



RECENT DEVELOPMENT ON BIOCOMPOSITES RESAERCH

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Abstract

Since the last decade, tissue engineering has shown a sensational promise in providing more viable alternatives to surgical procedures for harvested tissues, implants and prostheses. Due to the fast development on bio-material technologies, it is now possible for doctors to use patients' cells to repair orthopedic defects such as focal articular cartilage lesions. In order to support the three-dimensional tissue formation, scaffolds made by bio-compatible and bio-resorbable polymers and composite materials, for providing temporary support of damaged body and cell structures have been developed recently. Although ceramic and metallic materials have been widely accepted for the development of implants, its non-resorbability and necessity of second surgical operation, which induces extra for the patients, limit their wide applications. This review article aims at introducing (i) concept of cartilage tissue engineering, (ii) common types of bio-engineered materials and (iii) future development of biomaterial scaffolds.

Introduction

With the strong emphasis on environmental awareness, it has brought much attention in the development of recyclable and environmentally- sustainable composite materials since the last decade. Environmental legislation as well as consumer demand in many countries are putting more pressure on manufacturers of materials and end-products to consider the environmental impact of their final products at all stages of their life-cycle, including recycling and ultimate disposal. Natural fibers such as cellulose, and animal based fibers such as cocoon and spider silks have been widely recognized as strong natural reinforcements for soft recyclable polymers to form relatively high strength biodegradable composites compared with traditional polymers. Since these fibers are fully extracted from the nature, their advantages in terms of biodegradability and re-productivity are

outstanding. In general, many organic compounds require over 10^6 years associated with the application of high pressure and temperature to decompose them into simple forms of substances which can be directly absorbed by the nature or converted into other forms of useable materials such as crude oil and fertilizers. Due to the increasing demand of non-decomposable materials, particularly in the electronic product industry, the increase of solid wastes leads to the destruction of our environment because of the production of toxic gas during the manufacturing and/or decomposition process. In the United States, it encourages manufacturers to produce materials and products by practicing 3Rs, which are (i) to reduce the amount and toxicity of trash to be discarded (source reduction); (ii) to reuse containers and products, i.e. to repair what is broken or give it to someone who can repair it, and (iii) to reuse as much as possible, which includes buying products with recycled content. In Hong Kong and Mainland China, plastic wastes including polymer-based reinforced materials, are about 0.2 and 600 million tones each year, respectively. In the States, 4.4 million tones of metal wastes and agriculture and domestic wastes have been produced every year. It has been anticipated that these figures will be continuously increasing in the very near future if no proper measure is taken today. Although natural and animal fibers can be used to reinforce polymer-based materials, the amount of mechanical properties enhanced and the improvement of thermal stability are limited due to some weak properties of such fibers.

Most biodegradable polymers have excellent properties comparable to many petroleum-based plastics and they are readily biodegradable, and may soon be competing with commodity plastic. However, some of the properties such as brittleness, low heat distortion temperature, low melt viscosity restrict their applications in real life. Currently, there are two types of high strength green composites in the market, one is

the composites that are fully degradable which include natural and/or animal fiber reinforced biodegradable polymer composites. While another is partly biodegradable polymer composites. The base polymers used for these two types of composites are fully biodegradable such as PLA, PGA, PHB, TPS, PET and Chitosan. However, the reinforcements used for the former type are natural and animal fibers that include cellulose, fruit, ramie, kenaf, heap and Jute fibers, while the latter type involves non-degradable reinforcements such as carbon nanotubes, montmorillonite, in a layered form (hereafter called “nanoclays”) and nano-metallic particles. For the latter type, the use of ultra-strong nano-materials as nano-reinforcements for polymers is to enhance their mechanical strength and thermal stability. Since, in general, only a small amount of nano-materials is used for bio-degradable polymers to form grebe composites, the potential applications of the host bio-degradable polymers are then increased due to their enhancement of strength and thermal properties (for an example; glass transition temperature (T_g)).

