addition, those with a very proximal amputation may have residual limbs, which are not sufficiently long to secure a prosthetic limb. In these patients, direct skeletal fixation may be the only way to secure the prosthetic device to the residual limb.

1.5 Direct Skeletal Fixation

Osseointegration of titanium alloy screws inserted into rabbit tibias was first observed by Professor Per-Ingvar Brånemark in Gothenburg, Sweden in the 1960s [21]. These observations founded the development of a wide range of devices including dental implants and bone-anchored hearing aids. It also formed the basis for translation to osseointegrated bone-anchors for lower limb amputees [22]. The use of an osseointegrated prosthesis for reconstruction in amputees was a concept, first pioneered by Brånemark’s group in Gothenburg, Sweden [23]. At surgery, a commercially pure titanium implant was inserted into the medullary cavity of the long bone in the residual limb. The distal end of the osseointegrated implant was extended percutaneously, and a prosthetic limb could then be
attached to the external part, allowing the prosthesis to be secured directly to the residual skeleton of the amputated limb.

1.5.1 Benefits

Direct skeletal fixation using an osseointegrated implant not only addresses the cutaneous problems at the skin/socket interface, it also makes donning and doffing the prosthetic limb easier and affords the wearer a degree of ‘sensory’ feedback via osseoperception [24]. In addition, load bearing is more physiologic, since the mechanical forces are transmitted directly to the bone. Using an osseointegrated prostheses to secure an upper limb prostheses, allows heavier loads to be carried by the prosthesis (in traction) compared with a prosthesis secured using a socket [20]. Range of motion of the residual limb is also improved because the proximal joint is not constrained within a socket. This is particularly true if the residual limb is short, as a short residual limb may preclude fitting of a standard, socket-secured prosthetic device.

1.6 Bone-anchor Systems Already Used in Humans

There are many different osseointegrated systems which have been used for prosthetic reconstruction in humans (Figure 1.2). These include:

1. Osseointegration Prostheses for the Rehabilitation of Amputees (OPRA), Integrum AB, Mölndal, Sweden. This system was introduced in 1998 by Brånemark’s team. The system consists of an implanted “fixture”, where osseointegration takes place, and a percutaneous component known as the “abutment”, which is press-fit into the fixture. The prosthesis attaches to the abutment. The abutment is fixed to the fixture by way of an abutment screw. The OPRA system is a two-stage procedure, where the fixture part of the procedure is screwed into the bone (versus press-fit) and the abutment is inserted at a later operation (after 6 months) once the fixture has had time to osseointegrate [25].

2. Integral Leg Prosthesis (ILP – formerly Endo-Exo Prosthesis), Orthodynamics GMbH, Lübeck, Germany popularised by Aschoff et al. This single-stage bone-anchor was introduced in 1999, and initially used in transfemoral amputees; however, tibial and humeral implantations have been reported. The system consists of a cast
cobalt-chrome alloy stem which is inserted into the bony cavity as a press-fit. Previous versions of ILP included a bone-stabilising bracket, a design that was dropped in the latest iteration. The system is a two-stage procedure. In the first stage, the stem is inserted into the medullary cavity of the long bone and the skin closed over. At the second stage, performed 6 to 8 weeks later, a stoma is created in the skin overlying the distal part of the implant and a dual cone adaptor for fixation of the prosthesis is inserted [26], [27].

3. Osseointegrated Prosthetic Limb (OPL), Permedica s.p.a., Milan, Italy.
Design elements of the ILP system were refined by Al-Muderis et al., of the Osseointegration Group of Australia [28], to produce the OPL system. This system, introduced in Australia in 2013, was initially a two-staged procedure, much like the ILP system, before switching to single-stage surgery. Like the ILP system, it is a press-fit device. However, it is made from titanium alloy, and comes in standard sizes, with custom made devices available for transtibial and short transfemoral stumps. To date, more than 650 devices have been inserted, both in civilian and ex-military service men[1]. The application is in both upper and lower limb amputees.

4. Intraosseous Transcutaneous Amputation Prosthesis (ITAP). Initially developed in the UK by Stanmore Implants Worldwide, this single-component, single-surgery, press-fit device was used in a transhumeral amputee [20] and was part of a transfemoral clinical trial. A unique design characteristic of ITAP is a subdermal porous flange used to enhance soft tissue ingrowth and attachment, in an attempt to address the skin/implant seal.

5. COMPRESS System (Zimmer Biomet, Warsaw, USA). Initially developed as an endo-prosthesis in limb salvage, the design concept was developed further as a transcutaneous osseointegrated device. Bicortical transosseous pins are used to secure a medullary anchor plug, into which the intramedullary part is attached. External loading is applied to aid osseointegration by way of integral Bellville washers. Under custom device exception, 13 patients with above knee amputations received this implantable system, and there have been two cases of periprosthetic fractures [29]. To date this system remains in the clinical trials phase.

1.7 Infection Complications of Bone-Anchored Implants

Osseointegration is a reliable, reproducible process, which can be achieved successfully in most patients. However, achieving a stable skin-implant interface is more problematic and relies on how the soft tissues are managed. The main problem is epithelial downgrowth between the transcutaneous section of the prosthesis and the adjacent soft tissues; a process known as marsupialisation. Marsupialisation prevents the formation of a robust, infection-resistant skin-implant seal and creates a direct route for pathogens to enter the body [31]. Thus, concerns about infection and continuing discharge at the skin-implant interface remain one of the major obstacles to more widespread adoption of this technology. Rates of superficial skin infection of up to 61% have been reported for the OPRA system [32]. In a prospective follow up of 39 upper and lower limb OPRA implants, Tillander reported 14 patients (36%) with secretions from a skin pocket, of which 10 (26%) had purulent discharge [33]. Sooriakumaran reported 4 of 16 patients with an OPRA implant developed an implant-related infection during follow up [34]. Similar infection rates have been reported by other workers [35]. Superficial infection rates of 38% have been observed for patients using the ILP implant system [26]. Al-Muderis reported on a cohort of 22 transfemoral patients treated
with the OPL system using a two-stage approach (similar to OPRA) for insertion of the implant. There were 15 documented episodes of superficial infection in 12 patients requiring antibiotic therapy (and none requiring surgery), representing an infection rate of 55%. However, the mean follow up time of this cohort was only 14 months [36]. Clearly, longer-term outcome measures are needed to properly evaluate the outcomes using the OPL implant system.

The paucity of long-term outcome data, and the continuing perception that osseointegrated implants are associated with unacceptably high levels of complications (especially infection) has prevented the wider adoption of this technology. For these implants to be used routinely in amputees, a reliable, reproducible and robust skin-implant seal, capable of preventing marsupialisation and infection, is vital.

### 1.8 Intraosseous Transcutaneous Amputation Prosthesis (ITAP)

Natural analogues of osseointegrated implants exist successfully, without infection. These include deer antler, babyrussa tusks and the teeth of the naked mole rat [37]. Deer antler were studied as a biomimetic model for the development of ITAP with the aim of understanding the cutaneous integration which ensures the stability of transcutaneous interface [38], [39]. Pendegrass et al. found that a thin layer of dermal tissue (without fat) adhered to the pedicle (the porous transcutaneous bone segment of the antler) via collagen fibres extending from the dermis into the porous bone. They hypothesised that the resulting immobility of the epithelium immediately overlying the pedicle was a direct result of the tight dermal seal beneath. In addition, they postulated that the dermal-pedicle seal prevented epithelial migration and hence marsupialisation and resulting infection. ITAP was designed on the basis of these findings; consisting of an osseointegrated stem, with a porous, hydroxyapatite-coated, subcutaneous flange (designed to mimic the porous pedicle bone) and a smooth external abutment (to which the artificial limb could be attached).
Figure 1.3: ITAP used for a left transhumeral amputee. A) Prosthetic limb attached to the transcutaneous component of the bone-anchor. A circumferential strap holds skin-surface electrodes over the *m. biceps* and *m. triceps* to activate hand-open and hand-close in the myoelectric hand. The elbow joint was not powered but could be locked. B) Appearance of the skin-implant interface at 2 years after surgery. The bone-anchor has now been in place since 2007 and is still in full daily use by the patient, with no recorded episodes of superficial or deep infection since insertion.

Images courtesy of Mr Norbert Kang FRCS(Plast).

ITAP used for prosthetic reconstruction of a single transhumeral amputee (Figure 1.3) helped to prove the concept in humans [20]. It highlighted the advantages of an osseointegrated implant for the upper limb, demonstrating ease of donning and doffing the prosthesis, improvements in range of motion of the proximal joint and the utility of the prosthetic limb for carrying out the patient’s activities of daily living, with no morbidity for the patient.

### 1.9 Upper Limb Prostheses

There is a wide range of designs for upper limb prosthetics (also referred to as end devices) to serve the different needs of the end-user. The price of these devices reflects the complexity of their technology, however, broadly speaking, they can be divided into two categories: body-powered and myoelectric.
1.9.1 Body-Powered Prostheses

Body-powered end-devices continue to be one of the most common prosthetic solutions for the upper limb amputee [16]. They rely on a system of cables and pulleys that use surrogate movements of the body (e.g., pushing the shoulder forward) to open or close the prosthetic limb (Figure 1.4). The advantages of cable-operated systems include; low cost, reliability (since it contains no electronics), ease of maintenance (with only simple tools and training) and good levels of strength and control over the end device. However, the system requires considerable learning and effort on the part of the user. They also require non-intuitive, awkward movements to control the end device. For example, there is nothing normal or natural about moving a shoulder to control opening and closing of a claw hand.

In contrast, cosmetic prosthetic limbs serve no function other than to give the appearance of a normal limb (especially the outline of the shoulder area in glenohumeral or forequarter amputees) when wearing clothing. They are generally lighter in weight than body-powered prostheses and have no moving parts.

![Figure 1.4: Example of a body-powered prosthetic limb for a transradial amputee. The cable attached to the prosthetic hook-hand is routed to the contralateral shoulder, elevation of which results in opening of the end device. Reproduced from [40]. Figure redacted in online copy.](image)

1.9.2 Myoelectric Prostheses

The concept of a myoelectrically controlled prostheses was first described in 1919 in Germany [41]. However, it was not until 1948 when Reiter, at Munich University, created the first myoelectrically operated prosthetic limb using skin-surface detected EMG signals to control the end device [42]. However, the bulk and complexity of this revolutionary design,
(which was way ahead of its time) made it practically useless for amputees. It was not until the 1980s that myoelectrically controlled prosthetic limbs became a practical alternative to body-powered devices, mainly due to improvements in electronics and computing. Globally, there are now many manufacturers producing commercially available, upper-limb end devices (Figure 1.5).

![Figure 1.5: Examples of commercially available prosthetic hands. A) bebionic limb made by the Steeper Group (now part of Otto Bock), shaking hands with a Michelangelo hand also made by Otto Bock. B) i-limb ultra and partial hand, both made by Touch Bionics Inc. Figure redacted in online copy.](image)

### 1.10 Biosignals for Prosthetic Control

When muscles contract, EMG signals are propagated through the muscle fibres, producing a voltage difference in the order of a few millivolts. Human muscle EMGs generally fall in the range of 20 μV to 2000 μV depending on the size and number of motor units activated, with most of the EMG signals in the range of 10 Hz to 500 Hz [43]. In its simplest form, a myoelectric prosthesis relies on the electrical signals generated in a normally innervated muscle in the amputation stump, to control a movement in the prosthetic limb. The EMGs act as ‘switches’ to control the release of electrical power (stored in a battery pack) to electric motors in the end device. As the patient voluntarily contracts muscles in the residual limb, the EMGs generated by the residual muscles in the stump are detected by the respective electrodes. These are amplified, processed and used to trigger electric motors, resulting in movements of the prosthetic hand. Placing electrodes on agonist/antagonistic muscles results in opposing functions such as opening and closing of the hand or flexion and extension of the elbow.
1.10.1 Skeletal Muscle Physiology

Skeletal muscles consist of bundles of muscle fibres, or myocytes. These are elongated, multinucleated cells with a plasma membrane (sarcolemma), containing all the organelles and sarcoplasmic reticulum, in addition to myofibrils. It is the myofibrils within the muscle that generate the force of muscle contraction - through shortening of the sliding-filament mechanism. Muscle contraction is controlled by action potentials transmitted via a single motor nerve which innervates several motor fibres simultaneously – creating a motor unit. The interface between the nerve and the muscle forms the neuromuscular junction (NMJ) (Figure 1.6). This is a synapse which allows transmission of the nerve signal to the muscle, to initiate and control muscle contraction.

![Figure 1.6: Motor nerve termination on a muscle fibre.](image)

1.10.2 Skeletal Muscle Electrophysiology

The myoelectric signal is a measure of the potential difference (voltage) that ‘travels’ through the length of the muscle fibre as it is stimulated to contract by the motor nerve. This is represented by an electromyogram (EMG) signal.
Somatic motor nerves terminate at the NMJ, or motor end plate on the muscle fibre. Arrival of the signal from the axon at the motor end plate, results in the release of acetylcholine (Ach). Acetylcholine opens Na+ channels in the myocyte cell membrane, causing an influx of Na+ ions into the cell and reducing the transmembrane potential, resulting in depolarisation. Once the depolarisation exceeds the threshold point (-75 mV), a positive feedback mechanism occurs that results in further influx of Na+ until a full action potential is generated. This triggers the release of Ca2+ from the sarcoplasmic reticulum within the myocyte. The flood of intracellular Ca2+ activates ATP-ase, resulting in binding of myosin and actin elements in the myofibril which now shortens by way of the sliding filament mechanism [45]. The current flow (ion flow) described during depolarisation generates an electrical field. It is this difference in field potential at points distant from the membrane, which is detected by surface electrodes in the residual limb, which is then used to control movement in the prosthetic limb.

The summation of action potentials from individual muscle fibres innervated by the same single axon is termed a motor unit action potential (MUAP). When greater force of contraction is required, the rate of firing of neurons and the number of motor units recruited is increased. The summation of the MUAP for all active neurons results in an EMG signal. It is these signals that are detected by the skin-surface electrodes of a myoelectric prosthesis [45].

1.10.3 Peripheral Nerve Physiology

Peripheral nerves include all the neurons and their supporting cells found outside the central nervous system (brain and spinal cord). Peripheral nerves are made up of bundles of axons surrounded by an insulating connective tissue sheath called the epineurium. Epineurium is comprised of longitudinally arranged collagen and elastin fibres, allowing for flexing and stretching of a limb [46]. Nerve bundles (fascicles) are surrounded by an additional connective tissue layer called the perineurium. The perineurium controls substance transport into the fascicle, and forms a blood-nerve barrier. Each fascicle is comprised of several axons, each surrounded by another layer of connective tissue called the endoneurium. The endoneurium helps maintain the blood-nerve barrier, providing an optimal microenvironment for nerve fibres to function (Figure 1.7).
Peripheral nerves convey biosignals in the form of ENG signals (Electroneurographic/action potentials) that are propagated along the nerve. These electrical impulses travel from one neuron to another across a synapse, where the signal is converted from electrical to chemical forms and back to electrical as it is transmitted to the next neuron, or to an end organ (e.g., muscle), resulting in an action (e.g., muscle contraction).

Nerves are classified based on their diameter, myelinated state and conduction velocity. They are also classified as either sensory or motor. Myelinated axons are surrounded by a myelin sheath, which forms an electrically insulating layer, created by Schwann cells.

At regular intervals along the axon, there are breaks in the myelin sheath that form the Nodes of Ranvier, which increase conduction velocity. Conduction velocity is increased because the ions involved in the propagation of the action potential are only exchanged across areas of bare axon membrane (Figure 1.8) and not the areas covered with a myelin sheath. In this way, the action potential is propagated from node to node by a process of “saltatory” rather than linear conduction [47]. As a result, myelinated axons can transmit ENGs at a rate of 150 m/s compared to 0.5 to 10 m/s for non-myelinated axons.

Figure 1.7: Peripheral nerve anatomy. Reproduced from [44]. Figure redacted in online copy.
Figure 1.8: Sodium channels within an axon, showing opening of sodium channels, resulting in a rush of sodium ions into the axon, resulting in depolarisation of the membrane and the start of an action potential. Reproduced from [44]. Figure redacted in online copy.

1.10.4 Nerve Electrophysiology

The resting membrane potential of inactive nerves varies between -40 mV and -75 mV. This is achieved by maintaining a concentration gradient of, predominantly Na\(^+\), ions on the outside and K\(^+\) on the inside of the cell membrane. Opening ion channels in the axon membrane allows these ions to move across the cell membrane, generating an action potential, which is propagated down the axon as ion channels downstream from the site of initiation of the action potential open in succession.

In more detail, upon release of a neurotransmitter at a synapse, ligand-gated channels allow diffusion of Na\(^+\) and Ca\(^{2+}\) into the axon, until a threshold is reached (normally around -5mV). This triggers opening of voltage-gated Na\(^+\) channels, resulting in a rush of Na\(^+\) into the axon (Figure 1.8), creating a region of positive charge (typically to +40mV) or depolarisation of the cell membrane. The positive charge causes the voltage gated Na\(^+\) channels to close, and K\(^+\) channels to open, driving K\(^+\) out of the axon, with the aim of restoring the membrane potential back to its resting phase, ready for the next action potential to arrive. This process is referred to as repolarisation. The wave of depolarisation and repolarisation continues between adjacent nodes of Ranvier in a chain reaction, propagating the impulse along the axon. Finally, the Na\(^+\)/K\(^+\) ion pump restores the resting concentrations of Na\(^+\) and K\(^+\) to its pre-depolarisation state. These charge distributions create an electric potential, which can be
measured using electrodes. Recording of the neural potentials is known as electroneurography (ENG). The voltages are typically <100 μV, an order of magnitude less than their equivalent muscle potentials, which are around 1 mV [48].

1.11 Signal processing

Processing of the raw signal is necessary to extract useful information from EMG (and ENG) signals. The analogue signal is amplified, filtered, to attenuate the background electrical ‘interference’ picked up from domestic power supplies, and then digitised. Since the mean value of the EMG signals about the time axis is zero, non-linear processing (by way of full wave rectification) takes the negative portion of the EMG and turns it into a positive value. The processed signal is then averaged using a low pass filter, resulting in a processed myoelectric signal (PMES). Further processing of this signal enables extraction of the information needed to allows proportional control of the terminal device [45].

The term “signal-to-noise ratio” (SNR) is a measure of EMG signal fidelity. It is the ratio of the signal amplitude to noise amplitude. The higher this ratio, the better the signal ‘quality’. Noise constitutes any component that is not part of the biosignal one wished to measure. For example, electrical interferences from external sources (50Hz power cables), from adjacent muscles, and from movement artefacts, and noise inherent in the recording circuits [5], [49].

1.12 Limitations of Myoelectric Prostheses

Patients have two major complaints about their upper limb prostheses; their limited range of motion and their restricted number of degrees-of-freedom (DoF) especially for hand motion [50]. Restoring full hand motion is a major requirement for complete restoration of function for an upper limb amputee. A normal human hand has 22 DoF controlled by 38 muscles [51]. Therefore, as a minimum, for an upper limb prosthesis to fully recreate intuitive hand movements, 38 separate channels, or complex underactuations are required, each with its own independent, control signal.
1.12.1 Lack of Control Channels

Current, upper-limb, myoelectric prosthetic designs use control signals from just two antagonistic muscle groups in the stump to control 2 DoF, for example hand open-close and wrist rotation. These can be used sequentially to allow additional degrees of freedom to be controlled. This is achieved using a “mode switch”, either in the form of a physical switch on the prosthesis or through a coded signal (for example co-contraction of muscles) to instruct the prosthesis to switch control of the hand from one set of DoF (e.g., hand open/close) to another (wrist supinate/pronate). For example, in the case of the i-Limb Pulse, grip patterns are accessed by input signals described as hold open, double impulse, triple impulse and co-contraction [52]. Using Bluetooth technology (linked to the prosthesis via a graphical user interface (GUI)), the prosthetist can analyse the patient’s EMG signals. Based on this analysis, the prosthetist can then configure a customised control strategy and can change activation and switching thresholds for the individual patient [53]. More recent designs rely on control algorithms to lock out certain functions of the prosthetic hand thereby allowing the prosthetist and end user to create more specific actions (e.g., pointing a finger for typing on keyboards). Such settings can be pre-programmed into the prosthesis and the user can then select these settings depending on the task to be performed.

Despite the impressive levels of function that can be achieved using these approaches, present myoelectric designs are severely constrained using only 2 signals detected by skin-surface electrodes to control all the functions of the prosthetic hand. The cognitive burden of training to use these devices is enormous and many patients are simply unable to manage the complex learning needed to use these devices because the muscle contractions required to move the prosthesis are largely unintuitive and non-physiological. Essentially, with current systems, just two muscle groups (e.g., forearm flexors and extensors after a transradial amputation), are used to control all movements in the prosthetic hand instead of the 38 muscles in a normal hand. Furthermore, actions can only be executed in series, by switching from one control command to the other, making fluid hand motion impossible.

None of this can be described as “normal” (i.e. physiological for the end-user); none of these actions are intuitive. Intuitive suggesting that when the patient imagines a particular action (e.g., hand open or close) the prosthetic hand should also open and close. Instead, current control systems require patients to learn that muscle contractions previously associated with different upper limb functions (e.g., m. biceps and m. triceps after a transhumeral amputation
are normally intended to move the elbow) will now result in hand open and close instead of elbow flexion.

To move closer to normality, there is a need to increase the number of control channels available for control of the prosthesis. One way of doing this is to extract the biosignals directly from the nerves in the residual limb of the amputee, by detecting ENGs. This is investigated in Chapter 6. However, the technology for doing this may take longer to develop than systems that rely on EMG signals because of limitations inherent in the systems in current use for detecting ENGs. Fortunately, there is now a way for increasing the number of EMG signals in the residual limb through surgery by using targeted muscle reinnervation (TMR) and/or regenerative peripheral nerve interfaces (RPNIs).

**1.12.2 Problems with Surface Electrodes**

There are disadvantages inherent to surface electrodes that preclude their use as a solution for long-term, multichannel, intuitive control:

- Lack of permanence – the surface electrodes need to be replaced on a regular basis [30], [54].
- Lack of reliability it is difficult to place the electrodes in the same position over the muscles of the residual limb every time the patient uses their prosthesis, changing the recorded signal [30], [54].
- Impedance changes due to factors such as sweating, resulting in changes in signal amplitude.
- Electrode lift-off from the skin – especially when the patient moves the prosthesis or the rest of their residual limb.
- Cross-talk from deep or adjacent muscle signals. This can be particularly problematic in transhumeral amputees with short stumps where the EMG signals from the deltoid muscle can be detected inadvertently by surface electrodes placed over the remnants of the *m. biceps*/m. *triceps* muscles.
- Unintuitive control, contributing to a high rate of prosthesis abandonment among upper limb amputees [16], [55]–[58], with rates of up to 80% reported in the literature [16], [50], [59].
1.12.3 Proposed Solutions

To improve the function of upper limb, myoelectric prostheses, the quantity (and quality) of the signals that are available to control them must be increased beyond those currently available. This cannot be achieved by simply increasing the number of surface electrodes [60].

One way to address the shortcomings of skin-surface electrodes is to implant the electrodes directly onto or inside the individual muscles responsible for specific actions of the prosthesis. Once implanted, these electrodes get encapsulated within scar tissue and are then unlikely to move, thereby avoiding concerns about lift-off, unreliability of placement and lack of permanence [61]. Since implanted electrodes work in a constant environment, there will be fewer issues with varying impedance especially since the scar capsule that forms around the electrodes will not be detrimental to signal detection and the capsule actually enhances the voltage of the signal [62], [63]. Signal contamination and crosstalk will also be minimised due to accurate placement of the electrodes onto or into the target muscles and because of the insulating design of certain electrodes (e.g., epimysial electrodes with insulating silastic backing which helps focus the ‘pick up area’ to the muscle of interest). In addition, implantable electrodes have been shown to have classification accuracies at least equal to [64], if not greater than [65], surface electrodes when utilising pattern recognition as a way of controlling prosthetic limbs. The next sections contain a summary of both the nerve and muscle interfaces currently available to record biosignals from these two tissues.

1.13 EMG Recording Interfaces

The conventional method for detection of EMGs for upper limb prosthetics is to use electrodes placed onto the surface of the skin overlying the muscles in the residual limb, as described in the previous section. In contrast, implanted, intramuscular and epimysial electrodes have been used for many years for functional electrical stimulation (FES). FES is a technology used to deliver sequential and co-ordinated electrical stimuli to the muscles in the limbs of paraplegic patients which can allow them to regain certain functions like hand grip [66]. The use of implanted electrodes for FES has proven their long-term reliability and durability even after decades of use in vivo. More recently, the feasibility of using implanted
electrodes for prosthetic control has been described by Ortiz-Cataln et al. in several upper limb amputees [67],[68].

1.13.1 Needle Myoelectrodes

Percutaneous, fine-wire, bipolar, EMG electrode needles have been in use for decades for electromyographic studies in both health and disease. Although they can accurately detect EMG signals, their delicate structure and their transcutaneous path have precluded their use for long-term purposes. Instead, they are usually used for short term experiments [60], [64]. Ruff et al [69] modified an epimysial electrode based on a 10 μm thick polyimide structure that was 31mm long and 4mm wide. The electrode was implanted into the deltoid muscle of a rhesus monkey and the electrode cable were passed subcutaneously to the monkey’s head where they were attached to a transcutaneous connector. Using this system, EMG signals were successfully recorded during movements of the monkey’s arm. Similarly, Farina and his co-workers developed a multichannel, intramuscular EMG electrode using a silicon wafer as production platform for polyimide-based electrodes [70]. The electrodes were inserted perpendicular to the muscle fibres of the gastrocnemius muscle of rabbits and were then used to record EMG signals at various depths in the muscle. The recordings were taken with the animal anaesthetized after eliciting the righting reflex and crushing the sciatic nerve. All the experiments were carried out acutely prior to euthanasia of the animals. However, concerns about the longevity and durability of Farina’s system have yet to be answered. Moreover, no attempts were made to devise the means for long-term signal transmission across the skin barrier.

1.13.2 Intramuscular Electrodes

Implantable, intramuscular electrodes have been used for functional electrical stimulation (FES) of paralysed muscles, both in upper limb [66], [71], [72] and lower extremity [73], [74], patients with spinal cord injuries and strokes. These electrodes consist of Teflon®-coated stainless steel wire wound into a helix. The end of the electrode consists of bare metal with a barbed tip to anchor it within the muscle. Despite this, intramuscular electrodes have been observed to migrate beyond the point of their original site of insertion. The electrode is implanted by inserting a hypodermic needle through the skin and into the muscle belly [75]–[77]. The electrodes have the advantage of a compact design that can be inserted into the small muscles of the hand. Both percutaneous [78] and surgically implanted. monopolar
stimulating electrodes have been used [79]. However, they have not previously been used as chronically implanted bipolar EMG sensors.

Studies characterising the tissue response to long-term, intramuscular electrode implantation have only examined the use of this type of electrode for stimulating the muscles (e.g., for FES) and not when the electrodes are used passively for recording EMG signals. The tissue reaction around embedded steel electrodes is typically more severe than that around muscle surface, epimysial electrodes, with thicker capsule formation (535±401μm) and many active fibroblasts and macrophages present in the capsule [61]. These findings have been confirmed in other in vivo comparisons of epimysial and intramuscular electrodes, with intramuscular electrodes showing a much larger amount of tissue inflammation and scarring at the intramuscular implantation site compared with epimysial electrodes (see below) [80]. Furthermore, repetitive trauma from micromovement of the wire tip can alter the biosignal characteristics [81].

1.13.3 Epimysial Electrodes

These bipolar electrodes are positioned on the surface of the muscle and are held in place with sutures. They consist of 2 circular discs composed of Platinum-Iridium on a silastic backing. The cable leading from the electrode is composed of either 316 stainless steel or platinum-iridium. The latter has the added advantage of corrosion resistance. Epimysial electrodes have been in use in FES systems for more than 3 decades. Both monopolar stimulating electrodes as well as bipolar recording electrodes have been used for FES [66], [82], [83].

1.13.3.1 Longevity and Reliability

Studies by Kilgore et al. on epimysial and intramuscular electrodes used in FES have shown a 98.7% probability that an electrode will be intact and functional at 16 years after implantation [82]. Similar studies by other workers have found similarly high levels of long-term survival for implanted epimysial electrodes, with failures attributed to fatigue of the electrode cables over flexural surfaces (e.g., over the gluteus muscle during sitting) [74].

1.13.3.2 Tissue Reaction

Akers et al performed histological analyses on 24, long-term (11 to 50 months) implanted epimysial electrodes and found them to be associated with a minimally active or inactive fibrous collagen capsule (179±299μm), with very few or no macrophages present in the
tissues [61]. The tissue response was graded on a 6-point scale according to the inflammatory response (acute or chronic) to the implanted electrodes. The majority of the electrodes had minimally active or inactive fibrous capsules, respectively [61]. Ackers also noted that suture loss was associated with an increase in the thickness of the fibrous capsule. This could be explained by increased movement between the implant and the adjacent tissues for electrodes with fewer anchoring sutures, resulting in increased mechanical trauma and an increase in the local tissue reaction. Although an increase in the thickness of the capsule might be advantageous for increasing the measured voltage (see below), a stronger and more protracted tissue reaction around the implanted device might eventually impede the detection of biosignals.

1.13.3.3 Effect of Tissue Fibrosis on Potential Difference
Using volume conductor models, Lowery et al. simulated action potentials to explore the influence of the electrical properties of the surrounding tissues on action potential amplitude in implantable myoelectric sensors (IMES) [62]. These are hermetically sealed ceramic cylinder, 16mm long and 2.5mm in diameter with an electrode on each end capable of recording and transmitting EMG signals via wireless telemetry across the skin barrier. They found that the high resistance of the capsule around the implanted electrode restricted current flow, causing an increase in voltage amplitude. Similar findings have been observed in other studies; for example, when a layer of muscle below the electrodes is replaced with more resistive fat, an increase in the voltage is also observed [63], [84], [85]. Fibrosis is therefore advantageous for at least two reasons: for holding the electrodes in place, and for increasing the signal strength.

1.13.3.4 Correlation with fine-wire transcutaneous electrodes
Fine-wire electrodes, as used for electrophysiological studies, offer high-quality and selective EMG recordings. These can then be used as a benchmark to test the performance of implantable myoelectrodes. Hart et al [86] tested an implantable neuroprosthesis with myoelectric control in a dog model, using bipolar epimysial electrodes placed on to the triceps and brachialis muscles to capture EMG signals. To verify that the EMG recorded by the epimysial electrodes correlated with standard recording methods, EMGs were simultaneously recorded using fine-wire transcutaneous bipolar electrodes. The Pearson correlation coefficient was 0.85 between the 2 recording methods, demonstrating a good correlation between the 2 methods.
After considering the pros and cons of the different electrode designs, I elected to use epimysial electrodes for the EMG studies described in this thesis and for recording EMG biosignals for the implantable system. This work is presented in Chapters 2, 3, and 4.

1.13.3.5 Effect of Inter-electrode distance on EMG

The inter-electrode distance is defined as the centre-to-centre distance between all the conductive areas of the electrodes. Although no studies have been conducted to examine the effects of inter-electrode distance for implanted electrodes, the minimum recommended distance for sensor placement procedures and signal processing for surface EMGs is 20mm. However, for small muscles, this distance should not exceed a quarter of the muscle fibre length to avoid unstable recordings due to tendon and motor endplate effects [87].

Generally, the greater the inter-electrode distance, the greater the potential difference between the 2 points. However, since this also increases the distances between which any electrical activity can be detected, the potential for cross-talk may also be increased. Nevertheless, Baker implanted IMESs with an inter-electrode distance of 15mm into the forearm of a primate and found very little in the way of crosstalk from adjacent muscles [88]. Moreover, to date, there have been no in vivo experiments investigating the effects of increasing the inter-electrode distance and the use of tri-polar electrodes on signal to noise ratio.

Therefore, optimising the electrode configuration could be crucial in ensuring selective (electrical) recording of the muscles while minimising crosstalk. This is investigated in Chapter 6 of this thesis.

1.14 ENG Recording Interfaces

In theory, assuming that the proximal parts of the nervous system have not been injured, the peripheral nerve stumps in the residual limb of an amputee should carry all the necessary sensory and motor ENG signals needed to restore full biomimetic control of a prosthetic limb. These nerve stumps previously innervated the now amputated limb. The signals could be recorded by placing the requisite electrodes on or in the nerves. The same electrodes could then serve the additional purpose of providing an interface for direct stimulation of the nerves, providing feedback for the amputee. Studies have demonstrated the feasibility of this
approach in human amputees [89]–[91], allowing the patients to generate usable motor commands, decades after the original amputation.

In practice, active, implanted, nerve electrodes have been used for the treatment of neuromuscular disorders such as a dropped foot [92]–[94], bladder dysfunction [95], [96], and after spinal cord injuries to reanimate the paralysed muscles with functional electrical stimulation (FES) [97], [98]. However, the use of passive, recording, nerve electrodes to capture ENG signals for prosthetic control has remained primarily experimental and the idea that they could be used in this way has only become feasible in the past decade. This is because the technological barriers that have to be overcome have been formidable. For example, ENG signals are a thousand times smaller in amplitude than EMG signals [99]. Moreover, one of the major limitations of studies of implanted nerve electrodes for recording ENG signals is the way in which the signals degenerate quickly after implantation of the hardware. This occurs because nerve tissue is far more sensitive to surgical manipulation and the presence of foreign material (e.g., electrode implants) than muscle.

Below is a brief outline of ENG recording interfaces that have been investigated to date, and a summary of interfaces is shown in Figure 1.10.

1.14.1 Extraneural Electrodes

Cuff electrodes are extraneural electrodes, which are cylindrical in shape, and wrap circumferentially around the nerve. They contain the electrical contacts within the core of the electrode structure, with a silastic backing acting as an insulator. Since they are not as invasive as intraneural electrodes, they trade low selectivity for decreased trauma to the nerve, which (hopefully) generates less fibrotic, foreign-body reaction. Although mainly used in FES patients for nerve stimulation, they have also been used to record ENG signals from the sural nerve (sensory) in patients needing foot-drop correction [100]. They have also shown good long-term function after chronic implantation; 12 years in patients with hemiplegia [101] and 7 years for median and ulnar nerve stimulation for pain control [102]. The formation of scar tissue following implantation encapsulates the electrode and provides stability against displacement. Since they are not as invasive as other nerve electrodes, they are easier to replace in the event of failure. The main disadvantage is that they are less selective in comparison to more invasive electrode designs, especially when recording ENG signals from centrally placed fascicles in a nerve. Flat interface nerve electrodes (FINEs) area a modification of the cuff design, and serves to increase the selectivity of the electrode.
by physically flattening the nerve, thereby spreading the individual nerve fascicles over a wider area, giving the electrode more direct access to the individual fascicles [97], [103]. However, the additional trauma to the nerve increases the likelihood of long-term nerve damage [104], [105]. FINE electrodes have proven longevity when used for active stimulation of nerves in FES but are not a focus of prosthetic control research where the main imperative is the passive recording of efferent signals.

1.14.2 Intraneural Electrodes

Intraneural electrodes are implanted directly into the nerve and are more selective than extraneural electrodes since they are capable of recording ENG signals from individual nerve fascicles. One such example are Longitudinal Intra Fascicular Electrodes (LIFE electrodes), which are inserted parallel to the nerve fibres [106]. These electrodes are comprised of thin wires (<100 μm diameter), which are inserted along the long axis of the nerve, with metal contacts within the polymer-based core acting as electrical points for signal recording or stimulation. Stacking microwires within the LIFEs creates arrays which can record signals throughout the length of a nerve [107], [108]. Short-term implantation in two trans-radial amputees over a 2-week period allowed for stimulation of the nerves for sensory feedback in the form of touch and finger position [109]. In another study by the same group, 4 to 8 LIFEs were acutely implanted into the median nerves of 6 patients who had undergone below elbow amputations, with the electrode cables passed percutaneously. With minimal training, these patients were able to actuate motors in an experimental prosthetic limb to close the hand voluntarily [91]. While these experiments prove that LIFEs can be used to record ENG signals, there have been no long-term studies looking at the reliability of such an approach for chronic implantation. Therefore, the need for a robust method for transcutaneous signal transmission remains an unresolved issue.

One additional drawback to LIFEs is the longitudinal orientation of the wires, which limits the distribution of spatially selective sites to just one fascicle. This led to the development of transverse intra-fascicular multichannel electrodes (TIMEs). These electrodes are implanted at 90 degrees to the longitudinal axis of the nerve. The transverse orientation of the electrode allows for higher spatial selectivity as it can record ENG signals from several different fascicles simultaneously. TIMEs have been used for both control and sensory feedback for a single upper limb amputee in sub-chronic implantation [110].
1.14.3 Micro-Electrode Arrays

Micro-electrode arrays are another design for ENG recording devices and consist of arrays of needle electrodes that penetrate the neural tissue, to create an intrafascicular interface [111]. The Utah Array is the best example and consists of 100 needle electrodes, which were originally intended for use in the central nervous system. An adaptation of this design was created for use in the peripheral nervous system (PNS) [112], [113]. The adaptation used electrodes of different lengths to create a device which appeared slanted and could penetrate a peripheral nerve to different depths, allowing for selective stimulation/recording within different, individual, fascicles. This was tested in the median nerve of a single human subject (Dr K. Warwick). By 100 days, 80% of the electrodes had failed due to wire fatigue of the electrode cable as it exited the skin [114], [115]. Subsequently, the Utah Array was used in animal models, with chronic implantation in cat sciatic nerves (up to 1 year). It was noted that the tissue reaction to the electrode included chronic inflammation, fibrosis and reduced nerve fibre diameter [116].

1.14.4 Regenerative electrodes

These interfaces represent the most invasive of all the available designs, but with the potential for the greatest selectivity. Their design requires complete nerve transection and interposition of the nerve regenerative ‘sieve’ into the gap between the nerve ends. Importantly, the smaller the holes within the sieve, the greater the selectivity of the electrode as it can then record more signals from more axons as they regenerate through the implant. However, a key issue for the use of regenerative electrodes in the long term is how well the nerve regenerates following transection. If used in humans, the electrodes must also remain functional for the lifetime of the patient [117] without compromising any downstream functions of the transected nerves.

The electrodes are sited in micro-channels within the device and connections are made with the electrodes as the nerve fascicles grow through to reestablish continuity with their distal ends [118]. Early studies in a rat sciatic nerve model [117], [117] demonstrated failure of the sieve electrodes after 6-12 months due to micromotion and subsequent fibrosis damaging the axons as they passed through the small diameter sieve holes [119].
Moreover, small diameter channels (50 μm) quickly become blocked by connective tissue [120], a problem compounded by the tissue trauma caused to the nerve itself by the surgery to implant the electrode and the subsequent fibrosis this creates. This results in further reductions in the diameter of the channels for axonal regeneration [120], [121]. Soft polyimide sieves have shown better regeneration properties for short-term implantation in comparison to earlier designs using “stiff” silicon interfaces. However, there is still a functional decline with chronic implantation [117], [119], [122].

Furthermore, any delay in repairing a peripheral nerve results in a dramatic reduction in the number of axons which will regenerate (up to 67% in animal models) [123]. This is particularly relevant for amputees, where nerves require an end organ to regenerate correctly - although TMR (and possibly RPNI) may eventually provide a solution to this problem. To date, there have been no human studies using sieve electrodes.

Figure 1.9: A comparison of different nerve and muscle electrode interfaces in terms of invasivity versus selectivity. Adapted from [117].
Figure 1.10: A comparison of different peripheral nerve interface designs. Extraneural electrodes include; cuff or flat interface nerve electrodes (FINE). Intraneural electrodes are more invasive and include; the Utah slant electrode array (USEA), longitudinal intra-fascicular electrodes (LIFEs), and transverse intra-fascicular multichannel electrodes (TIMEs). Regenerative electrodes include; Sieve electrodes, microchannels (microchannel roll electrode, MCRE), or open on a flat surface (regenerative multielectrode interface, REMI). Images taken from [124]. Figure redacted in online copy.

Microchannel neural interfaces (MNI implants) are a type of sieve electrode. Their design overcomes some of the key issues encountered with other electrode designs, especially signal strength and the effects of the nodes of Ranvier. The design employs long (up to 5 mm), narrow (100 μm) microchannels to achieve the greatest signal gain [121]. MNI implants are a type of regenerative sieve electrodes. They require the transection of a nerve, interposition of the interface and subsequent regeneration of the nerve through holes in the interface. As the regenerating nerve axons grow, they pass through small channels within the interface. Electrodes present in these holes allow recording of ENG signals as they propagate along the regenerated nerve axons. However, MNI implant designs still need to be optimised to reduce fibrosis and encourage axonal regeneration.
1.14.5 Brain Machine Interfaces

Brain machine interfaces use electroencephalography (EEG) or electrocorticography (ECoG) to record brain electrical activity from surface electrodes positioned on the scalp [125] or placed directly onto the sensorimotor cortex of the brain [126], respectively, or record local field potentials or spiking activity using intracortical electrodes. ECoG signals have been used to allow human volunteers to gain 2-dimensional cursor control over individual finger flexion [127], [128]. These studies have demonstrated the potential for direct recording of cortical biosignals using electrodes placed on the scalp or directly on the surface of the brain. Since EEG and ECoG electrodes do not penetrate the brain, the risk of brain damage is smaller than for approaches using direct cortical implantation [129]. However, the methods described remain strictly experimental, and have been used mainly for patients with spinal cord damage where the peripheral nerves cannot be used as a source of the biosignals. A more detailed consideration of the use of direct, brain-machine interfaces is beyond the scope of this thesis and not directly relevant to my work. However, a detailed review of this approach can be found in [129].

