Biodegradable polymers and composites in biomedical applications: from catgut to tissue engineering
Part 2 Systems for temporary replacement and advanced tissue regeneration

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During the past century, and particularly in the past three decades, the application of conventional materials technology has resulted in clear advances in substitution medicine. For example, the development of artificial hips and knees have brought enormous benefits to patients. However, there are still no materials available that can adequately replace or aid the regeneration of functional tissues such as bones or large bone segments. In an increasingly aging population, malfunction or loss of tissue from injury or disease has led to reduced quality of life for many patients at significant socio-economic cost. There is thus demand for the development of new therapies through a multidisciplinary, biology driven approach in which biological tissues are engineered using both materials and bio-technologies. Tissue engineering has created a new field of application for biodegradable polymers, paving the way for the development of new classes of biomaterials from synthetic or natural origin and for the design of new materials formats for hybrid tissues. A general overview is provided of the main applications of biodegradable polymers in medicine, with particular emphasis on tissue engineering. The approaches available to tissue engineers and the requirements scaffold materials must fulfil to promote adequate interaction with cells and tissues are described; predictable trends and future developments are discussed. Processing routes for biodegradable polymeric scaffolds are also considered, presenting examples of three-dimensional materials fabricated by different methodologies.

Keywords: Tissue engineering, Biomaterials, Biodegradable polymers, Scaffolds, Temporary applications IMR/337b

Introduction

It is well established that biodegradable polymeric biomaterials are valuable in many short term medical applications that require the temporary presence of an implant, because they do not have to be surgically removed after fulfilling their functions in the human body. This advantage of degradable polymers has paved the way for a number of important biomedical applications.

Despite these enormous benefits, the limits of this approach are being reached and new breakthroughs can be expected only from novel hybrid technologies, such as tissue engineering, that can overcome the shortcomings of current materials technology. Tissue engineering involves the *ex vivo* culture of human cells, usually in polymeric scaffold materials, and allowing them to develop into three-dimensional 'living' constructions that can potentially integrate with the surrounding native tissue, after implantation.

Several biomaterials have recently been proposed as 'ideal' scaffolds for tissue engineering, but only a few have reached clinical application. For most applications, tissue engineering scaffolds must provide cell anchorage sites, mechanical stability and structural guidance; further, when implanted, they must provide an adequate interface to respond to physiological and biological changes, to promote integration with the surrounding native tissue. Formation of new tissue is deeply influenced by the three-dimensional environment provided by the scaffolds: composition, porous architecture and, of course, biological response to implanted cells

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and/or surrounding tissues. Therefore, the development of such scaffolds poses significant challenges. To meet all the necessary requirements, scaffold materials must be fabricated from polymers with adequate properties, but the establishment of basic properties and design constraints is not straightforward and requires a deep knowledge of the factors affecting cell/tissue–scaffold interactions. Many of these features are dictated by the scaffold fabrication methodology. However, the success of any tissue engineered construct also depends on complex issues such as cell sourcing and culturing methods.

Biomedical applications of biodegradable polymers

Biodegradable polymers have found many commercialised short term biomedical applications; other potential applications are still under investigation.

One of the most widely studied applications of biodegradable polymers is their use as implantable (or non-invasive) drug delivery carrier devices for controlled release of antibiotics, anti-inflammatory agents or other drugs.[1–17] More recently, these devices have been investigated for the release of specific growth factors,[18–20] or even, in the near future, for the delivery of isolated genes for *in situ* gene therapy.[1,21,22] Sutures are the oldest and one of the most important applications of biodegradable polymers.[23–26] Other current applications include orthopaedic fixation devices,[23,27–37] wound coverings,[38–40] nerve guides,[41–45] and artificial veins and arteries,[23,26] among many others.

These applications can be grouped into three categories: temporary barriers, drug delivery devices and temporary scaffolding materials. Biodegradable polymers used in these applications were detailed in the first part of this review.[46]

Temporary barriers

One of the major applications of a temporary barrier is in adhesion prevention after surgery that occasionally causes serious complications. The temporary barrier takes the form of a thin polymeric film or a mesh-like device that can be placed between adhesion tissues at the time of surgery,[47,48] functioning as a barrier to enable traumatised tissue to be separated from adjacent tissues during the tissue healing process. Therefore, these materials should be flexible and tough enough to provide a tight cover over the traumatised soft tissue and should biodegrade after the injured tissue is completely regenerated.

Temporary barrier-type devices can also serve as basis for the development of artificial skin for the treatment of burns and other skin lesions.[47,48] In fact, this is a widely investigated application, which has become the first tissue engineered product commercially available.

Drug delivery carriers and devices

Drug delivery devices are another exciting and widely investigated application for which biodegradable polymers offer tremendous potential since these devices are intrinsically temporary.[47–49]

Drug delivery systems are designed to release a bioactive agent in a specific location at a specific rate, facilitating optimum dosage and duration of the treatment, and consequently reducing dose frequency.[50,51] Therefore, these systems minimise harm to the patient and potentially improve human health in many ways. Delivery systems have been applied in several therapies,[52–57] to deliver insulin, anticancer drugs, anti-inflammatory agents, growth factors and contraceptives, for example. These systems exist as several types of vectors, used in accordance with the location at which they will act, the agent they carry, the administration method, the degradation rate and the physical and chemical properties, among other parameters.

A wide range of degradable polymers[1–17,47,48] of both synthetic and natural origin are useful for the production of drug delivery systems. Together with the wide range of bioactive agents that can be incorporated, the various sizes and shapes and the possible means of administration, have made such systems capable of being used in many therapeutic applications.

