Material types for tissue scaffolds

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Introduction

Most tissue scaffolds are manufactured from less than 20 different materials, which tend to be those that have already been approved by regulatory bodies for use in medical applications. These "biomaterials" are derived from both natural sources such as plants or animals and manufactured synthetically. Tissue scaffolds provide a structural framework on which cells seeded into the matrix can adhere to and in time populate it. The process of populating the scaffold requires the cells to be able to freely migrate through the structure such that a mass of differentiated cells that can function as a tissue is formed. These requirements place demands on the structure of the scaffolds in terms of having suitable surface characteristics to encourage cell attachment and subsequent deposition of an active extracellular matrix. The structure must also be able to support an increasing number of cells by permitting transport of sufficient amounts of nutrients and removal of waste products (Hutmacher, 2000).

The biomaterials used to produce tissue scaffolds are degradable *in vivo*, which depending on the type of material, the scaffold geometry, and local environment, can take place over a period ranging from a few weeks to several years. A key attribute of scaffold biomaterials is that they, and their degradation products, must be biocompatible, that is, elicit at most a minimal antigenic and inflammatory response after implantation. The range of potential scaffold materials that are available include polymers and ceramics, and the choice of which material to use is dependent on the requirements of intended application. Tissue scaffolds are typically highly porous matrices that can be well-defined regular structures, foamlike, which includes air-filled cryogels and aerogels, water-filled hydrogels, or ensembles of spun fibers which degrade over time either *in vitro* or *in vivo* after being seeded with cells.

Most scaffolds are manufactured from polymers, although bioglass (Fu et al., 2011) and naturally occurring inorganic materials such as coral, tricalcium phosphate, and hydroxyapatite are also used. Composites, such as a polymer containing a dispersed particulate ceramic phase, or mixtures of polymers have been and continue to be extensively used to fine-tune the properties of the scaffold, reflecting the increasing refinement of scaffold development that has occurred over the past decade.

A number of factors need to be considered when selecting a biomaterial to use as a tissue scaffold. Primarily, the material needs to be fit for purpose; that is to say, it must have similar mechanical characteristics of the tissue that it will replace.

Characterisation and Design of Tissue Scaffolds.

This is a necessary prerequisite as cells can and do respond to their local environment, for example, chondrocytes have been shown to de-differentiate in the absence of an appropriate mechanical load (e.g., Schnabel et al., 2002; Das et al., 2008). It is also essential to develop processing routes that can be used to create scaffolds with a range of different pore sizes and interconnectivities in any required shape or alternatively be machinable to produce the geometry needed. Furthermore, the scaffold may need to degrade over a limited timescale, which may range from months to years, a process that typically depends on the material or materials used to produce it, its structure, and overall geometry. Finally the cost of the material, processing, the ability to scale up manufacturing routes, sterilization, and storage are all critically important factors if commercialization is being considered.

Polymers

Most scaffolds either are made purely from polymers or polymers are used as the continuous phase in composite materials. The ability to tailor the chemical structure and molecular weight of these materials can be used to produce scaffolds that vary considerably in terms of their mechanical performance, degradation behavior, and biocompatibility. Changing the properties of the starting material may also be important for the process used to manufacture scaffolds, for example, enhancing the water solubility of a polymer will make it much easier to electrospin fibers from it. The chemical structure of the molecules can be tuned to control the degradation behavior, making them more bioinert or bioresorbable under physiological conditions. Similarly, the mechanism by which the polymer degrades is also affected by the chemical structure of the chains and may occur as a result of hydrolysis or through enzyme-mediated reactions or some combination of the two. A number of different terms are used to describe biomaterial polymers that degrade under physiological conditions:

Bioresorbable: Materials that can be metabolized by the body.
Bioabsorbable: Materials that can dissolve or disperse in body fluids and are eliminated by the body without chain scission, for example, poly(vinyl alcohol) (PVA), and poly(ethylene glycol) (PEG).
Biodegradable: Materials that degrade through the action of biological activity, for example, enzymes to form metabolizable or excretable fragments.

