

## Endochondral bone formation by adult human mesenchymal stem cells

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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. These were cultured for a duration of either 3 or 5 weeks *in vitro* in control, osteogenic or chondrogenic differentiation media. Following this period scaffolds were implanted subcutaneously into nude mice for either 4 or 8 weeks respectively. 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.

### 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. There are several advantages of this approach to tissue engineer bone tissue; firstly chondrogenic cells should be better able to withstand the initial hypoxic insult that occurs upon implantation of a construct. Secondly, endochondral ossification is characterised by attraction of blood vessels to the cartilage template as is desired in regenerative medicine. Conversely, intramembranous ossification is usually characterised by increased tissue vascularity followed by bone formation. Thirdly, the complex signalling message required for proper bone formation might be easier to generate using hypertrophic chondrocytes, known to be vital for successful endochondral bone formation in development. 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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