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Bioresorbable polymers for tissue engineering

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Abstract

Polymeric biomaterials are used as substitutes for damaged tissue and for the stimulation of tissue regeneration. One class of polymeric biomaterials are bioresorbable polymers that degrade both *in vitro* and *in vivo* and are used as a temporary support for tissue regeneration. Among the various types of bioresorbable polymers, α-hydroxy acids including the different forms of poly(lactic acid) (PLA), such as poly(L-lactic acid), poly(D-lactic acid) and poly(DL-lactic acid), as well as poly(glycolic acid) and polycaprolactone, have been extensively studied. These polymers are well known for their good biocompatibility, with their degradation products being eliminated from the body by metabolic pathways. Many reports have shown that the different PLA-based substrates do not present toxicity since the cells were found to differentiate over the different polymers, as demonstrated by the production of extracellular matrix components by various cell types. In this chapter, we describe the use of α-hydroxy acids, highlighting the different forms of PLA scaffolds used as cell culture substrates and their applications in clinical practice. The chapter is divided into (1) Introduction; (2) Bioresorbable devices as cell culture substrates; (3) Cell adhesion to polymer substrates; (4) Tissue engineering and bioresorbable polymers; (5) Cell growth and proliferation on bioresorbable polymers; (6) Bioresorbable polymers for cartilage engineering; (7) Bioresorbable polymers for bone tissue engineering; (8) Bioresorbable polymers for skin tissue engineering, and (9) Conclusion.

1. Introduction

For centuries, extensive tissue injuries normally originating from mechanical trauma or degenerative diseases have been a challenge because of the scarcity of therapeutic resources. Removal of the damaged part was the most common practice, which resulted in a series of limitations of the affected patient and in a significant decrease in quality of life. Thus, replacement and/or regeneration of the damaged body regions became the new target. The increase in life expectancy resulting from the discovery of antibiotics and chemotherapy, as

well as from improved sanitary and hygiene conditions, has encouraged the search for methods to replace damaged tissues [1].

There are two procedures used for the treatment of the failure or loss of tissues and organs: transplants and implants. In the case of transplants, tissues or organs are obtained from living donors (e.g., heart or kidneys) or from cadavers (e.g., lyophilized and frozen bone). In some cases, immunosuppressive drugs are necessary to prevent rejection of the transplanted organ, or other medications that minimize possible microbial contamination [1]. In addition, transplants have the disadvantage of raising a series of ethical and even religious issues. In contrast, devices developed to serve as implants do not present many of the problems reported above and are designed to act at the recipient tissue interface in the organism, interacting with these tissues [1, 2].

Biomaterials were first developed to remain inert in the organism. Thus, studies were aimed at investigating how to prevent or minimize undesired tissue reactions. At present, new materials are designed to elicit an effective interaction with tissues, provoking physiological responses such as cell growth and/or differentiation at the site of implantation [3]. Significant advances have been made over the past decades in the understanding of the mechanisms underlying the interaction of animal cells with their natural environment, i.e., the extracellular matrix, as well as of the influence of this matrix on cell growth and differentiation [4, 5]. This knowledge is frequently being used for the development of polymers that mimic the characteristics of extracellular matrix, thus playing an active role in tissue restoration.

The biomaterials used can be classified into permanent or temporary materials [6]. Permanent materials are used to replace damaged tissue for an undetermined period of time and are therefore designed to retain their mechanical and physicochemical properties for prolonged periods of time [6]. These types of devices are commonly employed experimentally as prostheses, replacing damaged joints, heart valves and intraocular lenses, among others. On the other hand, in some situations the support only needs to fill the damaged region temporarily until tissue recomposition is completed, or guides the regeneration process. Temporary biomaterials are an alternative in this case.

“Biodegradable” is a term that can be applied to polymers and solid devices that undergo dispersion *in vivo* as a result of macromolecular degradation, but without elimination of products and subproducts by the organism [7]. Biodegradable polymers can be attacked by biological elements in such a way that the integrity of the system is affected, forming fragments and other degradation products that can be removed from the site of action but not necessarily from the organism. “Bioabsorbable” is a term that can be applied to polymeric materials and devices that dissolve in body fluids without breakdown of the macromolecular chain or a reduction in molecular mass [7]. One example is the slow dissolution of soluble implants in organic fluids. “Bioresorbable” materials are polymers and solid devices that degrade through a reduction in size and that are resorbed *in vivo*, i.e., materials that are eliminated by metabolic pathways of the organism. Bioresorption is a concept that reflects the complete elimination of the material and of degradation products (compounds of low molar mass) in the absence of residual side effects [7]. The term “bioresorption” is applied when complete elimination occurs. A polymer can be bioresorbable if its macromolecules are excreted [7,8]. Bioresorbable polymeric materials are preferentially used as temporary devices [6].

2. Bioresorbable devices as cell culture substrates

A wide variety of temporary devices have been employed in biological systems, with the most widely used materials being based on α-hydroxy acid-derived polyesters, such as poly(L-lactic acid) (PLLA), poly(D-lactic acid) (PDLA), poly(DL-lactic acid) (PDLLA), poly(glycolic acid) (PGA), and polycaprolactone (PCL) [6,8]. During degradation, the polymer is broken down into smaller units by simple hydrolysis and its degradation products are eliminated from the body by metabolic pathways, such as the citric acid cycle, or directly by renal excretion (see Figure 1) [9-12].
Although the degradation of bioresorbable polymers mainly occurs by simple hydrolysis, there are reports in the literature indicating that degradation of PGA and PLLA, at least in part, is also mediated by enzymes [12,13]. Figure 1 is a schematic presentation of the degradation of PLLA by hydrolysis [8]. The elimination routes of the degradation products of some polyesters are shown in Figure 2.