Nanotube Polymer Composites

There is no doubt that carbon nanotubes are the world’s ultra-strong materials. Theoretically, their Young’s modulus and tensile strength reach 1 TPa and 200 GPa, respectively. By mixing these materials into polymers, they can serve as reinforced fibers (though a relative small scale) to reinforce the polymer to achieve its desirable mechanical and thermal properties. The nanotubes, generally speaking can be separated into three types, they are (i) zigzag, (ii) armchair and (iii) chiral. Many studies have reported that zigzag nanotubes can provide higher mechanical strength of their composites, while the risk of failure by a high interfacial shear stress increases [3-4]. Nanotubes can be manufactured in many different forms and shapes, such as singlewalled, multiwalled, coiled, bamboo and nanoclay-supported nanotubes. By applying these nano-materials, as nano-reinforcements, into bio-degradable polymers such as PLA and PGA, it would extend their applicability in different engineering industries. At least, these polymer-based composites can be used for producing some primary structural components.

Layered Silicate Green Composites

Polymer-based layered silicate nanocomposites have been well accepted in advanced composite industry since the addition of a small amount of layered silicates into polymer can substantially enhance the mechanical, electrical and thermal properties of host polymers [1]. The intercalated and exfoliated nanoclay structures can interrupt the cross-link of polymer

chains inside the composites when they are in the curing process, and thereafter generate both chemical and mechanical interaction between clay planar structures and matrix [2]. However, outside the laboratory, it is difficult to produce pure intercalated or exfoliated structures in real practice. Nanoclay clusters were also used to investigate their reinforcing ability to some polymers. Xu et al [2] have reported that modified nanoclay/Poly (propylene carbonate)(PPC) showed an increase of thermal decomposition temperature as well as storage modulus. Lam et al [5] has indicated that the hardness and wear resistance of nanoclay cluster polymer-based composites increased with an increase of the nanoclay content. However, it should have an optimal point.

Scaffolds for Tissue Engineering

Scaffolds used in tissue engineering applications, particularly for articular cartilage repair should demonstrate compatible biological and physical properties which match the physiological conditions in vitro and in vivo. The major function of the scaffolds is to provide a temporary support to body structures to allow the stress transfer over –time to injured sites, and facilitate tissue re-generation on the scaffolds. Recognition of this phenomenon raises the possibility that the scaffolds should have sufficient mechanical integrity to retain their shape and at implantation site until their function completed. The selection of appropriate materials for implant development requires satisfying some criteria, in which the effect to the human has to keep as minimal as possible. Besides, the biodegradability and the ability of cell growing from the scaffolds are highly depending on their physical and structural properties, such as interconnectivity, porosity and surface morphology. The design of the scaffold systems for articular cartilages, in human musculoskeletal system, is rather complicated due to the combination of repeated shear and compressive stresses co-exist. In the sack of simplicity, most of the previous studies were simply focused on a pure compressive stress acted on the articular cartilage system. In general, the design of scaffolds should allow the temporary support of body’s structures or injured sites through the stress transfer from one part to another, and thereafter be degraded over time. To select an appropriate type of materials for designing and manufacturing scaffolds, several key factors, listed as the follows, have to be studied in detail.

Biocompatibility

The biocompatibility of materials inside the human body plays a key role in tissue engineering, which ensures the materials are safe for use in the human body and in the endogenous fluids. Being an ideal implant

for repairing tissue defects and regenerating neo-tissue for wound sites, the materials used must be biocompatible, i.e. the materials must not induce any inflammatory response, extreme immunogenicity or cytotoxicity to native cells, tissues or organs in vivo. Since the implants are normally imbedded into the human body and last for a period of time, bi-products result from the degrading process of the implants should not produce any harmful material and/or element to the body also. For recent polymer-based scaffold design and development, the basic requirement is that the scaffolds must be naturally degraded with time, and gradually absorbed by the human body itself without generating any side effect. This process allows the functionality of the human body gradually recovers without too relying on the support of the scaffolds. Due to the demand on scaffolds and implants has been increasing, many comprehensive research on the biocompatibility of scaffolds and the toxicity of their decomposed products have been progressed rapidly in the past ten years.

