

## AN ENGINEERED VASCULARIZED GRAFT FOR LARGE BONE DEFECT

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**Background and Introduction:** Autologous bone grafting is the current golden standard for the repair of large bone defects, despite its drawbacks like limited availability of grafting material and donor site morbidity. Possible alternatives like allografts or xenografts have also serious limitations, like the risk of infections, possible immune reactions and ethical issues. Due to these problems, researchers in the area of bone repair have explored alternative solutions. Calcium and phosphate based materials as well as polymer scaffolds have shown some interesting osteoconductive properties. Nevertheless, the lack of osteoinductive potential prevents the healing of large bone defects treated only with such alloplastic materials. Many studies have shown that the lack of osteoinductive potential of such scaffolds can be partly overcome by seeding mesenchymal stem cells (MSC) onto the scaffold prior to implantation. However, a major problem still remains, namely the insufficient vascularization of the central part of these large grafts (>4cm).

**Rational:** Therefore, the aim of this study was to *in vitro* evaluate the interaction of MSC and endothelial cells (EC) combined within a 3D scaffold on MSC differentiation into osteoblasts and whether the EC were showing any early indications of vessels formation. Furthermore, in an earlier study, we have shown that activated platelet-rich plasma (PRP) had the potential to strongly promote osteoblastic differentiation of MSC (1). This osteoblastogenesis-promoting property of PRP combined with its ability to form a gel upon activation suggests that PRP could act as a carrier for cells, and could at the same time deliver autologous biological stimuli necessary to improve angiogenesis and bone formation within a construct seeded with the appropriate cells. We have therefore studied the potential of a complex 3D-construct composed of polyurethane (PU) scaffold seeded with MSC and EC (3) embedded in a PRP gel in a controlled *in vitro* environment.

**Methods:** Aspirates of bone marrow and blood were obtained from patients undergoing hip surgery after informed consent (KEK Bern 126/03). MSC were isolated using a Ficoll gradient

and were expanded in IMDM, 10% FCS and 5ng/mL FGF-2. Primary human umbilical vein endothelial cells (HUVEC) were purchased from Cascade Biologics (cat# C-003-5C) and were expanded in M200 (Cascade M200-500) supplemented with LSGS (S-003-10). PRP was produced by two consecutive centrifugation steps and was activated by 5U/mL bovine thrombin in CaCl<sub>2</sub>.

Four types of 3D-constructs were evaluated (Fig. 1) Constructs were analyzed for gene expression of typical osteoblastic and endothelial markers as well as genes involved in angiogenesis using real-time RT-PCR. Histological analyses were performed on cryosections using toluidene blue and van kossa staining, immunohistochemistry (laminin, vWf) and immunofluorescence (CD31, Osf2).

Results from two independent experiments performed in triplicates (n=6) are shown as mean±SEM. Statistical analyses were performed using the Mann-Whitney U-test, P<0.05 was considered to be statistically significant.

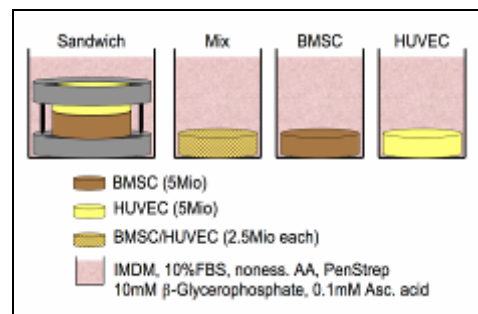


Figure 1. Experimental set-up.

**Results:** MSC and HUVEC embedded in PRP and seeded on PU scaffold were able to survive in shared culture medium for more than 35 days in IMDM, 10% FCS, without further supplementation.

Mix-constructs showed a significant up-regulation of osteoblastic markers compared to MSC alone or Sandwich-constructs after 35 days (Fig. 2). The same was true concerning endothelial and angiogenic markers (von Willebrand factor (vWf), VE-cadherin, EGFL7, VEGFR1,-2, and -3, PDGFRB, Tie1, Tie2, MMP-2 and MMP-9) compared to HUVEC alone or Sandwich-constructs after 35 days.