1.15 Control Strategies for a Myoelectric Prostheses

Although a number of myoelectric prosthetic limbs with multiple DoF are now commercially available (e.g., the Luke arm—approximately $150,000 per arm) [130], achieving reliable and intuitive control of these prosthetic limbs remains challenging. In the absence of sufficient numbers of myoelectric activation sites in the residual limb, users of the Luke arm are trained to use controllers attached to their shoes instead [131]. These are linked to the prosthetic limb by Bluetooth, wireless technology. Accelerometers in the controllers detect foot movements which are then translated into movements of the arm. However, there is nothing physiologic or intuitive about this method of control. Moreover, while this method of control is suitable for some patients, for others, the learning curve is simply too great and for those with no lower limbs, completely impossible. Most amputees will be unable to afford the cost of a Luke arm and will receive a lower-cost, less complex upper limb myoelectric prosthesis with many fewer DoF (typically only 2). For these patients, the current mainstay of control is direct.
1.15.1 Direct Control

This utilises EMG signals from antagonistic groups of muscles (e.g., flexors and extensors after a transradial amputation) to activate electric motors in the prosthetic hand to open and close. Proportional control is modulated by the strength of the EMG signal – more forceful muscle contractions result in larger amplitude signals which are translated into a stronger grip or faster movement in the prosthetic hand. In situations where alternative hand functions are required, a mode switch, either by way of muscle co-contractions or a mode switch button on the prosthesis, will then instruct the prosthetic hand to switch over to a different mode of action (e.g., wrist rotation), using the same flexor/extensor EMG signals as before. These actions can only happen in series, rather than simultaneously (as in a normal hand) and require high levels of concentration and learning on the part of the end user because such actions are not physiologic or intuitive [132]–[134]. The higher cognitive burden required to use prosthetic limbs with standard methods of control often leads to rejection of the prosthesis. This is because using the prosthesis may not be superior (and may sometimes be worse) than simply using the residual limb as a static post to assist the normal or less disabled upper limb. In contrast, if a control system is reliable and the end user can achieve good levels of functionality, then there is an incentive for them to persist with the complex training required to use the prosthesis. Eventually, control systems will be developed which should allow amputees to achieve completely natural, intuitive, simultaneous movements of all the degrees of freedom of their prosthetic limb. These systems do not currently exist.

1.15.2 Pattern Recognition

Determining the intention of the user by examining the pattern of EMG signals generated by the muscles in the residual limb can provide the basis for more intuitive control. Typically, for pattern-recognition systems, several skin-surface electrodes are placed circumferentially around the residual limb to capture EMG signals from all the underlying muscles. Computer algorithms are then employed to recognise particular morphological patterns amongst these signals which are then interpreted/assigned to specific hand or upper limb functions.

More recently, research in pattern-recognition has focused on deciphering the specific group of EMG signals produced when a normal hand/upper limb performs a particular action, e.g., prehension or wrist rotation. Computer algorithms are used to recognise the specific pattern
of EMG signals and can then use them to predict the intended movement of the amputee. This is then relayed to the prosthesis to produce that specific movement. Initially, problems with reliability limited the widespread acceptance and implementation of pattern-recognition in the rehabilitation community. However, increasingly, pattern recognition has been shown to be more successful than direct control methods. For example, Hargrove et al. randomised 8 transhumeral amputees to pattern recognition versus direct control, and found the pattern recognition patients scored significantly better on the Southampton Hand Assessment Procedure and the Clothespin relocation tasks ($p<0.05$) [135].

This is a rapidly advancing field, and pattern-recognition based methods for control of terminal devices (e.g., Co-apt Systems Inc) are now being used routinely in clinical practice since they are often easier for patients to manage than direct control systems [136]. In practice, patients often need little or no training to use these systems compared with direct control systems, in particular with transradial amputation where muscles controlling the fingers may be preserved. However, pattern-recognition systems still rely on skin-surface electrodes with all of their associated shortcomings. They also rely on the presence of multiple myoelectric activation sites within the residual limb, which are not always present, for example with transhumeral amputation. Moreover, they are expensive to purchase for most end-users. Therefore, direct control methods are likely to remain the mainstay of control systems for some years to come.

### 1.16 Surgical Solutions for Increasing Control Channels

In order to control a prosthetic arm more intuitively, there is a need to increase the number of physiologically appropriate EMG signals to control the end device. Two surgical techniques have been described which achieve this aim; Targeted Muscle Reinnervation (TMR), and Regenerative Peripheral Nerve Interfaces (RPNI).

#### 1.16.1 Targeted Muscle Reinnervation (TMR)

Targeted muscle reinnervation (TMR) is a surgical technique pioneered by Kuiken et al to redirect the peripheral nerve stumps in the amputated limb into residual muscles in the stump or trunk which are no longer in use [137]. For example, after transhumeral amputation, the $m.$
biceps and m. brachialis muscles are no longer required for elbow flexion while the m. triceps is no longer required for elbow extension.

The main purpose of TMR surgery is to create new muscle “switches” (i.e. new sources of EMG signals) that can be used to activate a myoelectric prosthesis. TMR surgery has two additional advantages;

1) Activation of these new sources of EMG signals is completely intuitive (i.e. contraction of the re-innervated “target” muscles occurs whenever the user imagines/expects these muscles to move, as if the reinnervated muscles were still in their normal limb).

2) Activation of these new target muscle “switches” can occur simultaneously (and not sequentially) – in a way that is similar to how groups of muscles in a normal limb would move simultaneously to produce specific functions such as holding a knife or fork.

TMR surgery can be performed at any level of amputation, proximal to the wrist, but is particularly useful for amputations above the elbow. The precise nerve transfers which are performed depend on the level of the amputation, the availability of suitable nerves for transfer and the availability of suitable muscle targets [138].

Pre-operatively, it is important (but not essential) to establish that the patient is able to exert voluntary control over the intended target muscle. This ensures that the target can receive a new nerve supply after division of its normal motor nerve. Moreover, there should be clinical signs that there are action potentials travelling down the intended donor nerves – as indicated by a Tinel’s sign (paraesthesiae) on palpation of the end of the nerve (i.e. the location of the neuromas). Absence of a Tinel’s sign on the intended donor nerve pre-operatively is a cause for concern if a TMR procedure is being considered, and this often happens in patients who have sustained a concomitant brachial plexus injury. If a donor nerve with no active axons is co-apted to a muscle target that was previously functioning normally then this represents a wasted opportunity for the patient who could have used that muscle for some other purpose (e.g., a pattern recognition system – Section 1.15.2).

Importantly, when there are no suitable target muscles in the residual limb, a free vascularized muscle transfer, with preservation of its motor supply, can be employed. For
example, the m. serratus anterior muscle has a segmental motor innervation and each muscle slip can be re-innervated with a separate donor nerve. Similarly, the m. gracilis and m. rectus abdominis muscles may also be used as these muscles also have a segmental motor nerve supply which allows multiple donor nerve stumps to be used to re-innervate different parts of each muscle [139], [140].

There are two, typical, scenarios for TMR in the upper limb:

1) The first is the patient with a transhumeral amputation.
2) The second is a patient who has sustained a more proximal, glenohumeral amputation.

For TMR surgery in cases of transhumeral amputation, the myoelectric signals needed to activate elbow flexion and extension in the prosthesis are normally generated by preserving the normal motor innervation to the long head of m. biceps (via the musculocutaneous nerve) and by preserving the motor branches of the radial nerve to the short or medial head of m. triceps. The EMG signals that allow for ‘hand close or open’ are then created by transferring the distal stump of the radial nerve to the motor branch of the lateral head of the triceps and by transferring the stump of the median nerve to the motor branch of the medial head of the m. biceps [137], [141], [142]. Once reinnervation is complete, when the patient imagines functions that require the contraction of muscles in the median nerve territory (e.g., wrist or finger flexion) then signals are transmitted down the median nerve which result in contraction of the medial head of m. biceps. Differential contractions of these muscles, when the patient is imagining either elbow flexion (long head of m. biceps) or wrist/finger flexion (medial head of m. biceps), are easily discerned under the skin of the residual limb in a transhumeral patient who has undergone TMR surgery. Using skin-surface electrodes, the EMG signals generated by the reinnervated medial head of m. biceps are (equally) easily distinguished from those generated by the lateral head of m. biceps. The same is true on the extensor side of the residual limb in relation to the reinnervated lateral head of m. triceps.

In TMR surgery for cases of shoulder disarticulation/glenohumeral amputation, any of the chest wall muscles can be used as a target for re-innervation since they no longer have any function to move the absent limb. Typically, it is possible to create three new sources of myoelectric EMG signals from the m. pectoralis major muscle by physically splitting the muscle into three separate neuromuscular units; an upper clavicular part and two lower, sternal, parts (Figure 1.11). Other potential targets for nerve transfers on the chest wall include the m. pectoralis minor, m. serratus anterior and m. latissimus dorsi muscles.
However, the latter can sometimes take a long time to reinnervate due to the length of its motor branch. Therefore, in TMR surgery, efforts are always made (wherever possible) to ensure that the donor nerves are co-apted as close as possible to the hilum of the target motor nerve so that the reinnervation distances are as short as possible.

In TMR surgery, efforts are also made to physically isolate the different muscles from each other, to minimize electrical crosstalk [143]. For example, adipofascial flaps can be interposed between the muscles to help isolate the myoelectric EMG signals arising from the different muscles. Moreover, in order to make it easier to detect the EMG signals with skin-surface electrodes, efforts are usually made to thin the subcutaneous fat over the reinnervated muscles, to reduce the conduction distance between the skin-surface and the underlying muscles.

Once TMR surgery has been performed, return of voluntary control over the reinnervated muscles may be evident as quickly as 8 weeks later but can take up to 6 months or more to be complete, depending on the reinnervation distance [144]. Although training of the re-innervated muscles is helpful from the perspective of strengthening the muscles to generate EMG signals for prosthetic control, one of the benefits of TMR, is that the reinnervation process occurs regardless of any specific training. Therefore, patients who undergo TMR can often learn to use a multiaxial prosthetic limb (especially with a pattern-recognition control system) within minutes, even without any specific training [135].

To make full use of the new sources of EMG signals, patients who have undergone TMR surgery are fitted with an array of surface electrodes which are then used by their prosthetists to “map out” the muscle areas which are responsible for specific upper limb functions. Equipped with such a map, a prosthettist is then able to adjust the end-device to respond in a way that allows the patient to regain intuitive and simultaneous elbow and hand control [140], [142], [145].
One observation made in TMR patients relates to the recovery of sensation from the missing hand in the skin overlying the reinnervated muscles [142]. The mechanism for this phenomenon is unclear since it seems unlikely that the sensory parts of the (mixed) donor nerves can physically grow through the target muscle and up into the overlying skin to make connections with the sensory end-organs. Nevertheless, this observation has led to the development of a technique called targeted sensory reinnervation (TSR) [146]. For TSR, a cutaneous branch supplying sensation to the skin of the chest wall is coapted in an end-to-side fashion with either the median or ulnar nerve. As with TMR, the recovery of sensation is not completely normal, with touch perceived more as paraesthesiae arising in parts of the absent limb but is more intense than in patients who undergo TMR alone. Despite this, functional MRI studies have shown that sensory cortical remapping does occur following TSR surgery [108]. Therefore, this observation creates the very real possibility that true sensory feedback from the prosthetic limb may (one day) become possible.

Other limitations of TMR include; its continued reliance on skin-surface electrodes (with their inherent disadvantages) and the (sometimes) limited availability of suitable target
muscles or donor nerves in the residual limb. As a result, it is sometimes difficult to create more than two or three additional myoelectric EMG sites for control of a prosthetic limb. This is a long way from the 38 muscles and 22 DoF present in a normal hand. Moreover, although the presence of a healthy soft tissue envelope is clearly advantageous, its absence is not an absolute contraindication to performing TMR surgery. Where necessary, TMR can be combined with the full range of adjunctive procedures performed by plastic surgeons – including the importation of skin and muscle from other sites, to provide improved skin cover and additional muscles as TMR targets in the residual limb [138]–[140].

Finally, one interesting observation is the ability of TMR surgery to improve the symptoms of both neuroma and phantom limb pain (PLP) in both upper and lower limb amputees. The reduction in PLP in particular is thought to arise because the target muscles provide feedback from an ‘end organ’ to parts of the cortex which still perceive the amputated part [147]. Certainly, in vivo animal models of TMR have demonstrated physical changes in the transferred nerves which regain a more normal architecture following coaptation with a motor nerve in the target muscle [148].

1.16.2 Regenerative Peripheral Nerve Interface (RPNI)

The regenerative peripheral nerve interface (RPNI) technique was developed by Dr Paul Cederna and colleagues at the University of Michigan. As with TMR, the RPNI technique uses skeletal muscle as a natural amplifier of biosignals from a donor nerve. Unlike TMR, RPNI increases the number of available EMG signals without needing to sacrifice valuable and limited donor muscles. Instead, RPNI uses small pieces of, non-vascularised skeletal muscle graft as targets for the donor nerves. The grafts are then neurotised by burying the ends of the donor nerve stumps from the residual limb into the center of the muscle [149], [150]. Initially, the muscle units are physically small enough to survive without a blood supply, through serum imbibition from the surrounding tissues. However, a blood supply is eventually established by normal vascular in-growth from the adjacent tissues. At the same time that the RPNI unit is fashioned, a biocompatible myoelectrode is sutured onto the surface of the muscle units to allow detection of EMG signals. This composite of donor nerve, muscle graft and electrode then provides the biosignals that can be used to control an artificial limb.
Used in this way, RPNI technology has the potential to massively increase the number of EMG signals available to control a prosthetic limb. Instead of using one whole donor nerve attached to just one target muscle (e.g., median nerve stump coapted to motor nerve of medial head of *m. biceps*), the donor nerve can be split into multiple fascicles. Each fascicle can then be buried into a muscle graft to create multiple RPNI units, each generating its own unique EMG signal. The data generated by each RPNI unit can be extracted from the residual limb through either a wireless or hard-wired system. This approach has the potential to provide the multitude of EMG signals required to reproduce and control fine hand and wrist movements - something that is currently impossible to achieve with standard TMR surgery. However, even if it were possible, current forms of prosthetic engineering do not have the means for processing this many signals and transforming them into the fine movements we attribute to a normal upper limb.

Currently, *in vivo* studies in animals have demonstrated long-term EMG signal reliability and selectivity of RPNI units and their associated muscle electrodes [150], [151]. However, to date the challenge remains of how to transmit these EMG signals across the skin barrier in a stable and durable manner that would survive outside the laboratory. Early work using an e-OPRA bone-anchor for signal transmission suggests that this may provide a long-term solution for upper limb amputees [personal correspondence]. However, these data have yet to be reproduced by other groups.

The RPNI concept has also been extended to provide a solution for sensory feedback. Termed sensory RPNI, a sensory nerve can be used to neurotised a muscle unit, in the same manner as for motor RPNI. An electrode is placed onto the surface of the muscle and this is used to stimulate contraction of the muscle. This in turn causes depolarization of the afferent nerve to provide sensory feedback. Electrophysiological testing at 3 months in a rat model has confirmed that sensory RPNI produced reliable signals with minimal cellular inflammatory responses on histological analysis [152]. Using muscle as the interface between a donor nerve and an electrode may then provide the means for avoiding the intense fibrosis and eventual loss of function observed when using electrodes embedded directly into nerves.
1.17 Transmission of Biosignals Across the Skin Barrier

In order to succeed in the real world of an amputee, an implanted, myoelectric, electrode-based system must have a robust, reliable and durable method for taking EMG signals from the internal electrodes to an external prosthesis. The absence of the hardware necessary to achieve this aim has contributed to the absence of any significant progress in developing inexpensive, easily available, multi-channel, myoelectric-controlled prosthetic devices. In principle, there are two solutions:

1. Extract the EMG signals through a hard-wired interface. For example, by using a bone-anchor as a physical conduit to take the signals out of the stump and into the prosthesis. This approach is investigated in more detail in Chapters 3 & 4.

2. Extract the EMG signals through wireless telemetry.

1.17.1 Hard-Wire Connections

Neural interfaces using electrodes embedded directly into nerve stumps are still far from being routinely available for clinical application. Therefore, in this thesis, I have focused my efforts on investigating the use of muscles as a naturally occurring interface and bio-amplifier for biosignals transmitted down the peripheral nerve stumps. This is the same approach as TMR and RPNI surgery. I postulate that the most reliable way for extracting usable EMG signals from the residual limb is to combine the hardware for attaching the prosthesis with the electrode system, to create a single, implantable, device.

Therefore, I created a modified, single-stage, osseointegrated implant. The central core of the device acted as a conduit for transmission of EMG signals from an internally implanted epimysial electrode. The performance of this combined implant was tested in an ovine model.

Since the initial work described in this thesis, the concept of using an osseointegrated implant for extracting EMG signals from internal electrodes has now been used on an experimental basis, in several human, upper-limb amputees [67], [68]. In each of these cases, an e-OPRA, osseointegrated bone-anchor was used to provide the means for direct skeletal fixation of a prosthesis [67]. Simultaneously, the bone-anchor allowed EMG signals from epimysial electrodes to be passed securely to the prosthesis using electrode cables passed through the
bone-anchor. This approach overcomes the problems associated with skin-surface electrodes; especially electrode failure in extremes of weather, loss of signals due to electrode “lift-off” caused by shoulder movements, and problems with reproducibility/reliability of placement of the electrodes over the intended muscle. It is likely that this, and other similar solutions, represent the long-term direction of research in this area.

1.17.2 Wireless Telemetry

Currently, there is only one system where wireless technology has been used to transmit EMG signals across the skin barrier that has been chronically implanted in humans. Implantable Myoelectric Sensors (IMES) consist of a hermetically sealed ceramic cylinder, 16mm long and 2.5mm in diameter with an electrode on each end. The core of the cylinder houses the differential amplifier, which digitises EMG signals and transmits these wirelessly to an external telemetry controller. IMESs receive power from an induction coil worn externally. Conventionally, the coil is built into the prosthesis socket (Figure 1.12). The concept of IMES is an extension of BION (bionic neuron), in which a single-channel implantable neurostimulator was used for FES with the hardware delivered by injection through a large-gauge hypodermic needle [153]. In a similar way, IMES can also be delivered through a 12-gauge hypodermic needle. This form of internal EMG capture offers the potential to target up to 18 muscles in the residual limb (e.g., after transradial amputation) that would normally control movements of the wrist and hand [154]. Preliminary results of the first, in-human (n=2) trial in transradial amputees have been published by Pasquina and colleagues based at the Walter Reed National Military Medical Center. They reported on the outcomes of just one of their subjects who was implanted with 6 active IMES with two redundant implants as backup. In this study, they reported that the subject (using a prosthesis) recovered the ability to perform 3 DoF movements simultaneously (i.e. wrist rotation plus finger and thumb movements) with greater fluidity and less fatigue when performing Southampton Hand Assessment Procedure (SHAP) activities [155]. This trial of the IMES approach is ongoing and the subject of the report was showing signs of continued improvement of function at 6 months after surgery. Outcomes from their second subject have yet to be released. However, several practical limitations of IMES are already evident. The first problem is the need for a power-data coil. Therefore, as currently envisaged, IMES could only be used in patients with a relatively long residual limb (e.g., long transradial or long transhumeral) and not all in patients with a shoulder disarticulation. This limits the use of IMES in combination with TMR for very proximal, upper-limb amputees. Furthermore,
IMES cannot be used if the target muscle(s) are too small or too thin relative to the size of the IMES implant.

Development of an implantable, wireless, system using epimysial muscle electrodes has been the subject of in vivo testing by Bergmeister et al. They used an n=2 sheep model in which a four-electrode system was connected to the central implant. The implant was then sealed intra-operatively - with fast-setting silicone sealant. They used standard epimysial electrodes with stainless-steel contacts and an inter-electrode distance of 10mm. The epimysial electrodes were placed over the brachiocephalicus, triceps, brachialis and latissimus dorsi muscles of each animal and EMG signals from triceps, brachialis and latissimus dorsi were monitored/analysed over a four-month period. To recover the signals from the electrodes, a wireless signal transmission system was created, using a custom-built jacket (containing the data transmitter and induction coil), which was worn by the animals. The induction coil was placed inside a sleeve of the saddle to provide power, trancutaneously, to the electrodes. The coil also allowed the EMG signals to be picked up and transmitted wirelessly - by radiofrequency [156]. Using this system, the authors report that it was possible to pick up high fidelity signals. However, problems with the reliability of the system were attributed to constant shifts in the position of the induction coil within the saddle. Nevertheless, this proof of concept study was promising since it confirmed that a wireless data-transmission system could work when combined with implanted muscle electrodes. This type of technology might be particularly applicable to very proximal amputees who have undergone TMR surgery, where there might be difficulties in fitting a circular coil around the residual limb to detect the EMG signals.
1.7.3 Rationale Behind Current Research

There have been several inventive steps that have led to the development of the work presented in this thesis:

1. The development of a bone-anchored device (ITAP) as a stable bone-anchor for direct skeletal fixation of an upper-limb prosthesis.
2. Recognition of the fact that stable, successful, fixation of an upper limb prosthesis to an amputation stump, on its own, is not enough to restore useful upper-limb function.
3. Recognition of the fact that EMG signals generated by muscles in the residual limb are already being used effectively to control the movement of myoelectric upper-limb prosthetic devices but that placing the detecting electrodes directly onto the muscles may increase the reliability of this interface compared with skin-surface electrodes.
4. Recognition of the value of skeletal muscle as a ‘natural’ amplifier of biosignals from donor nerves using the surgical technique of targeted muscle reinnervation. After reinnervation, the target muscle(s) provide a much larger number of myoelectric signals of greater amplitude than a normally innervated muscle.
5. Recognition of the fact that ENG signals obtained directly from a donor nerve may provide more information for more complex and intuitive prosthetic limb control.
6. Recognition that a bone-anchor can provide a physically robust structure that allows the EMG signals from muscle electrodes to be passed out of the stump and into a prosthesis on a permanent basis.

7. The recognition of the need to optimise the skin-implant seal for bone-anchors such as ITAP to reduce the long-term risks of soft and bony infection.

1.17.4 Aims of this Thesis

1. To determine whether internal (implanted) muscle electrodes can be used to provide a physically robust and reliable method for detecting efferent EMG signals arising in skeletal muscle.

2. To determine whether an osseointegrated implant can be combined with such electrode technology to provide a robust and reliable conduit to take myoelectric signals out of the limb of an animal, *in vivo*, over a long period of time.

3. To determine whether targeted muscle reinnervation (TMR) can be combined with bone-anchor + electrode technology to generate useful EMG signals.

4. To determine the optimal configuration for an implantable electrode system with regards to signal to noise ratio (SNR) and crosstalk.

5. To test several protein coatings for a microchannel neural interface (MNI), a type of peripheral-nerve sieve electrode, to determine whether these coatings can improve the functional, histological and electrophysiological function of these electrodes.

6. To determine whether soft tissue attachment to the ITAP might be improved by using a protein-coated, porous, implant.
2 Chapter 2 – Development of in vivo Animal Model and first Proof of Concept Study

This chapter is separated into two parts; the development of an in vivo animal model followed by an in vivo proof of concept study.

2.1 Introduction to Animal Model Development

The use of animal models in biomedical research is well established and offers advantages over in vitro models by investigating the effects of an intervention within the complex biology of the whole organism. Several species have previously been used in orthopaedic research, including dogs, cats, pigs, cattle, horses, non-human primates, rabbits, guinea pigs, rats and mice [157]. Small animals, such as rats and rabbits, are popular due to their ease of handling, low cost and availability. However, for this study, an animal model with similar size characteristics for bone, muscle and tendon, as a human was required. This would simulate more closely the healing characteristics of humans (smaller animals heal much faster) and allow surgical techniques and instruments that more closely reflect those used in humans to be used.

Although dogs have traditionally been used for orthopaedic research, their use is in decline due to the emotional aspects associated with their more common roles as working animals or pets. The same is true for primates, although they are still widely used for experiments that require animals to be trained to perform certain tasks. For example, implantation of IMES in monkeys [88] [88] and RPNI interfaces in rhesus macaques for control of hand prosthetics [151]. Further limitations to the use of primates are cost and the heavy ethical burden placed on their use in the United Kingdom.

Pigs share physiological similarities with humans but are difficult to handle because of their rapid body growth and weight when fully mature. Moreover, pigs are generally not as docile as other animals of a similar size. Such limitations may be disadvantageous with regards to ensuring the implantable device doesn’t get damaged inadvertently by the animal in the post operative period. Difficulty handling the animal could affect the ability to reliably obtain
physiologic EMG signals over the course of the experiment, and place investigators at undue risk.

Sheep and goats are often used as an alternative to pigs because of their general placidity and easy handling, as well as having weight and bone healing characteristics that approximate those in humans [158]. Moreover, previous animal work leading to the current design of the ITAP bone-anchor was performed in an ovine model [38], [54]. In these studies, the bone-anchor was placed transversely across the tibia to investigate methods for optimisation of the skin/implant seal. Therefore, there was already a wealth of experience in my host institution on the use of ovine models for bone-anchor testing.

2.2 Aims

To identify an appropriate animal model and muscle group for the purposes of recording physiologic (i.e. normal) EMG signals.

To achieve this, an appropriate animal model needed to be selected that met the following criteria most closely:

- Closely resembles the human musculoskeletal system.
- Docile and easy to handle – the animal would be expected to walk on treadmill after surgery to generate physiologic biosignals.
- Independence of movement of the hindlimbs. This would be important when walking the animal on a treadmill and inspecting the implant site.
- Possess large enough muscle groups to allow placement of one or more epimysial electrode.
- Possess motor nerves large enough (> 1mm diameter) to facilitate microsurgical coaptation for my studies involving TMR surgery.

2.2.1 Rationale for Ovine Model

Rodents did not meet the criteria listed above due to their small size and the relative difficulty involved in identifying and repairing the motor nerves of the muscles in their limbs. Most work involves the sciatic nerve and it’s major branches, which supply muscles in the hind
limb. Rabbits were not selected due to the difficulty involved in “walking” them on treadmills, as they are more likely to hop, even when walking at a slow speed. Therefore, a sheep model was selected as it most closely fulfils the criteria outlined above and because there was already extensive experience using sheep for ITAP research.

Following selection of an ovine animal model, an appropriate muscle needed to be identified from which EMG signals could be recorded, and on which targeted muscle reinnervation procedures could be performed. The following criteria had to be met:

- The selected muscle needed to be long enough to resemble the muscles in a human forearm.
- The selected muscle should be ‘expendable’, to ensure that subsequent denervation would not lead to significant lameness for the sheep, a necessary criteria to meet with regards to the ethical requirement for reducing harm and suffering. The selected muscle must not be the only muscle exerting a particular action over that joint, since denervating the muscle would otherwise result in a loss of one degree of freedom of movement over that joint and this might cause gait dysfunction.
- The selected muscle should have a defined mechanical action that crosses over a joint.
- Both the donor and recipient nerves used for the TMR study must supply ‘synergistic’ muscles (i.e. muscles with similar actions) to minimise the effect on gait. Hence, the use of antagonist muscles, such as gastrocnemius and tibialis anterior would not be appropriate.
- The target muscle should have at least 2, separate, motor-nerve branches supplying it, such that it would remain partially innervated when one of the motor branches was transected and coapted to a donor nerve to reinnervate it as part of the TMR experiments.

Although the anatomy of the sheep lower limb has previously been described [159], [160], these descriptions did not contain sufficient detail to allow me to select a muscle that was able to satisfy all the criteria listed. Therefore, I decided that a formal dissection of a fresh, cadaveric, limb was required.
2.3 Animal dissection

2.3.1 Materials and Methods

Previous ITAP research was performed using ovine tibia. For that reason, a suitable muscle in the hindlimb of a sheep was required. To find the most suitable muscle for the TMR studies, a fresh, cadaveric, hindlimb from a female Mule (ovine breed) was disarticulated at the knee joint and skinned to permit close study of both muscle action and innervation. Each muscle in the specimen was dissected out and the points of origin, insertion and innervation were identified and documented.

2.3.2 Results

The posterior compartment of the hindlimb consists of superficial and deep flexors, which join together to form the tendo-achilles. These muscles correspond with the gastrocnemius, soleus and deep flexor muscles in the lower limb of a human. The anterior compartment muscles consist of extensors that act on the hock and fetlock (Figure 2.1 & Figure 2.2).

From my dissection, the origins, insertions, actions and nerve supply of the anterior compartment muscles are summarised in Table 2.1.

Table 2.1: Summary of muscles in the anterior compartment of the sheep hindlimb.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Origin</th>
<th>Insertion</th>
<th>Action</th>
<th>Nerve supply</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis anterior</td>
<td>Tibial condyle</td>
<td>Medial and lateral Phalanges</td>
<td>Dorsiflexion at hock and fetlock</td>
<td>Peroneal nerve</td>
</tr>
<tr>
<td>Long extensor</td>
<td>Tibial condyle</td>
<td>Lateral part of metatarsals</td>
<td>Dorsiflexion at hock</td>
<td>Peroneal nerve</td>
</tr>
<tr>
<td>Peroneus tertius</td>
<td>Lateral tibial condyle</td>
<td>Medial part of metatarsals</td>
<td>Dorsiflexion at hock</td>
<td>Peroneal nerve</td>
</tr>
<tr>
<td>Peroneus longus/Extensor digitorum lateralis</td>
<td>Lateral tibial condyle</td>
<td>Lateral phalanges</td>
<td>Dorsiflexion at hock and fetlock</td>
<td>Peroneal nerve</td>
</tr>
</tbody>
</table>
Figure 2.1: A; Sheep on deckchair showing lower limbs. B; Close-up of lower limb anatomy.
Figure 2.2: A; Lateral view and B; anterior view of the left lower limb dissection of a sheep, with skin removed to expose underlying muscles, tendons and bone. In B, *m. tibialis anterior* is retracted with the right hand. PT; *m. peroneus tertius*. TA; *m. tibialis anterior*. PL; *m. peroneus longus*. 
2.4 Discussion

In humans, the peroneus muscles (longus and brevis) are located within the lateral compartment of the lower limb. Their main functions are to evert the foot and assist in plantarflexion. The peroneus tertius muscle in humans arises within the anterior compartment and takes its origin from the medial fibular shaft to insert onto the dorsal surface of the 5th metatarsal [161]. It acts as a weak extensor (dorsiflexion) of the ankle joint.

In the sheep hindlimb, I found 2 extensors that corresponded to the tibialis anterior and flexor digitorum longus (long extensor) in humans. There were also 2 laterally placed extensors. The first appeared to correspond to the m. peroneus tertius in humans, and was labelled accordingly. The other, located posterolateral to this, was identified as the m. peroneus longus/extensor digitorum lateralis – in agreement with Konno et al [159].

2.4.1 Posterior compartment muscles

I decided to avoid using any of the muscles in the posterior compartment for my TMR studies because:

1. The posterior compartment consists of 3 major flexor muscles – gastrocnemius, soleus and deep flexors. I found that the deep flexors were difficult to separate surgically as their fibres interdigitate extensively. Furthermore, accessing the motor nerves proved technically difficult as the motor nerves to these muscles branch extensively in the popliteal area. Therefore, accessing and identifying these nerves in vivo would require a prolonged and complicated dissection which would (in turn) result in a prolonged post operative recovery for the sheep.

2. I found that the motor nerves coming from the tibial nerve (to supply the muscles of the posterior compartment) are short in length. Therefore, when divided, I found that they did not reach the intended donor muscles directly. If selected for my TMR studies, this would have required the use of an interposition nerve graft to bridge the gap between the donor nerves and the target motor nerve. Reinnervation is suboptimal when a nerve graft
is interposed, compared with direct coaptation of a donor nerve with its target motor nerve [162].

3. I found that the posterior compartment muscles possessed a single motor branch entering their muscle bellies. If I used the gastrocnemius and soleus muscles as targets for my TMR studies, then both muscles would have to be denervated resulting in complete paralysis of most of the muscles in the posterior compartment - until reinnervation had occurred. This would have caused significant lameness of the animal, due to its inability to plantarflex at the fetlock (ankle) and hock (metatarsophalangeal joint).

![Retraction of the m. peroneus tertius revealing the segmental nerve supply (arrows) to m. tibialis anterior.](image)

Figure 2.3: Retraction of the *m. peroneus tertius* revealing the segmental nerve supply (arrows) to *m. tibialis anterior*. 
2.4.2 Anterior Compartment Muscles

The *m. peroneus tertius* muscle is the smallest in the anterior compartment. It is innervated by a single motor branch arising from the peroneal nerve. The action of this muscle is synergistic with the rest of the anterior compartment muscles, especially the long extensor, since it mirrors its point of insertion, on the contralateral side of the metatarsal. Therefore, loss of function in *m. peroneus tertius* would be unlikely to result in significant lameness of the animal.

The nerve supply to the tibialis anterior/long extensors comes from several branches which arise from the peroneal nerve (Figure 2.3). In the course of the dissection, it became apparent that one of these branches could be used as the donor nerve for *m. peroneus tertius*, whilst maintaining an adequate nerve supply to the rest of the anterior compartment muscles (i.e. tibialis anterior/long extensors).

Furthermore, surgical access to the anterior compartment muscles and nerves proved much easier because the motor nerves supplying the tibialis anterior/long extensors are long. With careful dissection, it was possible to dissect out a donor nerve from the tibialis anterior that would easily reach the target motor branch to *m. peroneus tertius* without the need for an interposition nerve graft.

2.5 Conclusion

Based on my findings, I decided to use the *m. peroneus tertius* as an appropriate muscle to investigate the function of implanted epimysial electrodes. My cadaveric dissections suggested that this muscle would also be ideal as the target muscle for my TMR studies, using one of the branches from the deep peroneal nerve (supplying the *m. tibialis anterior*) as the donor nerve. The second part of this Chapter investigates the use of an epimysial electrode to record EMG signals in a conscious sheep. I describe the process for implanting the electrode on the *m. peroneus tertius* and the methodology used to record, transmit and filter signals that could be used for control of a myoelectric prosthesis.
2.6 Introduction to in vivo Study to Record Physiological EMG Signals

It is important to select an appropriate type of implantable electrode for recording EMG over the long term. Epimysial electrodes have several advantages: their selectivity, safety and longevity of epimysial electrodes are well established [61], [62], [74], [82], [83]. They are easier to place accurately onto the desired muscle and are less likely to migrate compared with their intramuscular counterparts, especially in the initial weeks after implantation, since they are sutured to the epimysium. Their design has the added benefit of reducing crosstalk due to the inclusion of a silicone backing. This backing also confers an advantage as the mechanical properties of silicone are closer to those of the muscles than stiffer alternatives including polyimide and silicon. Therefore, mechanical stress to the tissues, especially during movement, can be reduced. This may reduce the inflammatory responses to the implanted device. For these reasons, epimysial electrodes were chosen for these experiments.

The following experiments were carried out to determine if a bipolar epimysial electrode could be used to capture EMG signals from the *m. peroneus tertius*. Similar, epimysial electrodes have been successfully used for FES to send electrical impulses from implanted stimulators to stimulate paralysed muscles and restore function in both upper [72] and lower limbs [74].

2.7 Aims

To assess the function of a bipolar epimysial electrode for recording EMG signals arising from the *m. peroneus tertius* in an ambulating sheep.

2.8 Materials and Methods

2.8.1 Electrode design

A bipolar, epimysial electrode (Ardiem Medical Inc., PA, USA) was used for this study. The electrode consisted of 2 circular Pt/Ir disks (surface area 10.64 mm²), with an interelectrode distance of 10.0 mm (centre to centre). The disks were mounted on to a silicone backing which was polyester-reinforced to prevent cheese-wiring of the silicone material when this
was sutured to the muscle. The electrode lead was composed of multistranded 316 stainless steel, 2-core, coiled cable with fluoropolymer insulation, that was completely encapsulated in silicone.

The silicone was stripped off the ends of the flying leads and soldered onto a two-channel socket (Figure 2.4). Heat-shrink, insulating polymer was used to insulate the contacts and the socket, together with an 8 cm length of lead. This assembly was then encased in medical grade silicone and cured for 12 hours at 50°C. The electrical integrity of the construction was tested using a closed-circuit multimeter, which demonstrated a closed circuit between each of the circular disks and the corresponding points of contact within the socket.

Figure 2.4: The modified external part of the wire is shown, with the epimysial electrode in the background.

### 2.8.2 Signal measuring equipment

The MP150 System with AcqKnowledge (Linton Instrumentation, Norfolk, UK) was used to capture, amplify and process EMG signals. The external hardware consisted of:

1. An amplifier cable consisting of 2 shielded leads (positive and negative) with a separate reference lead. The ends of the leads were modified by soldering a plug, corresponding to the electrode socket, onto the positive and negative ends of the leads. The electrical integrity of the connections was tested using a closed-circuit voltmeter.
2. An EMG amplifier box (MP 150 and EMG 100). This connected the amplifier cable to a recording laptop via an Ethernet cable.

3. A laptop computer running AcqKnowledge software for recording and processing EMG signals.

2.8.3 Operative Procedure

All animal procedures were carried out in accordance with the Home Office Animals (Scientific Procedures) Act 1986. A skeletally mature, female Mule (ovine breed) was used for this experiment. The animal was premedicated with xylazine hydrochloride half an hour prior to the operation. The animal was then intubated and ventilated under general anaesthesia.

Under anaesthesia, the left hindlimb was shaved and prepped with betadine and chlorhexidine and the animal was placed into a right decubitus position to facilitate access to the left hindlimb. A longitudinal incision was made, 5cm distal to the tibial plateau and 2cm from the lateral border of the tibia. Dissection through the fascia was performed and the peroneus muscle was quickly identified. The epimysial electrode was then sutured to the epimysial surface of the muscle with 4-0 prolene sutures (Figure 2.5). The socket part of the electrode was then passed through the skin using a separate stab incision, lateral to the original skin incision. The electrode lead was secured to the skin with a 2-0 silk suture (Figure 2.6). The wounds were washed with saline and closed in layers with 2-0 Vicryl (absorbable) suture (Ethicon Inc., NJ, USA). Mepitel (Mölnlycke Health Care Limited, Bedfordshire, UK). A dressing composed of sterile gauze and a bandage were fixed over the incision.

Antibiotics were given for 3 days post-operatively and fentanyl patches were used for one week. Full details of the perioperative and anaesthetic regimen can be found in the appendix section.

The animal was individually housed, and the first EMG signals were obtained 24 hours post-operatively.
Figure 2.5: Surgical exposure and identification of the *m. peroneus tertius* (left). Epimysial electrode sutured on to the epimysial surface of the muscle (right).

Figure 2.6: Appearance following skin closure and after securing the external part of the electrode lead using silk sutures.

### 2.8.4 Signal Recording

For these studies, the animal was walked on a treadmill 1.8km/hr, which is within the physiological walking speed of a sheep. Weekly readings were taken, with the first reading being taken one week post-operatively. For recording, the external part of the electrode was connected to the amplifier cable (plug and socket interface). Recordings were made using a BIOPAC EMG100C differential electromyogram amplifier and an MP150 data acquisition system with AcqKnowledge version 4.1.1 software (all from BIOPAC Systems, Inc., Goleta, Calif.). The recording parameters used were: 1000 samples per second, 100 Hz to 500 Hz band pass, 50 Hz notch filter, and 500× amplification. A reference electrode, consisting of a
21G hypodermic needle connected to the reference lead was inserted subcutaneously over the ankle, just before each recording was made. This part of the limb was chosen for the reference electrode since it does not contain any muscles (tendons only), minimising signal contamination. The EMG recording box was connected to a laptop running AcqKnowledge software for real-time recording of signals.

Signals were recorded for up to 3 minutes at a rate of 10,000 data points per second (10 kHz) at 500× gain, 1000 samples per second (sps). Since most of the EMG signals are in the 10 Hz to 500 Hz range [43], the band-pass was set at 100 Hz to 500 Hz. The lower end of the band-pass was selected to filter out artefacts (typically low frequency) and interference (typically 50 Hz power line interference). This selected band-pass is sufficient for identifying EMG signals associated with movement and to classify movement patterns [69].

Multiple reference electrode locations were assessed to find the best location for the reference electrode whilst trying to inflict minimal suffering to the animal. The measurements were repeated; once with a skin surface reference electrode placed onto the leg (over the ankle joint); and once with a needle reference electrode placed in the back of the animal (subcutaneously over the lumbar spine). The lumbar placement was intended to limit movement of the reference electrode and therefore movement artifact from that electrode.

Absolute values were taken prior to signal analysis. Using the AcqKnowledge software, the Mean Power was calculated over 0.25 second epochs in a manually selected 4-second section (3 or 4 gait cycles) of each recording. Epochs with greater than mean average Power were considered as EMG signals, all other epochs were considered as noise. Signal to noise ratios (SNR) were then calculated as the ratio between the mean absolute signal EMG and the mean absolute noise EMG. A more comprehensive description of the signal processing employed using AcqKnowledge software is outlined in detail in the appendix, in “EMG Recording Protocol”.