Biodegradability and Bioresorbability

The field of biodegradable polymers is a fast growing area of polymer science because of the interest of such compounds for temporary surgical and pharmacological applications. As referred to the definitions of these two phenomena, biodegradable materials, like polymers can be decomposed naturally but their degraded products will remain inside the human body. For bioresorbable materials, they will degrade after a certain period of time of implantation, and non-toxic products will be produced in the ways of elimination with time and/or metabolism. For the chemical degradation, two different modes are defined, they are (1) hydrolytic degradation or hydrolysis which is mediated simply by water and (2) enzymatic degradation which is mainly mediated by biological agents such as enzymes. Scaffolds should be biodegradable allowing ECM to occupy the void space when the biomaterial is degraded. Today, there are many types of materials that are widely used as bioabsorbable implants and their market is expanding rapidly worldwide. In fact, the advantages of using bio-degradable polymers over the traditional metallic materials for implant development include the reduction of stress bearing capability over time since the polymer will be degraded naturally, and the alleviation of pains, both physically and physiologically due to the need of second surgical operation for removing metallic implants.

Degradation Rate, Pore Size and Surface Morphology

The major function of a biodegradable polymer-based scaffold must be able to retain at the implantation site with maintaining its physical characteristics and ordinary mechanical properties, as well as supporting the attachment, proliferation and differentiation of cells, until the regeneration of tissues on an injured site. Ideally, the degradation rate of the scaffold should be matched with the rate of neo-tissue formation so as to provide a smooth transition of the load transfer from the scaffold to the tissue. However, based on recent research investigations, it was found that the degradation rate of different types of bio-degradation polymer has difference, depending on the composition of the polymer, conditions of loading and ambient environment. The enzymatic degradation rate proceeds from the surface of the polymer, therefore surface area and condition (porosity) of the polymer is one of the important factors to control the degradation rate.

Up-to-date, there are many ways to manufacture porous scaffolds, they are porogen leaching, emulsion freeze-drying, expansion in high temperature gas, 3D printing, phase separation techniques, thermal phase separation. In **Figure 1**, two porous films of PLA and PCL were fabricated by using the freeze-dry method is shown. It was found that the degradation rate of the films varied with the porous size and density.

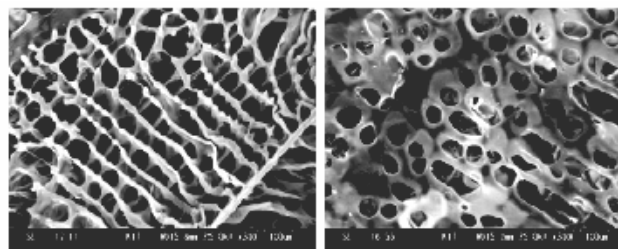


Fig. 1. SEM photographs of porous PLA (lef) and PCL (right) surface [8].

The two porous films decomposed faster than that of the films without pores (as shown in **Figure 2**). Large number of pores may be able to enhance the mass transport and neo-vascularization within the implants, whereas smaller diameter of pores is more preferable to provide larger surface per volume ratio. Moreover, instead of adjusting the pore size, the shape of the pores is also a key to affect the efficiency of tissue regeneration. It is important to note that some specific pore sizes can enhances the cellular activity, but optimal size and geometry are highly dependent on specific cell types grown on injured sites. For bone ingrowth, the optimal pore size is in the range of 75-250 μm . On the other hand, for in growth of fibrocartilaginous tissue, the recommended pore size ranges

from 200 to 300 μm . In Figure 3, it shows the percentage of cells attached onto scaffolds with different pore sizes and it further proves that the control of pore size for different scaffolds is necessary.

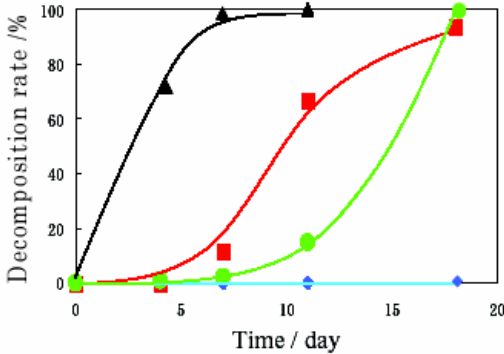


Fig. 2. Decomposition rate of porous (\blacktriangle) and original (\bullet) PCL films, and porous (\blacksquare) and original (\blacklozenge) PLA films [8].

