Local Transplantation Of CD31+ Cells From Peripheral Blood Improves Biologically Impaired Bone Healing By Modulation Of Early Inflammation And Angiogenesis

Anke Dienelt, Dr. rer. nat.1,2, Andrea Sass1, Katharina Schmidt-Bleek1, Sebastian Filter1, Agnes Ellinghaus1, Georg Duda1,2.
1Julius Wolff Institute and Center for Musculoskeletal Surgery, Charité-University Medicine, Berlin, Germany, 2Berlin-Brandenburg Center for Regenerative Therapies, Charité-University Medicine, Berlin, Germany.


Introduction: Delayed healing and false joint formation after long bone fracture are clinical orthopedic problems affecting more than 15% of all patients, particularly within the elderly population. The utilization of endogenous regenerative capacities offers a promising alternative approach to the conventional medical treatment of impaired bone healing conditions. Peripheral blood (PB) derived CD31+ cells are easily accessible and have been reported to play a role in vascularization and the regulation of the immune system. Since sufficient angiogenesis and a tightly controlled inflammation are crucial during tissue regeneration and hence essential in fracture repair, we hypothesize that a local administration of these cells to a fracture gap improves the healing process an early stage. Thereby, delayed healing or even the formation of non-unions might be prevented.

Methods: We analyzed availability, angiogenic and osteogenic potential of human CD31+ cells from PB in relation to age and gender. CD31+ cells were separated from PB via magnetic cell sorting. Flow-cytometric (FC) measurements were performed to check for availability and cell subset composition. Angiogenic properties were analyzed in tube-formation-assays in co-cultures with human endothelial cells. The paracrine effect of the progenitor cells on osteogenesis was investigated in differentiation studies of mesenchymal stromal cultivated under conditioned media derived from the progenitor cells. To prove a positive effect of CD31+ cells on fracture healing after local transplantation we performed in vivo analysis using an established rat model. The isolated cells were transplanted locally into a 2mm femoral defect created in aged rats with biologically impaired fracture healing. The effect of cell transplantation on bone regeneration was analysed histologically after 6 weeks and via µCT measurements after 2, 4 and 6 weeks.

Results: As evident from FC measurements, the circulation of CD31+ cells in the human PB is persistently high throughout all probands (60 - 70% of viable positive cells within the lymphocyte gate). The CD31+ cell population contained beside others large amounts of monocytes, naive T- and B-cells, as well as early and late endothelial outgrowth cells. Tube-formation-assays confirmed that CD31+ cells have a high angiogenic potential independent of the donor age or gender. Analysis of the osteogenic differentiation proved that CD31+ cells have a positive impact on osteogenesis. Finally, the regenerative potential of CD31+ cells could be proven in vivo by a higher bone tissue formation within cell-treated animals, compared to a control group treated with the entity of peripheral blood mononuclear cells. Protein-and gene expression analysis at early healing time points revealed an increased expression of angiogenic growth factors and an altered immune response in the CD31+ cell treated animals.
**Discussion:** We could prove that CD31+ cells from peripheral blood hold regenerative capacities, stimulates angiogenesis and modulates the early inflammatory healing cascade after local transplantation. Thus, a direct application of these cells to a fracture site during the first intervention is a promising approach for the prevention of impaired healing.

**Significance:** Since the population in developed countries grow older and older, patient numbers that suffer from continuous discomfort and prolonged hospitalization by impaired bone healing increases with the known consequences of high socio-economic costs. The study presented here indicate that CD31+ cells may be promising candidates for an autologous cell therapy facilitating fracture healing under impaired conditions, even in elderly and hence more often affected patients. Thus, hospitalization and rehabilitation times could be reduced by stimulation of the endogenous regenerative healing cascade.

*ORS 2015 Annual Meeting*
*Poster No: 0661*