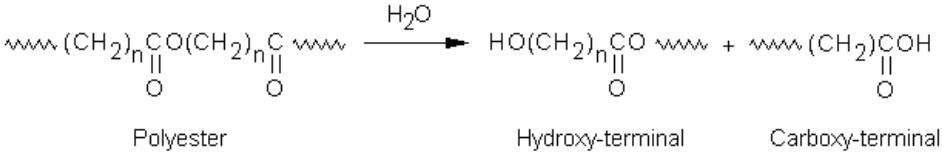

Fig. 1. Degradation of poly(α-hydroxy acids) by hydrolysis (From Barbanti *et al*. [8], with authorization).

Other bioresorbable polymers used are polyhydroxyalcanoates, polyesters produced by microorganisms. These compounds find applications as raw materials of different devices in the areas of biomedicine and tissue engineering [14]. Polyhydroxyalcanoates include poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer (PHBV), poly(4-hydroxybutyrate) (P4HB), poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) copolymer (PHBHHx), and poly(3-hydroxyoctanoate) (PHO). These compounds have been used for the development of sutures, devices to guide tissue repair, heart implants, orthopedic pins, stents, tubules for nerve regeneration, and membranes for skin regeneration [14]. The use of PLLA/PHBV blends has appeared as a new proposal in the literature. The biological evaluation of this compound is relevant for tissue engineering [15,16].

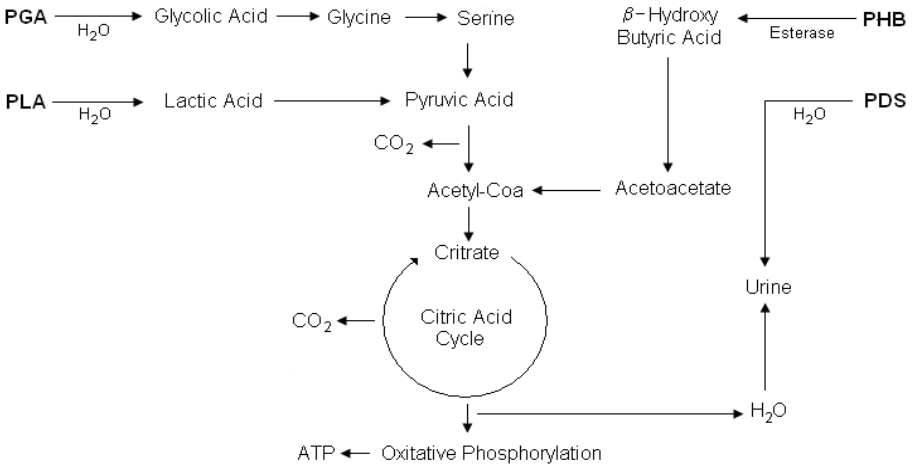

Fig. 2. Routes of degradation and excretion of some polyesters: poly(*p*-dioxane) (PDS), poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and poly(hydroxybutyrate) (PHB).

3. Cell adhesion on polymer substrates

Normally, cell adhesion to the substrate is necessary for a good polymer-cell interaction. Although the substrate does not need to present extracellular matrix-like characteristics for cell adhesion to occur, physicochemical similarity is often desired when the aim is to promote cell differentiation or a more effective interaction of a certain polymer at the implantation site [2-4,17]. Thus, polymers presenting physicochemical and/or mechanical properties as close as possible to those of the tissues into which they will be implanted are currently being developed. These properties include an adequate balance between hydrophilicity/hydrophobicity, electrical charge distribution, hardness, elasticity, and strength [18].

A relationship exists between hydrophilicity and cell adhesion. Among other parameters, more hydrophilic substrates tend to provide a better interaction with cells [19-21]. The relationship between cell adhesion and polar groups on the material surface has been demonstrated for polystyrene. Adhesion of cells was found to increase with increasing polarity of the substrate [22]. In a study investigating hepatic cells cultured on different substrates, cell adhesion increased proportionally to the surface energy of the growth membranes. Adhesion was even higher in the presence of serum proteins adsorbed to the substrates and the metabolic activity of hepatic cells increased on hydrophilic membranes [23]. Other studies demonstrated a relationship between wettability and cell adhesion. Lee *et al*. [24] prepared a wettability gradient on polyethylene surfaces to investigate the interaction of different types of cells (Chinese hamster ovary cells, fibroblasts and endothelial cells) with fetal bovine serum proteins, in terms of surface hydrophilicity/hydrophobicity. Better adhesion, spreading and growth of cells were observed on surfaces with moderate hydrophilicity. Maximum adhesion was found on substrates with a water contact angle of approximately 57º, irrespective of the type of cell used. Serum proteins also adhered better to substrates presenting moderate hydrophilicity [24].

Thus, cell adhesion is an extremely important factor for biomaterials research. Only after adhesion do the cells initiate the process of spreading, division and production of new extracellular matrix [18,19]. Cell spreading is a complex process that involves modifications in cell morphology as a consequence of alterations in the cytoskeleton, thus improving interaction with the substrate. These modifications are shown in Figure 3.

