An Introduction to Biodegradable Materials for Tissue Engineering Applications

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Abstract

Tissue generation by autogenous cell transplantation is one of the most promising treatment concepts being developed as it eliminates problems of donor site scarcity, immune rejection and pathogen transfer. Cultured cells are seeded onto a three-dimensional biocompatible scaffold that will slowly degrade and resorb as the soft and hard structures grow and assimilate* in vitro *and/or* in vivo*. The 3-D scaffold provides the necessary template for cells to proliferate and maintain their differentiated state. Ultimately, it defines the overall shape of the tissue-engineered transplant. The aim of this review is to describe and discuss the scaffold materials of natural and synthetic origin that are of specific interest to tissue engineers. This review is based on previous publications and our own experience in the use of biomaterials of natural and synthetic origin for tissue engineering applications. Biodegradable polymers which have been used for tissue engineering applications are mainly based on clinically established medical devices and implants. In the group of macromolecules of natural origin collagen, alginate, agarose, hyaluronic acid derivatives, chitosan, and fibrin glue have been used as scaffolds. Man-made polymers such as polyglycolide (PGA), polylactides (PLLA, PDLA), poly(caprolactone) (PCL), and poly(dioxanone) (PDS) have been studied as matrix material to guide the differentiation and proliferation of cells into the targeted functional premature and/or mature tissue. Appropriate selection of scaffold material with respect to the targeted tissue is essential. Today, biomaterials of choice remain to be those approved by the US Food and Drug Administration. In spite of that, novel biomaterials should be developed specifically designed for tissue engineering applications.

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Introduction

The concept of tissue engineering arises from the need to develop an alternative method of treating patients suffering from tissue loss or organ failure. Current therapies in use today are not only expensive but often do not adequately fulfil their intended purpose. In standard organ transplantation, a mismatch of tissue types necessitates lifelong immunosuppression, with its attendant problems of graft rejection, drug therapy costs, and the potential for the development of cancer. Even when one's own tissues are used, the types of tissues available for reconstruction or transplantation are often unsuitable. In such circumstances, surgical invasion of another part of the body leaves a patient in pain, in jeopardy of functional losses at the donor site, and in need of additional care. These problems will be solved only when human tissues can be patient-specific designed and grown—a prospect that is closer to reality than most surgeons realise. Tissue engineering is a truly multidisciplinary field that applies the principles of engineering, life science and basic science to the development of viable substitutes, which restore, maintain or improve the function of human tissues.[1,2]

Tissue engineering techniques generally require the use of a porous bioresorbable scaffold, which serves as a three-dimensional template for initial cell attachment and subsequent tissue formation both *in vitro* and *in* vivo.[3] The biodegradability or bioresorbility of the scaffold allows it to be gradually replaced by new cells to form functional tissues. Ideally, a scaffold should have the following characteristics: (i) highly porous with interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; (ii) biocompatible and bioresorbable with controllable degradation and resorption rate to match tissue replacement; (iii) suitable surface chemistry for cell attachment, proliferation, and differentiation; (iv) mechanical properties to match those of the tissues at the

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site of implantation; (v) be reproducible processed into variety of shapes and sizes by solid free form fabrication.[4] This paper reviews the scaffold materials which are made of natural and synthetic polymers.

Polymers of Natural Origin

Collagen

Collagen forms the most substantial group of structural proteins in connective tissue and represents about one-third of total body proteins. To date, more than 13 types of collagen have been isolated, some of which have been completely characterised, and others only partially characterised.[5] The individual types differ in their preliminary structure as well as in their macromolecular arrangement. However, a simplified category can be made into three groups: interstitial or fibril forming collagen, collagen of the basement membrane, and so-called "minor collagen", which appear in relatively small amounts in the tissue.[6]

