Int. J. Oral Maxillofac. Surg. 2004; 33: 325–332
doi:10.1016/S0901-5027(03)00199-1, available online at http://www.sciencedirect.com

International Journal of
Oral & Maxillofacial Surgery

Invited Review Paper
Osteobiology

Biological and biophysical principles in extracorporal bone tissue engineering Part I

U. Meyer, U. Joos, H. P. Wiesmann
Department of Cranio-Maxillofadal Surgery, University of Munster, Waldeyerstr. 30, D-48149 Munster, Germany

U. Meyer, U. Joos, H. P. Wiesmann:Biological and biophysical principles in extracorporal bone tissue engineering Part I. Int. J. Oral Maxillofac. Surg. 2004; 33: 325–332.

Abstract. Advances in the field of bone tissue engineering have encouraged physicians to introduce these techniques into clinical practice. Bone tissue engineering is the construction, repair or replacement of damaged or missing bone in humans or animals. Engineering of bone can take place within the animal body or extracorporal in a bioreactor for later grafting into the body. Appropriate cell types and non-living substrata are minimal requirements for an extracorporal tissue engineering approach. This review discusses the biological and biophysical background of *in vitro* bone tissue engineering. Biochemical and biophysical stimuli of cell growth and differentiation are regarded as potent tools to improve bone formation *in vitro*. The paper focuses on basic principles in extracorporal engineering of bone-like tissues, intended to be implanted in animal experiments and clinical studies. Particular attention is given in this part to the contributions of cell and material science to the development of bone-like tissues. Several approaches are at the level of clinical applicability and it can be expected that widespread use of engineered bone constructs will change the surgeon's work in the near future.

Key words: bone tissue engineering; osteoblasts; cell stimulation.

Accepted for publication 30 July 2003

Bone repair is a subject of intensive investigation in maxillofacial surgery. Current approaches in bone reconstructive surgery use biomaterials, autografts or allografts, although restrictions on all these techniques exist. These restrictions include donor site morbidity and donor shortage for autografts[26], immunologic barriers for allografts and the risk of transmitting infectious diseases. Numerous artificial bone substitutes containing metals, ceramics and polymers were introduced to maintain bone function[15]. However, each material has specific disadvantages, and none of these can perfectly substitute for autografts in current clinical practice. One important reason for the priority of tissue grafts over non-living biomaterials is that they contain living cells and tissue-inducing substances, thereby possessing biological plasticity.

Craniofacial research is currently in progress to develop cell-containing hybrid materials and to create replacement tissues that remain interactive after implantation, imparting physiological functions as well as structure to the tissue or organ damaged by disease or trauma[3]. Bone tissue engineering like in most other tissue engineering areas exploits living cells in a variety of ways to restore, maintain or enhance tissue functions[51,56]. There are three principal therapeutic strategies for treating diseased or lost bone in patients: (i) implantation of freshly isolated or cultured bone cells; (ii) implantation of a bone-like tissue assembled *in vitro* from cells and scaffolds and (iii) *in situ* tissue regeneration. Cellular implantation means that individual bone cells or small cellular

0901-5027/040325 + 08 $30.00/0

aggregates from the patient or a donor are injected directly into the damaged or lost region with or without a degradable scaffold. For tissue implantation, a complete three-dimensional tissue is grown *in vitro* using autologous or donor cells within a scaffold, which has to be implanted once it has reached 'maturity'[54,63,86]. For *in situ* regeneration, new bone formation is induced by specific scaffolds or external stimuli that are used to stimulate the body's own cells and promote local tissue repair. Bone growth by distraction osteogenesis is one classical example thereof[61].

Extracorporal bone tissue engineering requires the interaction of three biological components: bone cells, growth factors and the extracellular scaffolds. For engineering living tissues *in vitro*, cultured cells are grown on two-dimensional bioactive degradable biomaterials that provide the physical and chemical basis to guide their proliferation and differentiation. In bioreactors outside the body the biomaterial is assembled to a complex three-dimensional scaffold[40]. The assembly of cells into tissue substitutes is a highly orchestrated set of events that requires time scales ranging from seconds to weeks and dimensions ranging from 0.0001 to 10 cm. At the moment the techniques are moving from an experimental stage to the level of clinical application. We will consider extracorporal bone tissue engineering as a new approach to generate artificial materials used as substitutes and implants for reconstructive surgery. The review highlights the development of cell-based approaches for tissue engineering of bone, and offers perspectives on future treatment concepts.

Cells used for tissue engineering

Bone contains a variety of different cell types: vascular cells, marrow cells, preosteoblasts, osteocytes, chondroblasts and osteoclasts, all executing distinct cellular functions to allow the bone to work as a highly dynamic organ. Whereas all these cells are necessary to build up a 'real' bone, limited cell sources are regarded to be sufficient for engineering a 'bone-like' construct *in vitro*.

Osteoblasts

Bone tissue engineering requires at least living osteoprogenitor cells or osteoblast-like cells. Fortunately, it is possible to maintain and propagate various osteoblast-like cell types outside the human body for prolonged periods. Principal sources of cells for tissue engineering include autologous and allogeneic cells. Each category can be subdivided according to whether the cells are adult or embryonic stem cells capable of both self-renewal and differentiation into a variety of cell lineages. It has long been known that bone has a vast capacity for regeneration when autologous adult osteoblast-like cells are used[7]. There are no legal problems with their clinical use and no problems of immune rejection should be expected. Therefore, in current clinical practice autologous osteoblast-like cells are the most desirable cell source. However, these cells may be insufficient to rebuild damaged bone tissue in a reasonable time, and there are also some questions about the senescence of these cells.

