Tissue engineering of bone:
the reconstructive surgeon's point of view

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Abstract

Bone defects represent a medical and socioeconomic challenge. Different types of biomaterials are applied for reconstructive indications and receive rising interest. However, autologous bone grafts are still considered as the gold standard for reconstruction of extended bone defects. The generation of bioartificial bone tissues may help to overcome the problems related to donor site morbidity and size limitations. Tissue engineering is, according to its historic definition, an "interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function". It is based on the understanding of tissue formation and regeneration and aims to rather grow new functional tissues than to build new spare parts. While reconstruction of small to moderate sized bone defects using engineered bone tissues is technically feasible, and some of the currently developed concepts may represent alternatives to autologous bone grafts for certain clinical conditions, the reconstruction of large-volume defects remains challenging. Therefore vascularization concepts gain on interest and the combination of tissue engineering approaches with flap prefabrication techniques may eventually allow application of bone-tissue substitutes grown in vivo with the advantage of minimal donor site morbidity as compared to conventional vascularized bone grafts. The scope of this review is the introduction of basic principles and different components of engineered bioartificial bone tissues with a strong focus on clinical applications in reconstructive surgery. Concepts for the induction of axial vascularization in engineered bone tissues as well as potential clinical applications are discussed in detail.

Keywords: tissue engineering • bone replacement • vascularization • flap prefabrication • microsurgery • AV loop

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Introduction

Function and structure of bone

Bone is a dynamic, highly vascularized tissue with the unique capacity to heal and remodel without leaving a scar [1, 2]. It provides mechanical stability to the skeleton that is needed for load bearing, locomotion and protection of internal organs. Furthermore bone serves as a mineral reservoir and has the capacity to rapidly mobilize mineral stores if needed for homeostasis of the calcium blood level. The diversity of functional requirements of bone tissue is reflected by its complex architecture. In the adult skeleton bone tissue is either arranged in a trabecular pattern (cancellous bone) or in a compact pattern (cortical bone) [3]. Cortical bone is almost solid with less than 10% porosity and ubiquitously present in long, short and flat bones. In contrast, cancellous bone is organized in a porous sponge-like pattern. This type of bone harbors a large part of the bone marrow and is essentially present in the metaphysis of long bones, the iliac crest and the vertebral bodies [3].

Components of bone tissue

Although bone tissue is populated by a variety of different cells, its functional integrity is maintained by three different cell types: osteoblasts, osteocytes and osteoclasts [2, 4, 5]. These tissue-specific cells are embedded in a highly complex matrix that consists of a mineralized (hydroxylapatite) and a non-mineralized component [6, 7]. The non-mineralized, organic part contains collagens, glycoproteins, proteoglycans and sialoproteins that play an essential role in control of growth and differentiation of osteoblasts, osteocytes and osteoclasts and in bone remodeling [7–10]. Bone development and bone regeneration are complexly regulated processes that involve a plethora of different growth and transcription factors which coordinate the interaction of cells and matrix in response to external or internal stimuli [9, 11–20].

Bone regeneration

Bone regeneration is a highly efficient and tightly regulated process that involves all the above-mentioned components of bone tissue. Bone regeneration is the result of a continuous interplay between growth factors and cytokines for both initiation and regulation of the remodeling process. [7, 17, 21]. The majority of fractures heal well under standard conservative or surgical therapy. However, extended bone defects following trauma or cancer resection or non-unions of fractures may require more sophisticated treatment. In these cases bone grafting procedures, segmental bone transport, distraction osteogenesis or biomaterials are applied for reconstruction [22–25].

Established treatment of bone defects

Bone grafts

Today, autologous bone grafting is the gold standard for osteogenic bone replacement in osseous defects [26]. Autologous bone grafts reliably fill substance deficits and induce bone tissue formation at the defect site following transplantation. These grafts exhibit, depending on donor site, size, shape and quality, some initial stability. Chips, larger pieces and even blocks of several centimeters in size could be harvested. The use of other types of bone substitutes in combination with autologous bone grafts decreases the amount of bone tissue needed for reconstruction [27]. However, the clinical use of autologous osseous transplants is limited by a considerable donor site morbidity that increases with the amount of harvested bone. Bleeding, hematoma, infection, and chronic pain are common complications of bone graft harvest [28–30]. Processed allogenic or xenogenic bone grafts are also commonly used for repair of osseous defects when autologous transplantation is not applicable [26, 31–33]. Although the initial properties of allogenic or xenogenic grafts resemble those of autologous bone in terms of biomechanic stability and elasticity, the lack of osteogenicity represents a limitation even when osteoinductive factors are preserved during processing.

For specific indications vascularized bone grafts from different locations such as fibula, scapula, iliac crest and others are taken and transplanted into given bone defects using microsurgical techniques [34-36]. Large tissue defects with exposed structures like, bones, joints, tendons and nerves in regions of compromised perfusion do require a tissue transplant which brings good vascularity into

the affected zone and positively affects healing in the broader sense of the nutrient flap. However, free bone tissue transfer is associated with donor site morbidity [37]. Furthermore, availability is limited in quality and quantity.

Biomaterials

A variety of different biomaterials is currently being used for reconstruction of bone defects. Acrylate-based bone cements provide, after polymerization a high mechanical stability [38, 39]. They are widely used for fixation of total joint prosthesis, vertebroplasty and for craniofacial bone defects; they may be loaded with antibiotics for local drug delivery. However, despite sophisticated modes of application they do not possess osteogenic or osteoinductive properties and are, when at all, slowly resorbed. The long-term integration of non-porous bone cements into bone defects is not always warranted. Within the last two decades, many other biogenic and synthetic materials were evaluated for their use as bone substitutes. Calcium phosphate- and apatite-based bone cements, porous composites as well as other types of biomaterials have been clinically applied for treatment of fractures and bone defects [40–42]. They are, depending on their chemical composition and porosity osteoconductive, biodegradable and are integrated into given bone defects. However, in general terms their biomechanic stability is significantly lower in comparison to acrylate-based implants.

Osteoinductive substances

Although osteoinductive substances are clinically applied for reconstruction of bone defects or for acceleration of fracture healing, only small numbers of patients have been treated and application modes and indications are not completely standardized yet. Platelet rich plasma contains, besides platelet derived growth factor (PDGF), depending on processing and application modes, a variety of different growth factors [43–45]. It enhanced bone formation in experimental and clinical settings. Demineralized bone matrix (DBM) is prepared from allogenic or xenogenic bone and commercially available for clinical application in different formulations [46–49]. Its osteoinductive potential is

highly variable and depends not only on the donor but also on the processing protocols. DBM is commonly used in combination with other types of biomaterials [50]. Bone morphogenetic proteins (BMPs) have been identified as the most relevant osteoinductive factor in demineralized bone matrix [17, 51]. Right now there are two types of BMPs being clinically applied [52] [53–55]. Application of BMP 2 in open tibial fractures significantly improved bone healing in comparison to a conventionally treated control group in a randomized study [55]. However, heterotopic ossification, suboptimal release kinetics, and last but not least the high price pose a challenge to widespread application of these substances.

Tissue engineering

Definition

Tissue Engineering is a young field of research. Initially, it was defined as "… an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [56]. Tissue Engineering is based on the profound understanding of embryology, tissue formation and regeneration and aims to growing new functional tissues rather than building new spare parts. As mentioned above, it combines integral knowledge from physicists, chemists, engineers, material scientists, biologists and physicians to a comprehensive interdisciplinary approach. Tissue Engineering is tightly associated with the field of regenerative medicine.

