# CHAPTER II
# LITERATURE REVIEW

## 2.1 Tissue Engineering

### 2.1.1 Fundamentals of Tissue Engineering

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti, is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ"(Langer *et al.*, 1993). Tissue engineering has also been defined as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use" (Arthur *et al.*, 2005). A further description goes on to say that tissue engineering is the new approach to overcome the limitations of the existing therapies for the treatment of malfunctioning or lost organs. One of the goals of tissue engineering is to develop method to produce the biological substitutes that will restore, maintain or even improve tissue or organ function. Generally, biocompatible and biodegradable polymer is used in tissue engineering to allow the growth of the tissue surrounding the area of implantation and enable cells attachment, proliferation differentiation and maintenance of cell function. Therefore, cells and biomaterials are the two main components of tissue engineering.

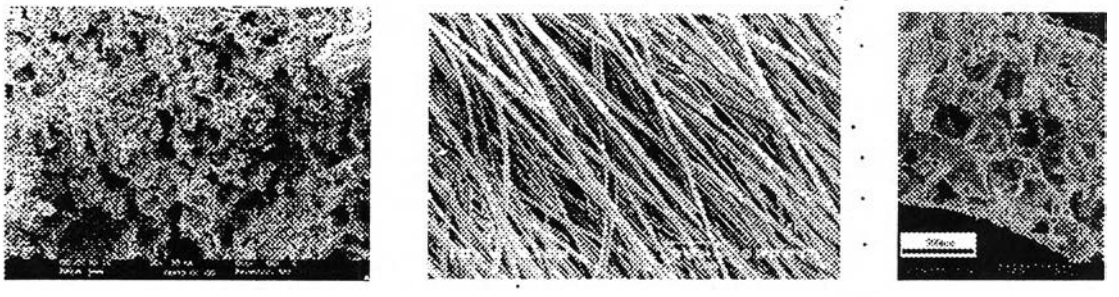
Tissue engineering scaffold is three dimensional structures that provide a site for cells to attach, proliferate, differentiate and secrete an extra-cellular matrix, eventually leading to tissue formation. The appropriate scaffold structure is also possible to guide cells into forming a tissue of predetermined, three dimensional shape and size. A scaffold cans be either permanent or temporary in nature, depending on the application and the function of the tissue. Temporary scaffold is made from biodegradable polymers, such as polyglycolic acid, polylactic acid, and polycaprolactone which degrade within the body to leave a purely biological tissue. Permanent scaffold remains within the body, working with ingrown tissue to form a polymeric/biological composite (Edwards *et al.*, 2004). Ideally, a scaffold should have the suitable characteristics for tissue regeneration (Table 2.1).

**Table 2.1** Ideal structural parameters of tissue engineering scaffolds (Edwards *et al.*, 2004)

| Scaffold Functions | Scaffold design parameters |
|---|---|
| Not to activate inflammatory response or toxicity *in vivo*. | Must be biocompatible, non-toxic and noncarcinogenic. |
| To assist in the growth of three dimensional tissue and organs. | Three dimensional scaffold of specific shape. |
| Give way to a uniform high cell seeding density. | High porosity and high interconnectivity between pores. |
| To provide the appropriate surface for cell attachment, proliferation and differentiation of function. | Optimum polymer surface chemistry and topography. |
| To allow significant cell surface interactions such as cellular attachment. | High surface area to volume ratio. |
| To promote cell proliferation and migration leading to tissue growth throughout the scaffold. | Optimum pore size to allow for cell penetration, with high porosity and interconnectivity between pores. |
| To direct the orientation of cells, ECM and new tissue. | Correct fiber orientation within the scaffold. |
| To allow for the movement of nutrients and waste in and out of the scaffold. | High porosity and interconnectivity between pores. |
| The scaffold may degrade to leave only natural tissue. | Rate of degradation to match rate of tissue formation. Polymer degradation products must not be toxic or promote inflammation *in vivo*. |
| Possess sufficient structural integrity to retain shape *in vivo*, with enough mechanical strength to support developing tissue and withstand *in vivo* forces. | Scaffold should equal mechanical properties of developing tissue. |

2.1.2 Scaffold Manufacturing Methods

The method used to produce a scaffold determines the key properties of that scaffold, such as porosity, pore size and mechanical strength. When choosing the scaffold manufacturing method, it is important to take into consideration these desired scaffold properties, and to ensure that the method does not adversely affect these properties, e.g. mechanical characteristics or biocompatibility. Another consideration is the use of high temperatures and harsh chemicals during scaffold manufacture, which can inhibit the incorporation of bioactive agents (e.g. growth factors) into the scaffold for drug delivery to the cells. Different manufacturing methods produce scaffolds of different configurations such as porous sponges (Ciapetti *et al.*, 2003), fibrous scaffolds (Sombatmankhong *et al.*, 2006), and tubular porous scaffolds (Wu *et al.*, 2006) (Figure 2.1)



(A) (B) (C)

**Figure 2.1** SEM micrograph of various scaffold; spongy scaffold (A), fibrous scaffolds (B), and tubular porous scaffolds (C).

