Biomedical Materials

The chicken eggshell membrane: a versatile, sustainable, biological material for translational biomedical applications

To cite this article before publication: Rosemond A Mensah *et al* 2023 *Biomed. Mater.* in press https://doi.org/10.1088/1748-605X/acd316

The chicken eggshell membrane: a versatile, sustainable, biological material for translational biomedical applications

Rosemond A Mensah, Kawther Salim, Katarzyna Peszko, Seyta Diop, Ting HR Wong, David YS Chau*

Division of Biomaterials and Tissue Engineering, Eastman Dental Institute, UCL, Royal Free Hospital, Rowland Hill Street, London, NW3 2PF, UK

*Corresponding author: d.chau@ucl.ac.uk

Keywords: biomaterial, eggshell membrane wound healing , tissue engineering, drug delivery,

Abstract

Naturally derived materials are often preferred over synthetic materials for biomedical applications due to their innate biological characteristics, relative availability, sustainability, and agreement with conscientious end-users. The chicken eggshell membrane (ESM) is an abundant resource with a defined structural profile, chemical composition, and validated morphological and mechanical characteristics. These unique properties have not only allowed the ESM to be exploited within the food industry but has also led to it be considered for other novel translational applications such as tissue regeneration and replacement, wound healing and drug delivery. a However, challenges still exist in order to enhance the native ESM: the need to improve its mechanical properties, the ability to combine/join fragments of ESM together, and the addition or incorporation of drugs/growth factors to advance its therapeutic capacity. This review article provides a succinct background to the native ESM, its extraction, isolation, and consequent physical, mechanical and biological characterisation including possible approaches to enhancement. Moreover, it also highlights current applications of the ESM in regenerative medicine and hints at future novel applications in which this novel biomaterial could be exploited to beneficial use.

Introduction

The chicken eggshell membrane (ESM) is a natural biomaterial that has gained increasing attention in the biomedical field due to its unique properties, versatility, and sustainability (Figure 1). It is a thin film-like structure that lines the interior surface of the eggshell and separates the albumen from the shell (Wang *et al*, 2017; Mensah *et al*, 2021; Shi *et al*, 2021;

Torres *et al*, 2010; Chai *et al*., 2013; Park *et al*., 2016; Zurita-Méndez *et al*, 2022; Torres-Mansilla *et al*, 2023). The eggshell membrane is composed of a complex matrix of proteins, glycosaminoglycans, and minerals that confer it with remarkable biodegradability and biocompatibility (Park *et al*., 2016; Ahmed *et al*, 2019 Torres-Mansilla *et al*, 2023). The eggshell membrane has been used for various applications, such as food supplements, nutraceuticals, and cosmetics. However, recent studies have identified its potential for translational biomedical applications, such as wound healing, tissue engineering, drug delivery, and regenerative medicine (Scatena *et al*. 2007; Mensah *et al*, 2021; Mendoza, Chavez and Araya, 2022; Mohammadzadeh *et al* 2019). The eggshell membrane can be easily isolated from waste eggshells generated by the poultry industry, making it a cost-effective and sustainable source of biomaterials (Morooka *et al*. 2009; Vuong *et al*. 2018; Cree and Pliya 2019; Ahmed, Suso and Hincke 2019; Saha *et al*. 2021). The aim of this review is to provide an overview of the chicken ESM as a versatile, sustainable, and biological material for translational biomedical applications. The composition, physical and biological properties, extraction methods and various applications, recent advances and limitations of ESM in the biomedical field are discuss.

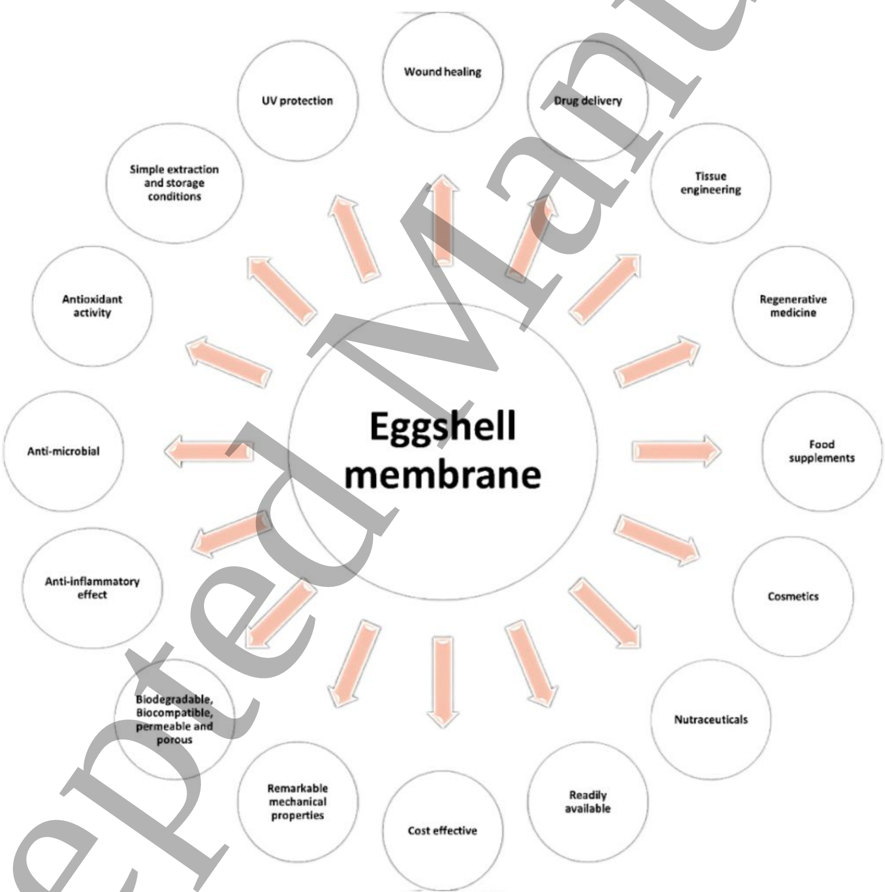

Figure 1: Chicken eggshell membrane properties as a unique biological material

Physical properties and components

The ESM lines the inner aspect of the eggshell and has a unique structure and biochemical composition consisting of a porous and fibrous mesh-like membrane composed of 3 layers: the outer membrane in contact with the eggshell; the inner membrane in contact with the albumen (egg white) and finally the limiting membrane (Yi *et al*, 2004; Park *et al.* 2016; Mensah *et al*, 2021; Torres-Mansilla *et al*, 2023) (Figure 2). The ESM is important in the eggshell mineralisation process by preventing the mineralisation of the albumin while inducing the mineralisation of the eggshell (Rose and Hincke 2009; Park *et al*, 2016; Han *et al*, 2023). Likewise, the ability of the ESM to prevent the internalisation of bacteria and thus its antibacterial properties are widely accepted (Ahlborn and Sheldon 2006). The presence of desmosine and isodesmosine crosslinks allows the ESM to be insoluble, allowing it to support the embryo during development (Torres-Mansilla *et al*, 2023).

The inner and outer layers of the ESM differ in their structural morphology and chemical composition. The outer membrane is mainly composed of type I collagen, meanwhile the inner membrane is predominantly composed of type V collagen in addition to type I collagen (Torres *et al*, 2010; Mensah *et al.*, 2021; Zurita-Méndez *et al*, 2022). Type X collagen is found in both the inner and outer membrane (Zurita-Méndez *et al*, 2022). In addition to the differences in the type of collagen present, the structural morphology also differs between the layers. The collagen fibres of the outer membrane closest to the eggshell is 1-7μm in diameter. Meanwhile, the collagen fibres of the inner membrane are smaller with a diameter between 0.1-3μm. Likewise, the general thickness of the three layers vary with the outer layer being the thickest (50-70μm) followed by the inner membrane with a thickness of 15- 30μm and finally the limiting membrane, the thinnest and is a non-fibrous layer interlaced within the inner membrane (Wang *et al*, 2017; Mensah *et al*, 2021; Shi *et al*, 2021). The collagen fibres of the inner membrane are more densely packed in comparison to the fibres of the outer membrane, which infiltrate the inner surface of the eggshell membrane (Chai *et al.*, 2013; Shi *et al*, 2022).

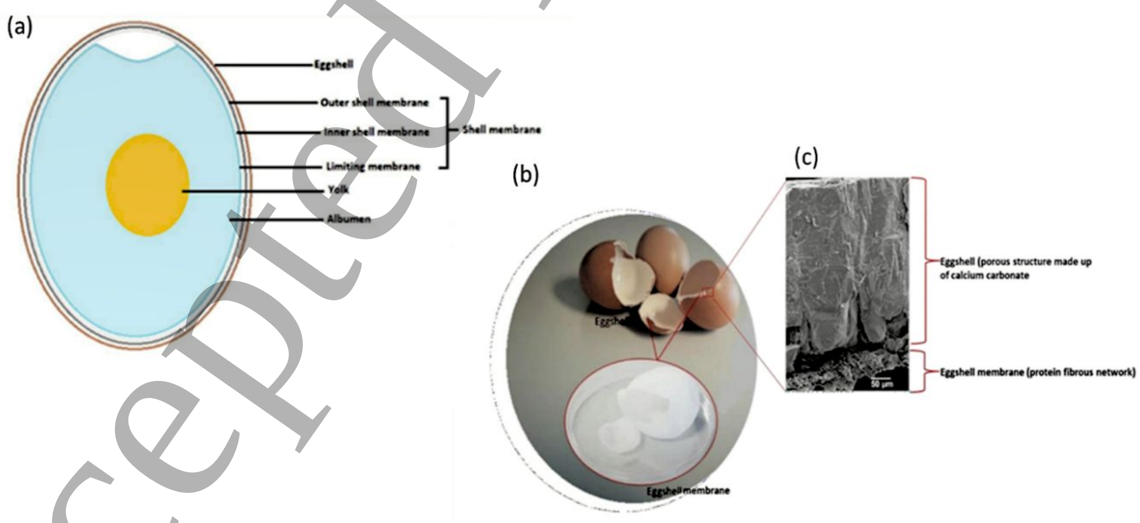

Figure 2: (a) Schematic diagram showing the anatomy of chicken egg; (b) a photograph of the eggshell membrane separated from the eggshell (c) cross-sectional of eggshell (Image adapted from Jonchere *et al.*, 2010; Mensah *et al.*, 2021)

Biological properties

It has been widely observed that the ESM is a protein-rich structure containing over 500 different proteins and acts as a natural source of collagen, fibronectin, proteoglycans, glycoproteins, hyaluronic acid and many amino acids such as arginine, glutamic acid, histidine, cystine, and proline which are found in high concentrations (Guru & Dash 2009; Ahmed *et al*, 2019; Mensah *et al*, 2021). In addition to the proteins and amino acids, $CaCO_3$ is also present in the ESM, this is due to the presence of a level of mineralisation in the outer membrane (Arias *et al*, 2020; Shi *et al*, 2021; Torres-Mansilla *et. al*., 2023). The close resemblance in structure between the ESM and extracellular matrix (ECM) and vast biological constituents allows the ESM to have tremendous usefulness in many applications in material science and tissue engineering. As mentioned previously, collagen is a key constituent of the ESM: however, collagen only makes up 10% of the 80-85% of the organic matrix the ESM contains (Kaweewong *et al.* 2013; Nakano *et al.* 2003; Shi *et al*, 2021; Han *et al*, 2023). Nonetheless, the collagen fibrils are a major morphological component of the ESM. It provides the ESM with the necessary structural support while also acting as a scaffold for biomineralization. The presence of collagen is exploited in tissue engineering and biomaterial formation.

