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BREAKTHROUGHS AND VIEWS

Bone Tissue Engineering: Hope vs Hype

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The requirement for new bone to replace or restore the function of traumatised, damaged, or lost bone is a major clinical and socioeconomic need. Bone formation strategies, although attractive, have yet to yield functional and mechanically competent bone. Bone tissue engineering has been heralded as the alternative strategy to regenerate bone. In essence, the discipline aims to combine progenitor or mature cells with biocompatible materials or scaffolds, with or without appropriate growth factors, to initiate repair and regeneration. This brief review outlines the concepts, challenges, and limitations in bone tissue engineering and the potential that could improve the quality of life for many as a result of interdisciplinary collaboration.

Key Words: **osteoprogenitor; bone marrow; biodegradable polymer; tissue engineering; scaffold; artificial bone; bone regeneration; stem cell.**

Tissue engineering has been defined as the application of scientific principles to the design, construction, modification and growth of living tissues using biomaterials, cells and factors, alone or in combination (1, 2). A plethora of newspaper headlines have captured the public (and scientific) imagination as to the potential of skeletal tissue engineering for bone regeneration (3). Skeletal tissue engineering requires, essentially, a scaffold conducive to cell attachment and maintenance of cell function, together with a rich source of osteoprogenitor cells in combination with selected osteoinductive growth factors. However, to date, a vascularised mechanically competent osteoconductive/inductive construct remains to be documented. Understanding how cells function and form matrix and the fabrication of materials to provide appropriate scaffolding, conducive to cell attachment and maintenance of cell function, are key concepts.

Tissue loss as a result of injury or disease, in an increasing ageing population, provide reduced quality of life for many at significant socioeconomic cost (4). This is compounded by the observation that, to date, artificial prostheses, which do not integrate with the surrounding normal tissue, are subjected to wear and, eventually, fail. Moreover, although joint replacement surgery in rheumatoid arthritis, osteoarthritis and osteoporosis has significantly improved the quality of life for many, biomaterial failure and incompatibility due to wear or corrosion have resulted in complications including, detachment of the stem coating and/or fracture of the cement–stem or cement–bone interface (reviewed in Spector (5)). At present, regimes that encourage bone formation, which hold the promise of significantly increasing bone density, have yet to become available. This major clinical requirement has stimulated interest in developing new therapies that involve bone regeneration and has led to the hope that bone tissue engineering may provide alternative solutions providing "living" constructs that possess the potential to integrate with the surrounding native tissue.

A common strategy employed for the generation of new tissue, for example cartilage, involves expansion *in vitro* of cells isolated from a biopsy, commonly using bioreactor technology and appropriate 3-D scaffolds (Fig. 1) (6). A permeable membrane allows gaseous exchange and the microgravity environment created by the rotation of the bioreactor reduces flow stress on the construct providing an environment for new tissue growth. Other approaches include implanting an un-seeded polymer into the defect, the scaffold is subsequently infiltrated with cells from the surrounding tissue or cells are injected after a few days and/or seeding the scaffold with cells and implanting this directly into the patient, using the body as a natural bioreactor (7).