Periosteal cells

A considerable number of cell divisions is needed to bulk the tissue to its correct size. Older studies regarded the ability of cells to propagate in culture as a serious problem, because it was thought that most adult tissues contained only a minority of cells capable of effective expansion. However, in numerous recent investigations it has been shown that bone cells proliferate in culture without losing their viability. Cells located within the periosteum and bone marrow can differentiate into fibroblastic, osteogenic or reticular cells[14,24,37,59,70,93]. Periosteum outgrowth techniques allow also the propagation of osteoclastic cells from monocytes located in the periosteum[91]. Periosteal-derived mesenchymal precursor cells generate progenitor cells committed to one or more cell lines with an apparent degree of plasticity and interconversion[10,69,78,85]. Outgrowth cultures of periosteum pieces favour the coculture of different cell types[58]. In culture expanded bone marrow and periosteum cells are able to heal a segmental bone defect after being reimplanted and induce osteogenic tissue when seeded into diffusion chambers[7,42,67,73].

Marrow cells

The first cell-based approaches for tissue engineering of bone used unfractioned fresh autologous or syngeneic bone marrow[41,72,99]. Because bone marrow is known to contain osteogenic precursors, its use was perceived to have the potential to lead to effective bone regeneration. Various preclinical investigations, and a limited number of clinical studies, have confirmed this to be true[46,72,73]. Human bone marrow osteoprogenitors can be isolated and enriched by using selective markers[88,89].

Despite the success that has been obtained using fresh marrow transfer, one biologic consideration limits its widespread application. Frequently, it is impractical to obtain sufficient amounts of bone marrow with the requisite number of osteoprogenitor cells. The reduction of healthy bone marrow components that occurs as a consequence of aging or disease is accompanied by a diminution of osteogenic precursors[33,82]. As the success of the *in vitro* use of bone marrow explants is critically dependent on the transfer of sufficient numbers of these progenitors, this approach may be least applicable in those situations where it is most needed. It was shown that osteoprogenitors represent approximately 0.001% of the nucleated cells in healthy adult marrow[18,44]. Therefore, techniques capable of selecting, expanding, and administering the progenitor cell fraction would be of great clinical benefit.

Stem cells

In different approaches, mesenchymal stem cells were harvested, expanded in culture, and then induced to differentiate into cells that are involved in the repair of damaged bone[80]. Osteoprogenitor and stem cells can be expanded in a reasonable time, which permits their potential use in tissue engineering[14,18,47,49,60]. The primitive stem cells renewing bony structures have been given a variety of names including connective tissue stem cells, osteogenic cells[43], stromal stem cells[76], stromal fibroblastic cells[93] and mesenchymal stem cells[19]. No nomenclature to date is entirely accurate based upon the developmental origins or differentiation capacities of these cells, but the latter term, although defective, appears to be in a favour at the moment.

The stem cell of bone tissue is a hypothetical concept with only circumstantial evidence for its existence, and indeed, there seems to be a hierarchy of stem cells each with variable self-renewal potentials. Bone cell populations may be derived from all bone surfaces by a variety of techniques, including mechanical disruption, explantation and enzyme digestion[94]. Mesenchymal stem cells have the capacity for extensive replication without differentiation, and they possess a multilineage developmental potential allowing them to give rise to not only

bone, but cartilage, tendon, muscle, fat and marrow stroma. Their isolation is generally based on density gradient centrifugation and cell culturing techniques. Techniques have been developed to allow mesenchymal stem cells (MSC) to be cultured and expanded in number without undergoing differentiation[18,68]. The phenotype of the cells is stable throughout culture and there is no loss in osteogenic, chondrogenic or adipogenic potential[18,80]. This expansion properties make MSCs a principally useful source of progenitor cells for tissue engineering of bone and other mesenchymal derivatives. Many attemps have been undertaken to optimize procedures for the amplification and differentiation of progenitor cells. Some studies have indicated that mouse marrow fibroblastic cells implanted locally or injected systemically may home to the bony site and persist to participate in regenerative processes[79,97].

Despite the various advantages of using intrinsic stem cells over other sources of cells, there is some debate as to whether large enough populations of differentiated cells can be grown *in vitro* rapidly enough when needed clinically. At present, stem cells are not able to differentiate definitively and to mineralize in a bone-like manner under *in vitro* conditions[47,81]. This must be considered as a severe limitiation for the use of stem cells in extracorporal tissue engineering. Genetic engineering to shape gene expression profiles may be, therefore, a future route for the use of allogeneic cells in human tissue engineering, but this approach is at the moment far away from clinical application.

Much more basic research is necessary to assess the full potential of cell therapy to reconstitute bone mass. It is expected that many future studies will be directed toward the development of gene therapy protocols employing gene insertion strategies[34]. The concept that members of the bone morphogenetic proteins (BMP) and the transforming growth factor-beta (TGF-beta) superfamily will be particularly useful in this regard has already been tested by many investigators[53,71].

Chondroblasts

Because endochondral bone formation, and frequently fracture repair, proceeds through a cartilaginous intermediate, some investigators have suggested that the transplantation of committed chondrocytes would also lead to bone regeneration[10]. Vacanti et al. compared the ability of periosteal progenitors and articular chondrocytes to effect bone repair[94]. Periosteal cells from newborn calves seeded on a scaffold and implanted in critical sized calvarial defects generated new bone. Specimens examined at early times contained material that grossly and histologically appeared to be cartilage. The scaffold seeded with chondrocytes also formed cartilage. However, no endochondral ossification was observed, since the transplanted specimens remained in a cartilaginous state. Therefore, chondrocytes proved ineffective as a cell-based therapy for tissue engineering of bone. Because mature cartilage is thought to produce factors that inhibit angiogenesis, implants seeded with committed chondrocytes may prevent the endochondral cascade by preventing vascular invasion. Cells derived from cartilage seem to be committed to retain their phenotype and, therefore, are unable to differentiate towards hypertrophic chondrocytes. In contrast, when precursor cells from the periosteum are provided, their primitive state allows them to proceed through the entire chondrogenic lineage, ultimately becoming hypertrophic chondrocytes. The molecular basis for the difference in the phenotypic potential of these different cell types remains mysterious and is an area under active investigation.

