

Invited Review Paper
Osteobiology

Biological and biophysical principles in extracorporeal bone tissue engineering Part II

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Abstract. The aim of this review is to characterise the biological and biophysical background of *in vitro* bone tissue engineering. The paper focuses on basic principles in extracorporeal engineering of bone-like tissues, considering parameters such as scaffold design, tissue construction, bioreactors, and cell stimulation *in vivo* and *in vitro*.

Scaffolds have a key function concerning cellular invasion and bone formation. The intra-architectural scaffold geometry, as well as the scaffold material, play an important role in the process of bone regeneration. Various types of bioreactors have been tested for their utility in bone substitute fabrication that is clinically effective and reproducible. Sophisticated bioreactor systems are those that mimic the three-dimensional morphology and the mechanical situation of bones. Mechanical stimulation as well as other biophysical stimuli appear to be critical factors for proliferation and differentiation of bone cells and for bone mineral and structure formation. Furthermore an enhancement of bone regeneration by application of chemical stimulation factors is discussed.

Key words: bone tissue engineering; scaffold design; biophysical stimulation; biochemical stimulation; bioreactor.

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Bone tissue engineering requires at least living osteoprogenitor cells or osteoblast-like cells in combination with suitable scaffolds⁷². In the previous review, literature on cells and cellular interactions were analysed and their potential for tissue engineering was discussed⁵³. Part II focuses on basic principles of scaffold design and bioreactor use as well as on cell stimulation *in vitro*.

Most bone lesions, such as fractures or small size defects, heal well with conventional therapy due to the high regeneration potential of bone⁷⁵. However, a bone graft is often required in maxillofacial surgery to assist healing of large

traumatic or post-surgical defects⁷⁷. The development of *ex vivo* bone grafts through tissue engineering approaches is directly related to changes in scaffold and bioreactor technologies. While the inclusion of materials requirements is standard in the design process of engineered bone substitutes, it also seems critical to incorporate biological and biophysical stimulation methods to engineer a clinically relevant cellular bone substitute. Several devices have been developed as scaffolds/matrices and/or bioactive factors delivery systems and have been tested for tissue engineering. The use of biomaterials to fabricate

three-dimensional scaffolds is a prerequisite to fill bone lesions^{23,32}. A critical analysis of the factors affecting scaffold structure and function is one step in creating an “optimized” bone substitute material. The ideal scaffold should be non-toxic, biocompatible, biodegradable and have an individually structured intra- and extrageometry²⁶. A proper scaffold should easily integrate with the adjacent tissue and favour new bone ingrowth, i.e. osteoconduction. An important aspect of extracorporeal bone tissue engineering is the physical and biological environment in which the tissue is produced. Bioreactors, cytokines or biophysical forces are

parameters that are directly involved in the tissue construction process. Bioreactors could improve the formation of bone by providing an efficient cell seeding in three-dimensional scaffolds, a cell migration within the scaffold and by allowing better diffusion of nutrients to the cells in the scaffold²¹. Tissue bioreactors allow the application of specific physical and chemical stimuli which can improve bone tissue growth and maturation²⁹. Following a paradigm similar to that used to solve an engineering design problem, the recent phase associated with successful tissue engineering is focused on characterising the specific design attributes and technology that will serve as the backbone of the construction under consideration. It therefore seems valuable to consider the design principles and targeted outcome as a function of single parameters.

Scaffolds

The naturally occurring bone tissue "scaffold" contains a considerable amount of non-living material such as organic minerals as well as various proteins of the extracellular matrix. The composition of matrix components (mainly collagen type I) determines the mechanical properties of bone, in particular the high stiffness and tensile strength of the tissue. For use in tissue engineering, scaffolds are commonly fabricated from bulk artificial or natural materials. Design and prototyping of scaffolds can be done individually on the basis of digital data formats^{42,71}. The material itself can be processed as sponge-like sheets, gels, or highly complex structures with intricate pores and channels²³. Bone scaffolds, like virtually all other scaffolds used in tissue engineering, are intended to degrade slowly and be replaced by new bone following transplantation.