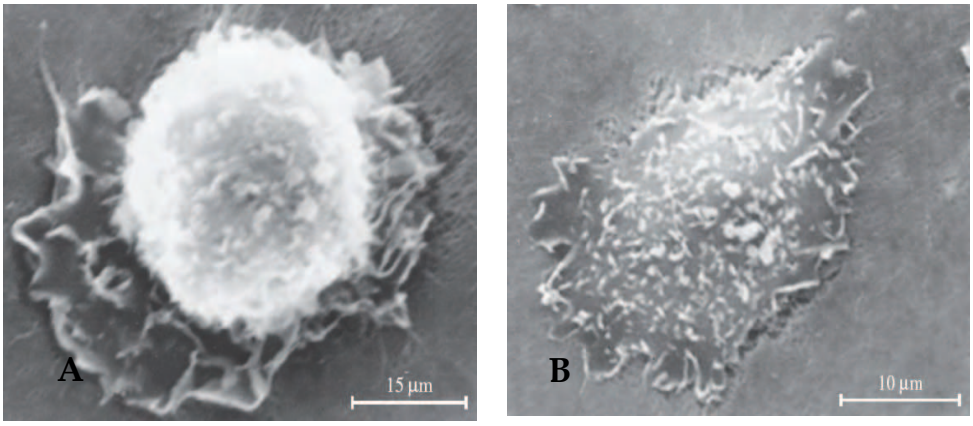

Fig. 3. Spreading of Vero cells on type I collagen gel. Round cells are observed during inoculation, a morphology that is adequate for the state of being in suspension. After adhesion, the cells start their interaction with the substrate. Note the onset of the phenomenon known as spreading in (A), which is characterized by modifications in the cytoskeleton and, consequently, in cell morphology as a result of interactions with the growth surface, with the cell flattening on the growth surface. (B) A cell spread on the substrate. Magnification bar: A = 15 µm; B = 10 µm (From Santos Jr and Wada [5], with authorization).

Good integration of the biomaterial with cells or tissues also depends on the structure of the devices itself. Our experience agrees with literature data indicating that porous materials promote cell growth and induce the production of extracellular matrix components by the cells [21,25]. A uniform distribution and pore interconnections are important to facilitate the formation of tissues in the form of an organized network, with a wide range of applications in tissue reconstruction [3,26,27]. *In vivo*, porosity and pore interconnection are essential for the proliferation of vessels, facilitating tissue nutrition around the implant. In this respect, different types of scaffolds containing PLLA have been developed and tested as substrates for cell growth [3]. Kwon *et al*. [28] evaluated nano- and microfibrous scaffolds with different compositions of PLLA, PCL and poly(L-lactic acid-co-caprolactone) (PLCL) (70/30, 50/50 and 30/70) as cell culture substrate. Human umbilical vein endothelial cells were found to adhere and proliferate well on the small-diameter fiber scaffolds (0.3 to 1.2 mm in diameter), whereas a marked reduction in cell adhesion and spreading, as well as a low proliferative capacity, was observed for large-diameter fiber scaffolds (about 7.0 mm).

Studies from our group have shown an initially slow adhesion to PLLA and PHBV scaffolds [21,25]. This finding does not necessarily indicate that the material tested would not be useful for tissue engineering. Mann *et al*. [29] showed that in the case of slow adhesion the materials may stimulate the early production of extracellular matrix components, permitting cell growth and proliferation. Our observations agree with these authors [21,25]. The extracellular matrix exerts a marked influence on the migration, proliferation and differentiation of cells cultured on biomaterials. Gunawan and coworkers [30] demonstrated the influence of the density of extracellular matrix components on the migration of rat intestinal cells (IEC-6 cells). The cells migrated in the direction of the gradient formed, with the demonstration of the particular role of laminin in this process.

Nevertheless, there is a trend to change the architecture of cell culture substrates used for tissue engineering [31]. The aim of these surface modifications is to improve the interaction with cells both *in vitro* and *in vivo*. With respect to cell cultures, increased cell interaction means to favor the initial interaction of cells with the substrate, i.e., to increase adhesion. In a recent study, nanofibrous PLLA scaffolds were fabricated and tested. The surface modifications introduced significantly increased cell adhesion and spreading. In addition, cell proliferation was maintained for more than 2 weeks and the modifications also increased the production of extracellular matrix components [32].

The surface properties of biomaterials can be altered in such a way as to make them more adequate for biomedical applications. The most commonly used techniques are chemical etching, gas plasma treatment and electron beam radiation [33]. Among these techniques, plasma treatment is particularly versatile because the modification is restricted to the surface without compromising the material properties as a whole. Plasma treatment can be used to modify the polymer surface in a nonspecific manner by introducing a variety of functional groups depending on the type of gas used [34,35]. Plasma is considered to be the fourth state of matter and consists of highly excited states of atoms, molecules, ions and radicals obtained from gases elevated to excited stages by radiofrequency, microwaves or electron discharge [35]. Thus, surface modification by plasma treatment is an economic and effective technique for biomaterials and is gaining space in the area of biomedicine, with its application becoming more frequent. The main advantage of plasma treatment is its capacity to modify the surface of materials, making them more biocompatible or permitting them to more closely mimic tissue without altering their properties. As a consequence, this technique permits a high degree of quality control, offering reliability and reproducing what would be difficult to achieve with conventional techniques. Another advantage of plasma treatment is that it renders the surface sterile by destruction of microorganisms such as bacteria and viruses and is therefore useful for biomedical devices, surgical instruments, tissue engineering and clinical applications [35]. Our experience shows that plasma treatment increases cell adhesion to PHBV matrices, rendering these scaffolds more receptive to cell growth [36].

Nakagawa *et al*. [37] submitted PLLA samples to plasma treatment in a CO_2 atmosphere and observed an increase in the hydrophilicity of the membrane. The cell response was also highly satisfactory for membranes submitted to plasma treatment compared to controls. Plasma treatment provided better cell adhesion and proliferation, although the authors had used short-term cell cultures (up to 3 days only). Ryu and coworkers [38] submitted poly(D,L-lactic acid-co-glycolic acid) (PLGA) membranes to plasma treatment. In that study, the PLGA surface was modified in order to increase the interaction of cells with the material

surface. The results showed that the surface modifications increased hydrophilicity and cell adhesion and proliferation.

Several studies have been conducted to develop materials with biomimetic characteristics. Surface modification of biomaterials using bioactive molecules is a relatively simple approach to produce biomimetic materials. Long chains of extracellular matrix proteins such as fibronectin, vitronectin and laminin have been used for this purpose. Biomaterials coated with these proteins normally present increased cell adhesion and proliferation [31,39]. Peptides have also been used for surface modifications, with the most frequently used sequence being Arg-Gly-Asp (RGD), the binding sequence derived from fibronectin and laminin. Other peptide sequences such as Tyr-Ile-Gly-Ser-Arg (YIGSR), Arg-Glu-Asp-Val (REDV) and Ile-Lys-Val-Ala-Val (IKVAV) have also been employed for substrates such as quartz, glass, metal oxide and polymers [31,39].