Vascular cells

The additional use of vascular cells offers several theoretical advantages over approaches of extracorporal bone tissue engineering exploiting only bone cells as a single cell type. As a cell-based strategy, endothelial progenitor cell therapy promises to deliver both substrate (endothelial cells) and the cytokines and growth factors important for cell/scaffold ingrowth. Endothelial progenitor cells are capable of homing to bone areas of neovascularization, thus exerting their effects in sites in need of new blood vessel growth[6]. Moreover, because these cells are ubiquitously present, they exhibit no unfavourable site effects when transplanted autologously. This makes them appealing components for bone tissue engineering in order to promote synergistic vasculogenesis and bone formation.

Since endothelial progenitor cells were first described by Asahara et al. in 1997, the number of published studies specifically addressing these cells has rapidly increased[5]. The initial studies with endothelial progenitor cells made significant progress in defining the origin and lineage of these cells. There is strong evidence to suggest that they originate in the bone marrow and are selectively recruited to sites of neovascularization.

The relative ease of isolating and expanding mature endothelial cells (from explanted blood vessels) or endothelial progenitor cells (from bone marrow) makes them an attractive source of autologous vascular cells for the generation of a vascularized scaffold complex *in vitro*. Some studies have demonstrated that endothelial progenitor cells form tubules in extracellular matrices *in vitro* and are able to induce vascular invasion by host tissue if implanted[2,66]. Although those studies were not intended to optimize the formation of vascular matrices for tissue engineering, the findings are encouraging in light of recent work showing the potential of axial vessels to vascularize cellular scaffolds *in vitro* and *in vivo*[22].

Endothelial progenitor cells may also have a potential role in the formation of complex tissue-engineered vascularized bone constructs. Tissue-engineered bone constructs may be fabricated by combining autologous vascular cells and bone cells in an optimized scaffold structure. Studies with various cell lines indicated that patency rates were strongly correlated with the amount of host cells (smooth muscle cells and endothelial cells) incorporated into the graft[32,48,52,95]. Endothelial progenitor cells are an attractive source of cells to line such complexes. It was shown that the success of grafts seeded with endothelial progenitor cells was significantly greater than that of non-seeded grafts[50]. Vacanti et al. demonstrated also an *in vivo* success with the placement of synthetic materials combined with autologous endothelial progenitor cells[94]. An additional advantage of endothelial progenitor cell use is the potential of thrombus regression, which has implications for the prevention and treatment of microvascular failures, once a tissue-engineered bone construct is transferred into the host site. Ongoing research with endothelial progenitor cells should help to improve the *ex vivo* formation of a 'mature' bone construct and give insight into the process of new blood vessel formation *in vivo*. With our recent understanding of the physiological roles of endothelial cells, reseachers should consider the importance of vasculogenesis in extracorporal bone tissue engineering, especially in light of the fact that cell survival balanced by nutrition is one of the main limiting steps in scaling up bone constructs for clinical use.

Cellular interactions on biomaterials

A crucial mainstay of bone tissue engineering is the biomaterial from which scaffolds are fashioned. The ideal biomaterial for a scaffold should selectively interact with specific transmembrane receptors expressed by osteoblasts and osteocytes. The underlying material of the scaffold guides the behaviour of these target cells and plays a crucial role in cell adhesion, proliferation, migration and cellular differentiation[64].

Implanted materials may be stable with time or ultimately degrade in response to matrix remodelling enzymes released by the cells[45]. Whether or not a material is biodegradable, its surface properties will determine the clinical fate after implantation. Two different features have impact on cellular responses towards the material: the three-dimensional topography and the physico-chemical properties of the surface. The three-dimensional topography can be conceptualized as the size, the shape and the surface texture of the material. Various studies demonstrated that bone cells are sensitive to the gross topography of the underlying material[25,31,39,55,69]. The finding that the shape of the substratum on which cells are growing affects their morphology and migration goes back at least to the 1930s, but it was not until the development of microfabrication methods that a wide range of defined surface structures could be generated on a micrometer scale[36]. Initially, the nanoscale materials were fabricated in silica or silicon by photolithography, but new methods of casting and embossing these surfaces have been emerged in the meantime[35,38].

Cells respond to the substrate topography by adapting their orientation, movement and attachment kinetics[21,30,100,102]. It has been suggested that the micrometre topography of a material alone determines whether the material will elicit an osteoblastic or a fibroblastic cell reaction. Some types of cells such as osteoblasts react to features as small as 10 nm, indicating their amazing ability to detect such small features[101]. The growth of epitenon in micrometer-sized grooves embossed onto a biodegradable polymer was exploited to aid tendon healing[100].

Recent developments in nanotechnology have enabled studies on the behaviour of cells in nanoscale dimensions. Engineered microscopic surface structures allow the control of interfacial forces with different effective ranges[38]. Predictions of the response of a cell to nanofeatures are at the moment difficult and the effects are often still unexpected. Many of the interfacial force effects average out when the scale of structures exceeds approximately 300 nm. Patterned surfaces on the nanometer scale are commonly produced using either electron beam lithography, microcontact printing, micromachining or vapour deposition.

It was demonstrated that a given type of cell reacts in very much the same way to a structured topography whether this is made out of silica or any one of a wide range of polymers or even metals. These materials are known to adsorb different kinds of macromolecule differentially. It seems that topography regulates the orientation and assembly of cytoskeletal components within the cell. Thus, it is not surprising that many cell functions can be related to topographical features.

