Chapter 9
Bone

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Abstract Commonly applied therapies to achieve bone reconstruction or function are restricted to the transplantation of autografts and allografts, or the implantation of metal devices or ceramic-based implants. Bone grafts generally possess osteoconductive and osteoinductive properties. They are, however, limited in access and availability, and harvest is associated with donor site morbidity, hemorrhage, risk of infection, insufficient transplant integration, and graft devitalisation. Research therefore focuses on alternative therapeutic concepts such as tissue engineering to aid bone regeneration. However, bench to bedside translations are infrequent as the process towards approval by regulatory bodies is protracted and costly. Approval requires both comprehensive in vitro and in vivo studies necessitating the utilization of well-standardized large preclinical animal models, fixation devices, surgical procedures and methods of taking measurements. Only then reliable data pools can be generated which consecutively serve as a base for further research directions.

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The following chapter gives insight into bone morphology and physiology, and describes the clinical background necessitating research for alternative treatments. Furthermore, basic principles of bone tissue engineering are introduced as well as key points to consider when discussing preclinical animal models. Finally, successfully translated bone regeneration concepts are summarized.

Keywords Bone regeneration • Tissue engineering • Bone defect • Stem cell • Scaffold • Growth factor • Large animal model

9.1 Bone Morphology and Physiology

Bone is a complex, constantly altering tissue and consists of cancellous and cortical bone. The specific architecture of bone allows the skeleton to fulfil its mechanical functions. In adults, cortical bone accounts for 80 % of the total bone mass. It responds slowly to changes in loads and aids to protect organs, provides levers for movement, and (together with cancellous bone) stores minerals. Cancellous bone is found interiorly and comprises of a trabecular network that reduces organ weight and provides space for blood vessels and marrow. Cancellous bone has a large surface area per unit volume and a greate rate of metabolic activity. The external surface of bone is covered by periosteum (Hutmacher and Sittinger 2003).

Specific collagen fibres (Sharpey) connect periosteum and bone. These fibres penetrate the cortex at sites exposed to high tensile forces. The periosteum comprises of an external, fibrous layer (collagenous and reticular fibres) and an inner, proliferative layer (cambium). The cambium layer hosts osteoblasts and osteoprogenitor cells. It is capable of lamellar bone apposition and of forming primary, woven bone after a fracture. The outer fibrous layer provides elasticity and flexibility facilitating the insertion of tendons, ligaments and muscles.

Bone has a rich vascular supply receiving 10–20 % of the cardiac output. In long bones, one or two principal diaphyseal nutrient arteries represent the most important supply of arterial blood. These arteries pass obliquely through the cortical bone and divide into ascending and descending branches to supply the inner two thirds of the cortex and medullary cavity. Numerous arteries supply metaphysis and epiphysis. These blood vessels mainly arise from arteries that supply the adjacent joint, anastomose with the diaphyseal capillaries, and terminate in bone marrow, cortical bone, trabecular bone, and articular cartilage. In growing bones, these arteries are separated by the epiphyseal cartilaginous plates. Periosteal arterioles supply the outer layers of cortical bone and the periosteum.

The majority of bone mass is made of extracellular bone matrix. It consists of an organic component, primarily composed of type I collagen, which provides tensile strength and an inorganic component, primarily hydroxyapatite, providing compressive stiffness. Specialized populations of bone cells form, maintain and remodel this matrix. Four types of bone cells are distinguished based on their location, morphology and function: Osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts. Osteoblasts develop from undifferentiated cells while osteocytes form from osteo-

blasts. Osteoclasts arise from hematopoetic stem cells and develop from blood-borne monocytes. Monocytes are attracted to the bone matrix by chemotaxis triggered by a range of stimuli such as cytokines released by resident cells. These cytokines then stimulate monocyte differentiation into osteoclasts. The processes of bone modelling and remodelling require osteoclastic resorption of bone matrix and deposition of a new matrix by osteoblasts. Modelling shapes and reshapes bones during growth and stops at skeletal maturity. Physiologic remodelling does not change bone shape and consists of bone resorption and subsequent bone deposition in approximately the same location. Since it continues throughout life, it appears to be important for the maintenance of the skeleton. Its exact function, however, remains unclear. Adaptive remodelling is the bone's response to altered mechanical conditions and may result in changes of strength, density and shape. In recent years, the understanding of the processes associated with the control of bone cell function has increased significantly.