A number of fabrication technologies have been applied to process biodegradable and bioresorbable material into three dimensional polymeric scaffolds of high porosity and surface area. Table 2.2, summarized the key characteristics and parameters of the techniques currently used (Hutmacher *et al.*, 2000). Table 2.3, compared the advantages and disadvantages of conventional scaffold processing techniques for tissue engineering (Buckley *et al.*, 2004).

**Table 2.2** Currently applied three dimensional scaffold fabrication technologies (Hutmacher *et al., 2000*)

| Fabrication | Processing | Material properties required | Pore size (μm) | Porosity (%) | Architecture |
|---|---|---|---|---|---|
| Solvent casting and particulate leaching | Casting | Soluble | 30-300 | 20-50 | Spherical pores. salt particles remain in matrix |
| Membrane lamination | Solvent bonding | Soluble | 30-300 | < 85 | Irregular pore structure |
| Fabrication of non woven | Carding. Needling | Fibers | 20-100 | < 95 | Insufficient mechanical properties |
| Melt moulding | Moulding | Thermoplastic | 50-500 | < 80 | |
| Extrusion in combination with particular leaching | Extrusion through dies | Thermoplastic | < 100 | < 84 | Spherical pores. salt particles remain in matrix |
| Emulsion freeze drying | Casting | Soluble | < 200 | < 97 | High volume of interconnected micropore structure |

| | | | | | |
|---|---|---|---|---|---|
| Thermally induced phase separation | Casting | Soluble | < 200 | < 97 | High volume of inter-connected micropore structure |
| Supercritical fluid technology | Casting | Amorphous | < 100 | 10-30 | High volume of inter-connected micropore structure |
| Supercritical fluid technology in combination with particle leaching | Casting | Amorphous | < 50<br>< 400 | < 97 | Micropore structure combined with interconnected. macropore structure |
| 3-D printing in and without combination of particle leaching | Solid free form fabrication | Soluble | 45-150 | < 60 | 100% interconnected macropore Structure |
| Fused deposition modeling | Solid free form fabrication | Thermoplastic | > 150 | < 80 | 100% interconnected macropore structure |

A number of different methods have been described in literature for preparing porous structures to be employed as tissue engineering scaffolds. Each of these techniques presents its own advantages, but none is devoid of drawbacks.

*2.1.2.1 Nanofiber Self-Assembly*

Molecular self-assembly is one of the few methods to create biomaterials with properties similar in scale and chemistry to that of the natural in vivo extracellular matrix (ECM).

*2.1.2.2 Textile Technologies*

These techniques include all the approaches that have been successfully employed for the preparation of non-woven meshes of different polymers.

*2.1.2.3 Solvent Casting & Particulate Leaching (SCPL)*

This approach allows the preparation of porous structures with regular porosity, but with a limited thickness. First the polymer is dissolved into a suitable organic solvent then the solution is cast into a mold filled with porogen particles. Such porogen can be an inorganic salt like sodium chloride, crystals of saccharose, gelatin spheres or paraffin spheres. The size of the porogen particles will affect the size of the scaffold pores, while the polymer to porogen ratio is directly correlated to the amount of porosity of the final structure. After the polymer solution has been cast the solvent is allowed to fully evaporate, then the composite structure in the mold is immersed in a bath of a liquid suitable for dissolving the porogen. Once the porogen has been fully dissolved a porous structure is obtained. Other than the small thickness range that can be obtained, another drawback of SCPL lies in its use of organic solvents which must be fully removed to avoid any possible damage to the cells seeded on the scaffold.

*2.1.2.4 Gas Foaming*

To overcome the necessity to use organic solvents and solid porogens a technique using gas as a porogen has been developed. First disc shaped structures made of the desired polymer are prepared by means of compression molding using a heated mold. The discs are then placed in a chamber where are exposed to high pressure $CO_2$ for several days. The pressure inside the chamber is gradually restored to atmospheric levels. During this procedure the pores are formed by the carbon dioxide molecules that abandon the polymer, resulting in a sponge like structure. The main problems related to such a technique are caused by the excessive

heat used during compression molding (which prohibits the incorporation of any temperature labile material into the polymer matrix) and by the fact that the pores do not form an interconnected structure.

