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Composite Scaffolds for Orthopaedic Regenerative Medicine

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1. Introduction

The goal of tissue engineering is to synthesise substitutes that mimic the natural environment to help guide the growth of new functional biological tissue *in vitro* or *in vivo*. Tissue engineering relies heavily on the use of porous 3D scaffolds to provide a supportive environment for the regeneration of tissues and organs, acting primarily as a template for *de novo* tissue formation. However, new advances in fabrication technologies and composite materials are facilitating the rapid development of many novel composites that are beginning to play a more active role in directing the regenerative process. It has been long recognised that the combination of two or more characteristically-distinct materials can often yield composite materials that possess many of the constituent materials mechanical and biological advantages with few of their disadvantages. When applied to regenerative medicine, these new composite materials are beginning to show real potential as bioactive, biodegradable substitute materials, capable of facilitating rapid orthopaedic tissue regeneration, while degrading in parallel with the advancing tissue repair process. These idealised tissue regenerative aids could finally offer clinicians the potential to completely regenerate damaged orthopaedic tissue, leaving no evidence that the tissue was ever damaged in the first instance.

At a simplistic level, biological tissues consist of cells, signalling mechanisms and extracellular matrix (ECM). Tissue engineering technologies are based on this biological triad and consist of (i) the scaffold that holds the cells together to create the tissue's physical form, (ii) the cells that create the tissue, and (iii) the biological signalling mechanisms (such as growth factors or bioreactors) that direct the cells to express the desired tissue phenotype (Figure 1). In native tissues, cells are held within an ECM which guides development and directs regeneration of the tissue. The ECM serves to organise cells in space and provides them with environmental signals to direct cellular behaviour. Consequently, the ECM is responsible for two of the three components in this tissue engineering triad, highlighting the critical role that this extracellular environment plays on tissue formation. What is becoming increasingly evident is that the combination of biomaterials into novel composite scaffolds can result in an engineered biomimicry of this extracellular environment that provide all the environmental cues to promote rapid development of *de novo* tissue. Consequently, these composites can be designed to act not only as carriers and supporting structures for the associated cells but to play a more active role in the initiation and development of the repair tissue.

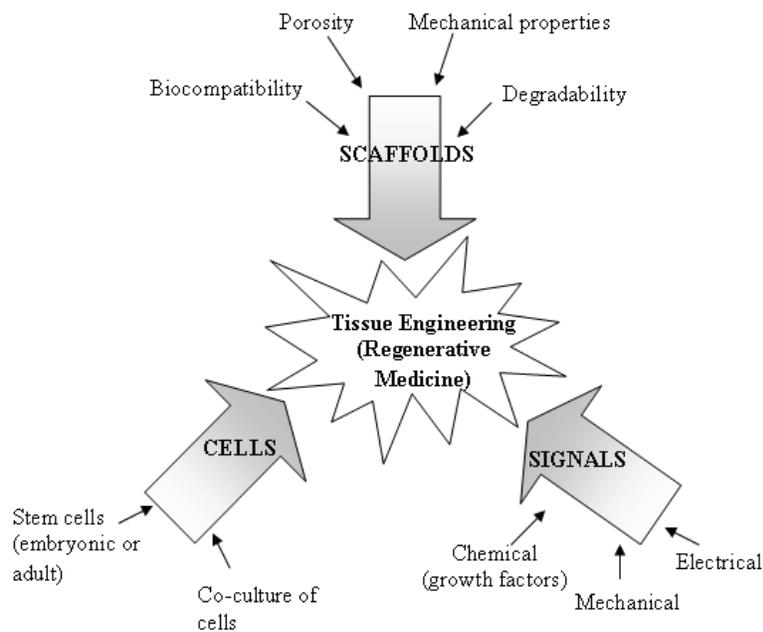


Fig. 1. The tissue engineering triad; factors that need to be considered when designing a suitable structure for tissue engineering applications

2. Scaffold requirements

Traditionally, scaffolds designed for tissue engineering attempted to meet a small number of common requirements that would allow them to be used safely for the *in vitro* production of engineered tissue or alternatively for *in vivo* implantation as regenerative aids. These included (i) providing an environment conducive to the facilitation of desirable cell-matrix interactions (e.g. cellular infiltration, attachment, proliferation and differentiation), (ii) support nutrient and waste product transport and encourage the movement of biochemical signals throughout the structure/matrix, facilitating long-term cellular survival and proliferation, (iii) biodegrade at an appropriate rate in parallel with the body's own natural healing process (i.e. supporting the healing phase while in tandem ensuring that the scaffold does not act as a barrier to the regenerative process, and (iv) to be bioinert and provoke a minimal inflammatory or immunological response as a result of its implantation or population with cells.

When designing or evaluating a scaffold for applications in tissue regeneration, there are a number of scaffold architectural, compositional and physical characteristics that have a deterministic influence on these four overlying tissue engineering scaffold requirements;

1. Biocompatibility

The word *biocompatibility* was mentioned for the first time in peer-review journals and meetings in 1970 by RJ Hegyeli and CA Homsy (Homsy et al., 1970). The definition of biocompatibility is "the ability of a material to perform with an appropriate host response in a specific application" and is a critical criterion for scaffold design. Not only must the scaffold material itself be biocompatible, but so too must its degradation products *in vivo*. It must not elicit toxic or injurious responses within biological systems once implanted or at any point throughout the degradation of the material *in vivo*. This characteristic alone rules out many synthetic or man-made materials as suitable components of tissue engineering matrices.

2. Biodegradability

Biodegradability refers to the ability of the physiological environment to breakdown or degrade an implanted material. Biodegradation is a critical characteristic of materials that are designed to regenerate tissue, as opposed to acting simply as inert substitutes for the native tissue. The overall goal of cutting edge regenerative tissue engineering therapies is to act as “smart” biomaterials by supporting the initial healing processes while also beginning to degrade in parallel with the advancing production of newly formed tissue matrix, ideally with no evidence of implantation once sufficient tissue regeneration has occurred, negating the need for subsequent clinical interventions to remove the implant from the body.

3. Bulk Mechanical Properties

The scaffold should provide an environment that is capable of surviving the implantation process and surgical manipulation required as part of the clinical procedure. It is widely believed that these materials must possess adequate mechanical integrity to survive the normal physiological loading environment at the site of implantation but this is currently an area of contention within the field. Traditional tissue engineering scaffolds prioritise a mechanically-competent scaffold, capable of supporting load bearing immediately upon implantation. Unfortunately, the characteristics of host tissue-like mechanical strength and the levels of porosity, permeability and pore interconnectivity, necessary for long-term scaffold in vitro and in vivo viability, are incompatible from a biomaterials perspective. Consequently current scaffolds are utilising cutting-edge advancements in composite biomaterials technology in an attempt to balance provision of bulk mechanical properties suitable for implantation and cellular support while retaining a material porosity high enough to encourage cell infiltration via diffusion throughout the scaffold.

4. Substrate Stiffness

Substrate mechanical properties of tissue engineering scaffolds plays a critical role in controlling and regulating a number of factors involved in directing cellular activity (Engler et al., 2006). Recent unpublished work from our laboratory has demonstrated that collagen-based scaffolds with a bulk stiffness of approximately 4 kPa exhibit increased cell attachment, proliferation and migration compared to less stiff scaffolds. Interestingly, recent studies have investigated the bulk and localised mechanical properties of highly porous scaffolds (Harley et al., 2007) and shown that the nature of high porosity structures means that their bulk mechanical properties are dramatically different to the mechanical properties of the individual struts within the open foam network. As a result, the substrate stiffness that a cell ‘feels’ while attached to one or multiple struts within the porous scaffold can be significantly higher than that predicted by bulk assessment of the material. Based on their study, it was estimated that the substrate stiffness experienced by a cell attached to a pore within a highly porous scaffold exhibiting a bulk stiffness of approximately 4 kPa would be of the order of approximately 50 to 100 MPa. Therefore, the effect of local substrate stiffness in a three-dimensional environment such as a porous tissue engineering scaffold is still an area that requires significant future investigation.

