Abstract

Aims

Massive endoprostheses rely on extra-cortical bone bridging (ECBB) onto the shaft of the implant to enhance fixation. The aims of this study are to investigate the role of selective laser sintered (SLS) porous collars in augmenting osteointegration of segmental bone prostheses.

Materials and Methods

The two novel porous SLS designs were a small pore (Ø700μm, SP) and large pore (Ø1500μm, LP) that were compared in an ovine tibial diaphyseal replacement. Osteointegration of these collars were compared to the clinically used solid grooved design (G). At 6 months tibiae were retrieved and underwent radiological and histological analysis.

Results

Porous collars provided a greater surface ($p<0.001$) for bone attachment than G. Significantly greater extra-cortical pedicle formation was seen radiologically around G (length $p=0.002$, thickness $p<0.001$, surface area $p=0.002$) when compared to porous collars. However for both porous collars bone ingrowth occurred from the transection site into the porous structure. A 5-fold increase in integration was exhibited in the SP with 3-fold increase observed in the LP design compared to G ($p<0.001$).

Conclusion

SLS porous collars allow for greater bone attachment by utilising direct bone ingrowth which is better than current designs that utilise surface ongrowth via ECBB.

Clinical Relevance

SLS collars are a viable alternative for segmental bone defect implants, providing greater osteointegration for massive segmental prostheses.

Introduction

The longevity of massive endoprostheses for the reconstruction of the bone following removal of malignant bone tumors is a major concern, especially in young and active patients who place high demands on their prostheses (1-3). Advances in chemotherapy leading to longer life expectancy means that reliable fixation is required in order for patients to avoid multiple revision operations. Aseptic loosening accounted for 25% of revisions in a series of 661 by Jeys et al (1), which included both upper and lower limb cases. Distal femoral and proximal tibial replacements performed the worst with respect to AL, with rates of 31% and 37% respectively. Loosening of these intramedullary cemented implants are associated with cortical bone loss initially occurring at the point of contact between the bone and the shoulder of the implant, followed by osteolysis, which progresses along the implant stem (4, 5).

An ingrowth region adjacent to the bone at the transection site has been shown to promote extra-cortical bone bridging (ECBB) (3, 6-10), where bone from the cortex adjacent to the transection site, grows out and over the shaft of the prosthesis. Potentially osteointegration at the shoulder of the implant may reduce the risk of aseptic loosening by improving stress transfer within the cement mantle, reducing mechanical loosening and may form a seal preventing migration of fluid and wear particles along the bone implant interface (5, 7, 10-12).

Coathup et al (13) showed the use of a grooved HA collar for ECBB enhanced osteointegration which occurred in 70% of patients. Survival of implants with bone growth onto the collar was higher than implants with no bone ingrowth. A further study in a patient matched series demonstrated that osteointegration of hydroxyapatite (HA) collars reduced the development of radiolucent lines around the cemented stem (14). McDonald 2013 (15) commented that the prime role of extra-cortical fixation was to prevent the migration of wear particles by sealing the interface and that could be achieved by soft tissue ingrowth alone.

Tanzer et al (12) reported that, although patient radiographs indicated bone ingrowth at the collar of porous-coated implants, histological analysis revealed no osteointegration with only fibrous tissue present in the porous coating. In the Coathup et al (13) study, histological analysis showed lamellar bone in direct contact with the hydroxyapatite coated grooves. In some cases, bony bridging may occur but osteointegration with the porous surface structure was not seen and in these cases aseptic loosening was increased. A more porous structure may allow greater bone ingrowth directly from the transected cortical surface and osteointegration may be improved(16).

Although block porous metal have been used to successfully treat metaphyseal defects around revision knee replacements (17) they have not been used to encourage ECBB. Selective laser sintering (SLS) is where a laser beam fuses thin layers of small metal alloy beads. In this study we have investigated the use of porous collars made by SLS to encourage ECBB. The HA plasma spray technique only coats the outer porous surface whereas, electrochemical deposition of HA is able to coat the entire internal and external surfaces of the porous alloy. We hypothesise that collars with a high porosity, manufactured using SLS and coated with an electrochemically deposited HA would have greater direct bone surface attachment (osteointegration) than current plasma sprayed HA coated solid collar.

