A review of biomaterials in bone defect healing, remaining shortcomings and future opportunities for bone tissue engineering


Despite its intrinsic ability to regenerate form and function after injury, bone tissue can be challenged by a multitude of pathological conditions. While innovative approaches have helped to unravel the cascades of bone healing, this knowledge has so far not improved the clinical outcomes of bone defect treatment. Recent findings have allowed us to gain in-depth knowledge about the physiological conditions and biological principles of bone regeneration. Now it is time to transfer the lessons learned from bone healing to the challenging scenarios in defects and employ innovative technologies to enable biomaterial-based strategies for bone defect healing. This review aims to provide an overview on endogenous cascades of bone material formation and how these are transferred to new perspectives in biomaterial-driven approaches in bone regeneration.

Clinical and Research Approaches to Treat Non-union Fracture.


PURPOSE OF REVIEW:

Impaired healing outcomes or even non-unions after bone injury are still a highly relevant problem in the daily clinical life. Especially within an aging population, the occurrence of bone fractures increases and thus novel treatment approaches to overcome compromised bone regeneration are needed.

RECENT FINDINGS:

The gold standard to treat delayed or non-healing bone injuries is still the use of autologous bone grafts to foster regeneration. Besides its successful treatment outcome, it also has disadvantages: a second surgery is needed in order to harvest the bone material and the material is highly limited. Looking into the recent literature, a multitude of different research approaches were already conducted to identify new possible strategies to treat impaired bone regeneration: application of mesenchymal stromal cells, platelet lysates, growth factors, interference in the immune system, or bone formation stimulation by ultrasound. This review gives an overview of the treatment approaches actually performed in the clinic as well as at the bench in the context of compromised bone healing. It clearly highlights the complexity of the nature of non-healing bone fractures as well as patient-dependent factors influencing the healing process.

Immunology Guides Skeletal Muscle Regeneration.


Soft tissue trauma of skeletal muscle is one of the most common side effects in surgery. Muscle injuries are not only caused by accident-related injuries but can also be of an iatrogenic nature as they occur during surgical interventions when the anatomical region of interest is exposed. If the extent of trauma surpasses the intrinsic regenerative capacities, signs of fatty degeneration and formation of fibrotic scar tissue can occur, and, consequentially, muscle function deteriorates or is diminished. Despite research efforts to investigate the physiological healing cascade following trauma, our understanding of the early onset of healing and how it potentially determines success or failure is still only fragmentary. This review focuses on the initial physiological pathways following skeletal muscle trauma in comparison to bone and tendon trauma and what conclusions can be drawn from new scientific insights for the development of novel therapeutic strategies. Strategies to support regeneration of muscle tissue after injury are scarce, even though muscle trauma has a high incidence. Based on tissue specific differences, possible clinical treatment options such as local immune-modulatory and cell therapeutic approaches are suggested that aim to support the endogenous regenerative potential of injured muscle tissues.

Leptin-deficiency eradicates the positive effect of traumatic brain injury on bone healing: histological analyses in a combined trauma mouse model.
The combination of traumatic brain injury (TBI) and long-bone fracture leads to increased formation of callus and mineral density in wild-type (WT) mice. However, this effect was not detected radiographically in leptin-deficient mice. Due to the complex interactions between hormonal and bone metabolism and the important role of leptin in this setting, our aim was to investigate morphologic properties and the tissue composition in the fracture callus comparing WT and leptin-deficient mice.

Leptin deficient mice showed a higher rate of non-union after osteotomy, less callus formation in the osteotomy gap, and unexpected bone and cartilage formation independent of the osteotomy region.

Leptin plays an important role in fracture healing and bone formation. Without Leptin, the positive effect of TBI on fracture healing ceases. The comprehension of the underlying pathophysiological process could sign important for novel strategies in stimulation of fracture healing.

Mechanobiologically optimized 3D titanium-mesh scaffolds enhance bone regeneration in critical segmental defects in sheep


Three-dimensional (3D) titanium-mesh scaffolds offer many advantages over autologous bone grafting for the regeneration of challenging large segmental bone defects. Our study supports the hypothesis that endogenous bone defect regeneration can be promoted by mechanobiologically optimized Ti-mesh scaffolds. Using finite element techniques, two mechanically distinct Ti-mesh scaffolds were designed in a honeycomb-like configuration to minimize stress shielding while ensuring resistance against mechanical failure. Scaffold stiffness was altered through small changes in the strut diameter only. Honeycombs were aligned to form three differently oriented channels (axial, perpendicular, and tilted) to guide the bone regeneration process. The soft scaffold (0.84 GPa stiffness) and a 3.5-fold stiffer scaffold (2.88 GPa) were tested in a critical size bone defect model in vivo in sheep. To verify that local scaffold stiffness could enhance healing, defects were stabilized with either a common locking compression plate that allowed dynamic loading of the 4-cm defect or a rigid custom-made plate that mechanically shielded the defect. Lower stress shielding led to earlier defect bridging, increased endochondral bone formation, and advanced bony regeneration of the critical size defect. This study demonstrates that mechanobiological optimization of 3D additive manufactured Ti-mesh scaffolds can enhance bone regeneration in a translational large animal study.

Longitudinal intravital imaging of the femoral bone marrow reveals plasticity within marrow vasculature


The bone marrow is a central organ of the immune system, which hosts complex interactions of bone and immune compartments critical for hematopoiesis, immunological memory, and bone regeneration. Although these processes take place over months, most existing imaging techniques allow us to follow snapshots of only a few hours, at subcellular resolution. Here, we develop a microendoscopic multi-photon imaging approach called LIMB (longitudinal intravital imaging of the bone marrow) to analyze cellular dynamics within the deep marrow. The approach consists of a biocompatible plate surgically fixated to the mouse femur containing a gradient refractive index lens. This microendoscope allows highly resolved imaging, repeatedly at the same regions within marrow tissue, over months. LIMB reveals extensive vascular plasticity during bone healing and steady-state homeostasis. To our knowledge, this vascular plasticity is unique among mammalian tissues, and we expect this insight will decisively change our understanding of essential phenomena occurring within the bone marrow.

In-situ tissue regeneration through SDF-1α driven cell recruitment and stiffness-mediated bone regeneration in a critical-sized segmental femoral defect

CipitrA. A. et al., Acta Biomater. 2017 Sep 15;60:50-63

Stromal cell-derived factor-1α (SDF-1α) is a chemoattractant used to recruit host cells for tissue regeneration. The concept that matrix stiffness can direct mesenchymal stromal cell (MSC) differentiation into various lineages was described a decade ago using in-vitro experiments. Recently, alginate hydrogels with an optimized stiffness and ex-vivo encapsulated MSCs were shown to have an effect in the regeneration of skull defects of nude rats. Here, we apply this material system, loaded with SDF-1α and without encapsulated MSCs, to (i) recruit endogenous cells and (ii) induce stiffness-mediated osteogenic differentiation in-vivo, using as model system a load-bearing femoral defect in immunocompetent rats. While a cell-free approach is of great interest from a translational perspective, the current limitations are described.