Of the naturally occurring or biological polymers used in surgery, collagen is by far the polymer most intensely studied.[7] It has been used as suture material for over a century. In general, it is derived from the submucosa of bovine or bovine intestine, hence the name "gut". The collagenous tissue derived is treated in an aldehyde solution, which cross-links and strengthens the preparation. In addition, this chemical treatment makes the collagen more resistant to enzymatic degradation. Suture material treated in this way is called "plain gut". If the suture is additionally treated in chromium trioxide, it becomes "chromic gut". Chromic gut suture is more highly cross-linked and, hence, is stronger and more resistant to biodegradation. Salthouse[8] has demonstrated that the mechanism by which gut or other collagen implant materials degrade is by sequential attack by lyosomal enzymes. In most locations, the initial attack is by acid phosphatase, with leucine amino peptidase activity increasing later during the degradation period. Collagenase is also thought to play a role in the enzymatic degradation of collagenous materials. In fact, the activity of collagenase is much higher for the processed, denatured protein than for the naturally occurring native collagen. The activity of collagenase can be reduced, however, if the collagen is cross-linked with either metal ions, which act as enzyme poisons, or by aldehydes. Consequently, treatment with gluteraldehyde, formaldehyde, or chromic salts greatly improves the degradation kinetics of these collagen-based materials.

The pure triple helical collagen molecule does not elicit a strong antigenic response. However, associated cellular debris, ground substance, or the associated nonhelical telopeptide region of the collagen molecule can evoke a rather strong antigenic response. In an effort to reduce the antigenic response of processed collagen-based materials, methods of dissociation, purification, and reconstitution of collagen have been developed.[9] The reconstitution process yields a pure, less antigenic collagen. Aqueous dispersions of reconstituted collagen may be cross-linked with an aldehyde to produce homeostatic collagen foam. Gelatin, one of the degradation products of collagen, can also be used to produce homeostatic gelatin foams. Injectable aqueous dispersions of reconstituted collagen are used in cosmetic surgery to ameliorate superficial skin defects. Collagen-based sutures, homeostatic sponges, cardiovascular implants, dressings for burns and wounds, and materials for correcting soft-tissue defects are of course well known to most surgeons and need not to be discussed in any detail here.

The diversification of collagen materials in tissue engineering applications was enhanced by two factors, the accumulation of the scientific knowledge that permitted proper engineering of collagen for as highly porous matrix, and the demand for scaffolds with characteristic biological properties, including interaction with cells. In the 1980s, Bell et al[10] were among the first to tissue-engineer bi-layered skin grafts. They showed that a collagen lattice seeded with autologous skin fibroblast contracts and forms dermal tissue, and suspensions of epidermal cells applied to these lattices *in vitro* led to differentiation of the epidermal cells. This skin equivalent has been used clinically in the treatment of venous ulcers, acute wounds and split thickness donor sites. It was reported to have similar behavior to human skin. More recently, Geesin et al[11] adopted a 3-dimensional, cross-linked bovine collagen matrix to develop a bi-layered skin model. Cross-linking was done using a dehydrothermal technique and prevented the matrix from contracting during the culture period.[11] Successes from the use of natural biodegradable cell matrices have been encouraging. However, the long-term fate of such grafts is not known. Shrinkage and the possibility of inter-species pathogen transfer are concerns.

Bovine type I collagen, presumably because of its availability in large quantities relative to other collagen types, has been utilised as a foam, sheet and gel template for cells in a number of orthopaedic tissue engineering studies. Bone-derived and bone-marrow cells have been cultured in three-dimensional collagen sponges in order to study the biology of bone cells *in vitro* and to determine the potential use of this biomaterial in transplantation and bone repair.[12] Collagen gels have been demonstrated to support chondrocyte proliferation.[13] The results of studies utilising collagen gels seeded with chondrocytes to repair articular cartilage defects in animal studies revealed some promising repair activity. One study tested collagen gels containing 36,000 cells/mm^3 implanted in 15-mm full-thickness articular

defects in the patellar joints of horses.[14] Histochemical analysis after 4 and 8 weeks revealed the deeper layers of the grafted defects contained an increased number of chondrocytes and cartilaginous components when compared to ungrafted control defects. However, structural analysis of the surface layers of collagen implanted defects appeared poorly organised. A type I collagen bi-layer scaffold was also developed and used with or without chondrocytes in rabbits.[15] Cell-seeded collagen scaffolds exhibited the best repair, in terms of histological appearance and amount of type II collagen synthesised. Gels composed of collagen and alginate were tested for their potential as vehicles for chondrocyte transplantation.[16] The chondrocytes proliferated within the collagen gels, but dedifferentiated toward a fibroblast phenotype after several days. Chondrocyte cell number in alginate gels exhibited an initial loss, but the chondrocyte phenotype was maintained. This result is consistent with data published by Beyna and Schaffer,[17] demonstrating that rabbit articular chondrocytes that dedifferentiated in mono-layer cultures will re-express a cartilage phenotype when maintained in anchorage-independent culture in agarose gels. Hence, the effect of collagen gels on chondrocyte phenotype remains an issue in tissue engineering cartilage research.

