Review article
CURRENT TRENDS IN THE ENHANCEMENT OF FRACTURE HEALING
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Repair of fractures involves a sequence of dynamic events which ultimately restores the integrity of the bone and its biomechanical properties. In some cases healing is compromised, leading to delayed union or nonunion. It is estimated that 10% of the fractures which occur annually will require further surgical procedures because of impaired healing. The preferred management of nonunion and bone defects is by autologous cancellous bone grafting, because this provides the essential elements for bone formation, namely living osteogenic cells, bone-inductive proteins which stimulate cell proliferation and differentiation, and a scaffold of apatite which supports the ingrowth of newly formed bone. The supply of suitable bone, however, is limited and its harvest results in additional morbidity to the donor site, leading to pain, haematoma, or infection.

For more than 30 years investigators have been developing alternative treatments, by physical or biological methods, aimed at providing the benefits of bone grafting without the complications related to harvest of an autograft. The physical strategy includes the use of mechanical stimulation, electromagnetic fields, and low-intensity ultrasound. The biological approach involves the use of osteoconductive biomaterials, growth factors which stimulate tissue repair (including bone morphogenetic proteins), and osteocompetent cells.

We will review the preclinical and clinical data on each therapeutic approach and discuss potential areas of development in the field of bone healing.

Physical methods

Mechanical stimulation. One of the fundamental concepts in orthopaedics is the understanding that appropriate mechanical loading accelerates the healing of fractures. This is based on the process of adaptation, according to which bone architecture is constantly optimised in response to the mechanical environment, and which occurs in response to dynamic rather than static loading. More specifically, it is related to the peak strain magnitude and the loading frequency. An osteogenic response is induced and bone formation is enhanced by peak strains above 0.4% (that is a deformation of 2 mm for an adult femur) and load cycles above 0.5 Hz. Bone formation occurring in response to a mechanical stimulus tends to saturate when the duration of loading is increased. Adaptation occurs when abnormal rather than normal strains are applied to the bone.

It is therefore not surprising that the mechanical milieu at the site of a fracture influences the pattern of fracture repair. Thus, when movement is prevented under conditions of rigid stabilisation, direct osteonal remodelling can occur with the formation of little or no external callus, leading to direct bone repair. By contrast, when a small degree of movement occurs under less rigid interfragmentary stabilisation, endochondral healing at the site of the fracture or intramembranous ossification within the periosteum is observed, resulting in direct bone repair. It is noteworthy that the rate of healing, the extent of the formation of callus and the amount of blood flow are all increased by applying appropriate cyclic interfragmentary movement in this type of repair. While excessive interfragmentary movement does favour the formation of callus it has deleterious effects on angiogenesis, resulting in hypertrophic nonunion.

The potential benefits of controlled mechanical loading on bone healing have been described in a prospective, randomised study of 102 tibial fractures stabilised by an external frame modified to apply controlled axial micromovement of displacement of 1.0 mm at 0.5 Hz for 30 minutes per day, starting one week after application of the frame. Axial dynamisation significantly reduced the healing time and was associated with a lower rate of secondary surgery.

Electromagnetic fields. When bone is subjected to mechanical stress, strain gradients are created, resulting in pressure gradients in the interstitial fluid. These drive fluid through the canaliculae from regions of high to low pressure, exposing osteocyte membranes to flow-related shear.
stress as well as to electrical potentials subsequent to the streaming process. These potentials could play a role in the process of mechanotransduction. In order to mimic these effects, several investigators have proposed administering an exogenous electrical field at the site of the fracture. Electromagnetic fields (EM) can be delivered by direct-current stimulation using implanted electrodes (invasive), inductive coupling produced by a time-varying magnetic field (non-invasive), or by capacitative coupling (non-invasive).

The underlying effects of EM on cellular processes are not well understood. In vitro exposure of osteoblasts to EM stimulates the secretion of numerous growth factors including bone morphogenetic proteins (BMPs) 2 and 4, transforming growth factor-β (TGF-β), and insulin-like growth factor-II (IGF-II). An indirect effect by way of an increase of temperature cannot be ruled out, however, and more study of this important question is clearly needed.