Porous PDLA and PLLA substrates containing 2-methacryloyloxyethyl phosphorylcholine (MPC) and *n*-butyl methacrylate (BMA) were investigated regarding their capacity to interact with fibroblasts. MPC is an analog of phosphatidylcholine, a typical lipid of the plasma membrane of cells. The results obtained were quite interesting. The number of adhered cells was correlated with PDLA and PLLA content, with the cells presenting a good pattern of adhesion and migrating into the substrate within only 24 h of culture. Furthermore, cell morphology was influenced by the contact with MPC [40], with the addition of MPC rendering the PDLA and PLLA scaffolds more receptive to the initial interaction with cells.

Despite promising results, some investigators have reported discrepancies in the findings regarding the addition of bioactive molecules to growth surfaces. In this respect, an interesting study was conducted by Harnett *et al*. [41] who coated various biomaterials with different adhesion molecules such as fibronectin, polylysine, polyornithine and collagen in order to evaluate changes in surface free energy and the hydrophilicity/hydrophobicity balance. Only fibronectin promoted a homogenous coating for all substrates tested, producing a monopolar acid surface, whereas polylysine, polyornithine and collagen coatings produced hydrophobic or hydrophilic surfaces depending on the underlying substrate they were coated on.

4. Tissue engineering and bioresorbable polymers

Once cell adhesion is established, the material tested is studied as a cell carrier in procedures aimed at the restoration of damaged tissues. Tissue engineering can be understood as the application of the principles of exact sciences to tissue creation and repair. Three general strategies have been adopted to obtain new tissues [17]:

1) Use of autogenous cells i.e., cells isolated from the individual himself; isogeneic cells, i.e., cells obtained from different individuals which, however, are genetically identical or belong to the same species; allogeneic cells, i.e., cells isolated from different individuals of the same species, or xenogeneic cells, i.e., cells obtained from different species [42]. These cells are expanded in culture and implanted into the body by infusion methods. However, limitations of this strategy include the limited capacity of the cells to maintain their differentiated characteristics *in vitro*, the difficulty in sufficiently expanding some cells in culture (for example, hepatic and neural cells cannot be expanded in adequate numbers for clinical use), and immunological rejection when allogeneic and xenogeneic cells are used.

2) Tissue culture for subsequent implantation and replacement of sick or damaged tissues. The most common example used in clinical practice is skin grafts [43]. The main advantage of this strategy is its high biocompatibility and biofunctionality. However, this strategy presents the same disadvantages as described above.
3) Use of substances that induce the regeneration of damaged tissue. The success of this strategy depends on the large-scale purification and production of appropriate signal molecules such as growth factors and adhesion molecules. The proliferation of many cell types (which may induce the formation of a new tissue) depends on a combination of various growth factors that are highly specific proteins. Some growth factors can be released slowly from polymeric capsules and may stimulate the growth of damaged tissue [44]. In contrast, adhesion molecules such as fibronectin, vitronectin and laminin are protein components of biological fluids and/or extracellular matrix that are adsorbed on the material surface and recognized by integrins (cell membrane receptors associated with the cytoskeleton) [4]. Integrins bind to the small domains of adhesion molecules [45], such as the RGD amino acid sequence of fibronectin and vitronectin or the YIGSR sequence of laminin. RGD and other oligopeptides have been incorporated into some biomaterials to stimulate adhesion and consequent cell proliferation.
Autogenous or autograft implants are of special interest. This technique consists of the use of healthy cells derived from the patient himself who will receive the polymer implant. Autogenous implants have some advantages over organ transplants. Since the cell population isolated is expanded *in vitro* by cell culture, only a small number of donor cells is necessary for preparation of the implant. In addition, the use of autogenous cells avoids immunological problems such as rejections or allergic processes [3,42,46].

5. Cell growth and proliferation on bioresorbable polymers

Bioresorbable devices have been used *in vitro* as a support for the growth and proliferation of different cell types. Endothelial cells have been shown to satisfactorily multiply on PLLA scaffolds in the absence of platelet activation [47,48]. Good adhesion to the material and an adequate multiplication rate were reported for NIH/3T3 mouse cells cultured on PHBV membranes. These parameters improved after the introduction of physicochemical modifications (change in polymer hydrophilicity) on the PHBV surface [49]. In another study, cell proliferation on a PHBV substrate continued to be similar to that observed for collagen sponges for up to 35 days of culture [50].
The capacity of a substrate to stimulate cell growth and proliferation is intimately related to its ability to absorb proteins. In a recent study, Zhu and coworkers [48] showed that coating of the surface of PLLA devices with free amine groups increases the spreading and proliferation of endothelial cells. Extracellular matrix proteins such as fibronectin, laminin and collagen have also been shown to stimulate the multiplication of cells on substrates used for tissue engineering [51]. We showed that Vero cells produce extracellular matrix rich in fibronectin and collagen when cultured on dense or porous PLLA membranes, PHBV scaffolds or PLLA/PHBV blends of different proportions [21,25]. This finding may explain the observation of a significant proliferation rate despite the initially slow cell adhesion to these scaffolds [25,52].