The bone scaffold engineered should, in principle, resemble the morphology of the bone to be replaced. The internal architecture should allow placing, orientation, spacing and maintenance of osteoblasts and other cells, as well as their synthesised products in the construct. By various techniques it is now possible to fabricate a biocompatible three-dimensional internal architectural structure with a desired material surface topography, pore size, channel direction and trabecular orientation⁴³.

The intra-architectural scaffold geometry has a major impact on new bone tissue formation. Coralline hydroxyapatite scaffolds, for example, with a pore

size of 500 µm were shown to allow the ingrowth of osteoblasts and small vessels, while bone formation and cellular invasion is hindered in scaffolds of 200-µm pore diameter⁴¹. Utilising macroporous biphasic calcium phosphate ceramics, GAULTHER et al. showed that a pore size of 500-µm better supported bone formation compared to 300-µm pore size¹⁹. The findings of TSURUGA et al.⁸⁴ and KUBOKI et al.⁴⁰ indicate that a scaffold geometry, which restricts vascular invasion, preferentially produces cartilage instead of bone, while geometries accommodating a Haversian system favour bone formation. RIPAMONTI et al. demonstrated that pore sizes of 150 µm do not support neovascularization⁷⁰. Altogether, these studies underline the fundamental effect of scaffold pore size on bone regeneration and vascular ingrowth.

An ideal bone scaffold material should be biodegradable, have degradation products that are non-toxic, support cell attachment, and can be remodelled by the local cells³⁵. The scaffold should be a good substrate for extracellular bone matrix (ECM) enzymes to modify and degrade it at rates that are clinically desirable. Furthermore, the scaffold should be degraded without lowering the physiological pH. This is especially of concern when synthetic polymers are used. The addition of carbonate was shown to be one way to stabilise the physiological pH in the vicinity of the implants. The substitute must be fully hydrated to keep the environment isotonic. The scaffold must allow cell motility and ingrowth of angiogenic elements, exhibit a low level of immunogenicity, and should be capable of being surgically fixated, if necessary, with screws or sutures.

When implanted in the body, scaffolds will have an influence on the bony implantation bed. Vice versa, the body acts on the implanted scaffold by cellular and non-cellular actions. As the scaffold interacts with its environment *in vitro* and *in vivo*, approaches were made to define more precisely scaffold properties. Based on the structural pre-requisites for bone tissue constructs, one major aspect in scaffold fabrication is to maintain a high level of accurate control over the three-dimensional macro- and microstructural properties. Despite the existence of a variety of conventional manual-based fabrication techniques, available for scaffold production⁹⁰, most of them could not meet the requirements for a control of the desired scaffold properties. Conventional fabrication

techniques of scaffolds, applied to the engineering of bone tissue^{30,82}, were limited by a low control over scaffold structure and properties, which restrict their promise in bone tissue engineering. The main limitations of conventional techniques are the manual intervention, accomplished with an inconsistent and inflexible processing procedures, the use of toxic organic solvents, the use of porogens, and the shape inaccuracy⁹⁶. The introduction of computer-based solid free form (SFF) fabrication technologies has improved scaffold design and manufacturing. The improved manufacturing capabilities of SFF have been successfully employed for bone replacement therapies¹⁴. Although the application of SFF for scaffold fabrication has not been used in clinical bone tissue engineering studies, its potential for producing scaffolds with highly complex macro- and microstructures is widely recognised. The advantages derivable with SFF include a customised external shape, computer-controlled fabrication, defined scaffold microstructures, and the processing conditions. At present, only a small number of SFF techniques have been exploited for bone tissue scaffold fabrication. A precise control of the external and internal structure is important in order to fabricate scaffolds with more defined and predictable scaffold/osteoblast interactions in the *in vitro* and *in vivo* environment⁴⁴.