*2.1.2.5 Emulsification Freeze-Drying*

This technique does not require the use of a solid porogen like SCPL. First a synthetic polymer is dissolved into a suitable solvent then water is added to the polymeric solution and the two liquids are mixed in order to obtain an emulsion. Before the two phases can separate, the emulsion is cast into a mold and quickly frozen by means of immersion into liquid nitrogen. The frozen emulsion is subsequently freeze-dried to remove the dispersed water and the solvent, thus leaving a solidified, porous polymeric structure. While emulsification and freeze-drying allows a faster preparation if compared to SCPL, since it does not require a time consuming leaching step, it still requires the use of solvents, moreover pore size is relatively small and porosity is often irregular. Freeze-drying by itself is also a commonly employed technique for the fabrication of scaffolds.

*2.1.2.6 Thermally Induced Phase Separation (TIPS)*

Similar to the previous technique, this phase separation procedure requires the use of a solvent with a low melting point that is easy to sublime. For example dioxane could be used to dissolve polylactic acid, then phase separation is induced through the addition of a small quantity of water: a polymer-rich and a polymer-poor phase are formed. Following cooling below the solvent melting point and some days of vacuum-drying to sublime the solvent a porous scaffold is obtained. Liquid-liquid phase separation presents the same drawbacks of emulsification/freeze-drying.

*2.1.2.7 CAD/CAM Technologies*

Since most of the above described approaches are limited when it comes to the control of porosity and pore size, computer assisted design and manufacturing techniques have been introduced to tissue engineering. First a three-dimensional structure is designed using CAD software, and then the scaffold is realized by using ink-jet printing of polymer powders or through Fused Deposition Modeling of a polymer melt.

**Table 2.3** Conventional scaffold processing techniques for tissue engineering (Buckley *et al.*, 2004)

| Process | Advantages | Disadvantages |
|---|---|---|
| Solvent casting and particulate leaching | -Large range of pore sizes<br>-Independent control of porosity and pore size<br>-Crystallinity can be tailored<br>-Highly porous structures | -Limited membrane thickness (3mm)<br>-Limited interconnectivity<br>-Residual porogens<br>-Poor control over internal architecture |
| Fibre bonding | -High porosity | -Limited range of polymers<br>-Residual solvents<br>-Lack of mechanical strength |
| Phase separation | -Highly porous structures<br>-Permits incorporation of bioactive agents | -Poor control over internal architecture<br>-Limited range of pore sizes |
| Melt moulding | -Independent control of porosity and pore size<br>-Macro shape control | -High temperature required for nonamorphous polymer<br>-Residual porogens |
| Membrane Lamination | -Macro shape control<br>-Independent control of porosity and pore size | -Lack of mechanical strength<br>-Limited interconnectivity |
| Polymer/ceramic fibre composite foam | -Independent control of porosity and pore size<br>-Superior compressive strength | -Problems with residual solvent<br>-Residual porogens |
| High-pressure processing | -No organic solvents | -Nonporous external surface<br>-Closed-pore structure |
| Freeze drying | -Highly porous structures<br>-High pore interconnectivity | -Limited to small pore sizes |

2.1.3 Parameter Investigation

The better synthetic scaffold should promote tissue regeneration. The important factors include obtaining an optimal porosity and size of interconnecting but maintaining scaffold mechanical strength, enabling complete penetration of cells and nutrients throughout the scaffold, preventing the formation of necrotic tissue in the center of the scaffold. There are many researches to control the result the porosity, pore size and interconnecting size but maintaining mechanical strength. The minimum recommended pore size for a scaffold is 100 μm based on the early work of Hulbert *et al.* in 1970, but subsequent studies have shown better osteogenesis for implants with pores more than 300 μm (Kuboki *et al.*, 2001). Relatively larger pores favor direct osteogenesis, since they allow vascularization and high oxygenation, while smaller pores result in osteochondral ossification.

Other factors, such as the rate of degradation of the scaffold for example, should be taken into account when porosity is assessed. Scaffolds fabricated from biomaterials with a high degradation rate should not have high porosities (>90%), since rapid depletion of the biomaterial will compromise the mechanical and structural integrity before substitution by newly formed bone. In contrast, scaffolds fabricated from biomaterials with low degradation rates and robust mechanical properties can be highly porous, because the higher pore surface area interacting with the host tissue can accelerate degradation due to macrophages via oxidation and/or hydrolysis. In vitro lower porosity enhances osteogenesis due to cell aggregation and suppressed proliferation (Karageorgiou *et al.*, 2005).

## 2.2. Bone Tissue Engineering

### 2.2.1 Bone

Bone is an amazing and a true nanocomposite. It is a complex and a highly specialized form of connective tissue involve to the formation of the skeleton of the body. Bone, not only provides mechanical support but also elegantly serves as a reservoir for minerals, particularly calcium and phosphate. It is a good example of a dynamic tissue, since it has a unique capability of self regenerating or self remodeling to a certain extent throughout the life without leaving a scar. The main compositions of the bone are organic (protein: collagen) and inorganic (mineral: hydroxyapatite) phase. An overall composition of the bone is given in Table 2.4.