6. Bioresorbable devices for cartilage engineering

The use of bioresorbable materials for articular cartilage repair is currently being investigated. Cartilage is an avascular tissue which basically consists of two cell types, chondrocytes and chondroblasts. Chondrocytes produce an extracellular matrix that mainly consists of collagen and glycosaminoglycans. The proportion of these components depends on the type of cartilaginous tissue [53]. Once damaged, cartilage presents little or no regenerative capacity and certain injuries may progress to severe degenerative joint diseases [46,53]. In addition to the fact that the mechanisms underlying the formation of articular cartilage are still unclear, there are few alternative clinical procedures to the replacement of joints with prostheses that can fill small defects resulting from trauma or degenerative diseases. Current treatments for articular cartilage engineering include 1) the creation of a defect by wear or perforation to permit the migration of progenitor cells; 2) arthroscopy; 3) autografts; 4) transplantation of perichondrium and periosteum to introduce undifferentiated cells with a chondrogenic potential; 5) autogenous cell transplants (chondrocytes or undifferentiated cells) previously expanded *in vitro* and reinjected as an autogenous periosteal graft that is able to maintain both chondrogenesis and osteogenesis [54]. Studies on cartilage engineering that combine different materials in the production of scaffolds used as a support for chondrogenic cells are necessary. In this respect, there is an intense search for materials that mimic the biomechanical behavior of articular cartilage and thus can be applied to joint repair [46]. Some polymeric materials including both temporary and permanent polymers are investigated for this application. Bioresorbable materials studied for their use as temporary cartilage matrix include PLLA and PGA polymers and their copolymers and blends.

Chondrocytes cultured on a fibrous PGA matrix and porous PLLA membranes have been studied [55]. The results showed the neoformation of cartilage tissue comparable to that observed for chondrocytes cultured on collagen substrates obtained from articular cartilage. Under these conditions, chondrocytes continued to grow on these polymers for up to 6 months, maintaining the shape of the original device and producing a tissue with characteristics similar to those of cartilage, including the synthesis of glycosaminoglycans and types I and II collagen [55]. In addition, it was observed that cartilage cells cultured on polyesters such as PLLA and PGA tend to show increased synthesis of proteoglycans and collagen when compared to cells cultured on collagen matrix [56]. Puelacher *et al.* [57] studied the *in vitro* and *in vivo* growth of chondrocytes on PGA and PLLA scaffolds that simulated the morphology of human nasal cartilage. The authors observed the formation of hyaline-like cartilage on these substrates. The experimental results indicate that, once improved, these tissue reconstruction techniques have potential applications in orthopedic, plastic and reconstructive and craniomaxillofacial surgery. In addition, the formation of hyaline-like cartilage was observed after 6 weeks when perichondrial cells were cultured on PLLA membranes and implanted into the femoral condyle of rabbits [55,58].

A study of cells obtained from human articular cartilage and maintained in culture on devices consisting of different bioresorbable polyesters showed that the process of adhesion was proportional to the hydrophilicity of the polymers. In addition, no variations in cell spreading were observed between the different biomaterials. Although the cells studied adhered less to the PLLA membranes when compared to PLGA, their proliferative capacity was better when grown on the PLLA membrane [59]. In addition, the production of cartilaginous matrix and type II collagen was found to be lower for human chondrocytes

cultured on PLLA membranes compared to those grown on PLGA membranes. On the other hand, cells grown on PLLA scaffolds presented a greater capacity of synthesizing type I collagen [60]. Interesting results were also obtained with PLLA scaffolds used for meniscus reconstruction. Porous implants were found to guide vascular growth into the injury region [61]. Canine meniscus reconstruction using lactic acid/ε-caprolactone copolymers have also been reported [62]. These results demonstrate that the principles of tissue engineering employing bioresorbable materials are a promising study area and certainly will yield significant results in the near future.

In an attempt to better mimic the natural environment of cartilage cells, Takagi and coworkers [63] developed a scaffold consisting of collagen and a copolymer mesh of PLLA and PLGA. Glucuronic acid is one of the components of glycosaminoglycans found in the extracellular matrix of tissues. The authors showed that cartilage cells cultured inside the scaffold consumed glucose from the culture medium and produced typical extracellular matrix components of cartilage tissue. Within this context, another study compared hyaluronic acid scaffolds and polyester scaffolds with different degradation rates [64]. The study showed that the degradation rate of the scaffolds is critical for the cartilage repair process. Cartilage formation was slow with dissolution of the materials, although presenting more cracks and discontinuities. Wang *et al.* [65] tested scaffolds of different origins, such as poly(L-lactide), poly(D,L-lactide) and collagen-hydroxyapatite, for the *in vitro* production of cartilage. Porcine chondrocytes were seeded onto the scaffolds. After 15 weeks of culture, a layer of viable neo-cartilage was produced on each material, with the collagen-hydroxyapatite constructs yielding better results in terms of cell viability and integration. Another approach was the use of a gelatin scaffold obtained from demineralized bone matrix inoculated with rabbit chondrocytes. Neoformation of hyaline-like cartilage was demonstrated [66].

In a well-conceived experiment, chondrocytes were cultured on PLLA microspheres in a bioreactor. In addition, the surface of the microspheres was modified by the addition of RGD peptides, small repetitive sequences of the amino acids arginine (Arg or R), glycine (Gly or G) and aspartate (Asp or D) which are known to stimulate cell adhesion. PLLA degradation was determined after different periods of time (7, 14, 21, 28, 35, 49 and 56 days). The authors observed that the materials continued to be stable to support cell growth after the periods studied. The use of a bioreactor resulted in a significant increase in the number of cells cultured on the biomaterials and the devices studied showed a good capacity to stimulate cell adhesion and proliferation. The authors also observed the formation of microaggregates, a finding that might indicate the production of extracellular matrix [67].

In another study, human mesenchymal stem cells were cultured on a PLDLA scaffold. This construct was maintained in chondrogenic medium and the other end was then loaded with cells of the same origin but previously induced to undergo osteogenesis. This system was then cultured under conditions able to maintain both chondrogenesis and osteogenesis, thus producing *in vitro* a hybrid osteochondral construct [68]. Taken together, these results are promising but the search for a polymeric material that better mimics the function of articular cartilage still continues.