9.2 Clinical Background

In general, bone displays a high intrinsic regenerative capacity following insult or disease. Consequently, the majority of bone defects and fractures heal spontaneously. Improved surgical techniques, advanced implant designs and adjustments of postoperative management have contributed to improve outcomes after complex injuries caused by high energy trauma, disease, developmental deformity, revision surgery, and tumour resection (Perka et al. 2000; Gugala and Gogolewski 2002; den Boer et al. 2003; Komaki et al. 2006; Laurencin et al. 2006; Wildemann et al. 2007). Extensive soft tissue injury, infections, and mechanical instability can, however, compromise the intrinsic regenerative potential and result in formation of large defects (Perry 1999). Their surgical treatment is challenging and associated with high socio-economical costs. To increase our understanding of factors and microenvironmental cues that favour the incidence of slow or non-healing defects therefore poses a major research challenge (DeCoster et al. 2004; Clements et al. 2008).

Fractures of cancellous bone are often impacted and result in defect formation after reduction (den Boer et al. 2003). Segmental cortical defects most commonly occur tibial diaphyseal as soft tissue coverage is marginal, which increases the risk of bone loss (DeCoster et al. 2004).

To date, the transplantation of autologous bone grafts still remains the gold standard treatment to augment or accelerate bone regeneration (Einhorn et al. 1984; Perka et al. 2000; Komaki et al. 2006) (Fig. 9.1). Nevertheless, considerable shortcomings are associated with bone grafting. Graft harvest requires prolonged anaesthesia and personnel for graft collection (Bucholz et al. 1989; Gao et al. 1996; Liu et al. 2008). Harvested graft amounts are often insufficient while donor site accessibility is limited (Stevenson 1998; Blokhuis et al. 2000; Oest et al. 2007; Liu et al. 2008). Persistent pain at donor sites or haemorrhage can occur, and the risk of infection is significantly increased. Once transplanted, grafted bone is associated with high failure rates (Sciadini et al. 1997; Blokhuis et al. 2000; den Boer et al. 2002;

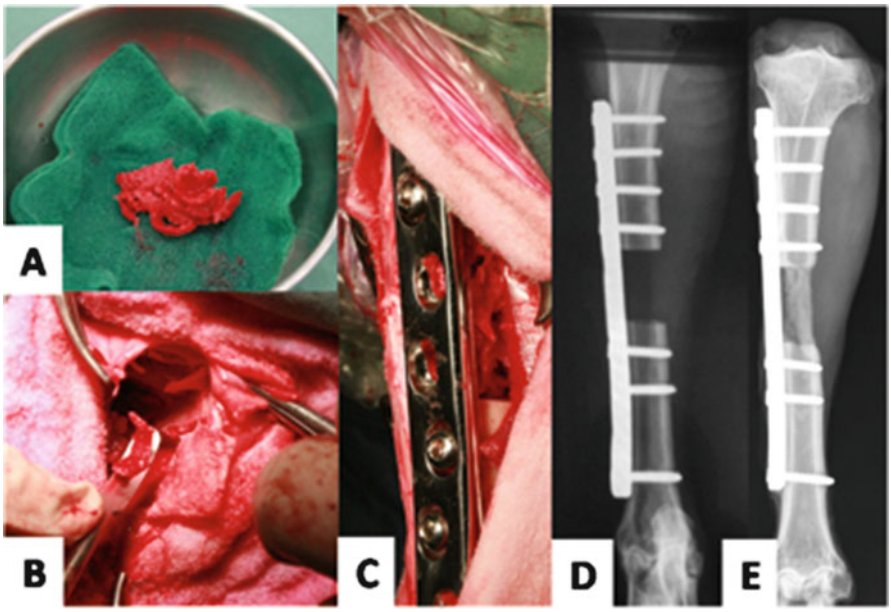

Fig. 9.1 Autologous, cancellous bone graft (**a**) harvested from the iliac crest (**b**) was used to reconstruct a 3 cm critical sized defect in an ovine tibia (**c**, **d**) Defects were stabilized with a 4.5 mm broad dynamic compression plate (Synthes). 12 weeks after surgery, new bone formation had resulted in solid bony union (**e**)

Liu et al. 2008) due to incomplete transplant integration. Graft devitalisation and subsequent resorption can compromise mechanical stability (Younger and Chapman 1989) and healing. Alternatively applied vascularised autografts are technically challenging whereas allografts and xenografts are prone to immune-mediated rejection, graft sequestration and transmission of infectious disease (Taylor et al. 1975; Dell et al. 1985; Gazdag et al. 1995; Puelacher et al. 1996; Chapman et al. 1997; Lindsey et al. 2006; Muscolo et al. 2006; Clements et al. 2008).

The high density of cortical bone allografts hinders both sufficient revascularization and cellular invasion from the surrounding host tissue (Oest et al. 2007). The limited revascularization and remodelling ability of allografts accounts for graft failure rates of 25 % and complication rates of 30–60 % (Cacchioli et al. 2006; Oest et al. 2007).

