Chapter 26

CONSIDERATIONS ON THE STRUCTURE OF BIOMATERIALS FOR SOFT- AND HARD-TISSUE ENGINEERING

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In the field of tissue engineering, various novel biomaterials have been developed in order to achieve regeneration of target tissues. Scaffolds for a specific tissue require specific characteristics such as mechanical properties, biocompatibility, and degeneration period. In terms of mechanical properties, one of the ultimate goals of these materials is to mimic naturally existing scaffolds such as tissue matrices. However, the physiological nature of naturally existing tissues is still difficult to mimic

The Handbook of Intelligent Scaffold for Regenerative Medicine
Edited by Gilson Khang

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by most artificially generated biomaterials. Controlling the degeneration period is also an important research interest for scaffold development. The degradation process *in vivo* is quite different from that *in vitro*. Furthermore, the degradation period is important, not only for the smooth replacement with regenerating tissue, but also for the long-term stability of the regenerated tissue. This idea is especially important for hard-tissue engineering, such as bone. In this chapter, the required properties of scaffolds for soft- and hard-tissue engineering are introduced and are intended to be a reference for designing novel scaffolds. There is a broad range of target tissues, so this chapter focuses on small-caliber blood vessels as an example of soft-tissue engineering and bone as an example of hard-tissue engineering. From a clinical point of view, several key features of scaffolds for these tissues are discussed and possible approaches to achieve these requirements are reviewed.

26.1 Introduction

To achieve efficient tissue engineering, various novel biomaterials and processing procedures have been developed. Recent developments in materials science have exponentially increased, and novel biomaterials have been introduced almost every year as a result. Scaffolds for tissue engineering should be designed from a clinical point of view since the ultimate goal of the materials is for clinical applications. Effective communication between material scientists and clinical doctors is one of the key factors for the development of successful biomaterials. Mechanical properties, biocompatibility, and degeneration period are other factors to be considered, which should also fulfill the clinical application requirements. In this chapter, we focus on two tissues, small-caliber blood vessels and bone. Through our preclinical *in vivo* and clinical studies, several key features of scaffold materials for these tissues are discussed, which aim to fill the gap between material scientists and clinical doctors.

Small-caliber blood vessels were selected as an example of soft-tissue engineering since generation of competent scaffolds for

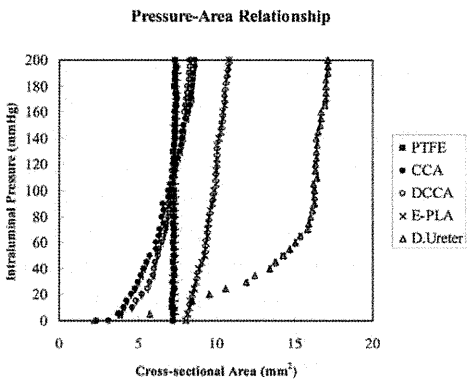

Figure 26.1. Pressure cross-sectional area relationship of each material. Polytetrafluoroethylene (PTFE) (■) showed a noncompliant response and almost no change in the cross-sectional area in response to intraluminal pressure. Canine common carotid artery (CCA) (•) and decellularized CCA (DCCA) (○) exhibited compliant responses, in a so-called J-shaped curve. Elastin gel combined with polylactic acid nanofiber tube (E-PLA) (×) evidenced cross-sectional area changes in response to an increase of intraluminal pressure, although the differences were relatively small compared with that of CCA, DCCA, or decellularized canine ureter (D.Ureter) D.Ureter (△) also showed a compliant response marked by more distensibility at a lower pressure range than CCA. and DCCA. Cross-sectional areas are expressed as mean values ± standard deviations (from Ref. 2, Murase *et al.*, 2006).

small-caliber blood vessels has been a challenge.[1] Despite enormous efforts to mimic natural blood vessels, the nature of the currently available materials is still not satisfactory (Fig. 26.1).[2] In order to overcome these difficulties, various approaches, including our own, are described.

Bone tissue engineering has been widely tested in the fields of orthopedic and oral surgery and has already been applied in clinics. In particular, regeneration of alveolar bone for dental implant surgery has attracted much attention since only a small volume of regenerated bone is required in most of the cases, which is advantageous in tissue engineering, and there is an increasing demand by elderly patients for dental implant surgery. Currently, animal-derived bioartificial bone substitutes have been widely used, but risks such as infection and immunoreactions against animal-derived proteins have

not been completely eliminated. Although the use of artificial bone substitutes has increased, animal-derived artificial bone may not be accepted as an equal alternative of autologous bone by most clinical doctors at present. Accordingly, the development of alternatives such as tissue-engineered bone is of immediate importance. From recent clinical studies, knowledge about the clinical fate of biomaterials has accumulated.[3–6] In this chapter, generation of scaffolds for bone tissue engineering, as an example of hard tissue, will also be considered. The required and favorable nature of scaffold materials for bone tissue engineering is discussed with reference to those findings obtained from recent clinical studies including at our own facility.