Polysaccharides

Polysaccharides form a class of materials, which have been neglected for many years in the field of biomedical engineering. Recently, recognition of the potential employment of this class of materials is growing in the field of tissue engineering.[18] Three factors have contributed to this growing recognition of polysaccharide-based scaffold materials. First is the large and growing body of information pointing to the critical role of saccharide moieties in cell signalling schemes and in the area of immune recognition in particular. Second has been the recent development of powerful new synthetic techniques with the potential for automated synthesis of biologically active oligosaccharides. These techniques may allow us to finally decode and exploit the language of oligosaccharide signalling. The third factor is the demand in tissue engineering research for new scaffold materials with specific, controllable biological activity, in combination with different degradation and resorption kinetics. Apart from their biological activity, one of the significant properties of polysaccharides is their ability to form hydrogels. Hydrogel formation can occur through a number of mechanisms and is strongly influenced by the types of monosaccharide involved, as well as the presence and nature of substituent groups. Polysaccharide gel formation is generally of two types: hydrogen-bonded and ionic. Hydrogen-bonded gels are typical of molecules such as agarose (thermal gellation) and chitosan (pH-dependent gellation), whereas ionically-bonded gels are characteristic of alginates and carrageenans. However, the distinction is limited, since some charged polysaccharides exhibit hydrogen-bonded gel formation under neutral conditions.

Alginate

Alginate is a crude product extracted from alga and contains several inflammatory components. (Salts of alginic acid are hydrophilic colloidal carbohydrates that are extracted from marine kelp.) Calcium, sodium, and ammonium alginates have been used as foam, clot or gauze for absorbable surgical dressings.[19] The purity of alginate has been found to be a pertinent factor in the biocompatibility of alginate-based poly-L-lysine capsules.[20] During recent years, a number of purification procedures for alginates have been described[21] that do not interfere with the molecular composition of the alginate as much. Virtually all these procedures are composed of filtration, precipitation and extraction steps. Purification substantially reduces the host tissue response. Applying purified alginate substantially reduces the host response but studies with purified alginates demonstrate two other pertinent obstacles in the clinical application. First, the duration of graft function is substantially prolonged but still limited to periods of 3 months to a year in spite of a virtually absent host response.[22,23] Second, fibrous tissue overgrowth is always found in a small portion of the capsules. This latter observation shows that biocompatibility of the microcapsules is influenced not only by systematic inadequacies, such as impurities in the materials applied, but also by individual imperfections of a more physical nature. Due to the constraints described above, alginate has been almost entirely replaced by the polysaccharides (see following) as matrix material in tissue engineering applications.

Agarose

Agarose is a natural-derived polymer primarily composed of alternating units of -galactopyranosyl and 3,6-anhydro-galactopyranosyl units. It is a seaweed-derived charged polysaccharide which forms a gel in the presence of supra physiological concentrations of calcium ions. It exhibits a temperature-sensitive water solubility that can be utilised to entrap mammalian cells. In a typical procedure, an agarose/cell suspension is transformed into liquid microbeads by extrusion or simply oil-in-water dispersion, which is hardened by a reduction in temperature. Agarose entered tissue engineering as an experimental material for encapsulating endocrine cells, such as insulin-secreting pancreatic islets, for transplantation by injection. A homogeneous phase of agarose layer, without a permeoselective barrier, was shown to be sufficient for cell entrapment. Coating the entrapped cells in agarose with an additional agarose layer can eliminate a possible drawback,

namely the possibility of cellular protrusion through the agarose membrane.[24]

Agarose gels seeded with chondrocytes have been utilised as a vehicle to apply biological and mechanical stimuli to test for factors that affect *in vitro* and *in vivo* engineering of cartilage. Cell agarose composites have been used to investigate factors that affect chondrocyte metabolism, including various growth factors, cell shape, synthesis of an extracellular matrix, *ex vivo* synthesis of cartilaginous material, cell phenotype and efficacy of cellular-agarose composites to promote repair of articular cartilage defects in animal models.[24,25]