Basic principles

How to grow new tissues? Independent of the type of tissue, cells, extracellular matrix, blood vessels, nerves, intercellular communication and cell-matrix interaction are only some "ingredients" to grow new tissues *in vivo*. These single components have to be combined in a well coordinated spatial and time dependent fashion. Besides the above-mentioned "ingredients", well elaborated surgical concepts are a prerequisite for successful *in vivo* application of tissue engineering concepts. Caution is needed with increas-

ing complexity of the engineered tissue composites, to prevent damage of the susceptible cells or bioactive substances during implantation. Depending on the specific concept, a facultative *in vitro* period prior to implantation may vary from 1 day up to several months. This period is commonly used for cell expansion or induction of tissue-specific cell differentiation. In the experimental setting Tissue Engineering concepts have been successfully applied to generate many different types of tissues such as bone, cartilage, liver, muscle, skin and others [57–62].

Properties of bioartificial bone tissues

The intended clinical use defines the desired properties of engineered bone substitutes. Defects of load-bearing long bones, for instance, require constructs with high mechanic stability whereas initial plasticity is not essential. On the other hand for craniofacial applications, initially injectable or moldable constructs are favorable. Although stable integration of the implants is imperative, the mechanic loads involved are not as high as in the former situation. Depending on the implantation site, initial vascularization may be essential for enhanced engraftment and prevention of infections. Mechanical stability, osteoconductivity (*i.e.* the capacity of a material to guide bone forming tissue into a defect [63]), osteoinductivity (*i.e.* the ability to induce bone formation by attracting and stimulating bone-forming cells of the recipient [64]), osteogenicity (*i.e.* the capability to form bone tissue *de novo* [65]), and ease of handling have to be well balanced in order to properly meet the clinician's needs.

Components of bioartificial bone

Tissues scaffolds

Any vital tissue consists of matrix and cells. The matrix acts as a biological three-dimensional scaffold for cells within tissues and provides cells with the tissue-specific environment and architecture [8]. Physiologically it serves as a reservoir for water, nutrients, cytokines and growth factors and allows cells to attach to it. For tissue engineering purposes, three main components could be extracted from this complex network of different interconnected functions: Mechanic support, cell attachment and participation in cellular communication pathways. A vast variety of materials has been used as matrix for bone tissue engineering applications [66, 67]. Porosity, surface chemistry, topography, three-dimensional architecture, immunogenicity and mechanic parameters are matrix properties which significantly influence formation of bone within bioartificial bone substitutes [68]. Gel-like matrices such as fibrin have been used for cell immobilization in combination with other scaffolds [69]. While a variety of highly innovative matrices are under *in vitro* and *in vivo* evaluation, clinically established and approved biomaterials are readily available for first applications of bioartificial bone tissues [70–77]. Computer assisted design – computer assisted manufacturing (CAD/CAM) and rapid prototyping techniques allow the generation of custom-made scaffolds for cell delivery that fit into certain bone defects [77, 78].

Osteogenic cells

Osteogenic cells are an integral part of any tissue engineering strategy. These cells are either transplanted along with the appropriate scaffolds into the bone defect or attracted from the host by osteoinductive factors. Osteogenic cells are not a homogenous cell population. A differentiation pathway similar to that of hematopoetic cells has been postulated [79, 80]. The affectors of bone remodeling, regeneration and fracture repair in the adult organism are the cellular components. Today it is still unknown which type of osteogenic cell will be the one most suitable for engineering of bone tissue. Mesenchymal stem cells, Bone marrow stromal cells, periosteal cells and osteoblasts have been successfully used for the generation of bone tissue [4, 75, 76, 81–83]. Isolation and expansion efficiency, stability of osteoblastic phenotype, *in vivo* bone formation capacity, and long-term safety are essential requirements that have to be met by any type of osteogenic cell for successful clinical application. Serum-free culture conditions or culture medium supplemented with autologous serum are preferable for cell expansion *in vitro*.

Recently, clinical applications of tissue engineered bone have been reported. Quarto *et al.* treated three patients with large bone defects of the tibia, ulna, and humerus, using macro porous hydroxyap-

atite scaffolds seeded with autologous *in vitro* expanded bone marrow stromal cells immobilized in collagen gel. All patients recovered limb function without major complications. Vacanti and coworkers reconstructed a patient's thumb using periosteal cells and a coral-based hydroxyl apatite scaffold [84]. Although the patient did well after implantation and hand function recovered significantly, quantitative histomorphometric analysis of a biopsy revealed that eventually only 5% of the implant volume was bone. The outcome of this clinical trial was not superior to that of conventional reconstructive approaches [85]. Periosteal cell-seeded polymer fleeces or mesenchymal stem cells and platelet-rich plasma, immobilized in beta-tricalcium phosphate scaffolds induced bone formation in sinus lift operations [86–88]. Despite anecdotal reports of successful implantation of engineered bone tissues, the small number of patients makes it difficult to eventually assess the efficacy of these constructs and comparison with conventional methods has not been performed yet.

Osteoinductive factors and gene transfer

Osteogenic substances augment the osteogenic capacity of tissue engineered bone constructs. They are either applied as a crude and hardly standardized mixture of proteins as described above (demineralized bone matrix and platelet rich plasma) or as isolated factors [21, 44, 47]. Osteoinductive growth factors have the capability to modulate proliferation and differentiation of implanted osteogenic cells. Furthermore these substances are able to attract precursor cells from the host to invade scaffold and induce osteoblastic differentiation. There is a large number of such proteins that stimulate proliferation and/or differentiation of osteogenic cells *in vitro* and *in vivo*. Some osteogenic factors have been cloned and are commercially available as recombinant proteins. Bone morphogenetic proteins (BMPs) are upon the most potent osteoinductive factors. BMPs belong to the TGF-β family and bind to extracellular matrix components such as heparan sulfate and type IV collagen [89, 90]. BMP2 and BMP-7 are being clinically applied for fractures and non-unions, but only a limited number of patients have been treated so far with long term follow up still pending [54, 55]. The effective *in vivo* use of isolated osteoinductive growth factors requires optimized pharmacokinetics. Intelligent delivery systems have proven to be of cru-

cial importance for reliable bone formation and economic application of BMPs [91]. Without controlled release systems, growth factors rapidly diffuse away from the constructs.

Gene therapeutic approaches potentially promote pertinent function of osteogenic cells and enhance performance of bioartificial bone tissues [92-95]. Expression systems for osteoinductive growth factors are therefore of high interest. There are different strategies to bring DNA sequences into cells. Viral vectors provide high transfection efficiency and some viruses even allow stable transfection of osteogenic cells [96]. Clinical use in tissue engineering concepts is however hampered by safety concerns. Non-viral gene transfer is achieved by either using transfection reagents, or by mediator-free techniques [97]. Matrix-mediated gene transfer is another innovative approach that avoids the use of any transfection reagent or potentially harmful vector [98]. In conclusion, gene transfer strategies may provide for efficient stimulation of bone formation within bioartificial bone tissues, but further insight into long-term effects, phenotypical stability and application techniques need to precede any wide-spread application in humans.

Vascularization of engineered bone tissues

Adequate vascularization is a prerequisite for formation of high quality bone. When *in vitro* engineered cellular constructs are transferred *in vivo* they have to rely on processes like interstitial fluid diffusion and blood perfusion. Here recites a core limitation for transfer of tissue engineering models from the *in vitro* to the *in vivo* environment. Diffusion is the initial process involved, but it can only provide for cell support within a maximum range of 200 μm into the matrix [99, 100]. The survival of cells in the center of large cell-containing constructs is therefore often limited by suboptimal initial vascularization [57]. Cell labeling experiments disclosed a considerable loss of osteoblasts within the first week following transplantation in porous cancellous bone matrices [101]. For this reason induction of vascularization is an integral element of any successful bone tissue engineering concept.