26.2 Material Design for Soft-Tissue Engineering: Small-Caliber Vascular Grafts

26.2.1 *Tissue Engineering for Cardiovascular Surgery*

Artificial organs, including vascular graft prostheses, have been investigated for more than 100 years in the field of cardiovascular surgery. Although state-of-the-art cardiovascular prostheses show increased patient survival rate, have minimized morbidity, and improved patient quality of life, currently available cardiovascular prostheses are still far from perfect due to immunogenicity (foreign-body reaction), thrombogenicity, lack of long-term durability, low patency rate of small-caliber prostheses, and growth inability. Tissue-engineered vascular grafts are a new promising alternative since they are expected to generate viable autologous tissue so that these shortcomings might be overcome. Despite such expectations, development of tissue-engineered vascular grafts is still in its early phase largely due to the lack of suitable scaffold materials. In general, two different scaffold materials have been used: (i) decellularized (natural) tissue matrix scaffolds and (ii) biodegradable synthetic polymer scaffolds. The latter can also be used as a slow-release device for various growth factors/cytokines since they are biodegradable.[7] In this section, we focus on tissue-engineered, small-caliber vascular grafts, which are one of the most challenging targets

for tissue engineering. In the last section, the prospects of scaffolds for cardiovascular tissue engineering are also discussed.

26.2.2 *Decellularized Tissue Scaffolds for Tissue-Engineered Small-Caliber Vascular Grafts: Methodology, Biocompatibility, and Mechanical Properties*

A decellularized technique has been developed to reduce immunogenicity and/or calcification of bioprostheses after implantation.[8,9] Advantages of using decellularized matrix for tissue engineering have been reported by many researchers.[10] The most favorable advantage of the decellularized scaffold is that it can be used to generate a completely identical structure of the target organ/tissue even if the structure is extremely complicated.

Various cell extraction methods, including detergent treatments, enzymatic digestion, and a combination of detergent and enzyme, have been reported for decellularization. Triton X-100,[11] sodium deoxycholate,[12] and sodium dodecyl sulfate (SDS)[13] are used as detergents, and trypsin[14] is used as an enzyme to disassociate the cells. As a unique method for decellularization, Fujisato *et al.* reported the use of ultrahigh pressure.[15] Each method has advantages and disadvantages, and optimization is necessary for each organ/tissue. For example, we found that esophagus treated with sodium deoxycholate showed superior mechanical properties, maintenance of extracellular matrix (ECM), and lower DNA content than that treated with Triton X-100.[16] Conversely, the degree of decellularization and maintenance of the matrix were best in the Triton X-100–treated ureters, while Triton X-100–treated and sodium deoxycholate–treated ureters had lower remnant DNA content and immunogenicity than the other treatments[17] (Fig. 26.2).

In vitro and *in vivo* biocompatibility of the decellularized scaffold was satisfactory (Fig. 26.3). Endothelial cells were easy to seed, and they adhered to the inner surface of the decellularized scaffold. The seeded cells functioned *in vivo* because cell-seeded grafts were found to have anti-thrombogenicity. Biocompatibility of the decellularized scaffolds depends on the native ECM, which may contribute

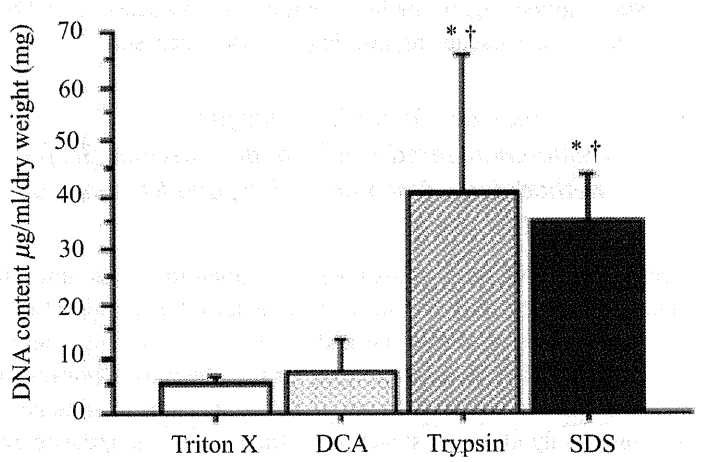

Figure 26.2. Residual DNA content of decellularized scaffolds prepared by four different methods. Triton X-100–treated and sodium deoxycholate–treated decellularized scaffolds had a significantly lower DNA content than SDS-treated and trypsin-treated scaffolds. DCA, deoxycholate. $*P < 0.05$ versus Triton X, $\dagger P < 0.05$ versus DCA (from Ref. 16, Narita *et al.*, 2008).

to cell adhesion and proliferation. The mechanical properties, for example, compliance, tensile strength, burst strength, and suture retention strength, of decellularized scaffolds were similar to those of the native tissue[17,18] (Fig. 26.1). Accordingly, it was suggested that cellular components may not play a major role in the mechanical properties of the decellularized scaffold but that the extracellular matrices could contribute to the mechanical strength and stiffness.