T Lymphocytes Influence the Mineralization Process of Bone


Bone is a unique organ able to regenerate itself after injuries. This regeneration requires the local interplay between different biological systems such as inflammation and matrix formation. Structural reconstitution is initiated by an inflammatory response orchestrated by the host immune system. However, the individual role of T cells and B cells in regeneration and their relationship to bone tissue reconstitution remain unknown. Comparing bone and fracture healing in animals with and without mature T and B cells revealed the essential role of these immune cells in determining the tissue mineralization and thus the bone quality. Bone without mature T and B cells is stiffer when compared to wild-type bone thus lacking the elasticity that helps to absorb forces, thus preventing fractures. In-depth analysis showed dysregulations in collagen deposition and osteoblast distribution upon lack of mature T and B cells. These changes in matrix deposition have been correlated with T cells rather than B cells within this
study. This work presents, for the first time, a direct link between immune cells and matrix formation during bone healing after fracture. It illustrates specifically the role of T cells in the collagen organization process and the lack thereof in the absence of T cells.

Impaired fracture healing with high non-union rates remains irreversible after traumatic brain injury in leptin-deficient mice


Patients with traumatic brain injury (TBI) and long-bone fractures can show increased callus formation. This effect has already been reproduced in wild-type (wt) mice. However, the mechanisms remain poorly understood. Leptin is significantly increased following TBI, while its role in bone healing remains unclear. The aim of this study was to evaluate fracture healing in leptin-deficient ob/ob mice and to measure any possible impact of TBI on callus formation. 138 female, 12 weeks old, ob/ob mice were divided into four groups: Control, fracture, TBI and combined trauma. Osteotomies were stabilized with an external fixator; TBI was induced with Controlled Cortical Impact Injury. Callus bridging was weekly evaluated with in vivo micro-CT (computer tomography). Biomechanical testing was performed ex vivo. Micro-CT (computer tomography) showed high non-union rates after three and four weeks in the fracture and combined trauma group. No differences were observed in callus volume, density and biomechanical properties at any time point. This study shows that bony bridging is impaired in the present leptin-deficient trauma model. Furthermore, the phenomenon of increased callus formation after TBI could not be reproduced in ob/ob mice, as in wt mice. Our findings suggest that the increased callus formation after TBI may be dependent on leptin signaling.

Tubular open-porous β-tricalcium phosphate polycaprolactone scaffolds as guiding structure for segmental bone defect regeneration in a novel sheep model


Large segmental bone defect reconstruction with sufficient functional restoration is one of the most demanding challenges in orthopaedic surgery. Available regenerative treatment options, as the vascularized bone graft transfer, the Masquelet technique or the lizarov distraction osteogenesis, are associated with specific indications and distinct limitations. As an alternative, a hollow cylindrical ceramic-polymer composite scaffold (β-tricalcium phosphate and poly-lactid co-ε-caprolactone), facilitating a strong surface guiding effect for tissue ingrowth (group 1; n = 6) was investigated here. In combination with an additional autologous, cancellous bone graft filling, the scaffold's ability to work as an open-porous membrane to improve the defect healing process was analysed (group 2; n = 6). A novel model of a critical size (40 mm) tibia osteotomy defect stabilized with an external hybrid-ring fixator, was established in sheep. Segmental defect regeneration and tissue organization in relation to the scaffold were analysed radiologically, (immune-) histologically, and with second-harmonic generation imaging 12 weeks after surgery. The scaffold's tubular shape and open-porous structure controlled the collagen fibre orientation within the bone defect and guided the following mineralization process along the scaffold surface. In combination with the osteoinductive stimulus, a unilateral bony bridging of the critically sized defect was achieved in one third of the animals. The external hybrid-ring fixator was appropriate for large segmental defect stabilization in sheep.

Demineralized Bone Matrix as a Carrier for Bone Morphogenetic Protein-2: Burst Release Combined with Long-Term Binding and Osteoinductive Activity Evaluated In Vitro and In Vivo


To allow bone defect regeneration, autologous bone grafting still represents the gold standard. However, autograft harvesting has limitations, including an additional surgery, donor site morbidity, and limited availability. Demineralized bone matrix (DBM) would represent an alternative, yet lacks sufficient osteoinductive properties. Combining DBM with a potent agent, such as bone morphogenetic protein-2 (BMP-2) might be a feasible alternative approach, optimizing an established grafting material with strong osteoinductive properties. A unique mixing device has been developed that enables perioperative handling to reach a homogeneous and standardized paste for bone defect filling. DBM proved in vitro to be a suitable carrier for BMP-2, with a documented release over 56 days at concentrations sufficient to stimulate osteogenic differentiation. At the end of the elution experiment, 56 days, bioactive BMP was still captured within the DBM. Using a sheep drill hole defect model, DBM peripherically mixed with BMP-2 showed strong osteoinductive properties comparable to those of autologous bone and outnumbering the one of DBM alone or empty defects. Bone defect healing was enabled at diaphyseal and metaphyseal defects and thus BMP-2-doped DBM represented an easy perioperative enriching method and an efficient carrier for BMP-2. With the comparability to the clinical gold standard autologous bone, DBM mixed with BMP-2 might serve as possible alternative grafting material enabling a controlled osteogenic stimulation.

A Pronounced Inflammatory Activity Characterizes the Early Fracture Healing Phase in Immunologically Restricted Patients

Hoff P. et al., Int J Mol Sci. 2017 Mar 8;18(3).

Immunologically restricted patients such as those with autoimmune diseases or malignancies often suffer from delayed or insufficient fracture healing. In human fracture hematomas and the surrounding bone marrow obtained from immunologically restricted patients, we analyzed the initial inflammatory phase on cellular and humoral level via flow cytometry and multiplex suspension array. Compared with controls, we demonstrated higher numbers of immune cells like monocytes/macrophages, natural killer T (NKT) cells, and activated T helper cells within the fracture hematomas and/or the surrounding bone marrow. Also, several pro-inflammatory cytokines such as Interleukin (IL)-6 and Tumor necrosis factor α (TNFα), chemokines (e.g., Eotaxin and RANTES), pro-angiogenic factors (e.g., IL-8 and Macrophage migration inhibitory factor: MIF), and regulatory cytokines (e.g., IL-10) were found at higher levels within the fracture hematomas and/or the surrounding bone marrow of
Fracture healing is a complex regeneration process which produces new bone tissue without scar formation. However, fracture healing disorders occur in approximately 10% of human patients and cause severe pain and reduced quality of life. Recently, the development of more standardized, sophisticated and commercially available osteosynthesis techniques reflecting clinical approaches has increased the use of small rodents such as rats and mice in bone healing research dramatically. Nevertheless, there is no standard for pain assessment, especially in these species, and consequently limited information regarding the welfare aspects of osteotomy models. Moreover, the selection of analgesics is restricted for osteotomy models since non-steroidal anti-inflammatory drugs (NSAIDs) are known to affect the initial, inflammatory phase of bone healing. Therefore, opioids such as buprenorphine and tramadol are often used. However, dosage data in the literature are varied. Within this review, we clarify the potential benefits of the haematoma as a source for inflammatory cells that release the cytokine pattern that directs cell recruitment towards the injured tissue. In reviewing the potential benefits of the fracture haematoma, the early development of angiogenic and osteogenic potentials within the haematoma are striking. Removing the haematoma during surgery could negatively influence the fracture healing process. In an ovine open tibial fracture model the haematoma was removed 4 or 7 days after injury and the bone that formed during the first two weeks of healing was significantly reduced in comparison with an undisturbed control. These findings indicate that whenever possible the original haematoma formed upon injury should be conserved during clinical fracture treatment to benefit from the inherent healing potential.