Angioinductive growth factors

Angiogenesis is a complexly regulated process [102–104]. Several mechanisms of regulation are involved throughout the angiogenetic cascade of events; the endothelial cell acting as the main mediator of neovascular growth is guided through space and time. A large number of angiogenic factors work together in a highly coordinated manner to induce endothelial cell outgrowth and the formation of functional vessels. These factors are promising tools for induction and acceleration of vascularization processes in three-dimensional scaffolds [105, 106]. VEGF and bFGF have been successfully used to improve vascularization of engineered tissues [107]. Immobilization of angiogenetic growth factors, for instance in fibrin gels or by using heparine-binding release systems, allows for optimized release kinetics and longer lasting effects [108, 109].

Endothelial cells

Endothelial cells, either derived from microvasculature, umbilical veins or large blood vessels, have been used for generation of capillary-like structures and vessles *in vitro* by different groups [110–112]. *In vivo* these cells are supposed to form networks of capillaries and gain access to the recipient's circulation [113]. Although the newly formed vascular networks display, depending on the experimental setting, a differentiated morphology, the processes that occur upon implantation are not completely understood at the moment and there are only scarce *in vivo* data on the efficiency of this approach with regard to enhance and accelerate vascularization of bioartificial tissues. Since cell-containing constructs require immediate supply with nutrients and oxygen after implantation and even with synchronous transplantation of endothelial cells some time is needed for formation of anastomoses between newly formed capillaries and the recipient's circulation, the potential benefits of endothelial cell transplantation for induction of vascularization are questionable [114].

Local factors at the recipient site

Local factors play a prominent role in tissue engineering concepts since the quality of the tissue at the recipient site influences vascularization of the scaffolds, cell engraftment and eventually bone formation. Neovascularization from the surrounding tissues, which is responsible for long-term survival of transplanted osteogenic cells, is a slow process so that constructs pre-seeded with tissue specific cells need to be sufficiently thin to ensure rapid vascularization and cell survival [115]. Even a perfectly engineered piece of tissue will fail to form bone when the local environment is inappropriate. In a clinical setting, for instance in cases of large bone defects following trauma or osteomyelitis, bacterial load and chronic scarring pose even more challenge to the transplanted constructs. Plastic surgical concepts may help to bridge the gap between perfectly *in vitro* engineered bone tissues and the sometimes disappointing *in vivo* performance.

Surgical angiogenesis

The majority of the above-mentioned tissue engineering approaches rely on the so called "extrinsic" mode of neovascularization [116]. The neovascular bed originates from the periphery of the construct which should be implanted into a site of high vascularization potential. Subcutaneous [69], intramuscular [117], and intraperitoneal [57] implantation has been reported. Although generation of vascularized bone tissue is feasible using this technique, this tissue is vascularized in a random pattern. Transfer to distant implantation sites is impossible without destruction of the vascular network. Reconstructive surgeons aim therefore to generate so called "axially vascularized" tissues that could be transferred to the defect site using microsurgical techniques of vascular anastomosis. These tissues are immediately vascularized upon implantation into the defect as free flaps do. In the following section different techniques for the induction of axial vascularization in bone grafts are discussed in detail.

Flap prefabrication and bone grafts

Conventional osteomyocutaneous flaps do not always meet the requirements of a composite defect. A prefabricated composite flap can be created according to the complex geometry of the defect. Prefabrication of multi component flaps is a well established procedure in plastic surgery [118–120]. This concept is based on the revascularization phenomenon directly related to host tis-

sue vascularity [121] and significantly expanded the frontiers of reconstructive surgery. Tissue prefabrication is essentially a method comprised by two steps. During the first procedure a tissue component is formed into the wanted shape and is thereupon implanted into a region with a vascular axis suitable for microsurgical transfer. During the second stage the autologous implant is harvested en-bloc with the surrounding tissue and the vascular pedicle as a free flap. The implant acquires its vascularization from the tissue block and the flap is connected to the local circulation by means of a microvascular anastomosis.

Prefabrication allows the transfer of preferred tissue composites suitable for reconstruction regardless of their native vascular origin as free or pedicled flaps and helps to reduce donor site morbidity [122]. Prefabrication of bone flaps using plastic surgical techniques may help to circumvent preceding problems of microsurgical bone transfer and to obtain previously non-existing tissue units that meet exactly the specific recipient site needs. Basically there are two strategies for flap prefabrication: either a bone graft is wrapped in axially vascularized tissues (cutaneous, fascioucutaneous or muscle flaps) or a vascular axis is implanted into the bone graft. The latter type of vascularization is called "intrinsic". In the "intrinsic" mode of vascularization the construct acquires an inherent perfusion and does not have to rely on favourable local conditions. This is achieved by inducing angiogenesis from a centrally located vascular axis. The configuration of the vascular axis could vary according to the reconstructive requirements [123]. After a facultative vascularization period the vascularized graft is transferred into the bone defect using conventional or microsurgical techniques. Prefabricated bone grafts have been clinically applied in different settings [124, 125].

Generation of vascularized bioartificial bone tissue

Flap prefabrication using conventional bone grafts allows generation of new types of flaps independent of the vascular anatomy of the bone transplant. However, the donor site morbidity after harvesting of the bone grafts is still a problem. Recently, biomaterials, osteogenic cells and osteoinductive growth factors have been used for generation of vascularized bone tissues in combi-

nation with a vascular axis or vascularized flaps. Revascularization of scaffolds is induced by an inflammatory wound healing response as a reaction to the surgical implantation. This, combined with the hypoxia within the implant evokes local expression of angiogenetic growth factors. Bone formation beneath "standard flaps" has been successfully induced using bioceramics seeded with autologous bone marrow stromal cells [126]. Induction of axial vascularization protected porous biomaterials from bacterial infection and transfer of this vascularized hard tissue as a free flap has been demonstrated [127]. Pedicled bone flaps based on collagen I scaffolds, bone marrow stromal cells and a PTFE membrane have been successfully generated using the carotid artery and jugular vein or the saphenous bundle as vascular axis [128]. Prefabricated vascularized bone grafts have even be used in a clinical setting for mandibular reconstruction following thorough *in vivo* evaluation in a pig model. This group buried granules of xenogenic bone minerals soaked with recombinant OP-1 in the latissimus dorsi muscle and transferred the neo-tissue using microsurgical techniques into mandibular defects [129, 130]. In the clinical setting a titanium cage was custom made according to CT scan and 3D reconstruction data, filled with bone marrow aspirate, xenogenic bone minerals and OP-1, and a large mandibular defect was successfully reconstructed following a prefabrication period of 7 weeks [131].