Temporary scaffolds

A temporary scaffold can be used when the natural tissue has been weakened by disease, injury or surgery and requires some artificial support until it heals and regains its strength.

A healing wound, for example, requires the use of sutures for helping regeneration. Sutures are the earliest, successful application of synthetic, degradable polymers in human medicine. The first sutures, made of poly(glycolic acid), became available in 1970 under the trade name Dexon.[47–49] It is therefore a very mature area, which is not expected to grow very rapidly in the future.

Biodegradable polymers have also found use as temporary scaffolds in dental applications.[49] In fact, porous polymeric particles can be employed as void filler after tooth extraction aiding in quicker healing.

Orthopaedic fixation devices[47–49] are another example of where biodegradable polymers can provide temporary support with the important additional advantage over metal implants of allowing transfer of stress over time to the injured area, facilitating tissue regeneration. However, current biodegradable polymers do not exhibit sufficient strength for bone plates to support long bones, such as the femur, or for other load bearing applications. Nevertheless, polymers have been used in several applications with less demanding mechanical properties, such as interference screws in the ankle, knee and hand areas, tacks and pins for ligament attachment and meniscal repair, and rods and pins for fracture fixation.

Biodegradable vascular grafts and stents are also examples of temporary scaffolds that are used when a blood vessel is damaged, for example. At present, only investigational devices are available for these applications where blood compatibility is a major concern.

Nowadays, the term 'biodegradable scaffold' is usually specifically associated with three-dimensional porous support materials used for cell growth *in vitro*, to build up a biological substitute for an organ or tissue that has lost its function.[47–49,58] This application of biodegradable polymers constitutes the basis of tissue engineering, one of the most exciting areas of biomedical research,[49,58] which will be considered in detail below.

There has been a trend towards the development of increasingly sophisticated applications for degradable biomaterials by combining several functions within a

single device.[47,59–61] These applications usually envision materials with a narrow range of properties designed with a very specific aim. For example, a biodegradable bone nail plate that holds a fractured bone in place can simultaneously stimulate the growth of new bone tissue and prevent infection at the fracture site by slowly releasing bone growth factors (e.g. bone morphogenic protein or transforming growth factor-β) and antibiotics, respectively, throughout its degradation.[47] The possibility of combining several functions is also very attractive for tissue engineering scaffolds, as these materials are required to perform a complex role in the development of tissue substitutes. Again, this issue will be further discussed below.

Tissue engineering: the great opportunity for biodegradable polymers?

The term *tissue engineering* was initially defined by the attendees of the first NSF (National Science Foundation, USA) sponsored meeting in 1988 as the 'application of the principles and methods of engineering and life sciences toward fundamental understanding of structure–function relationship in normal and mammalian tissues and the development of biological substitutes for the repair or regeneration of tissue or organ function'.[62] In 1993, after summarising the early developments in this field, Langer and Vacanti,[63] the so called 'fathers' of this branch of science, defined tissue engineering as 'an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain or improve tissue function'.

Tissue engineering offers the possibility of aiding regeneration of tissue damaged by disease or trauma and, in some cases, of creating new tissue and replacing failing or malfunctioning organs. This is achieved[21,64–71] through the use of degradable biomaterials to induce surrounding tissue and cell ingrowth or to serve as temporary scaffolds for transplanted cells to attach, grow, and maintain differentiated functions.[64,66,68–83] The role of the biomaterial is temporary, but crucial to the success of the strategy and therefore the selection of a scaffold material is critical.

The criterion of biodegradability excludes the use of all metals and most ceramics as scaffold materials.[70,84] Although biodegradable/bioresorbable ceramic materials, such as tricalcium phosphate and sea coral, have been used with some success[70,84] as scaffold materials (mainly in orthopaedic applications), they do have limitations: they are usually brittle and difficult to process into porous materials with complex shapes. Moreover, many of the corals with the best porosity distribution are covered by the CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) treaty, and there are concerns with the build up of heavy metal contaminants. More generally, it is difficult, if not impossible, to generate matrixes with clinically useful degradation rates from most available ceramics.

Polymers, on the other hand, are ductile and easily formed. Furthermore, there are many biocompatible polymers available, as is apparent from their numerous biomedical applications.[1–45,70,84] Nevertheless, the emerging field of tissue engineering[21,64–69,72–79,85,86] is creating new demand for such materials. Tissue engineering demands specific requirements that are not met by most current biodegradable polymers or those under investigation for biomedical applications.

Designing future biomaterials

As stated above, the ultimate goal of tissue engineering is to replace, repair or enhance the biological function of damaged, absent or dysfunctional elements of a tissue or an organ. This goal is accomplished using cells that are manipulated through their extracellular environment to develop engineered tissues that can function as living biological substitutes for tissues that are lacking.[87] Many different strategies may be used to develop these engineered tissues. The selection of the best strategy for developing hybrid materials for the regeneration of a specific tissue defect is determined by several factors, such as the technical feasibility, required properties of the implant and the interaction of the host with the graft.[87]