Materials and Methods

Manufacture of collars

The control collar was a plasma sprayed HA coated grooved Ti6Al4V collar (G) (Figure 1a, 1d). The longitudinally and circumferentially arranged 1mm deep grooves were integral with the implant shaft and stem of the diaphyseal tibial replacement. This design is similar to one used clinically and has previously been shown to enhance fixation (13). In the larger pore (LP) SLS collars the pores were cuboidal, 1500μm across with struts 750μm across (Figure

1c, 1f). These collars were plasma sprayed with a 70μm thick commercial HA coating (Plasma Biotal, Tideswel, Derbyshire, UK). Porous Ti6Al4V cylinders were made by SLS (Eurocoating, Italy). The smaller pores (SP) were 700μm across, were approximately cuboid in shape, with struts 300μm thick (Figure 1b, 1e). There were proximal and distal collars on each implant joined with a halving joint using bolts inserted in the transverse plane. After insertion, the bolts were swaged over to prevent them from backing out. The porous cylinder was press fit onto the stem of the diaphyseal implant.

Electrochemical deposition of HA on porous collar

A supersaturated 0.13M calcium phosphate (CaP) solution was prepared by adding 30g of $Ca(H_2PO_4)_2$ (C8017; Sigma-Aldrich, UK) to 1L distilled H_20. The solution was stirred for 1h and filtered using Whatman 540 filter paper (GE Healthcare, UK). Porous collars were submerged in the CaP solution and attached to a DC dual power supply pack (Peak Tech, Telonic Instruments Ltd, UK) to act as the cathode with a platinum ring as the anode. A current of 175mA measured by a FLUKE 867B Graphical Multimeter (Fluke Corporation, USA), was passed through the actively stirred solution for 40mins. The initial brushite precipitate was converted to HA by soaking the collars in 0.1M NaOH solution for 72h, rinsed with distilled water and air dried(18). The coating on the collar was analysed using energy dispersive x-ray spectroscopy (EDAX UK, Leicester, UK) and x-ray diffraction (XRD, Bruker, Massachusetts, USA) techniques.

In vivo model

Five centimetres of the right tibial diaphysis was resected. The canal of the remaining proximal and distal tibia was filled with cement under pressure after which stems of the diaphyseal implant were pushed into place. Each diaphyseal implant consisted of a proximal and distal collar, both of which were of the same design. 15 diaphyseal implants were inserted 15 skeletally mature female sheep with 5 sheep per group (G, SP and LP). All

animals recovered and were allowed to weight bear on the operated leg immediately post-operatively. All procedures were carried out in accordance with the UK Animal Scientific Procedures Act. Diaphyseal implants were retrieved after 6 months for radiological and histological analysis.

Radiological analysis

Antero-posterior (AP) and medio-lateral (ML) radiographs were taken and the ECBB around each collar was quantified in both the AP and the ML planes. ECBB resulted in a pedicle of bone that was measured from the shoulder of the collar using 3 different parameters; maximum thickness (mm), maximum length (mm) and surface area (mm^2). Measurements were calibrated on AxioVision LE64 software (v4.9.1.0, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) using known diameters of the implant intramedullary stem prior to tissue analysis. ECBB seen on radiography did not confirm whether there was direct bone attachment with the implant. We analysed this using microscopy of histological thin sections.

Histomorphometry

Following retrieval, samples were stripped of excess soft tissue, fixed, dehydrated, de-fatted, infiltrated and finally embedded in resin (LR White Resin, London Resin Company Ltd, Reading, Berkshire, UK). A coronal longitudinal section was taken of each collar through its centre and thin sections were prepared (approximately 80µm in thickness). Thin sections were stained with Toluidine Blue and Paragon to identify soft tissue and bone. Stained slides were viewed under a light microscope (Axioskop, Carl Zeiss, Welwyn Garden City, UK) and images analysed using ImageJ (v1.49, National Institutes of Health, USA).

The total surface available for bone attachment for each collar was quantified by measuring the length (mm) of the collar surface in each thin section. Osteointegration was quantified by measuring the length (mm) of the collar surface with direct bone attachment. For porous collars this included the outer and inner porous surfaces. The proportion of the surface with

direct bone contact (%) was calculated for each collar using these measurements. The amount of bone and soft tissue ingrowth was measured by calculating the area (mm^2) of these within the pores. Measurements were taken by using a combination of thresholding and manually drawing techniques using ImageJ.

Statistics

Data was tested (SPSS Statistics v22, IBM, New York, USA) for normality using the Shapiro-Wilk test and as the data was non parametric the Mann-Whitney U test was used to compare groups where $p<0.05$ was considered significant.

Results

No complications were encountered throughout the experiment.

Radiological analysis of extracortical bone growth

At 6 months greater bone pedicle formation adjacent to the G collars was seen. During surgery, all attempts were made to stop the cement extruding out of the canal and over the collar during stem insertion, preventing the collar from abutting directly against the cortex and creating a gap. Occasionally in both SP and LP groups this cement issue was noted but in all cases bone was able to grow across the gap from the transected cortex and into the porous collar (Figure 2). A similar gap was seen with the G group, and yet bone still grew over the gap onto the collar.

