Effect of bone graft substitute on marrow stromal cell proliferation and differentiation

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Introduction: Allogeneic and synthetic bone grafts are often used clinically, due to limited supply and donor site morbidity associated with autologous bone grafts. Seeding allogeneic bone and synthetic grafts with an osteogenic cell population can enhance their potential for bone regeneration. Marrow stromal cells (MSCs) are ideally suited for tissue engineered bone grafts since they have the potential to regenerate bone, but may also maintain the homeostasis of the repaired tissue through their ability of self-renewal and the generation large numbers of mature progeny. An ideal bone graft substitute should support MSC self-renewal as well as differentiation to ensure complete bone defect regeneration and maintenance. The purpose of this investigation was to determine the effect of calcium polyphosphate particles (CPP) on MSC expansion and differentiation. CPP has a relative high strength for a porous ceramic, which makes it attractive to use as a bone graft substitute [1]. MSC proliferation and differentiation on CPP, allogeneic bone and hydroxyapatite/tricalcium phosphate (HA/TCP) were compared in tissue culture.

Materials and Methods: Allogeneic bone was harvested from the femora and tibiae of seven rats. CPP was provided by Dr. Pilliar (University of Toronto) and HA/TCP was purchased from Berkley Advanced Biomaterials Inc. All scaffolds were prepared in particulate form in the range of 1-3mm for CPP and HA/TCP and 1-6mm for allogeneic bone chips. MSCs were harvested from the femora and tibiae of a transgenic GFP Sprague Dawley rat (NBRP, Japan) by virtue of their adherence to plastic, expanded to passage two and cryopreserved until they were used. The thawed MSCs were further expanded to passage five in Mesencult and 15% FBS (Stem Cell Technologies, Vancouver) and then seeded for 6hrs onto the three different particulate substrates. The seeded substrates were cultured in the same expansion condition as described above for a total of three weeks. After 0, 3, 7, 14 and 21 days cell numbers were determined by measuring their metabolic activity using a MTT assay and cell proliferation by 24hrs BrdU incorporation. Osteogenic differentiation of the MSCs was determined by alkaline phosphatase (ALP), cbfa1 (RUNX-2), pro-collagen I (Col-I), osteonectin (ON), osteopontin (OP) and osteocalcin (OC) expression using qRT-PCR. All measurements were made in triplicates. A 2-way ANOVA with the substrates and the time as factors was use for statistical analysis. A p value < 0.05 was considered as statistically significant.

Results: Cell numbers on bone and CPP initial declined but recovered later (Fig 1). In contrast, HA/TCP showed a gradually decreasing trend in cell number. BrdU incorporation increased with time for all substrate and was highest on bone and CPP (p<0.001). No BrdU positive cells were found for day 3 and 7 on HA/TCP. In general, gene expression was similar for the allogeneic bone and CPP. There was a substantial decrease in Col-I and ALP expression within the first 3 days on all substrates. HA/TCP had a significant higher expression of OP and lower expression of ON at day 7, 14 and 21 and lower Col-I expression on day 21 compared to CPP and the allogeneic bone (Fig 3, p<0.001).

Discussion: This comprehensive analysis demonstrated the effect of allogeneic bone, CPP and HA/TCP particles on MSC proliferation and differentiation under expansion condition. After an initial drop in cell numbers, CPP and the allogeneic bone graft supported an increase in proliferation activity at day 14 and 21. In addition, there was no upregulation of the mature bone markers OC or OP on CPP and bone, which suggests that these substrates support MSC expansion rather than differentiation. In contrast, MSC number on HA/TCP decreased with time and only rare BrdU positive cells were observed. This decrease in proliferation combined with the down regulation of Col-I and ON and the substantial increase in OP expression indicate that HA/TCP favours MSC differentiation and maturation along the osteogenic lineage (Fig 3).


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