Up to the present, four types of bone substitutes have been experimentally and/or clinically studied as a scaffold material for applications in tissue engineering: (A) various groups of synthetic organic materials including (i) biodegradable and bioresorbable polymers which have been used for clinically established products, such as polyglycolide, optically active and racemic polylactides, polydioxanone, and polycaprolactone; (ii) polymers which are currently under clinical investigation, such as polyorthoester, polyanhydrides, and polyhydroxyalkanoate; and (iii) entrepreneurial polymeric biomaterials, such as poly(lactic acid-co-lysine); (B) synthetic inorganic materials (e.g. hydroxyapatite, calcium/phosphate composites, glass ceramics); (C) organic materials of natural origin (e.g. collagen, fibrin, hyaluronic acid); and (D) inorganic material of natural origin (e.g. coralline hydroxyapatite^{9,48}). Most of these materials have promising properties in tissue engineering, but emphasis should be placed on the fact that slowly degradable materials may impair the dynamic bone remodelling.

Tissue construction

Technical problems in engineering tissue substitutes can be best appreciated by considering the cellular assembly of native tissues and organs. Although the boundary between a tissue and an organ is not precisely defined, an organ exhibits more complex functions and is usually composed of several tissues (e.g. the skeleton is composed of bone, cartilage and bone marrow). Tissue, as a lower level of body composition is an assembly of one or more types of cells and their associated intercellular material. This material is produced by local cells to fill in the space between them in a geometrically organised manner; for instance bone is composed of a mineralised extracellular matrix with interspersed bone cells. The structure and extent of the mineralised bone matrix has a great impact on the mechanical properties of bone tissue. Bone at this level is rather unusual in having very few other cells either in type or in number and therefore, bone tissue engineering should be easier to conduct than the engineering of other tissues.

Programmed cell death should be considered as an important process in the remodelling of bony tissues. Apoptosis, an induced and programmed cell death, is a phenomenon that is important for bone development and tissue homeostasis. In the last decade, many of the essential molecules and pathways that control the apoptotic process have been elucidated. Because apoptosis is involved in physiologic and pathologic bone processes, the understanding of its regulation has a significant impact in tissue engineering¹⁸. Bone cells are sensitive to a variety of extracellular signals inducing apoptotic cell death. Pro-apoptotic mediators include decreasing oxygen concentration, loss of cell adhesion to the matrix, and enhanced mechanical tension⁵⁴. *In vivo* tissue engineering studies have seldom been evaluated in the context of cell death. Most studies on apoptosis have been made under *in vitro* conditions in stem cell research. It is known, that bone cells derived from mesenchymal precursors need to be non-immunogenic, easy to harvest, and expanded to form a significant cell count¹⁷. Whereas difficulties in expanding and maintaining precursor cells represents one of the limiting factors in the scale-up of cellular constructs in the *in vitro* environment¹³, inhibition of apoptosis strategies such as through overexpression of Bcl-2 may be one way to overcome this problem through

reducing cell turnover and lengthening the cell cycle^{12,50}. Other stimuli inhibiting apoptosis have been identified, such as the hormone estrogen, firm adhesion to the cellular matrix, and formation of stable cell-cell contacts. By the evaluation of the exact pathway of apoptosis that restricts extracorporeal cell population growth, tailored anti-cell death manipulation may be formulated to overcome this problem. Such cell survival strategies may allow biologists and clinicians to use bone cells taken from easily accessible areas to improve the bioengineered bone tissue *in vitro* as well as in the *in vivo* situation⁸⁵.

As animal experimental and clinical tissue engineering studies have seldom investigated apoptotic processes, clinical studies from other scientific areas can therefore be assessed for their relevance to apoptosis. With the understanding of how programmed cell death is controlled⁶⁹ *in vivo*, combined with the improved ability to effectively influence the process of apoptosis, apoptosis is gaining clinical relevance in transplantation and tissue engineering approaches^{36,49}. Until recently, there have been only a few studies dealing with programmed cell death in tissue engineering^{17,54}, but there is now a growing interest in the scientific community to better understand apoptosis as it relates to their clinical practices⁶².