"Free-Style" vascularized bioartificial bone grafts

In the last decade the field of surgically induced angiogenesis has tremendously developed. Induction of "intrinsic" vascularization in biomaterials allowed the generation of vascularized tissues at different sites for transfer as pedicled or free flaps in several animal models. Although a number of vascular axes are available for intrinsically vascularized tissue compounds in humans, there remain limitations with regard to pedicle length and anatomic location. The creation of a vascular axis using vein grafts holds promise for generation of vascularized bone units relatively independent of anatomical limitations. Erol and Spira managed to produce a prefabricated skin flap by means of an arteriovenous vessel - loop using either artery or vein grafts in a rat model [132]. They observed an
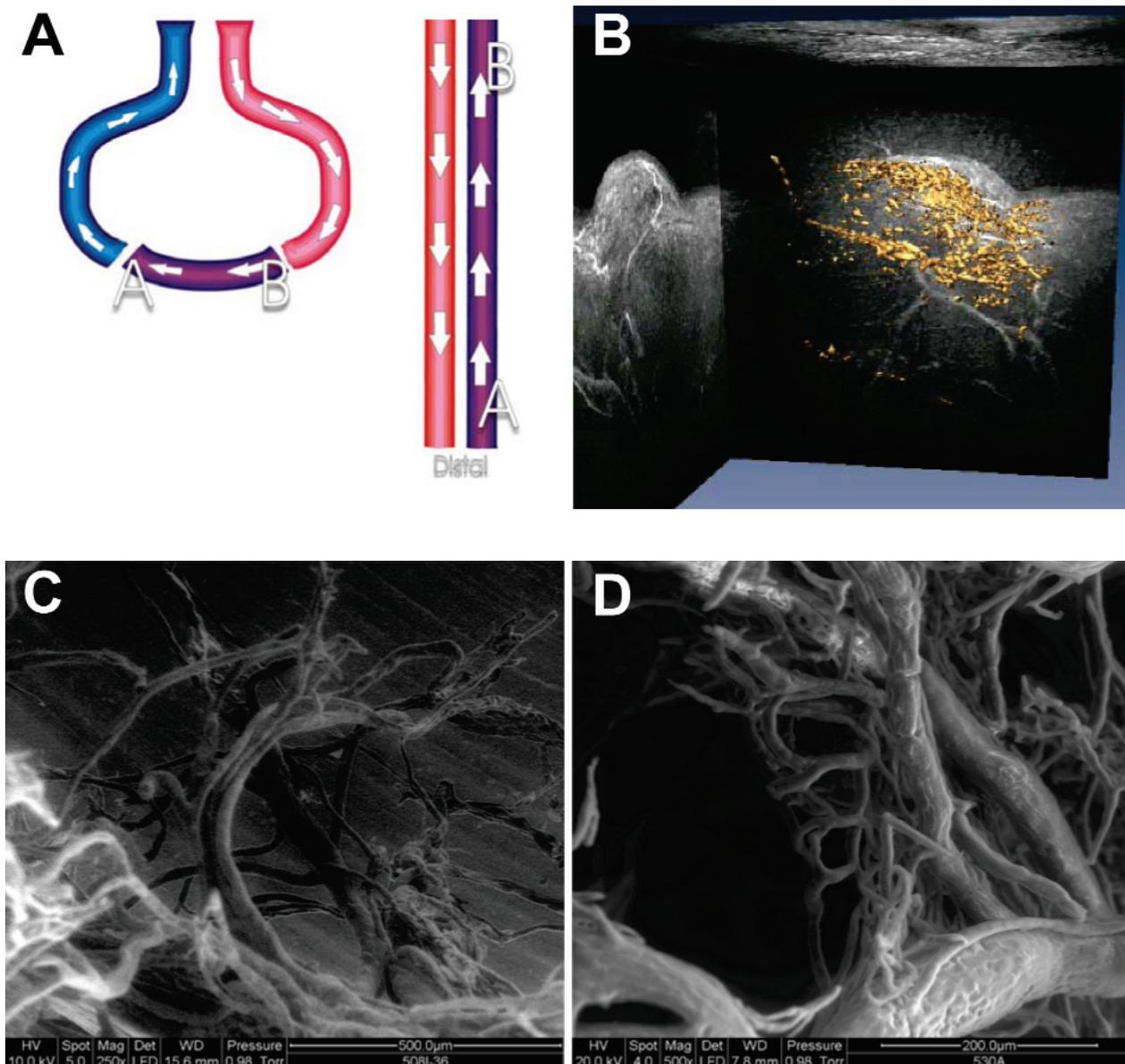
Fig. 1 Vascularization of porous matrices by implanting an arteriovenous loop. A. The arteriovenous loop consisting of an isolated artery and vein, and a vein graft of variable length is constructed using microsurgical techniques. In the rat model the loop is constructed between the femoral artery and vein. B. The significant degree of vascularity in the axially vascularized bioartificial tissue is demonstrated by intravital MRI angiography. High flow rates are highlighted by yellow staining. C&D. Differentiated morphology of the vascular system originating from the arteriovenous loop is demonstrated by sanning electron microscopy of vascular corrosion replicas. Vessels of different calibers invade the porous matrix and form a vascular bed for subsequent injection of osteogenic cells.

abundant neovascular outgrowth originating from the entire AV Loop. Morrison and co-workers augmented this model and implanted the AV-loop into polycarbonate isolation chambers. Furthermore they induced vascularization in polymer matrices and managed to generate large volumes of axially vascularized tissue [133–135]. In the era of tissue engineering this concept met with great interest. Recently, our group vascularized successfully a custom-made processed bovine cancellous bone matrix by means of an arteriovenous loop [136]. Meticulous analysis of the angiogenetic response by morphometry, high resolution
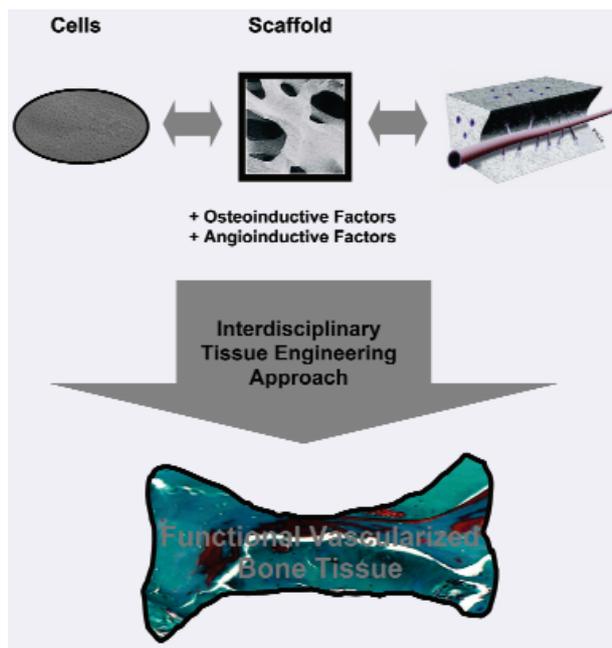
Fig. 2 Joint approaches are needed for efficient transfer of larger volume bone implants.

micro MRI and vascular corrosion casts revealed a vascularization of more than 90% of the scaffold within 8 weeks following implantation [Fig. 1]. The amount of vascularization was comparable with extrinsically vascularized control matrices; however, the inflammatory reaction was considerably less pronounced in the AV loop group [137]. Furthermore, prevascularization of porous hard tissue scaffolds by means of an arteriovenous loop significantly increased the number of initially engrafted osteoblasts in preliminary experiments [138]. Bone formation following osteoblast injection is right now being evaluated over a longer observation period in the same model.

Conclusion

Tissue engineering is a fascinating field of research and is bound to dramatically change clinical practice in reconstructive surgery. Osteogenic cells for bioactive implants are readily available following minimally invasive harvesting and *ex vivo* expansion. A plethora of highly innovative biomaterials with well tuned matrix properties have been developed to perfectly

meet the clinician's requirements. Osteoinductive substances may further enhance bone formation within engineered composites. Optimized implantation techniques are one more essential step towards clinical application of engineered bioartificial bone tissues. The answers towards upscaling of bioartificial devices will be delivered by advances in the area of vascularization. In the future, joint approaches are needed in order to transfer highly potent bioartificial osteogenic bone tissues into the demanding *in vivo* environment [Fig. 2]. Close cooperation between tissue engineers and reconstructive surgeons may eventually help to bridge the gap between bench and bedside.

