Strategies for Stimulation of New Bone Formation: A Critical Review

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Large bone defects, congenital or caused by diseases, trauma or surgery, do not heal spontaneously and are usually a clinical challenge in the orthopedic and dental practices. A critical review concerning strategies to substitute lost bone or stimulate osteogenesis was undertaken. Pivotal concepts ranging from traditional bone grafting and use of biomaterials to local application of growth factors and gene therapy were addressed, including critical comments on the efficacy and safety, difficulties, advantages and disadvantages of each method. The most predictable results are still obtained with autogenous bone graft, despite the inconveniences of morbidity and limited availability of graft material. Satisfactory results have been reported for recombinant bone morphogenetic proteins (rhBMPs)-2 and -7, which distinguish for their osteoinductive property, the difficulty being the need for a degradable carrier that allows its continuous release in a rate compatible to that of new bone formation. Other bone growth factors are currently under evaluation in preclinical models of bone defects; however their efficacy is also dependent on the competence of a delivery strategy and on an appropriate delineation of "which one", "which dose" and "when". Parameters of efficiency and safety for gene therapy are still being established. In conclusion, given the variety of growth factors involved in the complex cascade of bone repair and the biological interactions between them, it remains a challenge to accomplish the ideal strategy to stimulate reparational bone formation in specific conditions of the medical as in the dental practices.

Key Words: Biomaterials, bone morphogenetic proteins, bone growth factors, gene therapy.

BONE REMODELING AND HEALING

To fulfill adequately their functions of support, sustentation and protection for the internal organs and guarantee blood calcium homeostasis, bone tissue is in constant remodeling. Alveolar bone, the primary support structure for teeth, is particularly subjected to continuous and fast remodeling associated with tooth eruption and positional adaptations, as well as with the functional demands of mastication (1). The remodeling cycle comprises resorption of bone matrix, under osteoclasts responsibility, followed by formation and mineralization of a new matrix, in charge of osteoblasts. A complete cell differentiation, in addition to an appropriate functional activity, depends on intercellular communication between bone-forming and bone-resorbing cells. The osteoblasts control degradation of bone matrix by producing cytokines stimulatory of osteoclasts precursors (2,3). In the sequence, osteoclasts release acids and proteases to dissolve the mineral and degrade the organic matrix, releasing the stored growth factors. The bone growth factors, chiefly platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), transforming growth factor-β (TGF-β), insulin-like growth factors (IGFs), vascular endothelial growth factor (VEGF) and bone morphogenetic proteins (BMPs), control differentiation and functional activity of cells of the osteoblastic lineage (4).

Bone tissue has a recognized capacity of spontaneous regeneration which, in the same way as remodeling, is a complex and multifactorial cascade of biological events (cell migration, proliferation, adhesion and differentiation, besides vascular neoformation) regulated by distinct growth factors, secreted by bone cells and by reactive cells present in the damaged site (4,5). Nevertheless, large bone defects, congenital or caused by disease, trauma or surgery, do not heal spontaneously and are usually a clinical challenge in the

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orthopedic and dental practices. Such situations benefit from the use of strategies that may substitute for lost bone or stimulate bone formation.

BIOMATERIALS

A variety of biomaterials has been developed to fill bone defects, such as autogenous, homogenous (allograft) and heterogeneous (xenograft) bone grafts, and synthetic (alloplastic) substitutes (6-8). Biocompatibility, the minimum requisite for a biomaterial to interact with biological systems, is "the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy" (9).

In addition to biocompatibility, properties of osseointegration (the ability to chemically bond to the surface of bone without an intervening layer of fibrous tissue) and osteoconduction (the surface of the bone graft material serving as a scaffold for new bone growth) (6) may be sufficient when a prolonged replacement of bone structures are desired. However, characteristics of osteoinduction (the ability to induce differentiation of osteoprogenitor cells from surrounding tissues to differentiate into osteoblasts that begin new bone formation) and osteogenesis (formation of new bone by vital osteoblasts present within the graft material) (6) are required when it is expected that a biodegradable biomaterial temporarily fills the defect while stimulates new bone formation.

Despite the great variety of bone grafts available for medical and dental applications, the most predictable results are still obtained with autogenous bone graft (the "gold standard"), which possess vital osteoblasts and osteoinductive factors besides a porous structure that allows for fibro-vascular and osteogenic growth inside the pores; the inconveniences include morbidity related to surgery and limited availability of graft material. Allografts do not have osteogenic cells, but they may have osteoinductive properties if used in a demineralized form; the inconveniences include a potential risk of infection and immune reaction, besides the need of a special store, which results in the high costs of bone banks (6,7). Xenografts are obtained mainly from bovine bones and, given the abundance and low cost

of graft material, combined with a proper processing that minimizes the risk of infection, North American, European and Brazilian companies have produced them for use in medical and dental clinics (10).