The ESM provides a natural source of substances vital for tissue engineering and wound healing. For example, the ESM acts as a natural source of collagen but also glucosamineand hyaluronic acid which have important implications in biomaterial development and success. For instance, the collagen fibrils act as a 3D scaffold and can incorporate to form a new matrix which provides anchorage for new cells through cell surface adhesion meanwhile being biocompatible and biodegradable. Additionally, the type V collagen provides great tensile strength and structural support for the new matrix. The ESM also contains fibronectin which in addition to the arginine-glycine aspartic acid tripeptide glycoprotein motif facilitates cell adhesion. Meanwhile the fibronectin also facilitates cell growth, migration and repair which play a key role in wound healing and cell incorporation to ensure the success of the scaffold (Scatena *et al.* 2007; Mensah *et al*, 2021; Mendoza, Chavez and Araya, 2022). Furthermore, the presence of osteopontin in the ESM facilitates tissue repair and remodelling while also regulating cytokine release and macrophage recruitment (Scatena *et al.*, 2007; Han *et al*, 2023).

Historical outlook

Eggs and their products have been historically used for wound healing and beauty (Ohto-Fujita *et al.*, 2019). For example, the ESM has been historically used to cover wounds; meanwhile, the egg white has been used as an astringent to help wound closure (Forrest, 1982). More recently, eggshell powders are used as dietary calcium supplements (Bartter *et al.*, 2018). Eggshells and eggshell membranes (ESM) can be exploited for use rather than left to be destroyed, reigniting their historical applications in beauty and medicine (Ohto-Fujita *et al.*, 2019; Yoo, *et al.*, 2015). Likewise, in traditional Chinese medicine the ESM was historically used for healing burns, ulcers and tympanic perforations (Jia *et al.*, 2011). Meanwhile in Japanese cultures it continues to be used by sumo wrestlers for wound healing (Sah and Pramanik, 2014). In the early 2000s, researchers began to explore the potential of ESM in tissue engineering, where it could be used as a scaffold material to support the growth of new tissue (Mohammadzadeh *et al.,* 2019). One study published in 2023 demonstrated the ability of ESM to support the growth of bone cells *in vitro*, leading to the suggestion that it could be used as a bone graft material (Torres-Mansilla *et al.* 2023). Other studies have shown the potential of ESM for cartilage regeneration, nerve regeneration, and other tissue engineering applications (Ninov, Yun and Maximina Yun 2015; Kim 2020). More recently, researchers have explored the potential of ESM in drug delivery and as a platform for regenerative medicine (Chen *et al*, 2022). Studies have shown that ESM can be loaded with drugs and growth factors and used to deliver these agents to targeted tissue (Liu *et al*, 2017). In addition, ESM has been used as a platform for cell transplantation, where cells are seeded onto the membrane and transplanted into the body to regenerate damaged tissue (Vuong *et al*, 2018; Yan *et al*, 2020).

Extraction and Isolation

The ESM extraction methods (Table 1) can be divided into two major categories: (i) mechanical, and (ii) chemical. The mechanical approach requires manual removal of the eggshell by carefully peeling it from ESM with forceps Liu *et al.* 2019; Li *et al.* 2019a; Wan *et al.* 2022). However, the procedure is time-consuming and there is a risk of damage to the ESM or unintentional separation of its layers as the outer side is strongly embedded in the eggshell. To overcome this challenge, an alternative strategy has been proposed which is based on the dissolution of the $CaCO_3$-containing shell by placing the egg into highly acidic solution. The most common are acetic acid, hydrochloric acid and EDTA (Mensah *et al.* 2021; Sheish *et al.* 2021; Farjah *et al.* 2013). Mensah *et al.*, (2021) showed that the acid treatment resulted in thicker and more porous membranes compared to the manual stripping. Moreover, the membranes exhibited various wettability and swelling profiles depending on the separation method. This could be attributed to the effective preservation of the intact outer layer under the acidic treatment. Additionally, the treatment with acetic acid has been shown to improve biocompatibility of the membranes thanks to the introduction of carboxylic functional groups into the scaffold (Choi *et al.*, 2021). However, the efficiency of the chemical approach is strongly affected by the length of incubation, temperature, and the acid concentration (Santana *et al.*, 2016). In some studies, the two methods were combined, where the egg is first soaked in weakly concentrated acid solution to weaken the bonds between the ESM and the eggshell, which is then manually stripped (Sun *et al.*, 2022).

Nevertheless, the usability of natural ESM is limited by its poor solubility in aqueous conditions, which stems from the strong interactions between cystine, hydroxylysinonorleicine and desmosines present in the ESM fibres (Baker and Balch, 1962; Crombie *et al.*, 1981). Due to high concentration of disulphide bonds crosslinking the fibres, the manipulation of the shape and size of the membrane becomes a challenge. Therefore, a number of studies attempted to solubilize ESM and produce soluble eggshell membrane protein (SEP) in order to expand the potential of the material. Takahashi *et al.*, (1996) successfully obtained SEP by subjecting it to the performic acid oxidation and pepsin digestion, however, the yield was only 16-39%. Yi *et al.*, (2003) proposed a new method for SEP separation, which was based on incubation of ESM in 3-mercaptopropionic acid in the presence of 10% acetic acid. This strategy greatly improved the efficacy of isolation, increasing the yield up to 62%. However, it requires temperatures of at least 80 °C which could lead to protein denaturation. Nevertheless, it has remained as a prevailing method of SEP extraction (Yang *et al.*, 2015; Amirsadeghi, Khorram and Hashemi, 2021).

Table 1: The Advantages and limitations of different eggshell membrane extraction methods.

Extraction Method	Advantages	Limitations	References
Manual peeling	• Replication • of industrial setting • No chemical alterations	• Time-consuming • High risk of ESM damage and layer separation	Liu *et al.*, 2019 Wan *et al.*, 2022
Chemical dissolution (i.e., acetic acid, hydrochloric acid, EDTA)	• More porous and thicker ESM in comparison to manual peeling • Preservation • of outer layer • Enhanced biocompatibility	• Efficiency dependent on external factors: • Temperature • Incubation time • Acid concentration	Mensah *et al.*, 2021 Choi *et al.,* 2021 Santana *et al.*, 2016
Performic acid oxidation and pepsin digestion	• ESM Solubilization • Easier modification of scaffold's size and shape	• SEP yield at only 16-39%	Takahashi *et al.*, 1996 Yi *et al.*, 2003
SEP extraction in 3-mercaptopropionic acid and in 10% acetic acid	• Yield improved up to 62%	• Potential protein denaturation due to high temperature required (<80°C)	Amirsadeghi, Khorram and Hashemi, 2021 Yang *et al.*, 2015
Heat treatment (i.e., oven, microwave)	• Convenient and accessible	• Potential alterations to the biophysical properties of ESM	Hussain *et al.,* 2010
Machinery appliances	• Optimal for commercial scaling-up	• Pollution due to generated dust	Torres-Mansilla *et al*, 2023

		• Patented technologies	
Flash evaporation	• Simple and quick technique • Energy-efficient • Good yield of 69.2%	• Unknown ESM modifications	Chi *et al.,* 2022
Enzymatic reactions	• Stand-by methodology	• Efficiency dependent on external factors • Expensive	Torres-Mansilla *et al*, 2023

Application *Dermatology*

Millions are estimated to suffer from acute and chronic skin wounds yearly and these various wounds invariably bring aside the obvious health issues, potential emotional and financial implications to patients (Langemo and Brown, 2006; Shankaran, Brooks and Mostow, 2013). The centre of interest in intensive research on acute and chronic wounds is to find an effective treatment. The many proteins and peptides found in ESM make it an ideal candidate for wound healing. The application of chicken ESM for skin wound healing was first attempted by Maeda and Sasaki (1982). The initial study was conducted using rabbits. The results revealed that ESM was a suitable material for wound healing. Consequently, the ESM was applied as a skin graft in a patient and after seven days, the wound was well epithelialized. ESMs were further used in two cases, a 3-year-old female child with a severe burn on their foot and a 3-year-old female child with a scald burn on the elbow joint. In both cases, satisfactory epithelialisation was observed.

In wound management, the dressing must prevent bacterial infection, and stimulate angiogenesis and re-epithelialisation (Sivamani, Garcia and Rivkah Isseroff, 2007; Kim, 2018). In a study conducted by Li *et al.*, (2019), natural ESM was found to exhibit intrinsic antibacterial activity against both *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive). In order to enhance the bactericidal properties, the membranes were then immersed in a solution of silver nanoparticles, which were adsorbed onto their surface. The composites not only resulted in vastly superior antibacterial properties, but also demonstrated a sustained silver release over 4 days, which is important for a long-lasting protection. A similar profile was noticed with Briggs and colleagues who further adapted the ESM by modifying it with the thermoresponsive polymer, PNIPAAm (Briggs *et al.*, 2022).

In another study, a membrane consisting of polydopamine-modified ESM nano/microfibres with KR-12 antimicrobial peptide and HA was generated by Liu *et al.*, (2019). Accordingly, the *in vitro* biological results showed that the membrane had remarkable antibacterial activity and stopped the formation of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm on the membrane surface. In addition, the membrane increased the proliferation of keratinocytes and human umbilical vein endothelial cells and enhanced the secretion of vascular endothelial growth factor (VEGF). The *in vivo* animal model study revealed that the membrane is a suitable material for wound dressings.

