



Introduction

*Gilson Khang,
Moon Suk Kim and
Hai Bang Lee*

It has been recognised that tissue engineering offers an alternative technique to tissue transplantation for diseased or malfunctioned organs. Millions of patients suffer from end-stage organ failure or tissue loss each year. In the United States alone, at least eight million surgical operations are carried out annually, requiring a total national healthcare cost exceeding US\$400 billion per year [1, 2]. In the case of cardiovascular disease, approximately 500 000 coronary artery bypass surgeries are conducted each year in the United States [3]. Autologous and allogenic natural tissues, i.e. saphenous vein or internal mammary artery, are generally used for coronary artery replacement. The results have been quite favourable for these procedures, with patency rates generally ranging from 50% to 70%.

Failure in these procedures may be caused by intimal thickening, due in large part to the adaptation of the vessel in response to increased pressure and wall shear stress, compression, adequate graft diameter, and disjunction at the anastomosis. Successful treatment may also be limited by the poor performance of synthetic materials, such as polyethylene terephthalate (PET, Dacron®) and expanded polytetrafluoroethylene (ePTFE, Gore-Tex®), used for tissue replacement due to plaguing problems [4]. Despite improved patient outcomes, many of these materials possess serious problems including unpredictable outcomes, fibrous capsule contraction, allergic reactions, suboptimum mechanical properties, distortion, migration, and long-term resorption.

In order to avoid the shortage of donor organs and the abovementioned problems caused by the poor biocompatibility of biomaterials, a new hybridised method combining cells and biomaterials has been introduced: tissue engineering [5]. To reconstruct a new tissue by tissue engineering, triad components are needed. These include (1) cells which are harvested and dissociated from the donor tissue, including nerve, liver, pancreas, cartilage, and bone as well as embryonic/adult stem or precursor cell; (2) biomaterials as scaffold substrates in which cells are attached and cultured, resulting in implantation at the desired site of the functioning tissue; and (3) growth factors which promote and/or prevent cell adhesion, proliferation, migration, and differentiation by upregulating or downregulating the synthesis of protein, growth factors, and receptors (Fig. 1).

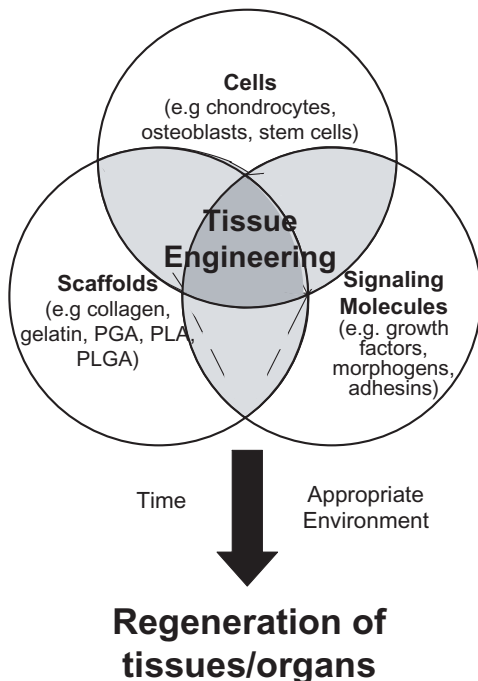


Fig. 1 Tissue engineering triad. Consisting of three key elements (i.e. cells, biomaterials, and signalling molecules), it regenerates tissue-engineered neo-organs.

Importance of Scaffold Matrices in Tissue Engineering

Scaffolds play a critical role in tissue engineering. The function of scaffolds is to direct the growth of cells either seeded within the porous structure of the scaffold or migrating from surrounding tissue. The majority of mammalian cell types are anchorage-dependent, meaning they will die if an adhesion substrate is not provided. Scaffold matrices can be used to achieve cell delivery with high loading and efficiency to specific sites. Therefore, the scaffold must provide a suitable substrate for cell attachment, cell proliferation, differentiated function, and cell migration.