Limitations concerning the use of bone grafts have prompted the development of a massive array of synthetic materials that posses some of the desired mechanical qualities of bone as well as osteointegrative and osteoconductive properties. The surface composition and structure of some materials, namely the calcium phosphate ceramics and bioactive glasses, have similarity with the mineral phase of bone matrix, conferring to them a bioactive property, as they bond to bone and enhance new bone formation. However, no matter what is the synthetic bone substitute, new bone growth is often limited because these materials are not osteoinductive or osteogenic (6).

Thus, despite the large variety of organic and synthetic materials available to replace bone or stimulate osteogenesis, and in spite of a growing progress in this area, there is no material at present that satisfies all the expected requirements. In addition to the conventional use of biomaterials, local biochemical stimulation with growth factors can represent an advantageous alternative in procedures aimed at stimulating bone healing by overcoming many limitations of the use of bone grafts.

BMPs AND CARRIER SYSTEMS

BMPs are bone growth factors synthesized and secreted by osteoblasts and incorporated into the organic matrix during bone formation. They are released during osteoclastic resorption and induce differentiation of mesenchymal cells into osteoblasts, stimulating osteogenesis in the remodeling and healing processes (3). Presently, 20 structurally related BMPs belonging to the TGF-β superfamily have so far been recognized, and two of them, the BMPs-2 and -7, distinguish for their osteoinductive property, emerging as an alternative for filling of bone defects (7,11). However, the difficulty for their clinical use is that, because they are rapidly diffusible in biological media, to achieve maximum efficacy without the need for excessively high doses they should be associated with a carrier system that allows its continuous release in a rate compatible to that of new bone formation. In addition to undergoing controlled biodegradation, other essential requirements for a potential carrier are biocompatibility, reduced immunogenicity and no toxicity; ideally they should be

osteoconductive, have mechanical stability and adequate porosity to allow infiltration of cells and support vascular ingrowth, and be sterile and user-friendly (5,7,12-15).

Autogenous and homogenous bone grafts, in addition to natural polymers (collagen, hyaluronans, fibrin, chitosan, silk, alginate, agarose), synthetic polymers (polylatic acid/PLA, polyglycolic acid/PGA and copolymer/PLGA), inorganic materials (calcium phosphate/ sulphate cements, bioglasses) and their composites, molded as membranes, sponges, granules, matrices, microparticules, hydrogels and foams, have been tested as carriers for BMPs and other bone growth factors (5,7,11-16). Nanotechnology, the technology that allows the construction of materials in a nanometric (1-100 nm) scale, has also been utilized to construct delivery systems for osteogenic growth factors (8,14,17).

Purified (mainly from bovine bones) and human recombinant BMP-2 and 7 combined with a variety of carriers have been proven effective in stimulating the healing of experimental defects in bones of different natures (calvaria, long bones, mandible and maxilla), including healing of defects large enough to preclude a spontaneous healing (critical-sized defects), despite some contradictory results, probably due to factors related to concentration/dose of BMPs and type of carrier (7,18-21). In fact, many carrier systems have limitations in terms of biodegradability and inability to promote continuous and controlled release of the protein in the site of injury (15).

The suitability of a carrier depends mainly on the coupling method used to bind the growth factor, which is most commonly through (i) physical entrapment in a reservoir type system or inside polymeric microparticles, liposomes, hydrogels, foams or bone cements, (ii) physical adsorption, as occurs in the impregnation of absorbable collagen sponge with a growth factor solution, and (iii) ionic complexation (5). A simple immersion into a growth factor solution, as provided by the carriers currently in clinical use, allows a mere adsorption of the protein to the carrier and therefore its prompt release in the biological media, which is not appropriate considering the kinetics of bone healing. Alternatively, incorporation of growth factor molecules into the biomaterial matrix for a gradual release during degradation would be highly desirable (16).