Potential shortcomings of decellularized scaffolds for small-caliber vascular grafts are long-term durability, aneurysmal formation, calcification, foreign-body (immunological) reactions due to residual allogenic or xenogenic proteins, and shortage of the donor if allogenic material is required. Sharp *et al.* demonstrated a case of aneurysmal formation using decellularized tissue matrix (SynerGraft, Cryolife) after peripheral arterial bypass surgery.[19] Other reports state that xenogenic decellularized matrix remnants contain xenogenic protein and may cause immunological foreign-body reactions.[20] Therefore, further studies will be necessary to confirm the safety and feasibility of decellularized scaffolds.

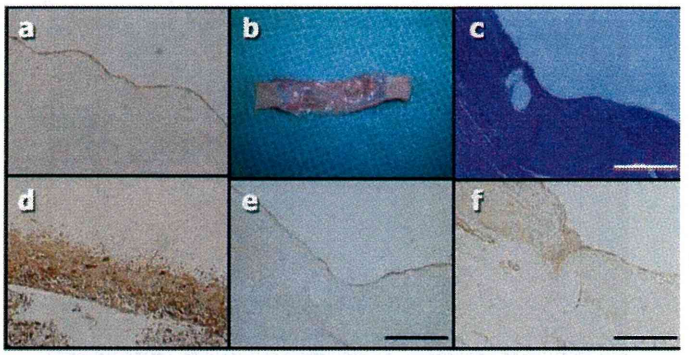

Figure 26.3. *In vitro* and *in vivo* biocompatibility of decellularized scaffolds. Endothelial cells (EC) and myofibroblasts were seeded inside and outside a decellularized ureter, respectively, and were cultured for 5 days. Immunohistochemical staining showed factor VIII–positive cells in a monolayer (a) and multilayered α-smooth muscle actin-positive cells (d) inside and outside the decellularized scaffold. Decellularized scaffolds removed 24 weeks after implantation (canine carotid arterial replacement model). The macro findings of EC-seeded grafts showed no thrombus formation and revealed a smooth and glistening surface (b). The section of the anastomotic site was stained with hematoxylin-eosin (c). Histological findings of decellularized scaffolds revealed complete re-endothelialization by immunohistochemical staining of factor VIII (e). However, the absence of a smooth muscle layer in the wall of decellularized ureters was confirmed by immunohistochemical staining with α-smooth muscle actin (f). Scale bar = 200 μm (from Ref. 17, Narita *et al.*, 2008).

26.2.3 *Biodegradable Synthetic Polymer Scaffolds for Tissue-Engineered Small-Caliber Vascular Grafts*

Scaffolds with biodegradable synthetic polymers can be manufactured artificially, which enables low-cost production compared with natural scaffolds such as decellularized scaffolds. We have developed a scaffold with electrospun nanoscaled fibers for cardiovascular tissue engineering, including small-caliber vascular grafts (Fig. 26.4). Scaffolds based on nanofibers offer great advantages for tissue engineering. They mimic the ECM (50–500 nm diameter fibers) and serve as a three-dimensional matrix for growing cells.[21–24] It is known that nanoscaled fibers also affect cellular

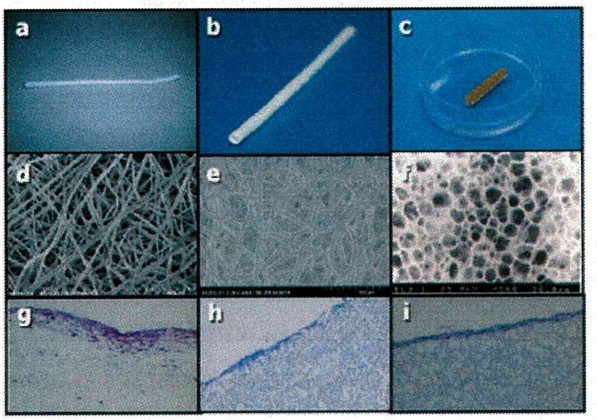

Figure 26.4. Appearance, SEM images, and biocompatibility of electrospun scaffolds with an inner diameter of 3 mm for small-caliber vascular grafts. PLA scaffolds (a, d, g), PCL scaffolds (b, e, h), and PLA with elastin scaffolds (c, f, i). SEM showed that PLA scaffolds had many crevices (d) and cells entered the wall of the scaffold (g) when stained histologically. On the other hand, PCL and PLA with elastin did not have many crevices (e, f), so the cells attached only to the surface of the scaffolds (h, i). *Abbreviations*: SEM, scanning electron microscopy; PLA, polylactic acid; PLC, poly(ε-caprolactone).

adhesion, morphology, proliferation, endocytotic activity, motility, and gene expression of various cell types.[25]

26.2.4 *How to Create Scaffolds for Tissue-Engineered Small-Diameter Vascular Grafts Using Electrospun Nanofibers*

We have generated four types of scaffolds for tissue-engineered, small-caliber vascular grafts using electrospun nanofibers.