Compressive strains applied to chondrocyte seeded agarose matrices affect synthesis of glycosaminoglycans, DNA, and total protein.[17] Firm agarose gels support chondrocyte proliferation and are reported to sustain the differentiated phenotype. Even after chondrocytes propagated as a mono-layer dedifferentiate as a consequence of serial passage, when propagated as a suspension culture in agarose gels, they re-express a differentiated phenotype as assessed by re-expression of type II collagen.[26] Rabbit articular cartilage full-thickness defects, implanted with agarose gels embedded with allograft chondrocytes, exhibited significant healing when compared with controls. New subchondral bone formed and the newly synthesised tissue appeared to integrate with host articular cartilage.[27]

Chitin/Chitosan

Chitosan is a semi-crystalline polymer and the degree of crystallinity is a function of the degree of deacetylation. Crystallinity is maximal for both chitin (i.e. 0% deacetylated) and fully deacetylated (i.e. 100%) chitosan. Minimum crystallinity is achieved at intermediate degrees of deacetylation. Because of the stable, crystalline structure, chitosan is normally insoluble in aqueous solutions above pH 7. However, in dilute acids, the free amino groups are protonated and the molecule becomes fully soluble below pH 5. The pH-dependent solubility of chitosan provides a convenient mechanism for processing under mild conditions. Viscous solutions can be extruded and gelled in high pH solutions or baths of non-solvents such as methanol. Such gel fibres can be subsequently drawn and dried to form high-strength fibres.[28]

In the 1990s, chitosan turned out to be a useful excipient for the pharmaceutical industry. The natural polymer is used in direct tablet compression, as a tablet disintegrant, for the production of controlled release solid dosage forms or for the improvement of drug dissolution. Chitosan has been used for production of controlled release implant systems for delivery of hormones over extended periods of time. Recently, the transmucosal absorption promoting characteristics of chitosan has been exploited especially for nasal and oral delivery of polar drugs to include peptides and proteins and for vaccine delivery.[28,30]

Depending on the source and fabrication technique, chitosan's average molecular weight can range from 50 to 1000 kDa. Commercially available medical grade materials have degrees of deacetylation ranging from 50% to 90%. Structurally, chitosan is a linear polysaccharide consisting of linked D-glucosamine residues with a variable number of randomly located N-acetyl-glucosamine groups.[14] It thus shares some characteristics with various GAGs and hyaluronic acid present in articular cartilage. Since GAG properties include many specific interactions with growth factors, receptors and adhesion proteins, this suggests that the analogous structure in chitosan may also have related bioactivities. In fact, chitosan oligosaccharides have been shown to have a stimulatory effect on macrophages, and the effect has been linked to the acetylated residues. Furthermore, both chitosan and its parent molecule, chitin, have been shown to exert chemoattractive effects on neutrophils *in vitro* and *in vivo*.[31] In general, these natural polymers have been found to evoke a minimal foreign body reaction. In most cases, no major fibrous encapsulation has been observed. Formation of normal granulation tissue, often with accelerated angiogenesis appears to be the typical course of healing. In the short term a significant accumulation of neutrophils in the vicinity of the implants is often seen, but this dissipates rapidly and a chronic inflammatory response does not develop. The stimulatory effects of chitosan and chitosan fragments on immune cells may play a role in inducing local cell proliferation and ultimately integration of the implanted material with the host tissue.

In vivo, chitosan is degraded by enzymatic hydrolysis. The primary agent is lysozyme, which appears to target acetylated residues. However, there is some evidence that some proteolytic enzymes show low levels of activity with chitosan. The degradation products are chitosan oligosaccharides of variable length. The degradation kinetics appears to be inversely related to the degree of crystallinity, which is controlled, mainly by the degree of deacetylation. Highly deacetylated forms (e.g. >85%) exhibit the lowest degradation rates and may last several months *in vivo*, whereas samples with lower levels of deacetylation degrade more rapidly. This issue has been addressed by derivatizing the molecule with side chains of various types. Such treatments alter molecular chain packing and increase the amorphous fraction, thus allowing more rapid degradation. They also inherently affect both the mechanical and solubility properties[31].