**CD31+ Cells from Peripheral Blood Facilitate Bone Regeneration in Biologically Impaired Conditions Through Synergistic Combined Effects on Immunomodulation and Angiogenesis**

Sass FA. et al., J Bone Miner Res. 2017 May;32(5):902-912

Controlled revascularization and inflammation are key elements regulating endogenous regeneration after (bone) tissue trauma. Peripheral blood-derived cell subsets, such as regulatory T-helper cells and circulating (endothelial) progenitor cells, respectively, can support endogenous tissue healing, whereas effector T-cells that are associated with an aged immune system can hinder bone regeneration. CD31 is expressed by diverse leukocytes and is well recognized as a marker of circulating endothelial (precursor) cells; however, CD31 is absent from the surface of differentiated effector T-cells. Thus, we hypothesized that by separating the inhibitory fractions from the supportive fractions of circulating cells within the peripheral blood (PB) using the CD31 marker, bone regeneration in biologically compromised conditions, such as those observed in aged patients, could be improved. In support of our hypothesis, we detected an inverse correlation between CD31+ cells and effector T-cells in the hematomas of human fracture patients, dependent on the age of the patient. Furthermore, we demonstrated the regenerative capacity of human PB-CD31+ cells in vitro. These findings were translated to a clinically relevant rat model of impaired bone healing. The transplantation of rat PB-CD31+ cells advanced bone tissue restoration in vivo and was associated with an early anti-inflammatory response, the stimulation of (re)vascularization, and reduced fibrosis. Interestingly, the depletion or enrichment of the highly abundant CD31+/14+ monocytes from the mixed CD31+ cell population diminished tissue regeneration at different levels, suggesting synergistic combined effects within the PB-CD31+ subsets. In summary, an intraoperative enrichment of PB-CD31+ cells might be a novel option to facilitate endogenous regeneration under biologically impaired situations by supporting immunomodulation and vascularization. This article is protected by copyright. All rights reserved.

**Immunological characterization of the early human fracture hematoma.**

Hoff P. et al., Immunol Res. 2016 Dec;64(5-6):1195-1206.

The initial inflammatory phase of fracture healing is of great importance for the clinical outcome. We aimed to develop a detailed time-dependent analysis of the initial fracture hematoma. We analyzed the composition of immune cell subpopulations by flow cytometry and the concentration of cytokines and chemokines by bioplex in 42 samples from human fractures of long bones <72 h post-trauma. The early human fracture hematoma is characterized by maturation of granulocytes and migration of monocytes/macrophages and hematopoietic stem cells. Both T helper cells and cytotoxic T cells proliferate within the fracture
hematoma and/or migrate to the fracture site. Humoral immunity characteristics comprise high concentration of pro-inflammatory cytokines such as IL-6, IL-8, IFNγ and TNFα, but also elevated concentration of anti-inflammatory cytokines, e.g., IL-1 receptor antagonist and IL-10. Furthermore, we found that cells of the fracture hematoma represent a source for key chemokines. Even under the bioenergetically restricted conditions that exist in the initial fracture hematoma, immune cells are not only present, but also survive, mature, function and migrate. They secrete a cytokine/chemokine cocktail that contributes to the onset of regeneration. We hypothesize that this specific microenvironment of the initial fracture hematoma is among the crucial factors that determine fracture healing.

Future treatment strategies for delayed bone healing - Arising from osteoimmunological research


Bone is a remarkable organ as it retains the capacity to regenerate [1]. If successful the healing of a fractured bone results in complete reconstitution of form and function. However, the healing process itself is quite complex and thus prone to failure. This explains that even today more than 10% of fracture patients experience a delayed or disturbed healing in industrialized countries. A high percentage of these patients are elderly people prone for fracture and compromised healing. Specific treatment options to prevent delayed bone healing in such patients is still missing. Currently, patient fracture fixation and treatment is widely handled without specialized concepts for elderly or for individual immune profiles.

Establishment of a preclinical ovine screening model for the investigation of bone tissue engineering strategies in cancellous and cortical bone defects.


BACKGROUND: New tissue engineering strategies for bone regeneration need to be investigated in a relevant preclinical large animal model before making the translation into human patients. Therefore, our interdisciplinary group established a simplified large animal screening model for intramembranous bone defect regeneration in cancellous and cortical bone. METHODS: Related to a well-established model of cancellous drill hole defect regeneration in sheep, both the proximal and distal epimetaphyseal regions of the femur and the humerus were used bilaterally for eight drill hole cancellous defects (Ø 6 mm, 15 mm depth). Several improvements of the surgical procedure and equipment for an easier harvest of samples were invented. For the inclusion of cortical defect regeneration, a total of eight unicortical diaphyseal drill holes (6 mm Ø) were placed in the proximal-lateral and distal-medial parts of the metacarpal (MC) and metatarsal (MT) diaphyseal bone bilaterally. Acting moments within a normal gait cycle in the musculoskeletal lower limb model were compared with the results of the biomechanical in vitro torsion test until failure to ensure a low accidental fracture risk of utilized bones (ANOVA, p < 0.05). The model was tested in vivo, using thirteen adult, female, black-face sheep (Ø 66 kg; ± 5 kg; age ≥ 2.5 years). In a two-step surgical procedure 16 drill holes were performed for the investigation of two different time points within one animal. Defects were left empty, augmented with autologous cancellous bone or soft bone graft substitutes. RESULTS: The in vitro tests confirmed this model a high comparability between drilled MC and MT bones and a high safety margin until fracture. The exclusion of one animal from the in vivo study, due to a spiral fracture of the left MC bone led to a tolerable failure rate of 8 %. CONCLUSIONS: As a screening tool, promising biomaterials can be tested in this cancellous and cortical bone defect model prior to the application in a more complex treatment site

Immune modulation as a therapeutic strategy in bone regeneration.