In a quest to generate a cost-effective wound healing product with anti-inflammatory properties, processed ESM powder (PEP) has been explored in several studies (Morooka *et al.*, 2009; Vuong *et al.*, 2018; Cree and Pliya, 2019; Ahmed, Suso and Hincke, 2019; Saha *et al.*, 2021). Guarderas *et al.*, (2016) evaluated the effectiveness of chicken ESM dressing on wound healing. The findings suggested that ESM significantly improves cutaneous wound healing. Vuong *et al.*, (2018) studied the effect of PEP on matrix metalloproteinase (MMP) activities *in vitro* dermal fibroblast cell culture and *in vivo* mouse skin wound healing models. The PEP treatments in both models increased the activity of MMP and the regulation of early cellular functions during wound healing. (Ahmed, Suso and Hincke, 2019) conducted a study to evaluate PEP for advancement of skin wound healing. A mouse wound model was implemented to assess the impact of the PEP on wound healing. The histopathological assessment of the wound at days 3, 7 and 10 showed that the PEP significantly enhanced the wound closure. Additionally, the histological studies revealed that the granulation tissue in the PEP treated wounds, a bilayered skin substitute was constructed based on PEP-crosslinked gelatine-chitosan cryogel (Saha *et al.*, 2021). The dressing exhibited high swelling capacity and porosity as well as enhanced flexibility and biodegradability compared to cryogels traditionally crosslinked by toxic glutaraldehyde. Additionally, the *in vitro* studies revealed that PEP creates a better microenvironment for fibroblast attachment and proliferation, whereas the *in vivo* testing showed accelerated wound healing comparable to the commercially available dressing.

Beside wound healing, ESM has been found to possess anti-aging properties. Ohto-Fujita *et al.*, *(*2019) demonstrated that the application of solubilised ESM to the mice skin resulted in increased expression of genes encoding for type III collagen, decorin and MMP2, which resembles the microenvironment of young papillary dermal skin. Moreover, the level of type III collagen was elevated, resulting in higher skin elasticity. Therefore, the ESM might be useful in preventing skin aging and maintaining its healthy state.

Nerve, bone, and cartilage regeneration

Nerve damage represents a major challenge in healthcare due to its devastating impact on the quality of life and the lack of effective treatments. Therefore, in recent years a huge interest has been generated in neural tissue repair and the development of novel regenerative strategies (Schmidt and Leach, 2003; Ninov, Yun and Maximina Yun, 2015; Kim, 2020). Due to its biocompatibility and high content of bioactive components, ESM constitutes a promising substrate for nerve regeneration. Farjah *et al.*, (2013) developed a conduit made out of an ESM tube that would connect severed nerves and guide their regeneration. The construct was placed between proximal and distal ends of sciatic nerves in rats. The *in vivo* study revealed that ESM supported the regeneration of peripheral nerves. Moreover, further study revealed that ESM is capable of not only boosting nerve repair, but also encouraging the operational improvement in an injured sciatic nerve of a rat (Farjah, Naeimi and Saberi, 2016). It was noticed that on the

90th day post-operation the ESM group exhibited a greater number of regenerated myelinated axons compared to the autograft group. The regenerative capacity of native ESM can be enhanced by combining the therapy with lycopene or ibuprofen, which have been shown to further accelerate the functional recovery of sciatic nerves (Farjah, Mohammdzadeh and Javanmard, 2020; Raisi and Mohammadi, 2019).

In recent years, bone tissue engineering has generated a substantial curiosity among the research community and strong effort has been devoted to the development of a cost-effective bioactive organic/inorganic hybrid materials capable of regulation of bone formation (Yoshikawa *et al.*, 2002; Tohma *et al.*, 2012). Arias *et al.*, (2008) proved the effectiveness of ESM as a biodegradable regulator of bone regeneration, where X-type collagen has been implicated as the main contributor. In this study, dried ESM was interposed into the osteotomy site in the rabbit ulna. The histological and radiographic examination of the ulna after 4 weeks revealed an intact ESM and lack of bridging of the osteotomized bone ends. After 16 weeks the bone was only partially bridged compared to the complete loss of the fracture line in the control group. This research demonstrated a great potential of biodegradable ESM in preventing the premature closures of bone, which could replace such conventional procedure as the interposition of an autologous fat grafts that require second incisions.

In recent years, several studies attempted to improve the potential of ESM for bone tissue engineering by introducing changes to its nanotopography or by incorporating bioactive agents (Park *et al.*, 2021; Kim *et al.*, 2021; Wan *et al.*, 2022). In the study by Park and colleagues, an ESM-based nanopatterned scaffold for bone regeneration was developed (Park *et al.*, 2021). In this study, the disulphide bonds between ESM fibres were broken down by double dissolution and the obtained ESM solution was subjected to nanoimprint lithography to mimic the naturally occurring extracellular matrix surrounding osteoblasts. The *in vitro* studies revealed that the nanopatterned ESM resulted in high attachment and complete alignment of osteoblasts. Moreover, the scaffold promoted growth factor secretion such as VEGF, which is crucial for vascularization. Further *in vivo* studies showed that the nanopatterned ESM is capable of accelerating bone regeneration in 3-mm-diameter cranial bone defects in mice (Park *et al.*, 2021). In another study, ESM was used as a base for periosteum-mimicking biomaterial (Wan *et al.*, 2022). Cerium (III, IV) oxide-mineralised ESM was fabricated based on the biomimetic mineralization principle. The cerium (III, IV) oxide provided ESM with enhanced immunomodulatory and neuro-vascularization capabilities. Moreover, the construct successfully prevented the infiltration of soft tissue cells and enhanced osteogenesis *in vivo*.

Chen *et al.*, (2019) proved the use of a versatile biomimetic mineralisation procedure to generate ESM/hydroxyapatite composite with the ESM as the model. The findings showed that both sides of ESM proved exceptional biomimetic mineralisation ability, with the hydrophilicity and thermal stability of ESM being efficiently better by the insertion of HA. Furthermore, *in vitro* experiments on MC3T3-E1 cells showed that the inmost side of the ESM benefited cell proliferation and adhesion more than the outer side. Incredibly, the processes of proliferation, adhesion and multiplying, along with the alkaline phosphatase (ALP) activity and demonstration of bone-related genes and proteins (runt-related transcription factor 2, ALP, collagen type I, and osteocalcin) on both sides of the ESM composites showed a suggestively advanced as compared to those of the original ESM. These results indicated that ESM-HA composites attained employing biomimetic mineralisation potentially could be new materials for future bone tissue repair.

Likewise, ESM could be combined with chitosan and silk fibroin into a functional hydrogel that could act as an articular cartilage replacement (Adali, Kalkan and Karimizarandi, 2019). The hydrogel proved to be suitable to support attachment and promote proliferation of chondrocytes. They were also capable of strong antibacterial response towards gram-positive-positive bacteria. Alternatively, SEP can be incorporated into agarose gel to facilitate cartilage regeneration as proposed by (Been *et al.*, 2021). Agarose in itself does not promote cell adhesion, therefore, the addition of SEP resulted in drastically higher numbers of attached and proliferating chondrocytes. Additionally, the presence of ESM resulted in the downregulation of immune response towards the scaffold.

Furthermore, in Oral and maxillofacial surgery, periodontitis is a primary cause of tooth loss in adults, and it affects 5 to 15 % of people worldwide (Petersen 2003). Guided tissue regeneration (GTR) is a technique employed in the regeneration of damaged periodontal tissues (Gentile *et al*. 2011). This technique involves the use of a barrier membrane to eliminate epithelial cells from the damaged surface and repopulate with the periodontal ligament cells (Salonen and Persson 1990; Dupoirieux *et al*. 2001; Jia *et al*. 2012). Synthetic GTR membranes have been shown to have poor biocompatibility and inflammatory effect due to the acidic degradation products (AlGhamdi and Ciancio 2009).. In another study by Kalluri and Duan (2022), ESM was electrospun and blended with poly(ε-caprolactone) and bioceramic nano-hydroxyapatite to create a novel GTR membrane. The study was focused on optimisation of parameters that influence the mechanical properties using Taguchi orthogonal arrays. No biological examination was performed of the obtained composite.

Ophthalmology

In ophthalmology, ESM was first utilized by Coover in 1899 for four different eye injuries namely symblepharon, burns on eyeball, cornea ulcer and iritis (Coover 1899). Before then, the ESM was not used due to fear of infection. In that study, raw ESM obtained by manually peeling from the shell was applied in each case study. In the case of symblepharon, after 10 days, the eyeballs and lids of the patients were smooth with no adhesions. Similar results were observed in patients with burns on eyeballs. The use of ESM in patients with corneal ulcers experienced no pain or irritation during the treatment. The ulcers were suitably healed after two weeks. Finally, ESM was employed in iridectomy for recurrent iritis and resulted in an effective wound healing with no infection. Mensah *et al.*, (2021) further explored native ESM as a potential material for corneal wound healing. The study demonstrated that the raw ESM is capable of successfully supporting the attachment and proliferation of immortalised corneal epithelial cells and corneal mesenchymal stromal cells. Additionally, Choi *et al.*, (2020) proposed the use of ESM for retinal pigment epithelium (RPE) regeneration. In their study, ESM was incorporated into gellan gum hydrogel, which resulted in improved biocompatibility and biodegradability. Moreover, ESM acted as an anti-swelling agent which allowed the implant to retain its shape. The *in vitro* study with RPE cells extracted from coloured rabbits revealed that ESM enhanced cell proliferation and caused no adverse effect on cell viability. No further studies have reported on the use of ESM in ophthalmic surgery or other eye applications.

Neurosurgery

In neurosurgical operations, it is important to protect the brain tissue from the hazardous effect of the metallic microsurgical instruments (Cokluk and Aydin, 2007; Spetzger *et al.*, 2011). The experimental study of Gokyar, Cokluk and Kuruoglu, (2017) evaluated the use of raw ESM as a therapeutic intervention for the protection of naked brain tissue. In their study, 13.3 % of the uncovered fresh cadaveric cow brains operated with ESM were minimally damaged as compared to 60 % of the brains without it. According to the findings, ESM has some promising effects as a material for brain tissue protection and essential in neurosurgery.

Otolaryngology

ESM has been shown as an effective patch for the treatment of moderate to large traumatic tympanic membrane perforation (TMP) in human (Jung *et al.*, 2017). TMP, a hole in ear drum is a condition that can be caused by infection or trauma (Afolabi *et al.*, 2009). In clinical practice, most TMPs have tendencies to heal on their own. Nonetheless, in large perforation, the spontaneous healing fails (Lou, Tang and Yang, 2011). Jung *et al.*, (2017) evaluated the effects of ESM patches on the healing time for TMP. Sterilized round disc ESM patches moisturised with saline were placed on the surface of perforation in patients. After 3 months, the healing time for patients with the ESM patches were significantly improved as compared to patients that received perforation edge approximation.