The prerequisite physicochemical properties of scaffolds are many: to support and deliver cells; induce, differentiate, and

channel tissue growth; target cell-adhesion substrates; stimulate cellular response; provide a wound-healing barrier; be biocompatible and biodegradable; possess relatively easy processability and malleability into desired shapes; be highly porous with a large surface/volume ratio; possess mechanical strength and dimensional stability; and have sterilisability, among others [2, 6]. Generally, three-dimensional porous scaffolds can be fabricated from natural and synthetic polymers, ceramics, metals, composite biomaterials, and cytokine release materials.

Natural polymers for scaffolds

Many naturally occurring scaffolds can be used as biomaterials for tissue engineering purposes. One example is the extracellular matrix (ECM), a very complex biomaterial controlling cell function that designs natural and synthetic scaffolds to mimic specific functions. Natural polymers include alginate, proteins, collagens (gelatin), fibrins, albumin, gluten, elastin, fibroin, hyaluronic acid, cellulose, starch, chitosan (chitin), scleroglucan, elsinan, pectin (pectinic acid), galactan, curdlan, gellan, levan, emulsan, dextran, pullulan, heparin, silk, chondroitin 6-sulfate, polyhydroxyalkanoates, etc. Much of the interest in these natural polymers comes from their biocompatibility, relative abundance and commercial availability, and ease of processing [8].

Synthetic polymers for scaffolds

Natural polymers are typically in short supply because they are expensive, suffer from batch-to-batch variation, and are susceptible to cross-contamination from unknown viruses or unwanted diseases. On the contrary, synthetic polymeric biomaterials have easily controlled physicochemical properties and quality, and have no immunogenicity. They can also be processed with various techniques and consistently supplied in large quantities. In order to adjust the physical and mechanical properties of tissue-engineered scaffolds at a desired place in the human body, the molecular structure and molecular weight are easily adjusted during the synthetic process.

Synthetic polymers are largely divided into two categories: biodegradable and nonbiodegradable. Some nonbiodegradable

polymers include polyvinylalcohol (PVA), polyhydroxyethylmethacrylate (PHEMA), and poly(N-isopropylacrylamide) (PNIPAAm). Some synthetic biodegradable polymers are the family of poly(α -hydroxy esters) such as polyglycolide (PGA), polylactide (PLA) and its copolymer poly(lactide-co-glycolide) (PLGA), polyphosphazene, poly(anhydride), poly(propylene fumarate), polycyanoacrylate, poly(ϵ -caprolactone) (PCL), polydioxanone (PDO), and biodegradable polyurethanes.

Of these two types of synthetic polymers, synthetic biodegradable polymers are preferred for the application of tissue-engineered scaffolds because they minimise the chronic foreign body reaction and lead to the formation of completely natural tissue. That is to say, they can form a temporary scaffold for mechanical and biochemical support.

Bioceramics for scaffolds

Bioceramics are biomaterials that are produced by sintering or melting inorganic raw materials to create an amorphous or crystalline solid body, which can be used as an implant. Porous final products are mainly used for scaffolds. The components of ceramics are calcium, silica, phosphorus, magnesium, potassium, and sodium.

Bioceramics used for tissue engineering may be classified as nonresorbable (relatively inert), bioactive or surface active (semi-inert), and biodegradable or resorbable (noninert). Alumina, zirconia, silicon nitride, and carbons are inert bioceramics; certain glass ceramics, such as dense hydroxyapatites [$9\text{CaO}\cdot\text{Ca}(\text{OH})_2\cdot 3\text{P}_2\text{O}_5$], are semi-inert (bioactive); and calcium phosphates, aluminium calcium phosphates, coralline, tricalcium phosphates ($3\text{CaO}\cdot\text{P}_2\text{O}_5$), zinc calcium phosphorus oxides, zinc sulfate calcium phosphates, ferric calcium phosphorus oxides, and calcium aluminates are resorbable ceramics. Of these bioceramics, synthetic apatite and calcium phosphate minerals, coral-derived apatite, bioactive glass, and demineralised bone particle (DBP) are widely used in hard tissue engineering.

Cytokine release system for scaffolds

Growth factors, a type of cytokine, are polypeptides that transmit signals to modulate cellular activities and tissue development

such as cell patterning, motility, proliferation, aggregation, and gene expression. As in the development of tissue-engineered organs, the regeneration of functional tissue requires the maintenance of cell viability and differentiated function, encouragement of cell proliferation, modulation of the direction and speed of cell migration, and regulation of cellular adhesion. The easiest method for the delivery of growth factors is via injection near the site of cell differentiation and proliferation. However, this direct injection method incurs a relatively short half-life, a relatively high molecular weight and size, very low tissue penetration, and potential toxicity at the systemic level [2, 9].