In clinical practice, EM have been investigated in delayed union and nonunion for 30 years in several departments, including that of one of the authors (LS), and various devices have already been approved by the American Food and Drug Administration. Stimulation by both inductive and capacitative coupling has displayed a rate of efficacy of 64% to 87% in the treatment of nonunion of the tibial shaft. In a prospective study performed on 35 nonunions treated by stimulation with inductive coupling, Sedel et al. reported positive effects in 14. There is, to our knowledge, however, only one prospective, randomised double-blind study which has been performed on the use of EM for treating pseudarthroses. This consisted of 23 patients with established nonunion at a mean of 28 months after injury. The control group was composed of 11 patients treated with a placebo unit. There were five cases of nonunion of the tibia in the active group and ten in the control group. Healing was achieved in 60% of the patients treated by capacitative coupling stimulation at a mean of 21 weeks, while none of the control group healed. Although this study suggests that there are some positive effects of the treatment of nonunion by electromagnetic stimulation, these results must be assessed in larger controlled clinical trials comparing this treatment with conventional autograft procedures.

**Low-intensity ultrasound.** The application of ultrasound (US) to the healing of fresh fractures was first introduced by Duarte. Although animal and clinical studies have confirmed the ability of US to enhance the healing of fractures, the exact physical mechanism has not been established. In vitro, low-intensity US has direct effects on cell physiology. It increases the incorporation of calcium ions in cultures of cartilage and bone cells, and stimulates the expression of numerous genes involved in the healing process, including aggrecan, IGF and TGF-β. Exposure to US increases the formation of soft callus and results in the earlier onset of endochondral ossification, suggesting that the most prominent effect is on the chondrocyte population.

Animal studies performed on fresh fractures in rats and rabbits showed a mean acceleration of the healing process by 1.5 times in the US-treated group, as assessed by radiography and biomechanical testing. It is likely that this effect is the result of several mechanisms, including an increase in bone formation as reported by Tanzer et al. In a study performed on canine femoral coated implants, US augmented bone ingrowth as compared with the contra-lateral untreated limbs.

Clinical experience with low-intensity US over 20 years has shown that it promotes healing in fresh fractures. Therapeutic US is currently used by physiotherapists with spatial-averaged temporal-averaged intensities ranging from 2 to 100W/cm². By contrast, the intensity required for the repair of fractures is very low and does not exceed 30 mW/cm². US is usually introduced during the first week after the fracture, and is applied for 20 minutes per day. In a multicentre randomised, controlled trial on the use of US in 67 fractures of the tibial shaft in humans, Heckman et al. reported a significant reduction (38%) in healing time. Similar results were reported by Kristiansen et al. in a prospective double-blind evaluation of 61 Colles’ fractures treated by closed reduction and application of a cast. The period required to achieve clinical and radiological healing was significantly reduced and treatment with US was associated with decreased loss of reduction. No adverse reactions or complications were observed. Based on these studies, the American Food and Drug Administration has approved the use of low-intensity US for the healing of fresh fractures.

Clinical investigations involving US have also provided promising results in the treatment of delayed union or nonunion. Duarte first reported a success rate of 85% in tibial nonunion at a mean of 14 months after fracture. Subsequent studies have supported these results and shown union in 80% to 91% of unhealed tibial fractures at a mean time of nine months after fracture. There is, to our knowledge, however, no reported prospective double-blind study comparing the efficacy of US with the conventional treatment of nonunion. Further investigations comparing costs and results of both treatments are necessary to show clearly the positive effects of US in this indication.

**Biological methods**

Strategies for the development of biological substitutes capable of mimicking the natural environment are based on a better understanding of the basic events in the healing of fractures. The biological approach aims to provide the key components which play a pivotal role in the repair of bone. The ideal material must be biocompatible, resorbable, and porous to facilitate rapid vascularisation and progressive replacement by newly-formed tissue. Several constructs have already been developed in animal studies by investigators, and some of these are currently used in clinical practice. Therapeutic strategies include the
use of osteoconductive biomaterials, osteoinductive biomaterials comprising a combination of growth regulatory molecules with carriers, and osteogenic biomaterials made of a scaffold seeded with osteocompetent cells.