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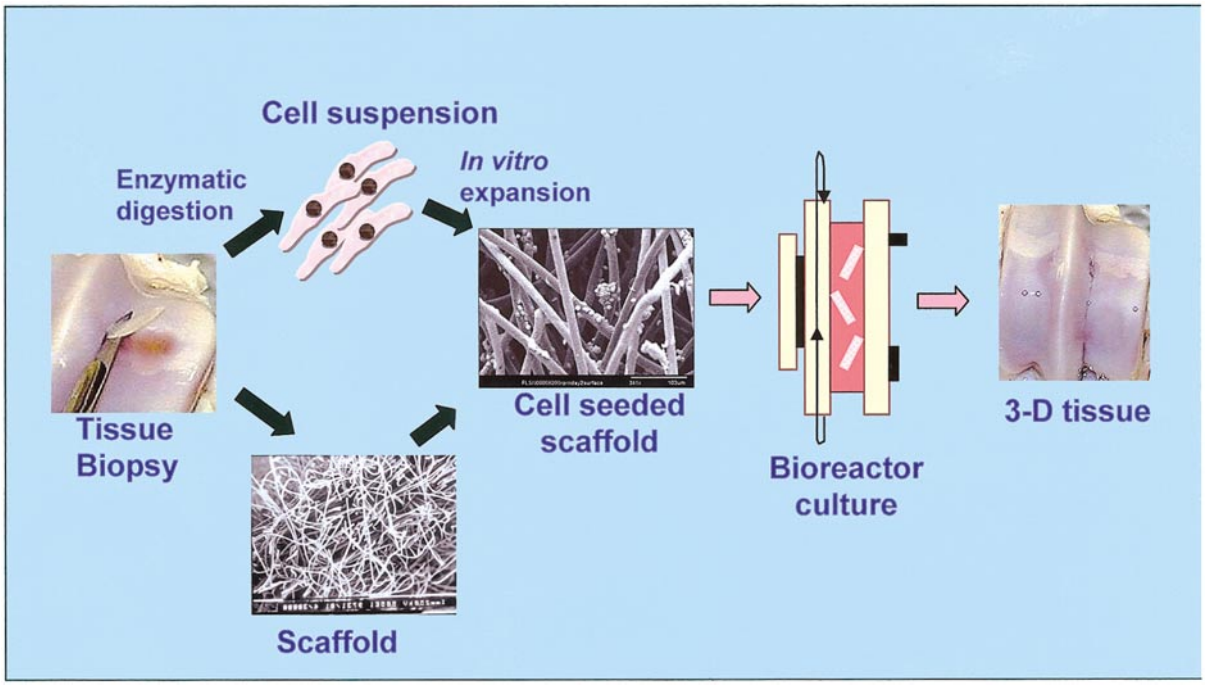

FIG. 1. Tissue engineering strategy.

CLINICAL NEED

The socioeconomic consequences in treating patients with bone fractures is a major concern, with health care in the United Kingdom alone set to cost over £900 million each year (8, 9). With an increasing ageing population, these health costs are set to rise. Each year in the United Kingdom there are some 150,000 fractures (wrist, vertebral and hip) due to osteoporosis. In particular, hip fracture is associated with high morbidity and mortality with fewer than half the patients returning home after surgery (9). Moreover, 30–50% of these hip operations will require subsequent revision surgery and in a significant proportion, bone augmentation will be necessary. The lack of techniques and approaches in reconstructive surgery emphasise the number of clinical applications that would benefit from tissue engineered bone (10). The need for better fillers in large defect reconstructive surgery, for improved

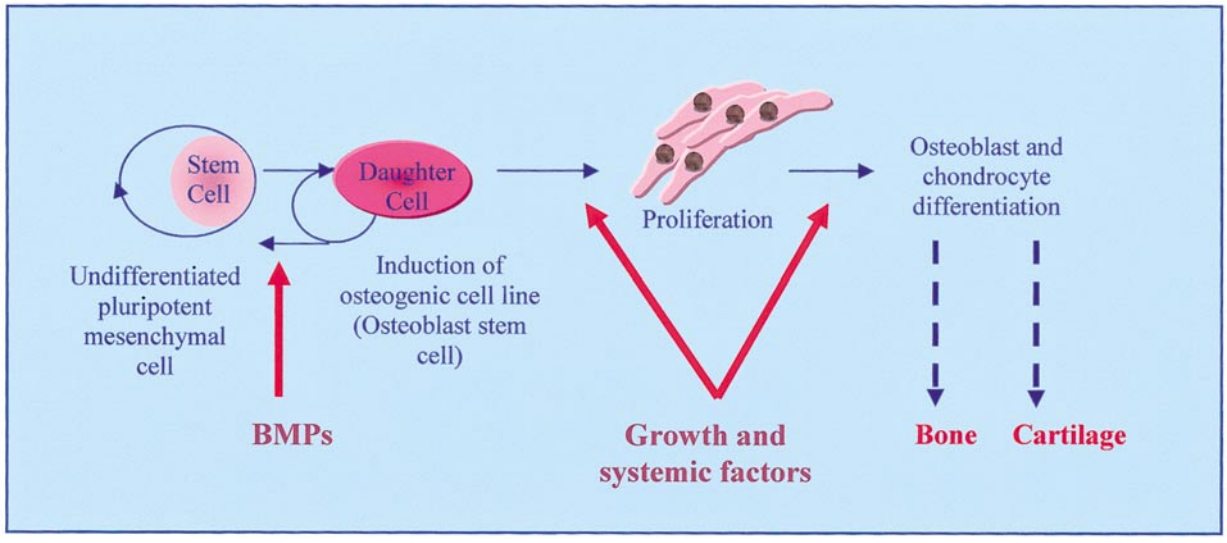

FIG. 2. Differentiation of mesenchymal stem cells into skeletal tissues.