Cardiology

Cardiovascular diseases (CVD) have become the leading cause of death worldwide, resulting in more than 19 million death per year (Health Intelligence Team, 2022). Conventionally, the replacement options for malfunctioning blood vessels are either allografts or autografts, however, they are associated with drawbacks such us availability or high donor morbidity (Fazal *et al.*, 2021). Therefore, there is high urgency for the development of new artificial vascular grafts. The intrinsic properties of ESM such as high gas permeability and antibacterial activity make it an attractive biomaterial for investigation in the CVD context. In one study by Yan *et al.*, (2020) ESM was used as a material mimicking the vascular intima surface in order to encourage endothelial cell growth. The membrane was incorporated into thermoplastic polyurethane, which provided mechanical support. The constructed vascular graft successfully promoted endothelial cell growth and rapid endothelialisation. Moreover, the grafts that contained heparin also resulted in antithrombotic activity. Further *in vivo.* study revealed that heparin-conjugated ESM can be successfully used as an arterial patch in a rat aortic angioplasty model (Sun *et al.*, 2022).

Limitations

Bacterial contamination

Widely consumed across the world, eggs are one of the leading causes of food poisoning in the UK (Adak *et al*. 2005), as the warm (42 °C), moist, and nutrient-rich environment of the egg is particularly favourable to rapid bacterial growth. Bacterial contamination of the egg, particularly of Salmonella, is a serious concern within the food industry for its food safety implications. It is therefore well documented how contamination may occur and which pathogens are commonly the causative agents. Trans-shell contamination in the first 30 to 60 seconds of laying, whereby eggshells with a wet surface can be penetrated by bacteria, has been heavily researched and confirmed to be the most likely route of infection (Bering *et al*. 1999). There is currently no literature found, however, which measures bacterial penetration of membranes alone, as for food purposes, the shell and membranes are usually considered together. Although antibiotics are routinely used in egg production and chickens vaccinated against salmonella, this is only partially protective, and infection of the egg still commonly occurs.

The risk of cross-contamination *in vitro* or more worryingly *in vivo* from using the ESM is evident, as it may cause cell death or sepsis, respectively. However, Guarderas *et al*. (2016) reported that their protocol included placing the ESM in solutions of antibiotics eliminates this risk. This seemingly easy solution poses its own risks, primarily, it may contribute to growing antimicrobial resistance which decreases the ability to treat infections. An alternative would be to screen all eggs before the ESM is used for biomedical purposes, but of course, this confers an extra processing step and cost. It should be noted that bacterial contamination is possible during storage of the ESM. Figure 3 shows the bacterial colony found on the inner ESM stored in PBS at ambient temperature. This clearly indicates that contamination remains a concern even if the egg is screened and shows no presence of bacteria, these can later be introduced if the ESM is handled in a non-sterile way and stored incorrectly with consequences later during its use.

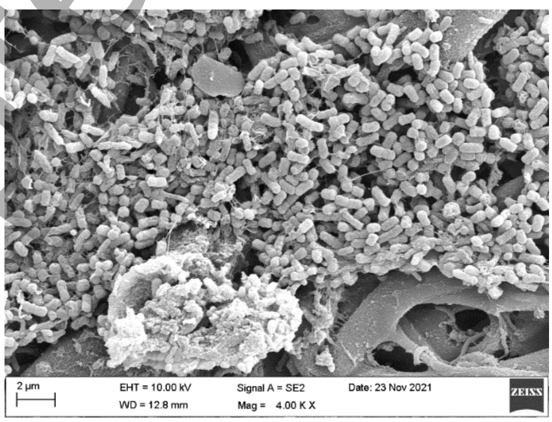

Figure 3: Bacterial colonisation of inner eggshell membrane

In summary, ESM has been found to possess antibacterial properties, particularly against Gram-negative bacteria such as Escherichia coli and Pseudomonas aeruginosa (Yoo *et al*., 2004). The antimicrobial activity of eggshell membrane is attributed to the presence of lysozyme, a naturally occurring enzyme that breaks down bacterial cell walls by hydrolysing the β-1,4-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine. It is important to note that the antibacterial properties of eggshell membranes do not provide complete protection against all types of bacteria and should not be relied upon as the sole means of preventing bacterial contamination. Proper handling and storage protocols, such as washing the eggshell before use and storing the eggshell membrane under sterile conditions, are necessary to minimize the risk of bacterial contamination. Additionally, using antibiotics to eliminate bacterial contamination in eggshell membranes may contribute to the growing problem of antimicrobial resistance, and should only be used when absolutely necessary.

Variation

Despite the use of the ESM for decades and in a wide variety of applications described herein, there remains a scarcity of biomechanical characterization in the literature, and any mention is almost always solely of the chicken ESM. This is further compounded by the high degree of heterogeneity within the ESM (Torres *et al*., 2010), as with many other naturally derived materials, which limits the reproducibility of results and the ability to draw significant conclusions. Torres, Trancos, and Montes (2013) demonstrated that this inhomogeneity resulted in a wide variability of results and made it difficult to accurately define its mechanical and biological properties or conduct further studies. For example, they found it challenging to precisely estimate the cross-sectional area of the ESM, which could explain the variability of ultimate tensile strength and pore volume. The heterogeneous nature of the ESM may also limit its applications as it may not behave in a consistent way each time it is used or depending on which part of the membrane is used. Without a standardized material, application *in vivo* and *in vitro* will remain limited to existing uses. However, crosslinking, or other tissue modifications, discussed within this review, are able to overcome this limitation to enhance the native properties of the ESM and generate uniformity in its properties.

Mechanical property

The ESM is fragile and mechanically weak, much like the amniotic membrane and other natural materials used for biomedical applications (Sari *et al*., 2020). This is an issue for wound healing purposes where a sufficiently strong material that can protect the underlying surface by maintaining a barrier between the wound and the outside environment is needed. Interestingly, Torres *et al*. (2010) reported the ESM to have a higher tensile strength when it is dry than when it's immersed in albumen or water. However, they also found that when dehydrated the structure of the fibrous network is lost so cannot be visualised making it challenging to ascribe the strength of the ESM to a particular structural. They showed that water acted as a plasticizer, interacting with the long-chain polymer molecules of the ESM and reducing the number of hydrogen bonds formed between them. This again is problematic if used as a wound dressing, any exudate that is produced will weaken the structural integrity of the ESM and leave it vulnerable to tearing and allowing infectious agents to access the healing wound, introducing the possibility for infection or development of a chronic wound (Mogoşanu and Grumezescu, 2014). Crosslinking of the ESM is a particularly useful avenue that should be further studied to identify the most efficient crosslinker and address the mechanical weakness to produce a more robust material.

The pore properties of the ESM are rarely investigated in literature despite the understanding that pore size is an important parameter in cellular migration, proliferation and nutrient diffusion on growth platforms (Han *et al*., 2021). Added to this, the little that is available, describes porosity in varying ways including as a percentage/ volume of the total material (Tsai *et al*., 2006; Mensah *et al*., 2021) or as an absolute pore size measurement (Hsieh *et al*., 2013). From what is known, the ESM is essentially nonporous on the inner membrane with macropores or voids within the outer side (Tsai *et al*., 2006) which is supported by images obtained by scanning electron microscopy (SEM) seen in Figure 4. Estimations by Torres *et al*. (2010) and Mensah *et al*. (2021) range from of 52.06% to 69.38% respectively depending on the method of extraction, whilst Hsieh *et al*. (2013) reported pore sizes of 3-10 μm. As different cell types have different preferences of pore size, fibroblasts for example prefer 5-10μm sizes, whilst osteoid and skin regeneration have optimal pores sizes at 20-125μm (Yang *et al*., 2001), depending on the application of the ESM, the native porosity can be an advantage or disadvantage for its function (Han *et al*., 2021). Fortunately, Hsieh *et al*. (2013) demonstrated that hydrogen peroxide is a useful tool in controlling pore size and was experimentally shown to reduce pore size to 1-5μm after treatment for 24h. Where necessary this could be used to achieve the desired porosity in the ESM.

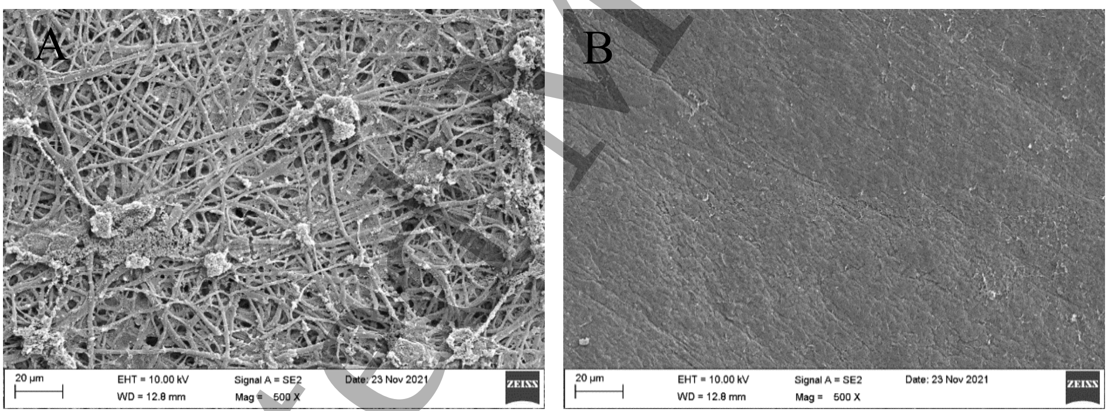

Figure 4: Scanning electronic microscopy of Eggshell membrane. (A) Outer eggshell membrane (B) Inner eggshell membrane

Modification of Eggshell membrane

Crosslinking has been established as a method to modify tissues, which can improve their mechanical and thermal stability and reduce degradation (Tolinski 2009). In the case of collagen or collagen-rich materials, various techniques are used, such as ultraviolet, physical treatment with heat, or chemical processes using 1-ethyl-3-carbodiimide hydrochloride. Caliari and Harley (2011) and Wang *et al*. (2015) suggest that crosslinking can reduce immunogenicity by masking antigenic markers. However, some literature suggests that crosslinking can impede intrinsic crosslinking and inhibit the breakdown of materials (Chapman 2007), and some crosslinking agents are cytotoxic or damage ECM components, such as glycosaminoglycans, which can affect the biocompatibility of treated materials (Hussein *et al*. 2017).