Callus distraction aims to circumvent these graft and integration related issues. It is successfully applied to treat large bone defects, infected non-unions and limb length discrepancy (Cierny and Zorn 1994). The procedure is, however, long-lasting, inconvenient for the patient (Goldstrohm et al. 1984; Ilizarov 1989) and recurrent pin track infections and pin loosening are common complications (Lindsey et al. 2006; Gugala et al. 2007).

To avoid the limitations related to current standard treatments, research interest has focused on bone graft substitutes, and the concept of tissue engineering has

emerged as an important alternative to regenerate compromised bone. Despite advances in the field and promising results achieved by the application of novel concepts in small and large animal models, translation of research results into clinical treatments remains rare.

9.3 Bone Tissue Engineering

Tissue engineering approaches can generally be classified into strategies that primarily aim at tissue conduction, induction or cell transplantation.

Tissue conduction utilizes graft materials such as hydrogels, microspheres/beads or scaffolds to mediate cell attachment. These materials usually provide an interconnected structure to support cell migration and blood vessel formation.

Tissue induction refers to the ability of a graft or substance (growth factors, lyophilized cell fractions, peptides, etc.) to induce progenitor cells migration, proliferation and differentiation into functionally mature cell types.

Cell transplantation aims at the replacement of limited structural and/or biochemical functions of the target tissue. Examples include the transplantation of chondrocytes in combination with a periosteal flap, the injection of myocardiocytes into heart muscle, haematopoetic bone marrow cell transplantation, and epidermal cell sheets for skin regeneration (Hutmacher et al. 2004).

Other approaches combine these principles with 3D cell culture to mimic natural extracellular matrices as closely as possible. Such 3D environments can be realized in form of sandwich cultures, by cell encapsulation into hydrogels, and by seeding solids or scaffolds with cells (Tibbitt and Anseth 2009).

In the past a multitude of matrices has for example been used as carriers to deliver MSCs. These include ceramics, collagen sponges and gels, and biodegradable polymers. A comprehensive report on scaffold design and fabrication for bone engineering is beyond the scope of this chapter and has been reviewed elsewhere (Hutmacher 2000; Hutmacher and Cool 2007). There is ample evidence that the nature and properties of a scaffold play an important role in bone engineering. It is, however, unclear what defines an ideal scaffold-cell or scaffold-neo-tissue construct as human tissues perform multiple functional roles. Consequently, it is unlikely that a single scaffold can serve as a universal foundation for the regeneration of a tissue.

At present bone tissue engineering efforts mainly concentrate on adult stem cells, which were found to undergo subsequent differentiation after in vivo transplantation in combination with scaffolds. These approaches rely on scaffold guided host cell and tissue in growth as well as transplanted cells as a part of an engineered device as the main factors regulating cell behaviour and performance in vivo are local cells and non-soluble factors within the extracellular matrix (ECM) (collagens, glycosaminoglycans (GAGs), cytokines, hormones, nutrients, minerals and waste products) (Table 9.1).

Table 9.1 Scaffold requirements

Scaffold requirements	
Biocompatibility	The material should not elicit an immunological or chemically detectable primary or secondary foreign body reaction
Biodegradability	Degradation at a controlled rate into non-toxic and easily excreted products
Mechanical strength	Maintenance of structural integrity during culture; support and transfer of loads after implantation
Ease of fabrication	
Porosity	Controlled and adequate porous architecture facilitating cell attachment, growth, tissue regeneration, vascularization and clearance of waste products
Osteoconductivity	Facilitation of vascular invasion, cell infiltration and attachment as well as appositional bone formation
Drug delivery	Allow release or attachment of active compounds
Ability to integrate	Integration with the host tissue following in vivo implantation
Availability	General availability; adequate shelf-life and handling properties
Sterilization	Ease of sterilisation without loss of characteristic properties

9.4 Pre-clinical Evaluation in Large Animal Models

To simulate human in vivo conditions and to assess the effects of bone grafts and tissue engineered constructs various large animal models have been developed. Most models, however, are not well described, defined, or standardized, and provide only rudimentary information on the process of model establishment.

Experimentally defects to study bone repair are postulated to be of dimensions to preclude spontaneous healing (Einhorn 1999). Such critical- sized defects can be defined as “the smallest size intraosseous wound in a particular bone and species of animal that will not heal spontaneously during the lifetime of the animal” (Gugala and Gogolewski 1999; Rimondini et al. 2005; Cacchioli et al. 2006) or as a defect which shows less than ten percent bony regeneration during the lifetime of an animal (Gugala and Gogolewski 1999).