Preparation for polylactic acid (PLA) or poly(ε-caprolactone) (PCL) tubelike scaffolds: A solution consisting of PLA or PCL and methylene chloride was loaded into a 1 mL syringe equipped with a blunt-end 18-gauge needle. The needle was clamped to the positive electrode of an electrospinning apparatus, and a voltage range of 10–15 kV was applied. The charged polymer was spun toward

the circular, cylinder-like counterelectrode, which was rotating at 60 rpm. The fibrous material collected on the counterelectrode formed a tubelike structure (Fig. 26.3a,b,d,e).

Preparation for a complex of PLA and water-soluble elastin[26]: A tubelike complex of PLA and water-soluble elastin was generated. In brief, water-soluble elastin was added to deionized water. A water-soluble cross-linking agent was added to the solution and stirred for a few minutes at room temperature. Triethylamine was then added to the solution and stirred for another few minutes. The mixture was poured into a cylindrical template already installed into the PLA tube and left to stand for two days until a gel had formed. The gel was then copiously rinsed with deionized water. Finally, a milky-white cylindrical molded article, which was a complex between the PLA tube and water-soluble elastin, was obtained. The complex was sterilized by autoclaving for all subsequent experiments (Fig. 26.3c,f).

Preparation of a three-layered electrospun tube: An artery is composed of three layers: intima, media, and adventitia. The intima is composed of endothelial cells and the ECM. The media is composed of smooth muscle cells and elastic tissues. The adventitia is made up of fibroblasts and connective tissues. Since the anatomical structure of the three-layered artery may be important to support the mechanical properties of the vessel, we tried to mimic the three-layered architecture using electrospinning techniques.

An electrospun tube was fabricated as follows: First, a mixture consisting of poly(D,L,-lactide-co-glycolide) (PLGA; 50/50) and poly(D,L-lactide-co-caprolactone) (PLCA; 76/24) was spun toward the circular, cylinder-like counterelectrode, which was rotating at 60 rpm. The fibers were discharged in the air using a neutralization apparatus during development of the tube. Since the speed for fiber spinning was reduced by neutralization, the fibers lay down softly on the counterelectrode and were rolled up to create the intima layer. For the media layer, PLCA was spun in a similar manner to give an elastic texture. Finally, a mixture of PLGA and PLCA were spun to generate the outermost layer (Fig. 26.5).

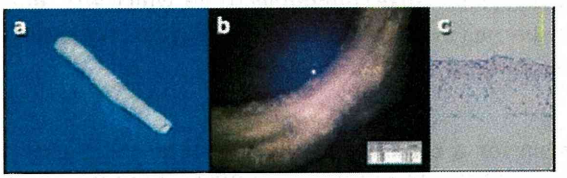

Figure 26.5. Appearance, loupe image, and biocompatibility of a three-layered electrospun scaffold. Histological findings revealed that three-layered scaffolds have good biocompatibility compared with PCL and PLA with elastin scaffolds.

26.2.5 *Biocompatibility and Mechanical Properties of Electrospun Synthetic Scaffolds*

To evaluate *in vitro* biocompatibility of electrospun nanofiber scaffolds, "cell-seeding tests" were performed. Cells were simply seeded onto the inside and the outside of the grafts and were evaluated histologically. When PLA was used as a material for electrospun nanofiber scaffolds, cells could populate the inside of the scaffold wall and form good adhesions to the scaffold (Fig. 26.3g). On the other hand, when a mixture of PCL, PLA, and elastin was used, cells attached only to the surface of the scaffold wall and did not populate the inside of the wall (Fig. 26.3h,i). The results from scanning electron microscopy (SEM) showed ultrastructural differences between the scaffolds made by these two materials (Fig. 26.3d–f). Although addition of PCL improved the mechanical strength of the scaffold (details of scaffold mechanical strength are described below), the fibers with PCL tended to attach to each other, which reduced the space between the fibers (Fig. 26.3e). Since the size of electrospun nanofibers is relatively small, this morphological change significantly affected the mobility of the cells.

Next, we hypothesized that a combination of PLA, which has good biocompatibility, and PCL, which has excellent mechanical strength, could form a satisfactory scaffold. Arteries have a three-layered structure, so we decided to create three-layered scaffolds to satisfy both mechanical strength and biocompatibility. In the case of three-layered scaffolds, the layers on both the inside and outside of the wall were made of PLA so that infiltration of the cells was satisfactory (Fig. 26.5). When generating electrospun nanofiber scaffolds,

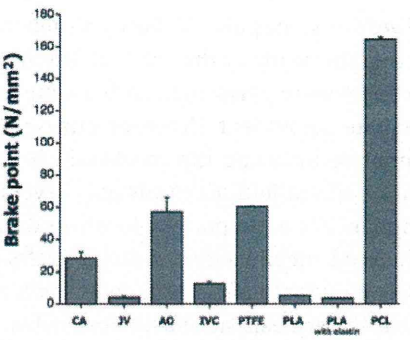

Figure 26.6. Maximum tensile strength of electrospun scaffolds and native tissues. PCL scaffolds were 3 times stronger than the aorta and PTFE. *Abbreviations*: CA, carotid artery; JV, jugular vein; AO, aorta; IVC, inferior vena cava; PTFE, polytetrafluoroethylene graft.

slight changes in materials could significantly affect nanoscaled structures. Since adhesion and infiltration of the cells depends on the structure of the scaffold, the difference in material constituents could affect biocompatibility of the electrospun nanofiber scaffold.