One of chitosan's most promising features is its excellent ability to be processed into porous structures for use in cell transplantation and tissue regeneration. Freezing and

lyophilising chitosan acetic acid solutions can form porous chitosan structures. A number of researchers have studied chitosan-based scaffolds in various tissue engineering applications.[32-37]

For future applications, it is important that the chitosan scaffold meet mechanical properties to withstand *in vitro* and *in vivo* forces in order to provide seeded cells with a proper biomechanical environment. Hence, the scaffold matrix should be able to withstand the contraction forces of blood clot, host tissue reaction, and finally tissue formation as well as remodelling. For example, development of a chitosan-based material that can support chondrogenesis may be significant not only in terms of the quality of tissue produced, but also in terms of the ability of that tissue to integrate with the host matrix.

Hyaluronan

Hyaluronan (HA) or hyaluronic acid is a polysaccharide of the extra cellular matrix (ECM). It is a main glycosaminoglycan (GAG) having many structural, rheological, physiological and biological functions in the body. It is a linear and monotonous anionic polymer, which is heterogeneously distributed, in various soft tissues. Two modified sugars, glucuronic acid and N-acetyl glucosamine form each of the disaccharide units. HA is a soluble molecule, forming highly viscous solutions in water and interacts with binding proteins, proteoglycans, growth factors, but also actively contributes to the regulation of the water balance, acting on the osmotic pressure and low resistance and selectively sieving the diffusion of plasma and matrix proteins. In the joints, it behaves like a lubricant supporting the articular cartilage surfaces under shear stress. At a molecular level, HA acts as a scavenger molecule for free radicals.[38]

In the last decade, the use of medical grade HA has been applied widely in joint surgery, corneal transplantation, treatment of cataract, intraocular lens implantation and treatment of vitro-retinal diseases. HA has shown that it can improve wound healing due to HA degradation products induce endothelial cell proliferation and angiogenesis. This new class of hyaluronan-based biopolymers has good biocompatibility and controlled biodegradability.[39]

At present, HA based scaffolds have been studied by a number of tissue engineers due to its excellent cell and tissue compatibility.[40-43] However, water solubility, rapid resorption and short residence time at the site of implantation, might be of disadvantage especially in hard tissue applications. For this reason, several attempts have been made to modify its molecular structure to fabricate a scaffold material with sufficient physical properties for tissue engineering applications. Cross-linking and coupling reactions were two of the ways considered in obtaining a modified, stable form of HA. New classes of insoluble polymers were developed using a variety of cross-linking agents. These chemical modifications were applied either to trap HA chains within a net of cross-linked proteins, or to create covalent bonds between HA chains. The production of all these derivatives was driven by a concept similar to that which led to the production of cross-linked collagen. However, in a number of cases, concern has been expressed for the potential toxicity of some of the cross-linking agents utilised, such as gluteraldehyde, formaldehyde and isocyanates. A new type of HA was obtained by creating cross-linking bonds by directly esterifying a certain percentage of the carboxyl groups of glucuronic acid along the polymeric chain with hydroxyl groups of the same or different hyaluronan molecules. Once esterifcation of the polymer has been obtained, the material can easily be processed to fabricate different scaffold types such as membranes, sponges, and microspheres via extrusion, lyophilisation or spray drying.[39]

Synthetic Polymers

Over the last four decades, aliphatic polyesters constituted the most attractive family among which poly(a-hydroxy acids) have been extensively studied. These macromolecules constitute a class of polymers represented by the general formula -(-O-CHR-CO-)-$_n$. Mechanical properties of polymers are strongly dependent on their molecular weight, orientation and crystallinity, material purity, the presence of defects, voids and/or reinforcing elements in the material, as well as the chemical structure of the polymer. The polymer tensile strength and moduli increase with increasing molecular weight up to a plateau of molecular weight, which differs for different polymers. The increase in polymer crystallinity and orientation enhances mechanical properties in the direction of the orientation. The presence of reinforcing structures like fibres and whiskers, improve the mechanical properties, while the presence of impurities and/or additives has an adverse affect. Aliphatic polyesters are viscoelastic materials. Independent of the degradation process, such materials show time related changes in their mechanical behaviour like creep and relaxation. This has to be taken into consideration for the function of scaffold/cells constructs, which are placed in load-bearing applications.[44]