Critical steps for engineering "bone" *in vitro* must take into account the spatial complexity, cellular heterogeneity and the scale-up for clinical use. The correct molecular to macroscopic architecture of bone is essential for a proper clinical function. Recent research indicates that bone cells grown on three-dimensional scaffolds, although secreting sufficient amounts of extracellular matrix molecules, still fail to acquire a complex bone architecture due to an impaired nutrition of the transplanted construct. A scale-up of the bone tissue vascular supply is of major importance for clinical application. One approach to overcome this serious and currently still not definitively solved problem is the development of cocultures of bone cells (osteoblastic and osteoclastic) and vascular cells³⁷. The creation of tissues containing self-assembled hierarchical cell-cell interactions will help to bring such approaches closer to clinical use.

Bioreactors

A recent challenge to bone tissue engineering is to lift research-scale products

up to a level of reproducible bone substitute fabrication that is clinically effective. Various types of bioreactors have been tested for their utility in bone tissue engineering. Most of the bioreactors were initially developed to test biomaterials⁵², but some of them were also constructed in order to allow extracorporeal bone tissue fabrication. The simplest and most widely used bioreactor for bone tissue engineering today is the culture dish. It provides an environment that is easy to handle and economical to manufacture. Although culture dishes and flasks are certainly the most commonly used bioreactors today⁹¹, they are of limited value when a three-dimensional bone construct has to be fabricated.

The cultivation of cell monolayers in culture dishes to multiply the initial cell number has various advantages. Cells in monolayer culture are not generally nutrient-limited. Passive diffusion is more than adequate to supply the osteoblast layers. The supply of oxygen and soluble nutrients becomes critical when the diffusion distance becomes wider than 100–200 μm . The diffusion can be, in part, improved by stirring the culture medium. The transport of the various cell metabolites, waste products, and other macromolecules within a cell-seeded matrix results, primarily, from diffusion generated by the existence of concentration gradients in culture dishes²⁴. The primary mechanism allowing transport of nutrients to the centre of three-dimensional scaffolds cultured in petri dishes is diffusion, which cannot meet the significant metabolic requirements of bone cells seeded on larger scaffolds, especially if cultured for longer time periods⁶⁰. Cells placed inside of porous scaffolds are assumed to migrate by chemotactic mechanisms towards the outer surface of the scaffold construct where nutrient concentration is higher. Different investigations demonstrated a low differentiation state of mesenchymal cells and a low expression of osteogenic marker proteins²¹ under petri dish cultivation conditions in larger cell/scaffold constructs based on an impaired nutrient supply, a condition known to have negative effects on the osteoprogenitor cell proliferation, differentiation, and matrix mineralization⁸⁶.

An improvement on bioreactor design was the spinner flask. Cell growth in a spinner flask provides continuous exposure of the bone cells to various nutrients. Scaffolds are usually positioned in spinner flasks by special devices holding

them in the centre of the flask. Convective forces are generated by a stirrer allowing continuous mixing of the media surrounding the scaffolds. Spinner flasks have been tested and were proven useful for the culture of cell/polymer constructs for cartilage^{87,88}, and bone tissue regeneration²². The improved bone cell behaviour observed in the cell/polymer constructs cultured in the spinner flask bioreactor is based on the enhanced nutrient supply for bone cells. When such bioreactors are connected to ports and filters for gas exchange, they should be regarded as more "opened systems" compared to conventional dishes and flasks. However, the fact that these bioreactor systems require individual manual handling for medium exchange, cell seeding, etc., ultimately limits their usefulness when large cell numbers are required.