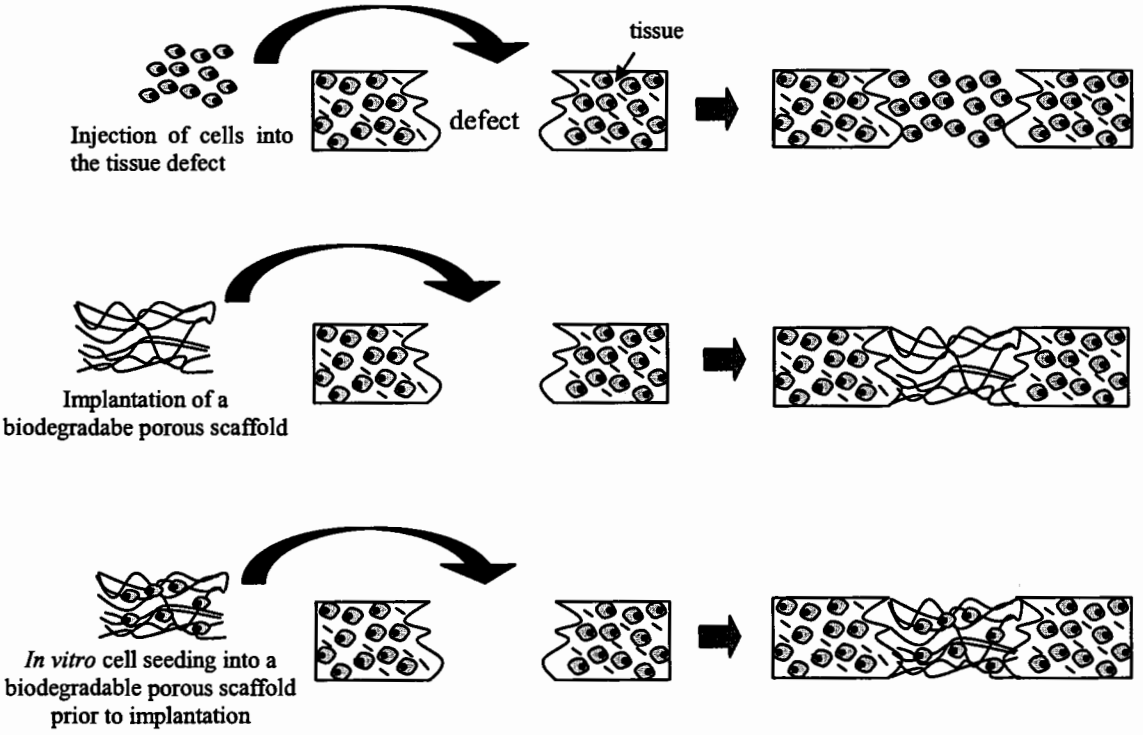
Basically, three general strategies (schematically represented in Fig. 1) have been adopted for the creation of new tissue;[63,88–90] these are described below.

Cell self-assembly

This approach corresponds to the direct *in vivo* implantation of isolated cells or cell substitutes[63,88–91] and it is based on cells synthesising their own matrix. This approach avoids the complications of surgery, allows replacement of only those cells that supply the needed function and permits manipulation of cells before infusion. Its potential limitations include failure of the infused cells to maintain their function in the recipient, and immunological rejection.[63,88,89] For other authors,[92] this approach involves a layer of cells secreting their own matrix, which over a period of *in vitro* culturing becomes a sheet and the formation of multiple layers can eventually result in the formation of skin substitutes or blood vessels, for example. However, it is well known that many cell types are *anchorage dependent* (their function is dependent upon specific cell–substrate interactions), and therefore their direct transplantation or *in vitro* culturing without a scaffold, as suggested in this approach, results in cell death or loss of function.[84,88]

Acellular matrix

This approach is based on the direct *in vivo* implantation of biomaterials[84,88–91,93] and relies on the ingrowth of tissue and cells into a porous material; this process, by which the regeneration is affected by ingrowth from surrounding tissue, is known as *tissue induction*.[84] In many cases, in this approach, the matrix (polymeric scaffold) is loaded with growth factors or some other therapeutic agent. With this approach, the issue of cell sourcing is eliminated, but its success depends on the infiltration and recruitment of the appropriate type of cells from the body in order to populate the construct and thus facilitate a proper tissue repair. However, as these are the patient's own cells, there is no concern regarding immunological rejection. In addition, such matrixes are readily available and usable 'off-the-shelf'.



top, cell self-assembly; *centre*, acellular matrix; *bottom*, cell-seeded polymeric scaffold

1 Schematic representation of generic tissue engineering approaches to regenerate a tissue defect

Cell-seeded polymeric scaffolds

In this approach, the temporary scaffold provides an adhesive substrate for the implanted cells and a physical support to organise the formation of the new tissue.[63,84,88–91,93,94] Transplanted cells adhere to the scaffold, proliferate, secrete their own extracellular matrixes (ECM) and stimulate new tissue formation. During this process, the scaffold gradually degrades and is eventually eliminated.[89,94,95] This is considered by many as the classic tissue engineering approach and is the most widely studied.

From the description of the main generic tissue engineering approaches it is possible to conclude that the regeneration/substitution of damaged tissue is strongly dependent on the interactions of three main components: the polymeric matrix, the cells and the growth factors. It is also obvious that more advanced tissue engineering strategies will result from the combination of these approaches. In this sense, tissue engineering will cross the field of drug delivery as drug delivery approaches can be applied to encapsulate living cells for incorporation within the scaffolds or scaffolds can be designed as drug delivery carriers to control a site and time specific release profile and also to protect the growth factor. The lack of an optimal carrier has been limiting the widespread implementation of strategies of this type. Many other recent and future developments in materials science (not to mention those in the biology related sciences) will bring important contributions to this multidisciplinary field. Some examples of these developments will be given in the next section.