To assess whether the properties and applications of the ESM can be enhanced through crosslinking, analysis of the mechanical properties of the native and modified ESM is required. The tensile strength or toughness of an ECM or growth medium is a factor in cell adhesion, differentiation, and proliferation (Engler *et al*. 2006; Anderson, Owens and Naylor 2014). The ability to control the mechanical strength of the ESM through crosslinking could provide influence over cell fate and proliferation rate and could be used in biomedical and clinical applications where specific cell niches are targeted or studied. A modified ESM could also be used as a platform for drug testing, reducing the dependence on animal models (Grela *et al*. 2020).

Dynamic scanning calorimetry (DSC) is a useful technique to determine the thermal stability of the different crosslinked membranes at differential temperatures (Fessel *et al*. 2014). DSC can show the ESM's behaviour at body temperature for biomedical applications such as wound dressing, as well as the membrane's ability to maintain integrity during storage at freezing (0°C), refrigerated (2-4 °C), or ambient (23-25 °C) temperatures (WHO and FAO 2009). Water contact angle (WCA) is another experimental technique that can be used to assess the wettability or hydrophilicity of a material. Hydrophilic membranes are better suited to promoting cell growth and proliferation, but hydrophobicity can be useful for cell detachment and fabric durability, particularly in cancer studies (Ferrari, Cirisano and Morán, 2019). Any tissue modifications to the ESM must consider the impact on hydrophilicity and therefore on protein adsorption.

Nevertheless, there is currently not enough literature or evidence exploring the interplay between the properties discussed herein. There needs to be a greater understanding of how other factors such as surface roughness directly impact the hydrophilicity or toughness both before and following tissue modification. This knowledge will allow for fine tuning of the membrane's properties to suit a wide range of applications and produce the desired outcome. Furthermore, modification will address some of the weaknesses that will be discussed in this review and will increase the efficacy of the ESM for some of the applications described below. Preliminary studies were conducted to evaluate the effects of physical crosslinking by boiling and chemical methods using biologically derived and synthetic agents; genipin and glutaraldehyde respectively. Figure 5 shows the appearance of the membranes following modification.

Each side of the modified membranes were mechanically tested for their tensile strength under strain, changes in physical properties at differential temperatures and their level of hydrophilicity. Once these physical characteristics had been measured, biological characterisation could be done. Gingival cells were seeded on the modified ESMs (mESM) and native ESM (nESM) and incubated for 1, 3 and 6 days under normal physiological conditions.

Initial results suggested that the use of chemical crosslinking agents, namely glutaraldehyde and genipin does indeed enhance the mechanical strength of the ESM. Genipin specifically also modifies the ESM to enhance cell viability and reduce cytotoxicity, making it a suitable construct for supporting cellular adhesion and proliferation. These results conformed with previous evidence reported in literature by Hussein *et al.*, (2017).

Further to this, it should be explored if the same crosslinking agent would be equally suited to combining several ESM membranes to form a large matrix. This would require DMA, DSC experiments and biological assays, particularly looking at the joining sites to determine if these have adequate properties relative to the rest of the membranes. Such a material would be most applicable to the translational purposes of wound dressing and 3D skin modelling for *in vitro* testing as it would standardise the material and allow custom sizes to be obtainable from one continuous sheet of modified ESM.

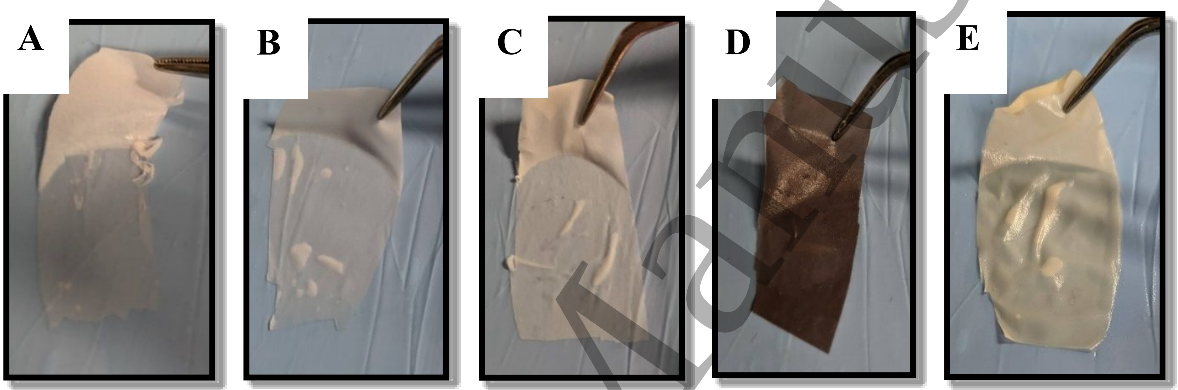

Figure 5: Images of the prepared ESM taken using a Google Pixel 3 camera phone. Non-crosslinked membranes: (a) control, manually peeled. (b) Fresh vinegar soaking extraction. Crosslinked membranes: (c) boiled ESM, (d) Genipin treated and (e) glutaraldehyde crosslinked

6. Conclusion

Historically, egg-derived components such as the egg white, eggshell and ESM have been commonly associated with wound healing and beautification strategies in Asian cultures, and suggest a pre-disposed acceptance of the scientific and cultural validation of the material. In addition, the current drive and promotion towards sustainability, ethical resourcing, and anti-animal testing movements have further raised awareness and popularity of alternatives to the current norm in the field of biomedical, clinical therapeutic and drug development pathways. An additional advantage of using this material stems from its encouragement of “green technology”- the conversion of a low-cost waste material to a product of significantly higher value. The ESM has shown to possess unique characteristics such as high biocompatibility, antimicrobial activity, appropriate mechanical and physical properties as well as additional parameters such as transparency, hydrophilicity/hydrophobicity, and porosity. In addition, the innate structure and composition of the ESM lends itself to additional enhancement which a number of examples have been described (e.g. crosslinking) and could result in a significantly more use in a variety of applications. To this end, the ESM has demonstrated a “pedigree” of usefulness- even in its native form- and shows promising characteristics which may be further exploited for not only biomedical applications but other areas of interest such as sustainable packaging, filtration systems, and horticultural platforms.

Funding

This work was supported by grants from the Rosetrees Trust [Seedcorn Award] and the Stoneygate Trust.

Conflict of interests

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank the technical/professional team members of the BTE group at the Eastman Dental Institute for their support during the preparation of this manuscript, and David Green, Fresh-pak Chilled Foods Ltd., UK, for his useful comments.

REFERENCES

Adak, G. K., Meakins, S. M., Yip, H., Lopman, B. A., & O'Brien, S. J. (2005). Disease risks from foods, Engl&&Wales, 1996–2000. *Emerging infectious diseases*, *11*(3), 365.

Adali, T., Kalkan, R. & Karimizarandi, L. (2019) The chondrocyte cell proliferation of a chitosan/silk fibroin/eggshell membrane hydrogels. International journal of biological macromolecules. 124, 541–547. doi: 10.1016/J.IJBIOMAC.2018.11.226.

Afolabi, O.A., Aremu, S.K., Alabi, B.S. & Segun-Busari, S. (2009) Traumatic tympanic membrane perforation: an aetiological profile. BMC research notes. 2. doi:10.1186/1756-0500-2-232.

Ahlborn, G. & Sheldon, B., (2006). Identifying the Components in Eggshell Membrane Responsible for Reducing the Heat Resistance of Bacterial Pathogens. Journal of Food Protection, 69(4), pp.729-738.

Ahlborn, G. J., Clare, D. A., Sheldon, B. W., & Kelly, R. W. (2006). Identification of eggshell membrane proteins and purification of ovotransferrin and β-NAGase from hen egg white. The Protein Journal, 25, 71-81.

Ahmed, T.A.E., Suso, H.P. & Hincke, M.T. (2019) Experimental datasets on processed eggshell membrane powder for wound healing. Data in brief. 26. doi: 10.1016/J.DIB.2019.104457.

Ahmed, T.A.E., Suso, H.P., Maqbool, A. & Hincke, M.T. (2019) Processed eggshell membrane powder: Bioinspiration for an innovative wound healing product. Materials Science &Engineering: C. 95, 192–203. doi: 10.1016/J.MSEC.2018.10.054.

AlGhamdi, A. & Ciancio, S., (2009). Guided tissue regeneration membranes for periodontal regeneration - a literature review. Journal of the International Academy of Periodontology, 11(3), pp.226-231.

Amirsadeghi, A., Khorram, M. & Hashemi, S.S. (2021) Preparation of multilayer electrospun nanofibrous scaffolds containing soluble eggshell membrane as potential dermal substitute.

Journal of biomedical materials research. Part A. 109 (10), 1812–1827. doi:10.1002/JBM.A.37174.

Anderson, L. R., Owens, T. W. & Naylor, M. J. (2014) 'Integrins in development &cancer', Biophysical Reviews, 6(2), pp. 191–202. doi: 10.1007/s12551-013-0123-1.

Arias, J. L., Fink, D. J., Xiao, S. Q., Heuer, A. H., & Caplan, A. I. (1993). Biomineralization and eggshells: cell-mediated acellular compartments of mineralized extracellular matrix. International review of cytology, 145, 217-250.

Arias, J. L., Nakamura, O., Fernandez, M. S., Wu, J. J., Knigge, P., Eyre, D. R., & Caplan, A. I. (1997). Role of type X collagen on experimental mineralization of eggshell membranes. Connective tissue research, 36(1), 21-33. Jia, J., Liu, G., Guo, Z.X., Yu, J. &Duan, Y. (2012) Preparation &characterization of soluble eggshell membrane protein/PLGA electrospun nanofibers for guided tissue regeneration membrane. Journal of Nanomaterials. 2012. doi:10.1155/2012/282736.

Arias, J.I., Gonzalez, A., Fernandez, M.S., Gonzalez, C., Saez, D. & Arias, J.L. (2008) Eggshell membrane as a biodegradable bone regeneration inhibitor. Journal of Tissue Engineering &Regenerative Medicine. 2 (4), 228–235. doi:10.1002/TERM.87.

Baker, J.R. & Balch, D.A. (1962) A study of the organic material of hen's-egg shell. Biochemical Journal. 82 (2), 352. doi:10.1042/BJ0820352.

Baláž, M., (2014). Eggshell membrane biomaterial as a platform for applications in materials science. Acta Biomaterialia, 10(9), pp.3827-3843.

Been, S., Choi, J., Cho, H., Jeon, G., Song, J.E., Bucciarelli, A. & Khang, G. (2021) Preparation &characterization of a soluble eggshell membrane/agarose composite scaffold with possible applications in cartilage regeneration. Journal of Tissue Engineering &Regenerative Medicine. 15 (4), 375–387. doi:10.1002/TERM.3178.