Naturally occurring polymers

Naturally occurring polymers derived from plants or animals may be more biocompatible than their synthetic counterparts, especially if they contain the tripeptide arginine-glycine-aspartate (RGD) sequences that are found in proteins such as fibroin. The RGD sequence plays a key role in cell attachment by acting as a receptor for cell adhesion molecules. Naturally occurring polymers can be extracted from tissues or used as seminatural matrices by removing all traces of cellular material

from them, for example, decellularized umbilical cords. The likely variability of these materials and potential difficulty of sourcing them as well as their possible higher costs compared with synthetic materials need to be considered versus the benefits that may be gained in using them.

Polysaccharides

Polysaccharides derived from natural sources are used as base materials for manufacturing tissue scaffolds. Polysaccharides may be subject to more variability than synthetic polymers, reflecting differences in the source of the material and other potentially uncontrolled factors such as the weather and length of the growing season. Most polysaccharide scaffolds form thermoreversible elastic hydrogels and have, because of their low modulus, been used to tissue engineer soft tissues such as skin, for example, xanthan gum, konjac gum, iota-carrageenan, and kappa-carrageenan. Cross-linking the hydrogels results in a significant increase in their stiffness. Pandit et al. (2013) have, for example, cross-linked chitosan in a mixture of methylcellulose, chitosan, and agarose using differing amounts of genipin to produce scaffolds that can be used for tissue engineering bone.

The materials described below are some of the more commonly used polysaccharides, but others such as cellulose, starch, dextran, and pullulan have and are being used to manufacture scaffolds.

Alginates

Alginates are derived from seaweeds. These linear polydisperse polymers are anionic polysaccharides and are binary copolymers of L-guluronic acid (G monomer) and D-mannuronic acid (M monomer). The G and M monomers can be linked together to form blocks of poly M and poly G or randomly intermingled depending on the origin of the material. The ratio of M to G varies with different types of seaweed as well as the growing season, which can pose practical problems when trying to source materials that have comparable characteristics. This is particularly important as the proportion, and the distribution, of the two monomers has a significant impact on the physicochemical properties of the alginate. To some extent, the natural variability can be reduced by blending polymers sourced from different seaweeds gathered from different locations at different times. However, although this approach will improve the consistency between batches, it must be noted that the cells themselves will be sensitive to local variability and may exhibit marked differences in behavior to scaffolds manufactured from comparable batches of starting material.

The alginates can be easily cross-linked by divalent ions such as calcium to improve the mechanical performance of scaffolds or to manufacture scaffolds with tailored geometries by moulding. The gelling process depends on the type of ion present (e.g., Ouwerxa et al., 1998) and follows the sequence $Mg^{2+} << Ca^{2+} << Sr^{2+} << Ba^{2+}$. The composition of the gel in terms of the amount and distribution of G and M monomers affects the mechanical performance of the resultant scaffold. High G content gels tend to be brittle with good thermal stability but are likely to

weep water on thawing should the product have been frozen. Polymers that have a high M content perform much better in a freeze−thaw cycle, but the gels that they produce tend to be weaker, although more elastic than their high G counterparts. Relying on ions to cross-link polymers can be practically challenging as the process relies on diffusion and the outer regions will gel first thereby increasing the time required for the ions to reach the core. This progressive cross-linking process will typically produce gels that have a relatively high level of internal or residual stress. This can easily be seen by cutting a gel into two, and observing changes in the dimensions of the two halves and monitoring how flat the cut surface remains over time. The overall divalent ion concentration should also be considered as it will affect the degree of cross-linking, producing a perhaps desirable increase in gel stiffness but at a cost of reducing the nutrient permeability through the structure (Wan et al., 2008).

The polymer chains are degraded by enzymes to form chains that are usually small enough for filtration and excretion, but in some cases may need a special alginase, not present in the body to do so. As with other naturally derived polymers, the purity of the alginate needs to be assessed prior to be used as a tissue scaffold, as contamination by endotoxins, heavy metals, and other impurities may have a detrimental effect on subsequent cell culture.

Chitosan

Chitosan is a high-molecular-weight cationic linear polysaccharide that is commercially made by deacetylation of the chitin found in shrimp and other crustacean shells as well as the walls of fungi. The polymer consists of randomly distributed D-glucosamine (deacetylated unit; D) and *N*-acetyl-D-glucosamine (acetylated unit; A). Nuclear magnetic resonance (NMR) spectroscopy is used to show the amount of deacetylation that has occurred (which is often reported as the degree of deacetylation). This is an important parameter, as the functionality of chitosan will be strongly influenced by it (ASTM F2260−03(2012)e1). The material is typically sold as a polydisperse water-soluble chloride or glutamate salt.