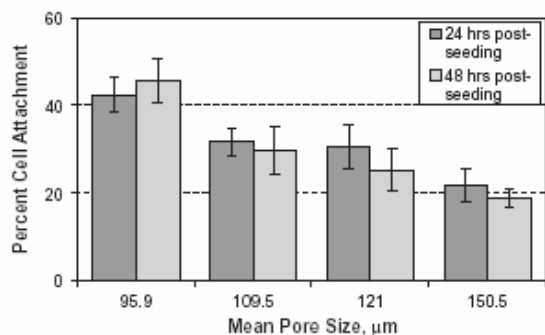


Fig. 3. Percentage of cells attached to the collagen-glycosaminoglycan (CG) scaffolds with different pore sizes [9].

Most organ cells require suitable substrate to retain their ability to adhere, proliferate and perform the later differentiated functions including spreading and differentiating seeded cells. Therefore, the design of scaffolds for tissue regeneration, the control of surface topography and roughness is important to allow the migration of the cells on the scaffolds' surface. The rougher surface also enhances the diffusion rates to and from the scaffolds as well as facilitates vascularization and improves the oxygen and nutrients supply and waste removal. In Figure 4, the osteoblast-like cells were grown on poly (L-lactic acid (PLLA) substrates with different surface morphologies, one was a smooth flatted surface while another with PLLA islands. The height of the cells on smooth surface was higher than that of the cells on the island-patterned PLLA, which implied that the cells preferred to stretch on a rough surface. The uniformity of the cells grown on the smooth PLLA substrate was also relatively poor as compared with that grown on the island-patterned

PLLA substrate. Besides in the figure, it also clearly indicates that the total contact area of the cells to the PLLA was much larger as compared with the one without the island-patterned structure built on the PLLA surface.

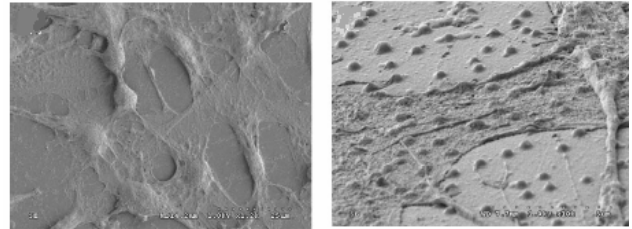


Fig. 4. SEM images of OCT-1 osteoblast-like cells attached on the (left) smooth surface of PLLA substrate and (right) island-patterned PLLA substrate [10].

Mechanical Integrity

In order to fulfil the physiological loading requirement, tissue engineered scaffolds should act as temporary physical supports (or called "structural members") to withstand the external and internal stresses until neo-tissues are generated. As for a native cartilage, its metabolism, synthesis, and the organization of ECM are affected by the mechanical environment experienced by chondrocytes. These forces are usually generated during both the implantation procedure and the mechanical forces experienced at the joint surfaces. In vitro, the scaffolds have to be able to resist the culturing mechanical environment such as direct compressive, hydro-static pressure and static loads. Especially, for the dynamic functional environment of the scaffolds, it can have a significant effect on the scaffolds in vitro degradation as well as release of bioactive agents. Consequently, the properties of the scaffolds should be as similar as the properties of neo-tissues generated, in order to provide a proper structural support in the stage of healing. At the later stage, all the loads have to be totally transferred to the neo-tissues since the scaffolds should be degraded gradually. Neves et al. [6] have studied the change of mechanical properties of starch with poly (lactic acid) (SPLA) with time. It is obvious that the strength of the SPLA dropped substantially after immersed into isotonic saline solution (0.154 M of NaCl) for 3 days in spite of the change of its deformation was small.

Types of Scaffold Biomaterials

As mentioned in the previous sections, the major concern in developing scaffolds for different surgical and orthopedic operations is the selection of suitable biomaterials, which must be biocompatible and biodegradable. Potential materials that have been proven with experimental data on their validity for biomedical applications are metal, ceramics, polymers

and the combinations of these materials. For metallic materials and ceramics, they have contributed to lists of medical applications, particularly in orthopedic tissue replacements. However, there are two major limitations, they are (i) not biodegradable except biodegradable bio-ceramics, and (ii) poor processability. For articular cartilage and scaffold design, normally the stiffness and coefficient of friction should be relatively low and the shape is highly irregular, in which the metallic materials cannot fulfil this requirement.