**Osteoconductive biomaterials.** Osteoconduction is the process which supports the ingrowth of newly-formed bone when the material is implanted in contact with the bone of the recipient host. The requisite pore size for bone ingrowth into porous implants is 100 to 500 μm. A variety of natural or synthetic osteoconductive biomaterials, such as calcium phosphate ceramics and coral, has been developed with these properties, and studied in several animal models over the past 30 years. Most investigations have focused on calcium-based ceramics, including hydroxyapatite (HA), tricalcium phosphate (TCP), and bioactive glasses. HA was first introduced because of the similarity between its formula and that of the mineral phase of bone. The rates of resorption of calcium phosphate ceramics vary inversely with the calcium/phosphate ratio, and also depend on the density, the size of the grain, and the porosity. TCP, with a calcium/phosphate ratio of 1.5, is highly resorbable compared with HA which has a calcium/phosphate ratio of 1.67 and is nearly undegradable when implanted in vivo. Macroporous biphasic calcium phosphate, a combination of HA and TCP (60% HA + 40% TCP), has an intermediate rate of degradation. All of these materials are well known for their excellent bone-bonding capacities but they are brittle and have poor resistance to compressive stress. HA and TCP have been widely used both in animal and clinical studies and have yielded good results in the treatment of small defects of bone when they are implanted in close contact with the host bone and the bone interface is not exposed to shear strain. Similar results have also been reported in operations for posterior spinal fusion. In a prospective, randomised trial on the use of blended HA/TCP in 58 patients with idiopathic scoliosis, Delecrin et al. reported a significant reduction of blood loss compared with the autograft group, with a comparable loss of correction in both groups.

Recently, several laboratories have developed a process which allows the formation of HA in situ. The bioactive bone-cement paste is produced by the combination of two ceramic phosphates and sets ten minutes after injection. Sanchez-Sotelo, Munuero and Madero performed a prospective, randomised clinical trial on the use of bone cement in the treatment of 110 Colles’ fractures and compared it with conservative treatment. They showed that patients treated with bone cement had better functional results and a lower rate of malunion when compared with the control group. This cement, however, is devoid of macroporosities where cellular ingrowth could occur, which is critical when dealing with larger defects of bone.

Natural coral exoskeleton derived from marine reefs is composed of calcium carbonate. It was introduced as a substitute for bone-graft in the mid-1970s, and has been used clinically to treat a variety of orthopaedic and craniofacial defects of bone. This natural ceramic has excellent mechanical properties (resistance up to 300 MPa) and an ideal interconnected porous architecture similar to that of spongy bone. Alternatively, the calcium carbonate of natural coral can be converted to HA by a chemical reaction termed the replamineform process. The interconnected porosity inherent in *Porites* coral is perfectly conserved. Partial conversion of the calcium carbonate exoskeleton into HA can also be achieved resulting in implants with an adjustable rate of resorption. Although these materials have very good osteoconductive properties, they have not been found to be suitable for the treatment of large defects of bone in clinical practice. Bone ingrowth in osteoconductive substitutes is often confined to the superficial surfaces and the extremities of the material; the substantial enhancement of bone formation necessary to replace a clinically significant defect can only be achieved with the addition of biologically-active substances such as growth factors or osteogenic cells.