Opportunities and challenges in tissue engineering scaffolding

The first aspect of the scaffold design to be considered is related to the selection of the biodegradable polymer that will be used for its construction. Traditionally, in spite of the wide range of biodegradable polymers available, there is a strong tendency to choose those that have a history of regulatory approval, using materials primarily developed for other applications, instead of letting the application guide the choice of the material.[58] This has created additional difficulties for the development of new materials with improved properties, specifically tailored for tissue engineering applications. Currently available natural and synthetic polymers were described in detail in the first part of this review.[46]

Nevertheless, it is quite obvious that the increased demands placed on scaffold performance will continue to fuel the necessity not only for improving the performance of the existing medical grade polymers, but also for developing new polymers.

The selection of the appropriate tissue engineering approach will define the most adequate scaffold design and the corresponding required properties, which must be able to induce the desired tissue response.[46,87] Although three-dimensional porous structures have been recognised as the most appropriate design to sustain cell adhesion and proliferation, several specific applications in tissue engineering may take advantage of other design formats or combination of different materials designs.[58] For example, it may be useful, in some cases, to enhance cell activity on one surface of a device while precluding transverse movement of surrounding cells onto that surface, which can be achieved using a barrier material, i.e. a membrane.[58] In other cases, it is helpful to use gels to encapsulate and, to a more limited extent, isolate, cells from surrounding tissues, especially to preclude antibody response to homograft and xenograft cells. Microspheres might also be used to encapsulate cells, growth factors or drugs and deliver them to a specific desired location.

In fact, as the demand for new and more sophisticated scaffolds develops, materials are being designed that have a more active role in guiding tissue development. Instead of merely holding cells in place, these matrixes are designed to accomplish other functions through the combination of different format features and materials.[83] A good example of this is the use of drug delivery devices that can act simultaneously as scaffolds for cell growth, as mentioned above. Other approaches include, for example, the combination (or incorporation) of microspheres (with encapsulated cells, growth factors or other therapeutic agents) with a polymeric matrix. Such multifunctional devices can also be designed as injectable materials, with the advantage of providing minimal invasive surgery procedures for their implantation in the body.

Another important field of current research in tissue engineering scaffolding is related to the development of external-stimuli-responsive matrixes,[96,97] i.e. matrixes that have in their composition and structure certain elements that allow them to respond to a certain stimuli that can be produced by different mechanisms, such as magnetism, electric effects, ultrasound, irradiation. This can enhance the ability of tissue engineering constructs to resemble natural human tissues and therefore to better perform their function *in vivo*, but also *in vitro*, if provided with adequate culture conditions.

Tissue engineering will also benefit in the future from the current studies on the development of smart polymers, engineered smart matrixes[97,98] and novel techniques such as molecular imprinting.[97,99,100] Engineered smart matrixes envision the use of several materials with different properties and/or consecutive coatings, or multilayer matrixes that produce multiple release phases. This allows the design of systems with multiple release times or even pulsatile release and preprogrammed delay periods, by selecting the initial properties of the polymers used. Molecular imprinting[97,99,100] is an emerging field that produces chemical architectures that can bind analytes and differentiate between closely established isomers, and thus constitute another potential source of new inputs to tissue engineering scaffolding.

Requirements for biodegradable tissue engineering scaffold: present situation and future outlook

The requirements for a scaffold material to be considered suitable for tissue engineering applications are complex and in many cases there is no consensus among the biomaterials research community about the specific demands that are required for a particular application. These requirements depend mainly on the tissue to be restored and on the location and size of the defect to be treated. Nevertheless, there are some general key characteristics that a scaffold material must possess.

1. *Biocompatibility* both in as implanted and degraded form, i.e. the scaffolds and their degradation products should not invoke an adverse immune response or toxicity (Refs. 49, 58, 62, 84, 88, 93, 101–107).

2. *Appropriate mechanical properties* to provide the correct stress environment for the neo-tissue; this is particularly important for the regeneration of hard tissues (Refs. 49, 58, 62, 91, 93, 101–105, 108–111).

3. *Controlled degradation rate*: because tissues regenerate at different rates, the degradation rate should be adjustable to match the rate of tissue regeneration, since strength decreases as the material degrades over time (Refs. 49, 58, 62, 84, 88, 93, 101, 104–107, 111).

4. *Appropriate pore size and morphology*: porosity, pore size and pore structure are important factors that are associated with nutrient supply to transplanted and regenerated cells (Refs. 62, 84, 88, 93, 101–105, 107, 111). Small diameter pores are preferable to yield high surface area per volume, as long as the pore size is greater than the diameter of a cell in suspension (typically 10 μm). There is a lack of consensus regarding the optimal pore size for maximum tissue ingrowth and/or for an optimal cell growth, but it is generally accepted that this depends on the tissue that is to be restored/substituted. In the case of bone regeneration, some authors maintain that a maximal tissue ingrowth is attained with a pore size ranging from 200 to 400 μm,[93] for others it should be from 100 to 150 μm[104] or from 100 to 350 μm,[112] for example. Interconnectivity between pores is highly desirable when compared to isolated pores, since an interconnected pore network structure enhances the diffusion rates to and from the centre of the scaffold and facilitates vascularisation (Refs. 62, 84, 88, 93, 101–105, 107, 111, 113), thus improving oxygen and nutrient supply and waste removal.