**Table 2.4** The composition of bone (Murugan *et al.*, 2005)

| Inorganic phase | wt % | Organic phase | wt % |
|---|---|---|---|
| 1. Hydroxyapatite | 60 | 1. Collagen | 20 |
| 2. Carbonate | 4 | 2. Water | 9 |
| 3. Citrate | 0.9 | 3. Non-collagenous proteins (osteocalcin, osteonectin, osteopontin, thrombospondin, morphogenetic proteins, sialoprotein, serum proteins) | 3 |
| 4. Sodium | 0.7 | 4. Other traces: polysaccharides, | |
| 5. Magnesium | 0.5 | lipids, cytokines | |
| 6. Other traces: $Cl^-$, $F^-$, $K^+$ $Sr^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Fe^{2+}$ | | Primary bone cells: osteoblasts, osteocytes, osteoclasts. | |

A complete biological mechanism involved in the bone building strategy is still unclear and thus research progresses in this direction significantly around the world. It is believed that key to the strength of the bone is the complex structural hierarchy into which it is organized in a self-assembling mode. It is important to know the biomechanical properties of the bone for producing the scaffold. The

compiled biomechanical properties of the bone are given in Table 2.5. (Murugan *et al.*, 2005)

**Table 2.5** Biomechanical properties of bone (Murugan *et al.*, 2005)

| Properties | Cortical bone | Cancellous bone |
|---|---|---|
| Young modulus (GPa) | 14-20 | 0.05-0.5 |
| Tensile strength (MPa) | 50-150 | 10-20 |
| Compressive strength (MPa) | 170-193 | 7-10 |
| Fracture toughness (MPa $m^{1/2}$) | 2-12 | 0.1 |
| Strain to failure | 1-3 | 5-7 |
| Density ($g/cm^3$) | 18-22 | 0.1-1.0 |
| Apparent density ($g/cm^3$) | 1.8-2.0 | 0.1-1.0 |
| Surface / bone volume ($mm^2/mm^3$) | 2.5 | 20 |
| Total bone volume ($mm^3$) | $1.4x10^6$ | $0.35x10^6$ |
| Total internal surface | $3.5x10^6$ | $7.10x10^6$ |

The cancellous bone has about 20% of the total bone. It is the spongy bone. It is lighter and less dense than compact bone (Figure 2.2). It has high porosity and higher concentration of blood vessels compared to compact bone. The porous architecture is easily visible under lower power microscopes and even to the naked eye if the pores are very large. The diameter of the pores may be from few micrometers to millimeters. The cortical bone is much denser than spongy bone. It is the compact bone. It has about 80% of the total bone. It has less porosity and thus less concentration of blood vessels. Its porous architecture is not visible to naked eye. The pores may be 10–20 μm in diameter and mostly separated by intervals of 200–300 μm. The cortical bone functions mechanically in tension, compression, and torsion, whereas cancellous bone functions mainly in compression. The cancellous bone is made of an interconnecting framework of trabeculae. At the nanostructural level, the bone is comprised mainly of collagen fibers and nanocrystals of bone minerals, particularly hydroxyapatite (Murugan *et al.*, 2005).

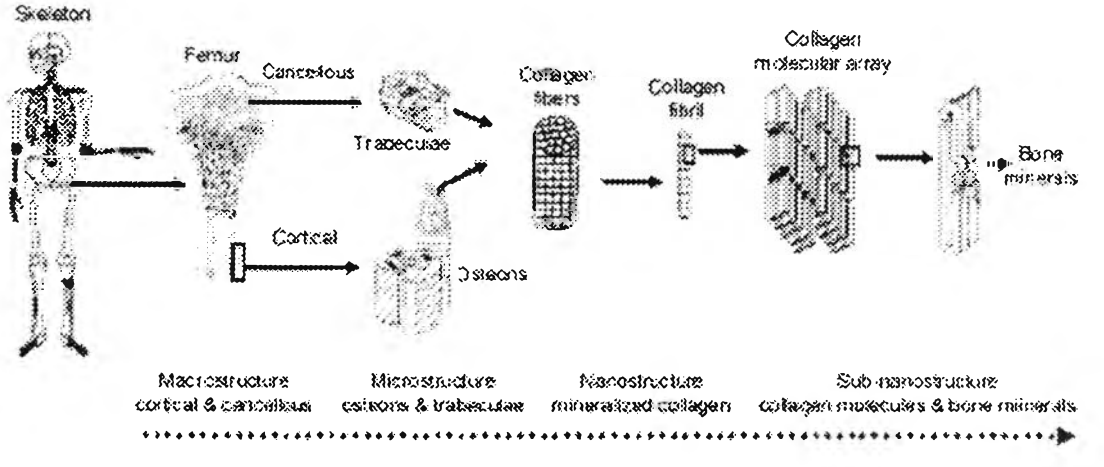



**Figure 2.2** The hierarchical structure of bone, from macro to nano assembly. (Murugan *et al.*, 2005)

2.2.2 Bone Tissue Engineering Program

The tissue engineering program for bone and cartilage has been classified into six phases (Table 2.6). Each tissue engineering phase must be understood in an integrated manner across the research program from the polymer material properties, to the scaffold architecture, to the cell, to the tissue-engineered transplant, to the host tissue. Hence, the research objectives in each phase are cross-disciplinary and the sub-projects are linked horizontally as well as vertically.