Technical innovations in extracorporeal engineering of bone *in vitro* has led to the development of more sophisticated bioreactor systems that mimic the three-dimensional morphology⁹² and the mechanical situation of bones⁹⁴. Bioreactors for mechanically supporting bone tissues have been custom-designed to provide defined deformations with strain amplitudes of up to 0.3%. Rotating wall vessel reactors, originally designed to simulate a microgravity environment, were introduced and assessed for their relevance in bone tissue engineering. Rotating wall vessels have been tested mainly in cartilage⁸⁸ and bone^{5,66} tissue engineering strategies. This type of bioreactor, comprised typically of two concentric cylinders, with the scaffolds placed in the annular space⁷⁶, rotates at a definable and controlled rate. By enhancing centrifugal forces in these bioreactors bone cells can be mechanically stimulated. Recent investigations on bone cell/polymer constructs grown in rotating bioreactors displayed minimal differentiation towards the osteoblastic phenotype. A low alkaline phosphatase activity compared to the static controls, a low extracellular matrix protein synthesis in the media throughout the long-term culture period, as well as a low calcium deposition, was found. The disappointing findings contradict earlier reports on an improvement of the behaviour of osteoblastic cells in rotating wall vessels^{5,66}. The underlying causes for the low inductive properties of rotating wall vessel bioreactors in bone tissue formation are not entirely known, but unphysiological forces may be responsible for the experimental findings.

It is known that under conditions of physiologic periodical strains, the mechanical properties of engineered bone-like tissues appear to improve significantly. In addition, the synthesis of bone-specific matrix proteins and collagen, both being components of the secreted bone cell environment, is enhanced by dynamic loading. The improved bone cell behaviour in spinner flasks may therefore be based on the existence of forces in the spinner flask exposing the cells to physiological strains. The sensitivity of osteoblasts to fluid shear stress is well established for cell cultures within flow chambers^{29,33,38,51,78}. The assumed mechanisms for the stimulation of osteoblasts by mechanical forces and electrical potentials generated through fluid shear stress include the activation of growth factor signalling pathways⁶³, cytoskeleton-integrin interactions⁸, and the formation of stress fibres and their attachment to focal adhesion points^{64,83}. All bioreactor systems that expose the cell/polymer constructs to physiological stimuli may lead to an enhanced differentiation of precursor cells towards the osteoblastic phenotype. Engineered bone tissue provides a good example that mimicking the native mechanical environment of cells can be beneficial for reproduction of tissues^{56,57}.

Closed bioreactor systems offer major advantages over open systems for manufacturing, since sterility can be assured and viability of the tissue product maintained⁸⁸. This approach has been used successfully in the manufacture of bone tissue-engineered products. The parameters that modulate growth in complex bioreactors include temperature, culture medium, biochemical and mechanical stimuli, fluid flow, and perfusion. Each of these factors can have a dramatic impact on the growth of the bone tissue substitute, and when controlled can be used as major positive modulators⁹¹. Providing the three-dimensional bone tissue with nutrients is of major importance and in complex systems nutrients are often actively delivered by direct perfusion. Neovascularisation may be reached by the use of a coculture system of bone cells and endothelial cells, however, since this approach introduces a new level of complexity, the technical challenges are significant.

Biophysical stimulation

Bones are continuously subjected to mechanical forces imposed by muscular

contractions, body movement and various other external loadings. From a variety of studies it is clear that externally applied mechanical forces elicit effects on osteoblast proliferation, cell orientation, gene activity and other features of cell activity¹⁶. Thus it is inherently obvious that mechanical forces applied from outside or organised from within the tissue render building tissues useful⁵⁹. BROWN et al. embedded cells in collagen gels that were subjected to cyclical tensional forces and found that cell orientation and gene activity were altered by cyclical mechanical loading. The most effective frequency of this loading is around 1 Hz^{6,7,57}.

Osteoblasts can sense small deformations that arise on the surface of the materials as a result of mechanical loading³⁴. It should be noted that the physiological strain environment of most bone cells is much lower than that of other types of cells and that bone cells are correspondingly far more sensitive to mechanical deformations than most other cell types⁵⁶. Obviously, load transfer through the scaffold and the substrate surface to osteoblasts induces surface strains⁵⁸ that have profound effects on cell behaviour. Deformations at the material/cell interface are sensed by osteoblasts through their attachment sites.