Chitosan has been used to manufacture scaffolds (e.g., Takeshi et al., 2014), repair intestinal damage (e.g., Zakhem et al., 2013; Croisier and Jérôme, 2013), form hybrid scaffolds, for example, with hydroxyapatite for use in bone tissue engineering (e.g., Brun et al., 2014) or with other polymers, for example, gelatin as a skin repair matrix (Pezeshki-Modaress et al., 2014) or with fibroin for nerve repair (Gu et al., 2014). Pok et al. (2014) have even used ground-up decellularized porcine heart valve mixed with chitosan to compare the electrophysiological function and viability of neonatal rat ventricular myocytes with a more conventional mix of gelatin/chitosan scaffold.

As with other naturally sourced materials, the properties of chitosan can vary with the source of the material. In general, material obtained from animals is likely to be more varied than that obtained from fungi such as mushrooms, where the growing conditions in commercial production are carefully managed (Kannan et al., 2010; Wu et al., 2004). It should also be recognized that processing of the material by gelling,

extruding, or some other route may result in some additional changes in its functionality; therefore, it is important not only to consider the characteristics of the as-supplied salts, that is, their purity, stability, ionic strength, degree of deacetylation, and viscosity (a measure of molecular weight) but also to characterize the material post processing. Chitosan is relatively stable when stored under suitable conditions but is prone to free radical attack on the glycosidic bonds, which cause a progressive decrease in molecular weight. The solid powder is much more stable, that is, will last years provided it is stored below 25°C, compared with solutions that are much less stable and need to be stored below 5°C. As with other naturally sourced materials, the purity of chitosan will need to be assessed. Typical contaminants include endotoxins, proteins, and heavy metals as well as bacteria and yeasts.

Guidance as to which tests can be used to physically and chemically characterize and to determine the purity of chitosan and performance of the material can be found in ASTM F2103−11.

Xanthan

The anionic polyelectrolyte xanthan gum is commercially prepared by aerobic fermentation from the bacterium *Xanthomonas campestris*. The molecule consist of a β-(1->4) D-glucopyranose glucan (cellulose) backbone with side chains of (3->1) α-linked D-mannopyranose-(2->1), β-D-glucuronic acid and (4->1) β-D-mannopyranose on alternating residues. Xanthan-based hydrogels have been prepared by heating a solution in the presence of a divalent ion and used in, for example, bone regeneration (Dyondi et al., 2014) and as potential materials for skin tissue engineering (Li et al., 2009).

Proteins

Collagen

Collagen is the most abundant protein found in mammalian tissue and is found in connective tissue and the extracellular matrix. To date, more than 20 different types have been identified, the most common form of which is type 1 collagen. This structural protein consists of a triple helix and readily forms fibers that have high mechanical strength. Type 1 collagen is found in tendon, bone, ligaments, dentine, and skin. It can be easily isolated and, because of its biomimetic nature, has been used widely in tissue engineering in the form of gels, sponges, or foamlike structures (De Kok et al., 2014; Fernandes et al., 2009; Fiorani et al., 2014; Acun and Hasirci, 2014). Collagen can be degraded to form gelatin, which also has been used extensively in tissue engineering (Gnavi et al., 2014; Kang et al., 1999; Siimon et al., 2014) to form, for example, electrospun fibrous matrices. Both collagen and gelatin stimulate minimal antigenic or inflammatory responses. Gelatin is mechanically weaker than collagen, but this can be improved through cross-linking, which can also be exploited to control the *in vivo* degradation rate (e.g., Yung et al., 2007).

As with other naturally derived materials, there will be variations in manufactured collagens such as amino acid content and purity. Typical impurities can include other proteins, for example, elastin or endotoxins, glycosaminoglycans, lipids, heavy metals, and/or host cell fragments. A guide to the physical and chemical tests that may be used to fully characterize batches of material and to ensure that they are fit for purpose can be found in ASTM F2212−08e1.