Several other material properties have also been proposed to guide the biological response of adherent cells. From cell culture experiments on different biomaterials it is known that the cellular behaviour of osteoblasts depends on the propensity of the physico-chemical surface, which can be described in terms of surface charge and surface energy (wettability)[13,28]. It was found that osteoblasts are most likely influenced by the properties of the surface charge[65]. The zeta potential of the surface is regarded as an important factor regulating the biocompatibility of the material. It has been suggested that the zeta potential of calcium phosphate ceramics is directly related to the surface reactivity governing osteoconductivity. Measurements of protein synthesis on different biocomposites have revealed that the amount of matrix protein production per cell is reduced on surfaces with low zeta potentials[27,65].

The interfacial tension, or wettability, is measured as a property of the interaction forces (or adhesion forces) between different materials and their interaction with the cohesion forces within the materials[4]. Thus, if the cohesion forces directed into the material are higher than the attraction forces to the other material, there will be little or no physical interaction. This property is only marginally related to the charge density on the surface and is thus not directly related to the zeta potential. A material with a positive or negative surface charge is assumed to be hydrophilic, whereas a surface with a neutral charge may be more hydrophobic in character. Osteoblasts at the material surface may alter their membrane potential by a low concentration of physiological ions at the surface. For instance, Dekker et al. used gas plasma treatment to change the wettability of polytetrafluoroethylene (PTFE) surfaces, but did not alter the zeta potential[29]. Moller et al. investigated the impact of wettabilities on the attachment and proliferation kinetics of osteoblasts and found no direct relationship[65]. Recent investigations have revealed that a reduced synthesis of collagen is often associated with low wettabilities of substrates[87]. These studies have suggested that increasing amounts of polar components improve cell attachment and matrix synthesis on artificial surfaces[83].

The material composition was found to have distinct effects on osteoblast behaviour[57,105]. Differences in the protein and ion composition of the outer most functional layer of a surface clearly affect the cellular response, although the exact mechanisms involved are not fully understood[20,39,65,84,87]. Because the chemical composition of the ionleachable materials is changed over time, the potential release of ions should be considered affecting the survival and growth of osteoblasts[58].

Numerous *in vitro* studies have demonstrated that the attachment of osteoblasts in the first hours after seeding differs significantly depending on whether the surface is protein-coated or not[31,60]. Among others, fibronectin and vitronectin are known to facilitate the adhesion of osteoblasts. These extracellular proteins act as a bridging element between artificial surfaces and osteoblasts. Especially, Arg-Gly-Asp (RGD)-containing peptides exhibit strong effects on osteoblast adhesion, matrix maturation and mineralization[84]. Some studies have suggested that in contrast to common convictions increasing the number of adhesion contacts between cells and the extracellular matrix may not always be advantageous[77]. If too few adhesive ligands are available, cells cannot get a strong enough grip to enable them to move. However, if there are too many ligands, cells adhere so firmly that they remain stuck in place. Thus, intermediate adhesion seems to be required for optimal cell migration[77].

Matrix mineralization

In the final stages of osteogenic differentiation *in vivo* a mature mineralized extracellular matrix is produced[9,62,81,90]. There is still much discussion as to how to achieve a 'bone-like' mineralization under *in vitro* conditions. Mineral accumulation found in the extracellular space

between cells may be only an artificial enrichment of calcium and phosphate and may not alone be representative for a bone-like apatite formation. Cell-mediated mineralized scaffolds enhance the mechanical stability of the construct and are advantageous in most clinical situations.

For the assessment of extracorporal bone-like mineral formation in tissue engineering the precise molecular nature of the crystallization process has to be elucidated[98]. It is known that newly formed mineral results from the synthesis of mineral spherites. It was demonstrated that matrix vesicles serve as initial sites of calcification in all skeletal tissues. These are membrane-invested particles of 100 nanometer diameter, located within the extracellular matrix[104]. These nodules are typical in mineralizing tissues and have been described at the mineralization front of woven bone[17], in mantle dentin[90], circumpulpal dentin[74,92], and mineralizing cartilage[75]. Mineral spherites have been identified at the initiation sites of mineral formation, suggesting that they function as nucleation core complexes for mineral formation[103]. In early studies, mineralizing osteoid appeared predominantly in multilayered structures that formed nodules after an extended period of time[11,12]. Although calcium phosphate crystals accumulate on different materials, a 'bone-like' mineral formation has not conclusively been demonstrated. Most studies on this issue do not differentiate between the precipitation of calcium phosphate and the formation of bone-like apatite structure[81]. For the refinement of bone-like substitutes the mineralization process has to be studied in more detail.

Biomineralization of cultured osteoblast-like cells is initially associated with cell surface globules, while *in vivo* it is closely associated with mineralizing matrix vesicle formation. The smallest mineral globuli produced by osteoblasts cultured on polystyrene surfaces have sizes comparable to the matrix vesicles found in bone tissue[97,98]. Size and crystal structure of newly formed mineral were found to be similar in matrix vesicle-mediated mineralization *in vivo* and *in vitro*. Whether the mineralization process found in cell culture systems resembles the physiological situation remains a matter of controversy. When maintained under suitable culture conditions, certain bone-derived cells form bone-like nodules in cell culture[96]. There is growing evidence that some cell types, such as primary periosteal osteoblasts, are able to differentiate terminally *in vitro*. The addition of glucocorticoids, ascorbic acid, beta-glycerophosphate or bone morphogenetic proteins was shown to induce matrix mineralization in different bone cell cultures[8,23]. Recent data from mineralization assays indicate that at least some of the actions of the above mentioned substances on matrix mineralization are dependent on the stage of cellular differentiation[16]. Beta-glycerophosphate, for example, has frequently been used as an exogenous phosphate source for cultured osteoblasts to synthetize mineralized material. It is a substrate for the alkaline phosphatase which precipitates calcium phosphate in the presence of calcium salts and phosphate esters. When β-glycerophosphate-stimulated osteoblast-like cells are exposed to higher than physiological levels of inorganic phosphate, it causes dystrophic mineralization rather than a bone-like mineral formation is induced[1]. A collagen associated bone-like extracellular mineral formation on artificial surfaces has not been demonstrated up to now, most likely because collagen fibres do not mineralizes under these conditions[62]. This should be considered as a recent problem of bone-like structure formation in extracorporal tissue engineering.