7. Bioresorbable devices for bone tissue engineering

Bone is a natural tissue which consists of an organic component (mainly collagen) and a mineral component composed of hydroxyapatite. Reconstruction of long bone defects is a clinical challenge. Normally, multiple surgeries are required to restore the structure and function of the damage site. The development of tissue engineering techniques has led to the use of new procedures for bone restoration. Polymeric materials may serve as a support for cell growth, permitting the penetration of blood vessels, and even exert morphogenetic activity in some cases. In the case of bioresorbable polymers, the materials are often enriched with hydroxyapatite, growth factors, bone morphogenetic proteins (BMPs) and other bone elements, a fact that renders them highly effective in the stimulation of bone neoformation in the damaged areas [10].

Transplants consisting of different types of isolated cells cultured on PLLA and PGA scaffolds have been investigated as temporary substitutes of damaged tissue portions [17]. Implantation of PLGA copolymers into bone resulted in bioresorption of the material and concomitant bone neoformation at the site of the implant. An additional advantage of PLGA is that its complete degradation is variable and may occur within weeks or years depending on the polyester ratio present in the copolymers [69].

Osteoblastic cells cultured on PLLA, PGA and PLGA films presented a satisfactory pattern of cell adhesion and spreading, in addition to the ability to grow and proliferate on the substrate. Furthermore, cells grown on these polymers presented increased alkaline phosphatase activity, a marker of osteoblast differentiation, and increased synthesis of collagen I [70]. Similar results were obtained when osteoblasts were cultured on PLGA scaffolds. In that case, mineralization of the bone matrix produced was also observed [71,72]. Interestingly, even bone marrow cells cultured on porous PLGA scaffolds and implanted into the rat mesentery were able to initiate ectopic bone formation [71].

It has been postulated that porous materials implanted *in vivo* present a better integration with the recipient tissue. Disagreement still exists regarding the ideal diameter of the pores for tissue growth. *In vitro*, low porosity stimulates osteogenesis, suppressing cell proliferation by forcing cell aggregation. On the other hand, *in vivo*, a higher porosity with larger pores promotes bone growth. However, these factors result in low mechanical properties and, therefore, a functional limit exists for porosity and pore size [73]. On the basis of these studies, a minimum pore size of approximately 100 μm was established considering cell size, migration requirements and transport. *In vitro* and *in vivo* results suggest pore sizes and pore interconnections > 300 μm to facilitate vascularization of the graft. Some investigators recommend diameter variations of 300 to 400 μm, whereas others propose even wider intervals of 200 to 400 μm [74]. Thus, the macrostructure (pre-existing macroporosity), microstructure (microporosity, enhanced surface microroughness or microtexture) and chemical composition of the material play an important role in osteoinduction guided by the biomaterial *in vitro*. In addition, the model used for *in vivo* study may also markedly influence the results. Osteoinduction is only occasionally observed in mice and rats, whereas the same material induces bone formation reproducibly in goats and dogs [75].

Another interesting approach is the adsorption of factors that stimulate cell differentiation on polymer surfaces. In 1965, ectopic bone formation was demonstrated after implantation of demineralized bone matrix into muscles of rabbits, rats, mice and guinea pigs [76]. A protein, called bone morphogenetic protein (BMP), was found to be involved in the cascade

of chemotaxis, mitosis, differentiation and bone formation [77]. Since these pioneering reports, the role of BMP in bone formation *in vitro* and *in vivo* has been extensively studied. A recombinant form of human bone morphogenetic protein type 2 (rhBMP-2) was added to bioresorbable PLGA substrates. In those studies, a higher production of bone matrix was observed for osteoblasts cultured on substrates covered with rhBMP-2 when compared to control [78,79]. A similar experiment was conducted by Hollinger *et al*. [80], who adsorbed rhBMP-2 to a collagen I matrix. When this collagen matrix was implanted into fractured bone segments, bone neoformation was observed, as well as integration of the implant into the damaged bone. Subsequently, various groups have shown that BMPs are able to induce endochondral bone formation when implanted at ectopic sites in experimental animals [81]. Although the results are promising, this therapeutic approach might be limited by the size of the fracture produced [78].

The exact mechanism of osteoinduction by biomaterials is still largely unknown. In addition, it is unclear whether the mechanisms of osteoinduction by BMPs and biomaterials are the same. In a recent review [81], it was demonstrated marked differences in osteoinduction mediated by BMPs and biomaterials: 1) biomaterials always induce intramembraneous bone formation, whereas BMP mainly induces endochondral bone formation; 2) in small animals such as rodents, bone formation is rarely induced by biomaterials but is easily mediated by BMPs; 3) newly formed bone is never observed at the periphery of biomaterials but always inside their pores, whereas bone formation induced by BMPs regularly occurs outside the carrier and soft tissue is observed distant from the surface of this carrier.

Gugala *et al*. [82] investigated the adsorption of proteins and activity of osteoblasts cultured for up to 3 weeks on porous and non-porous PDLA membranes. The presence of pores did not influence protein adsorption. The authors observed that the cells maintained their typical phenotype, formed mineralized nodules, i.e., regions where mineralized organic matrix is observed, and produced alkaline phosphatase, an enzyme commonly related to the process of biomineralization both *in vitro* and *in vivo*. The amount of protein, alkaline phosphatase activity and number of cells increased over time and were higher for the porous than for the non-porous membranes. In another study, osteoblasts were cultured on a gradient of PLLA and PDLLA in order to determine whether the gradient alters the pattern of cell interaction with the substrate [83]. Cell adhesion was similar at both ends of the gradient, but proliferation was more significant in the smoother PDLLA-rich region than in the rougher PLLA-rich region of the gradient. These results demonstrate that, in addition to the composition of the substrate, its topography also interferes with the behavior of bone cells. Thus, PLLA might not be the ideal substrate for the culture of bone cells. It was showed that murine osteoblastic cells more intensely adhered to both dense and porous PGA scaffolds than to PLLA scaffolds. Nevertheless, the pattern of cell spreading on the substrates was similar after 24 h of culture [84].