5. *Appropriate surface chemistry for cell attachment, proliferation and differentiation*: because most organ cell types are anchorage dependent, they require the presence of a suitable substrate to retain their ability to proliferate and perform differentiated functions since cell adhesion is the prerequisite for further cellular functions, such as spreading, proliferation, migration and biosynthetic activity (Refs. 58, 62, 84, 88, 101–104, 107, 111, 113, 114). Therefore, the surface characteristics of materials, whether their topography, chemistry, surface energy or wettability, play an essential role in cell adhesion on biomaterials.[115–118] However, it is very rare that any biomaterial with good bulk properties for a specific use in the biomedical field also possesses the required surface characteristics[119,120] for that application. It follows that most biomaterials need surface modification to acquire surface characteristics that allow adequate cell adhesion.[119–120] These surface modifications include, for example, roughening, coating, blending and grafting.[119]

6. *Easily sterilised* either by exposure to high temperatures, ethylene oxide vapour, or γ-radiation and remaining unaffected by one of these techniques.[49,84]

7. *Easily processed into three-dimensional shapes of irregular geometry* that can be maintained after implantation (Refs. 49, 58, 84, 88, 93, 101, 104, 105, 107). In some cases, a scaffold with unique three-dimensional geometry is required to fit an irregular defect. The regenerated tissue is therefore expected to take the shape of the initial scaffold.

Tissue engineering is clearly demanding more sophisticated scaffold materials that can ideally perform multiple functions and therefore help to create hybrid tissue substitutes more rapidly and with improved biological performance. Therefore, in future, to design adequate materials for these functions it will be necessary to address other aspects besides these basic requirements.

In addition, there are still technological constraints that need to be overcome in order to obtain scaffolds with the designed structure and properties. First of all, tissue engineers will need to comprehend exactly the target application of the materials that they are designing and tailor them for regeneration of that specific tissue defect. For this purpose, it is vital to understand the available choices among existing polymeric biomaterials that qualify for a given application and to use the possibility of local delivery of drugs/growth factors, hormones, etc. to induce tissue regeneration or a certain therapeutic effect, by using adequate carriers, or use smart materials that can accomplish additional advantageous features.

It is also important to create *in vitro* and *in vivo* complex models that can lead to accurate evaluations of the performance of tissue engineering constructs, revealing essential information about the performance of these constructs regarding their specific target application.

Processing of tissue engineering scaffolds

The development of matrixes to serve as templates for cell attachment/suspension and delivery has progressed at a tremendous rate in the past few years.

As discussed in the previous section, the materials to be used as scaffolds in tissue engineering must fulfil a number of complex requirements,[44,49,62,84] such as biocompatibility, appropriate porous structure, mechanical properties and suitable surface chemistry, for example. The selection of the most appropriate polymer to produce the scaffold is a very important step towards the construction of the tissue engineered product since its intrinsic properties will determine to a great extent the properties of the scaffold. However, the selected design and method of producing these scaffolds will deeply influence its final characteristics, as it can dramatically change the type and amount of porosity, the mechanical properties and degradation behaviour, the surface properties (critical for cell adhesion and proliferation) and even the biocompatibility of the scaffold material.

Therefore, various processing techniques have been developed to fabricate these scaffolds, of which a few are:

- solvent casting[93,102,121–124]
- particulate leaching[88,93,102,121,122]
- membrane lamination[93,125]
- fibre bonding[106,127–132]
- phase separation/inversion[93,133]
- high pressure based methods[93]
- melt based technologies[121,134–137]
- microwave baking and expansion.[138]

More recently, highly porous three-dimensional (3D) scaffolds have been obtained using advanced textile technologies and rapid prototyping technologies, such as fused deposition modelling (FDM) and three-dimensional printing (3DP).[106,139–141] These engineering technologies are highly controllable and reproducible and facilitate the manufacture of well defined three-dimensional structures.[142,143]

The major problem associated with scaffolds produced by the methods developed so far is their mechanical weakness (lack of stiffness and strength), which does not allow for their use in hard tissue regeneration where high strength scaffolds are required.[108] Therefore, the search for better methods of producing porous scaffolds, so that physical and chemical properties can be simultaneously optimised, is currently an important issue especially in hard tissue engineering research.[142,144] The optimisation and customisation of these processing technologies to design and manufacture the scaffolds, requires a thorough understanding of the materials and equipment that are used.[142,144]

In the following subsections, some polymer scaffold processing methods that have been proposed, and some examples of their application, are briefly described.

Fibre bonding

Fibre meshes consist of individual fibres either woven or knitted into three-dimensional patterns of variable pore size. The advantageous characteristic features of fibre meshes are a large surface area for cell attachment and a rapid diffusion of nutrients in favour of cell survival and growth[84,88,93,104,121] that usually results from a good interconnectivity among pores. A drawback of these scaffolds might be the difficulty in controlling accurately the porosity.[84,93,104,121]

For example, interconnected fibre networks have been prepared by a fibre bonding technique that involves the casting of a L-PLA (poly(L-lactic acid) or PLLA) solution over a non-woven mesh of PGA (poly(glycolic acid)) fibres.[84,88,93,104,121,126] Solvent evaporation results in a composite material that consists of non-bonded PGA fibres embedded into a L-PLA matrix. Fibre bonding occurs during a post-treatment at a temperature above the melting temperature of PGA. Finally, the L-PLA matrix is selectively dissolved in a non-solvent for PGA, and a network of bonded PGA fibres is released. Stipulations concerning the choice of the solvent, immiscibility of the two polymers, and their relative melting temperatures restrict the general application of the technique to other polymers.[84,93,121] In addition, this method of fibre bonding does not address the problem of creating scaffolds with complex three-dimensional shapes, but it has proven successful for producing hollow tubes that have been proposed for use in intestine regeneration.[84,93,121]