5. Pore Size and Pore Size Distribution

Pore size (Figure 2) is cell type specific (Murphy et al., 2010) and is arguably the most critical factor in the design of a tissue engineering scaffold optimised for a repair or regeneration of a specific tissue type. Pore size has a dramatic effect on cell seeding

efficiency within the scaffold (O'Brien et al., 2005) which can result in improved in vitro performance. If scaffold pore size is too small, cells are unable to rapidly infiltrate into the scaffold centre or homogeneously populate the matrix. Densification of cells around the matrix periphery occurs, acting as a barrier to further cell infiltration and leading to avascular necrosis within the scaffold centre (Phelps 2010, Ko 2007). Some investigators argue that having larger pores in the centre of an implant can help to support vascularisation of the implant when grafted onto a patient. It is believed that increased pore size with increasing depth is desirable (Mc Kegney et al., 2001). On the other hand, pores that are too big result in a significantly reduced specific surface area within the construct (Byrne et al., 2008).

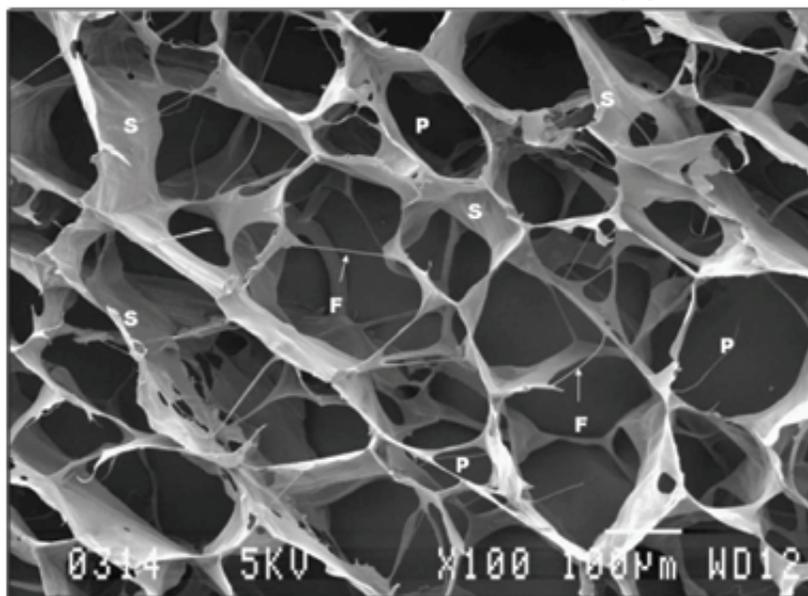


Fig. 2. SEM micrograph of a scaffold defining its pore structure. Wessels et al., 2008; S. Afr. j. sci. vol.104 no.11-12 Nov/Dec 2008

Cells interact with the pore substrate via ligands, chemical binding sites naturally associated with extracellular materials such as collagen. The availability of these ligands for promoting cell binding is directly related to the specific surface area, which is related to mean pore size. Consequently, pore size must be sufficiently large to provide the ideal ligand density to allow binding of a critical proportion of cells to occur. While the mean pore size is critical, the distribution of pore size range around this mean also plays an important role. Given that pore size is cell type specific, tissue engineering scaffolds with heterogeneous pore size distribution provide an environment with heterogeneous optimisation of the structure. Local ligand densities will differ substantially throughout the matrix, as will the ability of cells to infiltrate and attach throughout the construct. Therefore, development of a homogeneous structure with predictable cell adhesion, proliferation and differentiation characteristics requires a high degree of pore size homogeneity throughout and this is a characteristic offered by only a limited number of construct manufacturing processes. Figure 2 is an example of a porous structure of a collagen-based scaffold. An ideal scaffold is one which is open and has an interconnected pore network and a high degree of porosity (>90%), as it is easy for the scaffold to interact and integrate with the host (Freyman et al., 2001).

Clearly the optimisation of pore size is a critical design characteristic to allow optimisation, and consequently in vitro and in vivo performance, and the integration of cells within the developing extracellular matrix.

6. Porosity, Pore Interconnectivity and Permeability

Porosity is defined as the percentage of void space in a solid and its importance in tissue engineering scaffolds cannot be overstated. The degree of porosity in a scaffold with a given mean pore size will have a direct effect on the interconnectivity of the porous architecture and consequently the permeability/fluid mobility within the scaffold. These characteristics play an important role not only on the amount and rate of cell and fluid infiltration into the constructs but also facilitate the transport of nutrient and waste products throughout the cell-seeded construct, as well as construct vascularisation (Kuboki et al., 1998), for the duration of de novo tissue formation. These properties not only encourage complete tissue formation within the construct but also support the integration and mechanical interlocking of the implant. A scaffold which possesses an open and interconnected pore network, coupled with a high degree of porosity (>90 %) is ideal for cellular interaction and de novo tissue integration with the existing host tissue (Freyman et al., 2001).

3. Scaffold biomaterials for orthopaedic tissue regeneration

The first generation of biomaterials specifically designed for implantation into the human body appeared around the 1960s and 1970s. This first generation was characterised by attempting to “achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response in the host” (Hench, 1980). These materials were designed primarily around the principal of bio-inertness i.e. the idea of causing as little disruption to the physiological environment while facilitating a primarily structural role. Fuelled by the initial success of many of these devices, the field rapidly began to focus on improving on the concept of bio-inertness and began to aspire to creating biomaterials that exhibited a degree of bioactivity i.e. to elicit a positive and controlled response within the implanted physiological macro-environment that would aid the healing or regenerative process. It was at this point that a large move towards the use of ceramic based materials occurred.

3.1 Ceramics

Ceramics (inorganic, non metallic materials) include the calcium phosphates, bioglasses and glass-ceramics (Hench, 1998). Bioceramics can be further classified as being osteoconductive (supporting bone growth) or osteoinductive (stimulating bone growth). While osteoconductivity is common to all types of bioceramics, relatively few are osteoinductive, a property that although extremely coveted, is not fully understood or easily replicated in synthetic materials (Barrere et al., 2008). The calcium phosphate based bioceramics, bioglasses and glass-ceramics are commonly used as scaffolds for bone tissue engineering as they have a compositional similarity to the mineral phase of bone (Hing et al., 2005). Hydroxyapatite (HA) and tri-calcium phosphate (TCP) are two of the most commonly used calcium phosphate bioceramics in tissue engineering applications. TCP is commonly used as the basis of biodegradable scaffolds due to its relatively rapid degradation rate (Ducheyne et al., 1993) and its osteoconductivity. HA, the mineral that occurs naturally in bone tissue, has also been used extensively as a tissue engineering scaffold material due to its

osteoinductivity. It is typically used for coating biomedical implants to induce bone regeneration, allowing the implant to integrate with the surrounding tissue. While HA was originally popular for use as a scaffold for tissue engineering, it is non-resorbable in bulk form which has limited its popularity as a bone graft substitute material (Figure 3). However, recent work on micro- and nano-sized particles has led to a paradigm shift regarding the degradability of this material and has re-ignited interest in this ceramic as a critical component of tissue engineering composite scaffolds optimised for bone tissue regeneration. Ceramic materials offer a facilitative environment to bone forming cells, offering mechanical support that promotes mineralisation in the hope of achieving stability equal to the normal anatomical tissue. Synthetic calcium phosphates have been popular for a number of different applications, ranging from a simple coating layer on prosthetic devices (Klein et al., 1993) to being the implantable device itself, as porous bone graft substitutes in the repair or augmentation of bony defects. Many of these devices have been used clinically with some degree of success and are still widely used as bony void fillers. However, their clinical applications have been limited because of their brittleness and difficulty of shaping for implantation (Wang et al., 2003), low porosity and long term mechanical integrity issues (Bohner, 2010). Difficulties also exist in controlling the degradation rate of ceramics so as to ensure optimal resorption (Tancred et al., 1998). Although HA is a primary constituent of bone and might seem ideal as a bone graft substitute, problems include a slow degradation rate (Marcacci et al., 2007), poor mechanical properties and new bone formed in a porous HA network cannot sustain the mechanical loading needed for remodelling (Wang et al. 2003).

