BONE ENGINEERING
- BIOLOGICAL MATERIALS AND BONE MORPHOGENETIC PROTEINS -

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The biomaterials used in tissue engineering represent a major area of the current study. When the absorption of the biomaterial is too slow, bone formation is inhibited by itself. Their bioabsorption for bone engineering should, therefore, coincide with the rate of endogenous bone formation. Bone consists of two major components, collagen and hydroxyapatite, and is a repository of bone morphogenetic proteins (BMPs). In the field of tissue regeneration, pepsin-digested collagen (so-called atelocollagen) is used for tissue engineering as one of the most useful scaffolds. Its excellent biocompatibility, due to its biological characteristics such as absorbable properties and low antigenecity, has elevated collagen to become a primary resource in medical applications. On the other hand, hydroxyapatite is classified as a non-absorbable material and is not harmonized with bone formation and remodeling. Therefore, there is a need for absorbable bioceramics that allow for bone formation and which gradually biodegrade, to be absorbed by the body and replaced by new bone. The BMPs, osteoinductive molecules discovered from bone, are thought to contribute to signal the local mesenchymal cells to proliferate and differentiate to osteoblasts. In this paper, the characteristics of collagen and ceramics, and animal and human studies using BMPs for bone engineering are reviewed.

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TISSUE ENGINEERING

A new field, tissue engineering, is an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function1. The biomaterials used in tissue engineering represent a major area of the current study. The reconstruction of lost parts of the skeleton is generally carried out with autogenous bone grafts. Although autogenous bone graft is a gold standard for reconstruction, autograft requires a harvesting procedure that causes donor site morbidity and discomforts for the patients2. Therefore, a search for absorbable biomaterials with osteoconductive potential, signaling molecules, stem cell biology or bone production via bioengineering has begun for tissue-engineered regeneration.

The symbiosis of bone-inductive and conductive strategies is critical for tissue engineering, and is in turn governed by the context and biomechanics3. The context is the microenvironment, consisting of extracellular matrix, which can be duplicated by biomimetic biomaterials such as collagens, hydroxyapatite, proteoglycans, and cell adhesion proteins including fibronectins. Thus, the rules of
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architecture for tissue engineering are an imitation of the laws of developmental biology and morphogenesis.

BONE

Bone consists of two major components, collagen and hydroxyapatite, and is a repository of growth factors, including BMPs, transforming growth factor-β1 and β2, platelet-derived growth factor, insulin-like growth factor I and II, and acidic and basic fibroblast growth factor4,5 (Fig. 1). Osteoblasts produce calcifiable collagen matrix and the several growth factors, which become embedded in the matrix-network as osteocytes.

Bone formation in vivo occurs via two processes, one of which depends on pre-existing cartilage (endochondral ossification) and the other does not (intramembranous ossification). Cartilage serves as a template for bone and is eventually replaced by bone marrow in case of endochondral ossification, whereas intramembranous bone formation occurs in mesenchymal tissues without prior formation of cartilage6,7. It has been stated that oxygen concentration and mechanical factors could play an important role in chondro-osteogenic cell differentiation8,9,10. Bassett reported that mesenchymal cells differentiated into osteoblasts under the condition of compaction and high oxygen, chondroblasts in the condition of compaction and low oxygen, and fibroblasts in the condition of stretching and high oxygen8,9.

BIOLOGICAL SCAFFOLDS

- CELLULAR ANCHORAGE AND DELIVERY -

The basic approach to the treatment of bone defects involves the use of biological scaffolds to favor bone growth. It is well known that mesenchymal cells including bone-forming cells must attach to a solid substratum to spread and proliferate11,12. This property is named as anchorage dependence12. Natural materials are advantageous in that they contain information (for example, particular amino acid sequences) that facilitates cell attachment or maintenance of differentiated function1. Among many biomaterials, collagen is a natural substrate of various types of animal cells, and an excellent candidate of scaffolds as an absorbable biomatrix for bone regeneration. The principle advantages of collagenous materials are the low immunogenic response across species, the potential for complete replacement by tissue, and easy handling for preparing in any shape for contour formation. On the other hand, non-absorbable materials remain in tissues as foreign bodies for a lifetime and may trigger chronic or acute inflammation. The osteoconductivity of collagen and

Fig. 1. Scheme of bone.
Note natural BMPs, non-collagenous osteoinductive proteins, binding to collagen after HCl-demineralization. Rate: wt%
hydroxyapatite has been well shown, while polymers are not osteoconductive. The structure of biomaterials is very important for cell environment and drug delivery. We believe that the use of highly porous, absorbable and osteoconductive materials is, therefore, preferable. Bioabsorption of the biomaterials for bone engineering should, therefore, be harmonized with bone modeling and remodeling.