Acknowlegments

References

1. **Buckwalter JA, Glimcher MJ, Cooper RR, Recker R**. Bone biology. II: Formation, form, modeling, remodeling, and regulation of cell function. *Instr Course Lect.* 1996; 45: 387–99.
2. **Buckwalter JA, Glimcher MJ, Cooper RR, Recker R**. Bone biology. I: Structure, blood supply, cells, matrix, and mineralization. *Instr Course Lect.* 1996; 45: 371–86,.
3. **Ackerman LV, Spjut HJ, Abell MR**, Bones and Joints (Monographs in Pathology). Baltimore: Williams and Wilkins; 1976.
4. **Aubin JE**. Bone stem cells. *J Cell Biochem Suppl* 1998; 30-31: 73–82.
5. **Owen M**. The origin of bone cells. *Int Rev Cytol.* 1970; 28: 213–38.
6. **Heinegard D, Oldberg A**. Structure and biology of cartilage and bone matrix noncollagenous macromolecules. *FASEB J* 1989; 3: 2042–51.
7. **Robey PG, Fedarko NS, Hefferan TE, Bianco P, Vetter UK, Grzesik W, Friedenstein A, Van der PG, Mintz KP, Young MF.** Structure and molecular regulation of bone matrix proteins. *J Bone Miner Res.* 1993; 2: S483–7.
8. **Huang S, Ingber DE**. The structural and mechanical complexity of cell-growth control. *Nat Cell Biol.* 1999; 1: E131–8.
9. **Lian JB, Stein GS**. Development of the osteoblast phenotype: molecular mechanisms mediating osteoblast growth and differentiation. *Iowa Orthop J.* 1995; 15: 118–40.
10. **Nomura S, Takano-Yamamoto T**. Molecular events caused by mechanical stress in bone. *Matrix Biol.* 2000; 19: 91-6.
11. **Probst A, Spiegel HU**. Cellular mechanisms of bone repair. *J Invest Surg.* 1997; 10: 77–86.

12. **Olsen BR, Reginato AM, Wang W**. Bone development. *Annu Rev Cell Dev Biol.* 2000; 16: 191–220.

13. **Stein GS, Lian JB, Owen TA**. Relationship of cell growth to the regulation of tissuespecific gene expression during osteoblast differentiation, *FASEB J.* 1990; 4: 3111–23.

14. **Mundy GR**. Regulation of bone formation by bone morphogenetic proteins and other growth factors, *Clin Orthop Relat Res.* 1996; 324: 24–8.

15. **Stein GS, Lian JB, Stein JL, Van Wijnen AJ, Montecino M**. Transcriptional control of osteoblast growth and differentiation. *Physiol Rev.* 1996; 76: 593–629.

16. **Ducy P, Karsenty G**. Genetic control of cell differentiation in the skeleton. *Curr Opin Cell Biol.* 1998; 10: 614–9.

17. **Reddi AH**. Initiation of fracture repair by bone morphogenetic proteins. *Clin Orthop Relat Res.* 1998; 355: S66–72.

18. **Ducy P**. Cbfa1: a molecular switch in osteoblast biology. *Dev Dyn.* 2000; 219: 461–71.

19. **Karsenty G**. Minireview: transcriptional control of osteoblast differentiation. *Endocrinology* 2001; 142: 2731–3.

20. **Urist MR, DeLange RJ, Finerman GA**. Bone cell differentiation and growth factors. *Science* 1983; 13,220: 680–6.

21. **Lieberman JR, Daluiski A, Einhorn TA**. The role of growth factors in the repair of bone. Biology and clinical applications. *J Bone Joint Surg Am.* 2002; 84-A: 1032–44.

22. **Perry CR**. Bone repair techniques, bone graft, and bone graft substitutes. *Clin Orthop Relat Res.* 1999; 360: 71–86.

23. **Gugenheim JJ Jr.** The Ilizarov method. Orthopedic and soft tissue applications, *Clin Plast Surg.* 1998; 25: 567–78.

24. **Motoki DS, Mulliken JB**. The healing of bone and cartilage. *Clin Plast Surg.* 1990; 17: 527–44.

25. **Polykandriotis E, Stangl R, Hennig HH, Lennerz JK, Frank WM, Loos MD, Horch RE**. The composite vastus medialis-patellar complex osseomuscular flap as a salvage procedure after complex trauma of the knee—an anatomical study and clinical application. *Br J Plast Surg.* 2005; 58: 646–51.

26. **Gazdag AR, Lane JM, Glaser D, Forster RA**. Alternatives to autogenous bone graft: efficacy and indications. *J Am Acad Orthop Surg.* 1995; 3: 1–8.

27. **Sassard WR, Eidman DK, Gray PM, Block JE, Russo R, Russell JL, Taboada EM**. Augmenting local bone with Grafton demineralized bone matrix for posterolateral lumbar spine fusion: avoiding second site autologous bone harvest, *Orthopedics* 2000; 23: 1059–64.

28. **Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA**. Complications of iliac crest bone graft harvesting. *Clin Orthop Relat Res*. 1996; 329: 300–9.

29. **Banwart JC, Asher MA, Hassanein RS**. Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. *Spine* 1995; 1,20: 1055–60.

30. **Ebraheim NA, Elgafy H, Xu R**. Bone-graft harvesting from iliac and fibular donor sites: techniques and complications. *J Am Acad Orthop Surg.* 2001; 9: 210–8.

31. **Lobo Gajiwala A, Agarwal M, Puri A, D'Lima C, Duggal A**. Reconstructing tumour defects: lyophilised, irradiated bone allografts. *Cell Tissue Bank* 4: 109–18, 2003.

32. **Aho AJ, Ekfors T, Dean PB, Aro HT, Ahonen A, Nikkanen V**. Incorporation and clinical results of large allografts of the extremities and pelvis. *Clin Orthop Relat Res.* 1994; 307: 200–13.

33. **Cetiner S, Esen E, Ustun Y, Oztunc H, Tuncer I**. Long-term results of the application of solvent-dehydrated bone xenograft and duramater xenograft for the healing of oroantral osseous defects: a pilot experimental study. *Dent Traumatol.* 2003; 19: 30–5.

34. **Swartz WM, Banis JC, Newton ED, Ramasastry SS, Jones NF, Acland R**. The osteocutaneous scapular flap for mandibular and maxillary reconstruction. *Plast Reconstr Surg.* 1986; 77: 530–45.

35. **Shea KG, Coleman DA, Scott SM, Coleman SS, Christianson M**. Microvascularized free fibular grafts for reconstruction of skeletal defects after tumor resection. *J Pediatr Orthop.* 1997; 17: 424–32.

36. **Ozaki T, Hillmann A, Wuisman P, Winkelmann W**. Reconstruction of tibia by ipsilateral vascularized fibula and allograft. 12 cases with malignant bone tumors. *Acta Orthop Scand.* 1997; 68: 298–301.

37. **Babovic S, Johnson CH, Finical SJ**. Free fibula donor-site morbidity: the Mayo experience with 100 consecutive harvests. *J Reconstr Microsurg.* 2000; 16: 107–10.

38. **Lewis G**. Properties of acrylic bone cement: state of the art review, *J Biomed Mater Res.* 1997; 2: 155–82.

39. **Muh E, Zimmermann J, Kneser U, Marquardt J, Mulhaupt R, Stark B**. Lysineurethanedimethacrylate—a novel generation of amino acid based monomers for bone cements and tissue repair. *Biomaterials* 2002; 23: 2849–54.

40. **Zijderveld SA, Zerbo IR, van den Bergh JP, Schulten EA, ten Bruggenkate CM**. Maxillary sinus floor augmentation using a beta-tricalcium phosphate (Cerasorb) alone compared to autogenous bone grafts. *Int J Oral Maxillofac Implants.* 2005;20: 432–40.

