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Symbiosis of Biotechnology and Biomaterials: Applications in Tissue Engineering of Bone and Cartilage

A.H. Reddi

Laboratory of Musculoskeletal Cell Biology, Department of Orthopaedic Surgery, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2196

Abstract The three ingredients for the successful tissue engineering of bone and cartilage are regulatory signals, cells, and extracellular matrix. Recent advances in cellular and molecular biology of the growth and differentiation factors have set the stage for a symbiosis of biotechnology and biomaterials. Recent advances permit one to enunciate the rules of architecture for tissue engineering of bone and cartilage. The purification and cloning of bone morphogenetic proteins (BMPs) and growth factors such as platelet derived growth factors (PDGF), transforming growth factor-β (TGF-β), and insulin-like growth factors (IGF-I) will allow the design of an optimal combination of signals to initiate and promote development of skeletal stem cells into cartilage and bone. Successful and optimal bone and cartilage formation is a synergy of inductive and conductive strategies governed by biomechanics, optimal load bearing, and motion. BMPs function as inductive signals. Biomaterials (both natural and synthetic) mimic the extracellular matrix and play a role in conduction of bone and cartilage. Examples of biomaterials include hydroxyapatite, polyanhydrides, polyphosphoesters, polylactic acid, and polyglycolic acid. The prospects for novel biomaterials are immense, and they likely will be a fertile growth industry. Cooperative ventures between academia and industry and technology transfer from the federal government augur well for an exciting future for clinical applications.

Key words: bone, cartilage, BMPs, PDGF, TGF-β

Recent advances in the seemingly disparate fields of biotechnology and biomaterials are converging into the young discipline of tissue engineering. We define tissue engineering as the science of fabricating new tissues for replacement and total regeneration. Explosive developments in recombinant DNA technology have spawned the area of biotechnology of growth and differentiation factors. Biomimetic materials science has provided biomaterials for fabrication of bone and cartilage with growth and differentiation factors. This brief article will survey recent developments in this exciting frontier of tissue engineering of cartilage and bone and forecast trends in this field for the twenty-first century. The three principal ingredients for the successful tissue engineering of bone and cartilage are regulatory signals, cells, and extracellular matrix. The rules of architecture for tissue engineering can now be developed systematically. Among the many tissues in the body, bone has considerable potential for repair and regeneration. Hence, it is likely that many of the secrets of the principles of architecture for tissue engineering of bone repair may be discerned from bone. The accrued knowledge may then be applied not only to bone regeneration but to other tissues such as cartilage which are recalcitrant to repair.

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Address reprint requests to A. Hari Reddi, Ross Research Building—225, 720 Rutland Avenue, Baltimore, MD 21205-2196.

BMPs AND BONE INDUCTION

What is the molecular and cellular basis of the process for bone to repair and regenerate? The classical sequence of the bone repair cascade consists of hemostasis, migration of progenitor cells by chemotaxis, proliferation of stem cells, and differentiation of endochondral bone formation [1,2]. The entire cascade of bone repair is mimicked by extracellular matrix–induced bone induction in nonskeletal sites [3–5]. The phenomenon in brief consists of implantation of allogenic demineralized bone matrix into the subcutaneous space of a young (25–30 days old)

rat. This results in local induction of bone at the site of implantation. The responding cells are the local mesenchymal cells. The developmental steps include activation and migration of mesenchymal cells, attachment to collagenous matrix via fibronectin and integrins, mitosis of mesenchymal stem cells, differentiation of cartilage and bone, mineralization and remodeling, and hematopoietic marrow differentiation [5].

The demineralized extracellular bone matrix is in the solid state. Chaotropic agents such as 4 M guanidine hydrochloride, 8 M urea, and 1% sodium dodecyl sulfate solubilized 2–3% of the bone matrix. Neither the soluble extract nor the insoluble residual matrix was biologically active in bone induction. However, recombining the extract with the residue resulted in restoration of osteoinductive activity [6]. It would appear then that there is a collaborative interaction between soluble molecular signals and an insoluble substratum of extracellular matrix to initiate the cascade of bone morphogenesis. Therefore, the combination of matrix and growth and differentiation factors is optimal to promote bone repair. The active protein fraction was dubbed osteogenin and was purified by heparin affinity chromatography [7]. Osteogenin is identical to BMP-3 cloned by Wozney and colleagues [8]. The bone morphogenetic protein family (BMPs) now includes at least seven members [9,10] including BMP-2 through 7 and OP-2 (BMP-8). Table I lists all the BMPs known to date and their relationship to other TGF-β superfamily members including TGF-β_1, activins, *Drosophila* dpp and 60A, and *Xenopus* Vg 1 [2,10]. It is noteworthy that dpp and 60A gene products can induce bone in rats in the ectopic bone induction assay [11].