3.2 Synthetic polymers

Due to the numerous drawbacks of ceramic-based biomaterials, significant advances were made towards the development and use of bioresorbable second generation materials. These materials are polymeric-based and include many different biocompatible and bioresorbable materials, such as PLA and PGA scaffolds for use as implantable devices (Athanasίου et al., 1998). Polymeric-based biomaterials have a number of advantages such as high mechanical strength and biodegradability. Their mechanical, physical and biological properties can be tailored to give a wide range of properties that are desirable for bone tissue regeneration. In addition, their degradation rates can be controlled, as can their degradation by-products (Hennick and Van Nostrum, 2002). Among the many biodegradable synthetic polymers used for tissue engineering applications, there are numerous reports on the use of polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers poly (DLlactic- co-glycolic acid) (PLGA), which are approved by the US Food and Drug Administration (FDA). These polymers degrade by hydrolytic mechanisms and are commonly used because their degradation products can be removed from the body as carbon dioxide and water. Unfortunately, they are also associated with a number of problems, ranging from issues involving device rejection and resisting adequate resorption to promote vascularisation and ingrowth of new bone. Localised lowering of pH within the region of degradation can result in inflammatory responses. While these materials showed some promise, it is clearly evident from the lack of synthetic polymer-based products currently in use clinically as bone graft substitutes that synthetic polymers alone simply do not provide a sufficient degree of long-term biocompatibility or performance required for the clinically-successful regeneration of bone tissue.



Fig. 3. Porous HA implant used to fill bony void but no resorption after 2.5 years (Cancedda et al., 2007)

Polymeric materials however do offer a number of significant advantages over ceramics from a composite scaffold point of view. Biodegradable polymers can be used to encapsulate biologics and/or growth factors and can be designed to control the release kinetics of these substances via tailored polymer degradation properties for specific tissue regenerative applications. This ability presents a range of potentially controllable processes including cell growth, tissue regeneration and host response that can be influenced by developing suitable biopolymers (Hutmacher, 2000) for incorporation into novel composite materials. Appropriate selection and development of certain synthetic polymers for applications involving controlled biologic or growth factor release kinetics can be further advanced by the incorporation of 'surface eroding polymers', which biodegrade only at their surfaces. Poly(anhydrides), poly(orthoesters) and polyphosphazene exhibit this property and offer numerous advantages over bulk degradation polymers (Rezwan et al., 2006).

3.3 Natural polymers

Natural polymers offer a number of significant advantages over synthetically-derived polymers due to their biocompatible, biodegradable and bioactive nature. By using materials that form the basic building blocks of organic systems, host response due to immunocompatibility issues can be drastically, and sometimes completely, reduced. This approach also ensures that these materials are easily biodegradable via the body's own metabolic processes and that the resulting degradation by-products are non-toxic and can be easily assimilated or expelled from the tissue. Alternatively, natural polymers can usually be crosslinked using a number of means including physical or chemical crosslinking techniques. Physical crosslinking methods include UV radiation and dehydrothermal treatments, whilst cross-linking agents such as glutaraldehyde and carbodiimides (EDAC) can be used to produce chemically cross-linked natural polymers, allowing a customised degradation rate for different regenerative applications. This makes them ideal biomaterials for developing scaffolds with tailored biodegradation rates that can match the formation of

de novo tissue. Natural polymers used in bone tissue engineering include alginate, chitosan, fibrin, silk, glycosaminoglycans and collagen. Most natural polymers are biocompatible, degradable, and readily solubilised in physiological fluid (with exception of chitosan which is soluble under mild acidic conditions) (Seeherman and Wozney, 2005). This simple biomimetic approach means they can more closely mimic the natural extracellular matrix of tissues and can play a bioactive role in substrate cell interactions, such as promoting cell adhesion and proliferation.

Alginate and chitosan are two natural polysaccharides that are not found within the human body but due to their structural similarity to the glycosaminoglycans (GAGs), they have been the focus of a significant body of scientific work evaluating their applications in regenerative medicine (Terada et al., 2005; Chenite et al., 2000). While these materials are attractive due to their low toxicity and biocompatibility, there are significant issues with a lack of load bearing ability (Suh and Matthew, 2000) which has limited their clinical applications as single phase materials but chitosan still plays a major role as a composite component, due to its flocculating and chelating properties (Zhang et al., 2010) and its role in composite mechanical stabilisation (Jiang et al., 2006). Structural proteins such as fibrin have also been used in tissue engineering applications. Fibrin can be used as a natural wound healing material, and is most commonly used clinically as a sealant or adhesive. However, fibrin has very poor mechanical properties, degrades rapidly in vivo and cannot withstand physiological loading long-term when implanted into orthopaedic defects (Barrere et al., 2008). In spite of this, fibrin hydrogels have been used in muscle (Cummings et al., 2004) and cartilage tissue engineering (Hunter et al., 2004) in vitro. Silk has also been utilized as a tissue engineering scaffold for stem cell osteogenic differentiation of MSCs (Meinel et al., 2006). Silk as a biomaterial combines slow biodegradability, excellent mechanical properties, and biocompatibility. Native silk fibers are some of the strongest natural fibers known, rival synthetic materials such as Kevlar in terms of tensile strength (Cuniff et al., 1994) and have been used as suture materials for over 20 years (Vepari and Kaplan, 2007). Silk scaffolds have previously been shown to support tissue engineering of bone in vitro (Altman et al., 2003). Glycosaminoglycans are found in the natural extracellular matrix of tissues i.e. skin, bone, and blood vessels and are a form of proteoglycan; organic polymers that are found in cells and that are a major component of the natural ECM. Given the importance of GAGs in stimulating normal tissue growth, the use of GAGs as components of a scaffold for tissue engineering appears to be a logical approach for scaffold development. Glycosaminoglycans (GAGs) are long, unbranched polysaccharides that do not elicit an immune response and have been used extensively for tissue engineering applications and can be copolymerized with collagen to increase the stiffness and toughness and decrease its degradation rate.

Hyaluronic acid is one of the largest GAG components found in the natural extracellular matrix of all soft tissues and synovial fluid of joints (Drury and Mooney, 2003) and has been used in tissue engineering applications due to its role in structural organisation of the ECM components. However, its use has been limited due to rapid dissolution in water and fast biodegradation in biological environments. However, it can be chemically modified to produce a more hydrophobic molecule, thus reducing its solubility in water. Hyaluronic acid is a major component of cartilage matrix and is a viscoelastic material that forms random coils which entangle with each other at low concentrations and at high concentrations contains a very high viscosity dependant on shear stress making it pseudo-

plastic. Chondrocytes in cartilage have a high affinity for Hyaluronic acid via CD44 receptors and RHAMM (Lisignoli, 2001) and consequently it plays a more important role in cartilage tissue engineering constructs.