biocompatible orthopaedic implants (for non-union defects, replacement for diseased tissue, and maxillofacial surgery) that can integrate with native tissue, and prosthesis coatings (hip-implants), is evident (11, 12). Current therapies include autografting and allografting cancellous bone, applying vascularised grafts of the fibula and iliac crest, and other bone transport methods (13). However, although commonplace in orthopaedic surgery, these treatments have a number of limitations. Harvesting autografts, typically from the iliac crest, is expensive, constrained by anatomical limitations and associated with donor-site morbidity due to infection and haematoma. Allografts are limited by the potential risks of introducing infection or disease while vascularised grafts are prohibitively expensive (14, 15).

In essence, three elements are central in tissue engineering: (i) stem or precursor cells, (ii) an appropriate biological scaffold and, (iii) growth factors. The developments and limitations of each of these areas will be addressed in turn and their impact within bone tissue engineering strategies reviewed.

STEM CELLS AND GENE THERAPY

It has long been known that bone has a vast capacity for regeneration from cells with stem cell characteristics. These multipotential stromal stem cells located within the bone marrow can differentiate into fibroblastic, osteogenic, adipogenic and reticular cells (16–18). Furthermore, these stem cells generate progenitors committed to one or more cell lines with an apparent degree of plasticity or interconversion (19, 20). Culture expanded bone marrow cells can heal a segmental bone defect following reimplantation (21) and can give rise to osteogenic tissue within diffusion chambers in a variety of animal species (22–25). In addition, human bone marrow osteoprogenitors can be isolated and enriched using selective markers, such as STRO-1, from a CD34+ fraction (26, 27). These cells can be readily expanded, indicating their potential for marrow repopulation (18, 28–30). The lack of immunogenicity of mesenchymal stem cells has opened up the potential of these cells in cartilage and bone repair. Although true engraftment of human mesenchymal stem cells, long-term biological effects on the stem cells at the implant site as well as issues of cell plasticity remain unknown. Notwithstanding these caveats, (31) have shown, in preliminary studies, the therapeutic effects of human bone marrow derived osteoprogenitors transplanted into children with osteogenesis imperfecta while clinical studies by (32) and recently by Quarto and co-workers (33) illustrate the potential for autologous bone marrow stromal cells (with a porous bioceramic scaffold) in the treatment of large bone defects. However, mesenchymal stem cells alone are unlikely to be sufficient for bone regeneration. Although marrow injections are simple and provide a reduced risk of morbidity, for large skeletal defects, a scaffold of appropriate, shape, size and mechanical competence is required.

Developments in gene technology offer the possibility of genetic modification of isolated and expanded cells to produce populations of progenitor cells overexpressing selected signaling molecules. Lieberman and co-workers have shown regional cell and gene therapy using BMP-2 producing bone marrow cells on the repair of segmental bone defects in rats (34). Similarly, Breitbart and colleagues (35) have cultured periosteal cells retrovirally transduced with BMP-7 in a PGA scaffold in a critical sized calvarial defect model in rabbits. More recently other groups have indicated the potential to generate human bone marrow stromal cells expressing BMP-2 by adenoviral infection (36–38). To eliminate the problems associated with delivery of BMP-2 to the required site and the need for cell expansion, Musgrave and co-workers (39) reported on the use of direct adenoviral mediated gene therapy to deliver active BMP-2 and produce bone in skeletal muscle. Although the use of adenovirus vectors for gene therapy derives from their ability to transfect a variety of cell types including nondividing cells (40), this approach has a number of limitations not least issues of safety (immunogenicity *in vivo,* fate of adenoviral cells and long-term safety and requirement for cell expansion in culture prior to viral infection and reimplantation). The challenge will be to demonstrate bone repair in clinical models and subsequent absence of any immunological reaction. Irrespective of the cell population/delivery vehicle, central to the formation of new bone tissue is a scaffold to provide support and effective delivery.

SCAFFOLDS

The past 30 years has seen a staggering array of biomaterials proposed as “ideal” scaffolds for cell growth yet few have reached clinical efficacy. Biomaterials, either permanent or biodegradable, naturally occurring or synthetic, need to be biocompatible, ideally osteoinductive, osteoconductive, integrative and mechanically compatible with native bone to fulfil their desired role in bone tissue engineering. These materials provide cell anchorage sites, mechanical stability, and structural guidance and within an *in vivo* milieu, provide the interface to respond to physiological and biological changes and to remodel the extracellular matrix in order to integrate with the surrounding native tissue (4).