41. **Cassidy C, Jupiter JB, Cohen M, Delli-Santi M, Fennell C, Leinberry C, Husband J, Ladd A, Seitz WR, Constanz B**. Norian SRS cement compared with conventional fixation in distal radial fractures. A randomized study. *J Bone Joint Surg Am.* 2003;85-A: 2127–37.

42. **Jupiter JB, Winters S, Sigman S, Lowe C, Pappas C, Ladd AL, Van Wagoner M, Smith ST**. Repair of five distal radius fractures with an investigational cancellous bone cement: a preliminary report. *J Orthop Trauma.* 1997; 11: 110–6.

43. **Thorwarth M, Wehrhan F, Schultze-Mosgau S, Wiltfang J, Schlegel KA**. PRP modulates expression of bone matrix proteins in vivo without long-term effects on bone formation. *Bone* 2006; 38: 30–40.

44. **Sammartino G, Tia M, Marenzi G, di Lauro AE, D'Agostino E, Claudio PP**. Use of autologous platelet-rich plasma (PRP) in periodontal defect treatment after extraction of impacted mandibular third molars. *J Oral Maxillofac Surg.* 2005; 63: 766–70.

45. **Altmeppen J, Hansen E, Bonnlander GL, Horch RE, Jeschke MG**. Composition and characteristics of an autologous thrombocyte gel. *J Surg Res.* 2004; 117: 202–7.

46. **Moghadam HG, Sandor GK, Holmes HH, Clokie CM**, Histomorphometric evaluation of bone regeneration using allogeneic and alloplastic bone substitutes. *J Oral Maxillofac Surg.* 2004; 62: 202–13.

47. **Maddox E, Zhan M, Mundy GR, Drohan WN, Burgess WH**. Optimizing human demineralized bone matrix for clin-

ical application. *Tissue Eng.* 2000; 6: 441–8.

48. **Traianedes K, Russell JL, Edwards JT, Stubbs HA, Shanahan IR, Knaack D**. Donor age and gender effects on osteoinductivity of demineralized bone matrix. *J Biomed Mater Res B Appl Biomater.* 2004; 70: 21–9.

49. **Pietrzak WS, Perns SV, Keyes J, Woodell-May J, McDonald NM**. Demineralized bone matrix graft: a scientific and clinical case study assessment. *J Foot Ankle Surg.* 2005; 44: 345–53.

50. **Andreana S, Cornelini R, Edsberg LE, Natiella JR**, Maxillary sinus elevation for implant placement using calcium sulfate with and without DFDBA: six cases. *Implant Dent.* 2004; 13: 270–7.

51. **Reddi AH**. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. *Nat Biotechnol.* 1998; 16: 247–52.

52. **Einhorn TA**. Clinical applications of recombinant human BMPs: early experience and future development. *J Bone Joint Surg Am.* 2003; 85-A: 82–8.

53. **Kain MS, Einhorn TA**. Recombinant human bone morphogenetic proteins in the treatment of fractures. *Foot Ankle Clin*. 2005; 4: 639–50.

54. **Dimitriou R, Dahabreh Z, Katsoulis E, Matthews SJ, Branfoot T, Giannoudis PV**. Application of recombinant BMP-7 on persistent upper and lower limb nonunions, Injury 2005; 36: S51–9.

55. **Govender S, Csimma C, Genant HK, Valentin-Opran A, Amit Y, Arbel R, Aro H, Atar D, Bishay M, Borner MG, Chiron P, Choong P, Cinats J, Courtenay B, Feibel R, Geulette B, Gravel C, Haas N, Raschke M, Hammacher E, van der Velde D, Hardy P, Holt M, Josten C, Ketterl RL, Lindeque B, Lob G, Mathevon H, McCoy G, Marsh D, Miller R, Munting E, Oevre S, Nordsletten L, Patel A, Pohl A, Rennie W, Reynders P, Rommens PM, Rondia J, Rossouw WC, Daneel PJ, Ruff S, Ruter A, Santavirta S, Schildhauer TA, Gekle C, Schnettler R, Segal D, Seiler H, Snowdowne RB, Stapert J, Taglang G, Verdonk R, Vogels L, Weckbach A, Wentzensen A, Wisniewski T; BMP-2 Evaluation in Surgery for Tibial Trauma (BESTT) Study Group.** Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. *J Bone Joint Surg Am.* 2002; 84-A: 2123–34.

56. **Langer R, Vacanti JP**. Tissue engineering. *Science* 1993; 260: 920–6.

57. **Kneser U, Kaufmann PM, Fiegel HC, Pollok JM, Kluth D, Herbst H, Rogiers X**. Long-term differentiated function of heterotopically transplanted hepatocytes on threedimensional polymer matrices. *J Biomed Mater Res.* 1999; 47: 494–503.

58. **Kneser U, Schaefer DJ, Munder B, Klemt C, Andree C, Stark GB**. Tissue engineering of bone. *Min Invas Sur & Allied Technol.* 2002; 3: 107–16.

59. **Vacanti CA, Vacanti JP**. Bone and cartilage reconstruction with tissue engineering approaches. *Otolaryngol Clin North Am.* 1994; 27: 263–76.

60. **Stock UA, Vacanti JP**. Tissue engineering: current state and prospects. *Annu Rev Med.* 2001; 52: 443–51.

61. **Bach AD, Beier JP, Stern-Staeter J, Horch RE**. Skeletal muscle tissue engineering, *J Cell Mol Med.* 2004; 8: 413–22.

62. **Horch RE, Kopp J, Kneser U, Beier J, Bach AD**, Tissue engineering of cultured skin substitutes. *J Cell Mol Med.* 2005; 9: 592–608.

63. **Cornell CN, Lane JM**. Current understanding of osteoconduction in bone regeneration. *Clin Orthop Relat Res.* 1998; 355: S267–73.

64. **Hotz G, Herr G**. Bone substitute with osteoinductive biomaterials - current and future clinical applications. *Int J Oral Maxillofac Surg.* 1994; 23: 413–7.

65. **Cooper LF, Harris CT, Bruder SP, Kowalski R, Kadiyala S**. Incipient analysis of mesenchymal stem-cell-derived osteogenesis. *J Dent Res.* 2001; 80: 314–20.

66. **Shin H, Jo S, Mikos AG**. Biomimetic materials for tissue engineering. *Biomaterials* 2003; 24: 4353–64.

67. **Yang S, Leong KF, Du Z, Chua CK**. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Eng.* 2002; 8: 1–11.

68. **Logeart-Avramoglou D, Anagnostou F, Bizios R, Petite H**. Engineering bone: challenges and obstacles. *J Cell Mol Med.* 2005; 9: 72–84.

69. **Kneser U, Voogd A, Ohnolz J, Buettner O, Stangenberg L, Zhang YH, Stark GB, Schaefer DJ**. Fibrin gel-immobilized primary osteoblasts in calcium phosphate bone cement: *in vivo* evaluation with regard to application as injectable biological bone substitute. *Cells Tissues Organs.* 2005; 179: 158–69.

70. **Schantz JT, Teoh SH, Lim TC, Endres M, Lam CX, Hutmacher DW**. Repair of calvarial defects with customized tissue-engineered bone grafts I. Evaluation of osteogenesis in a three-dimensional culture system. *Tissue Eng.* 2003; 9: S113–26.

71. **Petite H, Viateau V, Bensaid W, Meunier A, de Pollak C, Bourguignon M, Oudina K, Sedel L, Guillemin G**. Tissue-engineered bone regeneration. *Nat Biotechnol.* 2000; 18: 959–63.