Fibrin and fibrinogen

Fibrinogen (Fbg) is a relatively small blood protein (340 kDa) that plays a key role in blood clotting and platelet aggregation, it has been widely used as a biomimetic tissue scaffold material (Brown and Barker, 2014; Balasubramanian et al., 2013; Dietrich et al., 2013). Fibrinogen forms fibrin (Fn) when mixed with thrombin in the presence of the chelating agent calcium. As might be expected, fibrinogen provides a very favorable surface for cell attachment and subsequent proliferation as the molecule contains two RGD integrin−binding sites. Furthermore, the molecule has a high affinity for growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) as well as other cytokines (e.g., Martino et al., 2013; Catelas et al., 2008). Fbg can be electrospun to form fiber-based scaffolds (e.g., Baker et al., 2012). Fibrin hydrogels are used as biological adhesives, and though they are intrinsically mechanically weak, this limitation can easily be overcome by blending with biocompatible synthetic or natural polymers, for example, hyaluronic acid (e.g., Lee and Kurisawa, 2013) or other reinforcing agents such as porous calcium carbonate (Lohse et al., 2012). Moreover, the fibrin network has a nanometric fibrous structure, mimicking extracellular matrix, and it can also be used in autologous applications (Dietrich et al., 2013; Barsotti et al., 2011).

Hyaluronic acid

Hyaluronan (HA) is a linear polysaccharide that consists of alternating D-glucuronic acid and *N*-acetyl-D-glucosamine. HA is present in virtually all human tissues and found in high concentration in bone, cartilage, and synovial fluid. HA plays an important role in healing damaged tissue and is recognized as having a major impact in controlling and regulating cell behavior, including cell proliferation and migration (Chen and Abatangelo, 1999; Knudson and Knudson, 1993). HA degrades *in vivo* by hyaluronidases to form glucuronic acid and oligosaccharides. It has been extensively studied as a scaffold material for tissue engineering cartilage. HA can be easily functionalized to enhance its performance, for example, by adding RGD sequences to improve cell adhesion or to improve its degradation profile. It has been shown that HA hydrogels improve the chondrogenesis of mesenchymal stem cells and cartilaginous matrix formation when compared to PEG hydrogels (Chung and Burdick, 2009). HA scaffolds have been made as hydrogels (Dvořáková et al., 2014), electrospun fiber mats (Arnal-Pastor et al., 2013), used as coating materials for other materials, for example, polycaprolactone

(Lebourg et al., 2013), and as composites, for example, with gelatin (Chang et al., 2013). HA has also been fabricated using solid freeform fabrication to produce scaffolds with user-specified spacing, geometry, and strut dimensions (Suri et al., 2011).

Synthetic polymers

Synthetic polymers were introduced into medicine in the late 1960s as degradable polyglycolide sutures under the trade name of Dexon. Since then, many more synthetic polymers have been used as implantable materials. These include polyethylene, poly(tetrafluoroethylene), silicone, polyurethanes, and copolymers of poly(lactic acid) (PLA) and poly(glycolic acid) (PGA). These materials have been selected and developed for medical applications because of their inertness, which *in vivo* translates into providing an acceptable level of biocompatibility. Obviously, it is possible to produce materials that are much more consistent when compared to those derived from natural sources. The manufacturing procedure can also be tuned to vary parameters such as molecular weight and molecular weight distribution as well as the degree of hydrophilicity/hydrophobicity. The first forays into tissue engineering used synthetic polymers extensively following the rationale that it is cheaper and easier to use materials for scaffolds that have a proven track record as implantable matrices. However, as the field has developed, it has become apparent that significant improvements in tissue growth and cell behavior can be obtained by, for example, coating synthetic scaffolds with specific proteins (Klein Gunnewiek et al., 2013; Udpa et al., 2013) or by using composite materials (Duan et al., 2014; Haider et al., 2014). This fusion of natural with synthetic materials perhaps offers the best route to enhancing the properties of scaffolds while managing production costs and ensuring high-quality.

Polyesters

PLA and PGA and their copolymers have been widely used as scaffold materials for tissue engineering as they are relatively straightforward to process and have a long history of being used as implantable materials in medicine. The degradation profile of these semicrystalline polyesters can be easily manipulated by altering the relative proportions of the PLA and PGA blocks, which can extend the degradation time from weeks to years depending on the structure and geometry of the scaffold and its local environment. Both PLA and PGA degrade by hydrolysis of hydrolytically unstable ester linkages within the polymer to form lactic and glycolic acids. Lactic acid is produced by the body as a result of incomplete oxidation of glucose, which occurs, for example, during hard or prolonged exercise. Glycolic acid is secreted in urine or further degraded to form serine. Both serine and lactic acid are precursors to pyruvic acid and can therefore enter the Krebs or tricarboxylic acid cycle, where they are metabolized to form carbon dioxide and water. The primary route of degradation of PGA and PLA is therefore through respiration.