The use of rapidly degrading synthetic or natural polymer matrices with low mechanical properties (high porosity) results in grafts with high biological activity but poor structural properties, such as low strength and rigidity. One approach to correct this problem is the combination of bioactive ceramics, such as calcium phosphate, with bioresorbable polymers in order to improve the mechanical properties of the scaffolds. A composite matrix also increases the osteoconductive properties of the scaffolds [73]. Various of these compounds are investigated for this purpose. Natural polymers combined with hydroxyapatite-

collagen, hydroxyapatite compounds (chitosan-hydroxyapatite), and PLA copolymers (PLA-polyethyleneglycol) have been tested.
Porous PLLA and PLLA-hydroxyapatite composite scaffolds were seeded with osteoblasts and cultured. The cells were found to penetrate deep into the PLLA-hydroxyapatite scaffolds and were uniformly distributed. Cell viability and proliferation, as well as the expression of bone differentiation markers, were higher for the composite scaffolds when compared to pure PLLA [85].
Other studies have shown that PCL-calcium phosphate composites confer favorable mechanical and biochemical properties (the ceramic confers strength and the polymer confers hardness and plasticity). In these scaffolds, the ceramic was homogenously distributed in the matrix as being exposed on the surface. The composite was more hydrophilic and degradation was accelerated when compared to the pure PCL scaffold [86]. In addition, the composite was more receptive to cell adhesion [87]. Another study investigating PLA-hydroxyapatite and PCL-hydroxyapatite composites showed that cells tended to adhere and spread among hydroxyapatite particles exposed on the surface. The presence of hydroxyapatite resulted in a higher activity of bone cells [88]. Despite these results, further studies are necessary to identify a material that serves as a substrate for the growth of bone cells for tissue regeneration since there is still no ideal compound that stimulates bone formation.

8. Bioresorbable devices for skin engineering

Several groups have investigated different methods for the creation of dermal equivalents using various substitutes based on biological materials such as collagen, fibrin, culture of epidermal layers, or synthetic materials [89]. However, in patients dermal substitutes have shown a slow growth of vascular structures into the dermal components. As a consequence, a second surgical procedure is necessary to transplant epidermal components into the regenerating wound [90].
Much of the knowledge about the mechanisms of skin regeneration by tissue engineering stems from three-dimensional scaffold-based fibroblast cultures. The mechanism of action of these grafts is based on 1) fibroblast colonization, 2) production of growth factors, 3) a substrate for keratinocyte migration, and 4) the wound immune response. Another important factor is the migration of keratinocytes to the site of injury. A large number of keratinocytes is observed around chronic wounds. However, these cells do not migrate onto the wound surface. One of the possible explanations is the degradation of extracellular matrix by proteases present at high concentrations in many types of injuries. The control of the synthesis and/or degradation of extracellular matrix seem to have marked implications in the outcomes of wound healing. Genes encoding extracellular matrix components are expressed during wound reconstruction. These compounds include tenascin, decorin and some types of collagen (I, III, V and VI). A provisional extracellular matrix is expected to provide a good substrate for the migration of cells such as keratinocytes and leukocytes. The maintenance of this matrix is therefore important [91].
Natural polymers, especially collagen, are extensively studied as a substrate for skin regeneration. Collagen has been obtained from different xenogenic sources such as cattle, swine and horses. Some types of human collagen are used in the United States with approval by the Food and Drug Administration (FDA). Collagen is extremely receptive to

the culture of fibroblasts. We showed that fibroblasts grown on collagen gels produce extracellular matrix components such as glycosaminoglycans and fibronectin, forming a tissue that resembles reconstituted connective tissue [92]. However, variations in culture conditions change the behavior of fibroblasts into that of epithelial cells, with a reduction in their migration behavior on the collagen matrix and the production of molecules such as collagen IV and laminin [92,93]. Thus, the use of collagen is an interesting model not only for the area of tissue reconstruction but also for the study of the differentiation itself of cells cultured on it.

Collagen presents a series of advantages such as availability, biodegradation, bioresorption, and resistance to distension. In addition, the properties of collagen can be altered by modifying its functional groups. However, there are disadvantages such as rapid degradation, high hydrophilicity that may cause significant swelling after implantation, low resistance to mechanical compression forces, and high cost of purification, factors limiting the use of collagen [94]. The dermal replacement layer of collagen serves as a matrix for the growth of macrophages, lymphocytes and endothelial cells derived from the wound. During the tissue repair process, an endogenous collagen matrix is deposited, whereas the dermal substitute is degraded within approximately 30 days. Furthermore, the skin graft is found to be flexible at the site of injury and does not adhere to deeper layers, thus permitting free and functional joint movement [95].

In view of the positive experiences obtained with collagen, development of an artificial dermis was the next target. This type of structure initially consisted of collagen I and chondroitin sulfate [96-98]. The so-called artificial dermis is composed of a bilayer membrane, in which a matrix of collagen I and chondroitin-6-sulfate is bonded to a layer of polysiloxane polymer (silicone). The collagen matrix serves to attract macrophages, lymphocytes, fibroblasts and capillaries. In addition to being bioresorbable, the collagen matrix is gradually eliminated, permitting the formation of a new dermal matrix. In the presence of adequate vascularization, the silicone layer is removed and an epidermal autograft is added to the newly formed dermis. The silicone layer represents an additional barrier that prevents or minimizes the risk of microbial contamination. The formation of this neodermis occurs within 14-21 days. After the completion of regeneration, the neodermis is histologically and functionally similar to normal dermis [98,99]. This dermal substitute is considered to be an effective treatment for burns and tissue reconstruction in patients with cancer [100,101].