In other studies, fibre meshes based on SPCL (a blend of starch with polycaprolactone, 30/70 wt-%) were obtained by a fibre bonding process, in this case consisting of the spinning, cutting and sintering of the fibres (Fig. 2). The SPCL scaffolds obtained by this method have highly interconnected pores and have exhibited a high potential for the regeneration of bone.[128,145]

Fibre meshes can also be prepared using textile processing techniques.[106,129,130] For example, Freed and co-workers[131,132] developed fibre meshes using PGA fibres previously obtained by extrusion. Non-woven meshes were produced from these fibres, using barbed needles to entangle them and lock them together. Heat setting further increased the dimensional stability of the mesh and smoothed the top and bottom surfaces.[129,132] These scaffolds, and others obtained from different polymers by similar methods, have been widely used in tissue engineering research (Refs. 128, 129, 131, 132, 146–150).

2 Three-dimensional microcomputarised tomography (μCT) image of fibre mesh based on SPCL (blend of starch with polycaprolactone) with 50% (top) and 75% porosity (bottom)

Solvent casting and particle leaching

This method consists of dispersing sieved mineral (generally water soluble, e.g. NaCl) or organic (e.g. saccharose) particles in a polymeric solution. This dispersion is then processed either by casting or by freeze-drying in order to produce porous two- and three-dimensional supports, respectively. The porosity basically results from the selective dissolution of the particles from the polymer–particles mixture, although phase separation of the polymer solution can also contribute to the porous structure.[104] A variation of this method includes the use of vibration during dissolution of the polymer in the solvent and during solvent evaporation.[151] The porosity and pore size can be controlled independently by varying the amount and size of the soluble particles, respectively. The surface area depends on both initial soluble phase weight fraction and particle size.[84,89,93,104,121] The disadvantages of this method, as it has been applied so far, include the extensive use of highly toxic solvents and the limitation that only thin wafers or membranes, up to 3 mm thick, can be produced (Refs. 84, 93, 102, 104, 106, 121).

This technique was validated for PLLA and PLGA but can be applied to any other polymer that is soluble in a solvent (Refs. 84, 88, 93, 102, 104, 106, 121), such as chloroform or methylene chloride. Scaffolds produced by this method have been used in a significant number of studies concerning their application in tissue engineering (Refs. 151–161).

Membrane lamination

The membrane lamination method uses membranes previously prepared by solvent casting and particle leaching. Membranes with the appropriate shape are solvent impregnated, then stacked in a three-dimensional assembly with continuous pore structure and morphology.[104] The bulk properties of the final three-dimensional scaffolds are identical to those of the individual membranes.[84,93,102,104]

This method may allow the construction of three-dimensional polymer foams with precise anatomical shapes, since it is possible to use computer assisted modelling to design templates with the desired implant shape.[104] However this fabrication technique is time consuming, because only thin membranes can be used. Another disadvantage is that the layering of porous sheets may only generate a limited number of interconnected pores.[106]

Melt moulding

Melt moulding has normally been used in combination with porogen techniques or to produce a preform of the final material, e.g. to produce fibres that will be used in fibre bonding methods, as described above, or in the high pressure method described below.

One application of this method consists of loading a mixture of fine PLGA powder and gelatine microspheres into a PTFE (polytetrafluoroethene) mould and then heating to above the glass transition temperature of the polymer.[84,93,104,121] The PLGA–gelatine composite is subsequently removed from the mould and gelatine microspheres are leached out by selective dissolution in distilled deionised water. In this way, porous PLGA scaffolds with geometry identical to the mould can be produced, and scaffolds of various shapes can be achieved by simply changing the mould geometry. This method also offers independent control of porosity and pore size[84,93,104,121] by varying the amount and size of microspheres used, respectively. In addition, it is possible to incorporate bioactive molecules in either polymer or gelatine microspheres for controlled drug delivery because this process does not utilise organic solvents and is carried out at relatively low temperatures for amorphous PLGA scaffolds. Other leachable components besides gelatine may be used.

This manufacturing technique may also be applied to PLLA or PGA. However, higher temperatures (above the polymer melting temperatures) are required because these polymers are semicrystalline, and this excludes the possibility of incorporating protein into these systems.[84,93,104,121]

Melt based techniques have also been extensively studied in the context of starch based scaffolds.[134,136,137] For example, a method based on compression moulding combined with salt leaching has been developed. A starch based polymer is blended with leachable particles of different sizes, in sufficient amounts to provide a blend with a continuous polymer phase and a dispersed phase of leachable particles. The mixture is compression moulded into a desired shape and then immersed in water to remove the salt particles, leaving an interconnected pore structure with controlled porosity and pore size.[136,137] Starch based scaffolds have also been produced using melt moulding alone, based on traditional technologies such as injection moulding or extrusion with blowing agents (Fig. 3). Here, the polymers are mixed with blowing agents, selected according to their decomposition temperatures, toxicity, etc., and processed in a extruder or injection moulder. These methods allow the production of highly reproducible scaffolds with very complex three-dimensional structures,[134,136,137] since it is possible to match the mould shape very precisely. This type of technology also offers the possibility of using currently available equipment to produce, for example, bi-material scaffolds, i.e.

3 Three-dimensional microcomputarised tomography (μCT) image of scaffold obtained by extrusion of SEVA-C (blend of starch with ethylene vinyl alcohol) with blowing agent

scaffolds combining two different polymers and/or two different structures.