Schlundt C. et al., J Exp Orthop. 2015 Dec;2(1):1

We summarize research approaches and findings on bone healing and regeneration that were presented at a workshop at the 60th annual meeting of the Orthopedic Research Society (ORS) in New Orleans in 2014. The workshop was designed to discuss the role of inflammation in bone regeneration in the context of fundamental biology, and to develop therapeutic strategies that involve immune modulation. Delayed or non-healing of bone is a major clinical problem, with around 10% of fracture patients suffering from unsatisfying healing outcomes. Inflammation is traditionally seen as a defense mechanism, but was recently found essential in supporting and modulating regenerative cascades. In bone healing, macrophages and T- and B-cells interact with progenitor cells, bone forming osteoblasts and remodeling osteoclasts. Among the cells of the innate immunity, macrophages are promising candidates for targets in immune-modulatory interventions that would overcome complications in bone healing and bone-related diseases. Among the cells of the adaptive immune system, CD8+ T cells have been shown to have a negative impact on bone fracture healing outcome, whereas regulatory T cells could be promising candidates that have a positive, modulating effect on bone fracture healing. This workshop addressed recent advances and key challenges in this exciting interdisciplinary research field.

The Role of Immune Reactivity in Bone Regeneration, Advanced Techniques in Bone Regeneration

Bucher H.C. et al., 2016


Bone is a complex organ with the capacity to regenerate. Even with this healing potential, healing results in fractured bone are unsatisfactory in a considerable patient cohort even with a good treatment regimen. These delayed healing cases encourage further research into possible new treatment approaches. The recently developed field of osteoimmunology addressing the tight interconnectivity of the skeletal system and the immune system could be a promising opportunity in this regard. In this review,
the complexity of bone and the bone healing process are highlighted with an emphasis on the early healing phase. Specific immune cell subsets are considered for their potential to enhance bone healing and thus to develop new treatment strategies for patients in need.

Traumatic brain injury and bone healing: radiographic and biomechanical analyses of bone formation and stability in a combined murine trauma model


INTRODUCTION:The combination of traumatic brain injury (TBI) and long-bone fractures has previously been reported to lead to exuberant callus formation. The aim of this experimental study was to radiographically and biomechanically study the effect of TBI on bone healing in a mouse model.MATERIALS AND METHODS:138 female C57/Black6N mice were assigned to four groups (fracture (Fx) / TBI / combined trauma (Fx/TBI) / controls). Femoral osteotomy and TBI served as variables: osteotomies were stabilized with external fixators, TBI was induced with controlled cortical impact injury. During an observation period of four weeks, in vivo micro-CT (computer tomography) scans of femora were performed on a weekly basis. Biomechanical testing of femora was performed ex vivo.RESULTS:The combined-trauma group showed increased bone volume, higher mineral density, and a higher rate of gap bridging compared to the fracture group. The combined-trauma group showed increased torsional strength at four weeks.DISCUSSION:TBI results in an increased formation of callus and mineral density compared to normal bone healing in mice. This fact combined with a tendency towards accelerated gap bridging leads to increased torsional strength. The present study underscores the empirical clinical evidence that TBI stimulates bone healing. Identification of underlying pathways could lead to new strategies for bone-stimulating approaches in fracture care.

BMPs in Bone Regeneration: Less is more effective, a paradigm-shift

Schmidt-Bleek K. et al., Cytokine & Growth Factor Reviews, 2016 Feb;27:141-8.

Worldwide, the clinical application of BMP2 (bone morphogenetic protein 2) has helped an increasing number of patients achieve bone regeneration in a clinical area lacking simple solutions for difficult bone healing situations. In this review, the historical aspects and current critical clinical issues are summarized and positioned against new research findings on efficacy and function of BMP2. Knowledge concerning how the dose of this growth factor as well as its interaction with mechanical loading influences the efficacy of bone regeneration, might open possible future strategies in cases where bony bridging is unachievable so far. In conclusion, it is apparent that there is a substantial need for continued basic research to unravel the details of its function and the underlying signaling pathways involved, to make BMP2 even more relevant and safe in daily clinical use, even though this growth factor has been known for more than 125 year

Macrophages in bone fracture healing: Their essential role in endochondral ossification


In fracture healing, skeletal and immune system are closely interacting through common cell precursors and molecular mediators. It is thought that the initial inflammatory reaction, which involves migration of macrophages into the fracture area, has a major impact on the long term outcome of bone repair. Interestingly, macrophages reside during all stages of fracture healing. Thus, we hypothesized a critical role for macrophages in the subsequent phases of bone regeneration. This study examined the impact of in vivo induced macrophage reduction, using clodronic liposomes, on the different healing phases of bone repair in a murine model of a standard closed femoral fracture. A reduction in macrophages had no obvious effect on the early fracture healing phase, but resulted in a delayed hard callus formation, thus severely altering endochondral ossification. Clodronate treated animals clearly showed delayed bony consolidation of cartilage and enhanced periosteal bone formation. Therefore, we decided to backtrack macrophage distribution during fracture healing in non-treated mice, focusing on the identification of the M1 and M2 subsets. We observed that M2 macrophages were clearly prevalent during the ossification phase. Therefore enhancement of M2 phenotype in macrophages was investigated as a way to further bone healing. Induction of M2 macrophages through interleukin 4 and 13 significantly enhanced bone formation during the 3week investigation period. These cumulative data illustrate their so far unreported highly important role in endochondral ossification and the necessity of a fine balance in M1/M2 macrophage function, which appears mandatory to fracture healing and successful regeneration.

Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners

Lei H. et al., Front Pharmacol. 2015 Sep 2;6:184.

Regulatory T cells (Tregs) offer new immunotherapeutic options to control undesired immune reactions, such as those in transplant rejection and autoimmunity. In addition, tissue repair and regeneration depend on a multitude of tightly regulated immune and non-immune cells and signaling molecules. There is mounting evidence that adequate innate responses, and even more importantly balanced adaptive immune responses, are key players in the tissue repair and regeneration processes, even in absence of any immune-related disease or infection. Thus, the anti-inflammatory and anti-apoptotic capacities of Treg can affect not only the effector immune response, creating the appropriate immune environment for successful tissue repair and regeneration, but growing evidence shows that they also have direct effects on tissue cell functions. Here we summarize the present views on how Treg might support tissue regeneration by direct control of undesired immune reactivity and also by direct interaction with non-immune tissue cells. We describe tissue-resident Treg and their specific phenotypes in skin, visceral adipose tissue, and skeletal muscle. In addition, we touch on the topic of osteoimmunology, discussing the direct interactions of Treg with bone-forming cells, such as osteoblasts and their mesenchymal stromal cell (MSC) progenitors—a field which is under-investigated. We hypothesize a cross-talk between Treg and bone-forming cells through the CD39–CD73-(adenosine)-
Improved bone defect healing by a superagonistic GDF5 variant derived from a patient with multiple synostoses syndrome


Multiple synostoses syndrome 2 (SYNS2) is a rare genetic disease characterized by multiple fusions of the joints of the extremities, like phalangeal joints, carpal and tarsal joints or the knee and elbows. SYNS2 is caused by point mutations in the Growth and Differentiation Factor 5 (GDF5), which plays an essential role during skeletal development and regeneration. We selected one of the SYNS2-causing GDF5 mutations, p.N445T, which is known to destabilize the interaction with the Bone Morphogenetic Protein (BMP) antagonist NOGGIN (NOG), in order to generate the superagonistic GDF5 variant GDF5(N445T).