Since these first results, various research groups have worked to improve the device in an attempt to optimize outcomes and to reduce production costs. Among the modifications introduced are the removal of chondroitin-6-sulfate and alterations in the storage format of the substrate for implantation [102]. Long-term studies have subsequently evaluated the efficiency of the implants fabricated, tissue receptivity, degree of regeneration, and strength of the skin formed. The data reported were highly satisfactory. The advantages of these implants include 1) that the inner layer of the implant spontaneously becomes a structure resembling connective tissue, 2) a good long-term postoperative appearance even in the case of thin implants, and 3) minimal damage to the donor tissue [43].

Intensive research into dermal substitutes and tissue engineering devices has inevitably led to bioresorbable polymers. The latter present some advantages over biological substrates: 1) since bioresorbable polymers are synthetic, their production can be standardized and variations between different production lots are therefore small; 2) these polymers can be

modified to better fulfill clinical requirements without the loss of their mechanical properties; 3) since the possibility of harboring viruses or prions is practically zero, these polymers are safer for patients [5,89]. Among the bioresorbable polymers available, the most widely used are variations of PLA, such as PLLA, PDLA, PDLLA, PGA, and PLGA. Fibroblasts cultured on three-dimensional dense and porous (different pore diameters) PLLA membranes were found to adhere to the polymers, to proliferate on them and to produce extracellular matrix molecules such as collagen IV and fibronectin [21]. This behavior suggests that PLLA can be used as a substrate for skin injuries. However, PLA presents low mechanical strength. One alternative would be the formation of copolymers or blends to modify the mechanical properties of the material. It was also evaluated the efficacy of PLGA membranes with and without plastifier as a dressing for skin injuries. The *in vitro* results showed that the addition of the plastifier reduced the vitreous transition temperature (Tg) of the membranes and increased their flexibility. *In vivo* analysis demonstrated that the polymer degraded rapidly when in contact with the skin without causing serious inflammation and protected the ulcerated area from the action of external agents. Wound healing was faster in the presence of the membranes, a fact indicating their potential use as skin dressings [103].

Another polymer that is currently gaining interest is poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) copolymer, a biodegradable and bioresorbable polymer derived from microbial activity. PLLA/PHBV blends are more resistant and, at the same time, more malleable than pure PLLA membranes. Since PHBV is more stable, its degradation is slow. Thus, the desired strength and degradability are obtained by varying the proportions of PLLA and PHBV in the blend. Fibroblasts cultured on different proportions of PLLA/PHBV blends (100:0, 60:40, 50:50, 40:60 and 0:100) were found to adhere to the substrates and to multiply on them. Again, these cells produced collagen IV and fibronectin on the supports [25].

New substrates such as poly(ethylene terephthalate)/poly(butylene terephthalate) (PEGT/PBT) are also being tested. A fragment of skin equivalent was reconstructed using PEGT/PBT scaffolds and cultures of fibroblasts and keratinocytes. The structure formed presented various characteristics of differentiated skin, such as an epidermal layer expressing several types of keratins on which grew layers of fibroblasts. In addition, basement membrane components such as collagen VII, laminin and nidogen were observed between these two layers [89].

Other alternatives also aimed at dermal regeneration and/or tissue engineering are being developed. Some investigators are carefully observing the use of chitosan. Chitosan, or *N*-carboxybutylchitosan, is derived from the deacetylation of chitin. Chitosan is a non-immunogenic compound that presents slow degradation in aqueous medium, even in the presence of lysozymes, and its degradation product, glycosamine, is not toxic [104]. Chitosan sponges should present, at least in theory, a stable shape and size during the period of cell culture. Porous chitosan membranes showed good integrity and favored cell spreading [104]. The use of collagen/chitosan blends was proposed taking advantage of the characteristics of chitosan and combining them with the properties of collagen. The blends were found to present a great water retention capacity, i.e., they were highly hydrophilic, and also markedly stimulated the growth of fibroblasts on their surface. Furthermore, the addition of chitosan did not reduce the interactivity of cells with collagen. These blends support fibroblast infiltration *in vivo*, a fact facilitating the formation of a neodermis [94].

Finally, our experience and the literature data available indicate that bioresorbable polymers are a suitable alternative as long as a growth substrate is chosen whose degradation coincides with the regeneration of the dermis-epidermis and that permits neovascularization of the skin formed at the site of the implant. Dense or porous PLGA membranes may show these characteristics. The search for materials with good properties might be a promising alternative for the reconstruction of damaged skin.

9. Conclusion

The advantages of the use of bioresorbable compounds for tissue engineering are numerous when compared to other more traditional surgical procedures. Internal fixation devices used in orthopedic surgeries lose their function of maintaining tissues together when structural recomposition is completed. The use of bioresorbable implants for internal fixation has the advantage of eliminating the second surgical intervention necessary for their removal. In addition, the risks of metal implants such as corrosion or friction with bone are avoided. Although still under study, the use of bioresorbable polymers is highly promising. New compounds are being developed and tested every year and their application perspectives are immense. The search for materials that are ideal for each tissue and clinical approach continues to represent a challenge.

10. References

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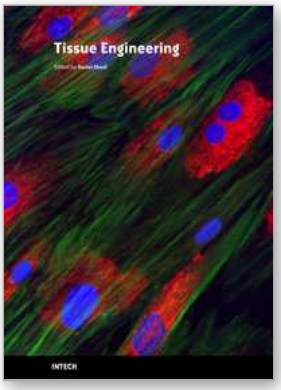

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The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues that closely match the patient's needs can be reconstructed from readily available biopsies and subsequently be implanted with minimal or no immunogenicity. This eventually conquers several limitations encountered in tissue transplantation approaches. This book serves as a good starting point for anyone interested in the application of Tissue Engineering. It offers a colorful mix of topics, which explain the obstacles and possible solutions for TE applications.

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