**Table 2.6** The research program for tissue engineering bone and cartilage classified into six phase (Hutmacher *et al.*, 2000)

| | |
|---|---|
| Phase I | Fabrication of bioresorbable scaffold |
| Phase II | Seeding of the osteoblasts/chondrocytes populations into the polymeric scaffold in a static culture (Petri-dish) |
| Phase III | Growth of premature tissue in a dynamic environment (spinner flask) |
| Phase IV | Growth of mature tissue in a physiologic environment (bioreactor) |
| Phase V | Surgical transplantation |
| Phase VI | Tissue-engineered transplant assimilation/remodeling |

2.2.3 Bone Graft Material

Over the past four decades, several biomaterials have been developed and successfully used as bone grafts. Bone and joint substitutes are commonly made of metals, ceramics, polymers, and their composites (Table 2.7). In most of the cases, metals and ceramics are used in hard tissue applications, whilst polymers in soft tissue applications due to their mechanical properties. Composites are widely used in both the applications.

**Table 2.7** Classification of biomaterials for bone graftings (Murugan *et al.*, 2005)

| Biomaterials | Advantages | Disadvantages | Applications | Examples |
|---|---|---|---|---|
| Metal and alloy | Too strong, tough, ductile | Dense, may corrode | Bone plates, load-bearing bone implants, dental arch wire, and dental brackets | Titanium, stainless steel, Co–Cr alloys, and Ti alloys |
| Ceramic | Bioinert, bioactive, bioresorbable, high resistance-to wear | Brittle, poor tensile, low toughness, lack of resilience | Hip joints and load-bearing bone implants bone filler, orbital implant, alveolar ridge augmentation, maxillofacial reconstruction, and bone tissue engineering | Alumina, zirconia |

| | | | | |
|---|---|---|---|---|
| Polymer | Flexible, resilient, surface modifiable, selection of chemical functional groups | Not strong, toxic of a few degraded products | Bone tissue scaffolds, bone screws, pins, bone plates, bone and dental filler, and bone drug delivery | Collagen, gelatin, chitosan, alginate, PLA, PGA, PLGA, PCL, PMMA, PE |
| Composite | Strong, design flexibility, enhanced mechanical reliability than monolithic | Properties might be varied with respect to fabrication methodology | Bone graft substitutes, middle ear implants, bone tissue scaffolds, guided bone regenerative membranes, and bone drug delivery | HA/collagen, HA/gelatin, HA/chitosan, HA/alginate, HA/PLGA, HA/PLLA, HA/PE |
| Nanocomposite | Larger surface area, high surface reactivity, relatively strong interfacial-bonding, design flexibility. | No optimized technique for material processing | Major areas of orthopedics, tissue engineering, and drug delivery | Nano-HA/collagen, Nano-HA/gelatin, Nano-HA/chitosan, Nano-HA/PLLA |

## 2.3 Polymer-Base Scaffold Materials

The meaning and definition of the words biodegradable, bioerodable, bioresorbable and bioabsorbable (Table 8) (Vert et al., 1992), which are often used misleadingly in the tissue engineering literature, are of importance to discuss the rationale, function as well as chemical and physical properties of polymer-based scaffolds.

**Table 2.8** Definition given by Vert (Hutmacher *et al.*, 2000)

| |
|---|
| **Biodegradable** are solid polymeric materials and devices which break down due to macromolecular degradation with dispersion *in vivo* but no proof for the elimination from the body. Biodegradable polymeric systems or devices can be attacked by biological elements so that the integrity of the system and in some cases but not necessarily, of the macromolecules themselves, is affected and gives other degradation by-products. Such fragments can move away from their site of action but not necessarily from the body. |
| **Bioresorbable** are solid polymeric materials and devices which show bulk degradation and further resorb *in vivo*; i.e. polymers which are eliminated through natural pathways either because of simple filtration of degradation by-products or after their metabolization. Bioresorption is thus a concept which reflects total elimination of the initial foreign material and of bulk degradation by-products (low molecular weight compounds) with no residual side effects. The use of the word bioresorbable assumes that elimination is shown conclusively. |
| **Bioerodable** are solid polymeric materials or devices, which show surface degradation and further, resorb *in vivo*. Bioerosion is thus a concept, too, which reflects total elimination of the initial foreign material and of surface degradation by-products (low molecular weight compounds) with no residual side effects. |
| **Bioabsorbable** are solid polymeric materials or devices, which can dissolve in body fluids without any polymer chain cleavage or molecular mass decrease. For example, it is the case of slow dissolution of water-soluble implants in body fluids. A bioabsorbable polymer can be bioresorbable if the dispersed macromolecules are excreted. |