The tensile strength of the scaffold was measured, and the break points (maximum tensile strength) of the PLA, PLA with elastin, and PCL were compared with canine carotid artery, jugular vein, inferior vena cava, aorta, and polytetrafluoroethylene (PTFE) tubes. The tensile strength of PCL was three times that of the aorta and PTFE. However, the tensile strength of PLA and PLA with elastin was more fragile than that of the vein (Fig. 26.6).

Compliance tests were performed by our original compliance measurement system (Fig. 26.1). This system is able to observe pressure and cross-sectional diameter or area relationship of the prostheses.[2] The cross-sectional area of PLA with elastin increased in response to high-pressure load, but the response was different than the native artery. In fact, the pressure-area relation curve of PLA with elastin was approximately linear. On the other hand, PCL and PLA showed a noncompliant response and almost no cross-sectional area change, which was similar to the PTFE tube. It is important to emphasize the fact that compliance tests by our original system can measure the compliance of material in conditions similar

to native blood vessels since pulsatile flow was generated, which created liquid movement inside of the scaffold. Unfortunately, most of the compliance tests were performed with a simpler system due to the lack of adequate equipment. However, our results indicate that the results cannot recapitulate the conditions *in vivo*, which may mislead evaluation of scaffold materials and development of novel materials. Compliance is an important factor in designing scaffolds for tissue-engineered, small-caliber vascular grafts since a compliance mismatch is thought to affect prognosis. Such a mismatch may cause neointimal hyperplasia at anastomotic sites, which, in turn, can lead to thrombosis and occlusion even at early stages after bypass surgery.[27]

We performed rat carotid arterial replacement using electrospun scaffolds to evaluate their function *in vivo*. Grafts were patent for 12 weeks, and no thrombus formation was observed. However, scaffold materials remained, and neither the smooth muscle layer, which is an important component of the vessel, nor regeneration of elastic fibers was observed (Fig. 26.7). When biodegradable materials for

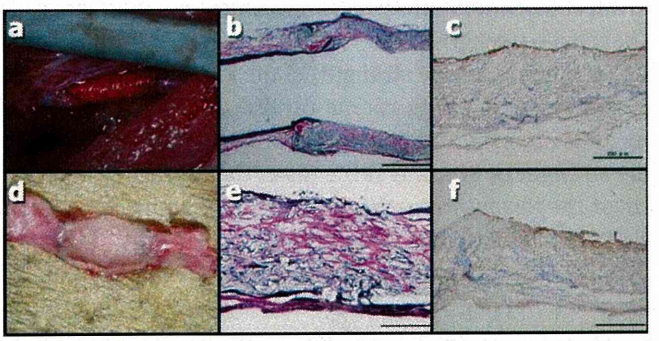

Figure 26.7. *In vivo* findings of electrospun scaffolds (PCL). Rat carotid arterial replacement was performed (a). The scaffold was removed 12 weeks after implantation. Grafts were in place for 12 weeks, and no thrombus formation was observed (d). Elastica van Gisson staining revealed that collagen was growing in the scaffold wall, but there was little elastin in the scaffold compared with native artery (b, e). Immunohistochemical staining for factor VIII showed single-layered endothelial cells covering the inside of the graft (c). However, α-smooth muscle actin staining revealed that the smooth muscle layer, which is important for vascular strength, was not regenerated (f). Scale bar = 200 μm (c and f) and 500 μm (b and e).

vascular scaffolds are used *in vivo*, optimization of the balance of material absorption and tissue regeneration is the most important and most difficult issue. Development of biodegradable elastomers, which do not cause compliance mismatches at early stages of implantation, is required.

26.2.6 *Prospects of Designing A Scaffold for Cardiovascular Tissue Engineering*

The requirements for scaffold materials for cardiovascular tissue engineering are excellent biocompatibility, adequate mechanical properties, good texture that has easy surgical handling and optimized timing of material absorption, and tissue regeneration. To improve scaffold materials, various manipulations have been tested such as ECM coating (e.g., collagen, fibronectin), drug coating (e.g., heparin, Argatroban), growth factor linking (e.g., basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), chemical treatment (e.g., argon-plasma), and peptides (e.g., the Arg-Gly-Asp [RGD] sequence). It is known that drugs can be capsulated directly into electrospun nanoscaled fibers, and these systems showed sustained release of the drug.[28,29] The use of electrospun fibers as drug carriers holds promise for future biomedical applications, especially scaffolds for tissue engineering. We developed a novel, controlled drug delivery device to prevent anastomotic stricture using electrospinning nanofibers, which were composed of biodegradable polymers and Tacrolimus.[25] In the future, intellectually multifunctional scaffolds may be developed for cardiovascular tissue grafts.