Scaffolds fabricated from type I collagen and a glycosaminoglycan have been used to study cell migration and contraction in vitro (Sethi et al., 2002) as well as to induce regeneration of the skin, conjunctiva, and peripheral nerves in vivo (Harley et al., 2004; Yannas, 2001). Collagen as a scaffold material is ideal as it possesses all the biological prerequisite for successful implantation such as biocompatibility, immunogenicity, cell adhesion and proliferation (Murphy et al., 2010; Byrne et al., 2008; Berry et al., 2004; O'Brien et al., 2005). Collagen is the most abundant ECM protein in the human body and is readily isolated and purified from various animal species by enzyme treatment. Since collagen type I is the main organic component that in human bone tissue and it is the substrate on which bone mineralisation occurs during osteogenesis, collagen has been extensively used as the material of choice in nearly all commercial orthobiologic bone tissue engineering applications currently used clinically in the repair and regeneration of bone tissue. Two of the biggest advantages of using collagen-based scaffolds for tissue engineering applications are that (i) they provide an extremely attractive substrate for cell adhesion and proliferation and (ii) collagen scaffolds do not alter the phenotype of seeded cells. Another significant attribute is the recent FDA approval and clinical success of collagen-based scaffolds used for skin and nerve regeneration (Yannas, 2001). However, despite the excellent biocompatibility, collagen, like all other natural polymers, is insufficient mechanically for orthopaedic applications and this has limited its use in load-bearing bone tissue defects.

4. Composite scaffolds for bone tissue regeneration

4.1 Synthetic polymer and ceramic composites

As a result of the problems associated with the use of single phase synthetic scaffolds, advances in the development and fabrication of composite scaffolds offered new materials using a combination of synthetic polymeric and ceramic phases. The development of these synthetically-based composites was believed to facilitate the development of biomaterials with all of the advantages of these single phase materials with none of their disadvantages. In spite of these efforts, these second generation bone graft substitutes have enjoyed limited clinical success (Ratcliffe, 2008) and simply do not possess all of the prerequisite characteristics of an ideal bone graft substitute (biocompatible, bioresorbable, osteoconductive, osteoinductive, structurally similar to bone, easy to use and cost-effective). The inability of synthetic materials to respond or adapt to changing physiological conditions means that they will always represent a compromise when used in the repair or regeneration of human tissue. As a result, this second generation of biomaterials have not been able to act as a viable clinical alternative to the gold standard, autogenous bone. Numerous innovative synthetic polymer-ceramic composites have been developed with porosities high enough to ensure cell infiltration and an environment potentially conducive to osteogenesis. While some have shown evidence of osteoinductivity when implanted into ectopic bone formation models (Barbieri et al., 2010), in in vitro conditions (Li et al 2010); and evidence of potent bioactivity (Deplaine et al., 2010), only a handful of these materials have been evaluated in large pre-clinical bone tissue defects.

4.2 Natural polymer and ceramic composites

The combination of natural polymers with a reinforcing and bioactive ceramic phase has shown significant promise over the last decade with a number of these composites progressing to widespread clinical use (Lee and Goodman, 2009; Carter et al., 2009; Kitchel, 2006; Muschler et al., 2005; Scabbia and Trombelli, 2004). While these materials fulfil many of the requirements of synthetic bone void fillers, none have shown conclusive clinical evidence of being a superior alternative to the clinical gold standard of autogenous bone. Such an ideal bone graft substitute must be capable of promoting rapid osteogenesis *in vivo*, encourage *de novo* bone formation and remodelling of the defect to restore anatomical normality and mechanical integrity, and biodegrade at the same rate as the progressing tissue regeneration process. Recent advances in composite biomaterials have led to a paradigm shift in this area towards the development and use of biomimetic composite scaffolds for orthopaedic regenerative medicine. Biomimicry, both in terms of composite composition and fabrication process may provide a compromise between the competing mechanical and the biological prerequisites needed to rapidly promote healing of bone tissue defects (Gleeson et al., 2010). The use of materials found naturally to occur within the human body allows the implantation of materials that are easily integrated, processed and degraded by the body. These materials form part of the normal “building blocks” of the human system and offer extremely favourable biological interactions. Advances in the processing and composite engineering of new composite materials comprised of these naturally-occurring materials offer exciting possibilities for not only meeting a compromise between the mechanical and biological prerequisites for implantation and bioactivity, but also for directing and controlling the chemical, biological and mechanical events that occur during the regenerative process.

There are a large number of natural materials currently used as the components of composite scaffolds for orthopaedic regenerative medicine. While there are many different materials used to promote bone tissue repair, bone’s native composition of predominantly type I collagen and hydroxyapatite makes these materials an obvious choice as the basis for a composite biomaterial capable of supporting and promoting the bone regenerative process (Dawson et al. 2008). Recent studies have shown that the interaction between osteoblasts and PLLA scaffolds can be improved by the application of a collagen-HA coating (Jiashen et al., 2010) clearly demonstrating the potential of a composite material composed of only collagen and hydroxyapatite for use as a bioactive bone graft. These composite scaffolds were traditionally fabricated using a number of different techniques involving some form of pre-processing step, with the scaffold architecture subsequently formed via a number of distinct processes, all of which possess numerous advantages and disadvantages (particulate leaching methods, phase separation, lyophilisation, foaming, emulsion templating, and solid free form (SFF) fabrication).

Current advances in composite fabrication have been driven by the desire to replicate the hierarchical scale of the naturally-occurring tissues. Bone is composed of mineralised tropocollagen molecules, arranged in a distinct quarter stagger arrangement, with nanometre-sized hydroxyapatite crystal intimately associated with these fibrils. The nature of these nanohydroxyapatite particles endows them with a number of distinct regenerative advantages relative to the micron-sized particles and this has been a driving factor in the move towards the hypothesis that biomimetic scaffolds fabricated using biomimetic processes may allow the development of composite scaffolds optimised to promote rapid

osteogenesis *in vivo*. As a result, many recent studies have utilised biocompatible or bioactive dispersants, such as chitosan (Zhang et al., 2010b) or biomimetic fabrication methods for the *in situ* mineralisation of collagen-HA scaffolds during the fabrication process (Zhang et al., 2010a; Xu et al., 2010; Yoshida et al., 2010; Kikuchi et al., 2004).

One of the earliest attempts to combine biologically-occurring composite constituents with biomimetic fabrication processes to form a bone graft substitute material was carried out by Kikuchi (Kikuchi et al., 2004). This approach involved a self-organisation mechanism designed to synthesise a bone-like collagen/hydroxyapatite nanocomposite material for use as a bone tissue engineering scaffold. There are many motivating factors that support the use of nano HA, most critically increased *in vivo* resorption rate, increased osteoblast adhesion and proliferation and increased bone formation *in vivo*. By using a self-organisation mechanism that involves the *in situ* mineralisation of a collagen solution, Kikuchi and colleagues were able to develop a collagen/HA composite that exhibited bone-like orientation of nano-sized hydroxyapatite crystals, aligned along the length of the collagen fibrils. However, although *in vitro* and preliminary *in vivo* data were promising, their application to orthopaedic regenerative medicine remains to be elucidated. In addition, control and regulation of this process and the resulting nature of the fabricated HA can be difficult with implications on the purity and crystallinity of the resulting mineral phase. Given that HA crystallinity and purity plays a significant role in promoting bone tissue formation *in vivo* (Zhang et al., 2010a; ter Brugge et al., 2002), the ability to produce pure collagen-HA scaffolds of high purity and crystallinity is desirable from a tissue engineering perspective.