In vivo degradation and resorption of aliphatic polyesters is described as a loss of physical and/or chemical integrity, resulting from the interaction of a material with living tissue, due to the hydrolysis process. Hollinger et al[45] reported that there is a difference in the metabolism of lactic and glycolic acids. Lactic acid takes part in the Krebs cycle and is consequently excreted by the lungs as CO_2, while glycolic acid is first acted upon by glycolate oxidase

and is transformed into glycoxylate. Then glycoxylate reacts with glycine transaminase to yield glycine, which may take part either in protein synthesis or in the Krebs cycle. From physical and physio-chemical viewpoints, enzymes that are large molecules cannot penetrate solid synthetic polymers. Poly(a-hydroxy acids) has ester linkages, which are likely to be hydrolysed by aqueous medium alone or by esterase enzymes. Salthouse et al[46] studied the effect of an aqueous medium on several suture materials. He concludes that the degradation and resorption kinetics depend upon the total time spent in an aqueous environment, regardless of whether the time is totally *in vivo* or a combination of *in vitro* and *in vivo*. This implies that the biodegradation process of a bioresorbable polymer is purely a hydrolytic process and that enzymes have no effect on it. Holland et al[47] has examined critically the literature dealing with the contribution of enzymes to aliphatic polyester degradation and resorption and came to the conclusion that, for glassy polymers, non significant enzyme involvement is expected in the early stages. The involvement can be more pronounced in the later stages however, when erosion and physical fragmentation occurs. In contrast, for polymers in the rubbery state, enzymes can play a significant role in their degradation and resorption process.

In general, when *in vitro* and *in vivo* degradation and resorption kinetics are compared, or when enzyme-free and enzyme-containing buffer media are compared, as is generally done in the literature, differences can be expected for many reasons other than enzymatic degradation of macromolecules.

Poly(glycolic acid) and Poly(lactic acid)

The simplest alpha polyester is polyglycolic acid. William Carothers, the 'father' of nylon, first synthesised poly(glycolic acid) (PGA) in the 1930s. At that time, it was noted that the major limitation of this polymer was its hydrolytic instability. It was this hydrolytic instability that attracted Schmidt and co-workers.[48] In the 1960s, the procedures for the synthesis of high molecular weight PGA and the next homologue in this series of alpha polyester, poly (lactic acid) were scaled up for commercialisation. PGA is polymerised from alpha acetic acid, commercially called glycolic acid. On mild heating, glycolic acids form cyclic diamers called glycolide. When subjected to a catalytic ring-opening procedure, these alpha glycosides polymerise to form high molecular weight PGA. The most distinctive characteristic of PGA is its high crystallinity which gives rise to a high melting point (230ºC) and low solubility in organic solvents. It was used in the production of the first synthetic bioresorbable suture by American Cyanamid.[49] PGA sutures have been commercially available under the trade name Dexon® since 1970. Dexon sutures resorb rapidly and tend to loose their mechanical strength over a period of 2 to 4 weeks after implantation. A co-polymer of glycolic and lactic acids in a 9:1 ratio is commercialised under the trade name Polyglactin 910. It has adequate mechanical strength and biocompatibility for use as a material for bioresorbable sutures. Polyglactin 910 sutures are marketed under the tradename Vicryl®.[50]

Lactic acid, with its asymmetric carbon atom, is optically active. It forms optically active cyclic diamers or lactides. As in the case of glycolides, a ring opening procedure is used to produce the polymer. Poly(lactic acid) (PLA) is more hydrophobic than poly(glycolic acid), due to the presence of an extra methyl groups. This limits its water uptake and reduces the rate of backbone hydrolysis as compared to PGA. In addition, PLA is more soluble in organic solvents than PGA. Since lactic acid is a chiral molecule, it exists in two stereoisometric forms which gives rise to four morphologically distinct polymers. Poly(D-lactide) and poly(L-lactide) are the two-stereoregular polymers. Poly(D, L-lactide) is the racemic polymer obtained from a mixture of D and L lactic acid, and poly(meso-lactide) can be obtained from D, L-lactide. The polymers derived from the optically active D and L monomers are semicrystalline materials, while the optically inactive poly(D, L-lactide) is always amorphous. This fact has important practical ramifications. The amorphous poly(D, L-lactide) is usually considered for applications such as drug delivery, where it is essential to have a homogeneous dispersion of the drug within a monophasic matrix. On the other hand, the semicrystalline poly(L-lactide) is preferred in applications where higher mechanical properties are required such as, for example, sutures, staples, and orthopedic devices. It is noteworthy that there is no linear relationship between the ratio of glycolic acid to lactic acid and the physicomechanical properties of the corresponding co-polymers. Whereas PGA is highly crystalline, crystallinity is rapidly lost in co-polymers of glycolic acid and lactic acid. These morphological changes lead to an increase and decrease in the rates of hydration and hydrolysis.[51]