TABLE I. Bone Morphogenetic Proteins (BMPs) and Members of the Extended Transforming Growth Factor Beta (TGF-β) Superfamily Comparison of the Amino Acid Sequence Identity With BMP-2 in the Carboxy-Terminal Mature Domain and Bioactivity of Bone Induction

BMP/TGF-β family	Other names	Carboxy-terminal mature domain (% identity)	Bone induction[a]
BMP-2[b]	BMP-2A	100	+
Osteogenin	BMP-3	49	+
BMP-4	BMP-2B	92	+
BMP-5	—	59	+
BMP-6	Vgr 1	62	+
Osteogenic protein-1	BMP-7	60	+
Osteogenic protein-2	BMP-8	55	+
TGF-β_1	—	32	–
Activin A	—	45	–
Activin B	—	44	–
Drosophila dpp	—	53	+
Drosophila 60A	—	58	+
Vg 1	—	58	?

[a]+ = positive; – = negative; ? = not known.
[b]BMP-1 is not a member of the TGF-β superfamily.

CURRENT STATUS OF BMP

BMPs in addition to initiating bone formation appear to be classic pleiotropic modulators. BMP-3 stimulated alkaline phosphatase activity and collagen synthesis in newborn rat calvarial periosteal cells and osteoblasts [2]. It is likely that BMPs in addition to initiating de novo bone formation may promote and maintain osteogenic phenotype.

It is also noteworthy that BMP-3 and BMP-4 stimulated the synthesis of sulfated proteoglycans of rat and rabbit chondrocytes. The homeostasis and repair of cartilage in bovine articular cartilage explants were maintained by both native BMP-3 and recombinant human BMP-4. This observation hints at the potential role of BMPs in cartilage.

BMPs bind extracellular matrix components, especially type IV collagen found in basement membranes [12]. It is well known that vascular invasion is a prerequisite for bone formation. It is plausible that the basement membranes around invading capillaries bind growth and morphogenetic proteins and initiate bone differentiation and morphogenesis. BMPs are thus presented in a matrix-bound form locally to responding mesenchymal cells. This principle of extracellular matrix affinity is likely to be critically important for tissue engineering of bone [1].

PROSPECTS: TISSUE ENGINEERING OF BONE AND CARTILAGE

The recent advances in the identification, isolation, cloning, and expression of BMPs have provided us with a menu for enunciating the rules of architecture for tissue engineering of bone and cartilage. The three critical ingredients for tissue engineering of bone and cartilage

are regulatory signals, cells, and extracellular matrix.

The primordial molecular signals for bone and cartilage are BMPs. BMPs can initiate bone and cartilage morphogenesis. Hence, the first rule is the provision of optimal regulatory signals for initiation. Once initiated the signals for propagation and progression of chondrogenesis and osteogenesis require growth factors to promote the phenotype. The prime candidates for promotion of bone formation are TGF-β_1 and -β_2. IGF-I and its cognate binding proteins are of paramount importance in both bone and cartilage maintenance.

There is a growing interest in the approaches of cell therapy and cell transplantation in bone and cartilage repair. For example, there is considerable interest in the application of mesenchymal stem cells [13–15]. It is likely that one can use mesenchymal stem cells from bone marrow stroma and prime the tissue construct to give it a head start to respond to molecular signals such as BMPs. Therefore, the second rule is to provide responding cells for the molecular signals.

Finally, it is well known that the biosynthesis and assembly of extracellular matrix is a prerequisite to restore the skeletal structures such as cartilage and bone. Each musculoskeletal organ has its own constellation of extracellular matrix components that comprises the specific composite for that particular tissue [16]. Optimal tissue engineering of cartilage and bone needs the synergy of both inductive and conductive strategies subject to governance by biomechanics and motion. Biomaterials mimic the extracellular matrix and play a crucial role in delivery of the growth and morphogenetic factors. At present considerable work is in progress in such biomaterials as hydroxyapatite, polyanhydrides, polyphosphoesters, and copolymers of polylactic acid and polyglycolic acid. The prospects for the development of novel biomaterials using principles of molecular biotechnology are immense. The third rule for tissue engineering, then, is the provision of the optimal extracellular matrix scaffolding for the responding cells and the use of biomimetic surface coated with matrix components such as fibronectin and laminin [16].

The recent breakthroughs in biotechnology and biomaterials may permit a synergy between the two frontiers. One can imagine the use of templates of biomaterials prefabricated to anatomical structures such as femoral head or articular cartilage of the knee joint loaded with responding cells or tissue and injected with molecular signals such as BMPs. In fact, such a potential practical application was achieved using vascularized muscle flaps placed in a silicone mold and injected with osteogenin, a bone morphogenetic protein [17]. This is an auspicious beginning for the realm of tissue engineering of skeleton. The only limitation for this exciting frontier is boundless imagination and development of novel biomaterials based on biotechnology. Cooperation between academia and industry and technology transfer from federal government laboratories augur well for clinical applications. We indeed are in a brave new world of prefabricating spare parts for the human body employing molecular signals, responding cells, and optimal extracellular matrix.

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