**Osteoinductive materials and growth factors.** Rapid and diverse events are activated by the fracture of a bone in order to limit the loss of blood and initiate cellular migration which will result in repair. Current concepts suggest that these cellular events are controlled to a large part by growth factors, low-molecular-weight glycoproteins, which induce migration, proliferation and differentiation of an appropriate subset of cells in the site of the fracture. The sources of growth factors include the clot and the bone itself. During clotting, platelets aggregate and release numerous regulatory molecules such as platelet-derived growth factor (PDGF) and TGF-β. Bone releases several growth factors at the site of the fracture including BMPs, TGF-β, PDGF, IGF-I, IGF-II and basic and acidic fibroblast growth factors (FGFs). The release of the last is likely to play an important role in the initial phase of the healing process, since they have shown angiogenic properties and mitogenic activity on the osteoblast lineage. It is generally accepted that once cells enter the wound, their proliferation and differentiation are most likely to be determined by the type and level of growth factor present at the site of the fracture. Of paramount importance is that BMPs are the only factors known to induce bone formation heterotopically by inducing undifferentiated mesenchymal cells to differentiate into osteoblasts. It is likely that increasing the concentration of growth factors in depleted areas will accelerate and improve the process of repair. Therefore, a number of investigators have proposed supplementary osteoconductive biomaterials with growth factors in order to boost the key steps of repair. We will illustrate this approach by summarising the data available on the use of BMPs for the enhancement of the healing of fractures.

BMPs were discovered in 1965 by Urist who pointed out their osteoinductive properties. Subsequently, they were cloned and are now produced by genetic engineering (rh-BMP). BMPs are members of the TGF-β superfamily. They
are present in many tissues including the kidney, the peripheral and central nervous system, the heart and the lungs, and have potent effects on morphogenesis and growth in tissues other than bone. BMPs have shown their ability to promote bone healing in large defects in rabbits, sheep and dogs, and in models of spinal fusion in dogs and monkeys. In a study performed at the authors’ institute on the use of bovine BMP in the healing of critical-size cranial defects in rats, the adjunction of bovine BMPs to coral alone (c) or coral plus fresh bone marrow (d), Figure 1e –Inset of area marked in Figure 1d. (Reprinted from Biomaterials, 20, 1910-1918, 1999, with permission from Elsevier Science.)

Table I. Clinical application of BMPs in nonunion

<table>
<thead>
<tr>
<th>BMP</th>
<th>Author/s</th>
<th>Number of patients</th>
<th>Bone</th>
<th>Mean length of bone defect (cm)</th>
<th>Period from initial fracture (mth)</th>
<th>Quantity of BMP (mg)</th>
<th>Scaffold</th>
<th>Number of healings achieved with one procedure</th>
<th>Mean time to healing (mth)</th>
</tr>
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<tbody>
<tr>
<td>h-BMP</td>
<td>Johnson et al52</td>
<td>6</td>
<td>Tibia</td>
<td>9.0</td>
<td>31.6 (8.1 to 68.3)</td>
<td>50 to 100</td>
<td>PLA/PGA/gelatin capsules + allograft (4 cases) or autograft (4 cases)</td>
<td>5/6</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>Johnson et al53</td>
<td>4</td>
<td>Distal tibia</td>
<td>2.0</td>
<td>24.8 (14.3 to 35.9)</td>
<td>50 to 100</td>
<td>PLA/gelatin capsules + autograft (1 case)</td>
<td>4/4</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Johnson et al54</td>
<td>25</td>
<td>Humerus</td>
<td>Tibia</td>
<td>Femur</td>
<td>4.0</td>
<td>30 (5 to 83)</td>
<td>100</td>
<td>AAA*</td>
</tr>
<tr>
<td></td>
<td>Johnson and Urist55</td>
<td>15</td>
<td>Femur</td>
<td>3.6</td>
<td>46 (9 to 240)</td>
<td>100</td>
<td>AAA</td>
<td>14/15</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>Johnson and Urist56</td>
<td>30†</td>
<td>Femur</td>
<td>3.3</td>
<td>39 (9 to 60)</td>
<td>100</td>
<td>AAA + autograft (13 cases)</td>
<td>24/30</td>
<td>6.0</td>
</tr>
<tr>
<td>rh-BMP?</td>
<td>Riedel and Valentin-Opran57</td>
<td>15</td>
<td>Tibia</td>
<td>?</td>
<td>27.2 (9 to ?)</td>
<td>?</td>
<td>Collagen</td>
<td>13/15</td>
<td>&lt;9.0</td>
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* allogeneic autolysed antigen free
† 30 procedures performed in 24 shortened nonunions, 4 nonunions of equal length and 2 malunions

Photomicrographs at eight weeks showing the application of BMPs to heal a critical-size cranial defect in rats. Figure 1a – Defects left empty did not heal. Figures 1b to 1d – Defects filled with coral alone (b) had less bone formation and calvarial thickening than those with the adjunction of bovine BMPs to coral alone (c) or coral plus fresh bone marrow (d). Figure 1e – Inset of area marked in Figure 1d. (Reprinted from Biomaterials, 20, 1910-1918, 1999, with permission from Elsevier Science.)