The early polymer scaffolds used for the purpose of cell transplantation and tissue regeneration were mesh or felt-like materials based on PGA and PLGA (Fig. 1). Over the last decade a great number of tissue engineers have applied non-woven or mesh-like scaffolds composed of polymer fibres of PGA, PGA/PDLA, and PGA/PLLA. This work has been reviewed by Freed et al[52] and will not be discussed here. Foam-like scaffolds can be designed and fabricated to meet the specific targets for tissue engineering applications, e.g. balancing high interconnectivity of pores (3D internal geometry) with overall structural integrity (mechanical

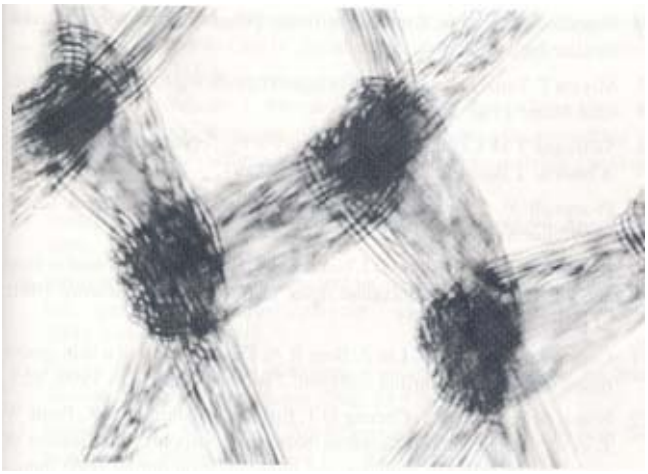

Fig. 1. ILM image of Vicryl mesh/fibroblast construct in vitro *cultured 2 weeks. Dermal fibroblasts proliferated along the interconnections of the non-woven.*

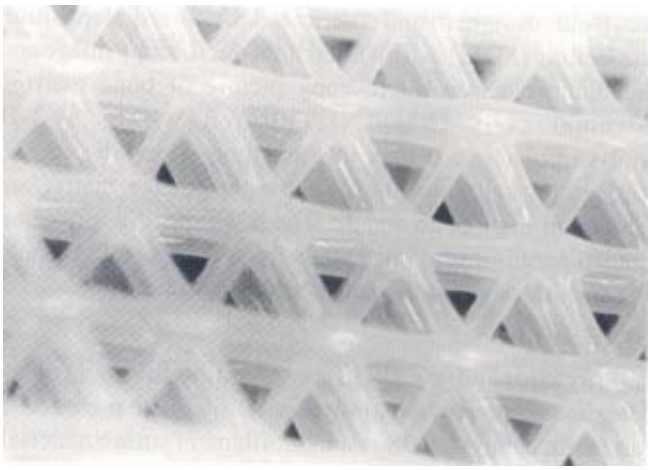

Fig. 2a. Phase-contrast light microscopy image of PCL scaffold with a lay-down pattern of 0/60/120º (x 25).