Appropriate matrices for the delivery of stem cells, which stimulate differentiation and bone conduction, have included hydroxyapatite and calcium phosphate and a range of ceramic biomaterials. However, hydroxyapatite and calcium phosphate are not them-

selves osteoinductive and are resorbed relatively slowly. Moreover, there are problems associated with biodegradability, inflammatory and immunologic reactions and in disease transmission when used as carriers for osteoinductive factors (reviewed in Oreffo and Triffitt (41–43). To circumvent these limitations, natural or synthetic materials and biodegradable composite scaffolds based on poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their co-polymer poly(lactic-co-glycolic acid) (PLGA) have been developed (44–46). These polymers are currently used for a number of orthopaedic devices, including suture anchors and interference screws (47). They have the advantage of FDA approval, can be chemically modified and their degradation rates can be controlled. Biodegradable scaffolds provide the initial structure and stability for tissue formation but degrade as the tissue forms, providing room for matrix deposition and tissue growth (15). They can be readily processed into three-dimensional porous structures, with desired pore morphological features, which fit the defect prior to surgery. They can be used either alone, in combination with growth factors (as discussed below), or with other osteoconductive materials, such as hydroxyapatite (48) and tricalcium phosphate (TCP) (reviewed in (47, 49, 50)). Despite these attractive properties, these polymers have one major disadvantage, not often addressed, and that is their lack of mechanical competence. Other biodegradable materials for bone tissue engineering include DegraPol-foam (α,ω-dihydroxy-oligo[(-3-hydroxybuterate-co-3-hydroxyvalerate)-block-ethylene glycol]-co-polymer) (51) and Polyactive, a polyethylene oxide-co-polybutylene terephthalate co-polymer (52). Both these polymers have been shown to support bone cell adhesion and proliferation. Surface eroding polymers (for example, poly(*ortho*-esters) may be advantageous in load-bearing bone applications as only the surface of these materials degrades, leaving the bulk of the material to provide mechanical strength; reducing the risk of implant failure (53).

Alternate scaffold strategies include the use of natural scaffolds based on the rationale that animal skeletons have been designed, through optimisation by natural selection, to physically support and physiologically maintain diverse tissue types. Biomimetic materials chemistry has sought to reproduce, in part, the complex structures that occur in nature, such as coral, nacre and calcite shells and spines of sea urchins (54–57), in purely synthetic systems and thus generate accurate and specifiable biomaterials. Generation of multipurpose hierarchical biomimetic scaffolds may aid cell, gene and growth factor delivery for bone regeneration (see below). While an attractive proposition, biomimetic scaffolds are a long way from clinical evaluation and little is known of the immunoreactivity, biocompatibility and effects on stem cell fate and host osseointegration of such constructs.

BIOMIMETIC MATERIALS AND SMART MATERIALS

The failure to identify either a single material or growth factor as the panacea for bone regeneration or indeed a biological scaffold that will promote integration and, most importantly, significant vascularisation has led to an increased interest in optimising biomaterials to promote specific cell–biomaterial interactions. For example, Arg-Gly-Asp (RGD) sequence peptides (involved in integrin-mediated cell adhesion) can be incorporated onto the scaffold surface to enhance cell adhesion and spreading (58). Yang *et al.* (59) have demonstrated the potential to promote human osteoprogenitor differentiation on RGD-coupled biodegradeable scaffolds. More recently drug delivery techniques such as entrapment within a hydrogel matrix allowing growth factors to be released in a controlled fashion from the scaffold to aid the regenerating tissue have been applied (60–63). Such approaches are appealing as they avoid the use of solvents, and high temperatures (and therefore protein degradation) and subsequent release of the growth factor is controlled, in response to environmental stimuli. This strategy has been employed in bone tissue engineering, where rhBMP-2 (64) basic fibroblast growth factor (62) and vascular endothelial growth factor (63) have all been successfully incorporated into a hydrogel prior to *in vivo* implantation.