In this study, we tested its capacity to support regeneration in a rat critical-sized defect model in vivo. MicroCT and histological analyses indicate that GDF5(N445T)-treated defects show faster and more efficient healing compared to GDF5 wild type (GDF5(wt))-treated defects. Microarray-based gene expression and quantitative PCR analyses from callus tissue point to a specific acceleration of the early phases of bone healing, comprising the inflammation and chondrogenesis phase. These results support the concept that disease-deduced growth factor variants are promising lead structures for novel therapeutics with improved clinical activities.

T and B cells participate in bone repair by infiltrating the fracture callus in a two-wave fashion

Fracture healing is a regenerative process in which bone is restored without scar tissue formation. The healing cascade initiates with a cycle of inflammation, cell migration, proliferation and differentiation. Immune cells invade the fracture site immediately upon bone damage and contribute to the initial phase of the healing process by recruiting accessory cells to the injury site. However, little is known about the role of the immune system in the later stages of fracture repair, in particular, whether lymphocytes participate in soft and hard callus formation. In order to answer this question, we analyzed femoral fracture healing in mice by confocal microscopy. Surprisingly, after the initial inflammatory phase, when soft callus developed, T and B cells withdrew from the fracture site and were detectable predominantly at the femoral neck and knee. Thereafter lymphocytes massively infiltrated the callus region (around day 14 after injury), during callus mineralization. Interestingly, lymphocytes were not found within cartilaginous areas of the callus but only nearby the newly forming bone. During healing B cell numbers seemed to exceed those of T cells and B cells progressively underwent effector maturation. Both, osteoblasts and osteoclasts were found to have direct cell-cell contact with lymphocytes, strongly suggesting a regulatory role of the immune cells specifically in the later stages of fracture healing.

Initiation and early control of tissue regeneration - bone healing as a model system for tissue regeneration

Tissue regeneration in itself is a fascinating process that promises repeated renewal of tissue and organs.

INTRODUCTION:

Tissue regeneration in itself is a fascinating process that promises repeated renewal of tissue and organs.

AREAS COVERED:

This article aims to illustrate the different strategies available to control tissue regeneration at a very early stage, using bone as an exemplary tissue. The aspects of a controlled inflammatory cascade to achieve a balanced immune response, cell therapeutic approaches for improved tissue formation and angiogenesis, guiding the organization of newly formed extracellular matrix by biomaterials, the relevance of mechanical signals for tissue regeneration processes, and the chances and limitations of growth factor treatments are discussed.

EXPERT OPINION:

The currently available knowledge is reviewed and perspectives for potential new targets are given. This is done under the assumption that early identification of risk patients as well as the application of early intervention strategies is possible.

In serumveritas-in serumsanitas? Cell non-autonomous aging compromises differentiation and survival of mesenchymal stromal cells via the oxidative stress pathway

Even tissues capable of complete regeneration, such as bone, show an age-related reduction in their healing capacity. Here, we hypothesized that this decline is primarily due to cell non-autonomous (extrinsic) aging mediated by the systemic environment. We demonstrate that culture of mesenchymal stromal cells (MSCs) in serum from aged Sprague-Dawley rats negatively affects their survival and differentiation ability. Proteome analysis and further cellular investigations strongly suggest that serum from aged animals not only changes expression of proteins related to mitochondria, unfolded protein binding or involved in stress responses, it also significantly enhances intracellular reactive oxygen species production and leads to the accumulation of oxidatively damaged proteins. Conversely, reduction of oxidative stress levels in vitro markedly improved MSC function. These results were validated in an in vivo model of compromised bone healing, which demonstrated significant increase regeneration in aged animals following oral antioxidant administration. These observations indicate the high impact of extrinsic aging on cellular functions and the process of endogenous (bone) regeneration. Thus, addressing the cell environment by, for example, systemic antioxidant treatment is a promising approach to enhance tissue regeneration and to regain cellular function especially in elderly patients.

The need for transparency and good practices in the qPCR literature

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Preoperative irradiation for the prevention of heterotopic ossification induces local inflammation in humans


Radiation of the hip is an established method to prevent heterotopic ossification (HO) following total hip arthroplasty (THA) but the precise mechanism is unclear. As inflammatory processes are suggested to be involved in the pathogenesis of HO, we hypothesized that the preoperative irradiation impacts local immune components. Therefore, we quantified immune cell populations and cytokines in hematomas resulting from the transection of the femur in two groups of patients receiving THA: patients irradiated preoperatively (THA-X-hematoma: THA-X-H group) in the hip region (7 Gy) in order to prevent HO and patients who were not irradiated (THA-H group) but were postoperatively treated with non-steroidal anti-inflammatory drugs (NSAIDs). Radiation resulted in significantly increased frequencies of T cells, cytotoxic T cells, NK cells and CD25+CD127-Treg cells, whereas the number of naive CD45RA-expressing cytotoxic T cells was reduced. These results indicate differential immune cell activation, corroborated by our findings of significantly higher concentrations of pro-inflammatory cytokines (e.g., IL-6, IFN?) and chemokines (e.g., MCP-1, RANTES) in the THA-X-H group as compared to THA-H group. In contrast, the concentration of the angiogenic VEGF was significantly suppressed in the THA-X-H group. We conclude that preoperative irradiation results in significant changes in immune cell composition and cytokine secretion in THA-hematomas, establishing a specific - rather proinflammatory - milieu. This increase of inflammatory activity together with the observed suppression in VEGF secretion may contribute to the prevention of HO.

Terminal differentiated CD8? T cells negatively affect bone regeneration in humans


There is growing evidence that adaptive immunity contributes to endogenous regeneration processes: For example, endogenous bone fracture repair is modulated by T cells even in the absence of infection. Because delayed or incomplete fracture healing is associated with poor long-term outcomes and high socioeconomic costs, we investigated the relationship between an individual's immune reactivity and healing outcome. Our study revealed that delayed fracture healing significantly correlated with enhanced levels of terminally differentiated CD8(+) effector memory T (TEMRA) cells (CD3(+)CD8(+)CD11a(+)CD28(-)CD57(+) T cells) in peripheral blood. This difference was long lasting, reflecting rather the individual's immune profile in response to lifelong antigen exposure than a post-fracture reaction. Moreover, CD8(+) TEMRA cells were enriched in fracture hematoma; these cells were the major producers of interferon-?/tumor necrosis factor-a, which inhibit osteogenic differentiation and survival of human mesenchymal stromal cells. Accordingly, depletion of CD8(+) T cells in a mouse osteotomy model resulted in enhanced endogenous fracture regeneration, whereas a transfer of CD8(+) T cells impaired the healing process. Our data demonstrate the high impact of the individual adaptive immune profile on endogenous bone regeneration. Quantification of CD8(+) TEMRA cells represents a potential marker for the prognosis of the healing outcome and opens new opportunities for early and targeted intervention strategies.