Collagen is the most commonly used and still the only organic BMP-carrier approved by American and European regulatory agencies for clinical use (13,16). The rhBMP-2 carried by absorbable type 1 collagen sponge, by particulate bone-derived type 1 collagen or by particulate collagen combined with carboxymethyl cellulose is commercially available in North America and Europe, restricted to specific needs depending on each country's regulatory authority (22). Despite the obvious success in promoting recovery of bone damage in the orthopedic and dental practices, including augmentation of maxillary sinus floor, induction of alveolar bone formation for placement of osseointegrated implants and augmentation/preservation of human alveolar ridge after tooth extraction (4,7,8,11), the clinical use of rhBMP-2 has raised concerns regarding its safety and efficacy, since excessive/ectopic bone formation and the possibility of toxicity, immunogenicity and carcinogenesis associated with excessive doses of the protein or with the collagen used as a carrier have suggested the need for further clinical trials (8,11,13).

In Brazil, it is commercially available a national product consisting of a pool of purified BMPs extracted from bovine fetal cortical bone. This material, adsorbed to synthetic microgranular hydroxyapatite, was tested for treatment of rat calvarial critical size bone defects, resulting in formation of a foreign body reaction that markedly inhibited new bone formation, allegedly because synthetic microgranular hydroxyapatite may not represent a good carrier for BMP (18). Moreover, when adsorbed to synthetic microgranular hydroxyapatite and added to granules of organic bovine bone matrix before implantation into human intrabony periodontal defects, this pool of purified BMPs did not provide added effects to guided tissue regeneration in terms of clinical parameters (23). The same material, added to granules of inorganic bovine bone and agglutinated with bovine collagen, inhibited bone regeneration in segmental defects in the radial diaphysis of rabbits (20). Using a microgranular resorbable hydroxyapatite as a carrier and mixing with bovine collagen, this material did not stimulate new bone formation in rat alveolar sockets (21); the authors suggested that the material did not warrant incorporation of the osteoinductive proteins to a slow-absorption system that would allow a BMPs release rate compatible to that of new bone formation.

The cubic phase of glyceryl monooleate (monoolein gel) is a synthetic compound extensively used in the pharmaceutical industry as a drug carrier in different formulations and routes of administration; the incorporated drugs, including peptides and proteins, are protected from enzymatic degradation, undergo slow release and do not lose biological activity and

stability (24). In a recent study, monoolein gel was used as carrier for rhBMP-2 in an experimental model of acute distraction osteogenesis in rat mandibles; the results suggested the effectiveness of this carrier as a sustained-delivery system for BMPs (25).

In conclusion, compilation of literature indicates the need to develop ideal carriers for specific clinical uses and to characterize the appropriate amount of BMPs at the damaged site, as the carrier systems available still require high doses of the protein, raising questions about safety and high cost.

OTHER BONE DERIVED GROWTH FACTORS

The modern "Diamond Concept" specifies 4 major elements to optimize bone repair: (i) osteogenic cells, (ii) osteoconductive scaffolds, (iii) mechanical stability and (iv) growth factors (4,13). A number of bone growth factors besides BMPs are expressed during bone repair and some of them are currently under evaluation, alone or in combination, mostly in preclinical models of bone defects (4,8,13,26,27). However, as mentioned for BMPs, their clinical efficacy will be dependent chiefly on the competence of a local delivery strategy as well as on an appropriate delineation of "which one", "which dose" and "when".

Some experimental studies have shown the beneficial action of PDGF, FGFs, VEGF, TGF-β and IGFs to stimulate the migration, proliferation and differentiation of osteoprogenitor cells, in addition to vascular ingrowth to the injury site (4). Exogenous VEGF enhanced blood vessels ingrowth, bone formation and callus maturation in an experimental model of long bone fracture healing (28). Preclinical studies have also pointed to a beneficial effect of FGFs in bone repair, although its action seems to be limited to the first 24 h after fracture (4,27).

Experimental evidence points to PDGF as an important mediator for bone healing and remodeling during trauma and infection, with positive effects on bone regeneration if used in conjunction with IGFs, TGF-β or BMPs (26). It seems to have a positive effect in accelerating long bone fracture healing and, when applied with IGF-1, can enhance the healing of periodontal bone defects (27). However, its therapeutic potential in humans is still unclear (4).

The potential of TGF-β to enhance bone formation has been suggested in some experimental conditions, mostly by stimulation of osteoblast chemotaxis, proliferation and matrix synthesis/mineralization (26,27). However, its promise to improve bone formation in clinical use deserves more investigation (26).

Experimental studies in calvarial critical-sized bone defects and long bone fracture repair have suggested the potential of IGF-1 to stimulate bone formation, mainly if used in conjunction with TGF-β1 (4,27). Additionally, a local application of IGF-I and PDGF appears to be promising in promoting bone regeneration in dentoalveolar defects around implants or after periodontal bone loss (26). The surface modification of metallic implants by coating with IGF-1 and TGF-β1 has accelerated bone formation and/or maturation in diverse rat and porcine experimental models (13).