There are two types of biodegradable polymers. The natural-based materials are one category, including polysaccharides (starch, alginate, chitin/chitosan, hyaluronic acid derivatives) or proteins (soy, collagen, fibrin gels, silk) and the second category, synthetic biodegradable polymers. Synthetic polymers can be produced under controlled conditions and therefore exhibit in general predictable and reproducible mechanical and physical properties such as tensile strength, elastic modulus and degradation rate. A further advantage is the control of material impurities. Possible risks such as toxicity, immunogenicity and favoring of infections are lower for pure synthetic polymers with constituent monomeric units having a well-known and simple structure.

2.3.1 Saturated Aliphatic Polyester

The most often utilized biodegradable synthetic polymers for 3D scaffolds in tissue engineering are saturated poly-α-hydroxy esters, including Polycaprolactone (PCL), poly(lactic acid) (PLA), and poly(glycolic acid) (PGA), as well as poly(lactic-coglycolide) (PLGA) copolymers. PLA exists in three forms: L-PLA (PLLA), D-PLA (PDLA), and racemic mixture of D,L-PLA (PDLLA).

*2.3.1.1 Polycaprolactone (PCL)*

PCL is a biodegradable polyester with a low melting point of around 60°C and a glass transition temperature of about −60°C. PCL can be prepared by ring opening polymerization of ε-caprolactone using a catalyst such as stannous octanoate. It is degraded by hydrolysis of its ester linkages in physiological conditions (such as in the human body) therefore it has received a great deal of attention for use as an implantable biomaterial. In particular it is especially interesting for the preparation of long term implantable devices, owing to its degradation which is even slower than that of polylactide. The structure of PCL is shown in Figure 2.3.

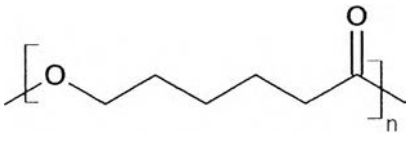



**Figure 2.3** The structure of polycaprolactone.

*2.3.1.2 Polylactic acid or Polylactide (PLA)*

PLA is an aliphatic polyester derived from renewable resources. Corn starch or sugarcanes are the common feedstock. Bacterial fermentation is used to produce lactic acid, which is oligomerized and then catalytically dimerized to make the monomer for ring-opening polymerization. It can be easily produced in a high molecular weight form through ring-opening polymerization using tin(II) chloride as a catalyst. Due to the chiral nature of lactic acid, several distinct forms of polylactide exist: poly-L-lactide (PLLA) is the product resulting from polymerization of L,L-lactide (also known as L-lactide). PLLA has crystallinity around 37%, a glass transition temperature between 50-80° C and a melting temperature between 173-178° C. The polymerization of a racemic mixture L- and D-lactides leads to the synthesis of poly-DL-lactide (PDLLA) which is not crystalline but amorphous. PLA is currently used in a number of biomedical applications, such as sutures, stents, dialysis media and drug delivery devices, but it is also evaluated as a material for tissue engineering. The structure of PLA is shown in Figure 2.4.

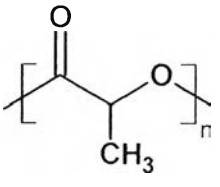



**Figure 2.4** The structure of polylactic acid.

*2.3.1.3 Poly(1,4-butylene succinate) (PBS)*

PBS is one of the commercially used biodegradable polymers with a range of interesting properties including good mechanical properties, melt processing, biodegradability and compostability. PBS is produced by the condensation reaction of the glycols 1,4-butanediol and aliphatic dicarboxylic acid, which is succinic acid. The structure of poly(1,4-butylene succinate), extended with 1,6-diisocyanatohexane is shown in Figure 2.5.