To successfully adopt biodegradable polymers for cartilage tissue engineering applications, understanding the compressive and shear stress transfer mechanisms at cartilage joints is essential to ensure the physical and mechanical properties of articular joints are strong enough to sustain different loadings. Basically, there are four types of polymer-based materials used for cartilage joints, they are (i) natural polymers, (ii) synthetic polymers, (iii) hydrogels and (iv) composites. Many natural polymers found in living organisms of known biocompatibility, actually are hydrogels and some are being popularly used in combination with other materials as composite scaffolds. Such polymers can be used to replace or regenerate native tissue structures and allows positive cell interactions with surrounding tissues. Conversely, synthetic polymers are formed through controllable chemical processes to achieve desirable material and chemical properties for a wide range of bio-medical applications. The synthetic polymers have a promising advantage over the natural polymers for scaffold developments because their mechanical and proliferation properties are comparatively more predictive and reproducible. Hydrogels are primarily composed of fluid that swells the polymer network to form a biphasic construct. Although the hydrogels can be synthesized, they are always formed naturally. Composite scaffolds are built by mixing two or more materials to achieve desirable properties and characteristics by taking advantages from each of the materials.

Natural polymers

Natural polymers used in cartilage engineering applications include collagen, alginate, agarose, chitosan, fibrin and hyaluronic acid-based materials. Natural polymers often possess highly organized structures and may contain an extracellular substance, called ligand, which can be bound to cell receptors. Although they are of known biocompatibility, lack of large quantities and difficult in processing into scaffolds limit them for clinical applications. Moreover, as natural polymers can guide cells to grow at various

stages of development, they may stimulate an immune response at the same time. This leads the concerns over antigenic and deliver of diseases for allograft. Since the degradation of these polymers depends on the enzymatic processes, degradation rate may vary from patient to patient.

Collagen is a fibrous protein that is the major component in connective tissues, which has been used widely for tissue regeneration applications, particularly for soft tissue repair. It provides cellular recognition for regulating cell attachment and function, but may lead to the concern of adverse immune response. Collagen favours cell adhesion that is normally found in joint tissues and those exogenous cells embedded in a collagen delivery device. Although it has to be purified before seeded with chondrocytes to make it less antigenic, it may transmit pathogen and induce immune reactions. On the other hand, it has poor mechanical properties, difficult to handle and fabricate, and is also less control over biodegradability.

Alginate is a polysaccharide extracted from algae. It can be formed as gels or beads, which are also used for cartilage repair, as a support to encapsulate cells within the scaffold. Through encapsulation, chondrocyte's phenotype during culture can be maintained and this allows cell that has been cultured in monolayer to be re-differentiated. Alginate, as a liquid, is injected and cross-linked with calcium to prevent the migration of the defect and allow the formation of a tissue with similar morphological characteristics as the native hyaline cartilage. Conversely, there are concerns on its slow degradation rate as it may cause problems when the neo-tissue starts to grow; insufficient mechanical integrity, and long term implants are impossible as alginate scaffolds will lose its functionality within a year.

Agarose is also a polysaccharide but it is extracted from seaweeds. It can be used to form hydrogels, which makes the seeded cells with more uniform distribution throughout scaffolds. It can be prepared as an injectable scaffold and provide a three-dimensional environment helps to maintain chondrocyte in a rounded shape during culture. However, it degrades slowly like alginate. Agarose exhibits a temperature-sensitive solubility in water for encapsulating cells. It has been used widely for chondrocyte behaviour studies in vitro and showed that agarose transmits the applied mechanical forces to the chondrocytes during compression, which excites the cells to produce more extracellular matrix proteins than during static controls.

Chitosan is a biosynthetic polysaccharide deacetylated derivative of chitin which is a naturally occurring polysaccharide extracted from crab shells, shrimps etc. or from a fungal fermentation process. Chitin and chitosan are semicrystalline polymers having a high degree of biocompatibility in vivo. Chitosan can be prepared as temperature-sensitive carrier materials and are injectable as fluids. It forms gels at body temperature and has the ability to deliver and interact with growth factors and adhesion proteins. Degradation of chitosan is controlled by the residual amount of acetyl content and it can degrade rapidly in vivo according to the deacetylation with the polymer. In addition, porosity of chitosan scaffolds can be controlled which can affect the strength and elasticity of the implants.