Polycaprolactone (PCL) is also a widely used scaffold material that also degrades into products that can easily metabolized. The *in vivo* degradation behavior of PCL depends on where it is located and on the application that it is being used for as well as the molecular weight of polymer. High-molecular-weight semicrystalline PCL, for example, has been shown to have a very long degradation time *in vivo*. Hydrolysis of the chains is an autocatalytic process resulting in the bulk material degrading at a faster rate than that present on the surface due to a build up of carboxylic acid end groups. The amorphous regions within the semicrystalline matrix degrade before the more resilient crystalline regions. During this initial phase of degradation, the polymer chains decrease in molecular weight, which doesn't result in any mass loss from the matrix. Subsequently, when the polymer chains are short enough, that is, they are effectively oligomers that diffuse rapidly through the matrix into the surrounding medium (Göpferich, 1996; Li, 1999). The loss of oligomers from the matrix results in a progressive decrease in the mass of the material and a decrease in the rate at which chain scission occurs.

Polypropylene fumarate (PPF) also has potential for bone tissue engineering (e.g., Fang et al., 2014; Dreifke et al., 2013); this material, like other polyesters, also degrades into nontoxic products: fumaric acid and polypropylene glycol. Similarly, polydioxanone has also been used as a matrix material for orthopedic tissue engineering (Lee et al., 2011) and for vascular applications (You et al., 2010).

Polyesters are relatively straightforward to process and can be solvent cast (e.g., Tse et al., 2010), prepared by nonisothermal supercritical carbon dioxide foaming (e.g., Gualandi et al., 2010), printed (e.g., Seyednejad et al., 2011), and electrospun (Seyednejad et al., 2011). There are challenges that need to be met to overcome their natural hydrophobicity but these can be met by careful use of small amounts of wetting agents.

Polyethers

The polyethers, PEG, and poly(ether sulfone) (PES) have been used as scaffold materials either on their own or as part of a composite material. Collagen-coated electrospun PES nanofibers, for example, have been shown to improve the infiltration of stem cells into a scaffold matrix (Shabani et al., 2009). Other examples of PES scaffolds include eximer laser channels created in hollow fibers for *in vitro* culture of nerve fibers (Brayfield et al., 2008).

Scaffolds for cartilage tissue engineering have been produced from PEG hydrogels that can support both chondrocytes and mesenchymal stem cells (Bryant et al., 2006; Nuttelman et al., 2004; Liu et al., 2010) and smooth muscle (Lin et al., 2014). PEG hydrogels have also been used as drug delivery vehicles for dispensing growth factors (Stukel et al., 2015). PEG, like many other scaffold polymers, can be modified, for example, to include lactic acid groups and RGD sequences (e.g., Guarnieria et al., 2010) to alter their degradation profile and to enhance cell−matrix interactions respectively.

Synthetic proteins

Using naturally derived materials is not an easy option; issues such as contamination by endotoxins, consistency, and the cost of sourcing materials and extracting the protein all need to be considered when deciding upon a scaffold material. The degradation behavior of the matrix also needs to be considered and the method used to produce a scaffold; it is challenging to create structurally homogenous scaffolds, especially hydrogels and the methods that are required to fully characterize them are not as robust as they might be. These challenges open up the field for self-assembling systems that form gels as a result of being exposed to an external stimulus, for example, a change in temperature or pH. Using click chemistry, small peptide sequences can self-assemble to form nanostructures that can be tuned for a given application to match the amino acid sequence required. Peptide-base copolymers, amphiphilic graft polyesters, and supramolecular polymers that contain cyclic oligomers among many others can all be created using click chemistry. The major benefit to tissue engineering is that these self-assembling systems can form hydrogels *in situ* after being injected into the body together with, for example, stem cells (e.g., Cigognini et al., 2014; Maude et al., 2013).

There are a number of examples of synthetic peptide based scaffolds and a number of good reviews on this topic, for example, Le Droumaguet and Velonia (2008), Matson and Stupp (2012), Zhang et al. (2005), and He et al. (2014).