Are pentraxin 3 and transsignaling early markers for immunologic injury severity in polytrauma? A pilot study


BACKGROUND:Inflammatory-related conditions and organ failure (OF) lead to late trauma mortality. Cytokine profiles can predict adverse events and mortality, potentially guiding treatment strategies (damage control surgery versus early total care). However, the specific cytokines to predict the clinical course in polytraumatized patients are not fully identified.QUESTION/PURPOSES:We investigated the early pentraxin 3 (PTX3), IL-6, soluble IL-6 receptor (sIL-6R), and transsignaling ratio (TSR) in polytraumatized patients to estimate immunologic injury severity and predict OF and survival.METHODS:We prospectively followed 58 patients with severe polytrauma, six patients with minor trauma, and 10 healthy volunteers. The mean Injury Severity Score (ISS) was 43 points and the mean Hannover Polytrauma Score (PTS) was 59 points, with a consequently high mortality rate (30%). Twenty-seven of the 58 polytraumatized patients (46%) developed OF, 67% systemic inflammatory response syndrome, and 38% sepsis.RESULTS:Mean sIL-6R concentrations in polytrauma initially were low. Mean PTX3 concentrations were high and peaked at 24 hours. The mean TSR peaked at 6 hours; at that time, the mean value was higher for nonsurvivors. PTX3 concentrations at admission were associated with injury severity calculated by ISS and PTS. Higher PTX3 serum concentrations 24 hours after admission correlated with lower probability for survival.CONCLUSIONS:PTX3, sIL-6R, and TSR were early markers for posttraumatic inflammatory status, OF, injury severity, and TSR for survival after polytrauma. The temporal profile of PTX3 and TSR might be used to anticipate the total injury severity and the clinical course and thereby guide decision making in polytraumatized patients.

CD133: enhancement of bone healing by local transplantation of peripheral blood cells in a biologically delayed rat osteotomy model


Sufficient angiogenesis is crucial during tissue regeneration and therefore also pivotal in bone defect healing. Recently, peripheral blood derived progenitor cells have been identified to have in addition to their angiogenic potential also osteogenic characteristics, leading to the hypothesis that bone regeneration could be stimulated by local administration of these cells. The aim of this study was to evaluate the angiogenic potential of locally administered progenitor cells to improve bone defect healing. Cells were separated from the peripheral blood of donor animals using the markers CD34 and CD133. Results of the in vitro experiments confirmed high angiogenic potential in the CD133(+) cell group. CD34(+) and CD133(+) cells were tested in an
in vivo rat femoral defect model of delayed healing for their positive effect on the healing outcome. An increased callus formation and higher bone mineral density of callus tissue was found after the CD133(+) cell treatment compared to the group treated with CD34(+) cells and the control group without cells. Histological findings confirmed an increase in vessel formation and mineralization at day 42 in the osteotomy gap after CD133(+) cell transplantation. The higher angiogenic potential of CD133(+) cells from the in vitro experiments therefore correlates with the in vivo data. This study demonstrates the suitability of angiogenic precursors to further bone healing and gives an indication that peripheral blood is a promising source for progenitor cells circumventing the problems associated with bone marrow extraction.

Human immune cells' behavior and survival under bioenergetically restricted conditions in an in vitro fracture hematoma model


The initial inflammatory phase of bone fracture healing represents a critical step for the outcome of the healing process. However, both the mechanisms initiating this inflammatory phase and the function of immune cells present at the fracture site are poorly understood. In order to study the early events within a fracture hematoma, we established an in vitro fracture hematoma model: we cultured hematomas forming during an osteotomy (artificial bone fracture) of the femur during total hip arthroplasty (THA) in vitro under bioenergetically controlled conditions. This model allowed us to monitor immune cell populations, cell survival and cytokine expression during the early phase following a fracture. Moreover, this model enabled us to change the bioenergetical conditions in order to mimic the in vivo situation, which is assumed to be characterized by hypoxia and restricted amounts of nutrients. Using this model, we found that immune cells adapt to hypoxia via the expression of angiogenic factors, chemoattracants and pro-inflammatory molecules. In addition, combined restriction of oxygen and nutrient supply enhanced the selective survival of lymphocytes in comparison with that of myeloid derived cells (i.e., neutrophils). Of note, non-restricted bioenergetical conditions did not show any similar effects regarding cytokine expression and/or different survival rates of immune cell subsets. In conclusion, we found that the bioenergetical conditions are among the crucial factors inducing the initial inflammatory phase of fracture healing and are thus a critical step for influencing survival and function of immune cells in the early fracture hematoma.

Deterioration of fracture healing in the mouse model of NF1 long bone dysplasia


Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disease resulting from inactivating mutations in the gene encoding the protein neurofibromin. NF1 manifests as a heritable susceptibility to tumours of neural tissue mainly located in the skin (neurofibromas) and pigmented skin lesions. Besides these more common clinical manifestations, many NF1 patients (50%) have abnormalities of the skeleton. Long bones are often affected (usually the tibia) and the clinical signs range from bowing to spontaneous fractures and non-unions. Here we present the analysis of bone fracture healing in the Nf1(Prx1)-knock-out mouse, a model of NF1 long bone dysplasia. In line with previously reported cortical bone injury results, fracture healing was impaired in Nf1(Prx1) mice. We showed that the defective fracture healing in Nf1(Prx1) mice is characterized by diminished cartilaginous callus formation and a thickening of the periosteal bone. These changes are paralleled by fibrous tissue accumulation within the fracture site. We identify a population of fibrous tissue cells within the Nf1 deficient fracture as alpha-smooth muscle actin positive myofibroblasts. Additionally, histological and in-situ hybridization analysis reveal a direct contact of the fracture site with muscle fascia, suggesting a possible involvement of muscle derived cells in the fracture deterioration.

Biomaterial delivery of morphogens to mimic the natural healing cascade in bone


Complications in treatment of large bone defects using bone grafting still remain. Our understanding of the endogenous bone regeneration cascade has inspired the exploration of a wide variety of growth factors (GFs) in an effort to mimic the natural signaling that controls bone healing. Biomaterial-based delivery of single exogenous GFs has shown therapeutic efficacy, and this likely relates to its ability to recruit and promote replication of cells involved in tissue development and the healing process. However, as the natural bone healing cascade involves the action of multiple factors, each acting in a specific spatiotemporal pattern, strategies aiming to mimic the critical aspects of this process will likely benefit from the usage of multiple therapeutic agents. This article reviews the current status of approaches to deliver single GFs, as well as ongoing efforts to develop sophisticated delivery platforms to deliver multiple lineage-directing morphogens (multiple GFs) during bone healing.