72. **Mankani MH, Kuznetsov SA, Fowler B, Kingman A, Robey PG**. *In vivo* bone formation by human bone marrow stromal cells: effect of carrier particle size and shape, *Biotechnol Bioeng.* 2001; 72: 96–107.

73. **Sikavitsas VI, van den Dolder J, Bancroft GN, Jansen JA, Mikos AG**. Influence of the *in vitro* culture period on the *in vivo* performance of cell/titanium bone tissueengineered constructs using a rat cranial critical size defect model. *J Biomed Mater Res A.* 2003; 67: 944–51.

74. **Timmer MD, Shin H, Horch RA, Ambrose CG, Mikos AG**. *In Vitro* cytotoxicity of injectable and biodegradable poly(propylene fumarate)-based networks: unreacted macromers, cross-linked networks, and degradation products. *Biomacromolecules* 2003; 4: 1026–33.

75. **Stangenberg L, Schaefer DJ, Buettner O, Ohnolz J, Moebest D, Horch RE, Stark GB, Kneser U**, Differentiation of osteoblasts in three-dimensional culture in porous cancellous bone matrix: quantitative analysis of gene expression based on real-time reverse transcription polymerase chain reaction. *Tissue Eng.* 2005; 11: 855–64.

76. **Groger A, Klaring S, Merten HA, Holste J, Kaps C, Sittinger M**. Tissue engineering of bone for mandibular augmentation in immunocompetent minipigs: preliminary study. *Scand J Plast Reconstr Surg Hand Surg.* 2003; 37: 129–33.

77. **Schantz JT, Hutmacher DW, Lam CX, Brinkmann M,**

Wong KM, Lim TC, Chou N, Guldberg RE, Teoh SH. Repair of calvarial defects with customised tissueengineered bone grafts II. Evaluation of cellular efficiency and efficacy *in vivo*. *Tissue Eng.* 2003; 9: S127–39.

78. **Yang S, Leong KF, Du Z, Chua CK**, The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques. *Tissue Eng.* 2001; 7: 679–89.

79. **Bruder SP, Fox BS**. Tissue engineering of bone. Cell based strategies. *Clin Orthop Relat Res.* 1999; 367: S68–83.

80. **Bruder SP, Caplan AI**. Terminal differentiation of osteogenic cells in the embryonic chick tibia is revealed by a monoclonal antibody against osteocytes. *Bone* 1990; 11: 189–98.

81. **Bruder SP, Fink DJ, Caplan AI**. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. *J Cell Biochem.* 1994; 56: 283–94.

82. **Aubin JE**. Advances in the osteoblast lineage. *Biochem Cell Biol.* 1998; 76: 899–910.

83. **Kuznetsov SA, Krebsbach PH, Satomura K, Kerr J, Riminucci M, Benayahu D, Robey PG**. Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation *in vivo*. *J Bone Miner Res.* 1997; 12: 1335–47.

84. **Vacanti CA, Bonassar LJ, Vacanti MP, Shufflebarger J**. Replacement of an avulsed phalanx with tissue-engineered bone. *N Engl J Med.* 2001; 344: 1511–4.

85. **Hentz VR, Chang J**. Tissue engineering for reconstruction of the thumb. *N Engl J Med.* 2001; 344: 1547–8.

86. **Ueda M, Yamada Y, Ozawa R, Okazaki Y**. Clinical case reports of injectable tissueengineered bone for alveolar augmentation with simultaneous implant placement. *Int J Periodontics Restorative Dent.* 2005; 25: 129–37.

87. **Schmelzeisen R, Schimming R, Sittinger M**. Making bone: implant insertion into tissue-engineered bone for maxillary sinus floor augmentation-a preliminary report. *J Craniomaxillofac Surg.* 2003; 31: 34–9.

88. **Schimming R, Schmelzeisen R**. Tissue-engineered bone for maxillary sinus augmentation. *J Oral Maxillofac Surg.* 2004; 62: 724–9.

89. **Reddi AH**. Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. *Tissue Eng.* 2000; 6: 351–9.

90. **Reddi AH**. Regulation of cartilage and bone differentiation by bone morphogenetic proteins. *Curr Opin Cell Biol.* 1992; 4: 850–5.

91. **Hollinger JO, Uludag H, Winn SR**, Sustained release emphasizing recombinant human bone morphogenetic protein-2. *Adv Drug Deliv Rev.* 1998;31: 303–18.

92. **Musgrave DS, Bosch P, Lee JY, Pelinkovic D, Ghivizzani SC, Whalen J, Niyibizi C, Huard J**, *Ex vivo* gene therapy to produce bone using different cell types. *Clin Orthop Relat Res.* 2000; 378: 290–305.

93. **Salyapongse AN, Billiar TR, Edington H**, Gene therapy and tissue engineering. *Clin Plast Surg.* 1999; 26: 663–76.

94. **Wu D, Razzano P, Grande DA**. Gene therapy and tissue engineering in repair of the musculoskeletal system. *J Cell Biochem.* 2003; 88: 467–81.

95. **Luo J, Sun MH, Kang Q, Peng Y, Jiang W, Luu HH, Luo Q, Park JY, Li Y, Haydon RC, He TC**. Gene therapy for bone regeneration. *Curr Gene Ther.* 2005; 5: 167–79.

96. **Chang SC, Chuang H, Chen YR, Yang LC, Chen JK, Mardini S, Chung HY, Lu YL, Ma WC, Lou J**. Cranial repair using BMP-2 gene engineered bone marrow stromal cells. *J Surg Res.* 2004; 119: 85–91.

97. **Nakashima S, Matsuyama Y, Nitta A, Sakai Y, Ishiguro N**. Highly efficient transfection of human marrow stromal cells by nucleofection. *Transplant Proc.* 2005; 37: 2290–2.

98. **Bonadio J, Smiley E, Patil P, Goldstein S**. Localized, direct plasmid gene delivery *in vivo*: prolonged therapy results in reproducible tissue regeneration. *Nat Med.* 1999; 5: 753–9.

99. **Goldstein AS, Juarez TM, Helmke CD, Gustin MC, Mikos AG**. Effect of convection on osteoblastic cell growth and function in biodegradable polymer foam scaffolds. *Biomaterials* 2001; 22: 1279–88.

100. **Folkman J, Hochberg M**. Self-regulation of growth in three dimensions. *J Exp Med.* 1973; 138: 745–53.

101. **Kneser U, Stangenberg L, Ohnolz J, Buettner O, Stern-Strater J, Moebest D, Horch RE, Stark GB, Schaefer DJ**. Reconstruction of critical size calvarial defects using processed bovine cancellous bone matrix and autologous osteoblasts. *J Cell Mol Med.* submitted: 2006.

102. **Sottile J**. Regulation of angiogenesis by extracellular matrix. *Biochim Biophys Acta.* 2004; 1654: 13–22.

103. **Madeddu P**, Therapeutic angiogenesis and vasculogenesis for tissue regeneration. *Exp Physiol.* 2005; 90: 315–26.

104. **Byrne AM, Bouchier-Hayes DJ, Harmey JH**. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). *J Cell Mol Med.* 2005; 9: 777–94.

105. **Tabata Y**. Tissue regeneration based on growth factor release. *Tissue Eng.* 2003; 9: S5–15.

106. **Elcin YM, Dixit V, Gitnick G**. Extensive *in vivo* angiogenesis following controlled release of human vascular endothelial cell growth factor: implications for tissue engineering and wound healing. *Artif Organs.* 2001; 25: 558–65.

107. **Ahrendt G, Chickering DE, Ranieri JP**. Angiogenic growth factors: a review for tissue engineering. *Tissue Eng.* 1998; 2: 117–30.