Ceramics

Something like 2.2 million bone graft procedures are performed annually in the world to repair bone defects and fractures (Giannoudis et al., 2005). Often defects are repaired using autografts harvested from another part of the patient, e.g., the pelvis, but such procedures add to the operating costs and, although an ideal material, is obviously limited in supply. Demineralized bone harvested from cadavers is an obvious substitute for allograft material but such material is also expensive and may trigger an adverse immune response. Using synthetic materials as bone tissue scaffolds would be an ideal replacement for natural bone matrices, but as yet none of the materials are able to accurately mimic the clinical success of graft material.

Bone architecture varies according to location, for example, the cortical bone found on the exterior of the femur, for example, is much more compact than the trabecular spongelike bone found in its core. The architecture also depends on the age and sex of an individual becoming much more open with age because of the onset of osteoporosis and reflects the diet and overall level of activity of the individual. However, the degree to which these factors need to be fully considered in developing bone tissue scaffolds is beyond the scope of this chapter, especially given that the mechanical response of bone has not yet been replicated by "the biodegradable polymers, ceramics, or alloys currently used in orthopedic applications" (Fu et al., 2011).

Calcium phosphate is the inorganic component of bone and is a biocompatible material that is both osteoconductive and degradable. Calcium phosphate ceramics support bone formation by promoting cell attachment, proliferation differentiation, and migration. Many types of calcium phosphate ceramics have and continue to be investigated for tissue engineering applications. The most commonly used material hydroxyapatite (HAP) is biocompatible and stimulates cell attachment, growth, and differentiation but degrades at a very slow rate. The second most widely used calcium phospate is beta tricalcium phosphate (BTCP) which has similar properties to HAP but with a faster degradation rate and is often blended with HAP.

Currently there is a lot of interest in coating polymer-based scaffolds with nanoparticulate HAP. Panda et al. (2014) have shown that the HAP coating improves the performance of an underlying silk-fibroin–based scaffold not only by increasing its stiffness and surface hydrophilicity but also improves the adhesion and subsequent differentiation of mesenchymal cells into osteoblasts. Li et al. (2014) found that the thickness of the HAP layer is important and needs to be optimized to maximize the osteogenic effect of the crystals. HAP coatings have been applied using a number of different coating technologies, which include plasma spraying, magnetron sputtering, and electrochemical treatments on implants (e.g., Daugaard et al., 2010; Gross et al., 2010; Roy et al., 2011) and scaffold polymers that include collagen, chitin, chitosan, and alginate (Qi et al., 2014; Peng et al., 2012; Jin et al., 2012). Given the potential number of variables that can occur in this type of experiment, that is, thickness of the HAP layer, size and geometry of the HAP nanoparticles, nature and geometry of the underlying scaffold, culture time/conditions, cell type and history, and methodology used to evaluate results, it is not easy to compare the findings of different publications in order to understand and therefore exploit the knowledge that has been generated.

Bioglasses are surface reactive silicon-based glass-ceramic biomaterials containing calcium and phosphorus that when dissolved stimulate expression of osteogenic genes and angiogenesis (Hench et al., 2000; Xynos et al., 2000a,b). Bioglasses tend to form bonelike apatite layers on the surface of the scaffold that mimics HAP in terms of its impact on cell behavior. They have been extensively researched since their discovery by Hench (Hench and West, 1996). There is no doubt that bioglasses have the ability to facilitate bone growth and to bond with both hard and soft tissues. The rate at which they degrade varies with the composition of the bioglass, and hence these materials have the potential to be specifically tailored to meet the requirements of different applications, that is, bone ingrowth and remodeling. Optimizing the composition of the matrix material and the method used to produce them results in scaffolds that have similar compressive strength and modulus to that of bone. However, these scaffolds are relatively brittle and have low levels of fracture toughness compared with cortical bone. An issue that is being addressed by using a biomimetic approach. Cortical bone itself is a composite material consisting of a collagen matrix (35% dry weight) reinforced by hydroxyapatite. Therefore, bioglass scaffolds have been prepared by compositing with a polymer such as polycaprolactone or PLA (Ródenas-Rochina et al., 2013, Han et al., 2014),

adding the glass as granules. A recent review by Jones (2013) provides an excellent overview of the history of bioglasses, where the field currently lies and potential new developments within it.