Most studies indicate that mechanical stress stimulates the proliferation of osteoblasts^{7,61}. The optimal tensional force *in vivo* and *in vitro* was found to be in the range of 1000–3000 μm strain^{56,57}. Mechanical stimulation was shown to result in an altered expression of bone-specific proteins, such as alkaline phosphatase, osteopontin, and osteocalcin. Whereas osteopontin synthesis is generally increased by mechanical stimulation⁵⁵, the effects on alkaline phosphatase, osteocalcin, or collagen expression vary depending on the techniques used for loading^{27,29}. The mechanisms whereby mechanical stimulation leads to proliferation and expression of bone-specific proteins are not entirely known³⁵. The application of micro-movements in extracorporeal tissue chambers can be considered to be a promising approach in tissue engineering.

Based on the discovery of piezoelectric potentials in bone tissue in the late sixties, it was assumed that osteoblast physiology is influenced by electrical fields²⁸. Many experiments have suggested that indeed electrical fields modify the behaviour of bone cells, but the exact molecular mechanisms involved have not been elucidated, yet. Recently,

it has been demonstrated that mineral formation in cultured osteoblasts is enhanced when cells are exposed to an electrical field. Long-term electrical stimulation of osteoblasts appears to alter the pattern of gene expression resulting in enhanced extracellular matrix synthesis which promotes bone tissue formation⁹³. In this respect the application of electrical fields in bioreactors seems to be a promising approach in extracorporeal tissue engineering. Besides external biophysical stimulation as one way to promote tissue formation, a different approach is the development of "mechano-active" scaffolds with optimised inherent physical properties⁹⁷. In summary, biophysical stimulation may be useful in tissue engineering in order to adapt a mature extracorporeal bone tissue.

Biochemical stimulation

Bone formation can be enhanced through the action of several cytokines and bioactive proteins (for review see SCHLIEPHAKE⁷³). Therefore, the addition of such molecules in bone tissue engineering may be beneficial⁴⁶. The release kinetics of different growth factors vary depending on their chemistry and the delivery system used. Selection of an appropriate carrier or delivery system has to take into account: (1) the ability of the system to deliver the growth factor at an appropriate rate and in the proper dose; and (2) the presence of a substratum that will enhance cell recruitment, attachment, and potentiate chemotaxis. Different carrier and delivery systems, including type-I collagen, synthetic polymers, and hyaluronic acid gels, have been used to deliver recombinant proteins in experimental and clinical models^{4,20,67,68,98}.

Numerous active molecules with different biological functions are expressed during bone formation. Some of these growth factors may serve as potential therapeutic agents to enhance the repair of bone also in tissue engineering^{73,81}. Among these growth factors are transforming growth factor-beta (TGF- β), bone morphogenic proteins (BMP), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and insulin-like growth factor (IGF). Transforming growth factor-beta belongs to a family of related proteins called the TGF- β superfamily which exhibits a broad range of cellular activities including growth, differentiation, and extracellular matrix synthesis. Bone morphogenic

proteins are also members of the TGF- β superfamily, and 13 individual molecules have been identified at this time⁷⁴. Currently, BMP-2, 4, and 7 are known to play a critical role in bone healing by stimulating the differentiation of mesenchymal cells to an osteoblastic lineage^{45,74}. In 1988, WOZNEY et al. identified the genetic sequence of bone morphogenetic protein, which led to the identification of its various isoforms⁹⁵. With this genetic information, it is now possible to produce various BMPs for use in recombinant gene technology. These proteins attached to various artificial scaffold materials formed the basis for therapeutic applications. Crosslinked gelatin hydrogels as well as collagens have been used to deliver rhBMP-2 to rabbit cranial defects in order to enhance bone formation³¹. Critical sized defects in rabbits treated with a scaffold of polylactide delivering rhBMP-2, demonstrated greater radiopacity as well as improved biomechanics as compared to untreated controls⁸⁹.