2.8.5 Repair of the socket

At week 7, excessive noise was observed, and on closer inspection of the electrode socket the soldered contacts were found to be broken (Figure 2.7). An attempt to repair this with the sheep under general anaesthesia was made, and then further signal recording was performed, both with the animal stationary, and walking. Both recordings showed excessive artifact with
no discernable EMG spikes. Therefore, after 7 weeks, the animal was sacrificed and the myoelectrode assembly was removed \textit{en-bloc} leaving the surrounding tissues intact. The myoelectrode and any adherent tissues were then prepared for histological analysis according to the protocol in the appendix and further detailed in section 3.4.13.

Figure 2.7: A; the broken external connector. B; electrode following repair of socket. A piece of plastic card was interposed between the soldered metal contacts to avoid a short circuit. C; a plastic sleeve was placed over the socket and filled with quick-curing silicone (right).

2.9 Results

2.9.1 Gross Morphology

Visual inspection of the exit point for the electrode cable showed some clear exudate but no evidence of infection. The surrounding muscles appeared to be healthy, with scar formation around the muscle from the implant surgery (Figure 2.8). There was also a well-formed layer of scar-tissue surrounding the electrode and its lead.
Figure 2.8: Gross appearance of the electrode on explantation, showing fibrosis covering the epimysial myoelectrode and the electrode lead. The wire has been (partially) exposed (arrows).

2.9.2 Histology

Histology showed that a dense fibrous capsule of between 600 μm and 680 μm thickness had formed around the electrode (Figure 2.9). The capsule consisted of collagen fibres arranged in a parallel orientation, with fibroblast nuclei interspersed within the fibres. A further layer of fibroblasts was observed at the interface between the electrode and the underlying muscle. During histological processing, the silicone backing was lost from the section as it failed to integrate with the resin used for embedding the specimen. Therefore, in subsequent histological processing, the electrode itself was removed to prevent tissue loss and resin fracture.
2.9.3 EMG Recordings

The socket connection eventually broke and an attempt to repair this failed. Therefore, the experiment was terminated at week 5. Initial readings taken at day 7 showed high levels of noise and low signal amplitude (Figure 2.10). However, subsequent readings showed an improvement in the SNR, presumably due to maturation of the scar tissue (capsule) around the electrode. The least noisy readings were taken 14 days after implantation, using a reference needle electrode placed in the limb (Figure 2.11). The calculated SNRs are presented in Figure 2.12 and Table 2.2. Due to the small sample size, descriptive statistics were used, without further inferential tests, as this is unlikely to provide statistical significance to infer robust conclusions.

Figure 2.9: Toludine blue staining of a longitudinal section through the electrode viewed under light microscopy, 10x magnification. Note the thick capsule seen directly above the muscle layer.
Figure 2.10: Example of noisy signal recording, taken at day 7 post-operatively.

Figure 2.11: EMG recordings 14 days post-implantation. The red signals are the raw EMG signals, blue represent the rectified signals using the Root Mean Square function, which is the square root of the arithmetic mean of the squares of the original values. This removes negative value deflections.
Table 2.2: SNR from epimysial electrode with reference electrode placed in back or limb.

<table>
<thead>
<tr>
<th>Days post-implantation</th>
<th>Reference Electrode</th>
<th>SNR (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Limb</td>
<td>2.31</td>
</tr>
<tr>
<td>7</td>
<td>Limb</td>
<td>2.54</td>
</tr>
<tr>
<td>7</td>
<td>Limb</td>
<td>2.37</td>
</tr>
<tr>
<td>14</td>
<td>Limb</td>
<td>6.05</td>
</tr>
<tr>
<td>14</td>
<td>Limb</td>
<td>6.30</td>
</tr>
<tr>
<td>14</td>
<td>Limb</td>
<td>9.31</td>
</tr>
<tr>
<td>23</td>
<td>Back</td>
<td>3.67</td>
</tr>
<tr>
<td>23</td>
<td>Back</td>
<td>3.64</td>
</tr>
<tr>
<td>23</td>
<td>Back</td>
<td>7.48</td>
</tr>
<tr>
<td>29</td>
<td>Limb</td>
<td>3.27</td>
</tr>
<tr>
<td>29</td>
<td>Limb</td>
<td>6.04</td>
</tr>
<tr>
<td>29</td>
<td>Back</td>
<td>1.63</td>
</tr>
<tr>
<td>35</td>
<td>Limb</td>
<td>2.95</td>
</tr>
<tr>
<td>35</td>
<td>Limb</td>
<td>2.56</td>
</tr>
<tr>
<td>35</td>
<td>Limb</td>
<td>4.07</td>
</tr>
</tbody>
</table>
Figure 2.12: SNR values over implantation period. Note deteriorating SNR over time, which failed after 5 weeks.

2.10 Discussion

In this study EMG signals were recorded from a walking ovine model for 5 weeks. Placing the reference needle electrode at the ankle seemed the most appropriate, as this configuration would most accurately reflect the location of a reference electrode in a fully implanted system (e.g., when used in the residual limb of an amputee). In practice, the SNR was indeed higher when the reference electrode was placed in the ankle versus the back of the animal. This might have been due to the absence of signal generating muscle tissue in the ankle (only tendons found at this level). Likewise, placement of the reference electrode in the back produced a greater amount of movement artefact, although this could also have been attributed to excess movement of the lead connecting the electrode with the signal recording equipment.

The signal was much less noisy by week 2 compared with week 1. Based on previous studies, it was anticipated that EMG signal quality would improve with time as a fibrous
capsule formed around the electrode, fixing it in place and reducing relative movements between the electrode and the underlying muscle. Moreover, the capsule that formed between the electrode and the muscle would have acted as an insulator, increasing impedance and the amplitude of the potential difference measured by the electrode [63], [84], [85]. Further, training effect and post-operative recovery are expected to cause a recovery in gait following surgery, to which low initial signal amplitudes are attributed.

For this study, a band-pass of 100 Hz to 500 Hz was used. This filters out low frequency noise, including the 50Hz generated by currents in the mains electricity supply. Signals were sampled at 1 kHz (the Nyquist rate), which is the lowest sampling rate that will permit alias-free signal sampling. Although signals could be sampled at a higher rate, this would place an additional processing burden (which slows down the response of an end-device) on any prosthesis without a significant gain in control accuracy.

However, in practice, the connectors were not robust enough to withstand the rigours of repeated disconnection and reconnection. Failure of transcutaneous electrode leads at the skin interface has been described by other workers. In addition, the ‘barn’ environment, and animal movements, especially repeated sitting and standing, contributed to mechanical failure of the electrode device. Attempts to repair the contacts were unsuccessful, as evidenced by the presence of excessive noise and the absence of discernible EMG signals following repair, after which the experiment was terminated.

Macroscopically, the electrode was encapsulated with scar tissue, a normal reaction to the implantation of any foreign body. Similar encapsulation with scar tissue formation is observed after implanting silicone based implants in humans – for example after using breast implants [163]. The silicone backing into which the Pt/Ir discs was embedded was lost during the process of preparing the histological specimen. However, histological examination of the tissue specimens confirmed that the scar that formed a capsule around the electrode was composed of fibrous connective tissue. This surrounded the former position of the electrode and was also situated between the electrode and the underlying muscle, forming a layer, which was up to 680 μm thick. This concurs with the findings of other groups investigating the use of epimysial electrodes [61], [164].

Although the device failed after 5 weeks in this experiment, the EMG signals were recorded successfully using this design for an implanted epimysial electrode. However, the transcutaneous design was too fragile to withstand the rigours of repeated
connection/disconnection and failed at the point of connection of the socket with the electrode lead. This emphasised the need for a more robust design both for the subsequent experiments and for use by amputees in the future. Specifically, any future design would need to cope with repeated attachment/detachment of the recording lead and would also need to tolerate the harsh barn environment.

2.11 Conclusion

In this experiment, I was able to show that my design for an implantable epimysial electrode sutured onto the surface of the *m. peroneus tertius* can be used successfully to record physiological EMG signals in a live animal model. The external part of the electrode connector eventually failed, resulting in an inability to obtain good quality EMG signals. Therefore, in the next Chapter, I investigate the design of a more robust interface, using a bone-anchor as a physical conduit for taking the EMG signals from the epimysial electrodes transcutaneously.
3 Chapter 3 – Design and Testing of the Bone-Anchor-Electrode Device

3.1 Introduction

Current upper limb prosthetics can be either body or myoelectric controlled. Body powered prostheses remain popular in low-income countries, because they are lightweight, cheap to build and can be maintained in regular use with little maintenance. However, the functions that they restore are limited and they do not provide intuitive control for the end device, relying instead on surrogate movements (e.g., shrugging of the shoulders) to apply a force to a wire that opens/closes the end device. Therefore, training to use body-powered prostheses can be problematic and many upper limb amputees quickly stop using them.

In contrast, current forms of myoelectric prosthesis rely on skin-surface electrodes to capture EMG signals that activate electric motors that produce the movements in the end-device. While some of these movements can be intuitive (e.g., hand open/close for a transradial amputee), others are not. Instead, the amputee must rely on mode switching to generate further DoF in the end-device Mode switching involves actions such as co-contraction of the muscles in the residual limb, physically pressing a switch button located on the prosthesis using the normal hand (impossible for a double amputee) or other complex, non-intuitive manoeuvres such as a double contraction in extension (like double clicking a computer mouse) [165].

There are many other limitations inherent to using a myoelectric system for controlling upper limb prosthesis. These are largely related to the inability of the skin-surface electrodes to distinguish electric signals emanating from superficial and deep muscles. To the signal processors, EMG signals from wrist flexors produce the same EMG signal as finger flexors. One solution is to use a system which can selectively record EMG signals from individual muscles. An implantable myoelectrode system has the potential to achieve this aim.
3.1.1 EMG Recording

3.1.1.1 Surface Electrodes
Current recording systems rely on skin-surface electrodes to detect EMG signals arising from muscles in the residual limb. There are many advantages to such a system including; the low cost of the electrodes, the non-invasive nature of the recordings and the ready availability of surface electrode recording systems. However, surface electrodes also have significant limitations; signal degradation caused by changes in skin impedance secondary to sweating, inconsistency in the optimal position for the electrodes because the skin moves around in relation to the underlying muscles, electrode lift-off when the amputee moves their residual limb (especially if the socket if not tightly fitting), and motion artifact – for example [16], [55]–[58].

3.1.1.2 Implantable Electrodes
Implantable muscle electrodes have been in use for FES, for more than three decades. Typically, FES systems use epimysial electrodes, which are sutured onto the surface of the muscle, or intramuscular electrodes, which are injected into the muscle, and are held in place with barbs Kilgore et al examined 204 epimysial and 34 intramuscular electrodes implanted between 1986 and 1999 for the purpose of FES to determine their survival over time. Kaplan-Meier analysis of the data showed a 98.7% probability that an FES electrode would still be functioning at 16 years after implantation [82]. Similarly, Uhlir et al reviewed 86 fully implanted, stimulating epimysial electrodes used for FES in patients with tetra or paraplegia. All electrodes were implanted in the lower extremities. There were four mechanical failures, all in the posterior muscles, which was attributed to shear forces and pressure exerted by the tissues on the electrodes with the patients in the seating position. Despite this, Uhlir reported that the overall probability of the electrodes remaining functional was high, with 4-year survival estimated at 90% [74].

The data on implant location also suggest that recording EMG signals with epimysial electrodes is as good as recording with intramuscular electrodes. Hart et al tested an implantable neuro-prosthesis for myoelectric control in a dog model and used bipolar epimysial electrodes placed on the triceps and brachialis muscles to capture EMG signals. To verify that the EMG signals recorded by the epimysial electrodes correlated with standard recording methods, EMGs were also recorded simultaneously, using fine-wire transcutaneous bipolar electrodes. Analysis of the EMG data showed strong correlation between the 2
recording methods confirming that an epimysial location is satisfactory [86]. More recently, Sando et al compared the tissue response between epimysial and intramuscular electrodes in rat hindlimbs coupled to RPNIs. In this study, the authors report that the SNR was greater for epimysial electrodes compared with the intramuscular type. Histological analysis suggests that one reason for the difference is the thickness of the capsule around the epimysial electrodes (<300 μm). In contrast, the intramuscular electrode sites showed substantially more inflammation, muscle necrosis and thicker fibrous capsule formation. They reported a significantly better SNR in the bipolar electrode arrangement compared to a monopolar configuration [80]. Other studies have confirmed that intramuscular electrodes migrate outside their target muscle and produce a greater tissue response (535±401 μm fibrous capsule), with many active fibroblasts and macrophages compared to epimysial electrodes [61].

3.1.1.3 Transmission of EMG Signals Across the Skin Barrier

Key to the success of any implanted electrode system is the development of a robust and reliable method for extracting EMG signals from implanted electrodes and transmitting them across the skin barrier. There are two approaches which have been intensively studied: wireless signal transmission, or the use of a hard-wired (physical) connection.

As previously described in my introduction, wireless signal transmission systems (using IMES) have been used for successful transcutaneous detection of EMG signals in humans for complex prosthetic control [155]. Similarly, animal experiments with an implanted, epimysial, four-channel system using wireless telemetry have been promising, achieving successful transcutaneous transmission of high fidelity EMGs [156]. However, the reliability of this particular system remains an unresolved issue. Although successful, in my view, these approaches were not sufficiently well characterised or reliable to be used for my experiments.

A simple example of a hard-wired connection passes the electrode leads from the epimysial electrodes, transcutaneously to the recording system. This is the approach used for the experiments described in Chapter Two. Using this approach, the electrode cables often fail mechanically due to fatigue of the wires at the point at which they exit the skin [114]. This is the same conclusion arrived at in my experiments. As an alternative, head connectors have been described in which a bone-anchor is used as a stable platform for passing signals from implanted electrodes across the skin. Head connectors are composed of a simple bone screw which is attached to the outer table of the skull of an animal and this provides a stable
platform through which the electrode cables can be passed across the skin barrier. Bone-
screws have been used in human trials of FES, where the electrode cables are passed through the bone-screw then subcutaneously to the muscles of interest. They have also been employed in animal experiments involving both rhesus monkeys [69], [151] and rats [80]. The bone screw reduces the amount of movement of the cable at the point at which it is passed through the skin and this reduces the amount of cable fatigue, thereby reducing the risk of mechanical failure and confirming the value of this approach.

As described in my Introduction, direct skeletal fixation of a prosthetic limb using an osseointegrated bone-anchor has revolutionised the way that a prosthetic device can be attached to the residual limb of an amputee [20]. [20]. Using a bone-anchor eliminates the vast majority of skin-related problems such as, sweating, chafing, and skin ulceration caused by interactions with the socket or straps used to secure the prosthesis to the residual limb. Furthermore, bone-anchors simplify the donning and doffing of the end device and improve the range of motion of the residual limb by eliminating the restrictions imposed on the proximal joint by a socket and straps. Other advantages of using a bone-anchor for securing a prosthesis include; osseoperception – the ability of the wearer to receive ‘feedback’ from the prosthesis through vibrations transmitted through the bone as well as an increased perception that the prosthesis is part of the wearer [166].

As stated in the Introduction, combining the two technologies together (i.e. osseointegrated bone-anchors and implanted epimysial electrodes) could provide a robust and reliable method for securing a prosthesis to an amputee while simultaneously providing the means for allowing EMG signals from the implanted electrodes to be passed transcutaneously to a recording device in the prosthesis. However, in order to use the osseointegrated bone-anchor for direct skeletal fixation routinely, we would also need to be able to create a robust skin/implant seal - reproducibly. Therefore, one of the key areas for investigation in this thesis was to determine if the reliability of the skin/implant seal could be improved through modifications in the porosity of the bone-anchor design and to examine the effects of surface coatings on soft tissue infiltration and dermal cell attachment. Details of my experiments in these areas are described in Chapter Seven.

The rapid mechanical failure of the external connector used for the experiment described in Chapter Two underlined the need for a more (mechanically) robust design to reduce the risk of subsequent failure of my efforts to record EMG signals from the implanted electrodes. To
achieve this outcome, the aim of the next experiment (Experiment 1) was to modify the existing design of the bone-anchor used in previous studies by my research group. The design would be modified to accommodate the electrode lead from my implanted epimysial electrode and the entire assembly (implanted electrode + electrode lead + bone-anchor) would then be tested in a conscious, walking, sheep model. This experiment would also allow me to investigate the performance of the bone-anchor and to determine the effectiveness of the electrode system in terms of signal contamination from adjacent muscles.

Therefore, this Chapter investigates the use of a bone-anchored device for transcutaneous transmission of EMG signals from an implanted epimysial electrode, in a sheep model. The first experiment was a proof-of-concept study with n=1 sheep. Experiment 2 of this Chapter replicates Experiment 1, but in n=6 sheep. In each sheep, the bone-anchor + electrode device was implanted into the left tibia, with two other bone-anchors inserted into the right tibia to investigate the effects of flange porosity and surface coatings on soft tissue adherence to the implant. These other experiments (which also form part of my thesis) are described in detail in Chapter Seven.

3.2 Aims: Experiment 1

- Design and test a bone-anchor + implanted electrode device, ready for use in a sheep model.
- Implant the device in a sheep model and obtain physiological EMG signals.
- Investigate the effects of EMG crosstalk by stimulating muscles adjacent to *m. peroneus tertius*.

3.3 Materials and Methods for Experiment 1 (N=1 sheep):

All *in vivo* procedures were carried out in accordance with The Animal (Scientific Procedures) Act UK, 1986 (revised 2013) and local guidelines, at the Royal Veterinary College, Hawkshead Campus.

The following experiment is described in Al-Ajam et. al. [54].
3.3.1 Design and Construction of a Bone-Anchored Device

The bone-anchored device was made from Ti-6Al-4V based on the original ITAP design [20]. The device consisted of an intraosseous component (stem) with a tapered end to allow it to be press-fitted into the bone following preparation of a suitable transcortical hole in the sheep tibia using a tapered reamer (Figure 3.1). Addition of a flange to the original design has previously been shown to encourage the overlying dermis to become adherent to the surface of the implant and to prevent down-growth of epidermal cells at the skin-implant interface [38]. The stem of the bone anchor was machined on a lathe and the flange was added afterwards using laser sintering. The flange consisted of porous titanium alloy with 700 μm diameter pores and a 300 μm strut diameter. A straight, 2 mm diameter hole was drilled through the device, passing obliquely from the top of the transcutaneous component of the bone-anchor and exiting immediately beneath the flange (Figure 3.1). The hole was designed to accommodate the electrode lead. A separate metal sleeve (to house the electrode lead socket) was press-fitted on top of the transcutaneous part of the bone-anchor, above the flange. The stem, flange and a 2mm section above the flange were HA-coated to encourage osseointegration of the parts of the device lying within the bone and cutaneous integration of the parts of the device lying within the soft tissues.

Figure 3.1: 2D CAD design of the implant device showing: the tapered stem; 15 mm diameter porous flange; 2 mm diameter hole (magenta) for passing the electrode cable; press fit sleeve for electrode cable connector. Image courtesy of Jay Meswania
3.3.2 Developing and Testing the Surgical Technique to Insert the Device

The bone-anchor device was designed to be press-fitted into a transcortical hole previously drilled through the tibia. To insert the bone-anchor, the device was gently tapped into the hole using a hammer. A trial insertion of the device was carried out using a freshly thawed, sheep hindlimb. The transcortical hole was drilled across both cortices of the tibia using a 4.6 mm drill. The outer cortex was further drilled with a 4.8 mm drill to accommodate the tapered shape of the stem. A tapered, 5 mm maximum diameter, reamer was then used to further enlarge the hole using careful, half-turns to prevent over-reaming (Figure 3.2). An acrylic pole was used to transmit axial force to the bone-anchor while hammering it into place. The pole was designed to fit over the top of the transcutaneous part of the device to protect the socket connector located within the top of the bone-anchor.

Figure 3.2: Insertion of the bone-anchor on an *ex vivo* sheep tibia. Note – the small gap left between the flange and the bone to allow space for the electrode cable to exit.

3.3.3 Pullout test of the bone-anchor

Following insertion, the strength of attachment of the bone-anchor was tested using a pullout test. For the test, the tibia was clamped into position (Figure 3.3) and attached to a Zwick/Roell Z005 tensile test jig (Zwick GmbH & Co. KG, Ulm, Germany). The bone-anchor was also clamped to the jig and a pullout force was applied at a speed of 5 mm/min until failure occurred. The force applied at failure was recorded over ten trials.
3.3.4 Assembly of bone-anchor + implanted electrode device

For this part of the study, a bipolar epimysial electrode (Ardiem Medical Inc., PA, USA) with Pt/Ir electrodes and an interelectrode distance 10 mm, as described in my previous experiments was used. To increase the mechanical strength of the external connector, the connector plug was encased inside a metal shell. The ends of the flying leads were crimped onto a 2-pin socket connector socket (PHG.00.302.CYMD22, LEMO UK Ltd, Worthing, U.K) positioned on top of the transcutaneous portion of the device. The electrode cable was passed through a borehole in the bone-anchor and the spaces adjacent to the cable were filled-in with medical-grade silicone (NuSil Med3-4013, NuSil Technology LLC, CA, USA) to prevent water ingress and to provide a seal between the internal and external environments. The silicone was injected using a syringe, degassed under vacuum, and cured for 24 hours at 50°C. The HA coated flange and stem were masked to prevent contact with uncured silicone. The silicone seal also helped to reduce flexing of the cable at the internal exit thereby reducing the risk of fatigue failure. To add further mechanical strength to the external part of the device, the socket was housed in a hollow metal sleeve which was also press-fitted onto the transcutaneous part of the bone-anchor and the sleeve was bonded in place with epoxy resin. The final device (bone-anchor, implant sleeve, electrode, electrode cable) was then sterilised using ethyl oxide gas. The components of the external connector for the electrode are shown in Figure 3.4 and the finished implant is shown in Figure 3.5.
Figure 3.4: Assembly of the electrode wire to the external socket. A; the flying (bare) leads of the electrode were fed through the borehole in the bone-anchor and brought through to the top of the device. The flying leads were crimped onto copper connectors, which were fed through an internal sleeve. B; the copper connectors were press-fitted into a plastic housing. Metal sleeves, forming part of the assembly, were used to encase the plastic housing, which was inserted into the external plug. The internal sleeve was press-fit into the external plug, and the entire assembly was inserted into the metal sleeve housing of the bone-anchor by pulling on the electrode wire until the external plug sat tightly inside the bone-anchor. Epoxy resin was later used to bond the socket to the press-fitted sleeve.

Figure 3.5: Final device assembled. A; showing the bone-anchor + implantable electrode. After passing the electrode cable through the bore hole in the bone-anchor, silicone was injected around the cable, then degassed under vacuum and cured for 24 hours at 50°C. B; bird’s eye view of the external socket connector.
3.3.5 Modification to the amplifier lead

A corresponding 2-pin plug connector – (FGG.00.302.CLAD22, LEMO UK Ltd, Worthing, U.K) was soldered to the amplifier lead wires (Figure 3.6), and the electrical integrity of the connection was confirmed using a voltmeter (Figure 3.7). The plug connector was used to connect the bone-anchor to record EMG signals using BIOPAC Equipment as described in Section 2.8.4.
3.3.6 Testing electrical integrity

Using a voltmeter, an arbitrarily selected, known potential difference (27mV), was applied across the 2 electrode discs. This potential difference was measured by attaching a 2-pin plug connector to 2 bare leads from the socket of the device (Figure 3.7).

3.3.7 Testing Water Resistance of the Electrode Channel

A separate bone-anchor + electrode construct was manufactured (using the method already described) using only the lead from the electrode, the terminal ends of which were attached to resistors to apply a potential difference of 27mV. The device was immersed in 0.9% saline above the level of the flange, to test for water ingress into the socket (Figure 3.8). Measurements were taken daily for 25 days.
3.3.8 Operative Procedure

The animal was positioned in a left decubitus position as described in Section 2.8.3. The limb was prepped with betadine and alcoholic chlorhexidine solutions and draped with sterile drapes. A sandbag was placed under the limb to provide a platform to stabilise the limb when drilling the tibia. The left decubitus position allowed for easy access to the medial aspect of the limb where the tibia is located subcutaneously, much like in humans. An incision was made 10 cm inferior to the knee joint on the medial aspect of the limb, overlying the tibia, and the soft tissues were dissected away to expose the tibia. Both cortices of the tibia were drilled with a 4.6mm drill. The outer cortex was further drilled with a 4.8mm drill bit and reamed to obtain a tight fit. The implant was then press-fitted into place with a mallet, leaving a 2mm gap between the flange and the underlying bone to accommodate the electrode cable, preventing potential crushing of the wire (Figure 3.9).
A separate skin incision was made over the lateral aspect of the left limb. *M. peroneus tertius* was identified through the incision. A subcutaneous tunnel was then created under the intervening skin between the 2 skin incisions - through which the electrode was passed. The electrode was secured into position onto the epimysial surface of the muscle using 4-0 Prolene (non-absorbable) sutures (Figure 3.10). The wounds were washed-out with normal saline. The subcutaneous fascial layer was closed over the flange with 5-0 monocryl suture (Figure 3.11) and the skin closed over this layer with 3-0 Vicryl (absorbable) sutures (Ethicon Inc., NJ, USA). Meticulous tissue handling was employed throughout to avoid damage to the soft tissue, and in particular to the skin. Mepitel (Mölnlycke Health Care Limited, Bedfordshire, UK). Sterile gauze and a bandage were used as a post-op dressing. The wound was redressed weekly for a period of 4 weeks. Thereafter it was left uncovered.
Figure 3.10: Intraoperative series for insertion of the bone-anchor + electrode construct. A; a separate incision was made over m. peroneus tertius, and the electrode was tunneled under the intervening skin bridge. B; electrode sutured onto the muscle, before closing the skin incision in layers. C; appearance post operatively.

Figure 3.11: Layered closure of wound over bone-anchor. A; placement of bone-anchor in medial aspect of left tibia. B; closure of soft tissue layer over flange prior to skin closure. C; appearance of skin/implant interface at week 12.

3.3.9 EMG Recording

The animal started treadmill walking and the data was recorded as per Section 2.8.4 as soon as possible after surgery (1-week post-operatively). Weekly readings were taken for 12 weeks, with the first reading taken one week post-operatively. The reference electrode was inserted subcutaneously at the ankle and consisted of a 21G hypodermic needle which was connected to the reference lead with a crocodile clip. All EMG signals were recorded using BIOPAC Equipment as described in Section 2.8.4.

Skin surface EMG signals were additionally recorded for comparison with the signals obtained from the implanted electrode. M. peroneus tertius in the contralateral limb was identified by palpation. The area was shaved and cleaned with alcohol, and surface
electrodes (Vermed Inc, VT, USA) were applied to the shaved skin overlying the muscle. The interelectrode distance for the surface electrodes was 20 mm, as per SENIAM recommendations [87]. The reference electrode for this experiment was placed over the bony prominence of the hock joint as described in Chapter Two.

All EMG signals were analysed using Acknowledge software (V4.2, BIOPAC Systems, Inc., CA, USA). The absolute values were taken prior to signal analysis. Mean power was calculated over 0.25 second epochs in a manually selected 4-second section (three or four gait cycles). Epochs with greater than mean average power were considered as EMG signals; all other epochs were considered as noise. The signal-to-noise ratio (SNR) was calculated as the ratio between the mean absolute signal EMG and the mean absolute noise EMG.

3.3.10 Impedance measurements

*In vitro* impedance measurements were made using the EVAL-AD5934EBZ (Analog Devices, MA, USA) using the potentiostatic method [167]. The frequency range for the impedance monitor was $10^3$ Hz to $10^5$ Hz and the impedance range was from 500 $\Omega$ to 1 M$\Omega$. The impedance converter system was calibrated using a 100 k$\Omega$ (1%) resistor before each measurement. The impedance of pre-implanted and explanted epimysial electrodes was measured in 0.9% saline (physiological saline) at 1 kHz to 77 kHz, 0.4 $V_{pp}$. The explanted electrode impedance was measured with a (approximately) 3 mm thick layer of tissue attached after preservation of the tissue in formal saline.

3.3.11 Failure of device

The device eventually failed at week 12, when there were no longer any clearly discernible EMG signals being received from the implanted electrode. Close examination of the external socket connector revealed that it was heavily contaminated with an accumulation of foreign debris which acted both as an electrical insulator between the plug and its socket, and to prevent complete mating of the plug with the socket (Figure 3.12). To test the electrical integrity of the device following explantation, the contact points in the socket were thoroughly cleaned of the debris using Industrial Methylated Spirit (IMS) and impedance measurements were taken.
By week 12, there was a build up of debris inside the external socket, occluding the electrical contacts and acting as electrical insulator between the plug and socket.

### 3.3.12 Co-stimulation of Adjacent Muscles to Investigate Crosstalk

Prior to undertaking any measurements to investigate signal crosstalk, the external socket was cleaned using IMS, and impedance measurements were taken to confirm the electrical integrity of the implant. Once confirmed, the relative selectivity of the epimysial electrode and the effects of crosstalk from adjacent muscles were investigated by stimulating the muscles adjacent to *m. peroneus tertius* with a nerve stimulator. At 12 weeks, with the animal under general anaesthesia, the motor nerve to *m. peroneus tertius* was identified. This was stimulated with Medtronic Vari-Stim III Surgical Nerve Stimulator (Medtronic Inc., MN, USA), set at 1 mA, resulting in a compound motor action potential. Contractions of the muscles adjacent *m. peroneus tertius* (*m. tibialis anterior, peroneus longus* and *gastrocnemius*) were then elicited by stimulating their respective motor branches. Six readings per muscle were obtained over a 60 second period. All EMG signals were recorded using the BIOPAC equipment as detailed above. Stimulus peak (V) and muscle signal peak
were analyzed to compare the strength of any signal from an adjacent muscle compared with the EMG signal from \textit{m. peroneus tertius}.

3.3.13 Histological Processing

After 12 weeks the animal was sacrificed by intravenous injection of 20\% pentobarbital. The implant was removed together with the electrode and a cuff of underlying muscle and a 5 cm segment of tibia. After surgical removal, all samples were individually submerged in formalin for one week. This was followed by serial dehydration of the samples in increasing concentrations of IMS (see appendix), until all the samples were fully submerged in 100\% alcohol. All solutions were then changed to chloroform for one week, with one change halfway through the week. The change was performed in a highly ventilated laboratory. The samples were then returned to 100\% alcohol for one week, with one solution change halfway through the week.

The specimens were then transferred to a solution containing 50\% resin: 50\% IMS for 5 days. During this time the specimens were kept in a darkroom and incubated in this solution under vacuum overnight and on a rotator during the day. This step was repeated once. Specimens were then submerged in 100\% Hard Grade Acrylic Resin (London Resin Company Limited, London UK) and incubated again in a darkroom under vacuum or on a rotator for 5 days and the step repeated again. Fresh, Hard Grade Acrylic Resin was put into the freezer for two hours before casting of the samples. For casting, each sample was placed into a new, labeled container (75ml volume). One droplet of LR White Accelerator (London Resin Company Ltd, London, UK) was placed into each container and this was spread around the inside of the container with a paper towel. The samples were then into their corresponding containers. When the Hard Grade Resin had cooled, it was transferred into a measuring jug. For every 10 ml of Resin, one droplet of LR White Accelerator was pipetted into the measuring jug. The resulting mixture was stirred evenly and gently to dissolve the accelerator into the resin and then poured into the containers containing the samples, making sure each sample was completely covered with the liquid mixture. The samples were then placed into a freezer at -20°C and left to harden, for a minimum of 24 hours.

The samples embedded in resin were sectioned transversely at the mid-point of each implant using an Exakt E310 diamond edged band saw (Medex, Frame, UK) against a clear Perspex.
backing. Sections were ground evenly using Exakt-Micro-Grinding System (Mederex, Frame, UK) to a thickness of 100μm with ascending abrasive papers (P400 – P4000) and polished with a pad lubricated with AP-A Suspension, a 5μm agglomerate α alumina suspension (Struers Ltd., Solihull, UK). Electrode samples were stained with toluidine blue for 20 minutes. Sections containing bone and the bone-anchor were stained with toluidine blue for 20 minutes followed by Paragon staining for a further 20 minutes. Histomorphological assessment was carried out using an Olympus BH2 microscope (Olympus Optical Company Ltd, Tokyo, Japan), linked to Zeiss KS300 3.0 image-analysis software (Imaging Associates, Thame, UK). Backscatter scanning electron micrography of the bone-anchored device was performed (JSM-5000, JEOL, Tokyo, Japan) to assess the success of the osseointegration and examine the skin/implant interface.

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### 3.3.14 Data Analysis

Unless otherwise stated, all data were analysed using SPSS version 22. Data were taken to be non-normally distributed because the conditions for parametric testing were not met, therefore non-parametric tests were used. The data are reported as median and interquartile range (IQR) unless otherwise stated. A Mann-Whitney U test was performed to identify differences between groups. A p value of <0.05 was considered significant.
3.4 Results

3.4.1 Water testing

The solution used for testing was 0.9% saline at 37°C. Readings were not taken at weekends. The median value was 27 mV (27.0-27.1 mV) (Figure 3.13).

![Figure 3.13: Line graph showing readings (mV) over a four-week period. Each data point represents a single measurement on each day.](image)

3.4.2 Pull-out test

The mean value at which the device was extracted from the tibia was 335.8N (328.6N to 346.7N). A typical stress-strain graph is shown in Figure 3.14. The strain distance that can be seen up to ~ 5 mm on the graph might represent the soft tissue deforming, as the soft tissue (muscle and fat) gets compressed against the overlying jig (Figure 3.3). This appears to be plastic deformation. After approximately 6 mm on the graph, the deformation appears to be more elastic, represented by a straight line, which implies that the bone is bending. The failure is rapid and singular (there isn't a series of small failures; the device pulls out in one go), as represented by the vertical drop-off on the graph.
3.4.3 Radiological assessment

Plain radiographs confirmed the correct position of the implant. This was shown on both the lateral and oblique radiographs (Figure 3.15). There was no evidence of loosening of the implant, which had osseointegrated into the surrounding bone. Evidence of osseointegration was further confirmed on histological examination.
3.4.4 EMG Signals

Clearly discernible EMG signals were obtained from the implanted electrodes while walking the sheep. These corresponded with the gait cycle of the animal (Figure 3.16). The SNR showed an increasing trend over time up to week 11. The greatest SNR was 7.05 at week 7. However, from week 5 to week 10, the SNR was greater than 5. The SNR was greater for the epimysial electrode compared with the surface electrode (5.1 vs. 1.6, see Figure 3.17). However, signal quality rapidly deteriorated after 11 weeks, which was attributed to accumulation of debris inside the connector (as previously described). At this point, no EMG signals could be distinguished from background noise.
Figure 3.16: A representative EMG recording from the implanted epimysial electrode during treadmill walking. Three distinct myoelectric signals can be seen, corresponding to muscle activation during the gait cycles.

Figure 3.17: A graph showing median SNR over time.

3.4.5 Co-stimulation of Adjacent Muscles to Investigate the Effects of Crosstalk

Peak stimulus voltage represents the size of the stimulus artefact from the nerve stimulator. Peak EMG is the magnitude of the muscle response to that stimulus. All the muscles tested
had a significantly lower peak EMG and stimulus voltage compared with *m. peroneus tertius* (*p*<0.004). There was a trend for a decrease in peak EMG with increasing distance away from the recording site. The muscles exhibiting the greatest amplitude EMG signals were those in the anterior compartment, supplied by the peroneal nerve, including; *m. peroneus tertius* 0.0355 V (0.031 V to 0.047 V), *m. peroneus longus* 0.0172 V (0.005 V to 0.019 V), and *m. tibialis anterior* 0.0077 V (0.006 V to 0.008 V). The *m. gastrocnemius*, a posterior compartment muscle, and the one furthest away from the recording implanted electrode, had the lowest peak EMG voltage: 0.0040 V (0.003 V to 0.004 V). Figure 3.18 shows the size of the peak EMG signals in each muscle tested.

With regards to stimulus artifact, the greatest amplitude voltage was seen amongst the anterior compartment muscles, which are all supplied by the peroneal nerve, including; *m. peroneus tertius* 0.209 V (0.188 V to 0.237 V), *m. tibialis anterior* 0.027 V (0.020 V to 0.030 V), and *m. peroneus longus* 0.059 V (0.055 V to 0.064 V). The gastrocnemius, which is supplied by the tibial nerve, had the smallest stimulus artifact: 0.018 V (0.016 V to 0.026 V), (Figure 3.19, Table 3.1).

![Figure 3.18](image)

**Figure 3.18:** Peak EMG signals during pre-terminal stimulation. Recordings taken from the implanted epimysial electrode overlying *m. peroneus tertius* with the adjacent muscles stimulated at their nerve supply with a 1 mV pulse. Significant differences are indicated by paired letters: a, *p* = 0.004; b,c,d, *p* = 0.002; e, *p* = 0.001; f, *p* = 0.003.
Figure 3.19: Peak stimulus artefact during pre-terminal stimulation. Recordings taken from epimysial electrode overlying *m. peroneus tertius* with the adjacent muscles stimulated at their nerve supply with a 1 mV pulse. Significant differences are indicated by paired letters: a,b,c,d,f, *p* = 0.002; e, *p* =0.004.

**Table 3.1**: Mann-Whitney U test comparing peak signals in the three test conditions

<table>
<thead>
<tr>
<th>Test Condition</th>
<th><em>P</em> Value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroneus tertius vs. Peroneus longus</td>
<td>0.004</td>
</tr>
<tr>
<td>Peroneus tertius vs. Tibialis anterior</td>
<td>0.002</td>
</tr>
<tr>
<td>Peroneus tertius vs. Gastrocnemius</td>
<td>0.002</td>
</tr>
</tbody>
</table>

### 3.4.6 Impedance Testing

The epimysial electrode impedance at 1 kHz increased from 1.6 kΩ pre-implantation to 2.3 kΩ after explantation. The impedance of the implanted epimysial electrode was measured in formal saline solution; 0.4 Vp-p, 1 kHz to 77 kHz, \( \delta =150 \) Hz where \( \delta \) was the step size between measurements (i.e. each measurement is at 150 Hz greater frequency than the previous), as shown in Figure 3.20.
3.4.7 **Gross Morphology**

The skin-implant interface at the time of explantation did not show evidence of infection or discharge (Figure 3.21). The skin overlying the flange appeared normal with normal hair growth.

![Image of skin-implant interface at 4 weeks (left) and at explantation (right, after shaving of the skin).]
The external socket connector showed a heavy accumulation of fine debris (likely small particles of dust/dirt from the barn environment and from the coat of the sheep) within the electrical connector which we suspected was acting as an electrical insulator. The surrounding muscles appeared to be normal, with a layer of fibrous tissue encasing the electrode and its wire – as expected from previous studies using FES electrodes and from my own earlier study (Chapter Two).

1.1.1 Histological Evaluation

The silicone layer of the electrode was lost during grinding and polishing of the sample embedded in resin. However, in the histological sections, the position previously occupied by the electrode left a distinct line at the interface with its surrounding capsule. So, it was relatively easy to simply imagine the electrode in its expected position.

At 12 weeks, the electrode interface showed encapsulation by fibrous tissue. The fibrous capsule was 250 μm at its thickest. The capsule consisted of parallel collagen fibers interspersed with fibroblasts. A well-demarcated layer of fibroblasts was seen at the interface with the electrode (Figure 3.22).

Histological evaluation of the interface between the stem of the implant and the bone showed bone formation consistent with osseointegration (i.e. fibrous tissue was absent at the interface between the bone and the metal surface). Epidermal and dermal attachment to the implant surface confirmed a stable soft tissue-implant seal. Note the bony bridging around the implant, and the downgrowth of the epithelium, which has then attached. (Figure 3.23).
(a) In this histological section, the electrode would have occupied the position indicated. A thin, fibrous layer (C) was seen between the epimysial electrode and the muscle fibers which are seen below this layer.

(b) Skin-implant interface showing soft tissue in-growth through pores in the flange. T – transcutaneous part and S – stem of implant. The epidermal layer (E) is seen growing down to the flange and then does not continue.

**Figure 3.22:** Soft tissue response to the epimysial electrode and bone-anchor.

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**Figure 3.23:** Backscatter scanning electron micrograph of the bone-anchored device. B - Arrows confirm that it has ‘osseointegrated’ because the bone is seen growing directly into the HA coating (H) which was plasma sprayed onto the metal stem (S) with a complete absence of any intervening fibrous soft tissue. C - Section through the skin-implant interface showing the keratinized epithelium (E) growing down to the junction between the flange (F) and the transcutaneous part of the implant (T) - and stopping at that point.
3.5 Discussion

This study describes the results of an experiment to record and analyse EMG signals acquired from an implanted muscle electrode in a conscious animal, walking over 12-weeks. To achieve this, I used a bone-anchor as a physical conduit for taking the EMG signals from the implanted electrodes across the skin barrier. Using a physical device allows the number of channels used for signal transmission to be increased without a disproportionate increase in the complexity of the implanted components. In contrast, using a wireless method of signal transmission requires a large bandwidth and consequently higher power consumption, to cope with a large number of channels (i.e. from multiple implanted electrodes) and high rates of data transfer [54], [155]. Crucially, using a bone-anchor-based approach permits the creation of a multi-channel control system that could be used to achieve more intuitive, voluntary control of a multi-axial prosthesis while simultaneously providing the means for direct attachment of the prosthesis to their residual limb.

