

***In vivo* formation of bone tissue by adult human mesenchymal stem cells depends on the stage of *in vitro* chondrogenic differentiation**

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INTRODUCTION: The use of a cartilage template to generate new bone tissue through an endochondral pathway represents an attractive tissue engineering approach, so far demonstrated for murine embryonic stem cells, but not for human adult mesenchymal stem cells (MSC)¹. We aimed this study at assessing whether the extent of *in vitro* cartilage maturation reached by bone marrow derived MSC (BMSC) regulates ectopic bone formation in nude mice.

METHODS: BMSC from two donors were expanded for 2 passages and cultured in transwell (5E10⁵ cells/insert) for 2 weeks in a *chondrogenic medium* (with TGFβ1; group1), or for 3 weeks in *chondrogenic medium* followed by 2 weeks in a *hypertrophic medium* (without TGFβ1 and with beta-glycerophosphate and thyroxine; group2). The resulting tissues were analysed histologically (Safranin-O, Von Kossa, Alizarin red), immunohistochemically (Collagen-I, -II, -X, bone sialoprotein –BSP–), biochemically (glycosaminoglycans –GAG– and DNA) and by real time RT-PCR (Collagen-II, -X, VEGF, Cbfa-1, BSP, osteocalcin –OC– and MMP-13). Group1 and group2 tissues were also implanted in subcutaneous pouches of nude mice and retrieved after 4 or 8 weeks. Extent of bone formation was quantified by microtomography (μCT) and verified histologically (H&E).

RESULTS: Group1 samples contained a dense matrix intensely stained for GAG and collagen II and a thin peripheral rim positive for von Kossa, negative for Alizarin Red and faintly stained for BSP (Fig1A) and collagen-X. Group2 samples contained cells of larger size embedded in a GAG and collagen-II rich matrix. These tissues displayed a notable increase in the dimension of the outer rim, which was intensely stained for Alizarin Red and BSP (Fig1B), and of the underlying area, which was intensely stained for collagen-X. As compared to group1, group2 tissues expressed higher levels of the hypertrophic and osteoblastic genes collagen-X, VEGF, MMP-

13, BSP, and OC (up to 430-fold). μCT analysis of explants showed that the mineralized tissue volumes and density increased with the *in vivo* incubation time and were higher in group2 samples (Table 1). Histological analysis demonstrated that all explants contained a rim of mineralized matrix increasing in size with the *in vivo* incubation time. However, only group-2 specimens displayed frank bone tissue formation. The cartilaginous centre of such explants underwent intense remodelling, resulting in the formation of cavities rich of infiltrating cells.

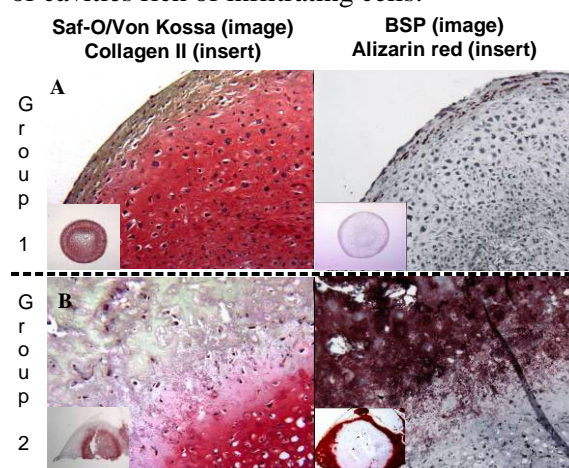


Fig. 1: Representative histological and immunohistochemical pictures of cartilaginous tissues generated *in vitro*. Objectives: 10x (image) and 4x (insert)

Table 1. Histomorphometric data of explants (MV = mineralized volume, MD= mineral density)

		Group 1	Group 2
MV (mm ³)	4 wks in vivo	0.12±0.06	1.07±0.10
	8 wks in vivo	1.06±0.10	2.09±0.49
MD (gray levels)	4 wks in vivo	87.8±3.5	105.8±1.1
	8 wks in vivo	103.3±1.4	116.8±3.4

DISCUSSION & CONCLUSIONS: Our results indicate that cartilage generated by adult human BMSC can efficiently remodel into bone when ectopically implanted in nude mice, but only after reaching a mature hypertrophic phenotype. Ongoing results are aimed at studying molecular pathways involved in such process and at assessing similarity with embryonic endochondral bone formation.

REFERENCES: ¹Jukes JM et al. Proc Natl Acad Sci U S A. 2008, 13;105(19):6840-5.