De-cellularized matrices

A primary research goal for developing tissue scaffolds is to manufacture something that is fit for its intended purpose, that is, it must be sufficiently biocompatible such that it does not elicit an unwanted inflammatory response, must provide an appropriate level of mechanical robustness, that is, a fibrous scaffold that is to be used for ligament repair must have comparable mechanical characteristics and function as a suitable matrix to host an increasing population of cells that are able to perform as in native tissue. This latter point is particularly important for the long-term functionality of the implant. This list of requirements represents a considerable challenge for tissue engineers and is further complicated by the fact that most tissues consists of different cell types and are mechanically heterogenous, for example, heart valves. A potential route to surmounting these challenges is to remove all traces of cellular material from native tissue to leave a collagen-rich acellular matrix that is close in composition to extracellular matrix, with the anatomical architecture and therefore mechanical performance intact. Cell adhesion to acellular scaffolds is generally superior to other scaffold material types because of the presence of the cell adhesion ligand RGD sequence. These can be produced from, for example, the bladder (Yang et al., 2010), the dermis (Moore et al., 2015), liver (Shirakigawa et al., 2012), umbilical veins (Hoenicka et al., 2013), and cardiac tissue (Momtahan et al., 2015). When implanted, the matrices are slowly remodeled by the implanted cells to potentially form new blood vessels (Jones et al., 2014), bladder (Hai-Ling et al., 2010), and cardiac tissue (Eitan et al., 2010).

Acellular matrices have also been coated with polymers to improve their mechanical characteristics and hemocompatibility, and there are a number of examples of matrices that have being approved by the regulatory bodies for use in humans.

Characterization of materials

Given that the physicochemical characteristics of the raw or starting materials used to manufacture tissue scaffolds play such an important role in determining cell behavior, it is important to understand them as fully as possible. A robust material specification will help to ensure that reproducible results are obtained when using different batches or sources of scaffold materials. An ASTM guide (ASTM F2027-08) provides an excellent compendium of those standards that cover the determination of the chemical, physical, and mechanical properties of synthetic and naturally sourced polymers as well as ceramics, metals, and composites. A similar document describes methods for characterizing tissue scaffolds (ASTM F2150-13).

The ASTM guide does not cover methods for assessing the biocompatibility of the starting material; this subject area is covered by the ISO 10993 suite of standards. ISO 10993, Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process, discusses the potential need for testing and extensive guidance as to which tests are needed and whether or not these can be carried out *in vitro* or whether animal models are required. Factors such as the potential toxicity of leachates and/or degradation products as well as the "bioavailability"[1] of the material are also discussed.

The Food and Drug Administration (FDA) in the United States has published a useful guide on how to apply the ISO 10993 standards to medical devices that are intended for use in the human body (Use of International Standard ISO-10993, 2013a,b). The guide is intended for those wishing to gain regulatory approval for commercialization of tissue scaffolds, which are considered as devices, but also contains very useful information for the research community. It is must be noted that the guidance specifically refers to the characteristics of the device[2] and not to the constituent materials per se, as it rightly states that the material characteristics can be affected by the processing method used as well as sterilization procedures and subsequent storage. The presence of other chemical species in or on the surfaces of the device that are associated with the manufacturing process, for example, mold release agents may also have an impact on the biocompatibility of the device itself. The guide also covers the issue of sampling, which is particularly important for batch testing of materials. It may also be necessary to consider biocompatibility tests that go beyond simple short-term *in vitro* cell cytotoxicity, that is, carcinogenicity, to potential effects on reproduction if there is potential commercial value in developing a tissue scaffold system.

Characterization of systems that contain nanoparticles or fibers is less straightforward that that of micrometer and larger particles or fibers, particularly when it comes to assessing the potential hazards associated with long-term exposure. Those who need to understand this area of developing science are advised to consult the literature and the websites of regulatory bodies such as the FDA and the UK Medicines and Healthcare products Regulatory Agency (MHRA) for current views and practices. Useful documents that provide additional background can be found at http://www.mhra.gov.uk/Howweregulate/Nanotechnology/, http://www.fda.gov/ScienceResearch/SpecialTopics/Nanotechnology/ucm309672.htm (Kunzmann et al., 2011, and Gil et al., 2010).