Although this is an important advance, it is also important to recognise that using skin-surface electrodes to detect EMG signals and combining this with pattern recognition algorithms has already made it possible to achieve at least part of this aim. Moreover, studies have shown no significant difference in the control accuracy of pattern recognition-based myoelectric controllers using skin-surface versus implanted electrodes [168]. However, these experiments were carried out in an ideal laboratory environment rather than in the real world, where problems related to the long-term consistency of signal acquisition might be more of a problem. As previously discussed, this could include problems such as; movement of the surface electrodes relative to the underlying muscle due to sweating, movements of the residual limb within the socket, electrode lift-off, changes in the shape of the stump over time resulting in inaccuracy of electrode placement, etc. Results from these experiments therefore suggest that implanted electrodes may confer a significant advantage over surface electrodes in the long-term.

The e-OPRA system described by Ortiz-Catalan et al has already confirmed many of the advantages of using implanted muscle electrodes over skin-surface electrodes [67]. Using implanted muscle electrodes, EMG signals can be readily recorded from small-sized or deeply sited muscles (e.g., \textit{m. flexor digitorum profundus} in a transradial amputee or \textit{m. pectoralis minor} for a through-shoulder amputee). In contrast to surface electrodes, the position of an implanted muscle electrode is constant and permanent with no movements due
to perspiration or changes in the shape or size of the residual limb. Similarly, the detection of EMG signals by implanted muscle electrodes is not affected by deformation of the skin or movements of the residual limb relative to a surrounding prosthesis socket. Using implanted muscle electrodes also eliminates the need to wear any external devices for holding the skin-surface electrodes in place, such as waistcoats, armbands or sockets [88]. Importantly, combining these advantages with the benefits of using a bone-anchor allows the device to provide both the means for attachment and the means for control of the prosthesis - in one operative step – as shown by e-OPRA.

Others have taken a similar approach to the one described in this Chapter. For example, Pitkin [169] reported on the use of a titanium-pylon, bone-anchored device, as a conduit to take signals from internal microelectrodes, across the skin barrier of a rabbit. The pylon was inserted into the tibia, and an additional hole was bored through the bone, through which the electrode cables were passed into the medullary canal of the bone and then through the pylon. Using this setup, just one reading was obtained (with the animal sedated) by eliciting the ‘righting reflex’. In contrast, for this study, ‘physiological’ signals over a 12-week period were recorded with a conscious sheep walking on a treadmill. Additionally, by passing the electrode cable through the bone-anchor itself, eliminated the need for an additional hole in the tibia (unlike the e-OPRA system, which requires drilling a hole in the bone to pass cables through), simplifying the operative procedure and reducing the risk of injury to the bone.

However, it is the e-OPRA system that comes closest to the main aims of this thesis by creating a device that combines the means for direct skeletal fixation of a prosthesis with a system for taking EMG signals from implanted electrodes across a skin-barrier. Despite the success of e-OPRA, there are many differences between it and my design for a bone-anchor-based system. In particular, the cable for my implanted electrodes is passed through a channel in the bone-anchor without the need for an additional hole in the bony cortex (see Figure 3.1, Figure 3.5 and Figure 3.9) to allow passage of the cable from the implanted electrode – as for Pitkin and e-OPRA. Ortiz et al do not give details of the operative steps involved in creating a hole in the bony cortex to allow the cables from the implanted electrodes to be passed through the medullary cavity of the bone and down into the bone-anchor [169]. However, informal discussions with the surgeons involved in implanting the e-OPRA bone-anchor and electrode system have confirmed that this step is extremely difficult to achieve (Dr Oskar Aszmann – personal communication). In contrast, the approach used for my design is much simpler since, apart from the stem of my device, all the hardware
remains outside the bone. Furthermore, my design requires only a single stage surgical procedure.

One weakness of my design is that the electrode cable was only sealed within the bone-anchor using injected, medical-grade silicone, although this was then reinforced with epoxy resin. Simple injection of silicone around the cable did not actually create a hermetic seal, creating a potential route for bacteria to pass along the electrode channel in the bone-anchor and into the sheep. Therefore, any similar device which might be used for humans in the future, would need to incorporate a better method for sealing the electrode cable into the bone-anchor in a more bacteriologically secure manner. However, by avoiding the need to pass the electrode cable through the bone, insertion of my design for a bone-anchored device would be a much simpler process than that involved in installing the e-OPRA device, reducing both the surgical and infection risks and avoiding the risks of damaging the electrode cable(s) when passing them through the bone. Moreover, since hydroxyapatite (HA) is osseoconductive, the bone surrounding my bone-anchored device was able to grow along the surface of the stem, up to the flange area (Figure 3.23), a phenomenon known as bony bridging [59]. Although this meant that, on explantation of my device, bone was found in and around the electrode cable as it passed through this point in the bone-anchor, this additional bone did not have any effect on transmission of EMG signals. Furthermore, since the electrode cable was encased in silicone, it did not become osseointegrated. Therefore, any revision of my bone-anchor device in the future (e.g., replacement of any broken electrode cables) would likely be much simpler than for e-OPRA, provided this does not require disruption of the hermetic seal.

Several tests of the bone-anchor + electrode assembly were performed before implantation. Water testing was performed to test for water ingress into the socket which could have resulted in corrosion or electrical shorting of the connections. This testing confirmed that a stable potential difference could be maintained throughout the 4-week test period, in vitro. Although this test period was short, it was felt to be sufficiently adequate in for this experiment to check for any early potential problems related to water ingress, since most failures are usually very early in the device life (within a week of immersion) when water first penetrates the device. A hermetic seal would be required to ensure full protection from water ingress.
The pull-out test showed that a mean force of >300 N was required to remove the bone-anchor from the tibia, immediately after insertion. The rationale behind performing this test was to find out whether inserting the device by means of press-fitting it in the manner described in the surgical section would be strong enough to withstand the likely trauma that a sheep would impose on the hardware. It is important that the device is secured sufficiently well, without it becoming dislodged, while it osseointegrates. The limitation of this test was that it was only carried out in one direction of force, and did not take into account lateral forces on the implant, which may have caused loosening of the device at a lower mean force.

Fortunately, there were no intraoperative complications and the animal made a good post-operative recovery, with no signs of any change in gait, pain or distress. Throughout the experiment, the quality of the signals was maintained, with a median SNR of 5.1. However, the device did eventually fail due to a build-up of debris inside the exposed external socket connector. This was undoubtedly due to the living environment of the sheep. The debris acted as an electrical insulator between the contact points of the plug and the socket which interfered with the conduction of electrical signals from the implanted electrode. Once the socket was cleared of debris, impedance spectroscopy was used to measure the impedance of the device. This was only slightly elevated compared to the pre-implant readings. The slight increase in impedance was in keeping with the increased resistivity brought about by the formation of an insulating capsule around the electrode. Capsule formation has been observed by other workers using implanted electrodes [84], [85]. Therefore, in all subsequent experiments, a flexible silicone dust cap was fitted to the external socket of the device to prevent similar contamination with debris.

Importantly, there were no clinical signs of infection around the implant (redness, heat, discharge, pain) after implantation and throughout the course of the experiment. Macroscopically, the skin overlying the flange was intact and appeared healthy, with a good colour and hair growth. Plain X-rays at 12 weeks at the termination of the experiment showed that the implant remained in good position throughout the experiment. Back-scatter, scanning electron microscopy of prepared specimens of the bone-anchor and the adjacent soft tissues showed keratinocytes growing down to the interface between the transcutaneous part of the bone-anchor and its flange. Importantly, the epidermal cells appeared to stop at that point, much as they have been observed to do at the skin-antler interface. I postulate that this demonstrates cutaneous integration had occurred between the skin and the implant. I also observed that the bone surrounding the stem had grown into the HA coating with no evidence
for any fibrous tissue (scar tissue) at this interface. Therefore, I interpreted this finding as an indication that osseointegration had occurred with the surrounding bone.

My EMG data showed that the SNR increased over the first 11 weeks of testing, from an initial value of 4.4 to a maximum of 7.0 at week 7. The SNR was greater for the epimysial versus surface electrodes (5.1 and 1.6 respectively). However, signal quality deteriorated rapidly after 11 weeks and it became difficult to identify muscle activation visually from the recordings, due to excessive noise. Therefore, I also investigated the significance of crosstalk.

Crosstalk refers to the contamination of EMG signals from one site by electrical activity from an adjacent site. Although this is a frequent problem for surface electrodes, especially when they are physically placed close together, crosstalk is likely to be reduced for internal electrodes. I accept that in using an ovine model for my experiments, skin differences might make comparisons of my results comparing epimysial and skin-surface EMG signals, inapplicable to human patients. Nevertheless, to test the effects of crosstalk, selective intra-operative, in vivo stimulation of muscles adjacent to *m. peroneus tertius* was performed and any EMG signals that were detected by the implanted electrode were recorded. Only the first 6 readings from each stimulated muscle were considered as valid because glycogen depletion would have resulted in diminished contraction strength with subsequent stimulations of the nerve, resulting in an EMG signal of weaker amplitude and a lower SNR. Although using a nerve stimulator to generate muscle contractions in a sheep under general anaesthetic is not representative of physiological stimulation of the muscle, my data showed that when the peak EMG signal strength from the implanted electrode was compared between *m. peroneus tertius* and the adjacent muscles, the amplitude of signals from *m. peroneus tertius* was significantly greater than the EMG signal from the adjacent muscles, under all 3 test conditions (*p*<0.05). The difference was greatest the further away the test muscle was from *m. peroneus tertius*. This was expected, since signal strength should get weaker, the further away the recording electrode is from the source of the contaminating EMG signal. However, all the EMG signals were still representing full activation of all the motor units in the muscle (maximal compound muscle action potentials). Whereas, in practice, the threshold for activating a prosthesis can be adjusted and can be set sufficiently high to avoid accidental activation by inadvertent contraction of adjacent muscles.
In a similar way, the ability to graduate grip strength in a prosthesis depends on being able to alter the recruitment of motor units in the specific muscle being recorded – more motor units recruited creates a higher amplitude EMG signal which is interpreted as a desire for the prosthesis to move more forcefully. If so, co-contraction of adjacent muscles might be problematic since even an implanted electrode might not be able to fully distinguish a change in the amplitude of the EMG signal occurring from just one muscle. While this might not be an issue for initiating simple functions such as hand open/close, it might be a problem when trying to create more nuanced functions. For example, trying to distinguish all the EMG signals generated simultaneously by the (extrinsic) forearm muscles of a transradial amputee when trying to recreate a function such as thumb opposition (normally requires simultaneous, graded, flexor pollicis, abductor pollicis, extensor pollicis longus and brevis functions, together with intrinsic muscle functions – now absent).

Implanted epimysial electrodes afford another notable advantage over surface electrodes; by significantly reducing the effort required to actuate the prosthetic limb, measured as a percentage of the maximal voluntary contraction of the muscle. Muscle activations can be observed using epimysial electrodes at a lower % contraction due to improved recording fidelity over surface electrodes. Ortiz-Catalan showed that when using epimysial electrodes, the activation threshold for controlling the prosthetic limb was set at 12% of maximum voluntary contraction, compared with 60% used for systems that utilised surface electrodes [67].

Experimental data suggest that selectivity is not as big a problem as might be expected. For example, Lowery et al [62], using a computer model to simulate the pickup area for IMESs, has shown that this is ellipsoidal in shape with only a 5mm in radius around the implant (assuming the electrode orientation is in the direction of the muscle fibres). In another in vivo experiment, Baker et al showed that EMGs from 8 adjacent IMESs implanted into the forearm of a monkey showed very little crosstalk [88]. Moreover, a rough estimate of the detection volume for an electrode is a sphere with a radius equal to the interelectrode distance (IED) [170]. For an IED of 10mm, this represents a sphere with a radius of 10mm, centered on the electrode. In the case of the epimysial electrodes used, I theorise that this detection volume more likely represents a hemisphere facing outwards from the non-insulated surface of the electrode discs. In effect, this side of the electrode acts as a window ‘looking’ for EMGs arising from the muscle with which it is in contact. At the same time, the silicone backing attenuates signals from the opposite side of the electrode. This configuration might
be an advantage when placing multiple electrodes near to each other within an amputation stump. Therefore, I speculated that the electrode design could be further modified to resemble a cuff electrode, wrapping around the muscle and insulating it from the adjacent muscles. However, equally, such a design might require more extensive dissection of the muscles, increasing the risk of more muscle damage and fibrous tissue formation.

There are many other ways in which crosstalk from implanted electrodes could be reduced in practice. These include; signal processing algorithms (such as pattern recognition) and the use of a tripolar electrode design. For tripolar electrodes, the potential difference is measured between 2 electrodes that are shorted together, with the central disc acting as the common cathode (quasi-tripole). Tripolar electrode designs have previously been used for FES where selective stimulation of nerves in close proximity to each other is required, such as the cauda equina [83]. I consider all the options for optimising electrode configuration in more detail in Chapter Five.

3.6 Conclusion

1. Experiment 1 of this part of my study demonstrated that it was possible to combine a bone-anchored device with an implanted epimysial muscle electrode system to permit EMG signal acquisition from a conscious, walking, sheep over a 12-week period.

2. The combined device could form the basis for a future solution that allowed direct skeletal attachment of a prosthetic limb together with control of the prosthesis.
3.7 Testing of Bone-Anchor + Electrode Device; Experiment 2 in N=6 sheep

In Experiment 1, the device failed at 12 weeks due to the build-up of debris inside the connector socket. To prevent this happening in future experiments, a flexible silicone dust cap was fitted to cover the socket between each recording session (Figure 3.24). Furthermore, each socket was inspected prior to each recording session to look for signs of any debris which might interfere with the electrical contacts. If debris were present, the connector was thoroughly cleaned before beginning the recording.

Importantly, the results of Experiment 1 confirmed that the overall design of my bone-anchor + implanted electrode system was able to function in the way that I hoped. Therefore, the subsequent experiment focused on achieving the same outcome but over a longer period of implantation (20 weeks) in a larger sample size. A power calculation shows that a group of n=6 will show a significant difference (p<0.05) with a mean difference between groups of 20%, a standard deviation of 5% and a power of 0.8. I therefore performed this experiment in n=6 sheep.

Figure 3.24: Fitting of silicone dust caps over the bone-anchor connector to protect the electrical contacts from contamination with debris from the barn.

3.8 Aims of Experiment 2:

- To investigate the ability of my bone-anchor + implanted electrode device to convey high quality EM signals to an external recording system over a 20-week period.
3.9 Materials and Methods of Experiment 2 in N=6 sheep:

Additional bone-anchor + implanted electrode devices were manufactured as described in section 3.4.4. For Experiment 2, the devices were implanted into n=6 sheep (female mule breed). Silicone dust caps (Greentree Shercon, Tewkesbury, UK) were applied to the external socket of the bone-anchors to protect them from accumulating debris from the barn. EMG recordings were carried out at weekly intervals for 20 weeks - as described in section 3.4.9.

A parallel study was conducted, using the same animal subjects, to investigate the effects of altering the porosity and surface coatings of the flange on the bone-anchor to try and achieve a reliable and stable skin/implant seal (see details of this part of the study in Chapter Seven). This parallel study involved the implantation of three further bone-anchors (total of two per tibia) in the right limb of each sheep, with the different surface coatings and flange porosities under investigation. One animal developed osteomyelitis (of an implant not involved in the study presently under discussion) and had to be euthanised, and another developed a vertically transmitted para-tuberculous disease requiring premature termination of the experiment. These events are considered in more detail in the relevant results section.

Euthanasia was performed with an intravenous injection of 0.7mg/kg IV Sodium Pentobarbitone (Pharmasol Ltd., Hampshire, UK) into the jugular vein.

3.9.1 EMG Recordings

Recordings were carried out as follows; the first recordings were made at one-week post-op. For the 1st month, recordings were made weekly. For the 2nd and 3rd months recordings were made fortnightly. For the 4th and 5th months, recordings were made monthly. This schedule of measurements meant that recordings were made on weeks 1, 2, 3, 4, 6, 8, 14 and 19. At week 19, surface electrode readings were performed as described in 3.4.9. Signal-to-noise ratio (SNR) was then calculated for 6 gait cycles per recording using MATLAB 2017b (The MathWorks, Inc., Natick, MA, USA). It was possible to identify the EMG signals visually because at 2 km/hr 1 the gait cycle occurs at a frequency of approximately 1 per second. The SNR was used as an estimate of signal quality.
3.9.2 Impedance Measurements

Impedance measurements were performed as described in section 3.4.10, with *in vivo* impedance measurements carried out immediately after euthanasia. Histological processing was performed as previously described in section 3.4.13.

3.9.3 Data Analysis and Statistical Assumptions

For all tests, Shapiro-Wilk tests of normality were carried out on each group to satisfy the assumption of parametric statistics. This showed a $p < 0.05$, indicating that the test data came from a non-normal distribution. This therefore required the use of non-parametric statistical tests, both in this section and subsequent chapters of this thesis. These include:

**Kruskal-Wallis Test** – A non-parametric one-way ANOVA test for k independent samples. $p < 0.05$ indicates that at least one group is significantly different from all the others.

**Mann-Whitney U Test** – A non-parametric test for 2 independent samples. $p < 0.05$ indicates that there is a significant difference between the two groups.

**Spearman’s Rank Correlation Coefficient (Spearman’s Rho)** – A non-parametric measure of bivariate statistical dependence. A +ve value indicates a positive correlation between x and y values. A value of 1 or -1 indicates that the data can be described using a monotonic function. Also known as Spearman’s $\rho$.

**Wilcoxon Signed-Rank Test** – A non-parametric test for 2 related samples. $p < 0.05$ indicates that there is a significant difference between the two groups.

Non-parametric tests typically have less power than the equivalent parametric tests. Since power is the probability of correctly rejecting the null hypothesis when it is false, non-parametric tests have an increased likelihood of making a Type II (or $\beta$) error, i.e. failing to reject the null hypothesis when it is in fact false. This limitation is acknowledged in this thesis.

For this chapter, Spearman’s rho was used to test correlation between time since implantation and SNR. Mann-Whitney U test was used to test the difference between surface and implanted electrode SNR. All the data are reported as median + interquartile range unless otherwise stated. A $p$ value of $<0.05$ was considered significant. Impedance and surface
versus epimysial EMG data are plotted as median, interquartile range and inner fences (1.5 times interquartile range).

### 3.10 Results

#### 3.10.1 Complications Requiring Euthanasia

There were 6 animals at the start of the study. These were identified by their unique ID number. Those highlighted in bold were terminated before the end of the 19 week study:

6027, **6034**, **6036**, 6046, 6059, 6064, 6073.

Animal 6034 developed chronic infection of the proximal pin on the right tibia, which became evident at week 3. This was not the bone anchor under consideration in this Chapter. The wound site was debrided under general anaesthesia on weeks 3 and 4 and the surgical wound was flushed with betadine. Intramuscular antibiotics were also administered. However, the infection did not improve, and the animal became lame by week 5. Therefore, the experiment was terminated at week 5 and the all implants were explanted. The infected pin was noted to be loose on examination and was easily removed with minimal force indicating an absence of osseointegration.

Animal 6036 was the intended replacement for animal 6034. Animal 6036 gradually developed anorexia, general apathy and weight loss. This was first reported at week 8 after surgery. Examination by a veterinary surgeon revealed a possible pneumonia, and the animal was started on antibiotics for one week. No readings were taken for 2 weeks to allow the animal to convalesce. The animal continued to suffer anorexia, dropping from a weight of 79 kg pre-op to 47 kg by week 12. In view of the deteriorating condition of the animal, the experiment was euthanised and the animal was submitted for post-mortem examination by the Animal Health and Veterinary Laboratories Agency Surveillance Centre at the Royal Veterinary College in Hatfield. The autopsy revealed multiple linear oesophageal erosions and a liver that was markedly pale. Histological examination of the ileum revealed severe, diffuse, autolysis. Clusters of multinucleated giant cells and coalescing aggregates of histiocytes were found multifocally in the lamina propria of the mucosa and underlying submucosa. Acid fast staining revealed abundant intracellular acid-fast organisms within giant cells and histiocytes within the ileal mucosa. These findings were consistent with a
diagnosis of Johne’s disease a multibacillary form of tuberculosis. This was a paranatally acquired disease, and therefore not the results of the present study.

The remaining 5 animals were euthanised after the final reading was performed – at week 19 after implantation.

3.10.2 Gross Morphology

3.10.2.1 Sieving of the flange of the bone-anchor

Four of the bone-anchor + electrode devices that were placed proximally on the tibia experienced large amounts of movement of the overlying skin. In some of these cases, a complication which will be referred to as ‘sieving’ was observed. This was first noticed at Week 4 post-operatively and involved necrosis of the skin immediately over the flange of the bone-anchor + electrode device. Initially, the skin immediately adjacent to the transcutaneous part of the anchor necrosed and then death of the skin progressed in a semi-circle to include the rest of the skin overlying the flange. Eventually, enough skin underwent necrosis for the flange to show through the skin (Figure 3.25). This was a dry ischaemic process of the skin with no evidence for infection as a causative factor (i.e. absence of redness and discharge). This phenomenon was encountered, to a much lesser degree, with the implants (four in total) placed in the distal limb, with thinning of the skin overlying the flange, although there was no sieving and these flanges remained covered by skin (Figure 3.26). However, admittedly, it is possible that these implants would have eventually eroded through the skin given enough time after implantation.
Figure 3.25: Animal 6027 at week 19, with exposure of the flange (referred to as sieving).

Figure 3.26: A; Animal 6046 at week 19 showing area of dermal loss, exposing a small amount of the flange (indicated by arrow). This implant was located proximally in the right limb. B; thinning of the skin overlying the flange in the proximal position of the left limb at week 19. These two examples show the precursor to sieving. Note that the flange shape is visible underlying the skin, clearly indicating thinning.

3.10.2.2 Broken Socket

Animal 6027 sustained damage to both the socket and the plug while walking on the treadmill (Figure 3.27). This affected the quality of the EMG recordings and necessitated omission of the last data point for this animal (week 19). The poor recording also correlated with a very high impedance value for the electrode part of the system on explantation, suggesting electrical discontinuity of the electrode and socket construct.
Figure 3.27: Animal 6027 at Week 19. A; Broken socket. B; damaged plug. This corresponded with poor quality EMG recordings requiring omission of the last data point.

3.10.3 EMG Signals

There was a significant and positive correlation between SNR and time since implantation as calculated using Spearman’s rho, with a coefficient of 0.389 ($p=0.006$). Using the median SNR per animal at each time point, a line of best fit was drawn, giving $R^2 = 0.151$ (Figure 3.28). The SNR was significantly higher for implanted electrodes: 6.56 (5.47 to 9.86) compared with surface electrodes 1.64 (1.34 to 1.88) at 19 weeks ($p=0.016$). These data are shown in Figure 3.30 and Figure 3.32, and a comparison of the EMG signals can be seen in Figure 3.31. All animals, with one exception (6036), showed a trend for increasing SNR over the 19-week study period. The falling SNR in 6036 was attributed to a gradual deterioration in the general health and severe weight loss of the animal caused paranatally acquired Johne’s disease, contributing to the poorer quality EMG signals.
Figure 3.28: Trend of SNR over the 19-week period of implantation. Animal 6034 was sacrificed after 30 days. Each data point represents the median recordings made on a particular day.
Figure 3.29: Change in individual animal EMG, SNRs plotted against time since surgery for $n = 7$ animals. One animal (6034) was sacrificed after 30 days. Each data point represents the median recording made on a particular day.
Figure 3.30: Raw EMG signals (a and c) and power spectrograms (b and d) of the EMG recordings from the epimysial electrodes (a and b) and skin surface electrodes (c and d) after 19 weeks. These traces were recorded simultaneously from the same animal. Spectrograms were created by Fourier transform with 50 sample Hamming windows [144].
Figure 3.31: Difference in SNR comparing implanted and skin surface electrodes at 19 weeks after surgery (from $n = 5$ animals). Due to the broken socket connector, results for animal 6027 were left out of the comparison. The difference is significant $p = 0.016$. 

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**Figure 3.31**: Box plot showing the difference in Signal-to-Noise Ratio (SNR) comparing implanted and skin surface electrodes at 19 weeks after surgery. The plot includes data from $n = 5$ animals, excluding animal 6027 due to a broken socket connector. The difference in SNR is statistically significant with $p = 0.016$. 

- **Implanted Epimysial Electrode** shows a higher median SNR compared to **Skin Surface Electrode**. 
- The range of SNR for implanted electrodes is wider, indicating more variability. 
- The box plot visually represents the central tendency and spread of the data, with the lower and upper limits indicating the range of values for each electrode type.
Figure 3.32: Comparison of epimysial (top) and surface electrode (bottom) EMG recordings taken simultaneously from the same muscle (*m. peroneus tertius*). The three distinct signals seen in the epimysial EMG recordings correspond to three gait cycles.

### 3.10.4 Impedance Spectroscopy

Pre-implantation mean impedance at 1 kHz was 1337 Ω (1325 Ω to 1352 Ω). This increased to 2238 Ω (1961 Ω to 2298 Ω) after 19 weeks *in vivo*, and 2620 Ω (2026 Ω to 4043 Ω) following explantation. There was a significant difference in impedance between pre-implant and *in vivo* impedance (*p*=0.003) and pre-implant and explanted impedance (*p*=0.008). There
was no significant difference between *in vivo* and explanted impedances \((p=0.245)\). Impedance values are shown in Figure 3.33.

![Figure 3.33: Change in 1 kHz impedance of epimysial electrodes before and after 19 weeks implantation for \(n=6\) animals. Significant differences are indicated by paired letters: a, \(p = 0.003\); b, \(p = 0.008\), (Mann-Whitney U Test \(p<0.001\)).](image)

### 3.11 Discussion

The results of Experiment 2 suggest that it is possible to maintain good signal fidelity and a high SNR over 19 weeks using my bone-anchor + electrode device. In all of the animals, the bone-anchor continued to provide a robust conduit for the passage of EMG signals from the implanted electrode, even when sieving of the flange occurred. The incident in which the external socket was damaged together with the corresponding plug attachment (Section 3.11.2.2.) led to a complete redesign of the device, so that it is one part, and the connector is fully enclosed, to offer better protection to this vulnerable link in subsequent experiments (detailed in Chapter Six). If a version of my device were to be used in humans, a fail-safe
mechanism [20] would be needed to protect the connector in case of a fall onto the end of the device (e.g., a fall with the prosthesis attached).

The cause of the phenomenon termed ‘sieving’ was unknown but was probably the result of ischaemia of the overlying soft tissues (either acute or chronic). It may have occurred because:

1. The post-operative bandages were applied too tightly, compromising the dermal blood supply leading to acute ischaemic skin necrosis. The ischaemia might have been made worse by ‘bunching’ of the dressings over the proximal parts of the limb, applying more pressure to the skin in these areas than to areas on the distal limb. Therefore, implants placed in the proximal limb were more vulnerable to this problem than implants placed in the distal limb. The evidence against this is that sieving did not become apparent for any implant until 4 weeks after implantation.

2. The edge of the flange structure was too sharp (rectangular cross section, with 90° angle at the corner) causing it to interfere with the blood supply of the skin as it made the transition from muscle onto the flange structure. This then cut its way into the overlying skin. This is a problem that might have been made worse by excessive movement of the skin relative to the flange during sitting/standing/walking. The excess skin movement was more noticeable for implants placed in the proximal rather than the distal limb. This seemed a more plausible mechanism for sieving since this would have caused a chronic insufficiency of the blood supply to the overlying skin which was seen here. A redesign of the flange with ‘softer’ rounded edges, as used in human implants [20] might have alleviated this problem and is used in Chapter Six.

3. There was insufficient soft tissue interposed between the skin and the underlying flange. More proximal implant placement could have provided increased soft tissue (including muscle) cover to protect the skin for the pressures applied to it by the overlying bandages. However, this may not have prevented ischaemia occurring according to Point 2 but would merely have delayed the time at which sieving subsequently occurred.

4. The flange structure was too wide. The skin immediately adjacent to the transcutaneous part of my bone-anchor became (in effect) a random-pattern skin flap. These flaps are subject to a strict 1:1 ratio (length: breadth). If the length of the flap exceeds its breadth, then the tip or leading edge of the flap dies due to ischaemic necrosis because of vascular insufficiency. Therefore, a flange with a narrower diameter might have been less prone to this problem. However, this possibility was not tested as part of this thesis.
5. Poor surgical technique. It was notable that sieving was not encountered in animals which had only one implant inserted at a time - which is what was done for the rest of the experiments described in this thesis. Therefore, it is possible that my surgical technique and soft tissue handling improved with each subsequent implant. Equally, in Experiment 2 where the animals had more than one implant inserted, perceived time pressure to perform each implantation more quickly, may have resulted in surgical technique being compromised and hence these adverse outcomes.

6. The presence of multiple implants (as for Experiment 2) might have affected the dynamics of skin movement. However, this seems unlikely given the distances between each implant on a given limb.

All the bone-anchor + electrode devices showed a linear trend for increasing SNR over time except for the device in animal 6036, which showed a decreasing linear trend over the same time period. This difference was attributed to Johne’s Disease affecting the overall condition of the sheep (Section 3.11.1). During Experiment 2, it was noted that the SNR for implanted electrodes was significantly higher at 19 weeks ($p=0.016$) compared with that for the surface electrodes, confirming my findings from Experiment 1. When comparing EMGs from surface and epimysial electrodes directly, there was also an obvious difference in signal morphology (Figure 3.28). For surface recordings, the EMG signals could be seen as relatively higher amplitude spikes which corresponded with the EMG signals recorded from the epimysial electrode. However, the surface electrodes also recorded additional EMG activity when only small or no EMG signals were being detected by the epimysial electrodes. This has been interpreted as indicating the presence of crosstalk from adjacent muscles, captured by the surface electrodes, that is absent from the epimysial recordings. This suggests that implanted electrodes provide greater selectivity of EMG recordings from their respective muscles, compared to surface electrodes.

7. Impedance is a measure of the resistance of a conductor to alternating current flow, and is applicable to the alternating signals comprising EMG. Any increase in impedance implied that there was an increase in resistance to the flow of current through the electrical parts of my bone-anchor + electrode device. Any increase in impedance noted post-operatively was attributed to encapsulation of the implanted electrode by collagen tissue - which has a high resistance to current flow [171]. This was observed in Experiment 2 ($n=6$) where the impedance at Week-19 was 2.2 kΩ compared to 1.3 kΩ before implantation. Moreover, my result for Experiment 2 was similar to the impedance
at Week-12 (2.3 kΩ) in Experiment 1. These data suggest that a stable, fibrous tissue interface forms relatively quickly and certainly by 12 weeks after implantation using the electrode system incorporated into my device [172]. Similar results were obtained by Lewis et al, who implanted epimysial electrodes in Macaque monkeys and found changes in electrode impedance which plateaued at four weeks after implantation and corresponded with strong fibrous (collagen) encapsulation of the electrodes over the same time period [173]. However, there was also an (non-significant) increase in impedance following explantation of my device (Figure 3.33). This was attributed to differences in the conduction properties of interstitial fluid and the normal saline which was used to bathe the device (to prevent desiccation) after explantation.

The data suggest that a bone-anchored based device can be used to transmit EMG signals across a skin barrier over a 20-week period and therefore possibly for longer. Therefore, this design could be used as a model for creating a similar device for use in humans. However, these experiments also highlighted many important drawbacks with the present design of the device that would need to be addressed before my device could be considered for use in humans:

1) Firstly, animal experiments would need to be carried out where the flange structure of the bone-anchor was designed to be dome-shaped with softer, rounded edges, as discussed above and as used previously for human implants [20] – to address concerns about sieving.

2) Second, the flange structure would need to be made (proportionately) much smaller in diameter to reduce the risk of ischaemic effects in the overlying skin – to address concerns about sieving.

3) Third, the channel drilled through the centre of the bone-anchor to permit passage of the electrode cable was not hermetically sealed. This created the possibility that the chancel could form a track for entry of bacteria. Although I used medical-grade silicone to seal this channel, human applications might require a hermetic seal to prevent the ingress of fluids and bacteria from the external environment into the human body. Conventionally, such seals are made from metal-in-glass or metal-in-ceramic [174]. Since my device was designed to be inserted into the bone by hammering, any glass or ceramic components would be vulnerable to cracking during insertion imposing a significant engineering challenge. In contrast, the e-OPRA system devised by Bränemark uses a hollow bone-anchor, which provides a passage
for cables from the implanted electrodes and seals the internal part of the bone-anchor using a modified abutment without a hermetic seal [67]. This system has now been used in several upper limb amputees with follow-up at 4 – 5 years, with no cases of deep infection. Therefore, it is possible to produce a bacterially clean seal at the interface between the electrode cables and the bone-anchor without needing to resort to conventional methods for producing a hermetic seal. It must be noted that the skin itself is not a hermetic barrier, allowing moisture to cross, but provides a physical barrier to bacteria. Silicone sealing in my device replicates this moisture permeable, but bacteria impermeable, barrier.

4) Fourthly, there is a physical limit to the number of channels that can be created through the bone-anchor without resulting in significant mechanical weakening of the structure if it is to be used for direct skeletal fixation of a prosthesis. For example, the e-OPRA system used by Brånemark is currently limited to four electrode cables due to the physical limitations of the size of the channel in the abutment. Ultimately, this could limit the value of a bone-anchor + electrode system when considering the much larger number of separate EMG signals that may be eventually generated by surgical techniques such as TMR or RPNI surgery.

To overcome the limitations imposed by Point 4 (above), one possible solution was to develop active implanted electrodes with signal amplification and recording at the electrode site followed by multiplexing of the signals inside the patient. Multiplexing allows multiple EMG signals (no theoretical upper limit to the number of signals) to be combined into a single digital signal that can be transmitted along a single cable. Once outside the patient, a de-multiplexer (e.g., located inside the prosthesis) would separate this single signal into the original input of multiple EMG signals. One can imagine a multiplexing as a switch, which swaps rapidly between channels sampling from each. Switching fast enough allows sampling from many channels, all at a data rate sufficient for recording EMG (1 kHz per channel in this work).

This approach could utilise simple implanted electrodes connected to a multiplexer box with implantable Craggs connectors [175]. The multiplexer would have a single input/output cable which would be used to power the multiplexer while simultaneously taking signals from the multiplexer through the bone-anchor and then on to the end device to be demultiplexed (Figure 3.34). This setup would have the added advantage of being serviceable. If any of the electrodes failed, they could be individually replaced by
unplugging them from the multiplexer box while a new electrode + cable was inserted and plugged into the multiplexer and resealed in-situ with medical-grade silicone. Moreover, if the multiplexer needed to be updated, it would be possible to remove the multiplexer box by simply disconnecting it from the electrode cables and replacing it with an updated unit (see schematic below of a ‘Smart Anchor’).

Figure 3.34: Schematic of my vision for a Smart Anchor, showing epimysial electrodes and multiplexer connections. For a more detailed discussion of this design - see section 7.6.1.

I speculated that my bone-anchor + implanted electrode device could also be used as a conduit for other purposes. For example, a sensory feedback loop could be created whereby electrical impulses created by pressure sensors in the prosthesis could be conducted to sensory nerves in the residual limb. It might also be possible to use the hardware to convey biosignals captured from the peripheral or central nervous system. Experiments have shown that direct recording of ENG signals from peripheral motor nerves using implanted nerve electrodes can (potentially) yield all the signals required to control a prosthetic limb [5], [117]. Since, the bandwidth requirements for ENG signals are much greater than for EMG
signals, a method for physical transmission of these signals out of the body could ensure more reliable and faster data transfer compared to current wireless technology. Wireless technology is likely to change in the future (e.g., compare 4G (200 Mbps) and 5G (> 1Gbps) transmission rates for mobile telephones). Despite this, I postulate that advantages of continuing to develop bone-anchor based systems will outweigh the disadvantages of continuing with the alternatives. This is primarily because a physical connection will always be more reliable than wireless systems and amputees need reliability and predictability from the control of their prosthetic devices.

3.12 Conclusions

- Experiment 2 of this part of my study confirmed that a bone-anchor based device could be used to convey EMG signals reliably from an implanted epimysial electrode in a conscious sheep model over a 20-week period.
- Experiment 2 confirmed that significant modifications to the design of the bone-anchor part of the device would be needed before it could be considered for use in humans.
Chapter 4 - Use of a Bone-anchor + Electrode Device for Recording EMG Signals from a Muscle Treated by Targeted Muscle Reinnervation

4.1 Introduction

The significant advantages of direct skeletal fixation over conventional methods of attaching a prosthesis to an amputation stump have been described in section 1.5. Direct skeletal fixation has revolutionised the way prostheses are attached to amputation stumps, eliminating the need for cumbersome and unsightly straps and increasing the range of movement, especially in proximal amputations. Just as direct skeletal fixation has addressed the problems associated with attachment of the prosthetic end device, implantable myoelectrodes have advantages over surface electrodes with regards to reliability of placement, reduction in crosstalk and increased selectivity (see sections 1.9 &1.12 and in Chapter Three). While this approach has the potential to work well in patients with distal amputations, those with a lack of adequate muscles to capture signals, such as high-level, above elbow amputees, require a different approach to achieve intuitive control.

As previously discussed (Section 1.16.1) targeted muscle reinnervation (TMR) is a surgical technique which can increase the number of EMG signals available for control of a prosthetic device, by rerouting blind-ending nerves to chest wall muscles. The number of EMG signals that can be created depends on the availability of suitable donor nerves and muscle targets [176]. Pre-operatively it is important (but not critical) to be able to demonstrate that the patient has voluntary control over the target muscle as well as active nerve conduction (as indicated by the presence of an active Tinel’s sign) over the donor nerve. Typical transfers for a high transhumeral amputee are shown in Figure 4.1. The use of an intraoperative nerve stimulator is essential for accurate identification of the motor nerves to the intended target muscles. In cases of shoulder disarticulation or very high transhumeral amputations, the chest wall muscles are often used as the target muscles since they no longer perform the function of moving the (now absent) arm.
Figure 4.1: Typical TMR transfers. Left - shows the situation prior to performing the nerve transfers. Right – shows the *m. pectoralis major* muscle has been split into three components and the *m. pectoralis minor* has been re-positioned into the axilla/lateral chest wall. Multiple nerves have been transferred including; the musculocutaneous nerve coapted to the motor branch controlling the middle part of *m. pectoralis major*. Median nerve 1 has been coapted to the motor branch of the lower part of *m. pectoralis major*. Median nerve 2 to has been coapted to sensory nerve branches of the supraclavicular nerves for Targeted Sensory Reinnervation (TSR). The ulnar nerve has been coapted to the motor branch controlling the *m. pectoralis minor*. The radial nerve was coapted to the thoracodorsal nerve (motor branch to *m. latissimus dorsi*) – not shown in this figure. Axillary nerve branches to the *m. deltoideus* muscle were left intact to preserve shoulder abduction. Similarly, the medial pectoral nerves to the clavicular part of *m. pectoralis major* was left intact to preserve shoulder flexion and abduction while the long thoracic nerve to *m. serratus anterior* was left intact to prevent problems with winging of the scapula. Image courtesy of Norbert Kang FRCS(Plast).

Although TMR is successful in generating new EMG signals, most amputees will still rely on skin surface electrodes to capture these signals. The limitations of surface electrodes has been discussed previously [99] in Section 1.12.2. Implanted epimysial electrodes have the potential to overcome many of the problems associated with surface electrodes. However, the biggest challenge has been to devise a way of conveying the EMG signals detected by an implanted electrode system across the skin barrier, in a reliable and reproducible manner.

Chapter Three describes how a bone-anchor device could be combined with an implanted electrode to create a physical conduit for transmission of EMG signals from an implanted
muscle electrode to an external recording device. In this Chapter the same device is used to capture EMG signals arising from a muscle that has been subjected to a targeted muscle reinnervation procedure, in a sheep model. The EMG signals were captured in real-time, over the course of reinnervation, providing insights into the reinnervation process which might be of clinical value to amputees undergoing TMR surgery in the future.

Importantly, this study replicates most closely the scenario of an amputee undergoing future prosthetic reconstruction, in which they decide to undergo prosthetic reconstruction using a bone-anchor based device. In the same operative procedure for insertion of the bone-anchor, a TMR procedure is performed to increase the number of EMG signals available for an implanted electrode system. The implanted electrodes are placed directly onto the target muscles, thereby ensuring accuracy of placement. The electrodes are then passed through the bone-anchor device to permit subsequent recording and analysis of EMG signals by electronics in the end-device.

4.2 Aims

To investigate the use of bone-anchor + implanted electrode device to record real-time EMG signals from a muscle subjected to a TMR procedure in a sheep model.

4.3 Materials and Methods

4.3.1 Surgical technique for the TMR procedure

A bone-anchor device was manufactured and combined with an epimysial electrode (as described in section 3.4.4) for implantation in an ovine model (n=1).