The minimum size that defines a defect as “critical” is not well understood. Nevertheless, it has been described as a segmental bone deficiency exceeding 2–2.5 times the diameter of the affected bone (Lindsey et al. 2006; Gugala et al. 2007). However, defect healing also depends on the species’ phylogenetic scale, anatomic defect location, associated soft tissue, and biomechanical conditions in the affected limb as well as age, metabolic and systemic conditions, and related co-morbidities (Lindsey et al. 2006; Rimondini et al. 2005).

The selection of a specific animal species as a model system requires consideration of multiple factors. The chosen animal model should clearly demonstrate close physiological and pathophysiological analogies with humans regarding the scientific question under investigation. Moreover, it must be manageable to operate and observe a multiplicity of study objects over a relatively short period of time

(Schimandle and Boden 1994; Liebschner 2004; Egermann et al. 2005). Further selection criteria include costs for acquisition and care, animal availability, acceptability to society, tolerance to captivity and ease of housing (Pearce et al. 2007).

Our own research group has established and applied a 3 cm measuring critical sized tibial defect model in sheep to compare the gold standard autograft with biodegradable composite scaffolds consisting of medical-grade polycaprolactone and tricalcium phosphate combined with autologous bone marrow–derived mesenchymal stem cells (MSCs) or recombinant human bone morphogenetic protein 7 (rhBMP-7). Critical-sized defects were treated with autograft, rhBMP-7, or MSCs.

Bridging was observed within 3 months for both the autograft and the rhBMP-7 treatment. After 12 months, biomechanical analysis and microcomputed tomography imaging showed significantly greater bone formation and superior strength for the biomaterial scaffolds loaded with rhBMP-7 compared to the autograft. Axial bone distribution was greater at the interfaces. With rhBMP-7, at 3 months, the radial bone distribution within the scaffolds was homogeneous. At 12 months, however, significantly more bone was found in the scaffold architecture, indicating bone remodeling. Scaffolds alone or with MSC inclusion did not induce levels of bone formation comparable to those of the autograft and rhBMP-7 groups (Fig. 9.2).

9.4.1 Clinical Applications in Orthopaedic Surgery

Vacanti et al. (2001) reported the replacement of an avulsed phalanx with tissue engineered bone. Treatment resulted in the functional restoration of a biomechanically stable thumb of normal length. Periosteal osteoblastic progenitor cells were obtained from sections of the distal radius and were seeded onto a coral based scaffold. A calcium alginate hydrogel encapsulating the cells was used to saturate the coral implant. During follow-up, MRI examination showed evidences of vascular perfusion and biopsy revealed new bone formation with a lamellar architecture. In a first clinical study, Quarto et al. have reported the use of cell based tissue engineering approaches to treat large bone defects in three patients suffering from various segmental defects (4 cm bone segment loss in the right tibia, 4 cm in the right ulna, and 7 cm in the right humerus) (Quarto et al. 2001). Prior to transplantation, bone marrow derived osteoprogenitor were isolated and expanded. The cells were then seeded onto a macroporous scaffolds designed to fit the missing bone fragment. Defects were stabilized with an external fixator. The radiographs on follow up showed abundant callus formation along the implants and good integration at the host bone interface. In all patients recovery was reported. However, conclusions were drawn solely based on radiographic evaluation; no confirming biopsies were taken. Due to the high radiopacity of the ceramic material the assessment of bone formation within the ceramics might have been difficult as the gain in radiopacity due to new bone formation would have been overshadowed by scattering. It is furthermore unclear if the callus formation was induced by the implanted human MSCs or by bone-forming cells of the periosteum.