BMP - OSTEOINDUCTIVE SIGNAL -

Demineralized bone matrix (DBM) (Fig.2) and demineralized dentine matrix (DDM) (Fig.3) induce bone and cartilage in non-skeletal tissues. The use of DBM in treating bone defects has been proven beneficial for bone regeneration, because BMPs, non-collagenous osteoinductive proteins, bind to bone matrix, mainly type I collagen, even after the HCl-demineralization of bone (Fig.1). Thus, extracellular matrix components tether active morphogens to confer the optimal conformation and perhaps protect them from proteolysis. The history of BMPs began with the observation that DBM can induce ectopic bone formation in subcutaneous and intramuscular pockets in rodents. BMPs were first discovered in bone in 1965 and have been investigated for its bone-inducing properties. BMPs need a carrier to allow slow release and a scaffold to encourage proliferation and differentiation of mesenchymal cells. In 2002, the approval by the US Food and Drug Administration (FDA; Rockville, MD, USA) of recombinant human BMP-2 or 7 for accelerating bone fusion in slow-healing fractures indicates that this protein family may prove useful in bone engineering.

Fig. 2. Photomicrographs of demineralized bone matrix (DBM).
A) SEM photomicrograph of DBM. B) Higher magnification of Fig. 2A. C) Photomicrograph of histological section of DBM alone at 4 weeks after implantation into rat subcutaneous tissues. Note absorption of collagenous matrix (↑) (HE staining).
Collagen is a major organic component in body and a major structural protein of bone. In human, collagen has been used for more than 20 years in the replacement of ligaments, tendons, and other soft tissues and as wound dressings, where it has proven its clinical safety. Now, collagen is widely used for regenerative therapy as one of the most useful of naturally-occurring scaffolds. Collagen-based biomaterial was first developed by Yannas and coworker in 1980 and has been modified in terms of antigenicity and structure for cellular response. Titers of antibodies against allogenic or xenogenic implants of collagen have been reported, but did not show interference with bone formation at the implanted site. The non-helical telopeptide region of the collagen molecule can produce a strong antigenic response by the host, but the immunogenicity of the collagen is reduced by a pepsin digestion. The telopeptide-depleted type I collagen, therefore, has been prepared from bovine skin, bone, tendon or pericardium for experimental and clinical uses (Fig. 4). The collagen carrier might form scaffold for cell attachment and migration, and it provides a three-dimensional porous network for the proliferation and differentiation of mesenchymal cells.

Nowadays, several collagenous materials are available in various types and forms, including solution, sponge, membrane, bead, mini-pellet and gel. Recently, a dry composite sponge of fibrillar and heat-denatured atelocollagen has been developed to reduce the inflammatory response and increase cellular affinity. The insolubility of collagen is relatively high for a fibrillar structure and superior in physical strength to denatured collagen (so-called gelatin). The cellular affinity of gelatin is highest among collagen derivatives. The absorption of collagenous materials depends on a combination of enzymes such as collagenase and proteinase, and cellular phagocytosis.

Role of Collagen in Bone Formation

Animal model studies have been carried out to investigate histological changes of implanted collagen material during healing of bone defects and bone induction by BMPs. As single component materials applied in bone, anionic collagen matrices from bovine pericardium were implanted in surgically
created mono-cortical defects (2 x 1mm) in rat tibias and subperiosteally in rat calvaria. Both results presented a low inflammatory response and bone formation. In addition, the fibrillar collagen reconstituted from pepsin-solubilized, bovine skin collagen was found to produce significantly more new bone than no graft or than heat-denatured fibrillar collagen in 4 mm diameter surgically created defects of rat calvaria. Moreover, collagenous composite materials were developed, and atelocollagen/heat-denatured collagen, phosphophoryn/calcium/collagen, hyaluronate/cross-linking collagen were estimated in the mandibular defects of rabbits, in the femur defect of beagle dogs, in the cranial defect of rats, respectively. These new materials demonstrated good biocompatibility and exhibited osteoconductive potential, whereas synthetic polymers are not osteoconductive.

Addition of BMPs to collagen affects the resorption rate of the collagen carrier. An enhanced resorption rate of the collagen carrier in the presence of BMPs has been observed in several studies.

**DEVELOPMENT OF CERAMICS**

In certain species, HAp alone appears to be osteoinductive. In the rectus abdominis of adult rabbits, dogs and baboons, a porous hydroxyapatite, obtained after hydrothermal conversion of the calcium carbonate exoskeleton of coral, induced bone at 90 days after implantation. One interpretation is that osteoinductive endogenous BMPs in circulation binds to the implanted porous HAp block. BMPs binds to HAp, type I and IV collagen, heparin and heparan sulfate. When an optimal threshold concentration of native BMPs is achieved, the HAp ceramics should become osteoinductive. Ceramics allow for increase initial flash spread of serum proteins compared to the more hydrophobic polymer surface. This characteristic is related with the cell compatibility. We proposed that diffusion of body fluid and blood into the inside of ceramics was an important factor for bioabsorbable characteristic. Cells never invade into dry bulk areas without body fluid (Fig. 5).