Bellairs, R. & Boyde, A., 1969. Scanning electron microscopy of the shell membranes of the hen's egg. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, 96(2), pp.237-249.

Berrang, M. E. *et al*. (1999) 'Bacterial Penetration of the Eggshell &Shell Membranes of the Chicken Hatching Egg: A Review', Journal of Applied Poultry Research, 8(4), pp. 499–504. doi: 10.1093/japr/8.4.499.

Boonprasert, P. & Tangboriboon, N., 2018. Electromechanical-Conductive Natural Rubber Doped Eggshell &Eggshell Membrane for Drug Delivery &Actuator Applications. Materials Science Forum, 934, pp.43-49.

Briggs E., Mensah R.A., Patel K.D., Mandakhbayar NE, Sharifulden NS, Erdogan ZK, Silva LVB, Salim K, Kim HW, Nguyen LTB & Chau DYS. Therapeutic Application of an Ag-Nanoparticle-PNIPAAm-Modified Eggshell Membrane Construct for Dermal Regeneration &Reconstruction. Pharmaceutics. Oct 11;14(10):2162. doi: 10.3390/pharmaceutics14102162. PMID: 36297596; PMCID: PMC9607136.

Caliari, S. R. & Harley, B. A. C. (2011) 'Collagen–GAG Materials', in Comprehensive Biomaterials. Elsevier, pp. 279–302. doi: 10.1016/B978-0-08-055294-1.00075-1.

Chai, Z., Li, Y., Liu, F., Du, B., Jiao, T., Zhang, C., & Leng, X. (2013). Outer eggshell membrane as delivery vehicle for polysaccharide/protein microcapsules incorporated with vitamin E. Journal of agricultural and food chemistry, 61(3), 589-595.

Chapman, R. A. (2007) 'Chemical bonding', in Handbook of Nonwovens. Elsevier, pp. 330–367. doi: 10.1533/9781845691998.330.

Chen, J., Zuo, K., Li, Y., Huang, X., Hu, J., Yang, Y., ... & Li, Q. (2022). Eggshell membrane derived nitrogen rich porous carbon for selective electrosorption of nitrate from water. Water Research, 216, 118351.

Chen, L., Kang, J. & Sukigara, S., (2014). Preparation &characterization of polyurethane/soluble eggshell membrane nanofibers. Bio-Medical Materials &Engineering, 24(6), pp.1979-1989.

Chen, X., Zhu, L., Wen, W., Lu, L., Luo, B., & Zhou, C. (2019). Biomimetic mineralisation of eggshell membrane featuring natural nanofiber network structure for improving its osteogenic activity. Colloids and Surfaces B: Biointerfaces, 179, 299-308..

Chen, X., Chen, Y., Fu, B., Li, K., Huang, D., Zheng, C., Liu, M. & Yang, D.P. (2022) Eggshell membrane-mimicking multifunctional nanofiber for in-situ skin wound healing. International Journal of Biological Macromolecules. 210, 139–151. doi: 10.1016/J.IJBIOMAC.2022.04.212.

Chen, Y., Feng, Y., Deveaux, J., Masoud, M., Chandra, F., Chen, H., Zhang, D. & Feng, L., (2019). Biomineralization Forming Process &Bio-inspired Nanomaterials for Biomedical Application: A Review. Minerals, 9(2), p.68.

Chi, Y. & Zhao, Y., (2009). Characterization of Collagen from Eggshell Membrane. Biotechnology (Faisalabad), 8(2), pp.254-258.

Choi, H.J., Kim, Y.M., Suh, J.Y. & Han, J.Y. (2021) Beneficial effect on rapid skin wound healing through carboxylic acid-treated chicken eggshell membrane. Materials science &engineering. C, Materials for biological applications. 128. doi: 10.1016/J.MSEC.2021.112350.

Choi, J., Lee, J., Shin, M.E., Been, S., Lee, D.H. & Khang, G. (2020) Eggshell Membrane/Gellan Gum Composite Hydrogels with Increased Degradability, Biocompatibility, &Anti-Swelling Properties for Effective Regeneration of Retinal Pigment Epithelium. Polymers. 12 (2941). doi:10.3390/polym12122941.

Cokluk, C. & Aydin, K., 2007. Maintaining Microneurosurgical Ability via Staying Active in Microneurosurgery. min - Minimally Invasive Neurosurgery, 50(6), pp.324-327.

Cokluk, C. &Aydin, K. (2007) Maintaining microneurosurgical ability via staying active in microneurosurgery. Minimally Invasive Neurosurgery. 50 (6), 324–327. doi:10.1055/S-2007-993159/ID/12.

Coover, D., 1899. The use of egg membrane in ophthalmic surgery. The ophthalmic record, 8, pp.222-224.

Cree, D. & Pliya, P. (2019) Effect of elevated temperature on eggshell, eggshell powder &eggshell powder mortars for masonry applications. Journal of Building Engineering. 26, 100852. doi: 10.1016/J.JOBE.2019.100852.

Crombie, G., Snider, R., Faris, B. & Franzblau, C. (1981) Lysine-derived cross-links in the eggshell membrane. Biochimica et Biophysica Acta (BBA) - Biomembranes. 640 (1), 365–367. doi:10.1016/0005-2736(81)90560-5.

Dupoirieux, L., Pourquier, D., Picot, M.C. & Neves, M. (2001) Comparative study of three different membranes for guided bone regeneration of rat cranial defects. International Journal of Oral &Maxillofacial Surgery. 30 (1), 58–62. doi:10.1054/IJOM.2000.0011.

Engler, A. J., & Sen, S. (2006). Sweeney hl, Discher DE. Matrix elasticity directs stem cell lineage specification. cell, 126, 677-89.

Farjah, G., Naeimi, M. & Saberi, A., 2016. Comparison Outcome of Nerve Regeneration across an Eggshell Membrane Guidance Channel &Autograft. Caspian Journal of Neurological Sciences, 2(4), pp.1-8.

Farjah, G.H., Heshmatian, B., Karimipour, M. & Saberi, A. (2013) Using Eggshell Membrane as Nerve Guide Channels in Peripheral Nerve Regeneration. Iranian Journal of Basic Medical Sciences. 16 (8), 901. /pmc/articles/PMC3786101/.

Farjah, G.H., Mohammdzadeh, S. & Javanmard, M.Z. (2020a) The effect of lycopene in eggshell membrane guidance channel on sciatic nerve regeneration in rats. Iranian Journal of Basic Medical Sciences. 23 (4), 527–533. doi:10.22038/IJBMS.2020.40228.9525.

Farjah, G.-H., Naeimi, M.-S. & Saberi, A. (2016) Comparison Outcome of Nerve Regeneration across an Eggshell Membrane Guidance Channel &Autograft. Caspian Journal of Neurological Sciences. 2 (1), 1–8. doi: 10.18869/ACADPUB.CJNS.2.4.1.

Fazal, F., Raghav, S., Callanan, A., Koutsos, V. & Radacsi, N. (2021) Recent advancements in the bioprinting of vascular grafts. Biofabrication. 13 (3). doi:10.1088/1758-5090/AC0963.

Ferrari, M., Cirisano, F. &Morán, M. C. (2019) 'Mammalian Cell Behavior on Hydrophobic Substrates: Influence of Surface Properties', Colloids &Interfaces, 3(2), p. 48. doi: 10.3390/colloids3020048.

Fessel, G., Cadby, J., Wunderli, S., van Weeren, R., & Snedeker, J. G. (2014). Dose-and time-dependent effects of genipin crosslinking on cell viability and tissue mechanics–toward clinical application for tendon repair. Acta Biomaterialia, 10(5), 1897-1906.

Gentile, P., Chiono, V., Tonda-Turo, C., Ferreira, A.M. & Ciardelli, G. (2011) Polymeric membranes for guided bone regeneration. Biotechnology journal. 6 (10), 1187–1197. doi:10.1002/BIOT.201100294.

Gokyar, A., Cokluk, C. & Kuruoglu, E. (2017) Using Eggshell Membrane in the Fresh Cadaveric Cow Brain for Brain Protection. Insights in Neurosurgery. 1 (3:23).

Grela, E. R., Knaga, S., Winiarska-Mieczan, A., & Zięba, G. (2020). Effects of dietary alfalfa protein concentrate supplementation on performance, egg quality, and fatty acid composition of raw, freeze-dried, and hard-boiled eggs from Polbar laying hens. Poultry science, 99(4), 2256-2265.

Guarderas, F., Leavell, Y., Sengupta, T., Zhukova, M., & Megraw, T. L. (2016). Assessment of chicken-egg membrane as a dressing for wound healing. Advances in skin & wound care, 29(3), 131-134.

Guru, P. S., & Dash, S. (2014). Sorption on eggshell waste—a review on ultrastructure, biomineralization, and other applications. Advances in Colloid and Interface Science, 209, 49-67.

Han, G. P., Kim, J. H., Kim, J. M., & Kil, D. Y. (2023). Transcriptomic analysis of the liver in aged laying hens with different eggshell strength. Poultry Science, 102(1), 102217.

Han, Y. *et al*. (2021) 'Effect of Pore Size on Cell Behavior Using Melt Electrowritten Scaffolds', Frontiers in Bioengineering &Biotechnology, 9. doi: 10.3389/fbioe.2021.629270.

Health Intelligence Team, B. (2022) Global Heart &Circulatory Diseases Factsheet.

Hsieh, S., Chou, H. H., Hsieh, C. W., Wu, D. C., Kuo, C. H., & Lin, F. H. (2013). Hydrogen peroxide treatment of eggshell membrane to control porosity. Food chemistry, 141(3), 2117-2121.

Hussein, K. H., Park, K. M., Lee, Y. S., Woo, J. S., Kang, B. J., Choi, K. Y., ... & Woo, H. M. (2017). New insights into the pros and cons of cross-linking decellularized bioartificial organs. The International journal of artificial organs, 40(4), 136-141.

Jia, J., Guo, Z., Yu, J., & Duan, Y. (2011). A new candidate for guided tissue regeneration: biomimetic eggshell membrane. Journal of Medical Hypotheses and Ideas, 5, 20.

Jonchère V, Réhault-Godbert S, Hennequet-Antier C, Cabau C, Sibut V, Cogburn LA, Nys Y, Gautron J. Gene expression profiling to identify eggshell proteins involved in physical defense of the chicken egg. BMC Genomics. 2010 Jan 21;11:57.