Acknowledgments. The authors acknowledge with thanks the financial support of the German Research Foundation and the German Federal Ministry of Education and Research.

References

1. Ahmad M, McCarthy MB, Gronowicz G. An in vitro model for mineralization of human osteoblast-like cells on implant materials. Biomaterials 1989: **20**: 211–220.
2. Al-Khaldi A, Eliopoulos N, Lachapelle K, Galipeau J, EGF-dependent angiogenic response induced bx in situ cultured marrow stromal cells. Anaheim, Calif: American Heart Association Scientific Session Nov. 2001: 11–14.
3. Alsberg E, Hill EE, Mooney DJ. Craniofacial tissue engineering. Crit Rev Oral Biol Med 2001: **12**: 64–75.
4. Andrade JD. Interfacial phenomena and biomaterials. Med Instrum 1973: **7**: 110–119.
5. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Wltzenbichle B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997: **275**: 964–967.
6. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999: **85**: 221–228.
7. Ashton BA, Allen TD, Howlett CR, Eaglesom CC, Hattori A, Owen M. Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. Clin Orthop 1980: **32**: 294–307.
8. Aubin JE. Osteoprogenitor cell frequency in rat bone marrow stromal populations: Role for heterotypic cell–cell interactions in osteoblast differentiation. J Cell Biochem 1999: **72**: 396–410.
9. Bagambisa FB, Joos U, Schilli W. A scanning electron microscope study of the ultrastructural organization of bone mineral. Cells Mater 1993: **3**: 93–102.
10. Bahrami S, Stratmann U, Wiesmann HP, Mokrys K, Dierichs R, Bruckner P, Szuwart T. Periosteally derived osteoblast-like cells differentiate into chondrocytes in suspension culture in agarose. Anat Rec 2000: **259**: 124–130.
11. Bellows CG, Aubin JE, Heershe JNM, Antosz ME. Mineralized bone nodules formed in vitro from enzymatically released rat calvaria cell population. Calcif Tissue Int 1986: **38**: 143–154.
12. Benayahu D, Kletter Y, Zipori D, Wientroub S. Bone marrow-derived stromal cell line expressing oteoblastic phenotype in vitro and osteogenic capacity in vivo. J Cell Physol 1989: **140**: 1–7.
13. Bertoluzza A, Simoni R, Tinti A, Morocutti M, Ottani V, Ruggeri A. Calcium phosphate materials containing alumina: Raman spectroscopical, histological, and ultrastructural study. J Biomed Mater Res 1991: **25**: 23–38.
14. Bianco P, Riminucci M, Gronthos S, Robey PG. Bone marrow stomal stem cells: Nature, biology, and potential applications. Stem Cells 2001: **19**: 180–192.
15. Binderman I, Fin N. Bone substitutes-organic, inorganic, and polymeric: Cell material interactions. In: Yamamuro T, Hench L, Wilson J, eds.: CRC Handbook of Bioactive Ceramics. Boca Raton, Florida: CRC Press 1990: 45–51.
16. Boskey AL. Matrix protein and mineralization: An overview. Connect Tissue Res 1996: **35**: 357–363.
17. Boyde A, Sela J. Scanning electron microscope study of separated calcospherites from the matrices of different mineralizing systems. Calcif Tissue Res 1978: **26**: 47–49.
18. Bruder SP, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. J Cell Biochem 1997: **64**: 278–294.

19. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991: **9**: 641–650.
20. Chehroudi B, Gould TRL, Burnette DM. A light and electron microscopic study on the effects of surface topography on the behaviour of cells attached to titanium-coated percutaneous implants. J Biomed Mater Res 1991: **25**: 387–405.
21. Chehroudi B, McDonnell D, Brunette DM. The effects of micro-machined surfaces on formation of bone-like tissue on subcutaneous implants as assessed by radiography and computer image processing. J Biomed Mater Res 1997: **34**: 279–290.
22. Chung S, Hazen A, Levine JP, Baux G, Olivier WA, Yee HT, Margiotta MS, Karp NS, Gurtner GC. Vascularized acellular dermal matrix island flaps for the repair of abdominal muscle defects. Plast Reconstr Surg 2003: **111**: 225–232.
23. Coelho MJ, Fernandes MH. Human bone cell cultures in biocompatibility testing. Part II: Effect of asorbic acid beta-glycerophosphate and dexamethasone on osteoblastic differentiation. Biomaterials 2000: **21**: 1095–1102.
24. Dahir GA, Cui Q, Anderson P, Simon C, Joyner C, Triffitt JT, Balian G. Pluripotential mesenchymal cells repopulate bone marrow and retain osteogenic properties. Clin Orthop 2000: **379**: 134–145.
25. Dalby MJ, Di Silvio L, Davies GW, Bonfield W. Surface topography and HA filler volume effect on primary human osteoblasts in vitro. J Mat Sci Mater Med 2000: **11**: 805–810.
26. Damien JC, Parson JR. Bone graft and bone graft substitutes: A review of current technology and applications. J Appl Biomater 1991: **2**: 187–208.
27. Davies JE, Causton B, Bovell Y, Davy K, Sturt CS. The migration of osteoblasts over substrate of different surface charge. Biomaterials 1986: **7**: 231–233.
28. Davies JE, Matsuda T. Extracellular matrix production by osteoblasts on bioactive substrata in vitro. Scanning Microsc 1988: **2**: 1445–1452.
29. Dekker A, Reitsma K, Beugeling T, Bantjes A, Feijen J, van Aken WG. Adhesion of endothelial cells and adsorption of serum proteins on gas plasma treated polytetrafiuoroethylen. Biomaterials 1991: **12**: 130–138.
30. Denbraber ET, Deruijter JE, Smits HTJ, Ginsel LA, Vonrecum AF, Jansen JA. Effect of parallel surface microgrooves and surface energy on cell growth. J Biomed Mater Res 1995: **29**: 511–518.
31. Dennis JE, Haynesworth SE, Young RG, Caplan AI. Osteogenesis in marrow-derived mesenchymal cell porous ceramic composites transplanted subcutaneously: Effect of fibronectin and laminin in cell retention and rate of osteogenic expression. Cell Transplant 1992: **1**: 23–32.
32. Deutsch M, Meinhart J, Fischlein T, Preiss P, Zilla P. Clinical autologous in vitro endothelialization of infrainguinal ePTFE grafts in 100 patients: A 9-year experience. Surgery 1999: **126**: 847–855.
33. Egrise D, Martin D, Vienne A, Neve P, Schouten A. The number of fibroblastic colonies formed from bone marrow is decreased and the in vitro proliferation rate of trabecular bone cells increased in aged rats. Bone 1992: **13**: 355–361.
34. Evans CH, Robbins PD. Possible orthopaedic applications of gene therapy. J Bone Joint Surg Am 1995: **77**: 1103–1114.
35. Fahrtmann M, Dambach S, Kiuegeskotte C, Wlesmann HP, Wittig A, Sauerwein W, Lipinsky D, Arlinghaus HF. Characterization of cell cultures with ToF-SIMS and laser-SNMS. Surf Interface Anal 2002: **34**: 63–66.
36. Fahrtmann M, Dambach S, Wittig A, Sauerwein W, Wiesmann HP, Arlinghaus HF. Subcellular imaging of freeze-fractured cell cultures by ToF-SIMS and laser-SNMS. Appl Surf Sci 2003: **203**: 726–729.
37. Friedenstein AJ. Precursor cells of mechanocytes. Int Rev Cytol 1976: **47**: 327–359.
38. Gleiche M, Chi LF, Fuchs H. Nanoscopic channel lattices with controlled anisotropic wetting. Nature 2000: **403**: 173–175.
39. Goshima J, Goldberg VM, Caplan AI. The osteogenic potential of culture-expanded rat marrow mesenchymal cells assayed in vivo in calcium phosphate ceramic blocks. Clin Orthop 1991: **262**: 298–311.
40. Griffith LG. Emerging design principles in biomaterials and scaffolds for tissue engineering. Ann N Y Acad Sci 2002: **961**: 83–95.
41. Grundel RE, Chapmann MW, Yee T, Moore DC. Autogeneic bone marrow and porous biphasic calcium phosphate ceramic for segmental bone defects in the canine ulna. Clin Orthop 1991: **266**: 244–258.
42. Gundle R, Joyner CJ, Triffitt JT. Human bone tissue formation in diffusion chamber culture in vivo by bone-derived cells and marrow stromal fibroblastic cells. Bone 1995: **16**: 597–601.
43. Ham AW. Histology. Philadelphia: J. B. Lippincott Co. 1969: 247.
44. Haynesworth SE, Goshima J, Goldberg VM, Caplan AI. Characterization of cells with osteogenic potential from human marrow. Bone 1992: **13**: 81–88.
45. Hubbel JA. Bioactive biomaterials. Curr Opin Biotechnol 1999: **10**: 123–129.
46. Jackson IT, Scheker LR, Vandervord JG, McLennan IG. Bone marrow grafting in the secondary closure of alveolar-palatal defects in children. Br J Plast Surg 1981: **34**: 422–425.
47. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 1997: **64**: 295–312.
48. Jarrell BE, Williams SK, Stokes G, Hubbard FA, Carabasi RA, Koople E, Greener D, Pratt K, Moritz MJ, Radomski J. Use of freshly isolated capillary endothelial cells for the immediate establishment of a monolayer on a vascular graft at surgery. Surgery 1986: **100**: 392–399.
49. Joyner CJ, Bennett A, Triffitt JT. Identification and enrichment of human osteoprogenitor cells by using differentiation stage-specific monoclonal antibodies. Bone 1997: **21**: 1–6.
50. Kaushal S, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW, Rabkin E, Moran AM, Schoen FJ, Atala A, Soker S, Bischoff J, Mayer JE. Functional small-diameter neovessels created using endothelial progenitor cells expanded in situ. Nat Med 2001: **7**: 996–997.
51. Langer R, Vacanti JP. Tissue engineering. Science 1993: **260**: 920–926.
52. L'Heureux N, Paquet S, Labbe R, Germain L, Auger FAA. A completely biological tissue-engineered human blood vessel. FASEB J 1998: **12**: 47–56.
53. Lieberman JR, Le LQ, Wu L, Finerman GA, Berk A, Witte ON, Stevenson R. Regional gene therapy with a BMP-2-producing murine stromal cell line induces heterotopic and orthotopic bone formation in rodents. J Orthop Res 1998: **16**: 330–339.
54. Loty C, Sautier JM, Boulekbache H, Kokubo T, Kim HM, Forest N. In vitro bone formation on a bone-like apatite layer prepared by a biomimetic process on a bioactive glass-ceramic. J Biomed Mater Res 2000: **49**: 423–434.
55. Lu JX, Flautre B, Anselme K, Hardouin P, Gallur A, Descamps M, Thierry B. Role of interconnections in porous bioceramics on bone recolonization in vitro and in vivo. J Mat Sci Mater Med 1999: **10**: 111–120.
56. Lysaght MJ, Reyes J. The growth of tissue engineering. Tissue Eng 2001: **7**: 485–493.
57. Matsuoka H, Akiyama H, Okada Y, Ito H, Shigeno C, Konishi J, Kokubo T, Nakamura T. In vitro analysis of the stimulation of bone formation by highly bioactive apatite-and wollastonite-containing glass-ceramic: Released calcium ions promote osteogenic differentiation in osteoblastic ROS17/2.8 cells. J Biomed Mater Res 1999: **47**: 176–188.
58. Meyer U DH, Szulczewski HD, Barckhaus RH, Atkinson M, Jones DB. Biological evaluation of an iono-