With the animal anaesthetised as described in Chapter Three, and placed in a right decubitus position, the bone-anchored device was inserted into the left tibia as previously described in Section 3.4.8. To reduce skin movements over the bone-anchored device, the device was placed in a more distal position than the devices used for Experiment 2 (Chapter Three). This position was chosen to reduce the complication of sieving of the flange portion of the bone-anchor. A separate skin incision was then made over the ipsilateral m. peroneus tertius. The muscle was retracted medially to expose the peroneal nerve. Motor nerve branches going to
the *m. tibialis anterior* and long extensors were identified using a nerve stimulator (Medtronic Vari-Stim III Surgical Nerve Stimulator, Medtronic Inc., MN, USA). Typically, the *m. tibialis anterior* has three motor branches, and these branches were identified and confirmed using a nerve stimulator. One motor branch was divided, and there was further confirmation that this was a motor branch as *m. tibialis anterior* was noted to contract during division of the nerve. This motor branch was dissected free of the adjacent tissues until its branching point from the peroneal nerve was reached, thereby creating sufficient length to allow it to reach *m. peroneus tertius* for a tension-free coaptation. The motor branch to the *m. peroneus tertius* was then divided, 1 cm proximal to its entry into the muscle, and coapted to the motor branch from *m. tibialis anterior* using 3, interrupted, 8/0 nylon sutures placed 120° to each other (Figure 4.2). The native motor branch to *m. peroneus tertius* was also dissected back to the point at which it branched off from the peroneal nerve and this was then excised to prevent it from accidentally regenerating. The epimysial electrode was sutured onto the surface of the *m. peroneus tertius* as before. All the wounds were washed with saline and the skin incisions were closed in layers.

Silicone dust caps (Greentree Shercon, Tewkesbury, UK) were applied to the external socket of the bone-anchors to protect them from accumulating barn debris, as previously described. Recordings of the EMG signals were made weekly, for 12 weeks, while walking the sheep on a treadmill as previously described in section 3.4.9. Impedance measurements were also performed as previously described in section 3.4.10, with further *in vivo* impedance measurements performed, immediately after euthanasia of the sheep.

This experiment was conducted over 12 weeks.
4.3.2 Force Plate Analysis

Recovery of nerve function was assessed using serial EMG recordings and force plate measurements. At weekly intervals before and after making the EMG recordings, force plate measurements were taken. Force plate measurements were taken 4 days pre-operatively to provide a baseline measurement. The force plate (left and right) was calibrated using a 50kg weight. The sheep was weighed before each measurement was made. The sheep was walked across the force plate and at least 6 recordings for each hindlimb (left and right) were taken each week. The force measurements were normalised for weight and reported as $F_{\text{max}}/\text{weight}$.

4.3.3 EMG recordings

At weekly intervals after surgery, the sheep was walked on a treadmill while connected to the recording device, to look for the return of any EMG signals from the denervated $m. \text{peroneus}$
tertius following reinnervation of the muscle with the motor nerve branch from m. tibialis anterior. A weekly time interval was chosen on the assumption that nerve regeneration would average 1 mm of growth per day. Since the nerve repair was made at 10 mm from the point of entry of the motor nerve into m. peroneus tertius, the earliest one would expect to see any discernible EMG signals would be 10 days after surgery. Therefore, the first EMG recording was performed at one week post-operatively.

4.3.4 Data Analysis

Unless otherwise stated all data were analysed using SPSS version 22. The data did not fit the assumptions for parametric testing, hence non-parametric tests were used. Spearman’s rho was used to test the correlation between time since implantation and the SNR. The data are reported as median values and interquartile range unless otherwise stated. Wilcoxon’s signed rank test was used to test for differences in force-plate measurements over time. This non-parametric pair-wise test was corrected for multiple comparisons using the Bonferroni correction, which is intended to reduce the likelihood of Type I error. A p value of <0.05 was considered significant. Force plate measurement data were plotted as median, interquartile range and inner fences (1.5 times interquartile range).

4.4 Results

4.4.1 Gross Morphology

At explantation, a fibrous capsule was observed to have formed around the electrode and its cable, similar to findings in chapters Two and Three. M. peroneus tertius was found to be intact with grossly normal looking muscle fibres ((Figure 4.3). The coapted nerve was noted to be in continuity (Figure 4.4).
Figure 4.3: Gross appearance of the *m. peroneus tertius* at week 12 during the pre-terminal stimulation with a nerve stimulator (here seen as the white probe in the *m. peroneus tertius*). Stimulation of the motor nerve to the muscle (seen with the gold probe) elicited strong contraction in the muscle, evidence of nerve regeneration in TMR.

4.4.2 EMG Signals

For the first 3 weeks after surgery, the EMG recordings showed predominantly artifacts with no clearly discernible compound motor action potentials (Figure 4.5). However, by week 4, clear EMG signals could be seen, with an increase in SNR from 1.86 (1.72 to 1.94) at 22 days post-op to 3.8 (2.65 to 4.06), at day 29 (Figure 4.6 Clear EMG signals could be seen in every recording after 29 days post-op. At 58 days post-op, the SNR peaked at 6.35 (5.26 to 7.86). However, by 12 weeks post implantation, the SNR had decreased to 4.27 (3.40 to 5.04) (Figure 4.5). Importantly, the SNR for the TMR treated muscle was similar to that for the non-TMR treated muscle by 12 weeks (see Figure 4.7 in Chapter Three). However, the mean frequency for the EMG signal in the TMR treated muscle was lower (mean 145 Hz) compared with the non-TMR treated muscle (mean 230Hz), as shown in Figure 4.7.
Figure 4.4: The appearance of the intact nerve repair at 12 weeks. The arrow indicates the site of the TMR nerve coaptation (i.e., donor motor nerve from \textit{m tibialis anterior} to motor nerve of target muscle \textit{m peroneus tertius}). The presence of the 8/0 suture material used to coapt the nerves was used to identify the site.
Figure 4.5: Raw EMG signals (a,c) and power spectrograms (b,d) of EMG recordings following targeted muscle reinnervation. The recordings were made at 3 week (a,b) and 10 weeks (c,d). Note the change in scale for the EMG plots from 0 mV to 0.05 mV at 3 weeks to 0 mV to 0.5 mV at 10 weeks, a 10× difference in scale. The spectrograms were created by Fourier transforms with 50 sample Hamming windows.
Figure 4.6: The change in SNR with time following TMR surgery showing the linear line of best fit. The trend is significant $R^2 = 0.355$, $p=0.003$. Each data point represents a separate recording on a particular day.

Figure 4.7: Example of the EMG power spectral density from a TMR treated muscle at 10 weeks after surgery (black, mean 145 Hz) compared with a non-TMR treated muscle at 19 weeks (red, mean 230 Hz).
Figure 4.8: SNR for a muscle treated with TMR (black line), compared with a muscle treated without TMR surgery (grey), measured in dB. The SNR data were plotted as means ± 95% CI. Each data point represents a single measurement made on a particular day.

4.4.3 Force Plate Analysis

Pre-operatively, there was no significant difference in $F_{\text{max}}/\text{weight}$ observed between the left and right legs ($p=0.478$). For the first month following the TMR procedure, the force was significantly greater in the unoperated limb, and this difference showed an increasing trend with time ($p<0.006$, $p_{\text{cut}}>0.007$). However, from 45 days post-op, the force was not significantly different between limbs ($p>0.039$, $p_{\text{cut}}<0.024$). The data for normalised force are shown in Figure 4.9.
Figure 4.9: Force per sheep weight in N/kg following targeted muscle reinnervation, n = 1, 6 measurement repeats per day. The left hind limb was treated with a TMR procedure involving a nerve transfer from a motor branch of \textit{m. tibialis anterior} to \textit{m. peroneus tertius}. Significantly, different pairs are indicated with an asterisk (*).

4.5 Discussion

This study presents the results of experiments to record EMG signals from a TMR treated muscle using a bone-anchored device as a physical conduit for the delivery of those EMG signals from an implanted epimysial electrode. The data show that reinnervation of the muscle occurred as quickly as one month after the reinnervation procedure - as evidenced by the return of discernible EMG signals. This also corresponded with a change in the peak difference in force distribution between the treated and untreated hind legs of my sheep model. Specifically, the data demonstrate that the difference in force between the two legs decreased with time after apparent muscle reinnervation, and by day 45 there was no
significant difference between the forces exerted by the right and left hind legs during walking.

The data demonstrated that there was only interference recorded by the implanted electrode at one week post-op, with no discernible EMG signals. This changed at day 29 when the EMG signals became detectable with a SNR of 3.8 (2.7 to 4.1), which stabilised to just above 4.0 (3.4 to 5.0) by the end of the experiment at week 12. For recognisable EMG signals to be detectable, there must have been successful growth of the motor axons across the TMR coaptation site followed by re-establishment of functioning links with the previous neuromuscular junctions. This is the only way in which normal muscle contractions could occur that were capable of generating the EMG signals. Moreover, since the motor nerve to *m. peroneus tertius* was divided as close as to its insertion into the muscle as possible (approx. 8 mm) this would have reduced the amount of time taken for the axons to reach the motor end plates and reinnervate the muscle – a factor that may have contributed to the speed of reinnervation. Finally, on the day of euthanasia, intra-operative, *in vivo* stimulation of the transferred nerve using a stimulator set at 1mV was performed. The stimulator electrode was applied just proximal to the site of coaptation of the motor branch from *m. tibialis anterior* to the motor branch to *m. peroneus tertius* and this resulted in visible muscle contractions in *m. peroneus tertius*, providing further confirmation that successful reinnervation had occurred.

This study coapted a motor nerve branch intended for *m. tibialis anterior* to the motor nerve of *m. peroneus tertius*. Both of these muscles act to extend the hock joint. Although the function of *m. tibialis anterior* was not monitored as part of this study, only 1 out of the 3 motor nerve branches to *m. tibialis anterior* was used for the TMR transfer to minimise the effects on gait for the animal. Subjective, visual inspection of the sheep walking on the treadmill before and after surgery suggested that this approach was successful. However, objectively, the force distribution data suggested that there were in fact detectable changes in gait although these eventually returned to normal. Therefore, the sheep model could represent a useful model for future TMR recovery studies. Moreover, evidence for atrophy of both *m. tibialis anterior* and *m. peroneus tertius* at autopsy of the sheep in comparison with the contralateral side was observed. However, since the force distribution data eventually returned to normal with no difference between right and left sides by the end of the study, this degree of atrophy was not functionally important.
Although the changes in the force distribution might have been due to the TMR procedure, equally, they might have been due to pain caused by the surgery and may not have been directly caused by the denervation and reinnervation process. The changes in the force distribution data might have been due to healing and learning, a training effect that might explain why the recovery in the EMG signals and weight-bearing corresponded so closely. If the musculature is altered, the animal may gradually learn to use the altered musculature until it is able achieve a normal gait once again. Therefore, to establish whether this sheep model was truly representative of the effects of treating *m. peroneus tertius* with a TMR transfer, one would need to perform a procedure in which the motor branch to *m. peroneus tertius* and a single branch of the motor nerves to *m. tibialis anterior* were divided and ligated (to prevent accidental reinnervation) to see if the same changes in force distribution occurred as in my TMR study – without returning to normal. However, this control experiment was not performed for this study.

An EMG signal is a composite of multiple MUAPs of various frequencies that are superimposed, one on top of the other. Despite the apparent complexity of the composite signal, it is possible to decompose the contribution of each of the frequencies to the total signal. One of the changes observed in the EMG signal from the TMR treated muscle was a reduction in the EMG frequency (Figure 4.7). This observation concurs with previous investigations [177]. After 10 weeks, the mean frequency of the EMG signal from the TMR treated muscle was 145 Hz, compared with a mean of 230 Hz for EMG signals from non-TMR treated muscles (e.g., Experiment 2, n=6, Chapter Three).

The muscle activation patterns were also qualitatively different with higher peak voltages (up to 0.4 mV) in the TMR treated versus the non-treated muscle (0.2 mV). This is best shown by comparing Figure 3.30 and Figure 4.5 which show the raw EMG traces for non-treated and TMR treated muscles, respectively. One potential explanation for these observations is that there was a reduction in the overall number of motor axons which reached the neuromuscular junctions in *m. peroneus tertius* following the nerve transfer. This could lead to a substantial reduction in the force generated by the muscle when it contracted [162], since muscle fibres that are not reinnervated eventually atrophy. Atrophy of the muscle would also lead to the loss of muscle mass, which is what was observed here (although not measured quantitively). Since there are now fewer active motor units in the muscle (i.e. fewer axons but more muscle fibres being innervated by the same axon), each individual action potential results in more muscle fibres being activated simultaneously, compared with a normal
muscle, generating higher amplitude EMG signals. Moreover, following denervation, there is usually a shift in the composition of the muscle fibres from a mix of slow-twitch (Type I) and fast-twitch (Type II) fibres to a predominance of Type II fibers [162]. This change in the morphology of muscle has been shown to be sustained, long after the nerve repair [178]. Surprisingly, fast twitch muscle fibres produce EMG signals with a higher frequency compared with slow twitch fibres [179]. Despite this, it seems as though the effect of denervation followed by immediate reinnervation (i.e. as for TMR) must have negated any potential increase in EMG signal frequency that might be expected if there were truly an increase in the proportion of fast twitch muscles. Therefore, considering all of this data, I expected the EMG signal of the TMR-treated muscle to show fewer, larger, voltage spikes occurring at a lower frequency within the compound trace compared with a normal muscle. This is what appears to be shown in Figure 4.7.

One further observation about the reinnervation of muscles (e.g., after repair of a motor nerve) is the loss which occurs in neural Ia afferent connections after the reinnervation process is complete. This leads to an impairment of proprioception, coordination, and stretch reflexes [180]–[182]. Although TMR and RPNI interface muscles no longer have roles in normal skeletal movement, maintaining proprioception might be beneficial. For example, in lower limb prosthetic control [183]–[185], where sensing the position of the ankle joint can be of great benefit. There might be similar value in the context of upper limb prosthetics where being able to sense the position of the prosthetic limb without having to resort to either aural or visual aids would further lower the cognitive burden for the patient.

Importantly, the data from this experiment show that the amplitude and quality of the EMG signals generated by the TMR treated muscle would be sufficiently good to be used for prosthetic control. Currently, amputees who have undergone a TMR procedure are (mostly) forced to rely on skin-surface electrodes which are embedded into the prosthesis socket or applied as an armband (transhumeral amputees) or waistcoat (through shoulder amputees). The data from this study also show that the quality of the EMGs recorded from the epimysial electrodes is similar for TMR and non-TMR muscles, and previous experiments showed these EMGs to be superior to those recorded from a skin surface electrode (Figure 3.14). Therefore, I would strongly anticipate incorporating implanted electrodes into any future developments for prosthetic control. These electrodes could be implanted at the same time that the TMR procedure was performed, thereby avoiding the need for a second operation. Implanting the electrodes at the time of the TMR surgery would also ensure that the
electrodes were placed more accurately onto the target muscles – especially if muscles were sited deeply (relative to the skin). For example, voluntary control to \textit{m. pectoralis minor} is often preserved, even in patients who have sustained a brachial plexus injury. Therefore, it often provides a useful target for TMR procedures. However, \textit{m. pectoralis minor} is situated beneath \textit{m. pectoralis major} and this usually prevents the detection of EMG signals from the smaller muscle using skin surface electrodes. Placing an implanted electrode directly onto \textit{m. pectoralis minor} means that the full value of this muscle as a target for a TMR transfer can be realised. Finally, implanting the electrodes at the time of the TMR procedure avoids the risk of accidentally damaging any of the nerve transfers. This is because (after healing is complete) the index TMR procedure usually generates copious amounts of scar tissue that obscures the tissue planes and this puts any nerve transfers at risk while dissecting out tissue tunnels for placement of the electrodes.

However, there are also good reasons for delaying implantation of the electrodes until the reinnervation process has occurred (i.e. after the TMR has performed rather than simultaneously). A delay ensures that the reinnervated muscles are actually generating useful EMG signals before implantation is performed. Once the TMR signals are established and have stabilised (earliest 1 month, but typically 3 – 6 months after surgery), signal mapping using surface electrodes can be performed [139]. With the exact location for the new EMG signals fully mapped out before surgery, implantation can be performed that ensures that the epimysial electrodes are only placed onto those specific sites rather than wasting efforts to try and detect EMG signals from muscles that are inactive or not useful for prosthetic control [141]. The added advantage of delayed implantation is that it gives the patient enough time to train the reinnervated muscles and to learn to use them for prosthetic control – prior to implantation. If the amputee never learns to use their TMR reinnervated muscles for prosthetic control using surface electrodes, then implanting the electrodes becomes pointless and this saves all parties the cost and risk of additional surgery. All surgery carries risks (including damage to the previous TMR nerve transfers) and the presence of additional implanted hardware further increases the risks of complications such as infection.

One frequent observation made after TMR is a change in the sensory perception of patients in the areas of skin overlying the reinnervated muscles. For example, the amputees shown in Figure 1.11 and Figure 4.1 all report being able to feel parts of their hands in the chest skin, when it is touched. The perception is not normal but more akin to paraesthesiae (pins and needles) corresponding to those parts of the hand or limb which were supplied by the donor
nerve used to reinnervate the underlying muscles. In the patient in Figure 4.1, the recovery of hand sensation was particularly intense because the surgeons performed a targeted sensory reinnervation (TSR) procedure coapting a part of the median nerve to the supraclavicular nerve (normally responsible for sensation to the upper part of the chest down to the nipple) [142][146]. However as with TMR, the sensory recovery after TSR is not completely normal. Touch is still perceived as paraesthesiae instead of normal pressure, there is no specific somatotopy for the referred sensation, and there is considerable overlap in the sensory territories between the TMR and TSR skin. Further work has been performed to dissect out the sensory fascicles within mixed nerves, for TSR only, and this has improved outcomes, producing more discreet sensory patches with no overlap [186]. To achieve these outcomes, it was necessary to use intraoperative, somatosensory, evoked potentials to guide the dissection of the donor nerves. The typical target nerves for TSR include the supraclavicular nerve (as shown in Figure 4.1) and the intercostobrachial cutaneous nerve, which are coapted to the donor nerves in an end-to-end fashion while the main nerve trunk (composed mostly of motor efferent axons) is used for standard TMR surgery as normal. Future developments of this type of surgery may create a promising way for establishing sensory feedback loops that are currently missing from myoelectric prosthetics. Instead, at present, patients have to rely on visual or aural cues for feedback from their prosthesis.

The main limitations of TMR surgery are the availability of a suitable target muscle and the availability of donor nerves. These limitations are discussed more fully in Section 1.16.1. However, from an amputees’ perspective, one of the additional benefits of TMR surgery is the significant improvement in both neuroma and phantom limb pain (PLP). The neuroma pain probably improves because TMR provides an end-organ for the nerves, preventing the formation of a new neuroma after coaptation to the target muscle [147]. Certainly, in vivo neuroma models in animals demonstrate that the donor nerves recover a more normal architecture following coaptation with the motor nerves of the target muscle [148]. The phantom limb pain probably improves because the target muscle provides feedback to the central nervous system which was lost when the amputation occurred. This allows the sensitivity of the feedback loops with the central nervous system to be reset at a lower level. This is certainly how amputees feel after TMR surgery since they notice that the biggest reduction in their PLP occurs at the same moment that they become aware that they have regained voluntary control over their reinnervated muscles (described by many patients as a “light-bulb moment”). These were not observations seen in this sheep model.
4.6 Conclusions

- A bone-anchored device can be combined with an implanted electrode system to detect EMG signals from a muscle treated with a TMR procedure.
- The surgery to implant the device and the electrode can be combined with the TMR procedure in one operative step.
- A sheep model can be used to study reinnervation of muscles after TMR, using implanted muscle electrodes.
- The EMG signals generated by a TMR treated muscle in a sheep model are similar to those that have been recorded in other studies confirming the value of this sheep model.
- At 29 days after the procedure, the quality and amplitude of the EMG signals generated by a TMR treated muscle were good enough for control of a prosthesis.
- There were differences in the quality of the EMG signals comparing a normal muscle and a TMR treated muscle, but these differences were not sufficient to prevent their use for control of a prosthesis.
5 Chapter 5 - Investigating Optimal Electrode Configuration

5.1 Introduction

The simplest design for a skin surface electrode uses bare metal pads that are held in place on the surface of the skin using self-adhesive or Velcro-secured straps [5]. When the underlying muscles contract, EMG signals are produced by the muscles and these are detected by the overlying electrodes. The EMG signals are filtered and amplified and are then interpreted as instructions to activate electric motors in a prosthetic limb, thereby producing useful movements. As indicated previously, surface electrodes suffer from many disadvantages (Sections 1.12.1 to 1.12.2).

Chapters Three and Four demonstrated that implanted, epimysial electrodes can detect EMG signals with a greater SNR compared to surface electrodes using a bone-anchor based device as a physical conduit for taking these signals across a skin barrier, and these findings have been previously published [54]. Implanted electrodes address many of the limitations of surface electrodes but, despite these advantages, crosstalk remains a concern. Therefore, some of the solutions for addressing this problem are discussed in Chapter Three (Section 3.6). One of the options considered was the effect of differences in the configuration of the implanted electrodes. This chapter will consider this solution in more detail.

5.1.1 Electrode Configuration

Selecting the optimum electrode configuration for the bone-anchor based device was a crucial determinant of the overall success of the design. The electrode design had to be robust enough to permit rough handling by a surgeon whilst ensuring long-term longevity in vivo. Simultaneously, the electrode had to be able to detect EMG signals continuously with high fidelity and with a high SNR while in use. As an example, percutaneous needle electrodes permit highly selective EMG readings to be recorded. However, they are not physically robust enough to tolerate chronic implantation in vivo and are prone to mechanical failure. In contrast, the epimysial electrode design that I selected for my bone-anchor based device has been in use for functional electrical stimulation (FES) for many decades. The experience of using this design for FES has confirmed that the design is mechanically robust.
and can remain functioning for many decades in vivo [74]. Previous studies have also confirmed that there is a good correlation in the quality and amplitude of the EMG recordings detected using bipolar epimysial electrodes and fine-wire transcutaneous bipolar electrodes [86]. Therefore, where possible, the more robust epimysial electrodes are always preferred since they are (bio-electrically) just as good.

However, even though epimysial electrodes are preferred, there is much than can be altered about their design. For example, the larger the size of the electrode discs, and the larger the inter-electrode distance (IED), the greater the strength of the EMG signal that can be detected. However, increasing the strength of the EMG signals that can be detected reduces muscle selectivity [187]. SENIAM guidelines (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles) recommend that a 10mm diameter electrode is used, to ensure adequate signal pickup, while reducing the detection area sufficiently to avoid signal contamination from adjacent or deep muscles [87]. Whilst these guidelines are applicable for surface electrodes, there are no such guidelines for implanted electrodes, since there are no commercially available epimysial electrodes currently available for human use.

Currently, there are three common surface electrode configurations which may help to inform my investigations of the optimum configuration for the design of an implanted electrode. Some of the properties of these different configurations are considered below.

5.1.1.1 Monopolar Electrodes
These electrodes rely on a single electrode placed over a muscle, with the reference electrode placed in an area with little to no EMG activity. The advantage with this setup is its ability to record a very large EMG signal. However, this configuration records EMG signals from all the muscles located between the two electrodes and therefore produces tracings with the greatest amount of crosstalk and the least specificity. Furthermore, monopolar recordings suffer from their inability to remove common mode signals (such as 50 Hz noise) without filtering or other post signal acquisition processing. Nevertheless, untargeted, monopolar, surface electrodes can perform well in combination with some control algorithms (pattern recognition), and these can offset the effects of electrode shift and reduced electrode number [188]. These properties, combined with appropriate control algorithms, may make implanted versions of untargeted electrodes very promising once these systems become reliable enough to be used commercially [189]. However, since direct control (as opposed to pattern
recognition) remains the most accurate and reliable method for prosthetic control, the use of multiple surface bipolar targeted electrodes remains the gold standard.

5.1.1.2 Bipolar Electrodes
These electrodes generally consist of two metal contacts held in close proximity to one another by embedding them in an insulating backing. As the muscle depolarises and the signal propagates along the myofibrils, the change in the potential difference across the two metal discs is recorded as an EMG signal. The main advantage of having two detecting elements over the monopolar design is greater selectivity, and the ability to reject common mode interference (that is signals which are the same at both electrodes), so for example vastly reducing 50 Hz noise. The detection volume of the electrode corresponds roughly with a sphere where the radius is equal to the inter-electrode distance (IED) [170]. Increasing the IED increases the amplitude of the EMG signal at the expense of increasing the amount of crosstalk detected from adjacent contracting muscles. Bipolar electrodes with an IED of 10 mm have been successfully implanted in humans for prosthetic control [67], and this configuration was used in all previously described experiments in this thesis.

5.1.1.3 Branched Tripolar Electrode
This setup (also referred to as pseudo-tripolar) consists of an array of 3 electrical contacts equally spaced in a linear pattern. The two outermost contacts are shorted (connected) together and the potential difference from these contacts is measured against the central contact point (Figure 5.1). Theoretically, this setup has the potential for the greatest selectivity, since it isolates the detection volume. This type of setup has also been used for neural prostheses to increase selectivity when stimulating specific spinal nerves for bladder control [95], [96]. Surface tripolar electrode arrangements exhibit greater reductions in crosstalk compared to bipolar electrode configurations [190]–[193]. For example, when a constant sloped voltage gradient is detected using a bipolar configuration, it is difficult to determine whether this potential is due to a local source producing a higher potential on one electrode or a distant source producing a constant potential gradient. With the addition of the third electrode it is possible to distinguish between a distant source producing a constant voltage gradient over all three electrodes, and a local peak in the bio-potential from the desired muscle [190].
Figure 5.1: Examples of monopolar, bipolar and pseudo-tripolar electrode configurations. The reference electrode is placed in a location with little to no muscle activity, to reduce any potential interference from muscle-generating EMG signals. Image courtesy of Dr H Lancashire.

5.2 Aims

To date, there have been no in vivo experiments investigating the optimal configuration for implanted EMG electrodes. In this chapter, the quality of the EMG signals recorded using the three electrode configurations described above is investigated, in a live, conscious sheep model over a 12-week study period. Furthermore, the effects of varying the IED on the quality and amplitude of the EMG signals using both bipolar and pseudo-tripolar implanted electrode designs are investigated. For simplicity, pseudo-tripolar electrodes are henceforth referred to as tripolar.

The aim of this part of my study was to:

- Investigate the SNR for EMG signals recorded using monopolar, bipolar and pseudo-tripolar electrode configurations in an attempt to find the optimal electrode design.
5.3 Materials and Methods

5.3.1 Design and Construction of Bone-Anchored Device

The design of the bone-anchored device used for the experiments described in this Chapter was based on the design of the devices used for the experiments described in Chapters Three and Four of this thesis and modified in response to the findings from those experiments. The new bone-anchor was made from Ti-6Al-4V using laser sintering (3T Additive Manufacturing Ltd., Newbury, UK). Laser sintering is a type of 3D printing in which a laser beam is used to fuse particles of a powdered base material (in this case a titanium alloy) particle by particle, building up the layers to create a solid, 3-dimensional structure. At the core of the design for the new bone-anchor was the original design for the ITAP bone-anchor. Therefore, as before, the device consisted of an intraosseous component (stem) with a tapered shape to allow it to be press-fitted into bone following preparation of a suitable hole with a tapered reamer. The stem was continuous with a transcutaneous part which was enlarged to accommodate a 9 mm threaded connector rather than creating a separate component to contain the connector as for earlier versions of the device (e.g., Figure 3.1). At the junction between the stem and the transcutaneous part was a dome-shaped flange structure. A “J-shaped”, 2 mm diameter channel was formed through the transcutaneous part of the device in the laser sintering process, and this channel passed through the flange and exited at the underside of the flange structure. The channel was created to accommodate the electrode cable. As previously, the stem, flange and a 2mm section above the flange were HA-coated to encourage osseointegration of the device

Figure 5.2). The modifications to the shape of the transcutaneous part of the device, the flange and the cable channel were all in response to the outcomes of the experiments described in Chapters Three and Four of this thesis.
Figure 5.2: Design of a bone-anchor based device for transmission of EMG signals. The central channel is 2 mm in diameter and is “L-shaped”. This allowed the cable from the implanted epimysial electrode to be passed through to the external connector housed in the expanded part of the transcutaneous part of the device. The cable exits the transcutaneous part of the bone-anchor through the edge of the dome-shaped flange. Note the change in the design of the flange structure compared with the designs used for earlier bone-anchor based devices (e.g., Figure 3.1) used for the experiments described in Chapters Three and Four.

5.3.2 Design of Electrodes

5.3.2.1 5-Electrode Array

Rather than create multiple electrodes with different IEDs and then implant them in multiple animal subjects, I designed one electrode array (shown in Figure 5.3) with 5 separate electrode discs. By using a switching system, it was then possible to record EMG signals from each disc separately and in different combinations. The switch diagram is shown in the appendix. For example, recording signals from the two outer discs created (in effect) a bipolar electrode with an IED of 40 mm. This design was manufactured to my specifications by Ardiem Medical Inc. (Pennsylvania, USA).

The array consisted of a polyester fibre reinforced silicone structure in which five circular Pt/Ir discs were embedded. The discs acted as the recording sites, with a total surface area of 10.64 mm² and an IED of 10mm centre to centre. The 5-pole connector at the end of the
flying lead was connected to a single 5-core coiled cable using a Letechipia spring-loaded connector. The entire connector assembly was then sealed with medical grade silicone elastomer (NuSil Med3-4013, NuSil Technology LLC, CA, USA) which was cured for 24 hours at 50°C. The electrode cable was then fed through the channel in the bone-anchor and sealed into place with the same medical grade silicone. The end of the 5-core cable was then soldered onto a socket connector (FGG.1B.306.CLAD62Z, LEMO UK Ltd, Worthing, U.K). These connectors were mechanically more robust than those used for the experiments described in Chapters Three and Four. To add further strength to the external part of the device, the connector was housed within the expanded part of the transcutaneous component of the bone-anchor and bonded into place with epoxy resin. The bone-anchor + electrode device was then sterilised using ethyl oxide gas.

Figure 5.3: The 5-pole and 2-pole electrode array specification.

5.3.2.2 2-Electrode Array

I designed and created a bone-anchor based implanted electrode system which included two, standard, 2-electrode arrays. One of the electrodes was intended for use as the reference electrode for all the experiments described in this Chapter and was located over the lateral aspect of the hock, while the other was used as an epimysial electrode to detect EMG signals.
from an antagonist muscle. In this case, *m. gastrocnemius* was selected as the antagonist muscle for my experiments. The rationale behind this was to understand which parts of the signal were due to cross talk (i.e. originated during a different part of the gait cycle when the antagonistic *m. gastrocnemius* was being activated). These two electrodes were combined with a bone-anchored device which was modified to create two channels in order to pass the cables from these two epimysial electrodes through the device. As for the 5-electrode array, the flying leads were soldered onto a socket connector (FGG.1B.306.CLAD62Z, LEMO UK Ltd, Worthing, U.K) which was then housed in the expanded part of the bone-anchor. The rest of the process for assembling and sterilizing the device was the same as for the 5-electrode array (as described in Section 5.3.2.1.

The final appearance of the assembled implants is shown in Figure 5.4.

![Figure 5.4: The final appearance of the assembled bone-anchors with their 2-pole and reference electrode (A), and 5-pole (B) electrode arrays.](image)

### 5.3.2.3 Electrode Configuration

The electrode configurations created and tested are summarised in Table 5.1, Table 5.2, and Table 5.3, for monopolar, bipolar and tripolar designs, respectively. Each of the recording electrode sites was tested individually. For the bipolar electrode setup, an IED of 10, 20, 30 and 40 mm was tested along the length of the 5-electrode array. A tripolar setup, was achieved by using the shortest IED of 10 mm between the 5 electrodes with the. The next intervals between the electrodes were as follows; long IED (20 mm), and unbalanced 10 mm and 30 mm and 10 mm and 20 mm (Table 5.3). To achieve these different configurations
from the 5-electrode array, a switch arrangement was used to record EMG signals from the different discs in varying combinations.

The switch box comprises 3 rotary switches and one toggle switch. The toggle switch chooses either monopolar, bipolar, or tripolar configuration and routes the signals to the relevant rotary switch. Each position of each rotary switch connects the signals appropriately to the BIOPAC inputs for the given configuration in the tables. The monopolar rotary switch turns two sets of connectors, one that selects the reference, and the other the monopole. The bipolar rotary switch turns two sets of connectors, each of which chooses one of the 5 electrodes, the connectors are lined up such that we can choose the different configurations. The tripolar rotary switch turns three sets of connectors (two for the outer and one for the inner); again, they are lined up such that we can choose different configurations.

The different combinations are indicated in Table 5.1 for monopolar, Table 5.2 for bipolar and Table 5.3 for tripolar configurations of the electrodes. Configuration order is enforced by the design of the switch box and was chosen to minimise connection path length and complexity, both in switch box design and during experimentation. Combinations are numbered in the order in which they occur on the switch box rotary switches.

Table 5.1: Configuration of the monopolar electrode, with a schematic of the 5-pole electrode indicating the electrode number used to create the particular combination.
Table 5.2: Configuration of the bipolar electrode. Each block represents an inter-electrode distance of 10 mm. Configuration of the bipolar electrode. Inter-electrode distances: 10 mm (b1, b6, b9 and b10); 20 mm (b2, b5, b8); 30 mm (b3, b7), 40 mm (b4).

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Table 5.3: Configuration of the tripolar electrode. Balanced short interelectrode distance of 10 mm (t1, t5, t6). Balanced long interelectrode distance of 20 mm (t3); unbalanced interelectrode distance of 10 mm and 20 mm (t7, t8); unbalanced interelectrode distance of 10 mm and 30 mm (t3, t4).

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<td>-</td>
</tr>
</tbody>
</table>

5.3.3 Operative Procedure

The operative procedure used to insert the bone-anchors was similar to that used for insertion of the bone-anchors in the experiments described in Chapter Three. One female sheep (mule breed) was chosen for this study. All animal procedures were carried out in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act 1986. A general anesthetic was administered using isoflurane (Isoflo) in oxygen. A 5 cm long skin incision was made over the right tibia, at 15 cm inferior to the medial aspect of the knee joint. The tibia was then exposed by dissecting away soft tissue to allow the creation of two drill holes, one for each of the two bone-anchor devices used for this experiment. For the holes in the bone, both cortices of the tibia were drilled using a 4.6 mm diameter drill bit. The hole in the medial cortex was then enlarged using a 4.8 mm diameter drill bit. Both cortices were then reamed, using a custom tapered reamer. The implant carrying the 2 electrode arrays was inserted and press fitted into the distal hole. As previously described, a 2 mm gap was left between the flange and the bone to allow passage of the electrode cable and to permit soft
tissue growth under the flange. The bone-anchor carrying the 5-electrode array was inserted into a bony hole which was situated 5 cm proximal to the 2-electrode bone-anchor, using the same operative technique.

Figure 5.5: Intra-operative pictures documenting the steps involved in inserting the two bone-anchored devices. The left-hand image shows the muscles in the lateral aspect of the left limb exposed, with the 2-pole and 5-pole epimysial electrodes sutured onto the surface of *m. peroneus tertius* and *m. gastrocnemius*. The center image shows the position of the reference electrode over the medial ankle region (mainly tendons rather than muscle). The right-hand image shows the relative positions of the two bone-anchors inserted into the medial aspect of the tibia, prior to closing the skin incisions (right).

To place the electrodes, a separate 10 cm skin incision was made over the lateral compartment muscles of the left limb. The *m. peroneus tertius* was identified through this incision (Figure 5.5). A subcutaneous tunnel was then created to connect this incision with the site of the bone-anchors and the 5-electrode array was passed through and sutured longitudinally over the *m. peroneus tertius* using 4-0 Prolene (non-absorbable) sutures. One of the 2-electrode arrays was then tunneled under the skin and sutured onto the epimysial surface of *m. gastrocnemius*, while the other 2-electrode array was tunneled subcutaneously to a separate skin incision in the hock area. The hock area corresponds with the ankle joint in humans which ensured that there would be only tendons underlying this reference electrode (Figure 5.5). The electrode was sutured to the deep fascia overlying the tendons using 4-0 prolene. The absence of muscles in this area helped to ensure that there would be no EMG signals generated that might interfere with the EMG signals generated by muscles in the proximal limb. The incisions were then washed with physiological saline solution and closed using Vicryl (absorbable) sutures (Ethicon Inc., NJ, USA) as previously described. Post-
operatively dressings were applied to the limb as previously described. Because of the larger amounts of hardware placed in the limb and the more extensive dissection, a decision was made to administer oral antibiotics for 3 days post-operatively and fentanyl patches were applied for one week, for analgesia. The animal was individually housed as previously described.

5.3.4 EMG Recordings

Before surgery, the sheep was acclimatised to walking on a treadmill. Post-operatively, the animal handler guided the animal to walk on the treadmill using food for positive reinforcement. The animal walked at a steady speed of 1.5 km/hr. For my previous experiments, the sheep walked at 2 km/hr on the treadmill - after surgery. Because of the smaller size of the animal used in this study compared with the previous experiments (52 kg vs. 67 kg), 2 km/hr was considered too fast to allow for normal gait.

Weekly recordings were made from week 4 onward for 12 weeks. The 4-week delay ensured that the electrodes were fully encapsulated in mature scar tissue and allowed the SNR to stabilise [173]. The EMG signals were recorded using the methods described in Section 2.8.4 and Section 3.4.9. A 15 second segment of the signals was analysed for each experiment. This corresponded to approximately 10 gait cycles. The signal-to-noise ratio (SNR) was calculated for 10 gait cycles per recording [194] using MATLAB 2017b (The MathWorks, Inc., Natick, MA, USA).

5.3.5 Impedance Measurements

Impedance spectroscopy was carried out as described in section 3.4.10. Measurements were made before implantation and immediately following explantation of the bone-anchor devices, *ex vivo*. All measurements of electrode impedance were taken without the cables being connected to the bone-anchor device.

5.3.6 Data Analysis

The conditions for parametric testing were not met, hence the data were not assumed to be normally distributed. The Mann Whitney U test was therefore used to test for differences in the SNR between electrode configurations. The SNR was reported with median and interquartile ranges unless otherwise stated. Impedance measurements are reported as median and interquartile range. A $p$ value of <0.05 was considered significant.
5.4  Results

5.4.1  Gross Morphology

At the end of the experiment at 12 weeks after insertion, both implants were noted to be fully intact, with no damage to the socket connectors, and with the dust caps intact. The interface between the skin and the implant was dry, with no evidence of erythema, infection or discharge (Figure 5.6). The skin overlying the flange appeared normal with normal hair growth. There was a dense layer of scar tissue surrounding the electrode and its cable – as noted in previous experiments. On removing the 5-pole electrode, the fifth disc (the distal-most electrode) was noted to be overlying the musculotendinous junction of the muscle, despite having been sutured directly over the muscle belly of *m. peroneus tertius* at the initial surgery, 12 weeks beforehand.

![Figure 5.6: Appearance of the two bone-anchor devices at 12 weeks after implantation, showing no evidence of sieving and a normal appearance of the overlying skin.](image)

5.4.2  Radiological Evaluation

Plain radiographs taken at 12 weeks post-operatively confirmed the correct position of both implants in the bone. This is shown on the lateral x-ray of the tibia (Figure 5.7). In cases where there is loosening of the implant, one would expect to see radiolucency around the implant stem, indicating absence of bone and failure of osseointegration. There was no evidence of radiolucency and radio-opaque bone tissue can be seen abutting the implant stem in both implants.
5.4.3 EMG Measurements

Beginning at 4 weeks after implantation, recordings of the EMG signals showed that the electrodes placed over *m. peroneus tertius* (5-pole electrode) and *m. gastrocnemius* (2-pole electrode) were detecting agonist/antagonist activation. An example of the EMG recordings obtained is shown in Figure 5.8. The timings from these recordings show that the EMG signals from the 5-pole electrode are only seen in the intervals when there is no signal from the 2-pole electrode and vice versa. In other words, when *m. gastrocnemius* is contracting there is no EMG signal from *m. peroneus tertius* which is relaxing. These observations were interpreted as confirmation that the two antagonistic muscles - as expected, were generating the EMG signals.
Figure 5.8: Recordings of the EMG signals from the 5-pole electrode and 2-pole electrodes. Note the timing of the EMG signals. The signals from *m. peroneus tertius* (5-pole electrode) are only present when *m. gastrocnemius* (2-pole electrode) is relaxing (i.e. showing no EMG signals) and vice versa. The blue shaded area indicates the stance phase when the limb is on the treadmill floor.