Jung, J., Yun, H., Kim, T., Joo, J., Song, I., Rah, Y., Chang, J., Im, G. & Choi, J., 2017. Analysis of Effect of Eggshell Membrane Patching for Moderate-to-Large Traumatic Tympanic Membrane Perforation. Journal of Audiology &Otology, 21(1), pp.39-43.

Jung, J.Y., Yun, H.C., Kim, T.M., Joo, J.W., Song, I.S., Rah, Y.C., Chang, J., Im, G.J. & Choi, J. (2017) Analysis of Effect of Eggshell Membrane Patching for Moderate-to-Large Traumatic Tympanic Membrane Perforation. Journal of audiology &otology. 21 (1), 39–43. doi:10.7874/JAO.2017.21.1.39.

Kalluri, L. & Duan, Y. (2022) Parameter Screening &Optimization for a Polycaprolactone-Based GTR/GBR Membrane Using Taguchi Design. International journal of molecular sciences. 23 (15), 8149. doi:10.3390/IJMS23158149.

Karuppiah D, Palanisamy R, Ponnaiah A, Liu W, Huang C, Rengapillai S, & Marimuthu S (2020). Eggshell-Membrane-Derived Carbon Coated on Li2FeSiO4 Cathode Material for Li-Ion Batteries. Energies 13(4): 786. doi: 10.3390/en13040786

Kaweewong, K., Garnjanagoonchorn, W., Jirapakkul, W., & Roytrakul, S. (2013). Solubilization and identification of hen eggshell membrane proteins during different times of chicken embryo development using the proteomic approach. The Protein Journal, 32, 297-308.

Kim, C.J. (2020) Neural Repair &Tissue Regeneration. International neurourology journal. 24 (Suppl 1), S1–S2. doi: 10.5213/INJ.2020EDI.003.

Kim, D., Gwon, Y., Park, S., Kim, W., Yun, K. & Kim, J. (2021) Eggshell membrane as a bioactive agent in polymeric nanotopographic scaffolds for enhanced bone regeneration. Biotechnology &Bioengineering. 118 (5), 1862–1875. doi:10.1002/BIT.27702.

Kim, H. (2018) Wound Dressing Materials: The Essentials. Journal of Wound Management &Research. 14 (2), 141–142. doi:10.22467/JWMR.2018.00458.

Kim, S., Youn, W., Choi, I.S. & Park, J.H. (2019) Thickness-Tunable Eggshell Membrane Hydrolysate Nanocoating with Enhanced Cytocompatibility &Neurite Outgrowth. Langmuir. 35 (38), 12562–12568. doi: 10.1021/ACS.LANGMUIR.9B02055.

Langemo, D.K. & Brown, G. (2006) Skin fails too: acute, chronic, &end-stage skin failure. Advances in skin &wound care. 19 (4), 206–211. doi:10.1097/00129334-200605000-00014.

Leach Jr, R. M., Rucker, R. B., & Van Dyke, G. P. (1981). Egg shell membrane protein: a nonelastin desmosine/isodesmosine-containing protein. Archives of Biochemistry and Biophysics, 207(2), 353-359.

Li, X., Cai, Z., Ahn, D.U. & Huang, X. (2019a) Development of an antibacterial nanobiomaterial for wound-care based on the absorption of AgNPs on the eggshell membrane. Colloids &surfaces. B, Biointerfaces. 183. doi: 10.1016/J.COLSURFB.2019.110449.

Li, X., Ma, M., Ahn, D.U. & Huang, X. (2019b) Preparation &characterization of novel eggshell membrane-chitosan blend films for potential wound-care dressing: From waste to medicinal products. International journal of biological macromolecules. 123, 477–484. doi: 10.1016/J.IJBIOMAC.2018.10.215.

Liu, M., Liu, T., Zhang, X., Jian, Z., Xia, H., Yang, J., Hu, X., Xing, M., Luo, G. & Wu, J. (2019) Fabrication of KR-12 peptide-containing hyaluronic acid immobilized fibrous eggshell membrane effectively kills multi-drug-resistant bacteria, promotes angiogenesis &accelerates re-epithelialization. International Journal of Nanomedicine. 14, 3345. doi:10.2147/IJN.S199618.

Lou, Z.C., Tang, Y.M. & Yang, J. (2011) A prospective study evaluating spontaneous healing of aetiology, size &type-different groups of traumatic tympanic membrane perforation. Clinical Otolaryngology. 36 (5), 450–460. doi:10.1111/J.1749-4486.2011.02387. X.

Maeda, K. &Sasaki, Y. (1982) An experience of hen-egg membrane as a biological dressing. Burns. 8 (5), 313–316. doi:10.1016/0305-4179(82)90029-8.

Mensah, R. A., Jo, S. B., Kim, H., Park, S. M., Patel, K. D., Cho, K. J., ... & Chau, D. Y. (2021). The eggshell membrane: A potential biomaterial for corneal wound healing. Journal of biomaterials applications, 36(5), 912-929.

Mogoşanu, G. D. & Grumezescu, A. M. (2014) 'Natural &synthetic polymers for wounds &burns dressing', International Journal of Pharmaceutics, 463(2), pp. 127–136. doi: 10.1016/j.ijpharm.2013.12.015.

Mohammadzadeh, L., Mahkam, M., Barzegari, A., Karimi, A., Kafil, H.S., Salehi, R. & Rahbarghazi, R. (2021) Preparation, characterization, &antibacterial properties of hybrid nanofibrous scaffolds for cutaneous tissue engineering. Human Cell. 34 (6), 1682–1696. doi:10.1007/S13577-021-00588-Y/FIGURES/14.

Morooka, H., Nagaoka, C., Nishimura, D., Ito, H. & Morooka, H. (2009) Effects of processed fabric with eggshell membrane on human skin properties. Journal of Fiber Science &Technology. 65 (7), 184–190. doi:10.2115/FIBER.65.184.

Nakano, T., Ikawa, N. I., & Ozimek, L. (2003). Chemical composition of chicken eggshell and shell membranes. *Poultry Science*, *82*(3), 510-514.

Ninov, N., Yun, M.H. &Maximina Yun, C.H. (2015) Current advances in tissue repair &regeneration: the future is bright. Regeneration (Oxford, England). 2 (2), 84–91. doi:10.1002/REG2.30.

Nys, Y., Gautron, J., Garcia-Ruiz, J. M., & Hincke, M. T. (2004). Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. Comptes Rendus Palevol, 3(6-7), 549-562.

Ohto-Fujita, E., Shimizu, M., Sano, S., Kurimoto, M., Yamazawa, K., Atomi, T., Sakurai, T., Murakami, Y., Takami, T., Murakami, T., Yoshimura, K., Hasebe, Y. &Atomi, Y. (2019) Solubilized eggshell membrane supplies a type III collagen-rich elastic dermal papilla. 376, 123–135. doi:10.1007/s00441-018-2954-3.

Park, S., Choi, K. S., Lee, D., Kim, D., Lim, K. T., Lee, K. H., Seonwoo, H., & Kim, J. (2016). Eggshell membrane: Review and impact on engineering. Biosystems Engineering, 151, 446-463.

Park, S., Gwon, Y., Kim, W. & Kim, J. (2021) Rebirth of the Eggshell Membrane as a Bioactive Nanoscaffold for Tissue Engineering. ACS Biomaterials Science &Engineering. 7 (6), 2219–2224. doi:10.1021/ACSBIOMATERIALS.1C00552.

Park, S., Kim, T., Gwon, Y., Kim, S., Kim, D., Park, H.H., Lim, K.T., Jeong, H.E., Kim, K. &Kim, J. (2019) Graphene-Layered Eggshell Membrane as a Flexible &Functional Scaffold for Enhanced Proliferation &Differentiation of Stem Cells. ACS applied biomaterials. 2 (10), 4242–4248. doi:10.1021/ACSABM.9B00525.

Parodi, B., Russo, E., Gatti, P., Cafaggi, S. &Bignardi, G., (1999). Development &*In vitro* Evaluation of Buccoadhesive Tablets Using a New Model Substrate for Bioadhesion Measures: The Eggshell Membrane. Drug Development &Industrial Pharmacy, 25(3), pp.289-295.

Petersen, P.E. (2003) The World Oral Health Report 2003: continuous improvement of oral health in the 21st century--the approach of the WHO Global Oral Health Programme. Community dentistry &oral epidemiology. 31 Suppl 1 (SUPPL. 1), 3–24. doi:10.1046/J.. 2003.COM122.X.

Pierschbacher, M. D., & Ruoslahti, E. (1983). Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature, 309, 30-33.

Pillai, M.M., Akshaya, T.R., Elakkiya, V., Gopinathan, J., Sahanand, K.S., Rai, B.K.D., Bhattacharyya, A. &Selvakumar, R. (2015) Egg shell membrane – a potential natural scaffold for human meniscal tissue engineering: an *in vitro* study. RSC Advances. 5 (93), 76019–76025. doi:10.1039/C5RA09959E.

Pillai, M.M., Dandia, H., Checker, R., Rokade, S., Sharma, D. & Tayalia, P. (2022) Novel combination of bioactive agents in bilayered dermal patches provides superior wound healing. Nanomedicine: nanotechnology, biology, &medicine. 40. doi: 10.1016/J.NANO.2021.102495.

Raisi, A. & Mohammadi, R. (2019a) Effects of local administration of ibuprofen on sciatic nerve regeneration &reinnervation after eggshell membrane conduit repair in rat. Journal of neurosurgical sciences. 63 (5), 531–540. doi:10.23736/S0390-5616.16.03217-X.

Raja, R., (2007). Wound re-epithelialization: modulating kerationcyte migration in wound healing. Frontiers in Bioscience, 12(8-12), p.2849.

Robinson, D.S. & King N.R. Carbonic Anhydrase &Formation of the Hen's Egg Shell (1963), pp. 497-498

Rose, M. L., & Hincke, M. T. (2009). Protein constituents of the eggshell: Eggshell-specific matrix proteins. Cellular and Molecular Life Sciences, 66, 2707-2719.

Sah, M. K., & Pramanik, K. (2014). Soluble-eggshell-membrane-protein-modified porous silk fibroin scaffolds with enhanced cell adhesion and proliferation properties. Journal of Applied Polymer Science, 131, 40138.

Saha, R., Patkar, S., Maniar, D., Pillai, M.M. &Tayalia, P. (2021) A bilayered skin substitute developed using an eggshell membrane crosslinked gelatin–chitosan cryogel. Biomaterials Science. 9 (23), 7921–7933. doi:10.1039/D1BM01194D.

Salonen, J.I. & Persson, G.R. (1990) Migration of epithelial cells on materials used in guided tissue regeneration. Journal of Periodontal Research. 25 (4), 215–221. doi:10.1111/J.1600-0765. 1990.TB00907. X.