Initial immune reaction and angiogenesis in bone healing


During hematoma formation following injury, an inflammatory reaction ensues as an initial step in the healing process. As granulation tissue matures, revascularization is a prerequisite for successful healing. The hypothesis of this study was that scarless tissue reconstitution in the regenerative bone healing process is dependent on a balanced immune reaction that initiates revascularatory steps. To test this hypothesis, cellular composition and expression profiles of a bone hematoma (regenerative, scarless) was compared with a muscle soft tissue hematoma (healing with a scar) in a sheep model. Upregulation of regulatory T helper cells and anti-inflammatory cytokine expression (IL-10) coincided with an upregulation of angiogenic factors (HIF1α and HIF1α regulated genes) in the regenerative bone hematoma but not in the soft tissue hematoma. These results indicate that the timely termination of inflammation and early onset of revascularization are interdependent and essential for a regenerative healing process. Prolonged pro-inflammatory signaling occurring in a delayed bone-healing model supports...
the finding that timely termination of inflammation furthers the regenerative process. Differing cellular compositions are due to different cell sources invading the hematoma, determining the ensuing cytokine expression profile and thus paving the path for regenerative healing in bone or the formation of scar tissue in muscle injury.

An experimental setup to evaluate innovative therapy options for the enhancement of bone healing using BMP as a benchmark--a pilot study


Critical or delayed bone healing in rat osteotomy (OT) models is mostly achieved through large defects or instability. We aimed to design a rat OT model for impaired bone healing based on age, gender and parity. The outcome should be controllable through variations of the hematoma in the OT including a bone morphogenetic protein (BMP) 2 guided positive control. Using external fixation to stabilise femoral a 2 mm double OT in 12 month old, female Sprague Dawley rats after a minimum of 3 litters healing was characterised following in situ haematoma formation (ISH-group)), transplantation of a BMP charged autologous blood clot (BMP-group) and the artificial blood clot only (ABC-group) into the OT-gap. In vivo micro-computer tomography (μCT) scans were performed after 2, 4 and 6 weeks. After 6 weeks specimens underwent histological analyses. In μCT examinations and histological analyses no bony bridging was observed in all but one animal in the ISH-group. In the BMP group complete bridging was achieved in all animals. The ABC-group showed less mineralised tissue formation and smaller bridging scores during the course of healing than the ISH-group. In this pilot study we introduce a model for impaired bone healing taking the major biological risk factors into account. We could show that the in situ fracture hematoma is essential for bone regeneration. Using BMP as a positive control the presented experimental setup can serve to evaluate innovative therapeutical concepts in long bone application

Inflammatory phase of bone healing initiates the regenerative healing cascade.


Bone healing commences with an inflammatory reaction which initiates the regenerative healing process leading in the end to reconstitution of bone. An unbalanced immune reaction during this early bone healing phase is hypothesized to disturb the healing cascade in a way that delays bone healing and jeopardizes the successful healing outcome. The immune cell composition and expression pattern of angiogenic factors were investigated in a sheep bone osteotomy model and compared to a mechanically-induced impaired/delayed bone healing group. In the impaired/delayed healing group, significantly higher T cell percentages were present in the bone hematoma and the bone marrow adjacent to the osteotomy gap when compared to the normal healing group. This was mirrored in the higher cytotoxic T cell percentage detected under delayed bone healing conditions indicating longer pro-inflammatorious processes. The highly activated periosteum adjoining the osteotomy gap showed lower expression of hematopoietic stem cell markers and angiogenic factors such as heme oxygenase and vascular endothelial growth factor. This indicates a deferred revascularization of the injured area due to ongoing pro-inflammatory processes in the delayed healing group. Results from this study suggest that there are unfavorable immune cells and factors participating in the initial healing phase. In conclusion, identifying beneficial aspects may lead to promising therapeutical approaches that might benefit further by eliminating the unfavorable factors CD73 and CD29 concurrently mediate the mechanically induced decrease of migratory capacity of mesenchymal stromal cells.


Assumption that mesenchymal stromal cell (MSC)-based-therapies are capable of augmenting physiological regeneration processes has fostered intensive basic and clinical research activities. However, to achieve sustained therapeutic success in vivo, not only the biological, but also the mechanical microenvironment of MSCs during these regeneration processes needs to be taken into account. This is especially important for e.g., bone fracture repair, since MSCs present at the fracture site undergo significant biomechanical stimulation. This study has therefore investigated cellular characteristics and the functional behaviour of MSCs in response to mechanical loading. Our results demonstrated a reduced expression of MSC surface markers CD73 (ecto-5'-nucleotidase) and CD29 (integrin 81) after loading. On the functional level, loading led to a reduced migration of MSCs. Both effects persisted for a week after the removal of the loading stimulus. Specific inhibition of CD73/CD29 demonstrated their substrate dependent involvement in MSC migration after loading. These results were supported by scanning electron microscopy images and phalloidin staining of actin filaments displaying less cell spreading, lamellipodia formation and actin accumulations. Moreover, focal adhesion kinase and Src-family kinases were identified as candidate downstream targets of CD73/CD29 that might contribute to the mechanically induced decrease in MSC migration. These results suggest that MSC migration is controlled by CD73/CD29, which in turn are regulated by mechanical stimulation of cells. We therefore speculate that MSCs migrate into the fracture site, become mechanically entrapped, and thereby accumulate to fulfil their regenerative functions

Immunologically restricted patients exhibit a pronounced inflammation and inadequate response to hypoxia in fracture hematoma


For patients who are known to have an impaired immune system, bone healing is often impaired. Therefore, it has been suggested that an effectively functioning immune system will have an influence on the quality of bone healing. Here, we demonstrate that cells within the fracture hematoma of immunologically restricted patients (1) exhibit a disturbed osteogenic differentiation (normal SPP1 but diminished RUNX2 expression), (2) show a strong inflammatory reaction (high IL8 and CXCR4),
and (3) react on local hypoxia (high expression of HIF1A) but with inadequate target gene responses (diminished LDHA and PGK1 expression). Thus, it is already within the early inflammatory phase of fracture healing that the local gene expression in fracture hematomas of immunologically restricted patients points toward a critical regeneration.