At 4 weeks after implantation, the EMG recordings from the 5-pole electrode showed that the SNR was maximum (median 7.2, range 5.6 to 9.7) with combination b9 (Table 5.4, Figure 5.9 and 5.10). In this combination, the 5-pole electrode had a bipolar electrode configuration with a 10 mm IED where the metal discs were centred over the muscle belly of *m. peroneus tertius*. The lowest SNRs were observed as the IED was gradually increased resulting in configurations where the metal discs were placed increasingly further away from the muscle belly. The tripole arrangement that showed the greatest SNR, t2 (median 3.1, range 2.36 to 4.74) had the discs placed in a similar location over the muscle belly as the best bipolar configuration, but with unbalanced 10 mm and 30 mm distances between the 3 electrodes (Figure 5.11). The monopolar configurations resulted in the lowest SNR (median 1.0, range 0.8 to 1.4), where many of the EMG signals were difficult to distinguish from interference (Figure 5.11, Table 5.5). The effects of all the different electrode combinations on the SNR are summarised in Figure 5.14.
Figure 5.9: SNR data for each bipolar electrode configuration. Configurations are indicated by numbers above the charts (see Table 5.2). Inter electrode distances are indicated. Electrode configurations with the greatest SNR were b9 (7.2, [5.6 to 9.7]), and b10 (5.0, [3.8 to 6.1]).

Figure 5.10: Diagrammatical representation of the electrode arrangements for the bipolar electrode setup. For ease of identification, the electrodes points that were tested are shown as dark discs, and the rest of the contact points are greyed out. The median SNR is shown under the respective electrode configuration.
Table 5.4: Median SNR and Interquartile Range (IQR) for the different bipolar electrode configurations. IED: interelectrode distance

<table>
<thead>
<tr>
<th>Electrode Configuration</th>
<th>IED (mm)</th>
<th>Median SNR</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>b1</td>
<td>10</td>
<td>3.5</td>
<td>2.8-5.5</td>
</tr>
<tr>
<td>b2</td>
<td>20</td>
<td>3.0</td>
<td>2.4-3.2</td>
</tr>
<tr>
<td>b3</td>
<td>30</td>
<td>2.5</td>
<td>2.2-3.1</td>
</tr>
<tr>
<td>b4</td>
<td>40</td>
<td>2.5</td>
<td>2.0-2.9</td>
</tr>
<tr>
<td>b5</td>
<td>20</td>
<td>1.8</td>
<td>1.4-2.3</td>
</tr>
<tr>
<td>b6</td>
<td>10</td>
<td>2.0</td>
<td>1.7-2.7</td>
</tr>
<tr>
<td>b7</td>
<td>30</td>
<td>1.8</td>
<td>1.7-2.0</td>
</tr>
<tr>
<td>b8</td>
<td>20</td>
<td>3.4</td>
<td>3.3-4.7</td>
</tr>
<tr>
<td>b9</td>
<td>10</td>
<td>7.2</td>
<td>5.6-9.7</td>
</tr>
<tr>
<td>b10</td>
<td>10</td>
<td>5.0</td>
<td>3.8-6.1</td>
</tr>
</tbody>
</table>
Figure 5.11: The SNR data for each individual tripolar electrode configuration. The different configurations are indicated by numbers above the chart (see Table 5.3). Note the greater variability in the SNR within readings.

Figure 5.12: Diagrammatical representation of the electrode arrangements for the tripolar electrode setup. For ease of identification, the electrodes points that were tested are shown as dark discs, and the rest of the contact points are greyed out. The two outermost discs were shorted together and the potential difference from these discs was measured against the central disc. The median SNR is shown under the respective electrode configuration.
Table 5.5: Median SNR and Interquartile Range (IQR) for tripolar electrode configurations.

<table>
<thead>
<tr>
<th>Electrode Configuration</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1</td>
<td>2.9</td>
<td>2.0-3.6</td>
</tr>
<tr>
<td>t2</td>
<td>3.1</td>
<td>2.4-4.7</td>
</tr>
<tr>
<td>t3</td>
<td>2.5</td>
<td>2.0-4.9</td>
</tr>
<tr>
<td>t4</td>
<td>3.0</td>
<td>2.4-3.5</td>
</tr>
<tr>
<td>t5</td>
<td>2.3</td>
<td>1.9-3.5</td>
</tr>
<tr>
<td>t6</td>
<td>2.0</td>
<td>1.8-2.4</td>
</tr>
<tr>
<td>t7</td>
<td>1.7</td>
<td>1.6-2.2</td>
</tr>
<tr>
<td>t8</td>
<td>2.1</td>
<td>1.6-2.8</td>
</tr>
</tbody>
</table>
Figure 5.13: SNR data for each individual monopolar electrode configuration, indicated by the numbers above the charts. Some values are missing, as it was not possible to distinguish the EMG signal from background noise.

Table 5.6: Median SNR and Interquartile Range for monopolar electrode.

<table>
<thead>
<tr>
<th>Electrode Configuration</th>
<th>Median</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1</td>
<td>1.0</td>
<td>0.7-1.2</td>
</tr>
<tr>
<td>m2</td>
<td>1.1</td>
<td>1.1-1.3</td>
</tr>
<tr>
<td>m3</td>
<td>1.1</td>
<td>1.0-1.5</td>
</tr>
<tr>
<td>m4</td>
<td>1.0</td>
<td>0.7-1.1</td>
</tr>
<tr>
<td>m5</td>
<td>0.9</td>
<td>0.8-1.3</td>
</tr>
</tbody>
</table>
5.4.4 Impedance Measurements

The median impedance pre-implantation was 184 Ω (176 Ω to 187 Ω). By explantation at 12 weeks, this had increased to 257 Ω (232 Ω to 265 Ω). These values are very much lower than the results reported in Chapter Three (Section 3.11.4). Although this was taken without the electrode cable attached, the impedance of the cable itself is approximately 10 Ω, a very small figure compared with the electrode impedance. Nevertheless, the change in impedance following implantation is comparable with the findings in Chapter Three and are similar to those observed in previous studies with implanted electrodes in rats [156].
Figure 5.14: Boxplot of the SNR for monopolar, bipolar and tripolar electrodes. The highest SNR values are indicated with black (dashed) lines – b9 and t3. Note the very low SNR for monopolar electrodes in all configurations. Statistical analysis showed that the difference in SNR between monopolar and bipolar, monopolar and tripolar, and between bipolar and tripolar configurations reached significance ($p<0.05$).
5.5 Discussion

For this study, I chose to record EMG signals from \textit{m. peroneus tertius} because that is the muscle which was used for all previous experiments in this thesis. Therefore, it was easier to make comparisons with my EMG data from previous experiments. Furthermore, it only has a single motor branch, which enters the muscle at its proximal end. By comparison, \textit{m. tibialis anterior} has multiple motor nerve branches and therefore multiple innervation zones. Therefore, I decided not to use this muscle to avoid the confusing effects that might be created by the propagation of electrical impulses from multiple locations within the muscle being studied.

Using the present design for a bone-anchor based, implanted electrode system, my data suggest that a bipolar electrode with the smallest IED of 10 mm (i.e. where the two electrode discs are placed directly over the muscle belly (b9)), was able to record the highest, median, SNR of 7.2 (5.6 to 9.7). The other 10 mm configurations for bipolar electrodes (b1 and b10) also performed reasonably well with median SNRs of 3.5 (2.8 to 5.5) and 5.0 (3.8 to 6.1), respectively. The exception to this trend was configuration b6, which also used an IED of 10 mm but showed a surprisingly low SNR value of <2.0. However, for b6, the 5-pole electrode used the metal discs located at points 4 and 5. As mentioned in Section 5.4.1, over the 12-week course of the experiment, the 5-pole electrode appeared to have migrated inferiorly, from its original position over the muscle belly of \textit{m. peroneus tertius}. Therefore, at explantation, disc 5 was found to be overlying the musculotendinous junction of \textit{m. peroneus tertius} at explantation rather than the muscle belly itself.

My data also appear to show that the SNR decreased with increasing IED. This was expected since the detection area is known to increase in size as the IED increases. Therefore, the possibility of crosstalk from adjacent muscles is likely to increase. The bipolar electrode configurations with an IED of 20 mm, 30 mm and 40 mm all yielded an SNR of \(\leq 3.0\), with the exception of b8, which had an IED of 20 mm and showed a SNR of 3.4 (3.3 to 4.7).

Another factor that could have influenced the SNR was the location of the electrodes with respect to the point of entry of the motor nerve into the muscle and the underlying neuromuscular junction (NMJ). For this experiment, the motor nerve entered the muscle at a distance of 10 mm proximal to disc 1, on the 5-electrode array. The close proximity of the NMJ to disc 1 could explain why the SNR was lowest when using this electrode point for the
10 mm IED configurations compared with the other bipolar 10mm configurations. It might also be related to the branching pattern of the motor nerve within the muscle, as the nerve terminated on the NMJ. Disc 1 might have been too close to this “innervation zone”, where axon terminals cluster as they give off several short branches before terminating in a motor end plate [195]. At this point on the muscle, MUAPs travel in both directions along muscle fibres, with the resultant effect of cancelling each other out if the electrode pair is placed perfectly over the innervation zone, or partially cancelling each out if placed at least part across the innervation zone. In fact, placement of the electrodes directly above the innervation zone has been shown to have an effect on the morphology of the recorded EMG signal [195], [196], often resulting in a decrease in amplitude [197]. Therefore, SENIAM recommendations for the siting of surface electrodes is to place them away from the innervation zone [87]. This view is supported by my own observation that bipolar electrode configurations centred on the middle portion of the muscle (i.e. away from the innervation zone) produced the highest fidelity EMG signals as measured by SNR.

Surprisingly, the “tripolar” electrodes in my experiment failed to show a higher SNR compared with a bipolar setup. The tripolar electrodes were expected to show the greatest selectivity, since they should be better able to isolate the detection volume. Certainly, previous studies using surface tripolar electrodes in healthy human volunteers showed that there was less crosstalk when compared with standard bipolar setups [190]–[193]. For example, Fortune et al. investigated evoked EMGs using surface bipolar and tripolar electrodes with an IED of 35mm, placed over the forearm flexors of healthy volunteers. They found that tripolar electrodes outperformed their bipolar counterparts in reducing crosstalk [190]. However, their study had many limitations including the use of evoked potentials (which rely on non-physiologic muscle stimulation), and the use of skin-surface electrodes (whose limitations have been discussed in detail). Since implanted epimysial electrodes were used for this study, it was assumed that many of the confounding factors would be made constant. Therefore, the benefits of tripolar setups might then be less obvious. That seemed to be true in my study, where the tripolar configuration with the highest SNR was t2 with a median 3.1 (2.4 to 4.7) compared to the best bipolar setup (b9) with a median SNR of 7.2. Importantly, in terms of their position on the muscle, the relevant discs for t2 were placed in a similar location to the best bipolar configuration (b9), but with unbalanced 10 mm and 30 mm distances between the 3 electrodes. Yet, this also failed to improve the SNR for the tripolar setup.
Finally, statistical analysis of the best median of the SNR for the three different electrode configurations (5.12) showed that this was significantly greater for bipolar electrodes compared with tripolar ($p=0.013$). Further comparisons showed that the SNR for bipolar and tripolar electrodes in this study was superior to that for monopolar ($p<0.001$) electrodes. However, these comparisons should be interpreted with caution given that they were the result of an experiment in a single sheep.

Despite the limitations of my study, there is a paucity of data from in vivo experiments investigating the effects of varying IED and electrode configuration on EMG signal fidelity and SNR. For example, Sando et al. implanted bipolar (3mm IED) and monopolar epimysial electrodes into the m. extensor digitorum longus of rats [80]. They found that bipolar electrodes recorded EMG signals with a higher SNR compared with monopolar electrodes. The SNRs for monopolar electrodes were also small (in the range of 1.0, similar to the findings in my own study). Interestingly, they also observed a reduction in recorded peak-to-peak voltage as well as SNR in bipolar epimysial electrodes, with the SNR averaging <2 by 4 months after implantation. They attributed this observation to fibrous encapsulation of the electrodes. However, a major limitation of their work is that the EMG signals they recorded were not generated in a conscious animal but rather by nerve stimulation that elicited a CMAP in the muscle. Importantly, the SNR readings from the best electrode configuration in my own study (b9) and the results I have reported in Chapter Three were both consistently higher (>5) than the highest SNR reported in Sando’s study. Moreover, it has been my observation that fibrous encapsulation does not result in a decrease in the signal amplitude, in contrast to their findings.

In another in vivo study, Farnsworth et al. described an implantable EMG recording system in a single rat model [198]. The authors used an intramuscular bipolar electrode, implanted into the muscles of the hindlimb of a rat. The electrode cables were routed under the rat’s skin, along its back, and into a metal cap attached to the rat’s skull. The EMG signals were recorded after tugging on the rat’s hindlimb. This elicited a withdrawal reflex that resulted in muscle contractions in the hindlimb muscles. Confusingly, although the authors described using an epimysial system, the methods section shows that they actually used an intramuscular electrode, presumably because this proved easier to implant. However, importantly, although they were able to record EMG signals with their electrode, these were not physiological signals from a conscious animal (although an argument can be made that
they are more ‘physiological’ than evoked CMAPs). Therefore, making comparisons of the results of their studies with those in conscious humans or other animal models is difficult.

One of the other limitations of studies investigating the effects of IED and electrode configuration on EMG signals is their use of surface electrodes [85], [190], [199]–[202]. This makes it difficult to make direct comparisons with the results of my own study. These previous studies have the additional limitations and confounding factors associated with the use of surface electrodes. The factors which might have influenced the outcomes include; the reliability and repeatability of electrode placement with respect to the innervation zones, orientation of the electrodes with respect to the muscle fibres, and skin preparation and impedance related to perspiration and temperature [203], amongst others. These factors might account for the conflicting reports from previous investigations regarding the effect of IED on EMG amplitude. For example, Beck et al compared IEDs of 20 mm, 40 mm, and 60 mm on signal amplitude in m. biceps brachii muscle in healthy individuals and found an increase in raw EMG amplitude with greater IED [199]. However, when the data were normalised [204], it appeared that there was no difference between the different setups. Furthermore, no attempt was made to record SNR, or the effects of crosstalk, so no comparison of these particular outcomes could be made with the results of my study.

Other investigators have reported either similar findings to my study [201] or contradictory results on the effects of varying IED. For example, Roeleveld et al and Vigreux et al found a linear relationship between IED and signal amplitude [205], [206] and argue that a larger IED has a greater pick-up area than an electrode arrangement with a smaller IED. However, these experiments were carried out on m. biceps brachii, where a large IED might be advantageous without the same concerns about crosstalk from adjacent muscles as in my study. Therefore, it is possible that the optimal IED may actually depend on the size and location of the muscle. As an example, a large IED of 40mm may provide greater EMG amplitude for m. biceps muscle EMG recordings. However, this larger distance might then result in problematic crosstalk in anatomical areas where a group of relatively small muscles are placed very close together, for example in the forearm or in the chest, after TMR surgery. In these situations, greater selectivity and reduced crosstalk might be achieved using electrodes with a smaller (10mm) IED [191]. Certainly, SENIAM have recommended that surface electrodes used for smaller muscles should have an IED that is one fourth of the muscle fibre length [87], and these guidelines have been met for the experiment described in this Chapter.
Although better electrode selectivity might be achieved by having a smaller IED resulting in a smaller detection volume, there are limits to how effective this strategy might be in practise. Indeed, Farina et al. hypothesised that decreasing the IED might not be an effective method for reducing crosstalk at all [202]. For example, the smaller the IED, the smaller the amplitude of the EMG signal recorded and the greater the noise levels in comparison to the size of the EMG signal [202]. While this can be offset by using signal processing algorithms to remove noise [190], [200], [207], [208], it is more advantageous to have a greater signal amplitude in the first place. Moreover, having an electrode setup, which minimises noise, in the first place reduces the downstream processing burden, and simplifies the design of any implanted system. This is particularly relevant if the implantable system requires in vivo multiplexing of signals prior to signal transmission via the bone-anchor. Generally, the simpler the system, the lower the cost, the greater the reliability, and the higher the chance of clinical success. For implantable electronics, one of the key considerations is the prevention of short-circuiting of the electronics and corrosion of the components due to water ingress. Use of an hermetically-sealed package reduces the chances of damage to the components [209]–[211]. However, the development of such a package was beyond the scope of this thesis, although it would need to be part of any future work if an implantable system were to become a reality.

There are a number of other concerns with using overly small electrodes with a small IED. These include their more delicate nature, which means that they might not tolerate intra-operative handling by surgeons and could be easily broken during surgery or break after implantation. Moreover, the smaller the electrodes, the greater the chance that they will shift position and migrate before they are fully encapsulated. In general, the smaller the electrode recording surface, the higher the impedance [212]. Further, electrode impedance also has an effect on noise levels since the greater the impedance the greater the amount of noise that is generated. Although, an extensive literature search failed to find any studies investigating the optimal surface area for EMG detection, it would appear that the size of the detection surface is not improved above a diameter of 5 mm [202]. The electrodes used in my study had a diameter of only 3.7 mm. Therefore, it is concluded that an IED of 10 mm probably represents a suitable compromise between selectivity and signal amplitude [213].

The work presented in this thesis represents one of only a few published collections of continuous, in vivo, physiological (i.e. normal) EMG recordings from multipolar implanted epimysial electrodes in a conscious animal. The only other study was by Bergmeister et al.
who tested a 4-pole, epimysial electrode system using stainless steel discs with an IED of 10 mm in 2 sheep. The epimysial electrodes were placed over brachiocephalicus, triceps, brachialis and latissimus dorsi and the EMG signals were monitored over a four-month period using a wireless signal transmission system to transfer data across the skin barrier by radiofrequency [156].

As in previous Chapters, the data presented in this Chapter suggest that the bone-anchor based, implanted electrode system that I designed and created was able to detect and record EMG signals reliably over a 12-week period. Further, these data suggest that the highest SNR was seen using a bipolar electrode setup with an IED of 10mm when the electrode was placed over the middle of the muscle belly. However, my study had several limitations, which mean that my conclusions must be interpreted with caution. These include:

1) The results presented were gathered from just one animal. It would be necessary to replicate the results in a larger number of animals to demonstrate consistency and reliability to confirm these outcomes.

2) There was migration of the 5-electrode array inferiorly from its original position over the muscle belly of m. peroneus tertius. This may have affected the results and my conclusions. To mitigate against the effects of migration, I would have to perform the experiment using a longer muscle with a longer muscle belly (e.g. peroneus longus).

3) More studies are needed to determine the true importance of placing the electrodes close to the innervation zones of the muscle.

4) No comparisons of the EMG signals detected with my implanted electrodes with skin surface electrodes in the three different configurations (i.e. monopolar, bipolar and tripolar) were made. These might have shown similar trends in terms of the SNRs even if the overall SNRs for the surface electrodes was higher than for the implanted electrodes.

5) No experiments with electrode discs of varying size were performed to see how this affects EMG signal recordings and SNR.

6) Impedance levels were measured after cutting off the electrode cables instead of leaving the assembly intact. Therefore, making comparisons between the results in this Chapter and those in previous Chapters was more problematic.

7) The design of my bone-anchor + implanted electrode system was mechanically more robust and reliable compared with the designs used in previous Chapters. This could have resulted in differences in the levels of background noise and therefore the SNR.
5.6 Conclusions

The data from this Chapter suggests:

- Comparing monopolar, bipolar and tripolar configurations of implanted epimysial electrodes, the configuration which recorded EMG signals with the highest SNR is bipolar.
- Comparing different IEDs for the bipolar configuration, a 10 mm IED with the electrodes located over the centre of the muscle belly recorded EMG signals with the highest SNR.
- Tripolar electrodes do not confer any advantage over bipolar electrodes with respect to SNR.
- Monopolar electrodes recorded EMG signals that were not meaningfully larger than the background noise (SNR = 1).
6 Chapter 6 – Investigating the Use of Microchannel Neural Interfaces

6.1 Introduction

The majority of electrically operated prosthetic limbs are myoelectrically controlled i.e. they use EMG signals to control their functions. While these artificial limbs promise to restore upper limb function, they fall well short of reproducing all the functions of the absent limb. Some of these limitations arise because of a lack of usable EMG signals from the amputee to control the artificial limb. Although the recent introduction of surgical techniques like TMR and RPNI go some way towards addressing this problem, it is unlikely that these techniques alone will achieve the goal of a fully biomimetic prosthetic solution with physiological degrees of freedom. Equally, the introduction of implanted muscle electrodes (epimysial or otherwise) is likely to be only part of the slow and steady progress towards achieving this goal.

Another way to increase the number of available biosignals for control of a prosthetic limb is to record them directly from the peripheral nerves in the amputation stump. In theory, the peripheral nerves already carry all the necessary biosignals required for full, normal control of a prosthetic limb. Recording these signals directly from the nerves will increase the amount of data available for control of a prosthesis in one simple step. A detailed review of the use of artificial interfaces for recording biosignals directly from the nervous system is detailed in Chapter 1.

In theory, MNI implants should be one of the most promising types of nerve interface available because of their ability to record the greatest number of signals from the (potentially) thousands of axons that could grow through the construct in three dimensions, rather than only at the surface of the electrode, or along a single line, as in LIFEs. In practice, animal studies have shown that there is only limited nerve growth through the sieve electrodes and MNI implants. Moreover, even after the nerve heals, this is followed by progressive axonal atrophy of the proximal nerve, resulting in poor functional recovery [113], [214]. However, all previous in vivo work on MNI implants has been in animal models
(mostly rats). To date, there have been no human studies involving the use of MNI implants, which might behave differently with a larger nerve structure.

As previously stated, using an approach based purely on increasing the number of available EMG signals is unlikely to be successful in the long-term if the final goal is the restoration of full biomimetic control over a prosthetic limb. In previous Chapters, I have demonstrated that a bone-anchor based implanted electrode system can survive and function for long periods in a sheep model. Moreover, based on a consideration of the available data, MNI implants appear to provide the greatest potential for achieving the goal of producing the largest number of usable biosignals compared with approaches that rely on EMG signals. Therefore, combining an MNI electrode system with my bone-anchor based device might represent an effective approach for future work. Since the main problem with MNI implants in their current form are poor axonal regeneration and longevity in vivo, I decided that it would be important to consider ways in which to address these difficulties to provide some context for any future attempts to combine a bone-anchor based system with MNI based electrodes.

6.1.1 Optimising Neurite Growth

One of the keys to success with MNI implants is to optimise nerve regeneration through the sieve structure. If this can be done, it might then be possible to record stable, high fidelity ENGs over long periods after implantation. There are many strategies that have already been tried to improve the biocompatibility of the hardware and to increase axonal regeneration through MNI implants. These include:

1) Augmentation of the surface of the hardware with coatings of trophic factors or other signalling molecules. Following nerve transection (and subsequent repair), Wallerian degeneration occurs within the parts of the nerve distal to the site of the injury. Axonal regeneration is then guided by the architecture of the extracellular matrix and the presence of trophic factors and support cells in the distal remnants of the injured nerve [215]. A similar situation is encountered when nerve grafts are used as conduits for repair. The grafts clearly do not contain viable axons and simply provide the support cells and extracellular matrix arranged in a way that is ideal for nerve regeneration [216]. Using these observations as a starting point, Lancashire showed that coating silicone with collagen IV, laminin and nidogen (the main constituents of the endoneural basement membrane) improved neurite outgrowths in vitro [217].
2) Optimising the surface topography of the hardware to channel axonal growth. A number of studies have shown that axons can be guided in vitro on surfaces with a micro-grooved topography. These appear to increase the alignment and outgrowth of neurites with increasing groove depth from 0.2 to 4 μm [218]–[222]. Surface roughness may also improve neurite outgrowth especially since basement membrane features range in size from 10 nm to 350 nm, with most features falling between 50 nm to 250 nm [223], [224]. Lancashire investigated the effects of micro-grooved topography and surface roughness of silicone substrates on neurite outgrowth. He found that creating surface roughness (in a range that mimics basement membrane) together with shallow grooves (15 μm width, 30 μm spacing, 10 μm depth) increased neurite length, alignment and number. This optimised topography may encourage cell attachment and increase the number and length of neural extensions when used in any future designs for MNI implants.

The long-term durability and performance of an MNI implant depends on our ability to direct the axons to grow consistently through the artificial electrode conduit, and to remain functioning, as well as our ability to record ENG signals from the regenerated axons. Ideally, testing of an MNI implant with the optimal topography and basement membrane protein coatings needs to be performed in vivo, in an animal model, to determine whether changes to these features truly improve axonal regeneration through the device.

In this Chapter, I describe the use of a multichannel, microneural nerve interface (i.e. MNI), modified with surface coatings (collagen IV, laminin and nidogen) which incorporates roughened surfaces to encourage neurite outgrowth. The process for creating this particular MNI implant is outlined in detail in Lancashire’s PhD thesis [217] and in [122]. Therefore, the work described in this Chapter is a summary of the findings of the collaborative work done together with H. Lancashire for his thesis.

My specific contribution to this collaborative effort related to:

1) Selection of an appropriate animal model.
2) Making a comparison of tissue glue versus suture for MNI implantation.
3) Formulating and carrying out the protocol for routine surgical implantation of the MNI implant in a rat model.
4) Formulating and carrying out the surgical protocol for MNI implant electrophysiology in a rat model.
The design and manufacture of the MNI implant itself, all histological processing, the gait analysis and analysis of the recorded signals were performed by H. Lancashire. However, in this Chapter I will present a summary of the main findings of the *in vivo* MNI experiments. A rat model was selected for all the *in vivo* work. In the majority of subjects, the MNI implant was implanted into the sciatic nerve [225]. Rats were selected for all of the *in vivo* experiments described in this Chapter because of their ease of handling and their fast healing times.

6.2 Assessing Nerve Regeneration

Assessment of the success of axonal regeneration after implantation of the MNI implant required:

1) Histological assessment of neural density. Histological assessments are the commonest outcome measure used in nerve regeneration studies. The outcomes most commonly measured include; the number of myelinated fibres, axon diameter and total number of nerve fibres [226].

2) Study of the ENG signals as a direct measure of nerve function. Electrophysiological measurements are of particular relevance in assessing nerve regeneration, since the purpose of an MNI implant is to record nerve signals. Serial electrophysiological readings can be carried out, either under general anaesthesia, or by routing the electrode cables through a head assembly. In the experiments that I describe below, all electrophysiological readings were performed under general anaesthesia.

3) Gait analysis of the animal, to observe functional outcomes. The sciatic function index (SFI) is a measure of rat hindlimb function, which has been used extensively in previous studies [227]–[231]. It is a method for assessing functional recovery following sciatic nerve injury by measuring the change in footprint shape during walking. Although SFI correlates weakly with other indicators of neural function, possibly indicating functional compensation as a confounding factor to regeneration [232], it is the only standardised method of assessing functional recovery in rats [226].

All three methods were used for this study.
6.3 Implantation Techniques

Conventionally, nerve repairs are performed using non-absorbable sutures such as nylon. More recently, non-surgical techniques, such as fibrin glue, have been used to coapt the ends of nerves [233]. Using fibrin glue to bring the severed ends of the sciatic nerve together with the MNI implant, instead of sutures, might confer certain advantages. For example, fibrin has been shown to improve regeneration within neural conduits [234]–[236]. Using fibrin glue also requires less manipulation of the nerve tissue, avoids the need to pass suture material through the nerve (reducing the risk of tissue damage) and avoids the need to leave a foreign material (sutures) in the nerve which may promote a fibrotic, foreign body reaction. Fibrin glues are also widely available commercially and are already widely used for internal haemostasis/sealing of tissues in humans.

6.4 Specification of the MNI implant

The multichannel MNI implant which was used for the experiments described in this Chapter consisted of a silicone tube containing the microchannels through which the axons are able to grow from the proximal to the distal end of the divided nerve (Figure 6.1). The channels were created using laser-cut layers of silicone sheets to produce microchannels 150 μm deep by 200 μm wide. The sheets were plasma bonded and stacked. Stainless steel foil was used to create electrical contact points within each channel to record any ENG signals. Four, stacked layers were assembled, with each layer containing 5 microchannels. Each layer contained one microchannel with a single, stainless steel electrode. The electrodes were connected to an oversized connector pad that was used to record any ENG signals. The whole construct was completed by the addition of short lengths of silicone tubing placed on either side of the MNI implant into which the cut ends of the sciatic nerve were placed before these were fixed in place with either fibrin glue or sutures. Figure 6.2 shows an example of the completed MNI implant.
Figure 6.1: Diagrammatic representation of a microchannel neural interface.
Figure 6.2: Top; stacked microchannel neural interface, at 20x magnification. The silicone tubing into which the nerve ends are placed before being fixed in place can be seen. Note the oversized tripolar connection pads used to record the ENG signals. Bottom; stacked MNI implant in cross section following explantation, at 50x magnification. The 4 microchannel layers with 5 channels per layer can be seen.
6.5 Aims

This Chapter describes an *in vivo* study which investigates the function of a microchannel neural interface in a rat sciatic nerve model. The aim of the study was to determine whether the addition of basement membrane coatings (as described in section 6.1.3) improves the function of an MNI implant compared with no coatings.

In this study, I will try to determine whether:

1. A rat sciatic nerve can regenerate and heal through a stacked MNI implant.
2. Basement membrane coatings of collagen-IV, laminin-2,-4, and nidogen-1 can improve the functional, histological and electrophysiological outcomes compared with uncoated control versions of the MNI implant.
3. Fibrin glue is a suitable method for fixing the nerve ends to the MNI implant compared with sutures, *in vivo*.

6.6 Materials and Methods

All *in vivo* procedures were carried out in accordance with The Animals (Scientific Procedures) Act UK, 1986 (revised 2013) and local guidance.

6.6.1 Cadaveric Implantation

A single cadaveric test was performed to determine the surgical protocol for implantation of the MNI implant for my experiments. An adult male Wistar rat was sacrificed by cervical fracture. The animal was placed into a right lateral position. An incision was made over the lateral aspect of the left thigh, through the skin and on through the underlying musculature to expose the sciatic nerve using blunt dissection of the *m. gluteus superficialis* and *m. biceps femoris*. The nerve was transected transversely, and a dummy implant was interposed between the two cut ends of the nerve. The implant was fixed in place with three 9-0 polyamide monofilament sutures (Ethicon, W2829) placed at 120° to each other (Figure 6.3). Other surgical approaches to the sciatic nerve were also explored, including placing the animal prone and approaching the sciatic nerve from the posterior aspect of the hind limb.
However, the lateral approach proved to be the easiest and least disruptive to the surrounding structures.

![Image: Figure 6.3: Cadaveric study to determine the optimum surgical protocol for implantation of the MNI implant. The image shows a skin incision made in the lateral aspect of the hind limb of an adult male Wistar rat which has been placed into the right lateral position with the left thigh muscles and sciatic nerve exposed. Note the presence of a dummy MNI implant sutured into place between the two cut ends of the sciatic nerve.]

6.6.2 Comparison of Tissue Glue versus Sutures for fixation of the MNI implant

Four adult Wistar rats were randomly assigned to one of two groups. Both groups then underwent the same operative procedure. General anesthesia was induced and maintained with 2% isoflurane (Isoflo, B506, Abbott) in oxygen (21/min). Each animal was then placed
into a right lateral position and the operative site was shaved and prepared with Hydrex Clear (0.5% w/v Chlorhexidine Gluconated in 70% v/v Denatured Ethanol B, Ecolab Ltd.) before draping the operative field. A skin incision was then made over the lateral aspect of the left thigh extending form the knee to the hip joint, and the sciatic nerve was identified as described in Section 6.5.1. The MNI implant was then implanted and fixed into position between the two ends of the sciatic nerve with either sutures or tissue glue. Following implantation, the skin was closed in layers with 4-0 and 2-0 polyglactin sutures (Vicryl, W9106). Opsite spray was used to seal the skin (Opsite, Smith and Nephew). Post-operative analgesia was achieved using 2.5 mg/kg Flunixin (50 mg/mL in saline, Noorbrook) delivered by subcutaneous injection. Each animal was housed individually for 7 days after surgery and thereafter in pairs in cages with enriched environments to reduce the likelihood of auto-mutilation.

6.6.2.1 Suture group
In group 1, the cut ends of the nerves were placed into the silicone tubing at either end of the MNI implant, to allow the cut ends to abut directly with the ends of the microchannels. The nerves were then fixed into position using 9-0 polyamide monofilament sutures (Ethicon, W2829). The technique involved passing the sutures through the full-thickness of the epineurium of each nerve and out to pass through the full-thickness of the wall of the silicone tubing of the MNI implant. A total of 3 sutures were placed into each nerve end, at 120° to each other.

6.6.2.2 Tissue-glue group
In group 2, the cut ends of the nerves were placed into the silicone tubing at either end of the MNI implant, to allow the cut ends to abut directly with the ends of the microchannels. Tisseel Fibrin Tissue Glue (Baxter, 2 mL, 1501764) was then prepared following the manufacturer’s instructions. A total of 0.5mls of Tisseel was then dropped circumferentially around the point at which the nerve entered the silicone tubing of the implant, encapsulating the entire area. The glue was then allowed to set for 3 minutes.

6.6.3 Comparison of Protein Surface Coatings for MNI Implantation
24 adult male Lewis rats were randomly assigned to one of 4 groups (n=6). All groups underwent the same operative procedure.
Under general anaesthesia, the sciatic nerves were exposed and transected as described in Section 6.5.2. For all groups, the nerve ends were fixed into the implant tubing with sutures. In two groups, protein coatings (10 μg/cm² Collagen-IV + 1 μg/cm² Laminin-2,-4 + 0.175 μg/cm² Nidogen-1) were applied to the MNI implant. For the two other groups, uncoated, control MNI implants were used.

Implants with protein coatings were first placed under vacuum for 5 minutes to remove any air prior to coating with protein. The protein coating of the MNI implants was achieved by prior incubation of the implants for 2 hours at 37°C in the relevant solutions to promote protein adsorption onto the silicone surfaces. The implants were then washed three times by immersion in sterile phosphate buffered saline for 5 minutes to remove any solution. A more detailed description of the protocols for protein coating can be found in [217].

The proximal and distal ends of the divided sciatic nerve were placed into the lumen of the silicone tubing attached to the MNI implant to abut the microchannels. The ends were fixed in this position using 3x epineurial 9-0 polyamide monofilament sutures (S&T, 03180, Interfocus Ltd, UK), which were also passed through the wall of the silicone MNI tubing. The wounds were irrigated with saline and closed in layers with 4-0 polyglactin 910 sutures (Vicryl, W9106, Johnson & Johnson International, UK). The skin incision was sealed with Opsite spray (Opsite, Smith and Nephew). Analgesia and post-operative recovery was carried out as described in Section 6.5.2.

One animal was lost during anaesthesia (Control, 4 week group); in all other cases recovery was uneventful.

6.6.4 ENG Recordings

Electrophysiological assessments were carried out using the electrodes within the implanted MNI implants. At four weeks and then eight weeks post-operatively, two groups each of 12 animals were anaesthetized (n = 6 per group) and prepared as previously described to access the site of the MNI implant. Dissection was performed to expose the MNI implant and a connector was attached to the recording portion of the MNI implant. Stimulation of the nerve proximal to the MNI implant was performed using a pair of stainless steel, hook electrodes. Neural signals were recorded using a neural signal amplifier (Iso-DAMA8A, World Precision Instruments, Sarasota, USA) connected to BIOPAC recording equipment (MP150 and AcqKnowledge software, BIOPAC Systems, Inc., CA, USA).
Hook electrode stimulation was carried out using the BIOPAC equipment to deliver a balanced square wave 1ms ±0.1 V pulse train at 10 Hz. Recording of any ENG signals were made at 10,000 samples per second (kSps), amplified 1000x, with a high pass filter of 100 Hz and a low pass filter of 10 kHz. In addition, the proximal nerve was stimulated using a Medtronic Vari-Stim III Surgical Nerve Stimulator (Medtronic Inc., MN, USA) set to a 1 mA pulse.

A second stimulation was applied directly to the sciatic nerve within the microchannel using the MNI implant to deliver the stimulation and EMG signals were then recorded downstream, from the tibialis anterior muscle. To record any EMG signals, stainless steel epimysial electrodes were placed onto the surface of the muscle belly of the exposed muscle. Simultaneously, ENG signals were recorded from the pads of the microchannel as previously described (above). The EMG (muscle) signals were recorded using BIOPAC amplifiers and recording equipment (MP150, EMG100 and AcqKnowledge software). The MNI implant stimulation was carried out using the BIOPAC equipment to deliver a balanced square 1 ms pulse train of 10 Hz between ±0.01 V and ±0.1 V was used. Recordings were made at 10 kSps, amplified 500x, with a high pass filter of 100 Hz and a low pass filter of 5 kHz. In addition, the proximal nerve was stimulated using a Medtronic Vari-Stim III Surgical Nerve Stimulator (Medtronic Inc., MN, USA) set to a 1 mA pulse.

The SNR was calculated as the ratio of peak-to-peak signal to the noise at one standard deviation.

### 6.6.5 Histological Analysis

The 4 animals used in the tissue glue versus suture experiment were sacrificed at 4 weeks post op (2 sutured, 2 glued). For the experiment investigating the effect of protein coatings, one batch of 12 animals (6 treated and 6 controls) was sacrificed at 4 weeks post-operatively and the other batch was sacrificed at 8 weeks post-operatively (6 treated and 6 controls).

All animals were sacrificed by cervical fracture. The MNI implant was excised together with a 1 cm portion of the nerve above and below the site of the implant. The samples were placed in 4% paraformaldehyde diluted with PBS. The nerve tissue was dehydrated in an ascending series of alcohol and was embedded in paraffin wax. A series of transverse sections of the nerve was made at 5 μm thickness and stored at 60°C for 24 hours to partially de-paraffinate the specimens. Each section was stained with Haemotoxylin and Eosin.
For the experiment investigating suturing versus tissue glue, 50% of the sections were stained with toluidine blue.

Sections within the proximal and distal 5 from the MNI ends were imaged at 10x, 20x and 40x using an Olympus microscope. Up to 50 axon cross sections per microchannel were counted [237]. Axon density (axons per μm²) and axon diameter (μm) were also measured [238]. Axon diameters were calculated using the axis parallel to the horizontal in the image and assuming a circular shape for each axon in cross-section. Axon densities (number of axons per μm²) were calculated for each section using the Cell Counter ImageJ plugin. Statistical analysis was carried out using data from one proximal and one distal section of each explanted interface.

6.6.6 Gait Analysis

Our method for gait analysis was based on a technique described by Brown et al. [239]. Track paper for capturing the paw prints was prepared by dipping it in Bromophenol blue followed by drying of the paper in an oven at 37°C overnight. This creates a strip of paper that becomes brilliant blue on contact with liquid water.

For each test, the animal’s hind feet were dipped in water and dabbed on absorbent tissue to remove any excess water. The animals were placed at one end of a test track lined with a strip of the track paper. The other end of the test track terminated in a darkened box. Once the animal had walked down the track and entered the box, the paper track was removed, and photographs of any footprints left on the paper were taken immediately.

The Sciatic Function Index (SFI) was used to calculate sciatic nerve recovery by measuring print length (PL); toe spread (TS – the distance between the first and fifth toes); intermediate toe spread (IT – the distance between the second and fourth toes). This was done for both the operated or experimental side (E) and the unoperated normal (N) limbs (Figure 6.4).
The following equation was used to calculate the SFI [230]:

\[
\text{Print Length Factor (PLF)} = \frac{\text{EPL} - \text{NPL}}{\text{NPL}}
\]

\[
\text{Toe Spread Factor (TSF)} = \frac{\text{ETS} - \text{NTS}}{\text{NTS}}
\]

\[
\text{Intermediate Toe Spread Factor (ITF)} = \frac{\text{EIT} - \text{NIT}}{\text{NIT}}
\]

Sciatic Function Index (SFI) = $-38.3 \times \text{PLF} + 109.5 \times \text{TSF} + 13.3 \times \text{ITF} - 8.8$

Figure 6.4: Rat footpad traces on bromophenol treated track paper with the following measurements marked: print length (PL); toe spread (TS); intermediate toe spread (IT). These measurements are taken for the operated or experimental side (E) and unoperated or normal side (N). Figure taken from Brown et al. [239].
6.6.7 Data Analysis
The gait data were analysed using SPSS version 21. The data were not assumed to be normally distributed, and so the conditions for parametric testing were not met. Therefore, non-parametric tests were used.

Any unpaired samples were tested using the Mann Whitney U test. This test was also used for analysis of:

a) muscle mass loss ratios.
b) comparisons of histological morphometry between different treatment groups at different time points.
c) rates of SFI increase per day.
d) signal to noise ratios between different treatment groups.

For related samples, the Wilcoxon signed-rank test was used. This was particularly useful for comparisons of the histological morphometry between the proximal and distal sections of the sciatic nerve.

The SFI was recorded as a continual bivariate data (SFI and days since implantation) and correlations were evaluated using Spearman’s rank correlation coefficient.

6.7 Results

6.7.1 Comparison of Suture vs. Fibrin Glue in fixing Nerve Ends to MNI

6.7.1.1 Cadaveric Implantation
This study confirmed that a lateral thigh approach to the sciatic nerve was the best for implantation of the MNI implant. This approach caused least muscle trauma, had the best access, was time efficient and did not require dividing any muscles or tendons in order to access the sciatic nerve. Furthermore, this study confirmed that the diameter of the sciatic nerve was appropriate for implantation into the MNI implant.

6.7.1.2 Gross Anatomy
At explantation, all the sciatic nerves were noted to be in-continuity with their respective MNI implants for all the animals (Figure 6.5). In one case, where fibrin glue was used, a
thick translucent capsule was also present around the nerve. This was likely to represent residual fibrin with early scar tissue formation.