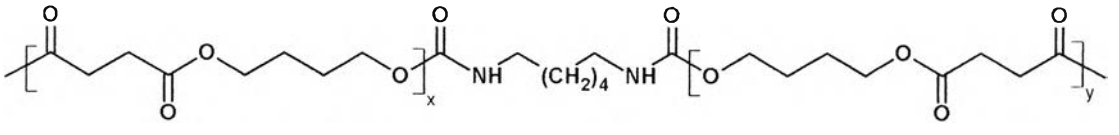


**Figure 2.5** The structure of poly(1,4-butylene succinate), extended with 1,6-diisocyanatohexane.

2.3.2 Polyhydroxyalkanoates (PHA)

Polyhydroxyalkanoates are aliphatic polyesters as well, but produced by microorganisms under unbalanced growth conditions (Doi *et al.*, 1995 and Li *et al.*, 2005). They are generally biodegradable and thermoprocessable, making them attractive as biomaterials for applications in medical devices and tissue engineering. Over the past years, PHA, particularly poly-3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV) were demonstrated to be suitable for tissue engineering and are reviewed by Chen *et al.*, 2005. Dependent on the property requirement by different applications, PHA polymers can be either blended, surface modified or composed with other polymers, enzymes or inorganic materials to further adjust their mechanical properties or biocompatibility. The blending among the several PHA themselves can change dramatically the material properties and biocompatibility (Chen *et al.*, 2005).

*2.3.2.1 Poly(3-hydroxybutyric acid) (PHB)*

Polyhydroxybutyrate was first isolated and characterized in 1925 by French microbiologist Maurice Lemoigne. PHB is produced by micro-organisms apparently in response to conditions of physiological stress. The polymer is primarily a product of carbon assimilation (from glucose or starch) and is employed by micro-organisms as a form of energy storage molecule to be metabolized when other common energy sources are not available. Microbial biosynthesis of PHB starts with the condensation of two molecules of acetyl-CoA to give acetoacetyl-CoA which is subsequently reduced to hydroxybutyryl-CoA. This latter compound is then used as a monomer to polymerize PHB (Steinbüchel, 2002). PHB is of particular interest for bone tissue application as it was demonstrated to produce a consistent favorable bone tissue adaptation response with no evidence of an undesirable chronic inflammatory response after implantation periods of up to 12

months. Bone is formed close to the material and subsequently becomes highly organized, with up to 80% of the implant surface lying in direct apposition to new bone. The materials showed no evidence of extensive structural breakdown in vivo during the implantation period of the study (Doyle *et al.*, 1991). The structure of PHB is shown in Figure 2.6.

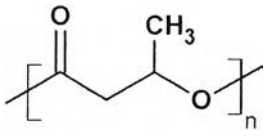



**Figure 2.6** The structure of Poly(3-hydroxybutyric acid).

*2.3.2.2 Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV)*

PHBV is one type of polyhydroxyalkanoate. It consists of copolymer between poly-3-hydroxybutyric acid and poly-3-hydroxyvaleric acid. The structure of PHBV is shown in Figure 2.7. PHBV is known to be biodegradable and biocompatible and its various properties such as natural origin (Gogolewski *et al.*, 1993, and Avella *et al.*, 2000), biodegradability, biocompatibility, streospecificity, piezoelectricity (Fukada et al., 1986), optical activity, and thermoplasticity make it suitable for a variety of applications in health industry. There are a number of studies about tissue responses to PHBV materials and their in vivo stability. It was found that porous PHBV materials were adequate as substrates for cell cultures (Gursel *et al.*, 2001 and Malm *et al.*, 1992). Rivard and co-workers (1996) demonstrated that PHBV 9 (9% 3-hydroxyvalerate in the structure) sustained fibroblast cell proliferation rate similar to that observed in collagen sponges for up to 35 days. The most important problem of PHBV is the lack of bioactivity of the PHBV so that the new tissue cannot bond to the surface of the polymer tightly (Chen *et al.*, 2002).