Fibrin glue is formed from the mixture of fibrinogen and thrombin and allows them to solidify. As the patient's own fibrinogen and thrombin can be used, it is not a concern on sterilization, biocompatibility and temperature change during setting. It is always being used as a carrier for cells and in conjunction with other scaffold materials. This material can be completely degraded and injected but with a drawback on lack of mechanical strength for articular cartilage engineering applications.

Hyaluronan (or hyaluronic acid, HA) is an anionic polysaccharide, which can be obtained from natural sources or through microbial fermentation and has often been used as carriers for cells to regenerate various tissues. It can be cross-linked to form scaffolds and seeded with chondrocytes. As it is injectable, it can be used in irregularly shaped defects and implanted with minimal invasion. Hyaluronic acid can be easily chemically modified, like collagen. And it is a desirable biomaterial since it is not antigenic and elicits no inflammatory or foreign body reaction. However, it has limited range of mechanical properties for applications.

Synthetic Polymers

Synthetic polymers are man-made polymers, which have the advantages over the use of natural origin polymers as they are more flexible, more predictable and processable into different size and shapes. The physical and chemical properties of a polymer can be easily modified and the mechanical and degradation characteristics can be altered by their chemical composition of the macromolecule. The functional groups and side chains of these polymers can be incorporated, i.e. the synthetic polymers can be self-cross-linked or cross-linked with peptides or other bioactive molecules, which may be a desirable

biomaterial for cartilage tissue engineering. Additionally, synthetic polymers are generally degraded by simple hydrolysis that is desirable as the degradation rate does not have variations from host to host, unless there are inflammations and implant degradation etc. to affect the local pH variations. The most extensively used synthetic polymers are Poly(glycolic acid) (PGA), Poly(lactic acid)(PLA) and their co-polymers; Polycaprolactone (PCL) and Polyethylene glycol (PEG).

Poly(lactic acid) (PLA) is an alpha polyester used widely in medical applications and it has been approved by the FDA for implantation in human body. PLA degrades slower than PGA due to its hydrophobic characteristic, which limits the water absorption of thin films and slows down the backbone hydrolysis rate. Based on available data to date, the duration of degradation can be ranged from 12 months to over 2 years. PLA can have a relatively low tensile strength and modulus of elasticity alone, depending on which isomer is used in the polymer. Although many researchers have tried to investigate porous PLA scaffolds for the usage in orthopaedic applications, PLA is primarily used as a non-woven mesh for tissue engineering applications. Murphy and Sambanis [7] have shown that fewer chondrocytes were attached to PLLA than to PGA at the initial stage, but as both surfaces allow extensive cells proliferation, giving the similar total number of cells at confluence. Lactic acid exists in two stereoisomerism forms, which can be separated into four morphologically distinct polymers namely D-PLA, L-PLA, D,L-PLA and meso-PLA. Degradation products of these materials reduce local pH, accelerate the polyester degradation rate and induce inflammatory reaction.

Poly(glycolic acid) (PGA) is the simplest linear aliphatic polyester. It has a highly crystalline structure with a high melting point and low solubility in organic solvents. PGA degrades too rapidly that makes it difficult to process and handle. The degradation period can be from 4 to 12 months, which is much shorter when comparing with PLA. The degradation products of PGA are naturally resorbed by the body makes it desirable to be a biomaterial. In comparison to PLA, PGA has a relatively high tensile strength and modulus of elasticity. However, these could not directly translate to the scaffold stiffness as the material is generally formed into a mesh or a felt for cartilage tissue engineering purposes. PGA is often extruded as thin polymer strands, which is around 13 μm in diameter, it must be moulded into non-woven mesh discs as a scaffold. This provides a high porosity environment for cells to grow and proliferate. To the contrary, this limits

its immediate applications on hard tissue engineering as the tissue need to fill much of the void space in order to maintain the construct with sufficient mechanical integrity. Consequently, the mechanical properties of PLA and PGA are relatively weak for high porosity scaffolds, which limit their usage on the hard tissue regeneration applications.

Conclusion

In this paper, a critical review on the use of nano-materials technology, based on the use of nano-reinforcements such as carbon nanotubes and nanoclays for bio-degradable polymers is given. Some nano-reinforcements are to be naturally degraded while some are not. However, the enhancements of the mechanical and thermal properties of these degradable polymer composites can definitely retard the damage of the environment.

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