Freeze-drying

Phase separation of polymer solutions may be induced in several ways.[104,162] The basic principle of freeze-drying is a thermally induced phase separation, which occurs when the temperature of a homogeneous polymer solution, previously poured into a mould, is decreased. Once the phase-separated system is stabilised, the solvent rich phase is removed by vacuum sublimation, leaving behind the polymeric foam. The foam morphology is of course controlled by any phase transition that occurs during the cooling step, i.e. liquid–liquid or solid–liquid demixing.[104–162] Current research shows that this method is very sensitive, i.e. the parameters must be very well controlled.[106] Furthermore, at present, only pore sizes in the region of 100 μm can be reproducibly obtained by this method.[106]

Porous PLLA and scaffolds loaded with small hydrophobic and hydrophilic bioactive molecules have been manufactured by this method:[102,121] the polymer is dissolved in a solvent such as molten phenol or naphthalene at a low temperature, followed by dispersion of the bioactive molecule in this homogeneous solution. A liquid–liquid phase separation is induced by lowering the solution temperature. The resulting bicontinuous polymer and solvent phases are then quenched to create a two phase solid. Subsequent removal of the solidified solvent by sublimation leaves a porous polymer scaffold loaded with bioactive molecules.[102,121] Proteins retained as much as 75% of their activity after scaffold fabrication with the naphthalene system. Since phenol has a lower melting temperature than naphthalene, it might be useful for entrapment of small drugs or short peptides into polymer matrix. However, the activity of alkaline phosphatase was lost completely after fabrication, even in the phenol system. Therefore, incorporating and releasing large proteins with defined conformations such as growth factors without loss of activity remains a challenge.[102,121]

Further, polystyrene foams, produced by phase separation from a naphthalene solution[163] have been used for hepatocyte culture experiments, after their derivation with lactose and heparin.

Aggregation of polymer microparticles

This method consists of aggregation, by physical or chemical means, of microparticles.[104] The porosity is solely the interstices between the aggregated microspheres and it is directly related to the microsphere diameter. The possibility of releasing previous encapsulated growth factors from the microspheres is an additional advantage of this technique.[104]

Macroporous PLA supports have been prepared in this manner. The microsphere aggregates can be stabilised by chemical crosslinking of a poly(vinyl alcohol) (PVA) precoating with glutaraldehyde.[104] Local fusion of the PLA particle aggregates at the point of contact is also possible, particularly in the case of plasticised particles with triethylcitrate.

High pressure processing with supercritical CO_2

In this technique, solid discs of the polymer, previously prepared by compression moulding or solvent casting, are exposed to high pressure CO_2 (5·5 MPa, 25°C) to saturate the polymer with the gas.[93,102,133] A thermodynamic instability is then created by reducing the CO_2 gas pressure to an ambient level, which results in nucleation and expansion of dissolved CO_2, generating macropores. This process yields a mostly non-porous surface, resulting from the rapid diffusion of the dissolved gas from the surface, and a closed-pore structure inside the polymer matrix, which may be problematic for cell seeding. The porosity and the pore structure are dependent on the amount of CO_2 dissolved, the rate and type of gas nucleation, and the rate of gas diffusion to the pore nuclei.[93,102,133]

High pressure processing has been used to obtain poly(D,L-lactic-co-glycolic acid) foams,[93,102,133] with 93% porosity and a pore size of about 100 μm.

In situ polymerisation

The polymer processing techniques discussed so far are methods to manufacture pre-fabricated scaffolds, which may then be used to regenerate the appropriate tissue. However, it is also possible to use polymeric hydrogels, which have the advantage of being injectable; this makes delivery of the construct less invasive and thereby reduces surgical risks.

Employment of hydrogels may allow delivery of an even distribution of a precise number of cells. Hydrogels can be configured to provide immediate mechanical support to the cells to maintain their specific phenotype, without inhibiting migration.[59] They can be crosslinked at the time of surgery to form a solid degradable scaffold; simultaneously with the crosslinking reaction it is possible to incorporate NaCl, which provides pores into which new tissue can grow. In this manner, a temporary scaffold is formed *in situ*, fulfilling the mechanical role of the malfunctioning tissue until new tissue, stimulated to form in the scaffold pores, can provide adequate support. In its liquid phase, the hydrogel can be injected or moulded into the defect, an additional advantage for this application, since many injuries result in defects that are relatively inaccessible.[121,163]

Use of hydrogels may represent an important step towards minimally invasive surgery.[163] However, most addition polymerisation reactions are exothermic and the heat generated may cause local tissue necrosis.

One example of this type of scaffold is poly(propylene fumarate) (PPF) based polymers.[121,164,165] An unsaturated linear polyester, PPF is a viscous liquid at room temperature. It can be crosslinked at the time of surgery

to form a solid degradable bone cement via addition polymerisation with N-vinyl pyrrolidone (N-VP). At the time of the crosslinking reaction, two other components are incorporated into the PPF: NaCl, which provides pores into which new bone can grow, and tricalcium phosphate, an osteoconductive material that stimulates new bone growth. During its liquid phase, the cement can be injected or moulded into the bone defect.[121,164] Furthermore, the crosslinking reaction between PPF and N-VP[121,164,165] was found to produce less heat than many addition polymerisation reactions, and no local tissue necrosis has been noted in *in vivo* studies.