26.3 Scaffold Design for Hard-Tissue Engineering: Alveolar Bone

26.3.1 *Bone Reconstruction/Regeneration in Orthopedic and Oral Applications*

Bone defects that do not heal spontaneously require bone reconstruction. Although autologous bone grafting is the current gold standard for reconstruction of relatively large bone defects,

autografts may cause donor site morbidity.[30] To avoid morbidity, several types of ceramic-based bone substitutes, which have similar mineral compositions to natural bone tissue, have been developed. Among them, synthetic hydroxyapatite (HA) and tricalcium phosphate (TCP) are the most commonly used ceramics as alternatives to autologous bone.[31] Block-type ceramics have proven useful for reconstruction of segmental bone defects, which are the major targets in orthopedic applications.[32] In contrast, relatively small and complicated-shape bone defects (alveolar bone) are the major targets in oral applications.[33] Since block-type bone substitutes are difficult to apply for small and complicated-shape bone defects, ceramic granules have been used. However, reconstruction of these defects with granules of HA and TCP is more difficult than block-type materials and inefficient partly due to the geometry of the substratum, which will directly affect the osteoconductive properties of the materials.[34] Accordingly, granules of natural materials such as allogenic/xenogenic freeze-dried bones are preferable for use in alveolar bone regeneration since these natural materials have better osteoconductive properties than synthetic HA and TCP.[33,35] However, use of allogenic/xenogenic bones cannot completely eliminate possible contamination risk (e.g., prions, viruses, zoonosis) or potential for immunological reactions against allogenic/xenogenic proteins.

Tissue engineering is an interdisciplinary approach to regenerate tissue through integration of cell biology and biomaterial/biomedical sciences. Since one of the concepts for tissue engineering is based on enhancement of the natural healing process of tissues, it can be considered an ideal therapeutic option for treating various tissue defects. For bone regeneration, tissue engineering is considered less invasive than autologous bone transplantation and more efficient than artificial bone substitutes.[36] Accordingly, bone tissue engineering has been studied extensively and has even reached the stage of clinical application in some facilities, including our own. Among various target diseases for bone tissue engineering, application to atrophic alveolar bone (oral application) has attracted much attention since the required bone volume is relatively small, a major advantage for tissue

engineering applications. Furthermore, there is an increasing demand for bone regeneration in patients who undergo dental implant placement. Our group has investigated the safety and efficacy of alveolar bone tissue engineering using autologous bone marrow stromal cells (BMSCs) and β-TCP granules as a scaffold. The interim results of this clinical study showed the feasibility of alveolar bone tissue engineering (Asahina *et al.*, manuscript under review). This clinical study also provided us information about the fate of transplanted scaffolds in the human body, which should help us to consider which scaffolds are truly useful for future bone tissue engineering.

26.3.2 *Ideal Ceramic Scaffolds for Bone Tissue Engineering from a Clinical Point of View*

Scaffolds for bone tissue engineering should act as a template for new bone growth and are expected to be eventually replaced by autologous bone tissue.[31] Therefore, the biodegradable properties of ceramics are of substantial importance when ceramics are applied for bone tissue engineering (Table 26.1). In this respect,

Table 26.1. Scaffolds used in current clinical trials are described.

Currently used ceramic-scaffold for cell-based bone tissue engineering		
Target tissue/area	Scaffold	References
large bone diaphysis defect	HA block (porosity:60% or 80% pore size: 613 or 430 μm)	marcacci et al.[44]
Upper and lower jaw bone defects	HA particles (porosity: 65%: pore size:?)	meijer et al.[45]
Infrabony periodontal bone defects	HA granules (porosity: ?; pore size:?)	yamamiya et al.[45]
Sinus floor augmentation	60%HA/40%TCP cubes (porosity:?; pore size: 300–500 μm)	Shayeteh et al.[46]
Maxillary defects	β-TCP granules (porosity: 65%; pore size:?)	Mesimaki et al.[46]
Femoral head defects	β-TCP granules (porosity: 75%; pore size: 100–400μm)	Kawate et al.[47]

synthetic HA is not an ideal ceramic for bone tissue engineering since HA does not degrade properly in the human body.[32] In contrast, TCP is a degradable calcium phosphate ceramic; thus, TCP is considered to be a better ceramic scaffold for bone tissue engineering. However, the osteoconductive properties of TCP are known to be less than those of HA.[37] In order to improve the osteoconductive properties of TCP, several biphasic calcium phosphate ceramics (HA/TCP) have been developed.[37,38] Although the optimal composition of HA/TCP remains controversial, this approach seems promising to develop a more reliable ceramic scaffold for bone tissue engineering.

