Recent Progress in Bone Induction by Osteogenin and Bone Morphogenetic Proteins: Challenges for Biomechanical and Tissue Engineering

A. H. Reddi

N. S. Cunningham

Bone Cell Biology Section,
National Institute of Dental Research,
National Institutes of Health,
Bethesda, MD 20892

Implantation of demineralized bone matrix results in local bone induction. Bone induction is a sequential biological chain reaction that consists of chemotaxis and proliferation of mesenchymal cells and differentiation of bone. Osteogenin, a bone morphogenetic protein has been purified and the amino acid sequence determined. Recently a family of bone morphogenetic proteins have been cloned and expressed by recombinant DNA technology. The availability of growth and morphogenetic factors will permit the rational design of new bone. The challenge for the biomechanical engineer is to attain mechanically optimal and functionally adaptive new bone for various skeletal prostheses. We are on the threshold for fabrication of new bone based on sound architectural design principles of tissue engineering based on cellular and molecular biology of growth and differentiation factors.

Introduction

It is well known that bone has an outstanding potential for repair and regeneration. There is a growing realization of the potential of demineralized bone matrix to initiate new bone formation [1–3]. However, implantation of mineralized matrix results in multinucleated giant cells and no bone induction. Demineralization is a necessary prerequisite to express the osteoinductive potential of bone matrix. The bone induction is a biological chain reaction consisting of chemotaxis and attachment of mesenchymal cells to collagenous demineralized matrix, proliferation of cells and differentiation of cartilage, bone and marrow (Table 1). This article is a brief progress report of this exciting frontier with special reference to the challenges for the biomechanical engineer.

Bone Induction is a Chain Reaction

The demineralized bone matrix-induced bone differentiation is a multistep biological chain reaction. The key steps are chemotaxis, mitosis and differentiation [2, 3]. Subcutaneous implantation of demineralized bone matrix initiates chemotaxis of mesenchymal cells to the implant. Fibronectin, a cell surface glycoprotein that is also present in blood plasma binds to the implanted bone matrix and promotes attachment of cells. Bone matrix is a local mitogen and stimulates proliferation of progenitor cells. The proliferation is followed by differentiation of bone and marrow. The matrix-induced bone formation is a useful experimental model and is reminiscent of embryonic development of limb in the fetus and stages of fracture repair in humans.

Table 1 Matrix-induced bone development is a multistep chain reaction

1 Chemotaxis of progenitor cells.
2 Mitosis of Mesenchymal Cells.
3 Differentiation of chondrocytes.
4 Hypertrophy and Calcification of Cartilage Matrix.
5 Angiogenesis and vascular invasion.
6 Differentiation of Bone.
7 Extracellular matrix biosynthesis and mineralization.
8 Bone Remodeling.
9 Hematopoietic marrow differentiation.
10 Functional Adaptation of Bone.

Isolation of Osteogenin and Cloning

The demineralized bone matrix is in the solid state. In order to isolate osteogenic proteins the matrix must be solubilized. The matrix was dissociatively extracted by chaotropic reagents such as 4M guanidine hydrochloride, 8M urea and 1 percent sodium dodecyl sulfate [4]. The extracted proteins were then reconstituted with the inactive collagenous bone matrix. The composite of soluble proteins and insoluble collagenous substratum induced new bone formation [4, 5]. The osteogenic protein, osteogenin was isolated by heparin affinity chromatography and preparative gel electrophoresis [6]. The majority of the activity was localized to the region between 30–40 kilo

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Daltons. It is a dimeric molecule held together by disulfide bonds. The amino acid sequence of the tryptic fragments revealed unique structure and this was similar to bone morphogenetic protein 3 (BMP-3). Bone morphogenetic proteins (BMP-2 and BMP-3) have been cloned and expressed [7]. The recombinant BMP-2 and BMP-3 are biologically active in inducing new bone formation. Very recently a new gene has been cloned, osteogenic protein-1 [8]. The various bone morphogenetic proteins including osteogenin are members of the superfamily of transforming growth factor-β (TGF-β). The bone morphogenetic proteins are related to developmentally important regulatory genes such as decapentaplegic (dpp) in *Drosophila* [9] and Vg-1 gene of the amphibian *Xenopus laevis* [10]. Recent work has implicated activins in induction of axial mesoderm in *Xenopus* [11].

Osteogenin Stimulates Osteoblasts

The mechanism of action of osteogenin and its target cells are not understood. As a first step we have investigated the influence of osteogenin on periosteal cells, osteoblasts, fibroblasts, chondrocytes and bone marrow stromal cells in tissue culture. In both periosteal cells and osteoblasts osteogenin stimulated alkaline phosphatase activity and collagen synthesis [12]. The production of proteoglycans by rat chondroblasts and rabbit chondrocytes was increased by osteogenin [12]. However, NIH-3T3 fibroblasts are not stimulated by osteogenin.

Osteogenin Binds to Developing Bone

In order to understand the possible developmental role of osteogenin during embryonic bone morphogenesis we examined the binding and localization of radioiodinated osteogenin by autoradiography in developing rats [13]. The results demonstrated maximal binding in mesodermal tissues such as cartilage, perichondrium, periosteum and bone. Osteogenin was also localized in developing membranous bones of the vault of the skull and craniofacial bones. These results imply a developmental role for osteogenin in skeletal morphogenesis [13].

Osteogenin Binds to Type IV Collagen

Although osteogenin was isolated from demineralized bone matrix, the interactions of osteogenin with other extracellular matrix macromolecules is not known. Osteogenin binds avidly to type IV collagen [14]. The binding occurred at physiological ionic strength and is reversible. What is the biomedical significance? It is well known that vascular invasion is a prerequisite for new bone formation during endochondral bone differentiation. It is likely that invading blood vessels and associated basement membrane components including type IV collagen bind osteogenin and related members of the TGF-β superfamily and perhaps orient them to an optimal conformation for initiation of bone differentiation locally. These observations provide mechanistic insights into the role of blood vessels in bone formation.

Osteogenin Requires a Substratum

For the optimal action of osteogenin and related bone morphogenetic proteins a collagenous substratum is required [15]. The role of the substratum was investigated by comparing insoluble collagenous bone matrix with granular (70–420 μm diameter) hydroxyapatite, β-tricalcium phosphate, polymethylmethacrylate and glass beads. Each of these substrata were implanted alone and in combination with osteogenin. The results revealed that new bone formation occurred only with a combination of collagenous substratum and osteogenin. Collagenous substratum alone is insufficient to trigger the bone development cascade. From an engineering standpoint further experimental investigations will elucidate the precise role of the substratum.

Clinical and Dental Applications: Challenges for the Future

The availability of the recombinant osteogenin and related bone morphogenetic proteins will permit the design of rational approaches to bone repair in orthopaedic surgery, oral surgery, plastic surgery and periodontal applications. It is also likely that osteogenin may be useful in promoting bone ingrowth into prosthetic implants to achieve optimal biological fixation and prevent the undesirable loosening. The major challenges for the biomechanical engineers are in the realms of the design of functionally adaptive bone from recombinant osteogenin and related bone morphogenetic proteins, collagenous matrix and responding cells. The influence of mechanical stimuli on bone forming cells is well known (see article by Cowin et al. in this issue). The other major challenge from the point of view of a tissue engineer is how to create and control the shape of new artificial bone that is compatible with natural biomechanical function. This is an exciting problem and requires the confluence of the minds of biomechanical and tissue engineers, biomaterials scientists and cellular and molecular biologists. Although it is a complex biomechanical problem the basic principles are in place and one can safely predict meaningful progress in the near future.

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