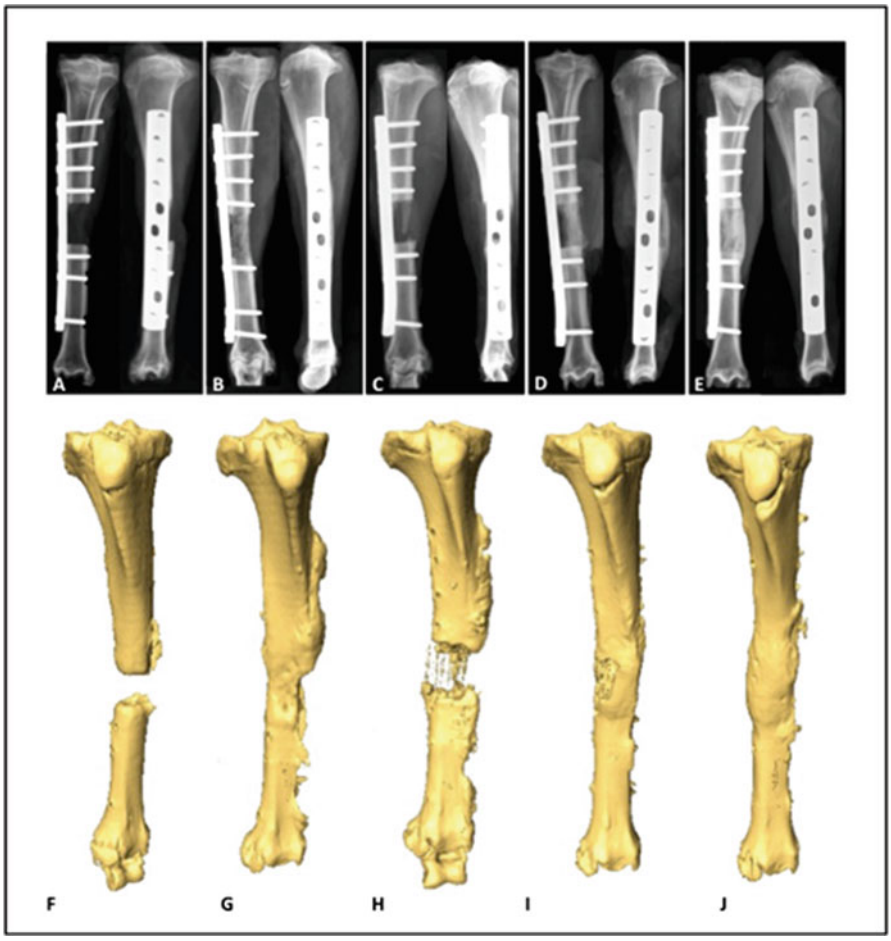

Fig. 9.2 X-ray images and CT 3D reconstructions after 3 months of an empty control defect (**a**, **f**), a defect reconstructed with cancellous bone graft from the iliac crest (**b**, **g**), a defect treated with a mPCL-TCP scaffold (**c**, **h**), and a defect augmented with mPCL-TCP + rhBMP-7 (1.75 mg D, I; 3.5 mg **e**, **j**). After 3 months, the images show clear radiographic signs of defect bridging for the autograft and rhBMP-7 group, with external callus formation in the rhBMP-7 groups. No bone formation was observed within the empty control defect and only little bone formation for the scaffold only group

The second published clinical study describes the augmentation of the posterior maxilla in 27 patients, using matrix derived from mandibular periosteum cells on a polymer fleece (Ethisorb; Ethicon,) (Schimming and Schmelzeisen 2004). In 12 patients, only radiographic and clinical assessments were performed. Limited conclusions can be drawn from the radiographic findings. The other 15 patients were treated in a biphasic approach. First, reconstruction of the host area was performed.

After a healing period of 3 months, prior to dental implant placement, a biopsy was taken. In 8 of these 15 patients a non-satisfying outcome was observed; the tissue engineered bone had been resorbed and replaced with connective tissue. In cases of positive biopsies (seven patients), the authors were unable to distinguish between bone formation induced by the implanted cells (osteoinduction) or by resident osteoblasts from the pre-existing bone (osteoconduction).

Bajada et al. (2007) reported on the successful healing of a 9 year old's left tibial mid-shaft non-union following a high-speed road traffic accident. The non-union had been resistant to various surgical procedures including the application of a monolateral external fixation, functional bracing, and two programmes of ring circular external fixation with autologous bone grafting. Using autologous bone marrow stromal cells (BMSCs) expanded in vitro to 5×10^6 cells within a period of 3 weeks combined with calcium sulphate ($CaSO_4$) in pellet form, the defect was reconstructed observing clinical and radiological convalescence 2 months after implantation.

Kim et al. investigated the effect of autologous osteoblast transplantation on healing of long bone fractures in patients (Kim et al. 2009). Autologous bone marrow derived osteogenic cells (1.2×10^7) were transplanted in combination with fibrin into fracture sites 2 weeks after internal fixation. After 8 weeks, the authors observe a significant acceleration of fracture healing compared with controls.

Due to their easy accessibility, peripheral blood progenitor cells represent a promising alternative cell source to marrow derived cells.

Kuroda et al. initiated a phase I/IIa clinical trial investigating the application of G-CSF-mobilized CD34+ peripheral blood cells for patients suffering from tibial or femoral nonunion (n = 7) (Kuroda et al. 2014). Five days following G-CSF injection, cells were magnet sorted to separate the CD34+ fraction. A number of 5.0×10^5 cells/kg body weight were incorporated into in atherocollagen gels (3 ml) and transplanted to the fracture site. Radiological fracture healing at 12 weeks was achieved in five of seven (71.4 %) patients, which exceeded the threshold (18.1 %) predefined by historical outcomes of standard care.