However, a significant flaw that exists in the biomimetic fabrication approach is the assumption that an ideal optimised bone graft substitute material must mimic the composition and structure of the final bone tissue. This postulation does not consider that a truly biomimetic composite scaffold for orthopaedic regenerative therapies must match the idealised environment that supports the earliest stages of osteogenesis. This presents the biggest current challenge within the field of composite scaffolds for orthopaedic regenerative medicine, namely to develop a composite material that meets the multitude of prerequisite characteristics necessary to induce, promote and support the process of osteogenesis. Such a composite scaffold must (i) be highly porous to facilitate rapid and complete cellular infiltration once implanted (ii) provide surfaces and an environment that encourages cellular adhesion and proliferation (iii) be highly permeable to facilitate the exchange of nutrients and waste products throughout the scaffolds and preventing avascular necrosis (iv) be sufficiently strong to withstand surgical manipulation and the implantation procedure (v) exhibit a high degree of pore interconnectivity (vi) must be osteoconductive and ideally osteoinductive (vii) biodegradable, biocompatible and bioactive and (viii) possess an optimised pore size and pore size distribution to facilitate homogenous mineralisation of the composite scaffold *in vitro* and *in vivo*.

Many of the traditional methods for fabricating scaffolds for tissue engineering are not ideal for the development of highly biocompatible scaffolds with the prerequisite pore network, porosity and pore interconnectivity characteristics that are increasingly being recognised as determining factors in the long-term *in vivo* viability of tissue engineering constructs. Problems associated with these techniques include, but are not limited to, poor control over internal architecture and a limited range of pore sizes, residual solvent and residual porogens, porosity limits of about 70% and highly heterogeneous nature of the pore

structure, limitations the development of photopolymerisable and biocompatible, biodegradable liquid polymer materials, high processing temperatures, use of toxic organic solvents and lack of mechanical strength. In addition, one of the major barriers to the successful development of a collagen-HA scaffold using these techniques is the difficulty in achieving a homogenous distribution of the HA throughout polymer-based matrices (Supova, 2009), an issue that can have a significant effect on a collagen-HA biomaterial's *in vivo* vascularisation and production of newly formed bone tissue (Lyons et al., (2010); Zhang et al. (2010a)). Recent attempts to overcome this issue have employed additional naturally-derived dispersants in an attempt to achieve a homogenous dispersion of the osteoinductive HA particles throughout the fabricated matrix (Zhang et al., 2010b) but radiological results do not provide evidence of the ability of these scaffolds to heal critically-sized bone defects. Freeze-drying is a process which can potentially solve many of these composite scaffold fabrication issues and is ideally suited to organic biomaterials such as collagen. This technology has a number of distinct advantages with regard to the production of high porosity, highly interconnected, homogenous biological constructs. Currently, our Tissue Engineering Laboratory produces a range of highly porous collagen-based scaffolds, using a constant cooling lyophilisation process, with specific applications including bone and cartilage tissue repair (Murphy et al., 2010, Farrell et al., 2009). These scaffolds have been optimised for bone tissue healing and have recently been shown to provide ideal substrates for supporting the process of osteo- and chondro-genesis *in vitro* (Farrell et al., 2006). Our laboratory's approach has involved the optimisation of these collagen-based scaffolds in terms of composition (Tierney et al., 2009), cross linking density (Haugh et al., 2009) and pore architecture (Murphy et al., 2010) for use in bone tissue engineering applications.

Case study: Highly porous collagen hydroxyapatite scaffolds for bone tissue repair

We have recently developed the ability to combine our highly porous, optimised collagen scaffolds with an osteoinductive HA phase in an effort to improve their osteogenic potential and provide the prerequisite mechanical integrity to promote rapid *in situ* bone tissue regeneration (Gleeson et al., 2010) (Figure 4). By combining the two primary constituents of human bone tissue, namely type 1 collagen and high purity, highly crystalline hydroxyapatite particles using a novel mixing process (WO200896334A2), a highly porous composite tissue engineering scaffold with a high degree of pore interconnectivity, improved mechanical strength, permeability and cellular bioactivity was developed, overcoming traditional HA dispersion problems and exhibiting a homogenous distribution of the osteoinductive HA phase. The combination of the extremely biocompatible and biodegradable collagen scaffold with an osteoinductive mineral component (Barrere et al., 2003) provides an ideal mechanical and biological environment to facilitate cell recruitment and maintain pore structure in order to promote healing.

By optimising these compliant and highly porous collagen-hydroxyapatite scaffolds to promote mineralisation upon implantation (Hutmacher et al., 2000), these materials have the potential to rapidly produce *de novo* bone tissue with a load bearing capacity within the newly mineralised bone tissue graft. These scaffolds possess all the ideal prerequisite characteristics of a biodegradable scaffold optimised for bone tissue regeneration, are comprised only of bone's natural constituent materials, and have demonstrate their ability to promote osteogenesis *in vitro* and *in vivo* repair of critical-sized bone defects (Figure 4).

These highly porous collagen hydroxyapatite scaffolds have also been used pre-clinically in the repair of critical-sized segmental bone defects in long bones and show a rapid return to anatomical normality (as evident by formation of a continuous marrow cavity through the regenerated segmental defect), as well as evidence of *de novo* tissue remodelling and rapid restoration of load bearing ability after only 6 weeks implantation (unpublished data). These materials appear to demonstrate real potential as a bone graft substitute materials, capable of facilitating and promoting osteogenesis *in vivo*.

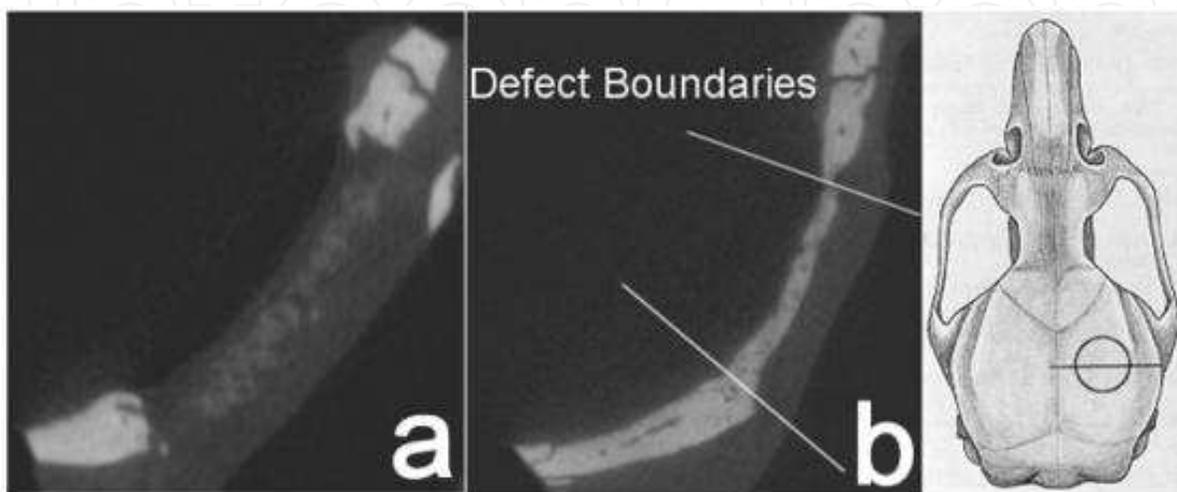


Fig. 4. MicroCT slice of representative level of mineralisation within rat calvarial defect centre showing defect boundary edges in (a) empty defect group, (b) collagen HA scaffold group after 28 days implantation. Almost complete defect bridging was observed in the collagen HA group, with mineralisation level comparable to surrounding native calvarial bone tissue (Gleeson et al., 2009)