Fig. 5. Photomicrographs of HAp ceramics. A) Photomicrograph of histological section of HAp alone at 4 weeks after implantation into rat.
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subcutaneous tissues. HAp particles sintered at 1,200°C (Sangi Co. Ltd., Tokyo, Japan). Note absence of body fluid permeation and cell invasion into inside of HAp (*) (HE staining). B) SEM photomicrograph of porous HAp particles sintered at 1,200°C (The Japan Steel Works, Ltd., Tokyo, Japan).

C) Photomicrograph of histological section. At 4 weeks after implantation into rat subcutaneous tissues, new bone induced by non-absorbable HAp/BMPs implant. Note absence of body fluid permeation and cell invasion into inside of HAp (*) (HE staining).

There are several in vivo studies involved in absorption rate of beta-tricalcium phosphate (β-TCP)⁴⁹,⁵⁰. In the presence of BMPs, the absorption rate of β-TCP has been enhanced. A BMPs/β-TCP composite was implanted into a critical size defect (1.4 cm) in adult dog skull. Better performance of the BMPs/β-TCP composite could be obtained for bone healing compared to controls. The un-absorbed β-TCP residues were, however, observed even at 4 months after implantation in the BMPs/β-TCP⁵⁵. An inorganic bovine bone and a porous β-TCP material were compared in the lateral bony mandibular bone defects (5 x 4 mm) in dogs. Both ceramics were still remained at 12 months postoperatively, but β-TCP particles were completely resorbed at 24 months⁵⁰. These reports indicate that further researches should be encouraged to obtain a formulation of sintered calcium phosphate that could be resorbed more rapidly. Thus, β-TCP can be classified as absorbable, but it shows so low absorption characteristics even after a few years. This point is the reason that why the ceramics is not a first candidate of scaffold for bone engineering.

Recently, unique and functional ceramics have been developed to promote bone formation. Negatively charged surface of electrically polarized HAp was proven to enhance osteobonding in canine bone tissues⁵¹. In a gap of 0.2 mm between HAp and bone, the negatively charged HAp conducted formation of unidirectionally oriented bone layer into direct contact with the HAp surface at 7 weeks. Interestingly, zinc-releasing calcium phosphate ceramics were developed⁵²,⁵³. The zinc-doped β-TCP, β-TCP, and HAp powders were mixed at a (Ca+Zn)/P molar ratio of 1.60. Implantation of the cylindrical ceramics containing zinc (the zinc content: 0.316 wt %) into rabbit femora resulted in favorable (a significant increase in intramedullary bone apposition rate at 6 weeks and in cortical bone at 24 and 60 weeks) and unfavorable (a significant increase of bone resorption in medullary cavity area at 60 weeks) effects on bone remodeling⁵³. We have been developing bioabsorbable and functionally graded HAp, characterized by the gradations in crystallinity and in grain size of HAp. The HAp supported by BMP-2 could be recognized as one of biomimetic scaffolds with osteoinduction for bone engineering⁴⁸.

HUMAN STUDIES - RHBMP-2 OR -7 AND ATELOCOLLAGEN -

In 1997, the first clinical studies have been published using rhBMP-2 and atelocollagen sponge⁵⁴,⁵⁵. Boyne and co-worker⁵⁴ implanted the BMP/atelocollagen complex in the sinuses of 12 edentulous or partially edentulous patients with severe atrophy of the maxilla. The subsequent increase in height of the treated maxilla varied between 2.3 and 15.7 mm. Howell and co-worker⁵⁵ reported that the BMP-2/atelocollagen complex could not form bone when applied in mandibular ridge augmentation, while all the BMP implants were replaced by new bone in tooth sockets. Groeneveld and co-worker⁵⁶ performed a histological estimation of the effect of the
BMP-7/atelecollagen complex in the maxillary sinuses of 3 patients with maxillary atrophy. Excellent bone formation was found in one of the patients, but the two other patients showed little or no bone formation at 6 months postoperatively. These clinical data suggest that future studies will be needed to focus on the development of biomaterials that have mechanical properties and practicality appropriate for controlled release of BMP-2 or 7.

SUMMARY

Bone formation can be inhibited when the absorption of the biomaterial is too slow. Bioabsorption of the collagen matrix and ceramics should, therefore, coincide with the rate of endogenous bone formation. This review discussed the characteristics of the biological materials and BMPs.

REFERENCES