One promising way to improve the efficacy of this technique is the locally controlled release of bioactive molecules for the desired release period by the impregnation into a scaffold. Through impregnation into a scaffold carrier, protein structure and biological activity can be stabilised to a certain extent, resulting in a prolonged release time at the local site. The duration of cytokine release from a scaffold is controlled by the types of biomaterials used, the loading amount of cytokine, the formulation factors, and the fabrication process. The cytokine release system may be designed for a variety of geometries and configurations, such as scaffold, tube, nose, microsphere, injectable forms, and fibre [10].

Fabrication and Characterisation for Scaffolds

Fabrication methods of scaffolds

Engineered scaffolds may enhance the functionalities of cell and tissue to support the adhesion and growth of a large number of cells by providing a large surface area and pore structure within a three-dimensional structure. Porosity provides adequate space, permits cell suspension, and penetrates the three-dimensional structure. These porous structures also promote ECM production, transport nutrients from nutrient media, and excrete waste products [6, 11]. Therefore, an adequate pore size as well as a uniformly distributed and interconnected pore structure are crucial to allow for easy distribution of cells throughout the scaffold structure. Scaffold structure is directly related to fabrication methods, many of which are listed in Table 1.

Table 1. The fabrication methods of scaffolds for tissue engineering.

Mechanism	Method	Remark
Leaching method	Solvent-casting/salt-leaching method	Chapter A
	Ice particle-leaching method	Chapter B
	Gas-foaming/salt-leaching method	Chapter C
	Gel-pressing method	Chapter D
Microsphere method	Biodegradable microsphere	Chapter E
	Macroporous bead	Chapter F
	Particle-aggregated scaffold	Chapter G
Phase separation method	Freeze-drying method	Chapter H
	Thermally induced phase separation	Chapter I
	Centrifugation method	Chapter J
Injectable gel	Polyphosphazene gel	Chapter K
Acellular scaffold	Decellularisation process	Chapter L
Keratin scaffold	Self-assembled process	Chapter M
Fibre-spinning method	Nanofibre electrospinning process	Chapter N
	Microfibre wet spinning process	Chapter O
	Nonwoven PGA fibre	Chapter P
Printing and prototyping method	Inkjet printing process	Chapter Q
	Melt-based rapid prototyping	Chapter R
Functional scaffold	Growth factor release process	Chapter S
Ceramic scaffold	Sponge replication method	Chapter T
	Simple calcium phosphate coating method	Chapter U

This book introduces detailed protocols for 21 different types of manufacturing methods for scaffolds in Chapters A–U. The most common and commercialised one is the PGA nonwoven sheet (Albany International Research Co., Mansfield, MA, USA), which has a porosity of approximately 97% and a thickness of 1–5 mm. In order to dimensionally stabilise and provide the mechanical integrity, the fibre-bonding technology by heat and by PLGA/PLA solution spray coating methods has been developed [12] (Chapter P).

Progen-leaching methods combine the polymerisation, solvent casting, gas foaming, or compression moulding of natural and synthetic scaffold biomaterials with the leaching of

pore-generating particles (e.g. sodium chloride crystal, sodium tartrate, and sodium citrate) sieved using a molecular sieve [2, 11]. PLGA, PLA, collagen, poly(ortho ester), or small intestine submucosa (SIS)-impregnated PLGA scaffolds have successfully fabricated a biodegradable sponge structure by this method with more than 93% porosity and a desired pore size of 1000 μm . Using the solvent-casting/particulate-leaching method, complex geometries such as tube, nose, and specific organ types can be fabricated as nanocomposite hybrid scaffolds by means of conventional polymer processing techniques like calendaring, extrusion, and injection. Complex geometries can be fabricated from the porous film lamination [13]. The advantage of this method is easy control of porosity and geometry. However, the disadvantages are the loss of water-soluble biomolecules or cytokines during porogen leaching, the possibility of remaining porogen as salt that can harmfully affect cell culture, and the different geometry surfaces and cross-sections (Chapters A–D).