5. Scaffold biomaterials for osteochondral tissue regeneration

Articular cartilage is a highly specialised tissue found covering the surfaces of the bony ends of all synovial joints in the human body. Its function is to lubricate joint movement and absorb small shock impacts within a joint. Articular cartilage is primarily composed of 70-80% water, 15% collagens (80% of which is type II collagen) and 5% cells. In synovial joints, the overlying lubricating articular cartilage is attached to the bone via a specialised tissue unit, known as osteochondral tissue. The structure and composition of osteochondral tissue is made up of a number of distinct but seamlessly integrated layers which vary in composition and structure according to their function and this serves to transfer mechanical forces at articulating surface down to the stiffer underlying subchondral bone via an intermediate tidemark layer, known as articular calcified cartilage. Cartilage is significantly different to bone from a regenerative point of view as it has a low regenerative capacity and damage to this tissue is irreparable and almost inevitably leads to the development of osteoarthritis (OA) within a joint and the eventual replacement (Arthroplasty) of the affected joint. Traditionally, the inherent lack of a natural regenerative capacity meant that autografting, allografting and joint replacement therapies offered the best potential outcomes for patients but recent advances in composite scaffolds are offering new regenerative possibilities.

Tissue engineering via in situ tissue regeneration may provide the prospect of regenerating cartilage and osteochondral tissue using a combination of an optimised tissue engineering scaffold and a source of potent progenitor cells used because of their ability to differentiate into a specific cell type and provide a source of extracellular matrix. This approach to cartilage and osteochondral repair is based on creating access to the most abundant source of nearby progenitor cells which reside in the underlying bone marrow. It is believed that this multi-tissue approach has the potential to offer improved restoration of the entire osteochondral unit and may potentially be more successful than simply trying to regenerate the avascular and largely non-regenerative mature articular cartilage tissue alone.

There are a number of critical factors that must be taken into consideration when designing tissue engineering scaffolds optimised for osteochondral repair via in situ tissue regeneration (Frenkel and Caesare, 2003). An ideal osteochondral graft substitute must (i) be biocompatible, (ii) provide adequate mechanical properties to withstand the implantation procedure and the subsequent mechanical and hydrodynamic loading within the joint, (iii) possess sufficiently high levels of porosity to allow ingrowth of host tissue and/or seeded cells, (iv) be retained at the site of implantation (Beris, 2005), (v) support and direct regeneration of the two predominant tissue types, namely bone and cartilage (Hutmacher, 2000) to ensure overlying repair cartilage is adequately supported, (vi) be optimised for cell attachment to favour colonisation by native cells (Coutts et al., 2001), (vii) promote integration with the existing tissues (Beris, 2005).

Composition is elementary in creating biologically active materials that can induce synthesis of new tissue in vitro and in vivo. As with bone graft substitutes, there exists a wide array of materials that have been used in an attempt to produce an optimised osteochondral graft substitute. Synthetic polymers have been widely used for tissue engineering. Polymers such as polylactide (PLA), polyglycolide (PGA) and their copolymers (poly(D,L-lactide-co-glycolide) are commonly used due to their degradability and US Food and Drug Administration (FDA) approval for clinical use. They have been investigated as scaffolds for cartilage tissue engineering since the early 1990s (Vacanti et al., 1991) and have formed cartilage-like tissue with good mechanical properties (Ma and Langer, 1999). The major advantages of the synthetic polymers include a wide range of tailored structural properties and the lack of disease transmission. PLA scaffolds have shown some promise in vivo but de novo tissue was found to be biochemically inferior to native cartilage tissue (Dounchis et al., 2000). PLA and PLA co-polymer composites have also been investigated for use in cartilage tissue repair (Niederauer et al., 2000) and shown evidence of hyaline cartilage and good bony restoration. However, there are many disadvantages with these materials, predominantly associated with the biological interactions that take place between the host and these synthetic materials over time and ultimately the translation of these synthetic polymer-based constructs into human clinical applications has not been seen due to concerns about their ability to illicit foreign body host responses and concerns of localized and systemic effects due to their toxic degradation by-products in vivo (Stoop, 2008)

Scaffolds developed using components of the ECM are generally more favourable than artificial polymers due to their ability to regulate cell function through specific cell-matrix interactions through ligand-integrin associations. Fibrin has been used extensively in the development of tissue engineered constructs for cartilage tissue engineering but the biochemical and morphologic features were not consistent with those of normal articular cartilage and exogenous fibrin may trigger an immune response (Kawabe and Yoshinao,

1991). Currently the use of fibrin is primarily limited clinically to securing perichondrial scaffold grafts. Agarose is another material that has been used for cartilage repair but does not resorb well and has been shown to elicit a foreign body giant cell response *in vivo* (Rahfoth et al., 1998). Alginate as a matrix for supporting chondrogenesis show improved biological performance seen histologically but resulting repair has been shown to be biochemically distinct from native tissue (Dausse et al., 2003).

Collagens and polysaccharides are the most commonly used components of the ECM in Tissue Engineering (TE). The ability of these materials to be fabricated into highly porous scaffolds allows enhanced diffusion of culture medium as well as an even distribution of ligands present for cell association and cellular migration (Murphy et al., 2010). The use of natural materials in TE reduces the risk of having products of wear and degradation that may elicit host tissue immune response. Collagen is commonly used in TE due to its abundance and ubiquitous nature which allows cellular biocompatibility. From a biomimetic point of view, osteochondral graft substitutes based on collagen and proteoglycans may facilitate the development of natural substrates capable of directing and regulating the chondrogenic process. Collagen-based matrices have been shown in a number of studies to be capable of maintaining a differentiated phenotype of chondrocytes and promoting appropriate proteoglycan synthesis *in vitro* (Wakitani et al., 1994). In combination with seeded chondrocytes and a standard microfracture technique, collagen-based scaffolds have been shown to be capable of regenerating hyaline-like cartilage tissue in an ovine model (Dorotka et al., 2004). This has also been seen in rabbit model where chondrocytes in collagen fibers induced a hyaline-like repair that was biochemically and mechanically similar to native tissue after 6 months (Frenkel et al., 1997) but hyaline-like tissue formation has long been accepted as only a temporary solution (Buckwalter and Mankin, 1998). In addition, integration with the existing host tissue in studies using single phase collagen type I scaffolds has been notably disappointing (Wakitani et al., 1998; Frenkel et al., 1997). Alternatively, collagen type II, a primary component in native articular cartilage, has been shown to promote a more chondrocytic phenotype when used as an *in vitro* substrate for chondrogenesis results in increase DNA and GAG content, compared to collagen type I-based scaffolds when seeded with chondrocytes while type I matrices show a more fibroblastic phenotype (Nehrer et al., 1997). Type II collagen has also been shown to provide chondroinductive signalling resulting in chondrogenic differentiation of adipose tissue-derived stem cells (Lu et al., 2010) and when combine with type I collagen and GAG, these scaffolds have outperformed ACI repair in pre-clinical trials (Breinan et al., 2001)