Giannotti et al. examined the long-term efficacy and safety of ex vivo expanded bone marrow MSCs, embedded in autologous fibrin clots, for the healing of atrophic pseudarthrosis of the upper limb (Giannotti et al. 2013). Bone marrow MSCs isolated from eight patients were expanded ex vivo and short-term osteodifferentiated. MSCs embedded in autologous fibrin clots were locally implanted in combination with bone grafts, calibrating their number on the extension of bone damage. Radiographic healing was evaluated with short- and long-term follow-ups (range averages: 6.7 and 76.0 months, respectively). All patients recovered limb function, with no evidence of tissue overgrowth or tumor formation.

The aim when treating femoral head necrosis is to preserve the femoral head and therefore avoid total hip replacement surgery. Core decompression has been shown to decrease intraosseous pressure, while additionally providing the opportunity to deliver bioactive materials and/or progenitor cells to enhance healing. We have [Nöth et al. 2007 #78] presented a therapeutic approach for patients suffering from femoral head necrosis stage ARCO II using bone marrow derived MSCs in

combination with a β-Tricalciumphosphate matrix. Kawate et al. (2006) reported on three cases of steroid-induced femoral head osteonecrosis stage Steinberg 4A (one patient) and C (two patients) treated with MSCs cultured with beta-TCP ceramics and with a free vascularized fibula. All hips showed preoperative collapse and radiographic progression was observed in two hips postoperatively, although osteonecrosis did not progress any further within the time frame reported. Moreover, Hernigou and Beaujean (2002) demonstrated that autologous bone marrow transplantations combined with core decompression before collapse of the femoral head made hip replacement surgery necessary in only 9 of 145 cases, compared to 25 of 44 cases when operated after manifest collapse. As well, Gangji et al. (2004) reported on successful treatment of 18 patients treated with bone marrow cells harvested from the iliac crest suggesting that the application of cell-based treatment concepts in case of femoral head necrosis might play a decisive role in future therapeutics.

Recently, Horch et al. reported on two cases of large bone defect reconstruction after debridement of an osteomyelitis. One of the defects was localized in the radius and one in the tibia (Horch et al. 2014). For osseus reconstruction, arteriovenous loops were created to serve as a vascular axis, and placed in the bony defects. In case 1, cancellous bone from the iliac crest in combination with fibrin glue was used to generate bone, in case 2 a clinically approved β-tricalciumphosphate/hydroxyapatite (HA), fibrin glue and bone marrow aspirated from the iliac crest. At final follow up after 36 and 72 months computed tomography (CT), magnetic resonance imaging (MRI) and doppler ultrasound revealed patent arterio-venous (AV) loops in the bone grafts as well as completely healed bone defects. The patients were pain-free and presented normal ranges of motion.

We report on a 50 year old, female patient. The patient was initially diagnosed with beginning osteoarthritis and underwent arthroscopic right knee chondroplasty at the age of 42. Despite anti-thrombotic prophylaxis, she developed a deep vein thrombosis postoperatively and ultimately an acute compartment syndrome of both flexor lodges of the lower leg. The compartment syndrome was subsequently treated by fasciotomy. Over the following 6 months she developed a progressive supination contracture of the right foot, which was corrected by subtalar arthrodesis. Consequently, the surgery related muscle imbalance in the operated foot resulted in the formation of claw toes DII-V, which were treated by tenotomy of the long and short flexor tendons, respectively. Furthermore, as another long-term consequence of the compartment syndrome, the patient increasingly suffered from developing knee stiffness with an overall extension deficit of 40° greatly affecting her mobility and quality of life. The ability to fully extend was restored by a combined arthroscopic ventral and open dorsal arthrolysis. Within 6 months after discharge a supination malposition of the right forefoot occurred accompanied by a contraction of the toes DIII-V. Together with the patient, the decision was made to perform an arthrodesis of the tibio-tarsal joint with one medial and two lateral cortical screws, open arthrolysis DII-V and tenotomy of the short flexor tendons plantar. Three months after corrective surgery, the patient suffered a traumatic fracture of the arthrodesis as one of her crutches broke while walking stairs. The fracture was stabilized with a retrograde intramedullary (IM) locking nail (12 mm diameter, 150 mm

length). As no progress towards bony consolidation was observed radiologically after a period of 5 months, revision surgery was scheduled to perform a thorough debridement. The pseudarthrosis was re-stabilized with an IM nail and cortico-spongious autograft from the right iliac crest was transplanted into the defect for bone augmentation. Six months later the two proximal locking bolts were removed, after another 6 months, the distal two. However, the desired effect of telescope-like sintering of the bone was not achieved and a persisting non-union had developed. Again, the non-vital bone in the pseudarthrotic region was resected and the resulting defect was reconstructed with a cortico-spongious autograft transplant taken from the iliac crest following thorough debridement of the non-union. Fifteen months later, still no bony union was achieved. As an *ultima ratio*, it was decided to pursue a newly developed, cell-based, experimental treatment concept. Bone marrow was aspirated from the posterior iliac crest and processed by Aastrom Biosciences Inc. (Stuttgart, Germany).