Significantly greater ECBB was measured adjacent to the grooved design when compared to porous collars (Table 1).

	Grooved collar	**Small pore collar**	**Large pore collar**	**p-value[a]**
Max. length of pedicle (mm)	7.02 (4.73-10.72)	2.09 (0-7.84)	3.05 (0-6.64)	*0.002*
Max. thickness of	3.31 (2.46-4.07)	1.33 (0-2.43)	1.11 (0-2.51)	*<0.001*

pedicle (mm)				
Surface area of pedicle (mm^2)	12 (5.89-20.31)	2.86 (0-15.28)	2.03 (0-11.85)	*0.002*

Table 1. Results of measurements of ECBB around each collar type with medians (IQR) shown. [a]Kruskal-Wallis test

Histological appearance of bone ingrowth into the collars

Osteointegration was seen in the distal and proximal collars of all implants.

Bone ongrowth onto the grooved HA collar was associated with ECBB and formed directly onto the implant surface. Only the part of the area adjacent to the transection site was osteointegrated. The bone that formed on the collar at the transection site was mature lamellar bone.

In the porous collar groups bone invaded the porous structure from the transverse transected cortex (Figure 3). Within the pores, bone grew peripherally on the pore surface rather than developing centrally within the pore. Adjacent to the transection site, in some animals and in both porous groups, cement penetrated the pores. The greatest quantity of cement penetration was in LP collars (Figure 5, Table 2) however, in all cases the cement never entirely blocked bone ingrowth from the transected surface of the cortex into the porous collars. Bone was formed by intramembranous ossification with no evidence of endochondral bone formation. HA coating was evident only on the outer surface of the LP porous metal struts and its position was consistent with the plasma sprayed line of site coating. In the SP collars the HA coating, which was applied using electrochemical deposition had resorbed. EDAX analysis of the electrochemical calcium phosphate coating reveals a Ca:P of 1.53 with XRD confirming both crystal and amorphous phases to present in the coating (Figure 4).

Histomorphometric analysis of collar surface size and pore content

Total collar surface available for bone contact (median, IQR) for SP collars (224.9mm, 203.5-236) and LP collars (105.7mm, 82.9-133.5) was significantly greater when compared to the G collar group (33.3mm, 28.5-41.6). SP collars showed a significantly larger surface even when compared to LP (Figure 5).

Greatest integration (median, IQR) with the implant surface was seen in the porous groups, with SP (72.4mm, 34.7-114.3) and LP (42.9mm, 4.5-84.5) collars showing significantly greater bone attachment than the G (13.1mm, 5-17.8) group (Figure 5).

Statistically greater soft tissue ingrowth was found compared to bone in the pores of the SP. Significantly greater amounts of bone and soft tissue were found compared to cement in both porous collar designs. A significantly greater amount of cement penetration was found within LP compared to SP. Results are summarised in Table 2 and Figure 6.

	Small pore collar	**Large pore collar**	**p-value[a]**
Bone area(mm^2)	8.72 (3.63-12.3)	7.85 (0.71-19.49)	0.970
Soft tissue area (mm^2)	19.35 (15.91-22.84)	17.58 (4.83-28.54)	0.543
Cement area (mm^2)	0.26 (0-1.03)	1.37 (0.45-2.55)	*0.015*
p-value[b]	*<0.001*	*<0.001*	

Table 2. Median(IQR) histomorphometric measurements quantifying areas of bone, soft tissue and cement found within collar thin sections together with p-values.
[a]Mann-Whitney U test: comparing the amount of bone/soft tissue/cement between collars.
[b]Kruskal-Wallis test: comparing the amount of bone/soft tissue/cement content within each collar.

Discussion

ECBB has been used in prostheses that replace large segmental diaphyseal defects (8). Osteointegration of the surface has only ever been demonstrated where a HA plasma coated structure has been used (2). When bone had osteointegrated onto the shaft of the implant, survival at 10 years was increased by over 20% (13). ECBB has previously been investigated in animal models. Virolainen et al (19) investigated the effects of a

corticocancellous onlay graft on bone and soft-tissue formation and showed the mechanical strength of the reconstruction, the area of the callus and the contact between the bone and the prosthetic shoulder were greater when a graft was used. In a similar animal model to the one we have used here other studies have shown a significant increase in extracortical bone and osteointegration when the HA collar was sprayed with autologous mesenchymal stem cells (20).

In our study we used SLS porous collars and showed that these significantly increased osteointegration. Completely porous collars are osteointegrated using direct bone ingrowth into the porous structure from the transection site with minimal ECBB. Massive segmental solid implants in clinical use today that have surface ongrowth areas or grooved surfaces only allow integration whereby ECBB must occur in order for bone to access these regions.