Clinical studies describing the use of BMPs are mainly dedicated to the treatment of nonunion, and segmental bone defects (Table I). Johnson, Urist and Finerman52-54 and Johnson and Urist55,56 described their experience with the use of h-BMP, extracted from human bone, on five occasions. In their latest report, 30 patients were treated with a
composite made of an allograft and h-BMP. When the femoral defect was greater than 2 cm, it was supplemented with bone autograft. Healing was achieved in 24 patients at a mean of six months after implantation of BMP. Two recent preliminary reports showed promising results with the use of recombinant BMPs in orthopaedic surgery. In a prospective trial on the use of rh-BMP2 in the healing of 12 open fractures of the tibia with a Gustilo classification of II or higher, bone healing was achieved in nine without further intervention. In a prospective, randomised evaluation of tibial nonunion in 30 patients treated by reamed nailing with either rh-BMP7 or bone autograft, Cook found no differences in the period of healing between the two groups. No adverse effects were observed. Although very encouraging, these results need to be validated on a larger number of patients. Moreover, the dose of rh-BMP implanted in each trial was excessively high at between 3.4 and 6.8 mg, when compared with the quantity present in bone. This may trigger unpredictable local or systemic reactions. Recent studies have concentrated on delivery systems which achieve a slow release of BMPs and thus reduce the dose implanted.

Several investigators have focused on the use of polyhydroxyester foams as carriers for growth factors. These polymers, specifically polyglycolic and polylactic acids (PGA, PLA), are already in use as resorbable sutures. They are bioreabsorbable and biocompatible, and their porosity can be controlled accurately during processing. Adjunction of polyethylene glycol in PLA/PGA polymers increases the rigidity of the material, allowing the development of new scaffolds which have the shape of human bones and joints. Degradation of the polymer, however, generates acid monomers which may be significant in their use in clinical practice. Consequently, the optimum delivery system for growth factors is as yet unknown and is still a subject of debate. One alternative is to use gene-delivery systems which maintain high local concentrations of growth factors at the site of the fracture. A possible method consists of transducing bone-marrow cells with a gene encoding for a growth factor, and implanting these genetically-modified cells into the site of the fracture using a suitable scaffolding material.

Osteogenic biomaterials. The efficiency of osteoinductive biomaterials relies on the recruitment of osteocOMPETENT cells from the surrounding tissues. Their use is therefore limited to clinical cases in which the bed of the wound can provide these cells. This precludes their use in necrotic areas and large defects of bone. To overcome this problem, the use of osteogenic material composed of a scaffold loaded with osteocOMPETENT cells has been proposed.

A number of investigators have experienced some success using autologous fresh bone marrow to augment bone formation in osteoinductive biomaterials since it contains osteogenic precursor cells. The quantity of newly-formed bone, however, is directly dependent on the number of osteocOMPETENT cells transplanted, prompting the use of techniques capable of selecting and expanding these cells. The preferred source of osteocompetent cells is the bone-marrow stroma from which a heterogeneous population of fibroblasts can be isolated and amplified in vitro while retaining its capacity to contribute to various tissues. These fibroblastic cells are called bone-marrow stromal fibroblasts (BMSFs). They can be induced to differentiate into bone cells when placed in appropriate culture conditions. BMSFs are often inappropriately referred to as mesenchymal stem cells, but they do not have the capacity for self-renewal, a prerequisite for stem cells. Clonal studies have shown the presence of a subpopulation of BMSFs with a capacity for at least tripotential differentiation into bone, cartilage or adipose tissue in man. The ability of BMSFs to form bone has been confirmed in various models in vivo.