Figure 6.5: Investigation of the difference in methods of fixation of the MNI into the sciatic nerve. Top; microsutures and Bottom; Tisseel fibrin glue. In both cases, the sciatic nerves can be seen to be in-continuity. Arrows indicate position of MNI.
6.7.1.3 Histology

In the suture group, axons were noted to have grown through the microchannels forming micro-fascicles (Figure 6.6). In one rat from the fibrin glue group, the nerve appeared to grow out into the glue rather than remain within the lumen of the implant (Figure 6.7).

![Image](image1.png)

**Figure 6.6:** Transverse sections through MNI implant of sciatic nerve following suture implanted interface (5 μm sections). Microfascicles are present in a spiral formation aligning with the spiral passive microchannel array design. Top: 5x, bottom: 20x magnification. Arrows indicate some of the microfascicles. H&E stain.
6.7.1.4 Sciatic Function Index

Based on the SFI, recovery of sciatic nerve function was greater in the group where the sciatic nerve was fixed to the MNI implant with tissue glue compared to the sutured group, at 3 weeks after implantation (SFI -66.6±21.3 (median ± I.Q. range) tissue glue group versus -77.9±19.3 in the sutured group). However, by week 4, the SFI was noted to have improved in both groups (SFI -64.1±30.7 in the tissue glue group; and -74.6±36.0 in the sutured group), though with increased variance, as shown in Figure 6.8.

(a)  
(b)  
(c)  
(d)
Figure 6.7: Sequential cross-sections of the sciatic nerve (a-f) after using tissue glue to fix the nerve ends to the dummy MNI implant. Each picture shows a 5 μm thick section of the nerve taken from proximal to distal and stained with haematoxylin and eosin. The nerve appears to exit the lumen of the dummy implant through a gap in the silicone wrapping and follow the fibrin glue outside the implant. SN; sciatic nerve. L; lumen of dummy MNI.

Figure 6.8: The SFI in the suture vs. Tisseel groups, with 3 measurements per time point.
6.7.2 Comparison of the effects of altering the surface coatings on axonal ingrowth

6.7.2.1 Gross Anatomy

In one control sample at 30 days after implantation I noted that there was a failure of the sutures used to hold the proximal end of the sciatic nerve into the MNI implant. Fortunately, the nerve remained within the lumen of the MNI implant. However, disappointingly, I noted that there was no axonal regeneration through the conduit. In all the other samples, the nerves were noted to be in continuity with the implant and the axons were seen to have grown through the MNI implant (Figure 6.9).

Twitching of the hindlimb was observed on transection of the nerve during explantation of the MNI implant in both the coated and control groups. This twitching was the result of reflex contractions of the muscles in the hind limb and was interpreted by me as an indication of successful reinnervation of the muscles. Moreover, neo-fascicles could be seen to have formed in the microchannels upon explantation. These were most easily seen after fixing the tissues with paraformaldehyde, which rendered the neo-fascicles sufficiently robust to allow them to be removed intact from within the microchannels after careful dissection (as seen in Figure 6.10).
Figure 6.9: Appearance of an MNI implant in situ at 8 weeks after implantation, showing that the sciatic nerve remains in-continuity with the device. Right of image: proximal part of nerve, left of image: distal part of nerve.
Figure 6.10: Top; MNI implant showing nerve in continuity. P; proximal, D; distal part of nerve. Below; Neofascicles removed from the MNI implant, indicated by white arrows. Linear growth pattern of the fascicles can be seen.
6.7.2.2 Histology

Axons were visible in transverse sections on histological processing. At 4 weeks after implantation, there was little difference between the two groups. The distal segment was seen to contain axons and blood vessels but in a disordered fashion. However, by 8 weeks after implantation, more organised fascicles could be seen in both the control group and the sECM coated group (Figure 6.11). Now, the axons and blood vessels could be seen within concentric layers of connective tissue. Axon diameters and densities were then measured. These results are summarized in Figure 6.12. Axon diameters were compared between the two groups, at points that were proximal and distal to the MNI implant, at 4 and 8 weeks after implantation.

Figure 6.11: Transverse sections of the sciatic nerves distal to the MNI implant at 40x magnification; (a) 4 weeks, Control. (b) 4 weeks, sECM treated. (c) 8 weeks, Control. (d) 8 weeks, sECM treated. Fracturing of sections is an artefact of histological processing. N indicates nerve fascicles.
6.7.2.2.1 Axon Diameters

At 4 weeks post-implantation, there was no significant difference between the proximal axon diameters in the control (3.91±2.23 μm, median ± interquartile range) and sECM coated (4.19±2.29 μm) groups, \( p=0.274 \). Distally, although the axonal diameter was smaller in the sECM group (3.12±1.37 μm) compared with the control (3.83 ±2.48 μm), this was not statistically significant \( (p=0.175) \).

At 8 week, this trend was reversed. At the proximal end, the sECM coated group showed slightly larger axon diameters (3.67±1.64 μm) compared with the control group (3.14±2.49 μm), although this difference was not statistically significant \( (p=0.394) \). Distally, the axon diameters were larger in the sECM treated group compared with the control group; 4.16±1.96 μm vs. 3.61±1.66 μm, although again this did not reach statistical significance; \( p=0.361 \).

Axon diameters were also compared within groups at ipsilateral ends of the nerve at 4 and 8 weeks. There was no significant difference in diameters between 4 and 8 weeks when comparing the proximal ends in the sECM coated group \( (p=0.931) \). This was also true when comparing the distal axon diameters \( (p=0.421) \). For the control groups, there was no significant difference in axonal diameter at both the proximal \( (p=0.886) \) and distal \( (p=0.429) \) locations at 4 and 8 weeks.

Finally, axon diameters were compared at different locations (proximal and distal) for the same treatment groups (sECM coated and control) at the same time points (4 weeks or 8 weeks). For the sECM group, there was no significant difference between the proximal and distal ends at 4 weeks \( (p=0.602) \) and 8 weeks \( (p=0.715) \). Similarly, for the control group, there was no significant difference between axon diameters proximally and distally at 4 weeks \( (p=0.730) \) and 8 weeks \( (p=0.762) \).

6.7.2.2.2 Axon Density

Axon densities were compared with respect to location, time point and treatment group, while keeping the other dependent variables constant. There were no significant differences observed across these tests, \( p=0.188 \) (Figure 6.13).

6.7.2.3 Sciatic Function Index

There was an increasing trend in SFI for both treatment groups (Figure 6.14). In both groups, the trend was weak but significant. However there was no significant difference in the trends between the two treatment groups \( (p=0.929) \).
6.7.2.4 Electrophysiology

ENGs were recorded from the microchannels following stimulation of the proximal nerve. Compound motor action potentials were recorded from *m. tibialis* anterior following stimulation using the MNI implant (Figure 6.16). Using these recordings, the SNR was calculated for the muscle and nerve signals. There was no significant difference in SNR between the two treatment groups or the two different time points for either nerve (*p*>0.265) or muscle (*p*>0.201) recordings (Figure 6.15).

![Figure 6.12: Boxplot of axon diameters. None of the differences were statistically significance (*p*>0.05).](image)

Section Location relative to Implant

Proximal  | Distal
---|---

Treatment Group

- eECM
- Control

Weeks since Implantation

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Weeks since Implantation

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Figure 6.13: Boxplot of axon density. None of the differences were statistical significance ($p\geq0.188$).

Figure 6.14: SFI post MNI implantation. Linear trend lines are shown with 95% confidence intervals of the mean. For the treated group, sECM, $R^2=0.394$, $\rho=0.665$, $p<0.001$. For the control group, $R^2=0.231$, $\rho=0.443$, $p<0.001$. There was no statistically significant difference in the trends comparing the two groups.
The data show that the ENG recordings had a larger SNR for the treatment group compared with the control group at 4 weeks (sECM treatment SNR = 7.29±2.31, control group SNR = 5.96±1.53, median±I.Q. range) and at 8 weeks (sECM treatment SNR = 8.93±3.73, control group SNR = 5.51±0.480). Furthermore, there was an increasing trend seen in the treatment group vs. the control. However, this difference was not statistically significant (p>0.533).

The SNR for the EMG recordings was greater for the control group compared with the treated group at 4 weeks (7.78±2.43 vs. 6.05±1.60). This trend was reversed by week 8, with a larger SNR for the treated group (6.69±1.27) compared with the control group (5.76±2.45). Neither of these differences reached significance (p>0.201). Trends in the SNR between the two time points also failed to reach significance; increasing in the treated group between 4 to 8 weeks (p=0.856) and decreasing in the control group (p=0.629). The data for both EMG and ENG SNRs are summarized in Figure 6.15.
Figure 6.16: Typical evoked nerve signal recordings from MNI implants (top), and muscle signals from *m. tibialis anterior* following stimulation through the MNI implant (bottom). The recordings were all obtained simultaneously and show that provision of a discreet stimulus through the MNI implant is followed either immediately (or a few milliseconds later) by a corresponding EMG signal from the muscle.
6.8 Discussion

In this Chapter, I present the results of my investigations of the use of a microchannel neural interface using a rat sciatic nerve model to determine its potential for bidirectional prosthetic control.

My preliminary cadaveric study confirmed that a lateral surgical approach was appropriate and that the size of the neural interface used for this study was a close match for the size of the rat sciatic nerve. Moreover, the technique I used for suturing the nerve to the interface proved robust and reliable over the implantation period. Importantly, my data suggested that suturing of the nerve was a more reliable method of securing the nerve to the implant compared with fibrin glue. In one case, the growing axons followed the path of the fibrin glue (which was applied to the outside of the nerve + MNI implant) rather than growing into the implant. In contrast, using a suture technique ensured that the nerve ends remained firmly lodged inside the silicone tubing of the implant despite the movements of the animal. This made it less likely for the sprouting nerve ends to go in a different direction other than through the conduit. Importantly, for this experiment comparing suturing and tissue glue, the design of the MNI implant included a small slit in the lumen to provide a potential alternative route for the axons to grow through. This slit was a construction consideration; it was easier to make devices with the slit. This was corrected following this observation and subsequent designs of the MNI implant did not include this slit. Fibrin glue is also more expensive than sutures and takes time to prepare before use (although this can be pre-prepared by a surgical assistant). Although in the context of end to end nerve repair, suture versus fibrin glue did not show superior outcomes [233], in the context of securely attaching the proximal and distal ends of the nerve in the MNI, suturing may be a more secure alternative. Finally, this part of the experiment was run over a 4-week period and the sample size was small (n=4). Nonetheless, there were no significant differences in the SFI comparing the sutured and fibrin glue group. In a previous study which was run over a 12-week period there was also no difference comparing sutures with fibrin glue [234]. However, the SFI eventually became more normal than in my study (12-week study; SFI range -35 to -55 compared with my 4-week study, SFI of -65 to -75). This could have been due to the longer time given for the axons to regenerate and for function to recover. Importantly, the absence of any difference in SFI suggests that suturing should be the preferred method for securing the nerve to the MNI.
implant since it is cheaper (~ £50 per ml versus £15 per suture), and makes no difference in terms of the long-term recovery of function of the nerve.

My data show that axons from a transected rat sciatic nerve are capable of regenerating through the MNI implant. Importantly, I was able to use the interface bidirectionally – for recording ENG signals and to stimulate the nerve to record EMG signals from *m. tibialis anterior*. This suggests that the same or similar designs could be used to create sensory feedback loops. However, any future design of the implant would also require many more channels and would require hardware/software that was able to select out those signals which are predominantly sensory rather than motor axons to achieve this aim. Alternatively, separate MNI implants could be used, one for purely sensory signals and another for motor signals.

In my study, axon diameters were similar to those reported in previous studies [48]. However, this parameter has not been uniformly reported in all previous studies [237], [240], making comparisons difficult. Given the more invasive nature of MNI implants (which require complete transection and regeneration of the nerve) MNI implants are expected to have a reduced axon diameter compared with other interfaces which do not require nerve transection (e.g. Utah array) [238].

There was no difference in the statistical significance outputs with regards to axon diameters for the comparisons made. However, there was an increase in the distal diameters for sECM at 8 weeks compared with 4 weeks. Further, distal diameters were larger in sECM group at 8 weeks both proximal and distal, but none of these trends were statistically significant.

Furthermore, axon density was not significantly different between the treated and control groups although the sECM treated group were observed to have axon densities that were more consistent than in the control group. This might have been due to the coating proteins acting as trophic factors to encourage more organised growth of axons down the microchannels. Moreover, previous *in vivo* studies of direct nerve repair (without interposition of an MNI implant) of rat sciatic nerves have shown an initial increase in the number of axons distal to the repair followed later by a gradual decrease in axon density. These changes were observed over 6 months, with a slow decrease back to pre-transection levels over the subsequent 2 years [241]. Since my experiments ran for a maximum of 8 weeks, the final axon count/density may not have had time to fully resolve. Therefore, over
such a short study interval, drawing any final conclusions about changes in axon density were necessarily limited.

My data for the SFI at 8 weeks showed that neither the treated or control groups returned to full function. However, importantly, there were also no significant differences in recovery of function between the two groups. Interestingly though, the rate of recovery in the untreated group appeared to lag behind the treated group, as shown in Figure 6.14. If these linear trends were to be extrapolated, a full recovery would be expected to occur in the treated group after 132 days compared with 183 days in the control group. However, a full recovery would only ever be theoretical, and the linear trend predicts a constant rate of recovery which is not expected physiologically. In practice, a full recovery would be unlikely to occur since the majority of axons never grow through the interface. This is one of the major limitations of MNI implants in their current form. Moreover, the longer the downstream structures remain denervated, the poorer the long-term functional recovery will be, due to a gradual loss of neuromuscular junctions with increasing time after denervation. Fortunately, in the context of prosthetic rehabilitation, as long as high fidelity, high SNR signals can continue to be recorded, reduced or absent muscle recovery becomes irrelevant because the intention is for this technology to be combined with TMR, or (in the absence of available local muscle targets) innervated free muscle flaps which then act as a source of neurotrophic factors to encourage nerve growth across the MNI implant.

In this study, I was able to record ENG signals from the MNI implant, with high SNRs, all of which were greater than the commonly accepted standard of >5 [242]. The amplitude of the neural spikes agreed with the in vivo results from other workers [48], [237], [240]. The large SNRs only consider observable neural spikes, and therefore inevitably exclude all neural activity with a low SNR as this was obscured by noise. Although this was still a proof of concept study, the data from my experiments suggest that MNI implants do have the potential to be used to record ENG signals with sufficient fidelity for prosthetic control. However, much more work would be needed to determine whether the implants would remain functional over much longer periods. It would also be necessary to assess the downstream nerve and muscle recovery over longer periods and to assess the effects of tissue fibrosis around the implant on signal degradation.

Since direct recording of ENG signals from peripheral nerves using MNI implants could provide a much greater number of usable signals compared with an EMG based interface, this
could be a more effective approach for future research. Furthermore, it would not be technically difficult to combine an MNI implant with a bone-anchor to create a device which has an even greater potential for controlling a prosthetic limb than one based on EMG signals alone. However, one fundamental limitation of MNI implants remains. In order for the axons to be encouraged to grow through the implant, it is necessary to create a neurotrophic drive for nerve growth arising from the downstream side of the implant. Currently, the only way to do this is to combine MNI implantation with a downstream source of neurotrophic factors. For my rat model, this was easily provided by placing the implant between the ends of the sciatic nerve. The neurotrophic drive was then provided by the mass of denervated muscle distal to the site of injury of the nerve. For an amputee, the problems are greater since such a neurotrophic drive would be absent. Therefore, it would not be possible to simply place an MNI implant into the gap between the cut ends of a nerve stump in the expectation that the axons will grow through since there would be no stimulus for such growth. Instead, MNI implants would always have to be combined with some way of encouraging the nerves to grow through to the distal end of the nerve. At present this means that MNI implants could only work in amputees if they were combined with TMR surgery or some other source of a neurotrophic drive.

6.9 Conclusions

In this Chapter, I present data suggesting that:

- rat sciatic nerves can grow through a stacked, microchannel neural interface.
- coating the silicone surface of the microchannels with collagen-IV, laminin-2,-4, and nidogen-1 (proteins which are native to neural basement membrane) has shown a trend to increase the diameter of the axons growing through the MNI implant (although this did not achieve statistical significance), but makes no statistically significant difference to axon density.
- it is possible to record ENG signals from the MNI implant with a high enough SNR for such a device to be considered for prosthetic control.
- it is possible to use the MNI implant to stimulate the downstream muscles and to detect a corresponding EMG signal.
the functional recovery of the sciatic nerves is not improved by the addition of protein coatings or by alterations in the topography of the microchannels when compared with controls.

while MNI implants remain an important area for research and development they are not ready for use in human amputees.
Chapter 7 - Optimising Skin-Implant Seal in Bone-Anchor

7.1 Introduction

7.1.1 Skin/Prosthesis Interface

Currently, the most common form of reconstruction after upper limb loss is a prosthetic device. As previously described various types of are used, from cosmetic devices that only serve to disguise the absence of the limb to highly advanced (and very expensive) electrically driven machines that can mimic many of the movements of the upper limb. Most prostheses rely on a combination of suction cups, sockets and straps to attach the device to the residual limb of the amputee. Generally, the socket is made of a rigid material (e.g., polymer) combined with a silicone liner which is custom-built for the patient. The distal end of the socket then provides a rigid point of attachment for the artificial limb. Precise fitting of the socket is critical if the prosthesis is to be comfortable for the user and much time and effort is expended by the prosthetist, working closely with the wearer, to optimise the fit. In general, the tighter the socket, the more stable the soft tissue/socket interface becomes, and the more efficient the energy transfer between the stump and the end device. While this is advantageous from a biomechanical perspective, it often leads to skin-related problems (Figure 7.1) [10]–[15]. The constant pressure on the soft tissue contained within the socket can also impair both vascular and lymphatic flow which prolongs healing times [11], [243]. This can be particularly challenging for patients with unstable skin due to scarring or skin grafts, a problem frequently encountered in high-energy and blast-type injuries which are commonly suffered by military patients. Some of these patients also suffer with painful heterotopic ossification, which can make it difficult or impossible to fit a socket. Dealing with skin-related issues is problematic because even small skin ulcers will only heal if the pressure on the soft tissues can be relieved, often for prolonged periods (months) - during which the prosthesis cannot be used. As a result, skin/socket complications contribute to the high rate of prosthetic disuse in the upper limb, which can be as high as 80% after 2 years [16].
7.2 Direct Skeletal Fixation

The concept of direct skeletal fixation of a prosthetic device to the bone in the residual limb of an amputee was first attempted in humans in the early 1980s [244]. Using a bone-anchor for direct skeletal fixation has the potential to address all skin and socket-related issues, while providing several other advantages over traditional methods of attachment. These include; ease of donning and doffing, greater mechanical advantage, physiological load bearing, and sensory feedback through osseoperception [20].

Many research groups are researching different designs of bone-anchors for direct skeletal fixation of a prosthesis. The goal is to create a device that is simple to insert, mechanically robust and complication-free [245]. A summary of the devices which are currently commercially available is summarised in Section 1.6. Brånemark’s group in Sweden were the first to use this approach routinely with their OPRA device (Osseointegrated Prostheses for the Rehabilitation of Amputees) [244]. Their approach has not been free from complications, with superficial infection rates as high as 61% [32]. Tillander et. al. reviewed data from 102 OPRA devices implanted from 1990 – 2010 and found 16 patients had osteomyelitis [246]. Tsikandylakis reported on 18 OPRA in transhumeral amputees performed between 1995 and 2010, with a minimum of 2 year follow up (median 8 years, range 2-19) and found only one case of osteomyelitis, which was treated with 3 months of antibiotics [247]. However, Brånemark’s group now have decades-worth of data on the behaviour of their implant, in many different anatomical sites. More importantly, they have

Figure 7.1: Skin irritation at the skin-socket interface in an above knee amputation from prosthetic use.

Images courtesy of Prof. Blunn.
published widely on the ways that amputee function can be improved by direct skeletal fixation of a prosthesis and it is reasonable to assume that these outcomes would be essentially the same for any bone-anchor system [18], [24], [245].

Aschoff et al. have been using an alternative bone-anchor system for the past 15 years which they have called an intraosseous limb prosthesis (ILP). Early iterations of Aschoff’s device exhibited high rates of granulation tissue formation at the stoma (the point at which the transcutaneous part of the bone-anchor comes through the skin). He attributed this to the presence of a rough surface for his implant, at the skin-implant interface. Therefore, the design was modified to create a smooth finish that appeared to reduce the rate of soft tissue complications. However, the overall, superficial, infection rate still remains high at 38% using the ILP system [26], [27], with similar rates reported by other workers [35].

More recently, Al-Muderis introduced the osseointegrated prosthetic limb (OPL) system. Initial outcomes were reported in a cohort of 22 transfemoral patients using a two-stage surgical approach (similar to OPRA). In this small sample, there were 15 infective episodes in 12 patients requiring antibiotic therapy (although none required surgery) representing an infection rate of 55%. Mean follow up time in this study was only 14 months [36]. Following this study, Al-Muderis altered his protocol to a one-stage approach which was accompanied by a dramatic fall in the overall infection rate [36], [248]. Importantly, longer-term follow-up has now shown that the deep infection rate for the OPL implant is <1.5% [36].

Despite the high infection rates associated with many of the current designs of bone-anchor in routine use, the benefits of direct skeletal fixation have been increasingly well documented, especially by the Brånemark team [25], [249]–[251]. For most patients, using a bone-anchor is life-transforming and multiple studies have now confirmed their profoundly positive impact on quality of life, physical activity and the emotional and psychological well-being of amputees [252]. This has been one of the main drivers for the continued effort to find the perfect bone-anchor solution.

7.2.1.1 Problems with the skin-implant seal
Superficial infections occur after transcutaneous bone-anchor implantation, primarily due to the breach in the skin barrier. The presence of the implant allows bacteria to gain entry to the
internal environment at the interface between the skin and the implant. This interface is commonly referred to as the “stoma”. In nature, there are naturally occurring bone-anchored, transcutaneous structures (e.g., antlers or sheep horns) but the interface between the skin and these structures does not routinely become infected. Studies of the interface between these structures and the adjacent skin suggests that this is because the dermal fibroblasts in the overlying skin become firmly attached to the transcutaneous structure making the skin immobile relative to the transcutaneous structure [253]. The immobility of the overlying skin encourages the epidermal cells to remain quiescent and stops them from migrating in an attempt to re-establish continuity of the skin (i.e. to heal). In the normal process of wound healing, a breach in the skin’s protective barrier to infection interrupts the epithelial cell layer. Epithelial cells begin to divide and proliferate, leading to cell migration. When cells contact each other, their migration is inhibited, as the epithelium continuity is re-established. This phenomenon is known as contact inhibition, which is a natural part of wound healing process. In contrast, in a percutaneous environment, when excessive movement of the skin-implant interface occurs, cells may migrate deep to the epidermis around the bone anchor (which is observed as downgrowth), as the cells strive to re-establish continuity. This results in marsupialisation (i.e. creation of a pocket of cellular debris and bacteria) at the skin-implant interface. This can lead to chronic, low-grade infection which is a recognised consequence of all bone-anchored devices [35]. Therefore, cellular downgrowth and (relatively) rigid attachment of dermal fibroblasts to the surface of the implant may be a critical determinant of the long-term success of a bone-anchored, transcutaneous device [38].

7.2.1.2 Optimising Skin/Bone-Anchor Seal
The intraosseous transcutaneous amputation prosthesis (ITAP) is a single-stage, press fit bone-anchor system with a unique design feature (a subcutaneous flange) that is intended to create a stable skin/implant seal [38], [39]. It has been used once in a single human patient who has continued to use the ITAP bone-anchor to secure a prosthetic limb since 2007 [20] (Figure 7.2)

To address the problem of the skin-implant seal, ITAP attempts to reproduce many of the properties of naturally occurring bone-anchored structures such as deer antlers. Antlers are composed of bone which is mainly made of hydroxyapatite. Antlers also have a roughened surface with multiple small pores of approximately 200 μm to 250 μm diameter. The skin
overlying the antlers becomes adherent to the bone because of the presence of numerous collagen fibres (known as Sharpey’s fibres), passing through the pores and into the dermis. These fibres ensure that the skin remains immobile relative to the antlers encouraging the epidermal skin cells to remain quiescent. Hydroxyapatite also induces a degree of contact inhibition in epidermal skin cells [253].

To reproduce these properties, ITAP includes a subcutaneous, dome-shaped flange structure which is intended to provide a surface for dermal fibroblasts attachment. To encourage attachment of the dermal fibroblasts, the flange surface is coated with hydroxyapatite, which also induces contact inhibition in any epidermal cells which may come into contact with it [253]. This is intended to reduce the inflammatory drive at any skin edge which comes into contact with the surface of the implant. The flange also has a porous structure to create a scaffold for soft tissue in-growth thereby reproducing the effect of Sharpey’s fibres, fixing the overlying skin to the surface of the implant [38].

Figure 7.2: ITAP implant inserted into the upper limb of a transhumeral amputee. A) Shows the subcutaneous flange structure onto which the defatted skin will be sutured. B) Shows the transcutaneous part of the implant passed through the skin. Images courtesy of Mr Norbert Kang FRCS(Plast)

1) Despite the clinical success of the ITAP implant in a single human case, there has since been additional clinical evidence over the last 10 years (unpublished) that suggests that the original design of the ITAP implant needs further improvement before it can be ready for routine use in humans. Moreover, the data from my own studies (Section 3.11.2.1) show that the design of my bone-anchor based, implanted electrode device needs to be improved to prevent problems with erosion of the flange
structure through the skin (a phenomenon that I have previously termed “sieving”). Therefore, on-going research aims to further improve the adhesion of fibroblasts and keratinocytes to metal surfaces. The goals have remained the same as when ITAP was first conceived. If the fibroblasts in the skin can be encouraged to adhere to the surface of the bone-anchor, it might be possible to immobilise the skin sufficiently to create a microbial seal. To achieve this aim, I considered two options:

2) coating the metal surface of a bone-anchored device with proteins that might encourage cellular adhesion.

3) modifications to the surface topography of the bone-anchored device.

7.2.1.2.1 Surface Coatings

Fibronectin (Fn) is a glycoprotein which is a key component of the ECM and is found within many tissues, including skin, where it plays a central role in dermal fibroblast attachment [254]. An important domain in fibroblast anchorage through Fn is a tri-peptide binding region consisting of Arginine-Glycine-Aspartate (RGD) [255]. This polypeptide promotes cell attachment by providing anchorage points between the cells [256]. Middleton et al. have previously shown that titanium implants silanised with fibronectin show significantly higher levels of dermal fibroblast attachment to metal surfaces compared with controls in vitro [31]. These findings were subsequently confirmed in an in vivo model[257].

Silanisation covalently bonds proteins to metal with an intermediate silane complex and this on its own can encourage cell attachment in vitro [258]. However, prior binding of Fn to the surface of titanium alloys by the process of silanisation has been shown to improve the durability of this attachment when the metals are soaked in protein-rich fluid (to mimic the biological environment) compared with adsorbed Fn [31]. Since fibronectin is sourced form animals or humans, its clinical use is highly regulated since it can induce an inflammatory response in the subject which is immune-related. Furthermore, sterilisation procedures may result in inactivation of the fibronectin by denaturing the proteins. Therefore, RGD may provide a better alternative to Fn since it can be artificially synthesised, thereby avoiding the problems of sourcing the material and reducing the risk of inducing an immunological response. It may also be less susceptible to damage from sterilisation while preserving its binding properties for cell adhesion.
In his PhD thesis, Dowling investigated the role of synthetic RGD-polypeptides and fibronectin on human dermal fibroblast (HDF) attachment to titanium alloy substrates *in vitro*. He found that silanised RGD-polypeptides significantly increased HDF attachment compared with controls. Importantly, the RGD-polypeptides were as effective as Fn in terms of cell adhesion. Dowling also showed that there was no difference between silanisation and adsorption as a technique for coating RGD-polypeptides to the surface of titanium alloys. However, silanisation provided a more durable coating [31] and might therefore be a more robust technique for *in vivo* applications. Furthermore, Dowling showed that silanisation of RGD-polypeptides produced coatings that were better able to withstand binding competition from serum proteins, leading to further, significant, increases in HDF attachment [259].

Hydroxyapatite (HA) is the most abundant constituent of bone, making up to 70% of its dry weight. It is well known that HA promotes osseointegration [260]–[262]. However, HA has also been shown to enhance the skin-implant seal around bone-anchors [38], [39], [263] by encouraging dermal fibroblast adhesions to metal surfaces. Pendegrass *et al.* investigated the effects of HA coatings and fibronectin on fibroblast attachment *in vitro*, and found that the combination of HA silanised with fibronectin further enhanced dermal fibroblast attachment [254], [263] compared to HA alone.

### 7.2.1.2.2 Surface Topography

Modifying the surface topography of a metal implant is known to result in better soft tissue integration [39]. Porous structures are better scaffolds for cell attachment compared with smooth surfaces since the pores allow for the movement of body fluids through the implant, which facilitates vascularisation and soft tissue in-growth [264]. In an attempt to find the optimal size for the pores in the surface of an ITAP implant, Chimutengwende-Gordon *et al.* implanted titanium alloy discs of varying pore and strut size in an *in vivo* model. In their study, they found that pore diameters greater than 700μm supported extensive tissue and blood vessel infiltration compared with less open structures [264]. Moreover, small (250μm) and large (400μm) strut sizes impaired blood vessel formation and fibroblast migration into the implant. Therefore, efforts to optimise the pore and strut size of any implant are likely to be important in any future designs for a bone-anchored device.

The combination of RGD-polypeptides with the optimum pore and strut size for the surface of a bone-anchored device has not previously been investigated. Therefore, in this Chapter, I
describe the use of a bone-anchored device which has been modified to incorporate an RGD-polypeptide surface coating with changes to the topography of the flange structure in terms of pore and strut size. The aim of this part of my study was to determine the effect of this combination of features on the skin-implant interface after implantation of the device in a sheep model. The work I describe is a summary of the collaborative work done together with by Dr Robert Dowling who was working on his PhD thesis. My specific contribution to the work included:

1) Selection of an appropriate animal model.
2) Surgical planning and surgical implantation of the bone-anchor devices.
3) Post-operative care of the animals.
4) Implant retrieval.

The design of the experimental setup and histological processing of any samples was performed by Dr Robert Dowling alone.

7.3 Aims

In this Chapter, I will try to determine:

1) The effects on soft tissue in-growth of altering the pore diameter and strut size of the flange structure of a bone-anchored device.
2) The effects on soft tissue in-growth of adding a silanised coating of RGD-polypeptides to a bone-anchored device with and without a modified porous structure.
3) Whether the addition of an RGD-polypeptide coating to the porous structure is better able to support dermal adhesion to the surface compared with a non-HA-coated, non-porous structure.
7.4 Materials and Methods

The four experimental groups to be tested are as follows:

1. Standard control flange.
2. Standard flange with RGD-polypeptides.
3. Porous control flange with pore diameter of 700μm and strut diameter of 300 μm.

The standard, control flange was a machined cylindrical collar perforated with 24 drill holes, 0.7 mm in diameter. The porous flange was a 3-D printed, porous structure incorporating the optimised pore and strut dimension described previously (700μm pores with strut diameter of 300 μm). The implants with a biological coating were silanised with 7 mM of RGD-polypeptides. HA coated structures were used as controls for both porous and non-porous flanges. All bone-anchors were implanted transtibially using the surgical technique described in Chapter Three. The 4 experimental groups tested in this study are summarised in Table 7.1.
Table 7.1: Study Description

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flange Control</td>
<td>Flange perforated with 24 drilled holes, each 0.7 mm in diameter. HA coated. This was treated as the clinical standard [20].</td>
</tr>
<tr>
<td>Flange RGD</td>
<td>Flange perforated with 24 drilled holes, each 0.7 mm in diameter. The drilled flange was then silanised with RGD polypeptides.</td>
</tr>
<tr>
<td>Porous Control</td>
<td>Porous Flange with pores of 700μm diameter and strut diameters of 300 μm. The implant was HA-coated</td>
</tr>
<tr>
<td>Porous RGD</td>
<td>Porous Flange with pores of 700μm diameter and strut diameters of 300 μm. The porous flange was then silanised with RGD polypeptides.</td>
</tr>
</tbody>
</table>
7.4.1 Operative Procedure

All *in vivo* procedures were carried out in accordance with The Animal (Scientific Procedures) Act UK, 1986 (revised 2013) and the local guidelines of the Royal Veterinary College, Hawkshead Campus.

The following experiment is described in Al-Ajam et. al. [54], and implantation of the devices was carried out as described in Section 3.4.8. These experiments were performed concurrently with the experiments carried out in Section 3.8. The porous, control, bone-anchor used for the experiments described in this Chapter was the same one that was implanted into the proximal part of the left tibia for the experiments described in Section 3.8. A further bone-anchor that was relevant to the experiments described in this Chapter was implanted 5cm distal to the site of this bone-anchor based implanted electrode device. In the right tibia, I implanted two further bone-anchors, each placed 5cm apart (Figure 7.3). The locations of all the implants, other than the uncoated porous implant, were allocated randomly between the right and left tibias. The implant positions are summarised in Table 7.2.

Table 7.2: *in vivo* implant positions

<table>
<thead>
<tr>
<th>Animal</th>
<th>Proximal Right Limb</th>
<th>Proximal Left Limb</th>
<th>Distal Right Limb</th>
<th>Distal Left Limb</th>
</tr>
</thead>
<tbody>
<tr>
<td>6034</td>
<td>Flange RGD</td>
<td>Porous Control</td>
<td>Flange Control</td>
<td>Porous RGD</td>
</tr>
<tr>
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<td>Flange Control</td>
<td>Porous Control</td>
<td>Porous RGD</td>
<td>Flange RGD</td>
</tr>
<tr>
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<td>Flange RGD</td>
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</tr>
<tr>
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<td>Flange Control</td>
<td>Porous Control</td>
<td>Porous RGD</td>
</tr>
<tr>
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<td>Porous RGD</td>
<td>Flange RGD</td>
<td>Flange Control</td>
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</tr>
<tr>
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<td>Flange RGD</td>
<td>Porous RGD</td>
<td>Porous Control</td>
<td>Flange Control</td>
</tr>
</tbody>
</table>
Figure 7.3: Different implant groups.  A) Top left; flange control.  B) Top right; porous control (bone-anchor with epimysial electrode) and RGD coating.  C) Bottom left; flange control.  D) Bottom right; porous flange and RGD coating.

### 7.4.2 Histological Processing

Implants were removed at 5 months after euthanasia of the animals as described in Section 3.4.13. All implants were retrieved with a cuff of soft tissue and bone and immediately placed in 10% formalised saline for 1 week. Histological processing was then performed as previously described in section 3.4.13. Longitudinal sections were cut through the centre of each implant and the surrounding tissue. Section were ground and polished to a thickness of approximately 70μm and stained with Toluidine Blue.
7.4.3 Histological and Data Analysis

Histological samples were examined under light microscopy to characterise the soft tissue implant interface. Four parameters were measured:

1. Epidermal downgrowth was measured by length, using the intercept method, previously described by Pendegrass et al. [38].
2. The percentages of epidermal attachment to the implants were calculated, based on the measurements of the epidermis peri-implant [38].
3. The degree of dermal tissue infiltration was measured within the central pores or drilled holes of the flange structures of each implant counting the number of cell nuclei as a function of area (μm²).
4. The number of blood vessels within the pores as a function of area (μm²).

The data were not assumed to be normally distributed, therefore non-parametric statistical analyses were performed. Kruskal Wallis Test was used to determine whether the groups were from the same population distribution. If they were, no further testing was required because the test demonstrates that there would be no significant differences between the individual groups. Where the Kruskal Wallis Test demonstrated that the groups were not from the same population distribution, pair-wise Mann Whitney U Tests were performed. Differences were considered significant at the $p<0.05$ level.

If one were to carry out a substantial number of these pair-wise tests, each with a significant level of 0.05, then even in the absence of any real effects, some of the tests could appear significant (Type I error). To avoid this; in high numbers of multiple-comparative case studies, the sample size can be increased, or the $p$ value can be divided by the number of comparisons being made (Bonferroni correction). The former option was not possible (due to abiding by the 3Rs and maintaining ethical justification of animal resources), and in this study, because the maximum number of pair-wise comparisons was 6, it was not considered necessary to reduce the significance level further than 0.05. Doing so with so few pair-wise comparisons would not offset the chance of still observing false positives.
7.5 Results

7.5.1 Complications requiring early euthanasia

One animal developed osteomyelitis of the implant, as described in Section 3.11.1. Early euthanasia was performed at the recommendation of the vet, as described in Section 3.4.10. This animal was replaced to limit the effects on any statistical analysis of the data.

7.5.2 Epidermal Downgrowth

In this test of epidermal downgrowth distance, Kruskal-Wallis statistical analysis demonstrated no significant difference between the 4 implant groups, \( p>0.05 \) (Figure 7.4). Median downgrowth lengths associated with RGD-polypeptide coated implants were higher compared with control implants. However, this difference was not statistically significant (Table 7.3).

7.5.3 Epidermal Attachment

When testing epidermal attachment, Kruskal-Wallis statistical analysis demonstrated no significant difference between the 4 implant groups, \( p>0.05 \) (Figure 7.5). Median percentage values associated with RGD-polypeptide coated implants were lower compared with control implants. However, the difference was not statistically significant. The results are summarised in Table 7.4.

7.5.4 Blood Vessel Density

A Kruskal Wallis test demonstrated a significant difference between the 4 implant devices: \( p <0.05 \) when testing blood vessel density. The highest blood vessel density was observed in the soft tissues of the porous controls. These data were significantly higher compared with the flange control implants (MWU \( p <0.05 \); Figure 7.6, Table 7.5). Significantly increased blood vessel density was also observed in the soft tissues within the porous RGD implants compared with control flange RGD implants (MWU \( p <0.05 \); Figure 7.6, Table 7.5). There was no significant difference in blood vessel density between flange controls and flange RGD
implant groups or porous controls and porous RGD implant groups (both MWU \( p > 0.05 \); Figure 7.6, Table 7.1).

### 7.5.5 Cell Nuclei Density

Kruskal-Wallis statistical analysis demonstrated no significant difference between the 4 implant devices, \( p > 0.05 \) (Figure 7.7). All cell nuclei densities remained consistent despite the addition of coatings to the implant (Figure 7.7, Table 7.6).

### 7.5.6 Dermal Tissue Infiltration

A Kruskal Wallis test demonstrated a significant difference in dermal tissue infiltration between the 4 implant groups: \( p < 0.05 \). The percentage of dermal tissue infiltration was significantly increased in porous RGD implants compared with flange RGD (MWU \( p < 0.05 \)). Porous RGD implants also showed significantly higher levels of dermal tissue infiltration compared with porous control implants (MWU \( p < 0.05 \); Figure 7.8, Table 7.7).

No other significant differences were observed amongst the groups. These data suggest that a porous flange design promotes soft tissue integration and this difference is further increased by the addition of a coating with RGD-polypeptides.
Table 7.3 Median Downgrowth Figures for ITAP Devices

<table>
<thead>
<tr>
<th>Group</th>
<th>Median (μm)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flange Control</td>
<td>7185.5</td>
<td>5941.3-7900.9</td>
</tr>
<tr>
<td>Flange RGD</td>
<td>8178.0</td>
<td>6255.1-11299.4</td>
</tr>
<tr>
<td>Porous Control</td>
<td>5359.0</td>
<td>4646.8-10409.2</td>
</tr>
<tr>
<td>Porous RGD</td>
<td>7878.0</td>
<td>5702.5-9195.5</td>
</tr>
</tbody>
</table>

Figure 7.4: Box and Whisker plots demonstrating the length of epidermal downgrowth (μm) for:

1) control flanges (red)
2) drilled flanges with silanised RGD-polypeptides (green)
3) porous flanges (blue)
4) porous flanges with silanised RGD-polypeptides (yellow).
Table 7.4: Median Figures for Epidermal Attachment for ITAP Devices

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Percentage Value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flange Control</td>
<td>50.9</td>
<td>32.5-63.3</td>
</tr>
<tr>
<td>Flange RGD</td>
<td>44.5</td>
<td>17.2-58.7</td>
</tr>
<tr>
<td>Porous Control</td>
<td>39.4</td>
<td>22.0-57.6</td>
</tr>
<tr>
<td>Porous RGD</td>
<td>27.2</td>
<td>21.8-49.0</td>
</tr>
</tbody>
</table>

Figure 7.5: Box and Whisker plots for percentage epidermal attachment for:

1) control flanges (red)
2) drilled flanges with silanised RGD-polypeptides (green)
3) porous flanges (blue)
4) porous flanges with silanised RGD-polypeptides (yellow).
Table 7.5: Pair-Wise (MWU) comparisons, showing p values.