Supercritical fluid technology has evolved as a promising approach in the development of porous biodegradable scaffolds for tissue engineering (65). The absence of solvents and thermal processing makes this an attractive approach to growth factor incorporation and Howdle and colleagues have demonstrated high protein (ribonuclease) loading into foamed PLA scaffolds which retained full activity on subsequent release from the PLA over 3 months (60, 66). This technology could provide a simple one-step process to the difficulties of incorporating growth factors and/or guest particles (such as hydroxyapatite—this would resolve the mechanical competence issue detailed above) into a controlled release delivery system. The challenge in tissue engineering will be the design of suitable scaffolds that incorporate and release, as appropriate, combinations of signaling molecules to promote vascularisation, osteoinduction with minimal inflammation at the construct site.

GROWTH FACTORS

Growth factors are cytokines that are secreted by many cell types and function as signaling molecules. They promote and/or prevent cell adhesion, proliferation, migration and differentiation by up-regulating or down-regulating the synthesis of proteins, growth factors and receptors. These molecules are essential for tissue formation and, vide infra, play an important role

in tissue engineering. In concert with osteoprogenitor and osteoblast populations, a plethora of growth factors have been implicated in osteogenesis. Major players in the skeletal tissue engineering are members of the TGFb superfamily notably the bone morphogenetic proteins (BMPs) (Fig. 2).

Since the cloning of the BMPs in 1988 by Wozney *et al.* (67), over 30 of these molecules have been identified and have promised much clinical efficacy as therapeutic molecules for bone formation, through recruitment, commitment and differentiation of bone progenitors (68–70). Their mechanism of action in cell signaling has been reviewed (70, 71).

Despite the commercial availability of recombinant human BMPs for over a decade, the efficacious (and cost-effective) cocktail for bone induction and regeneration in clinical practice remains unclear. Success has been hampered, in large part, by the failure to identifying a suitable carrier for these proteins and consequent failure in growth factor delivery, dosage and maintenance of biological activity. Thus, to date, only three clinical trials have been reported and, in each case, super physiological dosing was required to achieve efficacy. Given the unequivocal evidence for a role of BMPs in bone development there has been substantial interest in incorporating this cytokine into tissue engineering scaffolds and delivery systems (72–74). Examples include the use of porous PLGA with high molecular weight hyaluronic acid (the latter functioning as the BMP carrier) for rhBMP-2 delivery (75) and encapsulation of rhBMP-2 in poly(DL-lactide-co-glycolide). Implantation of such constructs into long bone defects promoted cortical bone formation *in vivo* (76). Another popular strategy has involved the use of collagen, the favoured carrier for BMPs, to generate composites, such as hydroxyapatite/collagen or PLA/collagen with rhBMP-2 for tissue engineering and efficacy *in vivo* has been demonstrated using both composites (64, 73). The challenge will be to identify the optimal mix of BMPs, dosage, release dynamics and matrix carrier, which will result in the long awaited therapeutic efficacy of these agents.

CLINICAL APPLICATIONS AND FUTURE PERSPECTIVES

Bone tissue engineering could provide suitable, efficacious alternative therapies for orthopaedic applications and is attractive on a number of fronts: (i) the ability to engineer tissue *in vitro* for transplantation would reduce the requirement for donor tissue as the number of skeletal cells required could be expanded in the laboratory prior to implantation; (ii) using biomaterials with similar mechanical properties to bone that could integrate with the surrounding native tissue has the potential to decrease the rate of implant failure and the need for revision surgery and; (iii) treatment of diseased tissue at an early stage with mesenchymal stem cells could alleviate or even cure the disease, reducing the need for life-long treatment and improving the quality of life of the patient. Clinical applications include for the augmentation of bone stock, in maxillo-facial surgery as well fracture and non-union fractures. However, it is apparent, that a single approach is unlikely to solve many of the bone tissue requirements and refined approaches targeted to a particular application site/problem will be required.

SUMMARY

The requirement for strategies for bone regeneration in an increasing ageing population is self-evident. Tissue engineering offers a number of possible approaches to the generation of "living" prosthesis that could integrate with host tissue reducing the need for further surgery or possible implant failure. Thus cell delivery vehicles, composite tissue engineering protocols and smart materials which can orchestrate the bone formation cascade will all undoubtedly have their place. However, it is clear many strategies are unlikely to reach clinical evaluation for the most basic of structural, biological or safety issues and that this discipline is still in its infancy. Paramount in each case will be the need to reintroduce an appropriate angiogenic response within the construct from, and integrated, with the host tissue. The next few years will undoubedly see dramatic technical innovations with corresponding media and market hyperbole. However, scientific rigour and clinical excellence should ensure that tissue engineering may, ultimately, improve the quality of life for many as a result of interdisciplinary collaboration.

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