INTRODUCTION: Generation of osteoinductive grafts in the context of a cell-based bone repair concept requires osteogenic cells which can be seeded into osteoconductive scaffolds, preferentially ceramic materials. Human bone marrow stromal cells (BMSC) can differentiate toward the osteogenic lineage in vitro, generate bone tissue in vivo [1], and were reported to support the repair of large segmental defects in some clinical cases [2]. Human jaw periosteal cells (JPC), more easily available than BMSC, have recently been proposed for cell-based bone repair [3]. With the ultimate goal of identifying a suitable cell source to generate osteoinductive grafts, in this work we compared the in vitro osteogenic differentiation and in vivo bone forming capacity of JPC and BMSC isolated from the same individuals.

METHODS: Iliac crest bone marrow aspirates and jaw periosteum biopsies were taken from 9 healthy donors (21-80 years) during maxillofacial routine intervention, following informed consent. BMSC were selected as the adherent and proliferating fraction of bone marrow nucleated cells following plating in plastic dishes. JPC were isolated by digestion of the periosteum biopsies in collagenase 0.5% for 4 hours. BMSC and JPC were cultured in alpha modified Eagle Medium containing 10% fetal bovine serum and further supplemented with dexamethasone and FGF-2, previously shown to enhance proliferation and commitment of osteogenic cells [4,5]. After 2 or 3 passages of expansion, the osteogenic capacity of BMSC and JPC was assessed using 3 different models, namely in vitro culture in 2D monolayers (Model I), in vitro culture in 3D scaffolds (Model II), and in vivo implantation (Model III). Model I (9 donors): Expanded BMSC and JPC were cultured were cultured in Petri dishes in osteogenic medium, containing dexamethasone, beta-glycerophosphate and ascorbic acid. Model II (5 donors): Expanded cells were resuspended in fibrin gel (Tisseel®, Baxter, Austria), statically loaded into porous ceramic scaffolds (Vitoss, Orthovita®, Belgium) or bovine bone derived granulates (Bio-Oss®, Geistlich, Switzerland), and cultured in osteogenic medium. Model III (5 Donors): Expanded cells were loaded into the scaffolds as described above and implanted subcutaneously in nude mice (CD-1 nude/nude, Charles River, Germany). Cells from Model I were assessed at 1, 7, 14 or 21 days for DNA amount, alkaline phosphatase activity (AP), calcium deposition and mRNA expression of bone sialoprotein (BSP), osteopontin (OP), cbfa-1 and Osterix using Real-Time RT-PCR [5]. Cells from Model II were assessed at 10 or 20 days for the mRNA expression of the genes listed above. Tissues from Model III were histologically assessed at 8 weeks for the presence of bone tissue following haematoxylin/eosin and Masson-Trichrom stain. Differences among experimental groups were statistically assessed by parametric (T-Tests) or non-parametric tests (Mann-Whitney) based on the normality of the distributions and considered significant with p < 0.05.

RESULTS: Model I: JPC proliferated faster, but had significantly lower AP activity than BMSC at all assessed time points. Calcium deposition by JPC was lower than by BMSC up to day 14 and similar at day 21. The mRNA expression of BSP (Figure 1) and OP was significantly lower by JPC than by BMSC at each time point, whereas cbfa-1 was similarly expressed. Osterix mRNA expression (Figure 1) was generally very low by BMSC, and negligible by JPC. Model II: Independently from the type of scaffold used, the mRNA expression of BSP and Osterix (Figure 2) was remarkably higher than in Model I and significantly lower by JPC than by BMSC. OP and cbfa-1 were similarly expressed by the two cell types. Model III: Bone formation was detected in 29 out of 49 (59%) implants loaded with BMSC, and in 9 out of 49 (18%) implants loaded with JPC. Independently from the type of scaffold used, the area of newly formed bone was higher in BMSC- as compared to JPC-loaded materials (Figure 3).

DISCUSSION: As compared to BMSC, JPC proliferate faster but have a delayed and overall reduced capacity to differentiate toward the osteogenic lineage in vitro and to form bone in vivo. Therefore, BMSC appear to be a more suitable cell source for cell-based bone repair approaches, even if their harvest would be less easy. Interestingly, our data underline that in vitro osteogenic differentiation of both cell types in a 3D system comprising fibrin and a calciumphosphate-based scaffold is greatly enhanced as compared to a 2D system using plastic dishes. Higher mRNA expression levels of BSP and Osterix, but not of OP and cbfa-1, by cells cultivated in the 3D system (Model II) appeared predictive of an increased in vivo bone formation capacity.


AFFILIATED INSTITUTIONS:
*** Osteoarticular Research Group, Institute of Pathology, University of Bern, Switzerland

51st Annual Meeting of the Orthopaedic Research Society
Poster No: 0289