<table>
<thead>
<tr>
<th></th>
<th>Flange Control</th>
<th>Flange RGD</th>
<th>Porous Control</th>
<th>Porous RGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flange Control</td>
<td></td>
<td>0.594</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Flange RGD</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Porous Control</td>
<td></td>
<td></td>
<td></td>
<td>0.854</td>
</tr>
</tbody>
</table>

Figure 7.6: Box and Whisker Plot demonstrating blood vessel density of soft tissues with:

1) control flanges (red)
2) drilled flanges with silanised RGD-polypeptides (green)
3) porous flanges (blue)
4) porous flanges with silanised RGD-polypeptides (yellow).
Table 7.6: Median and 95% Confidence Intervals for Cell Nuclei Density

<table>
<thead>
<tr>
<th>Group</th>
<th>Median/Percentage</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flange Control</td>
<td>0.000585</td>
<td>0.000254 – 0.00152</td>
</tr>
<tr>
<td>Flange RGD</td>
<td>0.000301</td>
<td>0.0000648 – 0.000813</td>
</tr>
<tr>
<td>Porous Control</td>
<td>0.000513</td>
<td>0.000385 – 0.000675</td>
</tr>
<tr>
<td>Porous RGD</td>
<td>0.00054</td>
<td>0.000386 – 0.000735</td>
</tr>
</tbody>
</table>

Figure 7.7: Box and Whisker plot plot demonstrating cell nuclei density of soft tissue with;

1) control flanges (red)
2) drilled flanges with silanised RGD-polypeptides (green)
3) porous flanges (blue)
4) porous flanges with silanised RGD-polypeptides (yellow).
Table 7.7: Pair-Wise (MWU) Comparisons

<table>
<thead>
<tr>
<th></th>
<th>Flange Control</th>
<th>Flange RGD</th>
<th>Porous Control</th>
<th>Porous RGD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flange Control</td>
<td></td>
<td>0.565</td>
<td>0.637</td>
<td></td>
</tr>
<tr>
<td>Flange RGD</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Porous Control</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 7.8: Box and Whisker plot demonstrating percentage of dermal tissue infiltration with control flanges (red) drilled flanges with silanised RGD-polypeptides (green) porous flanges (blue) porous flanges with silanised RGD-polypeptides (yellow).
7.5.7 Qualitative Assessment

Evidence of downgrowth was observed, evidenced by epidermal cells tracking along the implant. Gaps were observed between the implant shaft and soft tissue, resulting in pocket formation (Figure 7.9).

Wound debris, necrotic tissue and clots between the implant and tissue were observed impeding interface formation (Figure 7.10). However, where epidermal downgrowth was limited, contact between tissue and implant suggested a stable interface well above the flange (Figure 7.11).

There was good integration of the soft tissue of the dermis within porous structures, with infiltration seen throughout the interconnecting porous structure of the titanium construct (Figure 7.12), with collagen formation extending to the underside of the flange.

The presence of soft tissue within the porous structure was well vascularised (Figure 7.13 & Figure 7.14).

In instances where soft tissue infiltration was poor, gaps were observed at the tissue-implant interface (Figure 7.15)
Figure 7.9: Toluidine blue-stained light microscopy image of a transcutaneous section stained with of a Group 4 implant. Image shows epidermal downgrowth and sinus track formation along the implant shaft (arrows).
Figure 7.10: Toluidine blue-stained light microscopy image of a transcutaneous section stained Group 4 implant. Low magnification image of wound debris impeding epidermal attachment, forcing the cell sheet underneath the blockage before interfacing with the implant.
Figure 7.11: Toluidine blue-stained light microscopy image of a transcutaneous section of a Group 3 implant. Low magnification image of epidermal attachment (arrow), showing limited downgrowth.

Figure 7.12: Toluidine blue-stained light microscopy image of a transcutaneous section of a Group 4 implant within porous flange.
Figure 7.13: Toluidine blue-stained light microscopy image of a Group 3 transcutaneous section. High magnification image showing dense, well-ordered dermal tissue with a number of blood vessels formed within a pore.

Figure 7.14: Toluidine blue-stained light microscopy image of a Group 4 transcutaneous section. High magnification image showing dense well-ordered dermal tissue with blood vessels formed within a pore.
Figure 7.15: Toluidine blue-stained light microscopy image of a Group 1 implant. Low magnification image of dermal tissue infiltration within a flange showing poor contact at dermal/implant interface (arrow).
7.6 Discussion

My data show that there was epidermal downgrowth for all implant groups after 5-months of implantation. The addition of an RGD-polypeptide coating did not appear to reduce epidermal downgrowth. There was also no difference in the percentage of epidermal attachment comparing flanges that were coated or uncoated with RGD polypeptides. The addition of an RGD coating did not have a detrimental effect on the performance of the devices compared with the clinical standard of a previously implanted ITAP device [20].

The first phase of wound healing involves formation of a blood clot to plug the wound space. The clot acts as a scaffold for the migration of cells required for wound healing. In the same way, Farrell et al. described the formation of a serocellular crust at the junction of the implant and epidermis for their porous transcutaneous implants. The regenerating epidermal sheets followed the course of these cellular debris, which acted as a scaffold for the growth of the cells [265]. I observed similar findings in this study, with wound debris found at the transcutaneous interface both macro- and microscopically. This debris could have acted as a physical barrier at the epidermal-implant interface, preventing the epidermal cells from attaching to the implant and acting as a scaffold to direct downgrowth of the epidermal cells instead.

Epidermal keratinocytes do not express integrin αvβ3 receptors which have an affinity for the fibrinogen and fibrin that are found within a blood clot [266]. The absence of this receptor on the keratinocyte cell surface means that the clot acts as a physical impediment, forcing the regenerating sheet of epidermal cells to migrate under it rather than through it or over it. It is of course possible to minimise the clot that forms at the stoma through meticulous surgical technique and careful haemostasis followed by regular cleaning of the transcutaneous interface. In fact, this is the sort of cleaning regime that was implemented by our ITAP patient, including daily cleaning and re-dressing in the initial healing phase after implantation [20]. However, applying such a rigorous cleaning regime was not possible in our sheep model, resulting in an accumulation of wound debris. This may have contributed to epidermal downgrowth and may have negated the potentially beneficial effects of adding an RGD polypeptide coating or changing the morphology of the flange structure of the bone-anchor device.
Blood vessel density was significantly increased within all the bone-anchors with a porous flange compared with drilled ones. However, the addition of a coating of RGD-polypeptides had no effect (positive or negative) on the vascularisation of the soft tissue for either control of porous flange structures. Vascularisation of the flange structure relies on endothelial cell migration and cell attachment. Feng et al. demonstrated that ECM structures can influence the expression of integrin αvβ3 receptors by endothelial cells, upregulating blood vessel formation. Moreover, provision of a 3-dimensional fibrin matrix significantly increased expression of mRNA for integrin αvβ3 receptors compared with a single layer ECM protein coating [267]. In my experiments, the porous flange may have provided the 3-dimensional matrix, inducing integrin αvβ3 receptor expression on endothelial cells to a greater extent than for the control drilled flange.

There was no significant difference in cell density across the four groups at 5 months after implantation. Cell nuclei densities remained the same despite the addition of an RGD polypeptide coating to the implants. This might have been due to the time at which the implants were explanted in relation to the phases of wound healing. During the cellular phase of wound healing, fibroblasts initiate ECM formation by secreting collagen. As wound stability increases due to the structural support provided by the forming collagen matrix, the requirement for fibroblasts decreases and the wound then enters the maturation phase of healing. Typically, this process takes 3 months from wounding to maturation. By the time the implant retrieval had occurred at 5 months, the tissues had already entered the maturation phase, and an equilibrium must already have been achieved relating to the number of cells required to maintain the collagen matrix. This could explain why no effects were observed from the addition of an RGD polypeptide coating with uncoated bone-anchors. Farrell also observed similar outcomes. Specifically, he noted that there were significant differences in the degree of soft tissue infiltration of the transcutaneous structures with various porosities at 3-weeks, but that these differences was no longer present by the end of his experiment (4 to 6 weeks) [265].

Dermal tissue infiltration was significantly increased by the combination of RGD-polypeptides with a porous flange compared with the other implant groups. The early phase of wound healing involves the laying down of a structural framework to support cellular influx during the cellular phase of wound healing [268]. The porous flange silanised with RGD-polypeptides provided the initial wound matrix to support the cellular phase of wound healing, which was followed by a significant increase in dermal tissue infiltration. Previous
in vitro studies by Dowling et al. showed that silanised RGD-polypeptides enhance dermal fibroblast attachment \[259\]. RGD-polypeptides stimulate fibroblasts to express integrin receptors responsible for cell attachment. Integrins $\alpha_3\beta_1$ and $\alpha_5\beta_1$ receptors have been shown to be upregulated on the surface of fibroblasts in vitro \[269\]. These receptors have a high affinity for the RGD binding domain, and it is therefore postulated that this upregulation may be occurring in porous flanges silanised with RGD-polypeptides, resulting in the increase in dermal tissue infiltration observed in this study.

Although this experiment showed that the addition of RGD-polypeptides to a porous flange structure had a positive effect on early transcutaneous interface formation, these effects did not translate to all measurable outcomes. Previous in vitro\(^5\) experiments have shown a significant difference in HDF attachment to titanium alloy substrates to which an RGD-polypeptide coating has first been added. However, this effect was concentration dependent. Therefore, optimising the concentration used to coat the implants might have more effects, and this would need to be explored in future, in vivo, studies. There was also a significant increase in dermal tissue infiltration and blood vessel density in the RGD-polypeptide treated porous flange implants compared with controls. Since a blood supply is essential to support tissue in-growth and is critical in maintaining the integrity of the interface in the long-term, RGD-polypeptide coated porous implants may have advantages over standard designs of ITAP implants.

### 7.7 Conclusions

- The addition of an RGD-polypeptide coating to a porous flange structure resulted in a statistically significant increase in blood vessel formation and dermal cell attachment compared with controls.
- There was no significant difference in epidermal downgrowth or attachment comparing the 4 different implant groups.
- It is not clear whether these findings will have any practical implications for any future design of a bone-anchor in humans since multiple, additional, design modifications would be needed for any future human prototypes.
8 Chapter 8 – Discussion and Conclusion

This Chapter summarises the key findings and conclusions of this thesis. The main aim of my thesis was to investigate the *in vivo* feasibility of technologies for a bone-anchored device to provide upper limb amputees with a solution for both attachment and control of a prosthesis. Future work is also proposed including directions for the engineering, experimental, and clinical developments needed to turn the work presented in this thesis feasibility studies into a practical solution.

Chapter One presented an overview of the current solutions for reconstruction after loss of the upper limb, including a consideration of the prosthetic solutions that are most widely used. The limitations of the different solutions were described, with an emphasis on the problems encountered by patients when attaching a prosthesis to their residual limb. The skin-socket related issues and the potential advantages of direct skeletal fixation were described. The difficulties encountered by amputees in trying to control their prosthetic limbs was discussed in detail, including the limitations of surface electrodes and the need to internalise biosensors for both muscles and nerves to overcome these issues. The relevant biology and electrophysiology of muscles and nerves were detailed along with the methods used to record these biosignals. For this thesis, I considered that EMG signals to be the solution, which was most likely to be translated from bench to bedside most quickly. Therefore, I focused on the use of implantable epimysial electrodes for direct recording of EMG signals from individual muscles responsible for specific actions, to overcome the shortcomings with surface electrode (reliability and selectivity). In this Chapter, I also discussed the use of nerves to record biosignals for prosthetic control. While this technology has the potential to provide far greater information for a highly biomimetic end device, it is still in its infancy and its clinical application is likely to lag many decades behind the use of EMG signals due to the poor reliability of chronically implanted nerve interfaces. The use of MNI implants as a potential nerve recording interface were also discussed. Finally, the novel technique of TMR for increasing the number of EMG control channels in proximal amputees was discussed.

Chapter Two investigated the use of an sheep model to test the recording of EMG signals. A detailed cadaveric dissection identified *m. peroneus tertius* as a suitable muscle from which to record EMG signals as well as a muscle to be used for my investigations of signal regeneration after treatment with TMR - outlined in Chapter Four. I then described a proof-
of-concept study using an epimysial electrode passed transcutaneously to record EMG signals to demonstrate that this could be done. Although high fidelity signals with a high SNR were recorded initially, the external connector of my device failed due to mechanical stress, highlighting the need to use a more robust interface. The use of a more robust device was investigated in Chapter Three.

Chapter Three presented the results of further development and testing of a bone-anchored device for transmission of EMG signals from implanted muscle electrodes, in both a proof of concept study and in a larger study over 5 months. High fidelity EMG signals with a high SNR were recorded over the duration of the experiment. This work was published [54], leading to a discussion of the use of a hind-limb ovine model for the evaluation of muscle interface designs and signal transmission methods. This work improved on research previously carried out by Pitkin et al. [169] in that it recorded EMG signals from live, conscious animal. Similar ideas led to the development of signal transmission using a two-stage osseointegrated prosthesis [67]. The limitations imposed by crosstalk from adjacent muscles in the sheep hindlimb were also investigated in this Chapter and confirmed the selectivity of epimysial electrodes compared with their surface counterparts. Tripolar electrode configurations have the potential to further reduce SNR, and the same manufacturing methods for combining a bone-anchor with electrodes were implemented to investigate bipolar and tripolar electrode configurations in Chapter Five. A physical conduit such as a bone-anchor could also be applied for transmission of nerve signals for prosthetic control. The bandwidth requirements for ENG signals are greater than for EMG signals, and hard-wired transmission of these signals out of the body would ensure much faster data transfer without a drop in bandwidth (and the power requirements) associated with wireless telemetry. Therefore, accurate and reliable long-term ENG signal capture coupled with a bone-anchored device might provide the ideal solution for attachment and control of prosthetic limbs in the future.

Chapter Four presented the results of a study of the effects of TMR treatments in a sheep model. Reinnervation of the *m. peroneus tertius* after TMR treatment was investigated to determine the effects on EMG signal regeneration with time. The implanted epimysial electrode system created in Chapter Three made this possible. However, in Chapter Three, I pointed out that a system based purely on implanted electrodes alone would not provide the
intuitive control required for transhumeral (or more proximal) amputees, due to the absence of extrinsic hand muscles. In contrast, TMR has the potential to increase the number of control signals by rerouting blind-ending nerves that were once responsible for hand function and to surrogate muscles that are no longer needed for physiological function. This has been revolutionary in providing more intuitive prosthetic control in proximal amputees and in patients with shoulder disarticulation. In my TMR model, I observed a return of signal to the reinnervated *m. peroneus tertius* in real time, and was able to record EMG signals with high SNR comparable to non-reinnervated muscles, proving that implantation of the electrodes could be performed at the same time as TMR surgery. Importantly, the return of EMG signals corresponded to normalisation of the animal’s gait, as measured by force plate analysis.

Chapter Five investigated the optimal electrode configuration for maximising SNR in epimysial electrodes. Although high fidelity, high SNR EMG signals were previously recorded in previous Chapters with the standard bipolar electrode setup, further increasing this selectivity by using a tripolar setup has not previously been tested *in vivo*. Tripolar electrode setups promised greater selectivity over other electrode configurations since they isolate the detection volume, eliminating factors such as a constant sloped voltage gradient. However, surprisingly, tripolar electrodes failed to outperform their bipolar counterparts with regards to SNR, a measure of selectivity of EMG signal against background noise and crosstalk. These findings were in contrast to surface electrode work carried out by Fortune *et al.*, on healthy human volunteers, where they found tripolar electrodes outperformed bipolar electrodes with regards to crosstalk [190]. However, surface skin-surface electrodes have inherent limitations, which were reconsidered in detail.

A preliminary system testing a multichannel wireless EMG recording system was conducted concurrently with the implantable epimysial-bone-anchor system. The implantable electronics were kept outside the sheep’s body, with the system operating under the wireless telemetry protocol. Although EMG signals were successfully recorded, the system suffered reliability issues, the results are still being analysed and are not included in this thesis. Further work is currently underway to resolve the reliability issues ahead of future experiments. Once the reliability of the wireless system is optimised, a fully implantable
system will be planned since a wireless implantable system is an important option for patients that are unsuitable (or unwilling) to have a bone-anchor.

Chapter Six investigated the performance of stacked MNI implants in a rat sciatic nerve model. The use of sutures and fibrin glue as a method of securing MNI implants to sciatic nerve was investigated, and suturing was found to be a more reliable way of fixing the nerve ends into the implant. In the second experiment, all-in-one BM protein coatings were investigated on nerve regeneration, as measured by functional, histological and electrophysiological outcomes. My studies showed some weak evidence for the use of these protein coatings especially in regards to improvements in distal axon diameters, although this did not reach statistical significance. Importantly, this study was able to successfully record biosignals from the MNI, with SNR suitable for prosthetic control. Direct recording of ENG signals from peripheral motor nerves using MNI implants can potentially yield the much greater number of signals required to control a prosthetic limb compared with EMG interfaces and may be where future research should be targeted once the potential for increasing the number of EMG interfaces has plateaued. One issue that was not considered is how to achieve reliable, long-term transcutaneous signal transmission. To do this, MNI implants could be combined with a bone-anchor device.

Chapter Seven investigated the effects of changes to the porosity of the flange structure of my bone-anchor together with the addition of silanised RGD-polypeptide to the flange part of the device on formation of the transcutaneous-implant interface over a 5 month period. The interface was quantitatively characterised in terms of the length of any epidermal downgrowth, the percentage of epidermal attachment, percentage of dermal tissue infiltration into the flange, blood vessel density and cell nuclei within the infiltrated tissue. Although no significant difference was observed between the RGD polypeptide coated and control groups with regards to epidermal downgrowth and dermal attachment, there were significant increases in blood vessel density and dermal tissue infiltration. These were more notable in the porous flanges silanised with RGD-polypeptides. While an increase in blood vessel formation and dermal tissue infiltration might prove advantageous in terms of achieving the goal of improved dermal attachment, this is an area that will require further work. Initial in vitro experiments with RGD that informed the basis of the in vivo work outlined in this Chapter were performed in a 2-D culture system. Fibroblast morphology was shown to differ between 2-D and 3-D systems [270]. However, further work would be needed to better understand how dermal fibroblasts behave in 3-Dtissues, and an in vitro experiment could be
designed where dermal fibroblasts are introduced onto 3-D porous substrates to which differing concentrations of RGD-polypeptides have been added.

In this Chapter, I also discussed the specific engineering developments that would be needed for any future design of a bone-anchor + implanted electrode device to create a prototype that might be immediately ready for human implantation. The key areas for development would be in manufacturing, redesign of the flange structure of the bone-anchor, fixation technique, fail-safe devices, feed-throughs for any electronics and internal electronics,

I made recommendations on the use of electron beam melting, a type of 3-D printing used for the manufacture of metal objects with a complex three-dimensional structure was recommended for the manufacture of any future bone-anchor based device. This would allow greater flexibility in designing any internal structures, especially the curved channel that would be used to transmit electrode cables from any internal multiplexer box.

I suggested that the flange structure of the bone-anchor should be changed from its current dome shape to a pear-shape, similar to the implants used for clinical application in veterinary medicine [271]. This might reduce the pressure on the overlying dermal soft tissue, and prevent the ischaemic sieving that was observed in the experiments noticed in Chapter Three.

The surface of the implant stem that is in contact with bone should be roughened to aid osseointegration. HA coating increases both bony and soft tissue in-growth. However, the incorporation of a shoulder which serves as an abutment for the transected bone provides axial loading transfer onto the cortical bone is advantageous as it reduces stress shielding and provides for a more anatomical transfer of forces from the bone-anchor to the skeleton. Going forward with these findings, I would design a bone anchor incorporating these elements and translate it directly into a single patient exemption study over a two-year implantation timeframe, to further study both the osseointegration as well as the skin/implant seal. The success of this single study can then inform a larger clinical trial through the MHRA to obtain CE marking of the device for commercial use.

I suggested that efforts should be directed at designing a feed-through robust enough to withstand the forces of insertion of a press-fit bone-anchor into the medullary cavity. Since hermetic feed-throughs are generally made from glass or ceramic, the material has to be robust enough to withstand the hammering of the device during the press-fit of the anchor.
without shattering. Alternatively, a seal that does not rely on glass or ceramic may need to be considered.

I suggested that a fail-safe mechanism must be applied to the external part of the bone-anchor, which is designed to shear if the load applied to the prosthesis exceeds a specified limit. This is crucial to protect the bone-anchor and the bone. Such a device was fitted in the upper limb patient that received the first iteration of ITAP [20].

Finally, I have previously discussed the limitations of transmitting individual electrode cables from implanted epimysial electrodes through the bone-anchor. There is a limit to the physical space available in the bone-anchor to transmit electrode cables without compromising the mechanical strength of the device. As a result, previous attempts using this approach have been limited to just 4 electrode cables [67], frustrating any chances of achieving intuitive control due to the limitation on the number of control channels. Going forward, I would investigate the use of a fully implantable myoelectric system, using multiplexing to allowing multiple biosignals to be digitally combined into one signal, requiring only one electrode cable to be passed through the bone-anchor. This would allow demultiplexing (the conversion of the signal back into the original, separate signals) to take place in the prosthetic limb using an appropriate microprocessor. Furthermore, by taking the ‘envelope’ of the EMG signal instead of the entire raw signal, the amount of data transmitted can be reduced, further simplifying downstream data processing. By reducing the processing burden, a simpler microprocessor design can be instituted, which may increase reliability of the system in the long run. Although I recommended the use of this approach to overcome the problems highlighted above, I accept that incorporation of a multiplexer into any future designs will increase the difficulties of achieving regulatory approval for the device, placing it in the same category as FES devices (Class III). However, the other major advantage of an implanted electrode system based on a multiplexer is its serviceability. Should anyone (or more) of the individual components fail, it would be possible to replace the part or upgrade the entire system as new technologies are developed.

In this thesis I have shown that the combination of direct skeletal fixation with implantable EMG recording and TMR has the potential to provide an intuitive interface for prosthetic reconstruction after upper limb loss.
EMG Recordings Protocol

This protocol describes the procedure for recording epimysial (implanted) and skin surface EMG signals in an ovine model.

Epimysial Electrode Recording

1. Prepare the electrode recording equipment (MP150) with the following settings: 50 Hz High Pass (HP), 500 Hz Low Pass (LP), 100 x Gain.
2. Connect the recording equipment to the computer, turn on, and open AcqKnowledge Software.
3. Prepare AcqKnowledge software for signal acquisition:
4. Prepare the animal for walking on a treadmill.
5. Place the animal on the treadmill.
6. Place the ground electrode
7. Connect the recording equipment to the device socket.
8. Begin recording on the computer.
9. Start the treadmill.
10. Increase the speed to 2.0 km/h.
11. Maintain a speed of 2.0 km/h for 120 seconds.
12. Observe the recorded signal.
13. The recording may be repeated 3 times for each animal.
Skin Surface Electrode Recordings

1. Prepare the electrode recording equipment (MP150) with the following settings: 50 Hz High Pass (HP), 500 Hz Low Pass (LP), 100 x Gain.
2. Connect the recording equipment to the computer, turn on, and open AcqKnowledge software.
3. Prepare AcqKnowledge software for signal acquisition:
4. Prepare the animal for skin surface electrodes: shave and clean the skin over the m. peroneus tertius.
5. Palpate the lower limb to locate the epimysial electrode.
6. Place two skin surface electrode 2cm cm apart (centre to centre) over the epimysial electrode site.
7. Place the ground electrode.
8. Connect the recording equipment to the electrodes.
10. Start the treadmill.
11. Increase the speed to 2.0 km/h.
12. Maintain a speed of 2.0 km/h for 120 seconds.
13. Observe the recorded signal.
14. The recording may be repeated 3 times for each animal.

Simultaneous Recordings

Simultaneous recordings are made following the protocols for skin surface and epimysial electrode recordings, connecting both electrode sets simultaneously. Two MP150 recording systems are used, and care taken to ensure that the epimysial electrodes are connected to channel 1 and the skin surface electrodes are connected to channel 2.

Recording Times

This outlines the recording times for Chapter Three, second experiment with n=6. The first recording is made no less than 24 hours after implantation. For the first month recordings are made weekly. For the second and third months recordings are made fortnightly. For the fourth and fifth months recordings are made monthly. This is summarised in table I.
<table>
<thead>
<tr>
<th>Week</th>
<th>Recording</th>
<th>Week</th>
<th>Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Y</td>
<td>10</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>Y</td>
<td>11</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>Y</td>
<td>12</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>Y</td>
<td>13</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>Y</td>
<td>14</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>15</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>Y</td>
<td>16</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>N</td>
<td>17</td>
<td>Y</td>
</tr>
<tr>
<td>9</td>
<td>Y</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table I: Recording Times
Method for calculating Signal to Noise Ratio Using Acknowledge Software

1. Select a 4 second section. Typically this will contain 3-4 signals corresponding with the gait cycle of the animal.

2. Save section.
3. Open the saved file.

4. Go to Analysis in the menu bar, select Electromyography → EMG Frequency and Power Analysis.
5. When prompted, select 0.25 seconds epoch.

6. Second from bottom graph represents Mean Power.
7. Save document as excel spreadsheet (for data points).
8. Open Excel spreadsheet with data points. There are 4000 data points in total taken at 0.001 second intervals.
10. Our readings are in column B: CH1, Analogue Volts. I also need column F, Mean Power (Volts squared divided by Hz). Power is calculated in 0.25 second epochs.

11. Paste values in new spreadsheet.

12. A formula is produced to assist with data analysis.
For calculating the absolute values of signals (since these can be either positive or negative) in column I. Average Mean Power is also calculated (column E). Any value above this value is taken as signal; below this value is regarded as noise. Average noise is calculated as the mean of all values below Average Mean Power (column K). Average signal is calculated as the mean of all values above Average Mean Power (column J). SNR is taken as Average signal ÷ Average noise (column L).
Specifications for plugs and sockets

Socket - PHG.00.302.CYMD22
Plug - FGG.00.302.CLAD22

LEMO UK Ltd, Worthing, U.K
Specification Sheet for Epimysial Electrode (ARDIEM)

Epimysial Myoelectric Signal Recording Electrode (EPI-MES)

Distal Termination – Dual disk, bipolar electrode encapsulated in polyester reinforced silicone suture apron

Electrode material
Platinum alloy

Number of electrodes
Two

Exposed contact area per electrode
3.68 mm (0.125”) diameter (10.64 mm²)
Spacing between contacts
10.0 mm (0.3937") center of distal disk to center of proximal disk

Lead wires
Fluoropolymer insulated 316 SS lead wires, coiled and tubed in silicone

Lead OD
0.050" (1.27 mm) OD of silicone tubing

Lead Length
Standard - 30 cm (12")
Special 4 - 90 cm (1.5 - 35")

2 Proximal termination options
Flying lead (Unterminated bare wires)
Letechipia connector (Specialized connector)
IS-1 Bipolar (One IS-1 Bipolar)
IS-1 Unipolar (Two IS-1 Unipolar)
Special (Per customer request)

Dimensional illustration of Epimysial EMG electrode
Protocol for Histology Processing

All samples were individually submerged in formalin after surgical removal from the animal subjects for one week. This was followed by serial dehydration with alcohol (Industrial Methylated Spirit, IMS)(see table below), until all the samples were fully submerged in 100% alcohol. All solutions were then changed to chloroform for one week, with one change halfway through, this was performed in a highly ventilated laboratory. The samples were then returned back into 100% alcohol for one week, with one solution change halfway through.

**Processing Protocol for EOS Porous Implants**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three days</td>
<td>50% IMS : 50% dH₂O</td>
</tr>
<tr>
<td>Three days</td>
<td>75% IMS : 25% dH₂O</td>
</tr>
<tr>
<td>Three days</td>
<td>85% IMS : 15% dH₂O</td>
</tr>
<tr>
<td>Three days</td>
<td>95% IMS : 5% dH₂O</td>
</tr>
<tr>
<td>Three days</td>
<td>100% IMS</td>
</tr>
<tr>
<td>Three days</td>
<td>100% IMS</td>
</tr>
<tr>
<td>Three days</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Three days</td>
<td>Chloroform</td>
</tr>
<tr>
<td>Three days</td>
<td>100% IMS</td>
</tr>
<tr>
<td>Three days</td>
<td>100% IMS</td>
</tr>
</tbody>
</table>

Each sample was submerged in Hard Grade Acrylic Resin (London Resin Company) and stored in the dark at all times. The samples were kept under vacuum or on a rotator and swapped over daily. All the samples were left in these conditions until the Hard Grade Acrylic Resin had completely penetrated through each sample, this took a minimum of one week.
Fresh Hard Grade Acrylic Resin was put in the freezer for two hours pre-casting of the samples. To cast, each sample was given a new, labelled container (75ml volume). One droplet of LR White Accelerator (London Resin Company Ltd) was placed into each container and spread with a paper towel around the inside of the container. The samples were individually placed in their corresponding containers. When the Hard Grade Resin had cooled, it was transferred into a measuring jug. For every 10ml of Resin, one droplet of LR White Accelerator was pipetted into the measuring jug. The mixture was stirred evenly and gently to dissolve the accelerator into the resin and then poured into the containers, making sure each sample was completely covered with liquid. The samples were then placed into the freezer and left to cast, for a minimum of four hours.
Specification for Bone-Anchored Device
Water Test Results

Day 0 = 15/08/11

1% saline solution at 37 degrees Celsius. Where readings were not taken corresponds to weekends.

<table>
<thead>
<tr>
<th>Day</th>
<th>Reading</th>
</tr>
</thead>
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<tr>
<td>0</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>27.0</td>
</tr>
<tr>
<td>3</td>
<td>27.0</td>
</tr>
<tr>
<td>4</td>
<td>27.1</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
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<td>7</td>
<td>27.1</td>
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<tr>
<td>8</td>
<td>27.0</td>
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<tr>
<td>9</td>
<td>27.0</td>
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<tr>
<td>10</td>
<td>27.1</td>
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<tr>
<td>11</td>
<td>27.0</td>
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<tr>
<td>12</td>
<td>-</td>
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<tr>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-----</td>
</tr>
<tr>
<td>15</td>
<td>27.0</td>
</tr>
<tr>
<td>16</td>
<td>27.0</td>
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<tr>
<td>17</td>
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<td>18</td>
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<tr>
<td>20</td>
<td>-</td>
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<tr>
<td>21</td>
<td>27.0</td>
</tr>
<tr>
<td>22</td>
<td>27.1</td>
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<td>23</td>
<td>27.0</td>
</tr>
<tr>
<td>24</td>
<td>27.0</td>
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<tr>
<td>25</td>
<td>27.1</td>
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</table>
## Experiment 1 SNR

<table>
<thead>
<tr>
<th>Days from implantation</th>
<th>SNR mean (epimysial)</th>
<th>SNR mean (surface)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.22</td>
<td>1.58</td>
</tr>
<tr>
<td>8</td>
<td>4.10</td>
<td>1.62</td>
</tr>
<tr>
<td>15</td>
<td>4.35</td>
<td>1.89</td>
</tr>
<tr>
<td>22</td>
<td>4.86</td>
<td>1.44</td>
</tr>
<tr>
<td>38</td>
<td>5.91</td>
<td>1.69</td>
</tr>
<tr>
<td>44</td>
<td>7.00</td>
<td>1.54</td>
</tr>
<tr>
<td>50</td>
<td>5.14</td>
<td>1.67</td>
</tr>
<tr>
<td>57</td>
<td>6.50</td>
<td>1.43</td>
</tr>
<tr>
<td>63</td>
<td>5.84</td>
<td>1.47</td>
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<tr>
<td>71</td>
<td>5.62</td>
<td>1.65</td>
</tr>
<tr>
<td>77</td>
<td>4.73</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Mean SNR of Epimysial Electrode in Ovine Model over 12 weeks
### Experiment 1 Co-Stimulation

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Repeat</th>
<th>Stimulus Peak (V)</th>
<th>EMG Peak (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroneus tertius</td>
<td>1</td>
<td>0.25223</td>
<td>0.03319</td>
</tr>
<tr>
<td>Peroneus tertius</td>
<td>2</td>
<td>0.27946</td>
<td>0.03113</td>
</tr>
<tr>
<td>Peroneus tertius</td>
<td>3</td>
<td>0.20897</td>
<td>0.03044</td>
</tr>
<tr>
<td>Peroneus tertius</td>
<td>4</td>
<td>0.2829</td>
<td>0.02174</td>
</tr>
<tr>
<td>Peroneus tertius</td>
<td>5</td>
<td>0.2314</td>
<td>0.02884</td>
</tr>
<tr>
<td>Peroneus tertius</td>
<td>6</td>
<td>0.21973</td>
<td>0.03159</td>
</tr>
<tr>
<td>Peroneus longus</td>
<td>1</td>
<td>0.06432</td>
<td>0.01923</td>
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<tr>
<td>Peroneus longus</td>
<td>2</td>
<td>0.06157</td>
<td>0.01579</td>
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<tr>
<td>Peroneus longus</td>
<td>3</td>
<td>0.05676</td>
<td>0.0174</td>
</tr>
<tr>
<td>Peroneus longus</td>
<td>4</td>
<td>0.05424</td>
<td>0.02266</td>
</tr>
<tr>
<td>Peroneus longus</td>
<td>5</td>
<td>0.07805</td>
<td>0.02083</td>
</tr>
<tr>
<td>Peroneus longus</td>
<td>6</td>
<td>0.04715</td>
<td>0.01717</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>1</td>
<td>0.16342</td>
<td>0.00618</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>2</td>
<td>0.20348</td>
<td>0.0087</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>3</td>
<td>0.11055</td>
<td>0.00755</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>4</td>
<td>0.09705</td>
<td>0.00916</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>5</td>
<td>0.11719</td>
<td>0.0103</td>
</tr>
<tr>
<td>Tibialis anterior</td>
<td>6</td>
<td>0.10986</td>
<td>0.00847</td>
</tr>
<tr>
<td>Muscle</td>
<td>Trial</td>
<td>Peak Value</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>--------------</td>
<td>-------</td>
<td>------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1</td>
<td>0.0174</td>
<td>0.00664</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>2</td>
<td>0.02953</td>
<td>0.00458</td>
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<tr>
<td>Gastrocnemius</td>
<td>3</td>
<td>0.02174</td>
<td>0.00343</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>4</td>
<td>0.05241</td>
<td>0.00412</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>5</td>
<td>0.01328</td>
<td>0.00412</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>6</td>
<td>0.01556</td>
<td>0.00595</td>
</tr>
</tbody>
</table>

Peak value recordings following stimulation of various muscle groups
Anaesthetic and Perioperative Regime – Sheep

Pre-med

Xylazine Hydrochloride (Rompun 2%) 0.1mg/kg
Bayer Health Care
Bayer House
Strawberry hill
Newbury
Berkshire
RG14 1JA

Induction

Ketaset (Ketamine) 2mg/kg
Fort Dodge Animal Health Ltd
Southampton
SO30 4QH

Hypnovel (Midazolam) 2.5mg flat rate
Roche Products Ltd
Welwyn Garden City
Hertfordshire
AL7 3AY

Maintenance

Isoflurane
IsoFlo 100% Isoflurane
Abbott Laboratories Ltd.
Abbott House
Vanwall Business Park
Vanwall Road
Maidenhead
Berkshire
SL6 4XE

Analgesic
Durogesic 75mcg/hr (Fentanyl) 2 patches 12 hours prior to surgery followed by 2 patches 60 hours post surgery

Janssen-Cilag
50-100 Holmers Farm Way
High Wycombe
Bucks
HP12 4EG

Vetergesic (Buprenorphine)

Alstoe Animal Health
Pera Innovation Park
Nottingham Road
Melton Mowbray
LE13 0PB

Vetergesic (Buprenorphine) 0.6mg 72 hours after 2nd application of fentanyl patches

Antibiotic Cover

Betamox LA (Amoxicillin) 150mg/ml used when taking aspirates 1ml/10kg

Norbrook Laboratories (GB) Limited
The Green
Great Corby
Carlisle
CA4 8LR

OR

Ceporex (Cefalexin)
Schering-Plough Animal Health
Division of Schering-Plough Ltd
Welwyn Garden City
AL7 1TW

5ml/day for 4 days

Euthanasia

Pentobarbital solution 20%
Pharmasol Ltd.
North Way
Andover
Hampshire
SP10 5AZ

0.7mg/kg (Normally 40ml/animal)
<table>
<thead>
<tr>
<th>Days P.O.</th>
<th>SNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1.642178717</td>
</tr>
<tr>
<td>8</td>
<td>2.508400943</td>
</tr>
<tr>
<td>8</td>
<td>1.406517775</td>
</tr>
<tr>
<td>14</td>
<td>1.792765489</td>
</tr>
<tr>
<td>14</td>
<td>1.446237647</td>
</tr>
<tr>
<td>14</td>
<td>1.404631772</td>
</tr>
<tr>
<td>22</td>
<td>2.143813463</td>
</tr>
<tr>
<td>22</td>
<td>1.735369319</td>
</tr>
<tr>
<td>22</td>
<td>1.704594573</td>
</tr>
<tr>
<td>29</td>
<td>1.462510628</td>
</tr>
<tr>
<td>29</td>
<td>3.834509896</td>
</tr>
<tr>
<td>29</td>
<td>4.29395951</td>
</tr>
<tr>
<td>36</td>
<td>3.086873591</td>
</tr>
<tr>
<td>36</td>
<td>2.644978023</td>
</tr>
<tr>
<td>36</td>
<td>4.292979974</td>
</tr>
<tr>
<td>36</td>
<td>1.871840094</td>
</tr>
<tr>
<td>44</td>
<td>21.10415143</td>
</tr>
</tbody>
</table>
Table showing Force Plate Analysis data with Wilcoxon signed rank test with Bonferroni correction.
Force plate analysis. The first reading was taken four days pre-operatively as baseline measurement. Wilcoxon signed rank test was used as the data was dependant. A Bonferroni correction was employed to counteract the number of times the test was repeated (in this instance 7 times).

<table>
<thead>
<tr>
<th>Days</th>
<th>-4</th>
<th>9</th>
<th>15</th>
<th>23</th>
<th>31</th>
<th>45</th>
<th>92</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilcoxon signed rank test</td>
<td>0.627</td>
<td>0.002</td>
<td>0.001</td>
<td>0.064</td>
<td>0.001</td>
<td>0.126</td>
<td>0.108</td>
</tr>
<tr>
<td>P-Cut (0.05/7) ..ni correction</td>
<td>0.00714</td>
<td>0.00714</td>
<td>0.00714</td>
<td>0.00714</td>
<td>0.00714</td>
<td>0.00714</td>
<td>0.00714</td>
</tr>
<tr>
<td>Sig. diff. between legs?</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

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Protocol for Epimysial Electrode Recordings

1. Prepare the electrode recording equipment (MP150) with the following settings: 100 Hz High Pass (HP), 500 Hz Low Pass (LP), 100 x Gain.
2. Connect the recording equipment to the computer, turn on, and open AcqKnowledge Software.
3. Prepare AcqKnowledge software for signal acquisition:
4. Prepare the animal for walking on a treadmill.
5. Place the animal on the treadmill.
6. Place the ground electrode
7. Connect the recording equipment to the device socket.
8. Begin recording on the computer.
9. Start the treadmill.
10. Increase the speed to 1.5 km/h.
11. Maintain a speed of 1.5 km/h for 40 seconds.
12. Observe the recorded signal.
13. The recording may be repeated 3 times for each animal.
Circuit Diagram for Switch Box – Chapter 5
Table: SNR for the various electrode configurations. IQR – interquartile range.

<table>
<thead>
<tr>
<th>Electrode Configuration</th>
<th>Median</th>
<th>IQR</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>m1</td>
<td>1.0</td>
<td>0.70-1.20</td>
<td>1.0</td>
<td>0.27</td>
</tr>
<tr>
<td>m2</td>
<td>1.1</td>
<td>1.05-1.26</td>
<td>1.1</td>
<td>0.11</td>
</tr>
<tr>
<td>m3</td>
<td>1.1</td>
<td>0.99-1.54</td>
<td>1.2</td>
<td>0.29</td>
</tr>
<tr>
<td>m4</td>
<td>1.0</td>
<td>0.73-1.08</td>
<td>0.9</td>
<td>0.18</td>
</tr>
<tr>
<td>m5</td>
<td>0.9</td>
<td>0.75-1.29</td>
<td>1.0</td>
<td>0.29</td>
</tr>
<tr>
<td>b1</td>
<td>3.5</td>
<td>2.84-5.48</td>
<td>4.1</td>
<td>1.88</td>
</tr>
<tr>
<td>b2</td>
<td>3.0</td>
<td>2.37-3.18</td>
<td>2.8</td>
<td>0.68</td>
</tr>
<tr>
<td>b3</td>
<td>2.5</td>
<td>2.22-3.05</td>
<td>2.7</td>
<td>0.73</td>
</tr>
<tr>
<td>b4</td>
<td>2.5</td>
<td>1.96-2.86</td>
<td>2.5</td>
<td>0.48</td>
</tr>
<tr>
<td>b5</td>
<td>1.8</td>
<td>1.43-2.31</td>
<td>1.9</td>
<td>0.48</td>
</tr>
<tr>
<td>b6</td>
<td>2.0</td>
<td>1.68-2.68</td>
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<td>0.48</td>
</tr>
<tr>
<td>b7</td>
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<td>1.67-2.00</td>
<td>1.8</td>
<td>0.36</td>
</tr>
<tr>
<td>b8</td>
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<td>3.25-4.72</td>
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<td>0.87</td>
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<tr>
<td>b9</td>
<td>7.2</td>
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10 Bibliography


[259] Robert Dowling, ‘To Develop Techniques that will Enhance Dermal Cell and Tissue Attachment in Order to Create a Seal and Prevent Infection of Implant Biomaterials used for ITAP’, University College London, 2015.