108. **Wong C, Inman E, Spaethe R, Helgerson S**. Fibrin-based biomaterials to deliver human growth factors. *Thromb Haemost.* 2003; 89: 573–82.

109. **Sakiyama-Elbert SE, Hubbell JA**. Development of fibrin derivatives for controlled release of heparin- binding growth factors. *J Control Release.* 2000; 65: 389–402.

110. **Wenger A, Stahl A, Weber H, Finkenzeller G, Augustin HG, Stark GB, Kneser U**. Modulation of *in vitro* angiogenesis in a three-dimensional spheroidal coculture model for bone tissue engineering. *Tissue Eng.* 2004; 10: 1536–47.

111. **Mertsching H, Walles T, Hofmann M, Schanz J, Knapp WH**. Engineering of a vascularized scaffold for artificial tissue and organ generation. *Biomaterials* 2005; 26: 6610–7.

112. **Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, Langer R**. Functional arteries grown *in vitro*. *Science* 1999; 284: 489–93.

113. **Tremblay PL, Hudon V, Berthod F, Germain L, Auger FA**. Inosculation of tissueengineered capillaries with the host's vasculature in a reconstructed skin transplanted on mice. *Am J Transplant.* 2005; 5: 1002–10.

114. **Mooney DJ, Mikos AG**. Growing new organs. *Sci Am.* 1999; 4: 60–5.

115. **Eiselt P, Kim BS, Chacko B, Isenberg B, Peters MC,**

Greene KG, Roland WD, Loebsack AB, Burg KJ, Culberson C, Halberstadt CR, Holder WD, Mooney DJ. Development of technologies aiding large-tissue engineering. *Biotechnol Prog.* 1998; 14: 134–40.

116. **Cassell OC, Hofer SO, Morrison WA, Knight KR**. Vascularisation of tissueengineered grafts: the regulation of angiogenesis in reconstructive surgery and in disease states. *Br J Plast Surg.* 2002; 55: 603–10.

117. **Beier JP, Kneser U, Stern-Strater J, Stark GB, Bach AD**. Y chromosome detection of three-dimensional tissue-engineered skeletal muscle constructs in a syngeneic rat animal model. *Cell Transplant.* 2004; 13: 45–53.

118. **Schipper J, Ridder GJ, Maier W, Horch RE**. The preconditioning and prelamination of pedicled and free microvascular anastomised flaps with the technique of vacuum assisted closure. *Laryngorhinootologie* 2003; 82: 421–7.

119. **Khouri RK, Upton J, Shaw WW**. Principles of flap prefabrication. *Clin Plast Surg.* 1992; 19: 763–71.

120. **Kimura N, Hasumi T, Satoh K**. Prefabricated thin flap using the transversalis fascia as a carrier. *Plast Reconstr Surg.* 2001; 108: 1972–80.

121. **Khouri RK, Upton J, Shaw WW**. Prefabrication of composite free flaps through staged microvascular transfer: an experimental and clinical study. *Plast Reconstr Surg.* 1991; 87: 108–15.

122. **Abbase EA, Shenaq SM, Spira M, el-Falaky MH**. Prefabricated flaps: experimental and clinical review. *Plast Reconstr Surg.* 1995; 96: 1218–25.

123. **Gill DR, Ireland DC, Hurley JV, Morrison WA**. The prefabrication of a bone graft in a rat model. *J Hand Surg [Am].* 1998; 23: 312–21.

124. **Homma K, Himi T, Hoki K, Ezoe K, Shintani T, Yamaguchi H, Fujita T**, A prefabricated osteocutaneous flap for tracheal reconstruction. *Plast Reconstr Surg.* 2003; 111: 1688–92.

125. **Safak T, Akyurek M, Ozcan G, Kecik A, Aydin M**. Osteocutaneous flap prefabrication based on the principle of vascular induction: an experimental and clinical study. *Plast Reconstr Surg.* 2000;105: 1304–13.

126. **Casabona F, Martin I, Muraglia A, Berrino P, Santi P, Cancedda R, Quarto R**. Prefabricated engineered bone flaps: an experimental model of tissue reconstruction in plastic surgery. *Plast Reconstr Surg.* 1998; 3: 577–81.

127. **Bernard SL, Picha GJ**. The use of coralline hydroxyapatite in a "biocomposite" free flap. *Plast Reconstr Surg.* 1991; 87: 96–105.

128. **Mankani MH, Krebsbach PH, Satomura K, Kuznetsov SA, Hoyt R, Robey PG**. Pedicled bone flap formation using transplanted bone marrow stromal cells. *Arch Surg.* 2001; 136: 263–70.

129. **Terheyden H, Warnke P, Dunsche A, Jepsen S, Brenner W, Palmie S, Toth C, Rueger DR**. Mandibular reconstruction with prefabricated vascularized bone grafts using recombinant human osteogenic protein-1: an experimental study in miniature pigs. Part II: transplantation. *Int J Oral Maxillofac Surg.* 2001; 30: 469–78.

130. **Terheyden H, Menzel C, Wang H, Springer IN, Rueger DR, Acil Y**. Prefabrication of vascularized bone grafts using recombinant human osteogenic protein-1 - part 3: dosage of rhOP-1, the use of external and internal scaffolds. *Int J Oral Maxillofac Surg.* 2004; 33: 164–72.

131. **Warnke PH, Springer IN, Wiltfang J, Acil Y, Eufinger H, Wehmoller M, Russo PA, Bolte H, Sherry E, Behrens E, Terheyden H**. Growth and transplantation of a custom vascularised bone graft in a man. *Lancet* 2004; 364: 766–70.

132. **Erol OO, Spira M**. New capillary bed formation with a surgically constructed arteriovenous fistula. *Surg Forum.* 1979; 30: 530–1.

133. **Mian R, Morrison WA, Hurley JV, Penington AJ, Romeo R, Tanaka Y, Knight KR**. Formation of new tissue from an arteriovenous loop in the absence of added extracellular matrix. *Tissue Eng.* 2000; 6: 595–603.

134. **Hofer SO, Knight KM, Cooper-White JJ, O'Connor AJ, Perera JM, Romeo-Meeuw R, Penington AJ, Knight KR, Morrison WA, Messina A**. Increasing the volume of vascularized tissue formation in engineered constructs: an experimental study in rats. *Plast Reconstr Surg.* 2003; 3: 1186–92.

135. **Cassell OC, Morrison WA, Messina A, Penington AJ, Thompson EW, Stevens GW, Perera JM, Kleinman HK, Hurley JV, Romeo R, Knight KR**. The influence of extracellular matrix on the generation of vascularized, engineered, transplantable tissue. *Ann N Y Acad Sci.* 2001; 944: 429–42.

136. **Kneser U, Polykandriotis E, Ohnolz J, Heidner K, Grabinger L, Euler S, Aman KU, Hess A, Brune K, Greil P, Sturzl M, Horch RE**. Engineering of vascularized transplantable bone tissues: induction of axial vascularization in an osteconductive matrix using an arteriovenous loop. *Tissue Eng.* in press. 2006.

137. **Polykandriotis E, Horch RE, Arkudas A, Labanaris A, Brune K, Greil P, Bach AD, Kopp J, Hess A, Kneser U**. Intrinsic versus extrinsic vascularization in tissue engineering. *Adv Mol Biol Med.* in press. 2006.

138. **Kneser U, Arkudas A, Polykandriotis E, Heidner K, Ohnolz J, Beier JP, Bach AD, Kopp J, Hess A, Horch RE**. Axial prevascularization of porous matrices by means of an arteriovenous loop significantly increases initial survival of transplanted autologous osteoblasts. *Tissue Eng.* Abstract: 2006.