Fig. 2b. ILM image of PCL scaffold/osteoblasts construct in vitro *cultured 4 weeks. Osteoblasts proliferated, migrated, linked with their filaments and secreted dense nodules (arrows) within extra-cellular matrix in the scaffold (x 100).*

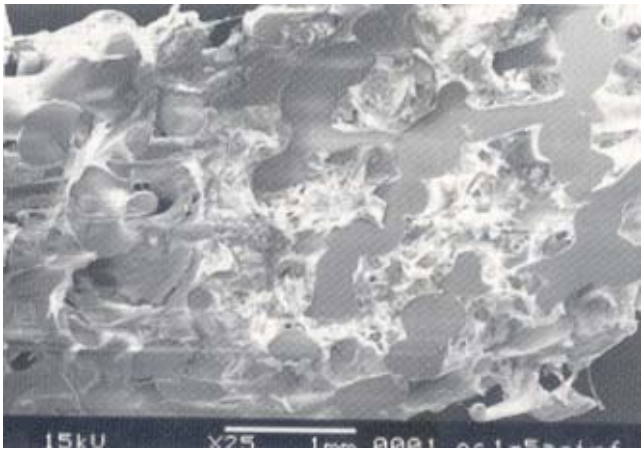

Fig. 2c. SEM image of the scaffold/osteoblasts construct in vitro *cultured 8 weeks. The scaffold was filled with extra-cellular matrix richly produced and secreted by osteoblasts (x 25).*

characteristics). Therefore, several foam fabrication methods have been applied to provide PGA. PLA and PLGA based scaffolds with good mechanical properties to generate mainly hard tissues. Several reviews have discussed this approach.[4,53-55]

Poly(caprolactone)

Poly(caprolactone) was one of the earliest polymers synthesised by the Carothers group in the early 1930s.[56] It became available commercially following efforts to identify synthetic polymers that could be degraded by microorganisms. Poly(caprolactone) can be prepared by either ring-opening polymerisation of caprolactone using a variety of anionic, cationic and coordination catalysts or via free radical ring-opening polymerisation of 2-methylene-1-3-dioxepane. Poly(caprolactone) is a semicrystalline polymer. Its crystallinity tends to decrease with increasing molecular weight. The high solubility of poly(caprolactone), its low melting point (59 to 64ºC) and exceptional ability to form blends has stimulated research on its application as a biomaterial. Poly(caprolactone) have slow degradation and resorption kinetics and can therefore be used in drug delivery devices that remain active for over one year. The Capronorä system, a one-year implantable contraceptive device, has become commercially available in Europe and the US. The toxicology of poly(caprolactone) has been extensively studied as part of the evaluation and clinical approval of Capronorä. Based on the literature, poly(caprolactone) is currently regarded as a non-toxic and hard and soft tissue compatible material.[57,58]

Several groups[59-61] could show the good biocompatibility of two-dimensional PCL specimens in human osteoblast-like cell cultures. Marra et al[59] concluded from their 2-D cell studies that PCL is superior to PLGA for bone cell growth. Cell migration requires a biomolecular healthy and dynamic interaction between the cell, the scaffold surface and its cytoskeleton. Human osteoblast-like cell culture data evidence the good biocompatibility of PCL for hard tissue formation. Figure 2a shows a novel PCL scaffold designed and fabricated by fused deposition

modelling (FDM). An inverted light microscopy image and scanning electron microscopy picture of bone marrow stromal cells (BMSCs) cultured within a 3-D polycaprolactone matrix in a medium which directs the progenitor cells into the osteoblastic lineage matrix are shown in Figures 2b and 2c. The bone specific formation and mineralisation of the extracellular matrix (ECM) could be seen throughout the entire scaffold architecture.

Poly(dioxanone)

Poly(dioxanone) (PDS) is a homopolymer of p-dioxanone, prepared by polymerisation in the presence of an Et_2Zn catalyst. PDS is a monofilament suture material, which elicits a low tissue reaction it can be regarded as a modified PGA. It retains a resorbable character due to the presence of the ester bond, and its flexibility is improved due to the change of one ester linkage to an ether linkage. It retains 60% strength after 4 weeks and the total strength loss is after 8 weeks. The time of complete resorption is approximately 4 to 6 months. Other medical device applications of PDS are fixation pins, staples, braided mesh, and as foil to reconstruct the orbita wall.[51] A PDS fleece was applied as a synthetic ECM in tissue engineering articular and flexible cartilage.[62-64]

Conclusion

Appropriate selection of a suitable biodegradable material is an essential component in the field of Tissue Engineering. Although there are numerous variety of biomaterials to choose from, it appears that the biomaterials of choice remain to be those approved by the US Food and Drug Administration (FD). This is because approval of new biomaterials for medical use is usually a lengthy and costly process. Nevertheless, with the advancement of Tissue Engineering, novel biodegradable materials will have to be developed.

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