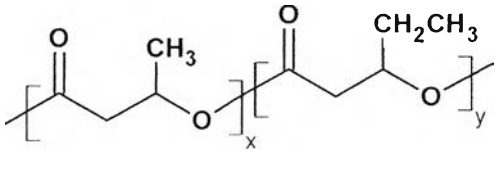



**Figure 2.7** Poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid).

## 2.4 Degradation

Once implanted, a scaffold material should maintain its mechanical property until it is no longer needed and then be absorbed and excreted by the body, leaving no trace. There are two types of biodegradation and both are discussed. A simple chemical hydrolysis of the hydrolytically unstable backbone is the prevailing mechanism for a polymer's degradation. This occurs in two phases. In the first phase, water penetrates the bulk of the device, attacking the chemical bonds and converting long polymer chains into shorter water-soluble fragments. This occurs in the amorphous phase and initially there is a reduction in molecular weight without a loss in physical properties, since the device matrix is still held together by the crystalline regions. The reduction in molecular weight is followed by a reduction in physical properties, as water begins to fragment the device. In the second phase, enzymatic attack and metabolization of the fragments occurs, resulting in a rapid loss of polymer mass. This type of degradation, where the rate at which water penetrates the device exceeds that at which the polymer is converted into water-soluble materials, is called bulk erosion. This results in erosion throughout the device. All commercially available synthetic devices and sutures degrade by bulk erosion. A second type of biodegradation, known as surface erosion, occurs when the rate at which the water penetrates the scaffold is slower than the rate of conversion of the polymer into water-soluble materials. Surface erosion results in the device thinning over time while maintaining its bulk integrity. In general, this process is referred to as bioerosion rather than biodegradation. In principle, the degradation rate of the scaffold should match the rate of tissue formation. Therefore, the degradation behavior of a scaffold has crucial impact on the long-term performance of a tissue-engineered cell/ scaffold construct (Babensee *et al.*, 1998). Several factors, such as polymer molecular weight, polydispersity (Recum *et al.*, 1995), crystallinity (Pistner *et al.*, 1993), shape and morphology (Grizzi *et al.*, 1995), are known to affect the rate of hydrolytic degradation of polyester. Other factors, such as pH, ionic strength, temperature and buffering capacity of the medium in which the degradation occurs, also influence the degradation kinetics (Vert *et al.*, 1990). Moreover, the chemical environment of the cleaved bonds, rigidity of the polymer chain, the molar mass of

the polymer, adsorption and surface activation of the enzyme, removal and dissolution of fission products from the surface etc. are discussed to control the degradation process (Huang *et al.*, 1994, Chandra *et al.*, 1998, and Scherer *et al.*, 1999).

2.4.1 Enzymatic Degradation

The degradation of polyesters by microorganisms is initiated by extracellular hydrolases, which are secreted by the organisms to reduce the molar mass of the polymeric substrate and to make it bioavailable. It was first demonstrated by Tokiwa *et al.*, 1977 that synthetic polyesters also can be attacked by hydrolases (lipases). Detailed and systematic studies on the influence of polyester-specific parameters on the biodegradation with lipases were reported by Marten *et al.*, 2003. As a basic assumption, it is anticipated that the degradation of synthetic polymers such as polyesters and polyamides follows a scheme similar to that of natural polymers. As shown in Figure 2.8, the presence of polymers induces or enhances the microbial production of enzymes which are excreted into the environment and are capable to cleave specific bonds in the polymer chain being available for the enzymatic system on the surface of the polymeric material. Thus, the solid polymer is destructed layer by layer (surface erosion) and short chain and water soluble intermediates and monomers are generated, which can be assimilated into the cells. In an ideal situation, the polymer is finally completely decomposed and converted into biomass, $CO_2$, and $H_2O$.

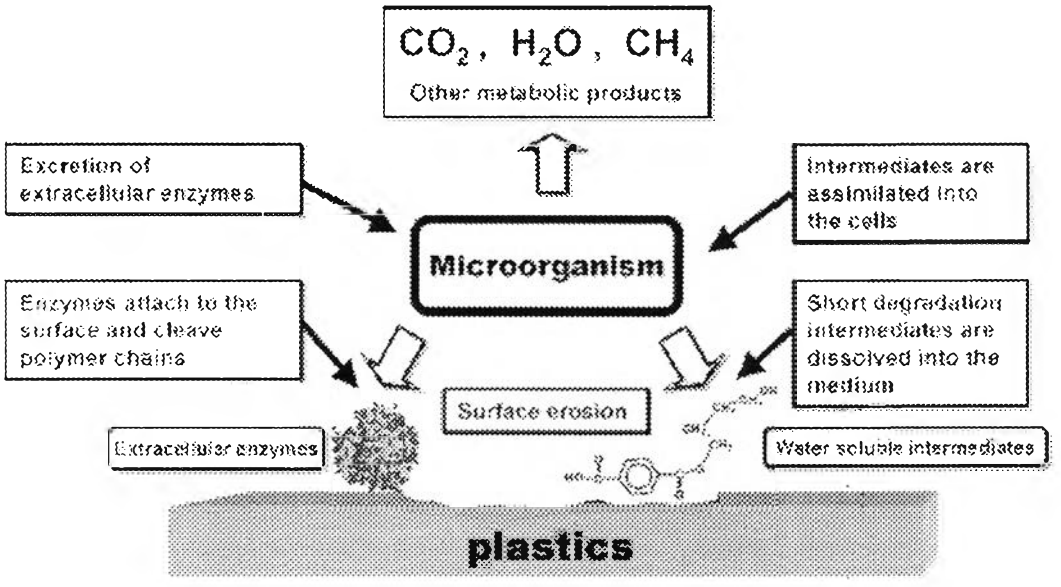



**Figure 2.8** Principle of microbial polyester degradation (Marten *et al.*, 2003).

Tsutsumi *et al.*, 2003 studied on enzymatic degradation of commercial biodegradable polymeric films by eleven kinds of lipases and they found that aliphatic polyesters were most degradable by lipase from *Pseudomonas sp.* The serum lipase concentration in healthy adults is 30-190 units/l. (Chawla *et al.*, 2002).