Porosity of the ceramic scaffold has a great influence on the efficacy of bone tissue engineering since it directly affects cell adhesion, migration, and proliferation of the osteogenic cells.[31,39,40] Anatomically, cortical bone has 3%–12% porosity, while trabecular bone has porosity in the range of 50%–90%.[41] Since the primary target of bone tissue engineering is the regeneration of trabecular bone and ceramic scaffolds should have enough strength to provide physical support for the cells, 65%–75% porosity might be ideal for ceramic scaffolds. Pore diameter is also known to influence cell migration, proliferation, and eventually the ability to support bone regeneration. The minimum pore size to regenerate bone is generally considered to be 50–100 μm.[38,40] Accordingly, β-TCP granules, which have 75% porosity and a pore size ranging from 100 to 400 μm, were utilized in our clinical trials. Interconnectivity of the pore and geometry of the scaffold are also known to influence the efficacy of bone tissue engineering, and these factors might cause a difference in osteoconductive properties between block- and granule-type ceramic scaffolds.[34]However, the influence of these factors on osteogenic cells has not been well investigated.

Although the development of novel biomaterials is a rapidly expanding area of science, basic understanding of cell-to-material interactions should be carefully considered to develop an ideal ceramic scaffold for bone tissue engineering. From a clinical point of view, degradability, composition, porosity, pore size, interconnection of the pore, and geometry are factors that need to be considered.

26.3.3 *Fate of Transplanted Scaffolds in the Human Body: A Clinical Study of Alveolar Bone Tissue Engineering Using Bone Marrow Stromal Cells and β-TCP*

Among the biomaterials used for bone tissue engineering, the fate of ceramic-based materials has been relatively well described since these materials can be used alone as bioartificial bone substitutes such as β-TCP.[3–6] However, the degradation process of β-TCP *in vivo* is much different from that *in vitro*. Transplants are immersed in an aqueous solution with ions and enzymes at various pHs. Furthermore, cells such as osteoclasts are known to interact with the materials and play some role during the degradation process. Biodegradation of TCP is considered to be mediated in two different ways: (1) resorption by osteoclasts and (2) dissolution by interstitial fluid.[4] Interestingly, when TCP was transplanted alone without cells, Zerbo *et al.* reported that osteoclastic activity did not precede osteogenic activity and most of the degradation may have happened as a result of a local drop in pH because of the production of acidic by-products and poorly developed vascularlization in the regenerating tissue.[4]

Ceramic-based materials have been used not only as bioartificial bone substitutes, but also as scaffolds for bone tissue engineering. Since only a limited number of publications are available on clinical bone tissue engineering, information about the fate of β-TCP as a scaffold is rather limited. However, cell-to-material interactions should play important roles during the degradation process of the scaffolds since the materials are transplanted with cells.

We have performed a clinical study of alveolar bone regeneration using autologous BMSCs and β-TCP granules as a scaffold. BMSCs were harvested from the iliac crest under local anesthesia and cultured with α-minimum essential medium (MEM) containing 10% autoserum and antibiotic/antimicotic reagents (Fig. 26.8). Nonadherent cells were discarded. After osteogenic induction for seven days, adherent cells were detached from the flasks and suspended in platelet-rich plasma (PRP), which was turned into a gel using autologous thrombin. The gel was then mixed with β-TCP granules as a scaffold and transplanted to the sinus floor and alveolar ridge to obtain enough bone volume to support the dental implant.

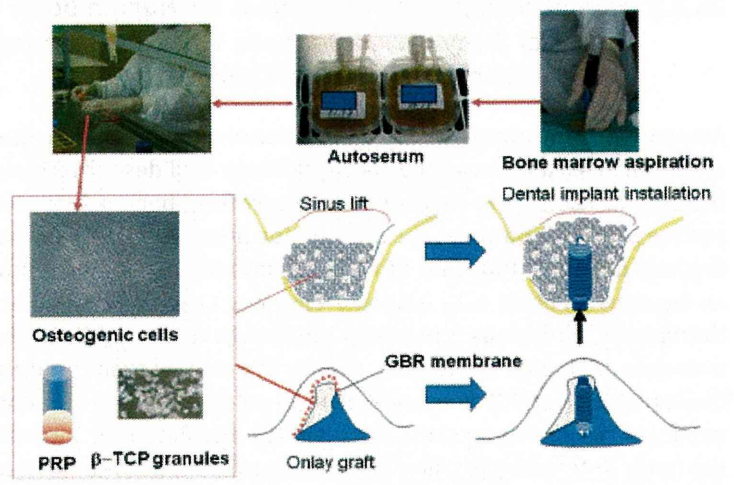

Figure 26.8. Schematic figure showing the procedure for alveolar bone tissue engineering. BMSCs were harvested from the iliac crest under local anesthesia and cultured in a cell culture facility. Nonadherent cells were discarded. At the time of surgery, cells were detached from flasks and suspended in PRP, which was turned into a gel using autologous thrombin. The gel was then mixed with β-TCP granules as a scaffold and transplanted into the sinus floor and/or alveolar ridge.

The results showed that bone regeneration using autologous BMSC-derived osteogenic cells was feasible (Asahina *et al.*, manuscript under review).