Time kinetics of bone defect healing in response to BMP-2 and GDF-5 characterised by in vivo biomechanics


This study reports that treatment of osseous defects with different growth factors initiates distinct rates of repair. We developed a new method for monitoring the progression of repair, based upon measuring the in vivo mechanical properties of healing bone. Two different members of the bone morphogenetic protein (BMP) family were chosen to initiate defect healing: BMP-2 to induce osteogenesis, and growth-and-differentiation factor (GDF)-5 to induce chondrogenesis. To evaluate bone healing, BMPs were implanted into stabilised 5 mm bone defects in rat femurs and compared to controls. During the first two weeks, in vivo biomechanical measurements showed similar values regardless of the treatment used. However, 2 weeks after surgery, the rhBMP-2 group had a substantial increase in stiffness, which was supported by the imaging modalities. Although the rhGDF-5 group showed comparable mechanical properties at 6 weeks as the rhBMP-2 group, the temporal development of regenerating tissues appeared different with rhGDF-5, resulting in a smaller callus and delayed tissue mineralisation. Moreover, histology showed the presence of cartilage in the rhGDF-5 group whereas the rhBMP-2 group had no cartilaginous tissue. Therefore, this study shows that rhBMP-2 and rhGDF-5 treated defects, under the same conditions, use distinct rates of bone healing as shown by the tissue mechanical properties. Furthermore, results showed that in vivo biomechanical method is capable of detecting differences in healing rate by means of change in callus stiffness due to tissue mineralisation.

A 5-mm femoral defect in female but not in male rats leads to a reproducible atrophic non-union


INTRODUCTION: The objectives of this study were to (1) establish a reproducible atrophic non-union model in rats by creation of a segmental femoral bone defect that allows, (2) in-depth characterization of impaired healing, and (3) contrast its healing patterns to the normal course. Hypothesis was that a 5-mm bone defect in male rats would deviate from uneventful healing patterns and result in an atrophic non-union.MATERIALS AND METHODS: A femoral osteotomy was performed in two groups of 12-week-old male rats (1 vs. 5 mm gap) stabilized with an external fixator. Bone healing in these models was evaluated by radiology, biomechanics, and histology at 6 or 8 weeks. The evaluation of the 5-mm group revealed in some cases a delayed rather than a non-union, and therefore, a group of female counterparts was included.RESULTS: The creation of a 5-mm defect in female rats resulted in a reproducible atrophic non-union characterized by sealing of the medullary canal, lack of cartilage formation, and negligible mechanical properties of the callus. In both gap size models, the male subjects showed advanced healing compared to females.DISCUSSION AND CONCLUSION: This study showed that even under uneventful healing conditions in terms of age and bone defect size, there is a sex-specific advanced healing in male compared to female subjects. Contrary to our initial hypothesis, only the creation of a 5-mm segmental femoral defect in female rats led to a reproducible atrophic non-union. It has been shown that an atrophic non-union exhibits different healing patterns compared to uneventful healing. A total lack of endochondral bone formation, soft tissue prolapse into the defect, and bony closure of the medullary cavity have been shown to occur in the non-union model.

The early fracture hematoma and its potential role in fracture healing


Research regarding the potency and potential of the fracture hematoma has begun to receive increasing attention. However, currently there is a paucity of relevant literature on the capability and composition of the fracture hematoma. This review briefly summarizes the regenerative fracture healing process and the close interplay between the skeletal and immune systems. The role of immune cells in wound healing is also discussed to clarify their involvement in immunological processes during regeneration. We attempt to describe the current state of knowledge regarding the fracture hematoma as the initial stage of the regenerative process of fracture healing. The review discusses how a better understanding of immune reactions in the hematoma may have implications for bone tissue engineering strategies. We conclude the review by emphasizing how additional investigations of the initial phase of healing will allow us to better differentiate between deleterious and beneficial aspects of inflammation, thereby facilitating improved fracture treatment strategies.

Insight into the molecular pathophysiology of delayed bone healing in a sheep model


Delayed and nonunions are still challenging problems. In this study, we examined the endogenous mRNA expression of genes regulating cartilage formation, bone formation, endochondral ossification, and bone remodeling during mechanically induced delayed bone healing in a large animal model. A tibial osteotomy was performed in two groups of sheep and stabilized with either a rigid external fixator leading to standard healing or with a rotationally unstable fixator leading to delayed healing. At days 4, 7, 9, 11, 14, 21, and 42 after surgery, total RNA was extracted from the callus. Gene expressions of several molecules functionally important for bone healing were studied by quantitative reverse transcriptase-polymerase chain reaction. The expression profiles were related to callus tissue composition, analyzed by histomorphometry. Histomorphometry demonstrated a delayed, prolonged chondral phase and a reduction in bone formation in the experimental group. There was no differential expression of Runx2 between both groups until day 42, but mRNA expression levels of BMP2, BMP4, BMP7, noggin, Col1a1,
IGF1, TGFβ1, OPN, MMP9, MMP13, TIMP3, TNFα, MCSF, RANKL, and OPG were lower in the delayed healing group at several time points. This study provides insight into the temporal periods during which various factors may be deficient during a compromised bone-healing situation.

Cellular composition of the initial fracture hematoma compared to a muscle hematoma: a study in sheep


Bone fracture leads to a cycle of inflammation, cellular migration, and proliferation to restore tissue integrity. Immune cells at the site of injury are involved especially in the early phase of the healing process, but little is known about the cells present in the initial fracture hematoma. The hypothesis of this study was that the cellular composition in a fracture hematoma differs from that found in a muscle hematoma and that these divergences get more pronounced over time. By using a reproducible osteotomy model and muscle trauma in sheep the distributions of the immune cell subpopulations were evaluated 1 and 4 h after surgery. The cell amount within the first 4 h increased in both hematoma. The number of dead cells was higher in the muscle hematoma. One hour postoperatively the initial fracture hematoma revealed a lower granulocyte percentage compared to the muscle hematoma. The ratio of T helper to cytotoxic T cells was higher in the fracture hematoma compared to the muscle hematoma at both investigated time points. B cell percentage increased in the fracture but not in the muscle hematoma from 1 to 4 h. This is the first study that compares the immune cell subpopulations of a fracture and muscle hematoma.

Differential regulation of blood vessel formation between standard and delayed bone healing

Lienau J. et al., J Orthop Res. 2009 Sep;27(9):1133-40.

Blood vessel formation is a prerequisite for bone healing. In this study, we tested the hypothesis that a delay in bone healing is associated with an altered regulation of blood vessel formation. A tibial osteotomy was performed in two groups of sheep and stabilized with either a rigid external fixator leading to standard healing or with a highly rotationally unstable one leading to delayed healing. At days 4, 7, 9, 11, 14, 21, and 42 after surgery, total RNA was extracted from the callus. Gene expressions of vWF, an endothelial cell marker, and of several molecules related to blood vessel formation were studied by qPCR. Furthermore, histology was performed on fracture hematoma and callus sections. Histologically, the first blood vessels were detected at day 7 in both groups. mRNA expression levels of vWF, Ang1, Ang2, VEGF, CYR61, FGF2, MMP2, and TIMP1 were distinctly lower in the delayed compared to the standard healing group at several time points. Based on differential expression patterns, days 7 and 21 postoperatively were revealed to be essential time points for vascularization of the ovine fracture callus. This work demonstrates for the first time a differential regulation of blood vessel formation between standard and mechanically induced delayed healing in a sheep osteotomy model.

Dr. rer. nat. Katharina Schmidt-Bleek
Principal Investigator