Santana, A., Melo, A., Tavares, T. & Ferreira, I.M.P.L.V.O. (2016) Biological activities of peptide concentrates obtained from hydrolysed eggshell membrane byproduct by optimisation with response surface methodology. Food &Function. 7 (11), 4597–4604. doi:10.1039/C6FO00954A.

Saratale.R, Ghodake.G, Shinde.S, Cho.S.-K., Saratale.G, Pugazhendhi. & A, Bharagava.R, (2018). Photocatalytic activity of CuO/Cu $(OH)_2$ nanostructures in the degradation of Reactive Green 19A &textile effluent, phytotoxicity studies &their biogenic properties (antibacterial &anticancer). Journal of Environmental Management., 223, pp. 1086-1097. doi: 10.1016/j.jenvman.2018.04.072

Sari, R., Larasati, G. S., Kuncorowati, N. G., & Syaify, A. (2020). Platelet-rich fibrin (PRF) membranes accelerate open wound healing better than amniotic membranes: A histological study on the proliferation phase. Wound Medicine, 31, 100190.

Scatena, M., Liaw, L., & Giachelli, C. M. (2007). Osteopontin: A multifunctional molecule regulating chronic inflammation and vascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology, 27, 2302-2309.

Schmidt, C.E. & Leach, J.B. (2003) Neural Tissue Engineering: Strategies for Repair &Regeneration. http://dx.doi.org/10.1146/annurev.bioeng.5.011303.120731. 5, 293–347. doi: 10.1146/ANNUREV.BIOENG.5.011303.120731.

Shankaran, V., Brooks, M. & Mostow, E. (2013) Advanced therapies for chronic wounds: NPWT, engineered skin, growth factors, extracellular matrices. Dermatologic therapy. 26 (3), 215–221. doi:10.1111/DTH.12050.

Sheish, S.G., Emadi, R., Ahmadian, M., Sadeghzade, S. & Tavangarian, F. (2021) Fabrication &Characterization of Polyvinylpyrrolidone-Eggshell Membrane-Reduced Graphene Oxide Nanofibers for Tissue Engineering Applications. doi:10.3390/polym13060913.

Shi, Y., Zhou, K., Li, D., Guyonnet, V., Hincke, M. T., & Mine, Y. (2021). Avian eggshell membrane as a novel biomaterial: a review. Foods, 10(9), 2178.

Sivamani, R.K., Garcia, M.S. & Rivkah Isseroff, R. (2007) Wound re-epithelialization: modulating keratinocyte migration in wound healing. Frontiers in bioscience: a journal &virtual library. 12 (8), 2849–2868. doi:10.2741/2277.

Spetzger, U., Von Schilling, A., Brombach, T. & Winkler, G. (2011) Training models for vascular microneurosurgery. Acta neurochirurgica. Supplement. 112 (112), 115–119. doi:10.1007/978-3-7091-0661-7_21.

Strnková, J., Nedomová, Š., Kumbár, V. & Trnka, J., (2016). Tensile Strength of the Eggshell Membranes. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 64(1), pp.159-164.

Sun, P., Yan, S., Zhang, L., Zhang, C., Wu, H., Wei, S., Xie, B., Wang, X. & Bai, H. (2022) Egg Shell Membrane as an Alternative Vascular Patch for Arterial Angioplasty. Frontiers in bioengineering &biotechnology. 10. doi:10.3389/FBIOE.2022.843590.

Takahashi, K., Shirai, K., Kitamura, M. &Hattori, M. (1996) Soluble Egg Shell Membrane Protein as a Regulating Material for Collagen Matrix Reconstruction. Bioscience, Biotechnology, &Biochemistry. 60 (8), 1299–1302. doi:10.1271/BBB.60.1299.

Tang, J., Han, L., Yu, Y., Kang, J. & Zhang, Y., (2011). A label-free immunoassay using eggshell membrane as matrix & poly(diallyldimethylammonium chloride) as light-scattering enhancer. Luminescence, 26(6), pp.634-639.

Tohma, Y., Dohi, Y., Ohgushi, H., Tadokoro, M., Akahane, M. & Tanaka, Y. (2012) Osteogenic activity of bone marrow-derived mesenchymal stem cells (BMSCs) seeded on irradiated allogenic bone. Journal of Tissue Engineering &Regenerative Medicine. 6 (2), 96–102. doi:10.1002/TERM.401.

Tolinski, M. (2009) 'Crosslinking', in Additives for Polyolefins. Elsevier, pp. 215–220. doi: 10.1016/B978-0-8155-2051-1.00015-7.

Torres, F. G., Troncoso, O. P. & Montes, M. R. (2013) 'The effect of temperature on the mechanical properties of a protein-based biopolymer network', Journal of Thermal Analysis &Calorimetry, 111(3), pp. 1921–1925. doi: 10.1007/s10973-012-2915-0.

Torres, F. G., Troncoso, O. P., Piaggio, F., & Hijar, A. (2010). Structure–property relationships of a biopolymer network: The eggshell membrane. Acta biomaterialia, 6(9), 3687-3693.

Torres-Mansilla, A., Hincke, M., Voltes, A., López-Ruiz, E., Baldión, P. A., Marchal, J. A., ... & Gómez-Morales, J. (2023). Eggshell Membrane as a Biomaterial for Bone Regeneration. Polymers, 15(6), 1342.

Tsai, W. T., Yang, J. M., Lai, C. W., Cheng, Y. H., Lin, C. C., & Yeh, C. W. (2006). Characterization and adsorption properties of eggshells and eggshell membrane. Bioresource technology, 97(3), 488-493.

Vuong, T., Rønning, S., Ahmed, T., Brathagen, K., Høst, V., Hincke, M., Suso, H. &Pedersen, M., 2018. Processed eggshell membrane powder regulates cellular functions &increase MMP-activity important in early wound healing processes. PLOS ONE, 13(8), p.e0201975.

Vuong, T.T., Rønning, S.B., Ahmed, T.A.E., Brathagen, K., Høst, V., Hincke, M.T., Suso, H.P. & Pedersen, M.E. (2018) Processed eggshell membrane powder regulates cellular functions &increase MMP-activity important in early wound healing processes. PLoS ONE. 13 (8). doi: 10.1371/JOURNAL.PONE.0201975.

Wan, Q.Q., Jiao, K., Ma, Y.X., Gao, B., Mu, Z., Wang, Y.R., Wang, Y.H., Duan, L., Xu, K.H., Gu, J.T., Yan, J.F., Li, J., Shen, M.J., Tay, F.R. & Niu, L.N. (2022) Smart, Biomimetic Periosteum Created from the Cerium (III, IV) Oxide-Mineralized Eggshell Membrane. ACS applied materials &interfaces. 14 (12), 14103–14119. doi:10.1021/ACSAMI.2C02079.

Wang, X. *et al*. (2015) 'Procyanidins-crosslinked aortic elastin scaffolds with distinctive anti-calcification &biological properties', Acta Biomaterialia, 16, pp. 81–93. doi: 10.1016/j.actbio.2015.01.028.

Webb, B.C.W., Rafferty, S. & Vreugdenhil, A.J. (2022) Preparation &Characterization of Antibacterial Films with Eggshell-Membrane Biopolymers Incorporated with Chitosan &Plant Extracts. Polymers. 14 (3). doi:10.3390/POLYM14030383.

WHO & FAO (2009) Food Hygiene. Rome.

Wong Liong, J. W., Frank, J. F., & Bailey, S. (1997). Visualization of eggshell membranes and their interaction with Salmonella enteritidis using confocal scanning laser microscopy. Journal of food protection, 60(9), 1022-1028.

Wu, B., Zhang, G., Shuang, S. & Choi, M., (2004). Biosensors for determination of glucose with glucose oxidase immobilized on an eggshell membrane. Talanta, 64(2), pp.546-553.

Yan, S., Napiwocki, B., Xu, Y., Zhang, J., Zhang, X., Wang, X., Crone, W.C., Li, Q. & Turng, L.S. (2020) Wavy small-diameter vascular graft made of eggshell membrane & thermoplastic polyurethane. Materials Science &Engineering C. 107. doi: 10.1016/J.MSEC.2019.110311.

Yang, S., Leong, K. F., Du, Z., & Chua, C. K. (2001). The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue engineering, 7(6), 679-689.

Yang, T., Li, Y., Ma, M., Lin, Q., Sun, S., Zhang, B., Feng, X. & Liu, J. (2015) Protective effect of soluble eggshell membrane protein hydrolysate on cardiac ischemia/reperfusion injury. Food &Nutrition Research. 59. doi:10.3402/FNR.V59.28870.

Yang,Di, Qi,L. & Ma, L (2002) Eggshell membrane templating of hierarchically ordered macroporous networks composed of TiO2 tubes Adv. Mater., 14 , pp. 1543-1546

Yi, F., Yu, J., Guo, Z.X., Zhang, L.X. & Li, Q. (2003) Natural bioactive material: A preparation of soluble eggshell membrane protein. Macromolecular Bioscience. 3 (5), 234–237. doi:10.1002/MABI.200390030.

Yoshikawa, T., Nakajima, H., Uemura, T., Ichijima, K. & Takakura, Y. (2002) Osteogenic Activity of FK506-Treated Cultured Bone Tissue. Key Engineering Materials. 218–220, 391–394. doi: 10.4028/WWW.SCIENTIFIC.NET/KEM.218-220.391.

Yoshikawa, T., Nakajima, H., Uemura, T., Ichijima, K. & Takakura, Y., (2001). Osteogenic Activity of FK506-Treated Cultured Bone Tissue. Key Engineering Materials, 218-220, pp.391-394.

Zaman, H. U., Islam, J. M. M., Khan, M. A., & Khan, R. A. (2011). Physico-mechanical properties of wound dressing material and its biomedical application. Journal of the mechanical behavior of biomedical materials, 4(7), 1369-1375.

Zhang, Hongding, Sifei Wu, Zhenhua Xing, & Hai-Bo Wang. (2021). Turning Waste into Treasure: Chicken Eggshell Membrane Derived Fluorescent Carbon Nanodots for the Rapid &Sensitive Detection of Hg 2+ &Glutathione. The Analyst 146(23): 7250–56. doi: 10.1039/D1AN01582F

Zurita-Méndez, N. N., Flores-Merino, M. V., & Espinosa-Medina, M. A. (2022). Development of Bioactive Glass-Collagen-Hyaluronic Acid-Polycaprolactone Scaffolds for Tissue Engineering Applications. Frontiers in Bioengineering and Biotechnology, 10, 83.