meric bone cement by osteoblast cell culture methods. Biomaterials 1993: **14**: 917–924.
59. Meyer U, Szulczewski HD, Moller K, Heide H, Jones DB. Attachment kinetics and differentiation of osteoblasts on different biomaterials. Cells Mater 1993: **3**: 129–140.
60. Meyer U, Meyer T, Jones DB. Attachment kinetics proliferation rates and vinculin assembly of bovine osteoblasts cultured on different pre-coated artificial substrates. J Mat Sci Mater Med 1998: **9**: 301–307.
61. Meyer U, Meyer T, Vosshans J, Joos U. Decreased expression of osteocalcin and osteonection in relation to high strains and decreased mineralization in mandibular distraction osteogenesis. J Craniomaxillofac Surg 1999: **27**: 222–227.
62. Meyer U, Wiesmann HP, Meyer T, Schulze-Osthoff D, Jasche J, Kruse-Losler B, Joos U. Microstructural investigations of strain-related collagen mineralization. Br J Oral Maxillofac Surg 2001: **39**: 381–389.
63. Meyer U, Joos U, Szuwart T, Wiesmann HP. Mineralized 3D bone tissue engineered by osteoblasts cultured in a collagen gel. Tissue Eng 2001: **7**: 671.
64. Meyer U, Meyer T, Jones DB. No mechanical role for vinculin in strain transduction in primary bovine osteoblasts. Biochem Cell Biol 1997: **75**: 81–87.
65. Moller K, Meyer U, Szulczewski DH, Heide H, Priessnitz B, Jones DB. The influence of zeta potential and interfacial tension on osteoblast-like cells. Cells Mater 1994: **4**: 263–274.
66. Murayama T, Tepper OM, Silver M, Ma H, Losordo DW, Isner JM, Asahara T, Kalka C. Determination of bone marrow-derived endothelial progenitor cell significance to angiogenic growth factor-induced neovascularization in vivo. Exp Hematol 2002: **30**: 967–972.
67. Nakahara H, Bruder SP, Haynesworth SE, Holecek JJ, Baber MA, Goldberg VM, Caplan AI. Bone and cartilage formation in diffusion chambers by subcultured cells derived from the periosteum. Bone 1990: **11**: 181–188.
68. Nakahara H, Bruder SP, Goldberg VM, Caplan AI. In vivo osteochondrogenic potential of cultured cells derived from the periosteum. Clin Orthop 1990: **259**: 223–232.
69. Nakahara H, Goldberg VM, Caplan AI. Culture-expanded periosteal-derived cells exhibit osteochondrogenic potential in porous calcium phosphate ceramics in vivo. Clin Orthop 1992: **276**: 291–298.
70. Nuttall ME, Patton AJ, Olivera DL, Nadeau DP, Gowen M. Human trabecular bone cells are able to express both osteoblastic and adipocytic phenotype: Implications for osteopenic disorders. J Bone Miner Res 1998: **13**: 371–382.
71. Oakes DA, Lieberman JR. Osteoinductive applications of regional gene therapy: In situ gene transfer. Clin Orthop 2000: **379**: 101–112.
72. Ohguschi H, Goldberg VM, Caplan A. Repair of bone defects with marrow cells and porous ceramics. Acta Orthop Scand 1989: **60**: 334–339.
73. Ohgushi H, Goldberg VM, Caplan AI. Heterotopic osteogenesis in porous ceramics induced by marrow cells. J Orthop Res 1989: **7**: 568–578.
74. Ohma N, Takagi Y, Takano Y. Distribution of non-collageneous den tin matrix proteins and proteoglycans, and their relation to calcium accumulation in bisphosphonate-affected rat incisors. Eur J Oral Sci 2000: **108**: 222–232.
75. Ornoy A, Langer Y. Scanning electron microscopy studies on the origin and structure of matrix vesicles in epiphyseal cartilage from young rats. Isr J Med Sci 1978: **14**: 745–752.
76. Owen M, Friedenstein AJ. Stromal stem cells: Marrow-derived osteogenic precursors. Ciba Found Symp 1988: **136**: 42–60.
77. Palecek SP, Loftus JC, Ginsberg MH, Lauffenburger DA, Horwitz AF. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature 1997: **385**: 537–540.
78. Park SR, Oreffo RO, Triffitt JT. Interconversion potential of cloned human marrow adipocytes in vitro. Bone 1999: **24**: 549–554.
79. Perka C, Schultz O, Spitzer RS, Llndenhayn K, Burmester GR, Sittinger M. Segmental bone repair by tissue engineered periosteal cell transplants with bioresobable fleece and fibrin scaffolds in rabbits. Biomaterials 2000: **21**: 1145–1153.
80. Pittinger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999: **284**: 143–147.
81. Plate U, Arnold S, Stratmann U, Wiesmann HP, Hohling HJ. General principle of ordered apatitic crystal formation in enamel and collagen rich hard tissues. Connect Tissue Res 1998: **38**: 149–157.
82. Quarto R, Thomas D, Liang T. Bone progenitor cell deficits and the age-associated decline in bone repair capacity. Calcif Tissue Int 1995: **56**: 123–129.
83. Redey SA, Nardin M, Bernache-Assolant D, Rey C, Delannoy P, Sedel L, Marie PJ. Behavior of human osteoblastic cells on stoichiometric hydroxy-apatite and type A carbonate apatite: Role of surface energy. J Biomed Mater Res 2000: **50**: 353–364.
84. Rezania A, Healy KE. The effect of peptide surface density on mineralization of a matrix deposited by osteogenic cells. J Biomed Mater Res 2000: **52**: 595–600.
85. Schantz JT, Hutmacher DW, Chim H, Ng KW, Lim TC, Teoh SH. Induction of ectopic bone formation by using human periosteal cells in combination with a novel scaffold technology. Cell Transplant 2002: **11**: 125–138.
86. Schliephake H, Knebel JW, Aufderheide M, Tauscher M. Use of cultivated osteoprogenitor cells to increase bone formation in segmental mandibular defects: An experimental pilot study in sheep. Int J Oral Maxillofac Surg 2001: **30**: 531–537.
87. Schwartz Z, Boyan BD. Underlying mechanisms at the bone-biomaterial interface. J Cell Biochem 1994: **56**: 340–347.
88. Simmons PJ, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. Blood 1991: **78**: 55–62.
89. Stewart K, Walsh S, Screen J, Jefferiss CM, Chainey J, Jordan GR, Beresford JN. Further characterization of cells expressing STRO-1 in cultures of adult human bone marrow stromal cells. J Bone Miner Res 1999: **14**: 1345–1356.
90. Stratmann U, Schaarschmidt K, Wiesmann HP, Plate U, Hohling HJ, Szuwart T. The mineralization of mantle dentine and of circumpulpal dentine in the rat: An ultrastructural and element-analytical study. Anat Embryol (Berl) 1997: **195**: 289–297.
91. Szulczewski DH, Meyer U, Moller K, Stratmann U, Doty SB, Jones DB. Characterisation of bovine osteoclasts on an ionomeric cement in vitro. Cells Mater 1993: **3**: 83–92.
92. Takano Y, Sakai H, Baba O, Terashima T. Differential involvement of matrix vesicles during the initial and appos-itional mineralization processes in bone, dentin, and cementum. Bone 2000: **26**: 333–339.
93. Triffitt JT, Oreffo ROC. Osteoblast lineage. In: Zaidi M, ed.: Advances in Organ Biology. Molecular and Cellular Biology of Bone, Advances in Organ Biology Series. Connecticut: JAI Press, Inc. 1998: **5B**: 429–451.
94. Vacanti CA, Kim W, Upton J, Mooney D, Vacanti JP. The efficacy of periosteal cells compared to chondrocytes in the tissue engineered repair of bone defects. Tissue Eng 1995: **1**: 301–308.
95. Weinberg CB, Bell E. A blood vessel model constructed from collagen and cultured vascular cells. Science 1986: **231**: 397–400.
96. Wiesmann HP, Tkotz T, Joos U, Zierold K, Stratmann U, Szuwart T, Plate U, Hohling HJ. Magnesium in