Hyaluronic acid (HyA), abundant in the synovial fluid and ECM, plays an important role in structural organisation of the ECM components, maintenance of ECM space, transport of ions and nutrients, and maintenance of tissue hydrodynamics. Studies have shown that HyA addition in collagen scaffolds has resulted in a change of the matrix stiffness, fibrillogenesis and matrix viscoelasticity (Tang et al., 2007). Hence, the addition of HyA in collagen scaffolds initiates biophysical cues that influence cell response. The properties and roles of hyaluronic acid are however dependant on its molecular weight. Low molecular weight hyaluronic acid has been shown to induce angiogenesis whereas high molecular weight hyaluronic acid is more chondroinductive *in vivo* (Loken et al., 2008). A number of commercial scaffolds are currently available and are based predominantly on Hyaluronic acid and have shown very positive short term results. Hyalograft C (Fidia Advanced Biomaterials) is a 3-dimensional engineered scaffold made of Hyaff 11, the benzyl ester of

hyaluronic acid and has been used in clinical studies and has resulted in over 95% of patients returning with normal or nearly normal cartilage after arthroscopic examination after 4 years with another study showing no statistical significant between patients receiving ACI and Hyalograft C (Grigolo et al., 2005). Hyaluronic acid has been used in combination with Chondroitin sulphate and gelatin, forming tri-copolymer scaffolds and used to investigate in vitro chondrogenesis using porcine chondrocytes. These scaffold show excellent results, with even distribution of chondrocytes, evidence of newly secreted ECM and collagen type II, and good phenotype retention for up to 5 weeks (Chang et al., 2003). While many of these materials possess the basic requirements of biocompatibility and can be manipulated so as to provide adequate mechanical support, porosity, cell attachment and retention at the site of implantation, the regeneration of osteochondral tissue requires a multiplicity of biological and biochemical functions that are difficult to provide when using only a single phase material. Indeed, the possibility of a single phase scaffold promoting both chondro- and osteo-genesis and replicating the natural anatomical structure of native osteochondral tissue seems highly problematic (Niederauer et al., 2000). Native osteochondral tissue exhibits a multi-phase anatomical structure, comprised of multiple layers seamlessly interwoven and integrated on a molecular level. Given this more multiphase anatomical structure, it would seem clear that a more advanced biomimetic strategy may be required to comprehensively regenerate the tissue and ensure long-term repair of a defect. This has been recognised within the field and more and more we are seeing the emergence of multi-layered scaffolds, attempting to regenerate multiple tissue types in vivo. The use of bi-layered scaffolds allows the development of optimised, tissue-specific biological environments within each respective layer via variations in mechanical, structural, and chemical properties (O'Shea et al., 2008). These scaffolds can be designed to better mimic the native ECM for each tissue type independently rather than trying to fabricate a construct that attempts to compensate for the functional requirements of both cartilage and bone in a single structure but fail to address the mechanical and biological need for an intermediate calcified cartilage phase to comprehensively integrate these distinct tissue types in vivo.

A number of recent studies provide convincing evidence supporting the need for a scaffold comprised of a number of distinct constituents, contained within a gradient scaffold architecture. Gradient scaffold pore architectures have been used to investigate the effect of a gradient structure on in vitro chondrogenesis (Woodfield et al., 2005). In vitro culture on these scaffolds can lead to zonal distributions of glycosaminoglycans (GAGs) and collagen type II while changes in the permeability or fluid mobility of scaffolds (that would occur within scaffolds with varying pore size gradients) have also been shown to preferentially favour chondrogenic differentiation of BMSCs and cartilaginous ECM production of chondrocytes (Kemppainen and Hollister, 2010). Computational models of osteochondral repair (Kelly et al., 2006) using a mechano-regulation algorithm point to the importance of a depth-dependent mechanical properties and permeability as optimum for osteochondral repair. The use of bi-layered scaffolds was first pioneered by Schaefer and colleagues (Schaefer et al., 2002) and provided promising results as the first attempt at using multi-phase scaffolds to regenerate osteochondral defects in rabbit knees. The results showed good integration with the underlying bone but not with the peripheral cartilage, although the constructs did show evidence of engineered cartilage that remodelled into osteochondral tissue. Subsequent studies applying this biomimetic approach have resulted in distinct

tissue healing within respective layers of a bi-layered construct (Tampieri et al., 2008) with evidence of a mineralised interface (Schek et al., 2004).

Although multi-layered scaffolds can be fabricated by the combination of individually fabricated layers, standard techniques for combining these layers (such as suturing and gluing) are problematic from both a mechanical and a biological point of view. Currently, bi-layered scaffolds with a seamlessly integrated structure are only available composed of synthetic materials (Ghosh et al., 2008) with one exception (Chondromimetic, Tigenix) and although these multi-layered constructs have shown promise *in vitro*, a significant amount of *in vivo* data needs to be gathered regarding their clinical efficacy (O'Shea et al., 2008). Consequently, the ability to fabricate multi-layer natural scaffolds exhibiting layer-specific composition, porosity, pore size, mechanical properties, degradation rate and permeability as part of a seamlessly integrated construct is of significant interest. Our laboratory has recently developed a novel multi-layered natural scaffold for osteochondral tissue repair, developed using our existing collagen-based technologies previously used as novel bone graft substitutes. These novel multi-layered scaffolds for use as osteochondral graft substitutes are designed to promote regeneration that replicates the structure and composition of healthy anatomical osteochondral tissue. These scaffolds exhibit a seamless integration between the distinct layers, ensuring rapid cellular infiltration, creation of optimised, tissue-specific biological environments in each respective layer via variations in mechanical, structural, and chemical properties and are designed to better mimic the native ECM of each tissue type independently. These scaffolds may provide a characteristic interfacial region that may help to inhibit the phenomenon of growth factor-induced angiogenesis and the consequential up growth of osseous tissue into the cartilage region. In addition, the use of collagen as a basic component in all three layers provides the opportunity to load distinct bioactive molecules within each distinct scaffold layer.

Case study: Multilayer composite scaffolds for osteochondral tissue regeneration

Our laboratory's approach has been the development of a number of novel lyophilisation fabrication techniques that facilitate a large degree of control over the manufacture of each layer, while still resulting in a completely integrated construct, free from interfacial barriers to cell infiltration and migration. This multi-layered polyphasic scaffold exhibits a structure and composition designed to replicate the environments of the three major layers of anatomical osteochondral tissue, namely subchondral bone, calcified cartilage and cartilage. This material is currently being optimised to provide a substrate for high quality hyaline cartilage repair tissue, seamlessly integrated with a supporting calcified cartilage and bone layers. Each of the three layers possesses a unique composition. The top or cartilage layer contains type I collagen, type II collagen and glycosaminoglycan. Glycosaminoglycans can easily be cross-linked to collagen and the level of cross-linking can alter the mechanical properties of these scaffolds as well as improving their degradation rate (Lee et al., 2001). Chondroitin sulphate (CS) is the most abundant type of GAG in the natural cartilage tissue and is fundamental in the maintaining the structure of cartilage tissue as well as generating electrostatic repulsion via highly charged sulphate groups in the structure. CS manipulates chondrocyte morphology, proliferation and proteoglycan production due to the GAG molecules acting as ligands for the regulation of metabolism and gene expression in chondrocytes. This biochemical is widely used both *in vitro* and *in vivo* due to its anti-

inflammatory activity as well as the decrease in catabolic activity of proteolytic enzymes such as Nitric Oxide which degenerate cartilage matrix (van Susante et al., 2001). Ko and colleagues (Ko et al., 2009) investigated the effect of collagen-chondroitin sulphate-hyaluronan (CCH) composites on chondrogenesis and their results demonstrated that CCH scaffolds showed upregulated cartilage specific gene expression of collagen-2 and aggrecan compared to scaffolds with no GAGs. Furthermore their results revealed that the CCH scaffolds provided the best microenvironment for the preservation of chondrocyte phenotype.