The fibroblast growth factors (FGFs) comprise a family of structurally related polypeptides that are characterised by their affinity for the glycosaminoglycan heparin-binding sites on cells. They are known to induce angiogenesis and mesenchymal cell mitogenesis^{15,47}. The most abundant FGFs in normal adult tissue are acidic fibroblast growth factor (FGF-1 or α -FGF) and basic fibroblast growth factor (FGF-2 or β -FGF). Both, FGF-1 and FGF-2 promote growth and differentiation of a variety of cells, including epithelial cells, myocytes, osteoblasts, and chondrocytes²⁵. The mitogenic effects of FGF-1 have been associated with chondrocyte proliferation, while FGF-2 is expressed by osteoblasts in which it is generally more potent than FGF-1. Crosslinked gelatin hydrogels with β -FGF incorporated via electrostatic interaction⁷⁹ implanted into rabbit cranial defects enhanced bone regeneration as compared to free β -FGF of the same dose without carrier⁸⁰. Various other cytokines or growth factors have also been used successfully in tissue engineering approaches for the enhancement and acceleration of craniofacial bone formation^{1,10,11,39}. Platelet-derived growth factor-BB, frequently used protein in maxillofacial surgery, incorporated into PLA/PGA meshes, increased new bone formation in rat calvarial defects and completed bony reunion after 2 weeks of implantation⁶⁵.

Although several molecules may soon be available as recombinant or non-

recombinant growth factors, there is concern that a single dose of exogenous protein will not induce an adequate biologic response in patients, particularly in situations in which the viability of the host bone and the surrounding soft tissues is compromised. To overcome this problem another approach for protein delivery may be gene therapy. Gene therapy involves the transfer and expression of genetic information to target cells. DNA-based therapies for tissue regeneration blend the technologies of gene therapy and tissue engineering³. With gene therapy the genetic message is delivered to a particular cell, which then synthesises the transfected gene product. In general, the duration of protein synthesis after gene therapy depends on the techniques used to deliver the gene to the target cell. Both short-term and long-term expressions are possible. Extracorporeal tissue engineering is well suited for this strategy because cells can be transfected *ex vivo*. Genetic methods for tissue engineering of bone in the craniofacial area are based on the delivery of DNA (gene therapy) to the cell/matrix construct¹. Typically, recombinant vectors encoding therapeutic molecules are formulated with porous biomaterial carriers/scaffolds³. The biomaterial fills the wound bed, holding the DNA vector *in situ* until endogenous repair cells arrive. As these cells migrate within the material, they are transfected/transduced, essentially becoming local *in vivo* bioreactors that produce the therapeutic factor encoded by the DNA. Thus, genetic approaches to tissue engineering involve the migration of bone cells on artificial scaffolds and local gene delivery and expression. Most of the approaches use a passive process, that is, target cells encounter the DNA as they migrate within the biomaterial scaffold. Gene therapy is being applied to a variety of tissue engineering applications such as bone tissue engineering, and several reviews have been published recently that discuss these efforts^{2,3}. The use of bone morphogenic proteins (as the most classic example) has proven successful for enhancing bone formation in long as well as in craniofacial bones^{1,4,10,11,81}. Alongside the potential use of gene therapy in tissue engineering, gene therapy also involves some technical and legal problems that have yet to be solved.

In conclusion, extracorporeal bone tissue engineering is an emerging approach in maxillofacial surgery. A suitable intra- and extra-scaffold geometry as well as biocompatible and biodegradable scaffold

materials will, in combination with biophysical and/or biochemical stimuli, provide a fast and complete bone regeneration even of complex geometries. The very first point is, however, that the cell and tissue handling is state of the art. Multidisciplinary approaches will most likely solve current limitations in the near future, suggesting that this treatment option will soon be employed in clinical practice.

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