ImmunoTools *multiplex* Award 2014

Anke Dienelt, PhD

Berlin-Brandenburg Center for Regenerative Therapies and
Julius Wolff Institute, Charité – Universitätsmedizin Berlin

Development of a cell therapy for the treatment of impaired fracture healing

Beside liver, only bone tissue possesses the unique capability of scarless self-renewal in the human body. This renewal process is highly regulated and involves a multitude of different cells, distinct types of extracellular matrix and diverse signalling molecules. The fracture healing cascade is initiated by a hematoma formation that goes along with the secretion of pro-inflammatory cytokines. While the granulation tissue within the fracture site maturates, anti-inflammatory signalling augments, fibroblasts start to secrete new matrix, new blood vessels appear and a soft callus is formed. Subsequently, intramembranous ossification at the periosteal regions and endochondreal ossification within the fibrous tissue of the callus start to consolidate the injured area. This is accompanied by a trafficking of stem and progenitor cells with regenerative potential via the emerging vasculature or by migration out of the periosteum and the bone marrow. During healing progression the emerging cartilage islands become hypertrophic, woven bone appears and the so called “hard callus” is formed. Finally the woven bone is remodelled according to the mechanical demands and the bone tissue returns to its original lamellar structure.

However, more than 15% of all fractures show a delayed regeneration with no healing progression in the first three months. Moreover, 5-10% of all fractures do not heal at all and result in the formation of a so called pseudarthrosis or non-union. Amongst others, this impaired healing outcome can be due to disturbances in the early control of the inflammatory cascade (timely anti-inflammation) or a deficiency in angiogenesis. Particularly elderly patients suffer from impaired healing conditions accompanied with continuous discomfort and prolonged hospitalization, with the known consequences of high socio-economic costs. Since we are facing an aging society, there is even a putative increase in the number of occurrences. Clinically available therapies focus on the treatment of established non-unions only.

Thus, I am focusing on the development of an autologous intraoperative cell therapy to provide an early intervention strategy for the treatment of delayed fracture repair. The utilization of endogenous regenerative capacities offers a promising alternative approach to the conventional medical treatment of such pathologies. Different progenitor cells that are characterized by their distinct expression of surface markers,

e.g. CD133, are easily accessible from peripheral blood and have been reported to play a role in vascularization and immune modulation. As angiogenesis and a highly orchestrated immune response are crucial to initiate fracture healing, I hypothesized that an application of these peripheral blood derived progenitor cells to the fracture site can improve the healing outcome in impaired healing situations.

To prove this hypothesis angiogenic and immunological properties of peripheral blood derived progenitor cells were analyzed *in vitro*. I could prove that even in elderly, and hence more often affected people, the regenerative properties of these cells are conserved. Subsequently, first proofs of concept *in vivo* studies were performed. Therefor a rat osteotomy animal model with impaired fracture repair was used. Freshly isolated progenitor cells were locally transplanted into the osteotomy site and the healing outcome was observed via µCT evaluation. This analysis revealed that an application of peripheral blood derived progenitor cells significantly improves bone regeneration.

To further analyze the impact of the transplanted cells on bone healing, I am planning to perform analyses of early healing stages after cell transplantation. I suppose that cell transplantation favors the development of an anti-inflammatory milieu in the fracture hematoma and thereby stimulates early regenerative processes as described above. The multiplex array for human cytokines and different CD markers is an ideal tool to get first insights into the composition of the fracture hematoma after cell transplantation. A comparison to the control group will reveal whether there are changes in the proportion of pro- and anti-inflammatory cytokines as well as different cell types associated with the innate and adaptive immunity.