Endochondral Bone Formation by Chondrogenic Priming of Adult Human Mesenchymal Stem Cells but not with Premineralisation

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Introduction: The use of bioengineered cell constructs for the treatment of bone defects is becoming increasingly common, particularly in research. Often bone marrow stromal cells (BMSCs) are used that are in vitro-stimulated towards the osteogenic lineage aiming at intramembranous bone formation. To date, the success of this approach has been lacking and currently does not reach or surpass the current gold standard of autologous bone grafting. A major concern in these constructs is core degradation and necrosis caused by a lack of vascularisation. We hypothesize that stimulation of cells towards the endochondral ossification process would be more successful. In this study we test how in vitro priming of human BMSCs along osteogenic and chondrogenic lineages influences survival and osteogenesis in vivo.

Methods: MSCs were culture expanded and seeded onto collagen glycosaminoglycan scaffolds. Samples were cultured in vitro in control, osteogenic or chondrogenic differentiation media. Also chondrogenic samples were switched to an osteogenic or glycerophosphate containing medium to mimic the mineralisation phase of endochondral ossification. Following this period scaffolds were implanted subcutaneously into nude mice for either 4, 8 or 14 weeks. Following this period samples were assessed for cartilage and bone forming capacity. We further characterised this process in vitro using pellet culture to better understand the differences between culture conditions that might lead to observed differences in vivo.

Results: Scaffolds that were pre-cultured on chondrogenic culture medium showed collagen type II and collagen type X production. Furthermore, vessel ingrowth from the host was observed after 4 weeks in vivo. Priming along the osteogenic lineage led to a mineralized matrix of poor quality with few surviving cells and no bone formation after 4 weeks. Following a longer in vitro culture period of 5 weeks, followed by an extended in vivo period (8 weeks), mineralization was observed in all conditions. There was however a marked difference in the quality of this mineral between the osteogenically primed vs chondrogenically primed samples. Interestingly, the chondrogenically primed samples were capable of bone formation, whereas the osteogenically primed samples were not, forming a mineralized matrix of poor quality. Of note, samples that were switched from chondrogenic medium to a phosphate containing medium for 1 week also showed no bone formation in vivo despite extensive mineralization.

Discussion & conclusions: These data suggest that chondrogenically primed adult human bone marrow stromal cells are indeed progressing along the endochondral route of bone formation as opposed to the usual route of intramembranous ossification. Importantly, the stage of differentiation at which implantation occurs appears to be critical to the outcome since the presence of any mineralised tissue prior to implantation seemed to prevent true bone formation. The data thus far suggest that chondrogenic priming of BMSCs might provide a superior approach for bone generation in tissue engineering and regenerative approaches.

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