Biodegradation of scaffold materials should be considered as part of the characterization process. This is by no means a straightforward task, as the rate of degradation of a given material is usually affected by a number of variables that include temperature, pH, as well as, for example, copolymer composition, enzyme concentrations, and types for naturally occurring polymers. A survey of the literature that

[1] Bioavailability takes into account the duration and degree of expoure of the device within the body and under what conditions it will be used.

[2] The term medical device is used to describe an object that does not contain cells i.e. an un-seeded tissue scaffold.

deals with material degradation will also reveal many inconsistencies in the methodologies that are used to investigate this area of materials science, namely:

- The geometry and size of the sample used
- Whether or not the sample is porous
- The volume of liquid surrounding the sample and the frequency at which that this is changed.
- Whether the pH of the liquid that surrounds the sample is controlled or not
- What concentration of enzyme is used (for natural polymers) and the frequency at which this is replenished
- Whether any antimicrobial agents are used
- The temperature at which the tests are conducted—in many instances temperatures higher than 37°C are used to accelerate the process of degradation
- Whether the samples are agitated during the experimental period and, if so, what form does it take and what frequency is used?

These issues all relate to a controlled *in vitro* environment; a further layer of complexity will be need to be considered for *in vivo* testing, where many of these factors will be difficult to control. Protein adsorption, cell attachment, the development of an extracellular matrix may also further complicate matters.

Reporting of the material used and the results should also be carefully considered to ensure that the data recorded are as accurate and extensive as possible, covering aspects such as impurity types and concentrations. Guidance on this topic can be found in the FDA guide to using ISO 10993 part 1.

Sterilization

Identifying a robust procedure for sterilizing tissue scaffolds can be challenging, particularly if the scaffolds have been doped with labile molecules such as growth factors. This topic is discussed in more detail in Chapter 10, so only an overview of the main challenges will be provided here. For polymer-based scaffolds, the high levels of heat and moisture associated with autoclaving is likely to prematurely degrade the material or, in some cases, may lead to melting, distortion, or collapse of the scaffold. Gamma radiation is also widely used to sterilize medical equipment, but most polymers will suffer from some form of radiation damage where the polymer chains cleave thereby reducing their molecular weight and altering the level of polydispersity. These effects may have a detrimental impact on the scaffolds mechanical performance and the materials degradation profile.

Scaffolds can also be sterilized by exposing them to ethylene oxide gas, which is a known carcinogen. Although effective as a sterilizing agent the high levels of toxicity of this material requires long periods of degassing and monitoring to ensure that no residual gas remains in the scaffold. This method along with gamma radiation works with polymers that are degraded by heating, although the caveats remain that the material may be changed or contaminated as a result of sterilization.

Future trends

The composition of scaffolds both in terms of the type and mixtures of materials has changed considerably from the late 1980's when the field of tissue engineering came into existence. This enhancement in sophistication reflects our increased understanding of the needs of cells and in managing their behavior, which are fundamental to engineering functional tissues. Successful culture of functional tissue is not only reliant on the mechanical characteristics of the scaffold on which it is cultured or indeed the chemical environment that surrounds it, but also on its detailed surface chemistry and topography. These latter two characteristics are not that easy to characterize at the levels that seem to be important to cells, that is, the chemical composition of the first few tens of nanometers and the topographical characteristics ranging from the micrometer to nanometer scale or to reliably control these parameters during manufacture. Such developments will, no doubt, continue into the future with the emergence of increasingly complex scaffolds that can support viable populations of different cell types and overcome the challenges of producing them.

Sources of further information

There is a plethora of information on the web regarding materials that can and are being used to manufacture tissue scaffolds, how they are processed, and some assessment of performance. PubMed (http://www.ncbi.nlm.nih.gov/pubmed), for example can easily be searched to find publications on a particular material and its use in tissue engineering; similarly, current activities, thematic groups, and forthcoming conferences can be found at http://www.termis.org, the web site of the Tissue Engineering and Regenerative Medicine Society.

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ASTM F2212–08e1, Standard Guide for Characterization of Type I Collagen as Starting Material for Surgical Implants and Substrates for Tissue Engineered Medical Products (TEMPs).

ASTM F2103−11, Standard Guide for Characterization and Testing of Chitosan Salts as Starting Materials Intended for Use in Biomedical and Tissue-Engineered Medical Product Applications.

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