The intermediate or calcified cartilage layer contains type I collagen, type II collagen and hydroxyapatite while the bottom or bone layer is identical in composition to our optimised bone graft substitute material (Gleeson et al., 2010), specifically type I collagen and hydroxyapatite. We have shown previously that the inclusion of the osteoinductive hydroxyapatite phase in discrete amounts within the collagen-based scaffolds allows a degree of control on the osteogenic process in vitro. The composition of the intermediate layer is designed to act as an interface between the cartilage and bone layers while at the same time facilitating a degree of integration between the tissues engineered within each layer. The fabrication process produces a seamlessly integrated construct. Mechanical testing of the interfacial strength has shown excellent interfacial bonding, with no delamination occurring at scaffold yield and evidence of collagen fibre pullout at the yield point. Interestingly, failure of these construct in tension does not occur at the interface but within the mechanically weakest layer, strong evidence of the seamless integration at the layer interfaces.

We have shown that the addition of the GAGs to the top cartilage layer appears to increase the levels of cartilage ECM specific sulphated GAG production when these constructs are cultured for up to 28 days in vitro. In addition, the type of GAG added to the top layer of the multi-layered construct has a dramatic effect on cellular infiltration, specifically the addition of hyaluronic acid significantly improves cell distribution throughout the constructs when seeded with MSCs and cultured in chondrogenic media (Matsiko et al., 2010). We have also shown that the addition of type II collagen results in increased production of sulphated GAG assessed using Safranin-O staining within the constructs after culture with rat MSCs in chondrogenic media for up to 28 days (Levingstone et al., 2010). These constructs have also been investigated using a preliminary in vivo pre-clinical rabbit osteochondral defect model in a small cohort of animals (n=2). This work was carried out with ethical approval from the RCSI research ethics committee. A critical-size osteochondral defect (3 mm diameter, 4 mm deep) was created in the femoral condyle of 2 New Zealand White Rabbits. The multi-layered scaffold was cut to size and placed into the osteochondral defect with no fixatives and following the procedure the rabbits were allowed to weight bear with appropriate analgesia. 12 week healing compared to empty defects can be seen in the MicroCT images in Figure 5.

The femoral condyles are currently being assessed histologically to investigate the level of healing and composition of the regenerated tissue. Although MicroCT does not allow a detailed analysis of the overlying chondral repair tissue in these samples, it is clear that a significant amount of subchondral bone healing has taken place and that the defect has been completely sealed at the condylar surface. Additional images taken of the defect surface after 12 weeks (Figure 5) show evidence of integration between the in situ regenerated tissue within the defect and the peripheral hyaline cartilage and the macroscopic appearance of

this tissue closely matches the surrounding cartilage in terms of colour and glistening appearance.

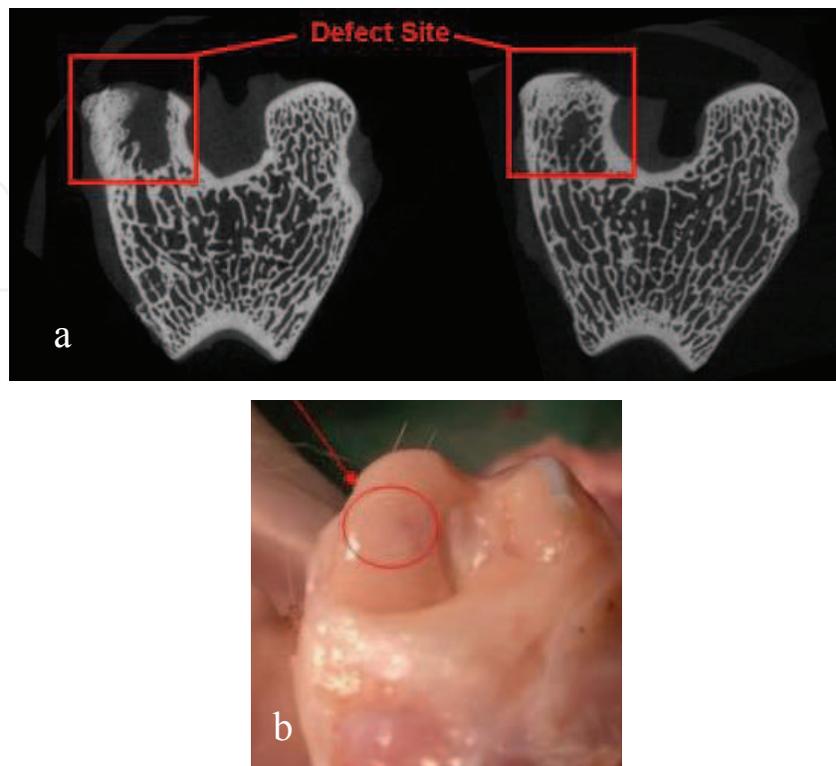


Fig. 5. (a) MicroCT slice of empty control (Left) and scaffold-treated group (Right) after 12 weeks implantation. Note the extensive subchondral remodeling and appearance of cartilage-like repair tissue at joint surface (b) Appearance of treated condyle after 12 weeks implantation. Good repair tissue integration with surrounding cartilage and hyaline-like appearance of de novo tissue

While there remains much work left to do on these constructs and indeed within the field of multi-layered constructs for osteochondral tissue repair, preliminary *in vitro* and *in vivo* studies have been extremely promising. The natural composition of the structure, in combination with the layer specific control of composition, structure and mechanical properties endow these materials with great potential for facilitating the *in situ* repair of osteochondral tissue and may finally offer a clinical therapy that may finally offer patients a real regenerative possibility for damaged or degenerated osteochondral tissue.

6. Conclusions and future directions

The regenerative capabilities of mature bone and osteochondral tissue differ significantly and this has resulted in the two distinct approaches, summarised in this chapter, in an attempt to repair or regenerate these tissues either in the lab or within the patient themselves. Interestingly, despite their final differences, the genesis of these tissues begins in a common cartilaginous anlage present during limb development. The future of bone and osteochondral tissue repair may therefore lie in our understanding of the process of endochondral ossification, and an ability to control and direct this process using improved advances in composite materials and technology. Angiogenesis plays a critical role in

endochondral ossification, during which the avascular cartilaginous tissue precursor is gradually transformed into vascular osseous tissue by the migration of blood vessels via cartilage channels. This ingrowth and advancement of vasculature provides the first set of cells capable of disintegrating the cartilage ECM, preventing avascular necrosis and supporting osteogenesis and subsequent bone development and growth. The ability to direct or control tissue vasculogenesis using cutting-edge composite materials and scaffolds may lead to new ways to develop both de novo bone and osteochondral tissue, possibly from a common composite scaffold.

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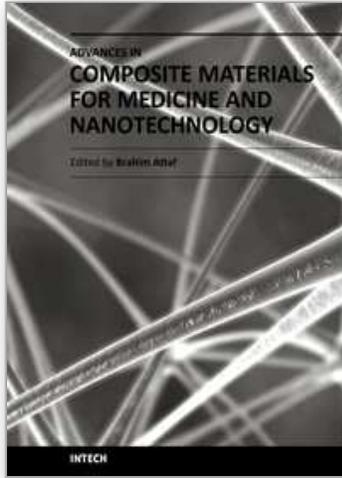
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Due to their good mechanical characteristics in terms of stiffness and strength coupled with mass-saving advantage and other attractive physico-chemical properties, composite materials are successfully used in medicine and nanotechnology fields. To this end, the chapters composing the book have been divided into the following sections: medicine, dental and pharmaceutical applications; nanocomposites for energy efficiency; characterization and fabrication, all of which provide an invaluable overview of this fascinating subject area. The book presents, in addition, some studies carried out in orthopedic and stomatological applications and others aiming to design and produce new devices using the latest advances in nanotechnology. This wide variety of theoretical, numerical and experimental results can help specialists involved in these disciplines to enhance competitiveness and innovation.

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