Histological analyses after 21 and 35 days revealed early mineral deposition only in Mix-constructs (by Van Kossa staining). Furthermore, cells in Mix-constructs showed formation of inter- and intracellular lumen (Fig. 3) as well as formation of tube-like structures, as assessed by immunostaining for vWf and laminin (Fig. 4), as well as immunofluorescence staining for CD31 (data not shown).

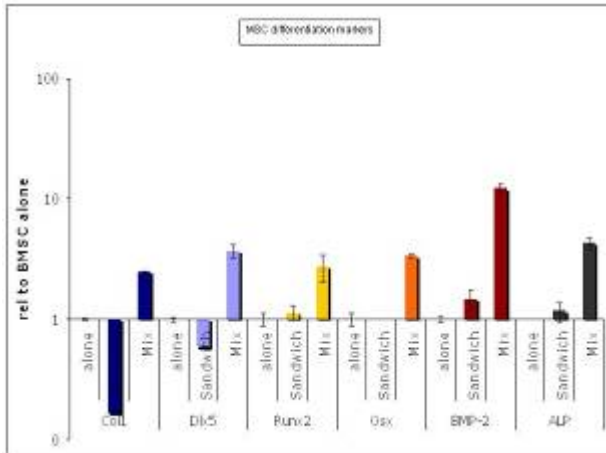


Figure 2: Mix-constructs are showing an up-regulation of osteoblastic differentiation markers.

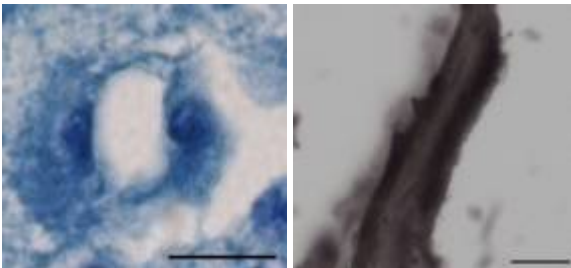


Figure 3: Intercellular lumen. Figure 4: Tube-like structure

**Discussion and Conclusion:** The aim of this study was to define an optimal construct *in vitro* to serve as a potential alloplastic bone graft *in vivo* that could overcome the problem of insufficient vascularization in large bone defects. Our constructs consisting of a polyurethane scaffold seeded with MSC and/or HUVEC in PRP showed an up-regulation of genes involved in osteoblastic and endothelial differentiation, as well as angiogenesis. Furthermore, constructs consisting of a mixture of MSC and HUVEC in PRP (Mix-construct) showed a much higher expression level of the above genes than the other evaluated construct types. This suggests that direct contact of MSC and HUVEC enhances osteoblastogenesis and vessels formation, at least at an early stage. In this respect, histological analyses revealed intra- and intercellular lumen formation as well as tube-like structures only in Mix-constructs. All these

observations were done up to culture day 35, proving the high stability of these tube-like structures. These *in vitro* results suggest that our 3D-construct consisting of a mix of MSC and HUVEC in PRP seeded on a PU scaffold might have the potential to significantly improve vascularization and therefore bone formation within the construct when implanted into a large bone defect *in vivo*.

**References:** (1) Meury T, Kupcsik L, Heini P, Becker S, Stool T, Alini M. Effect of platelet-rich plasma on *in vitro* osteoblastic differentiation of mesenchymal stem cells. *J Cellular Biochem.* 2007 (submitted); (2) Gogolweski S, Gorna K, Turner S. Regeneration of bicortical defects in the iliac crest of estrogen-deficient sheep, using new biodegradable polyurethane bone graft substitutes. *J Biomed Mater Res A.* 77A:802-810, 2006; (3) Lippross S, Verier S, Hoffmann A, Alini M. Platelet release growth factors boost expansion of endothelial progenitor cells. ORS abstract, 2007.

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