Rapid prototyping technologies

Rapid prototyping (RP) technologies have the potential to produce three-dimensional constructs of complex geometries in a multilayer design within the same gross architectural structure, from a computer aided design (CAD) model of an object, without part specific tooling or knowledge.[106] Some RP machines deposit and bond liquid, powder and sheet materials to form parts, layer by layer, directly from a computer generated model. Rapid prototyping technologies such as three-dimensional printing (3DP) and fused deposition modelling (FDM) are manufacturing processes capable of creating porous scaffolds that mimic the microstructure of living tissues.[106]

Three-dimensional printing technology is based on the printing of a binder through a print head nozzle onto a polymer powder bed, with no tooling required. The part is built sequentially in layers. Binder is delivered to the powder bed representing the first layer; the bed is then lowered by a fixed distance and further polymer powder is deposited and spread evenly across the bed, building up a second layer. This is repeated until the scaffold is fabricated. The entire process is performed at room temperature, which offers great potential for tissue engineering by allowing the incorporation of biological agents[106] such as growth factors without inactivation.

Fused deposition modelling involves extrusion of a polymer filament through a heated nozzle and its deposition as thin layers on a platform.[106] First, a conceptual geometric model is designed on a CAD workstation. The design is imported into software that mathematically slices the conceptual model into horizontal layers. Toolpaths are generated before the data is downloaded to the FDM hardware. The FDM extrusion head operates in the x and y axes while the platform moves along the z axis to enable each layer to be formed. In effect, the process draws the designed model (scaffold) one layer at time.

Hutmacher and co-workers[139,140] designed and fabricated novel polycaprolactone (PCL) scaffolds by fused deposition modelling. These scaffolds have low degradation rates and a structure fully interconnected with honeycomb-like pore architecture that revealed mechanical properties suitable for bone tissue engineering. These matrixes have shown ability to support *in vitro* adhesion and proliferation of osteoblasts and the *in vivo* implantation of these scaffolds/osteoblast constructs in nude mice led to the formation of bone-like tissue. Other studies have shown the potential of these scaffolds to support the formation of elastic cartilage-like tissue.[141]

Examples of scaffolds based on different biodegradable polymers, which were developed using one of the processing methods for tissue engineering applications described herein, are given in Table 1.

Concluding remarks

There is a significant, well known clinical need to establish alternative treatments for tissue loss or end-stage organ failure resulting from injury, since the transplantation of tissues or organs in these circumstances is

Table 1 Some examples of scaffolds based on different biodegradable polymers that have been studied for different tissue engineering applications and which were developed using one of processing methodologies described in this review*

Processing method	Polymers used†	Aimed tissue	Ref.
Fibre bonding	L-PLA/PGA	Intestine	104, 121
	Hyaluronic acid	Cartilage	166
	SPCL	Bone	128, 145
Solvent casting–particle leaching	PLLA, PLGA	Bone	88, 104, 121, 123, 124
	PLGA/PEG	Skeletal tissues	169
	SCA	Bone	136
Melt based technologies	PLLA, PLGA, PGA	Not specified	104, 121
Injection moulding and extrusion with blowing agents	SEVA-C, SCA	Bone	134, 136,
Compression moulding–particle leaching	SCA	Bone	136, 137
High pressure CO_2	PLGA	Not specified	93, 102, 133
	PLA, PGA	Not specified	170, 167
Freeze-drying	PLLA	Not specified	102, 121
	PE	Liver	163
	PLGA	Bone	166
Aggregation of microparticles	PLA	Bone	104
In situ polymerisation	PPF	Bone, cartilage	121, 163, 164
Microwave processing	SEVA-C	Bone	138
Rapid prototyping‡			
FDM	PCL	Bone, cartilage	139, 140, 141
3DP (TheriForm)	PLLA	Several	168

*Cited references are selected from works published between 1997 and 2003.
†PGA: poly(glycolic acid); PLA: poly(lactic acid); L-PLA/PLLA: poly(L-lactic acid); PLGA: poly(L-glycolic acid); PEG: polyethylene glycol; PE: polystyrene; SEVA-C: starch–ethylene vinyl alcohol blend; SCA: starch–cellulose acetate blend; SPCL: starch–poly(ε-caprolactone); PPF: poly(propylene fumarate); PCL: poly(ε-caprolactone).
‡FDM: fused deposition modelling; 3DP: three-dimensional printing.

severely limited by donor scarcity and strongly associated with the risk of rejection and disease transfer. The developing field of tissue engineering has already brought significant advances to regenerative medicine, but tissue engineers have still a large field to explore for new and enhanced solutions.

Attempts to achieve breakthroughs in tissue engineering and regeneration will continue to focus on the interplay between cells, scaffolds and therapeutic agents and will need to address:

- the development of adequate human cell cultures to produce the tissues (cells and matrix) in suitable polymeric scaffold materials for subsequent use as a medical device
- the development of culture technology whereby human tissue can be grown *ex vivo* in three-dimensional polymeric scaffold matrixes
- the development of materials processing technologies to produce degradable, three-dimensional polymeric matrixes suitable for cell culture (proliferation, differentiation) and having mechanical properties similar to those of the natural tissue.

Much of the discussion above has focused on the challenges of producing three-dimensional multifunctional structures from biodegradable polymers, as these materials present outstanding potential (from the materials science point of view) to provide the necessary support to new and emerging tissue engineering strategies. It is clear that the need to develop new and improved devices based on biodegradable polymers will continue to challenge researchers to come up with new polymers and processing methodologies. The advances in biomaterials science (particularly in tissue engineering) engendered by this research will lead to enhanced medical therapies that will benefit the quality of life of thousands of patients throughout the world.

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