ECBB reduces aseptic loosening possibly by sealing the bone implant interface, theoretically preventing wear debris and joint fluid from accessing the implant interface, decreasing the risk of osteolysis (5). Alternatively it has been shown that osteoclast activation from pressurised joint fluid causes bone resorption (21, 22) and the bone-implant interface may be in contact with pressurised joint fluid leading to early loosening. In a large segmental implant where osteointegration at the shoulder does not occur there is a membrane surrounding but not adherent to the collar that is in continuity with the synovium. Therefore greater osteointegration seen with porous collars in this study would result in a more robust seal. Another possibility is that ECBB and osteointegration diverts the load from the stem resulting in reduced stresses and mechanical failure.

Ti foams have recently been made by foaming and sintering, producing controlled interconnected pores and complex surface topography (23), however; with these materials it is difficult to change the pore size and to alter the geometry of the implant. Titanium alloy implants made by SLS allow for different mesh sizes that can be used in a single implant

where appropriate. In order to provide the appropriate strength, these may be joined to solid metal made in the same process. In our study we used a collar that had a cross-sectional shape similar to the resected bone shape and it is conceivable that customised implants matching the shape of the transected bone in humans could be easily and cost effectively made using this technology.

Several investigators have studied bone ingrowth into porous systems with different porosities (24, 25). Pore size on solid implants of 100–400 μm (26-31) for example produced using sintered beads, have been investigated. In our study we used pore sizes of 700 and 1500 μm and there was little difference in the amount of bone formation. Porous ingrowth collars situated in the femoral diaphyseal region are subject to different forces compared to porous backings on acetabular shells for example and therefore the collar design must reflect this in terms of porosity, strut thickness and length. The design of the porous collar structure must be stiff enough to withstand compression under weightbearing yet compliant enough to prevent stress shielding. These porous implants rely on cortical bone growth to penetrate deep within its structure and its design must encourage this to occur by avoiding stress shielding and allowing the development of a vascular system(32). Porous layers on the backs of acetabular shells are reliant on superficial penetration of bone tissue or in some instances surface attachment with no penetration of the cancellous bone.

In our study one of the porous implant surfaces was plasma sprayed with HA coating whilst the other had a biomimetic calcium phosphate coating that was electrodeposited according to Redepenning et al (18). After 6 months the plasma sprayed coated implant retained its coating which was restricted to the outer strut surfaces but the electrodeposited coating had been resorbed. This can be explained by the low crystillinity of the electrochemical coating compared to commercial plasma sprayed HA. Even with complete resorption of our electrochemical coating there was no apparent difference in bone formation between the porous collars at 6 months.

A cement layer at the shoulder of the implant may have been more of an issue for the porous collars than for the grooved collar, which relied on ECBB. This is because bone ingrowth for the porous collars originated from the transected cortical bone surface. A layer of cement between the implant and the collar in this position would impede bone ingrowth and a solution may be to use uncemented implant stems or to press fit the collar onto the implant once the cemented stem had been implanted.

In summary we have investigated two SLS collar types, both of which allow for osteointegration using ECBB. The SP design provides the greatest surface area and hence osteointegration when compared to the LP design and the solid grooved collar. We have shown that porous collars provide a viable alternative ingrowth region to the current solid grooved design as part of massive segmental prostheses

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FIGURE LEGENDS:

Fig.1 (top) Pictures of grooved collar (a) and SLS small pore (b) and large pore (c) collars with respective radiographic appearance (d, e and f).

Fig. 2 Lateral radiograph of cemented tibial diaphyseal implant in situ with proximal and distal SLS large pore collars.

Fig. 3 Microscope image showing interface between porous collar and transection site. x2.5 magnification. Toluidine blue and Paragon stain used. B; Bone (pink), ST; Soft tissue (purple), C; Collar (black). Scale bar shown.

Fig. 4 XRD spectra of electrochemically deposited calcium phosphate coating on Ti6Al4V discs identifying peaks for hydroxyapatite (HA) and titanium (Ti).

Fig. 5 Boxplot showing histomorphometric results for total surface of collar available for integration together with actual osteointegration for each of the collar types. Significant p-values of pairwise comparisons are $^{a}0.016$, $^{b}<0.001$, $^{c}<0.001$, $^{d}<0.001$.

Fig. 6 Boxplot showing histomorphometric results for inner pore contents of SLS collars. Significant p-values of pairwise comparisons are $^{a}0.003$, $^{b}<0.001$, $^{c}<0.001$, $^{d}0.015$.