In this study, six months after cell transplantation, bone biopsies were performed using a trephine bur at the site of implant placement. Histology of the regenerated bone was analyzed. Newly formed bone was observed adjacent to the scaffold as well as between the scaffolds (Fig. 26.10). The available tissue sample from patients was from only one time point, so the time course of scaffold degradation could not be analyzed. However, there might be two differential types of scaffold degradation, as reported previously.[4] When the newly formed bone was adjacent to the scaffolds, it presented as brushlike borders, which may support the idea that β-TCP granules had started to degrade due to resorption by osteoclastic cells prior to bone regeneration (Fig. 26.9). Some of the scaffold seemed like it was degraded spontaneously but not

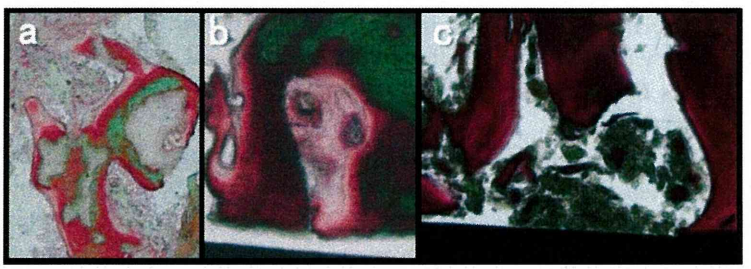

Figure 26.9. Histology of regenerated bone. Six months after cell transplantation, a bone biopsy was performed using a trephine bur at the site of implant placement. Nondecalcified tissue sections were cut, and the sections were stained with Villanueva-Goldner stain. Newly formed bone was observed adjacent to the scaffold, and the scaffolds seemed like they were absorbed by osteoclastic cells (a, b). Bone also formed between the scaffolds. In these cases, it seemed like the scaffolds were degraded spontaneously (c).

actively by osteoclastic cells since cells were not observed close to the degrading scaffold (Fig. 26.9). Actual tissues display a combination of two types of degradation. Compared with the transplantation of bone substitute alone, the presence of new bone adjacent to the scaffold was more frequent, which may reflect the role of transplanted cells. However, quantitative analyses were not performed, and these observations require further investigation.

26.3.4 *Considerations for Designing Scaffolds for Clinical Bone Tissue Engineering*

Factors to consider for successful scaffold materials include biocompatibility, degradation time, and mechanical properties. For bone tissue engineering scaffolds, morphology is important. In terms of ceramic-based scaffolds, porous structure is important and a certain size of pore is essential for osteoconductivity, as described above. In terms of fibrous scaffolds, fiber diameter also affects the success of bone regeneration. Alveolar bone defects do not bear large physiological loads until after the implant placement. Accordingly, mechanical strength of the scaffold may not be critical for dental implants. However, mechanical strength is important for most orthopedic applications. The shape of the scaffold is also important. Bone defects

in alveolar bone are small and complex in shape and so are difficult to treat with block-type scaffolds. On the other hand, segmental bone defects may require solid, block-type scaffolds to support the mechanical strength.

The process of clinical bone tissue engineering can be divided into at least three phases: generation of tissue grafts for transplantation, scaffold degradation as bone regeneration occurs, and normal remodeling of the newly formed bone. The initial phase of bone tissue engineering is the generation of tissue-engineered grafts, which includes cell seeding, culture, induction, and transplantation (Fig. 26.10). Cultured cells can be seeded directly onto the scaffold, induced into osteogenic cells, and then transplanted. Alternatively, cells cultured in flasks can be induced into osteogenic cells and detached from the flask at the time of surgery. These are then mixed with the scaffold for transplantation. For the granular-type scaffolds,

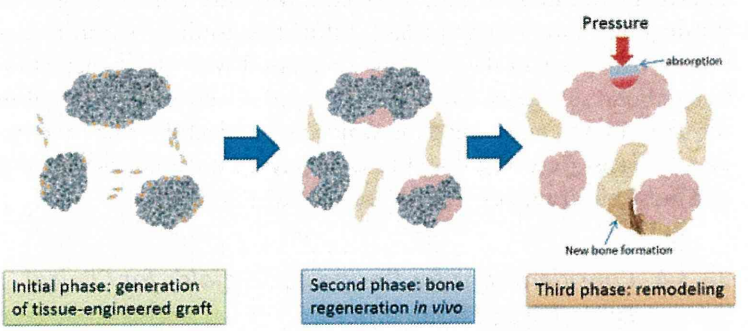

Figure 26.10. The process of clinical bone tissue engineering can be divided into at least three phases. The initial phase of bone tissue engineering is generation of tissue-engineered grafts. Autologous BMSCs are seeded on scaffolds before or after osteogenic induction. The scaffolds with cells are then transplanted. The second phase is the process of bone regeneration. During this phase, scaffolds are degraded as new bone formation occurs, which continues until the scaffolds are completely degraded. The third phase is the process of bone remodeling after bone regeneration and scaffold absorption. The volume of regenerated bone is regulated by coordinated bone resorption and formation, which are affected by various local and systemic factors such as pressure from surrounding soft tissues and physiological loads from the dental implant.