Thus, although different bone growth factors have shown efficacy in some experimental conditions, a small number of clinical trials with inconclusive results do not guarantee its effectiveness in the medical or dental settings. Further studies are needed before their clinical use, especially given the high cost of human recombinant growth factors.

The platelet rich plasma (PRP) emerges as an alternative for local treatment with human recombinant growth factors, with the advantage of reproducing a physiological condition by the combination of these factors. PRP is a platelet-rich blood product produced by a simple and inexpensive method, which can release a variety of growth factors. However, the effectiveness of their experimental or clinical use, alone or in combination with bone grafts, synthetic biomaterials and/or mesenchymal cells from bone marrow, has been queried (4,26,29,30), allegedly due to absence of an osteoinductive property, to the low amount of growth factors or to the presence of significant amounts of TGF-β, which is one of the main stimulators of collagen deposition, filling the defect with fibrous tissue (13,29).

GENE THERAPY

Instead of the traditional bone grafting and the direct application of growth factors into bone defects (protein therapy), gene therapy, in which desired genes are delivered to target cells resulting in a sustained *in vivo* production of osteogenic proteins, emerges as a potential alternative. The encoding gene can either be introduced directly into the target site, so as host cells are transfected and express the protein (*in vivo* transduction), or delivered through transfection of cultured cells (obtained from the recipient itself or from pre-existing

osteoprogenitor cell lines) implanted at the regeneration site (*ex vivo* transduction) (4,8,15). Vectors have been used to optimize the transduction and expression of encoding genes. Viral vectors have the advantage of high frequency of transduction but also the major disadvantage of immunogenic potential. Alternatively, non-viral vectors (DNA plasmids, lipoplexes, polyplexes etc) avoid the problems associated with viral vectors but still did not achieve the intrinsic efficiency of viral vectors (4,15).

An up-to-date review (15) listed experimental studies conducted to evaluate the *in vivo* and *ex vivo* gene transfer, using viral and non-viral vectors to deliver rhBMP-2 and induce bone formation at sites of bone injury, and thus establishing parameters of efficiency and safety, major difficulties, advantages and disadvantages of each method. The authors referred to expected future improvements in gene therapy, emphasizing that although promising results have been achieved in animal models, human trials have not yet been reported.

CONCLUDING REMARKS

The wide variety of organic and synthetic biomaterials available to stimulate osteogenesis does not satisfy all the expected requirements for a bone graft substitute. Local stimulation with rhBMPs and other growth factors still needs a proper carrier and a definition of dose and time sequence appropriate for the kinetics of bone healing. Regarding the growth factors, a small number of clinical trials with inconclusive results do not guarantee its effectiveness in the medical or dental settings. Gene therapy, so far in the preclinical phase of evaluation, has emerged as a potential alternative.

RESUMO

Defeitos ósseos de grandes dimensões, congênitos ou causados por doenças, traumas ou cirurgias, não se regeneram espontaneamente e são, no geral, um desafio para médicos e dentistas. O presente trabalho apresenta uma revisão crítica sobre estratégias para substituir tecido ósseo ou estimular a osteogênese reparacional. São apresentados conceitos relevantes relativos aos métodos tradicionais de enxertos/implantes ósseos e uso de biomateriais até a aplicação local de fatores de crescimento e a terapia gênica, incluindo comentários críticos sobre eficácia, segurança, dificuldades, vantagens e desvantagens de cada método. Os resultados mais previsíveis ainda são obtidos com enxertos ósseos autógenos, apesar das inconveniências de morbidade e disponibilidade limitada de material. Resultados satisfatórios têm sido relatados com o uso de proteínas ósseas morfogenéticas humanas recombinantes (rhBMPs)-2 e -7, que se distinguem pela capacidade de osteoindução, apesar da necessidade do uso combinado com um carreador biodegradável que permita sua liberação em um ritmo compatível com o da neoformação óssea. Outros fatores de crescimento ósseo estão presentemente em fase pré-clinica de avaliação e sua eficácia também depende de uma estratégia adequada de liberação, além da definição de parâmetros como "qual fator", "em que dose" e "quando". Ainda estão sendo estabelecidos os parâmetros de eficiência e segurança para aplicação da terapia gênica em defeitos ósseos. Concluindo, diante da grande variedade de fatores de crescimento envolvidos na complexa cascata do reparo ósseo e das interações que eles estabelecem, definir a melhor estratégia para estimular a formação óssea em situações específicas das práticas médica e odontológica permanece um desafio para cientistas e clínicos.

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