The gas-foaming method refers to the exposure of a solid scaffold matrix to a sudden expansion of CO_2 gas under high pressure, resulting in the formation of a sponge structure due to nucleation and expansion in the dissolved CO_2 scaffold matrix. PLGA scaffolds with more than 93% porosity and about 100 μm median pore size have been developed using this method [14]. The significant advantage is no loss of bioactive molecules in the scaffold matrix, given that there is no need for the leaching process and no residual organic solvent; whereas the disadvantage is the presence of skin film layers on the scaffold surface, resulting in the further removal process of this skin layer (Chapter C).

The phase separation method is divided into freeze drying, freeze thawing, freeze immersion precipitation, and emulsion freeze drying [15]. Phase separation by freeze drying can be induced by a polymer solution with an appropriate concentration by rapid freezing. The used solvent is then removed by freeze drying, resulting in porous structure as a portion of the solvent. Collagen scaffolds with pores of 50–150 μm , collagen–glycosaminoglycan blend scaffolds with an average pore size of 90–120 μm , and chitosan scaffolds with a pore size of 1–250 μm have been developed; the sizes vary with the freezing condition.

In addition, scaffold structures of synthetic polymers such as PLA and PLGA have been successfully made using this method, with over 90% porosity and 15–250 μm size.

The freeze-thawing technique induces phase separation between a solvent and a hydrophilic monomer upon freezing, followed by the polymerisation of the hydrophilic monomer by means of UV irradiation and removal of the solvent by thawing. This leads to the formation of macroporous hydrogel. A similar method is freeze immersion precipitation. The polymer solution is first cooled before being immersed in a nonsolvent and then a vapourised solvent, leading to porous scaffold structure. The emulsion freeze drying method is also useful for the fabrication of porous structure. In this case, a mixture of polymer solution and nonsolvent are thoroughly sonicated, quickly frozen in liquid nitrogen at -198°C , and then freeze-dried, resulting in sponge structure. The advantage of these phase separation techniques is the loading of hydrophilic or hydrophobic bioactive molecules, but the disadvantages are a relatively small pore size and difficulty in controlling the precise pore structure (Chapters H–J).

Injectable gel scaffolds have also been reported [11]. Injectable, gel-forming scaffolds provide several advantages: they can fill any shape of defect due to flowable materials, load various types of bioactive molecules and cells by simple mixing, do not contain residual solvents that may be present in a performed scaffold, and do not require surgical procedure for placement. Typical examples are thermosensitive gels such as Pluronics and polyethylene glycol (PEG)–PLGA–PEG triblock copolymer; pH-sensitive gels such as chitosan and its derivatives; ionically cross-linked gels such as alginate; fibrin gel; hyaluronan gel; etc. In the near future, multifunctional and tissue-specific gels, very fast sol–gel transition, and injectable scaffold materials that are fully degradable for the desired period are expected to be available (Chapter K).

Nanoelectrospinning of PGA, PLA, PLGA, PCL copolymers, collagen, elastin, etc. has been extensively developed. For example, electrostatic processing can consistently produce PGA fibre diameters at or below 1 μm . By controlling the pick-up of these fibres, the orientation and mechanical properties can be tailored to a specific need of the injured site. Collagen electrospinning has also been performed, utilising type I collagen

dissolved in HFP with 0.083 g/mL concentration. The optimally electrospun type I collagen nonwoven fabric appeared with an average diameter of 100 ± 40 nm, resulting in biomimicking of fibrous scaffolds (Chapters N–P).

Moreover, newly hybridised fabrication techniques, such as organic/inorganic and synthetic/natural at the nano-sized level, are being continuously developed for the application of tissue-engineered scaffolds.

Physicochemical characterisation of scaffolds

For the successful achievement of three-dimensional scaffolds, several characterisation criteria are required. They can be divided into four categories: (1) morphology (e.g. porosity, pore size, surface area); (2) mechanical properties (e.g. compressive and tensile strength); (3) bulk properties (e.g. degradation and its relevant mechanical properties); and (4) surface properties (e.g. surface energy, chemistry, charge).