newly formed dentin mineral of rat incisor. J Bone Miner Res 1997: **12**: 380–383.
97. Wiesmann HP, Nazar N, Klatt C, Szuwart T, Meyer U. Bone tissue engineering by primary osteoblast-like cells in a two- and three-dimensional space. J Craniomaxillofac Surg 2003 (in press).
98. Wiesmann HP, Cm L, Stratmann U, Plate U, Fuchs H, Joos U, Hohling HJ. Sutural mineralization of rat calvaria characterized by atomic-force microscopy and transmission electron microscopy. Cell Tissue Res 1998: **294**: 93–97.
99. Wolff D, Goldberg VM, Stevenson S. Histomorphometric analysis of the repair of a segmental diaphyseal defect with ceramic and titanium fibermetal implants: Effects of bone marrow. J Orthop Res 1994: **12**: 439–446.
100. Wojciak-Stothard B, Curtis ASG, Monaghan W, Sommer I, Wilkinson CDW. Role of the cytoskeleton in the reaction of fibro-blasts to multiple grooved substrata. Cell Motil Cytoskeleton 1995: **31**: 147–158.
101. Wojciak-Stothard B, Curtis A, Monaghan W, Macdonald K, Wilkinson C. Guidance and activation of murine macrophages by nanometric scale topography. Exp Cell Res 1996: **223**: 426–435.
102. Wojciak-Stothard B, Denyer M, Mishra M, Brown RA. Adhesion, orientation and movement of cells cultured on ultrathin fibronectin fibers in vitro. Cell Dev Biol Anim 1997: **33**: 110–117.
103. Wu LN, Yoshimori T, Genge BR, Sauer GR, Kirsch T, Ishikawa Y, Wuthier RE. Characterization of the nucleational core complex responsible for mineral induction by growth plate cartilage matrix vesicles. J Biol Chem 1993: **268**: 25084–25094.
104. Wu LN, Ishikawa Y, Sauer GR, Genge BR, Mwale F, Mishima H, Wuthier RE. Morphological and biochemical characterization of mineralizing primary cultures of avian growth plate chondrocytes: Evidence for cellular processing of Ca^{2+} and Pi prior to matrix mineralization. J Cell Biochem 1995: **57**: 218–237.
105. Zreiqat H, Howlett CR. Titanuim substrata composition influences osteoblastic phenotype: In vitro study. J Biomed Mater Res 1999: **47**: 360–366.

Address:
Ulrich Meyer
Department of Cranio-Maxillofadal Surgery
University of Munster
Waldeyerstr. 30
D-48149 Munster
Germany.
Tel.: +49-251-83-47-201
Fax: +49-251-83-47-203
E-mail: ulmeyer@uni-muenster.de