The bone marrow sample taken from the patient in the first step of the process contains a range of cells including hematopoietic and mesenchymal cells. These cells are known to play important roles in the natural healing mechanisms of the human body (Bruder et al. 1997; Cui et al. 2007). Aastrom's patented single-pass perfusion technology controls gas and cell culture media exchange to enable the replication of these naturally occurring cells. The cellular therapy resulting from this process contains expanded populations of mixed stem and progenitor cells to support the regeneration of tissue (Tissue Repair Cells, TRC). This approach reduces the risk of rejection and increases the likelihood of integration with the surrounding tissues, eliminating the need for immunosuppressive drugs.

The patient's bone marrow derived cells were expanded to a total number of 104.81×10^6. During final revision surgery, the IM nail was removed, and the TRC were applied at a concentration of 7.65×10^6/ml (total volume 13,7 ml) to the pseudarthrotic area and the cavity caused by the IM fixation device in combination with 20 cc of a synthetic cancellous bone void filler (Vitoss, Orthovita, Malvern, USA).

Vitoss is composed of β-tri-calcium-phosphate (β-TCP) and is stable at physiologic pH. It resorbs during the natural remodeling process of bone. Evidence suggests that β-TCP resorbs in the most relevant time frame in comparison to other bone substitutes such as hydroxyapatite (HA) and calcium sulfate (CaS) (Anker et al. 2005). Vitoss has an open-interconnected structure that facilitates 3-D bone regeneration and is composed of nano-particle construction, allowing for physiologically relevant cell mediated resorption. Vitoss is highly porous with an overall porosity of 90 % (Hinz et al. 2002).

Postoperatively, the patient was supplied with a dorsal lower leg splint for a period of 2 weeks, afterwards with a circular cast. The patient was asked not to weight-bear for a period of 4 weeks after stem cell transplantation. Gradually, she was allowed to partially weight bear with 20 kg (4 weeks), subsequently with half of her body weight (4 weeks) to then fully weight bear after all. The patient was regularly followed up in our outpatient department, lastly, 4 years after stem cell transplantation surgery. After a period of 6 months solid bony consolidation of the pseudarthrosis was observed radiologically, half a year later, signs of cortical remodeling as an indicator of successful, biomechanically stable bone healing

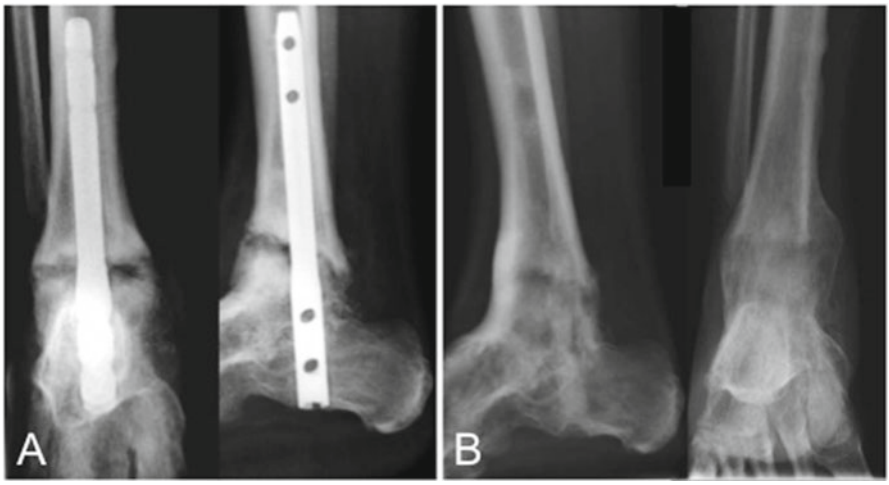

Fig. 9.3 Conventional x-ray images showing the pseudarthrotic tibial non-union in two standard planes (anterior-posterior view *left*, lateral view *right*) prior to autologous stem cell transplantation (**a**) and increasing bony consolidation with signs of cortical remodeling 1 year post mesenchymal stem cell application (**b**) forming a mechanically stable arthrodesis

(Fig. 9.3). The patient was additionally supplied with orthopaedic footware and henceforward free of complaints.