Porosity is defined as the fraction (i.e. percentage) of the total volume occupied by voids. The most widely used methods for measuring porosity are mercury porosimetry, scanning electron microscopy, and confocal laser microscopy.

Mechanical properties are extremely important when designing tissue-engineered products. To determine the mechanical properties of a porous structure, conventional testing instruments may be used. Mechanical tests can be divided into creep tests, stress–relaxation tests, stress–strain tests, and dynamic mechanical tests. These test methods are similar to those of conventional biomaterials.

The rate of degradation of manufactured scaffolds is one of the most important factors in designing tissue-engineered products. Ideally, the scaffold construct provides mechanical and biochemical support until the entire tissue regenerates without any change, and then it completely biodegrades at a rate consistent with tissue generation. Immersion studies are commonly conducted to track the degradation of biodegradable matrices. So, the changes in weight loss and molecular weight can be evaluated by the chemical balance, scanning electron microscopy, and gel permeation chromatography. From these results, the mechanism of biodegradation can be determined.

It is generally recognised that the adhesion and proliferation of different types of cells on polymeric materials depend largely on surface characteristics such as wettability (hydrophilicity/hydrophobicity of surface free energy), chemistry, charge, roughness, and rigidity. In particular, three-dimensional applications of tissue engineering are more important for cell migration, cell proliferation, DNA/RNA synthesis, and phenotype presentation on the scaffold materials. Surface chemistry and charge can be analysed by electron scanning chemical analysis and streaming potential, respectively. The wettability of the scaffold surface can be measured by the contact angle with static and dynamic methods.

References

- [1] Langer R, Vacanti J. *Science* **260**: 920–926, 1993.
- [2] Khang G, Lee SJ, Kim MS, Lee HB. Biomaterials: tissue engineering and scaffold, in Webster J (ed.), *Encyclopedia of Medical Devices and Instrumentation*, 2nd ed., Wiley Press, New York, 366–383, 2006.
- [3] Mann BK, West JL. *Anat Rec* **263**: 367–371, 2001.
- [4] Lee HB, Khang G, Lee JH. Polymeric biomaterials, in Park JB, Bronzino JD (eds.), *Biomaterials: Principles and Applications*, CRC Press, Boca Raton, FL, 2003.
- [5] Petit-Zeman S. *Nat Biotechnol* **19**: 201–206, 2001.
- [6] Chaignaud BE, Langer R, Vacanti JP. The history of tissue engineering using synthetic biodegradable polymer scaffolds and cells, in Atala A, Mooney DJ (eds.), *Synthetic Biodegradable Polymer Scaffolds*, Birkhauser, Boston, MA, 1996.
- [7] Wong WH, Mooney DJ. Synthesis of properties of biodegradable polymers used as synthetic matrices for tissue engineering, in Atala A, Mooney DJ (eds.), *Synthetic Biodegradable Polymer Scaffolds*, Birkhauser, Boston, MA, 1996.
- [8] Baldwin SP, Saltzman WM. *Adv Drug Deliv Rev* **33**: 71–86, 1998.
- [9] Khang G, Kim MS, Min BH, Lee I, Rhee JM, Lee HB. *Tissue Eng Reg Med* **3**: 376–395, 2006.
- [10] Seal BL, Otero TC, Panitch A. *Mater Sci Eng R* **34**: 147–230, 2001.

- [11] Thompson RC, Wake MC, Yasemski MJ, Mikos AG. *Adv Polym Sci* **122**: 245–274, 1995.
- [12] Khang G, Shin P, Kim I, Lee B, Lee SJ, Lee YM, Lee HB, Lee I. *Macromol Res* **10**: 158–167, 2002.
- [13] Leibmann-Vinson A, Hemperly JJ, Guarino RD, Spargo CA, Heidaran MA. Bioactive extracellular matrices: biological and biochemical evaluation, in Lewandrowski KU, Wise DL, Trantolo DJ, Gresser JD, Yasemski MJ, Altobeli DE (eds.), *Tissue Engineering and Biodegradable Equivalents: Scientific and Clinical Applications*, Marcel Dekker, New York, 2002.
- [14] Khang G, Jeon JH, Cho JC, Lee HB. *Polymer(Korea)* **23**: 471–177, 1999.