Once the expansion of BMSFs is achieved, the next step in the process of building an osteogenic material is to load BMSFs onto a scaffold to which they will adhere, allowing a spatial limitation of bone formation. We are uncertain as to the physicochemical characteristics and mechanical performance of an ideal material, but there is general agreement that the scaffold should favour adhesion, proliferation and differentiation of BMSFs as well as encourage rapid vascular invasion. The new extracellular matrix should take the mechanical function of the template as bone forms, allowing the scaffold to disappear at a rate commensurate with the formation of new bone. A number of carriers have been evaluated for delivering BMSFs including collagen, alginate, polylactic polyglycolic acid polymers or calcium phosphate ceramics. However, the ideal scaffold remains to be found.

The primary clinical use of osteogenic materials is in the repair of large defects of bone. A number of experimental studies have assessed their efficiency in the repair of critical-sized defects of femoral bone in the rat. The bone-regenerating ability of osteogenic implants has always been greater than that of either the scaffold alone or of the empty defects. In this model, however, the size of the defect was only 6 mm. Therefore, to show clinical applicability, the ability of osteogenic materials to repair defects of appropriate size was evaluated in situations of load-bearing in large animals. In a study on segmental bone defects in dogs the use of an osteogenic material made of a synthetic HA-TCP ceramic loaded with BMSFs led to an increase in osteogenesis when compared with the use of a scaffold alone. However, numerous fractures of the ceramic were observed. Another study was carried out at our institute to explore the possibility of using natural coral as a cell-delivery system. This material combines good mechanical properties with a rapid rate of resorption and an open porosity, making it an interesting candidate for the repair of large skeletal defects under predominantly compressive loads. The bone hybrid was assessed in a large segmental model in sheep. The tissue-engineered bone elicited more bone formation than that obtained with the scaffold alone or the scaffold plus fresh bone marrow (Fig. 2). It under-
went morphogenesis leading to complete recorticalisation and the formation of a medullary canal with mature lamellar bone in the most favourable cases. Unfortunately, clinical union was obtained in only three of the seven limbs. These results are encouraging but further studies are needed to obtain optimum results in animal models before moving to clinical trials.

Conclusions

The development of alternative techniques to treat non-union or bone defects offers promising perspectives for a large number of patients. Physicians treating fractures will have an increasing variety of options available, some of which will probably soon supersede harvest of bone autograft. Physical methods are non-invasive and have been shown to offer beneficial effects in the healing of fresh fractures (mechanical loading, ultrasound stimulation), and in the treatment of hypertrophic nonunion and congenital pseudarthroses (electromagnetic fields). These methods may be useful clinically, especially since some have been shown to reduce substantially the costs of treatment.75 More recently, the development of techniques of tissue engineering which aim to construct bone tissues in the laboratory, have shown great promise for the replacement of massive bone defects. These techniques are invasive and expensive, however, and further problems must be overcome before they can be adapted for widespread clinical use. The long-term effects of implanted growth factors must be carefully evaluated, and suitable delivery systems

Fig. 2

Microradiographs and photomicrographs at 16 weeks showing the efficiency of an osteogenic material to heal a critical-size defect in a sheep model. Figures 2a and 2e – There was no bone formation in defects left empty. Figures 2b, 2f, 2c, 2g, 2d and 2h – The tissue-engineered bone (2d, 2h), made of coral seeded with BMSFs, elicited more bone formation than that obtained with coral alone (2b, 2f), or coral plus fresh bone marrow (2c, 2g). (Reprinted from Nat Biotechnol, 18, 959-963, 2000, with permission from Elsevier Science.)
developed to provide the slow release of active molecules which can target specific areas of the construct. The enhancement of fracture healing is not only a major issue of health-care, with potential benefit for millions of patients every year, but is also a potential market for the industry. Further independent research must be conducted to make quantified measurements in vivo, in animal models and in controlled clinical trials undertaken to ascertain the role of these techniques in improving the healing of fractures with identification of the most effective material.

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