Bone repair and regeneration with bone morphogenetic proteins (BMPs) have advanced as an alternative treatment option in orthopaedic and trauma surgery. A number of animal studies and subsequent clinical trials have demonstrated the osteogenic potential of BMPs. The result has been the commercialisation of two of the early BMPs, BMP-2 and BMP-7 (also called osteogenic protein-1 or OP-1).

OP-1 is an attractive adjunct in the treatment of fractures and atrophic long bone non-unions.

The use of OP-1 in the treatment of open tibial shaft fractures was evaluated by the Canadian Orthopaedic Trauma Society. One hundred and twenty-four open tibial fractures (62 controls and 62 OP-1) were included in the study. After irrigation, debridement and intramedullary nailing, at the time of definitive wound closure, patients were randomised to standard wound closure or standard wound closure with the addition of OP-1 to the fracture site. Patients were followed up radiographicly, clinically and serologically until union. Outcomes showed that the number of secondary interventions for delayed union and non-union was significantly lower in the OP-1 group than in the control group (8 vs. 17; $p=0.02$). A significantly greater number of patients in the OP-1 group was able to fully weight bear without pain at 12 months compared to the control group. No OP-1 related adverse effects were clinically evident. The study investigators suggested that the use of OP-1 is safe for use in open tibial shaft fractures, and its use decreased the number of secondary procedures for delayed or non-union.

More recently, Ristiniemi et al. have evaluated OP-1 in the treatment of distal tibial fractures (Ristiniemi et al. 2007). Twenty patients with distal tibial fractures were treated with external hybrid fixators and OP-1 and compared to 20 matched patients treated without BMP. Outcome measures included time to radiographic union, duration of application of the external fixator, the number of secondary interventions due to delayed healing and length of absence from work. The mean time to union as well as to fixator removal was significantly shorter in the BMP group (15.7 weeks vs. 23.5 weeks, $p=0.002$; 15 weeks vs. 21.4 weeks, $p=0.037$). Revisions for delayed union were required in two patients in the BMP group and seven patients in the control group. Average time off work was significantly lower in the BMP group than in the controls.

The use of OP-1 in the treatment of tibial non-union was studied by Friedlaender et al. in a randomised controlled, prospective clinical trial (Friedlaender et al. 2001). Clinical and radiographic results were compared to assess the efficacy of OP-1 versus autograft in the treatment of tibial non-unions that had persisted for at least 9 months. One hundred and twenty-four tibial non-unions in 122 patients were randomised to either intramedullary nail and autograft or intramedullary nail and implantation of OP-1 at the non-union site. Nine months after surgery, 81 % of the OP-1 group and 85 % of the autograft group had achieved clinical union. Radiographic analysis indicated that 75 % of the fractures treated with OP-1 and 84 % of the autograft-treated group had healed. There was no statistically significant difference between groups clinically or radiographicly. More than 20 % of the bone graft group complained of persistent donor site pain. The authors concluded that OP-1 was a safe and effective alternative to bone grafting in the treatment of tibial non-unions. This study led to multiple regulatory approvals worldwide. Numerous studies in the literature suggest that OP-1 is a safe and effective treatment option for fractures and atrophic non-unions not only of the lower but also of the upper extremities.

9.5 Summary

The reconstruction of complicated fractures and large segmental bone defects remains a significant clinical problem. Large bone defects may occur as a result of extensive bone loss resulting from pathological events such as trauma, inflammation, and tumour resection. Present therapeutic approaches include the application of bone graft transplants (autologous, allogenic, xenografts), fixation devices consisting of different synthetic and natural biomaterials, and segmental bone transport. However, to date, no existing therapy has been fully satisfactory. A number of research groups therefore work on the development of new bone grafting materials, carriers, growth factors, and tissue engineered constructs for bone regeneration. To optimize cell-scaffold combinations and the application of locally or systemically active stimuli remains a complex process. It is characterized by a highly interdependent set of

variables with a large range of possible variations. Consequently, the evaluation of new developments in the field of bone tissue engineering must base on clinical experience, knowledge of basic biological principles, medical necessity, and commercial practicality. The area of bone tissue engineering relies on animal models to evaluate both experimental and clinical hypotheses. To overcome current limitations associated with bone tissue engineering, researchers must rely on the functional assessment of biological and biomechanical parameters of generated constructs. For comparison of different studies and their outcomes, the standardization of animal models, fixation devices, surgical procedures and methods of taking measurements is essential to accumulate a reliable data pool guiding further directions to orthopaedic and tissue engineering developments. Only then we can overcome the lack of translation in the field of bone engineering which is closely related to difficulties in integrating individual technical discoveries in model tissue engineering systems, in manufacturing scale up, in funding, and in regulatory approval.

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