Combinatorial Cassette for Screening of Osteogenesis by Transplanted Human Bone Marrow Stromal Cells

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Statement of Purpose: The use of in vivo transplantation assays conditions has become a valuable standard for evaluating osteogenic potential of stem cell populations and scaffold formulations. However, preclinical models are low throughput and can have high animal to animal variability. To address these needs, we have developed an *in vivo* combinatorial transplant model that uses a cassette with multiple slots for screening osteogenesis in an immunodeficient mouse subcutaneous model. The combi-cassette approach was tested using a series of positive and negative controls with different combinations of human bone marrow stromal cells (hBMSCs) with hydroxyapatite/tricalcium phosphate (HA/TCP) or gelatin sponge (Gelfoam) scaffold formulations.

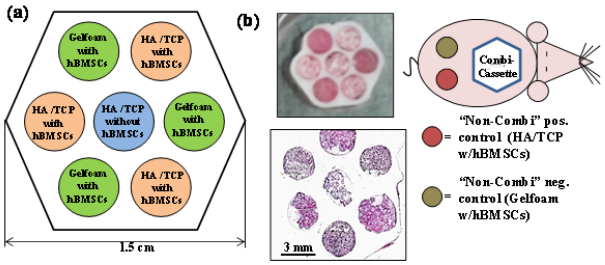

Figure 1. (a) Schematic showing the positions of different hBMSC-scaffold formulations in the Teflon combi-cassette. Each cassette contained 7 formulations including 3 positive controls and 4 negative controls. (b) Top Left: Photo of the combi-cassette loaded with hBMSC-scaffold formulations prior to implantation. Right: Illustration of subcutaneous implant positions in each mouse. Each mouse received 3 implants: 1 combi-cassette containing 7 formulations, 1 "non-combi" positive control implant and 1 traditional "non-combi" negative control implant. Bottom: Histological section of combi-cassette is efficient since all 7 transplants can be handled at once and are present in 1 section.

Methods: Combi-cassettes with 7 wells were fabricated from Teflon using a laser cutter (Fig. 1). The cassettes were 3 mm thick and each microwell was 4.3 mm in dia. with a 44 μL volume. Three of the wells were loaded with a positive control HA/TCP-hBMSC formulation (60:40 hydroxyapatite:tricalcium phosphate powder, Zimmer) which previously yeilded bone in the mouse subcutaneous model [1,2]. Three wells were loaded with a negative control Gelfoam-hBMSC formulation (gelatin sponge, Upjohn) which does not yeild bone [1]. The last well was loaded with a different negative control, HA/TCP without hBMSCs, which also does not yeild bone. Each mouse received 3 implants, one combi-cassette plus two traditional "non-combi" transplants (positive control HA/TCP-hBMSCs and negative control Gelfoam-hBMSCs). Immuno-compromised NOD.Cg-Prkdcscid female mice (age 2 mos.) served as transplant recipients using 3 mice each for 3 time points (4, 8 and 14 weeks).

Results: Histology revealed abundant bone formation for positive controls (HA/TCP-hBMSCs) in both the combi-cassettes and the traditional "non-combi" implants at 4 weeks (not shown) and 8 weeks (Fig. 2). Bone morphology was comparable between combi-cassette and "non-combi" positive controls and good vascularization was found for each condition. In contrast, no bone formation appeared in the Gelfoam-hBMSC negative control samples for either the combi-cassette or the traditional "non-combi" transplants at 4 weeks (not shown) or 8 weeks (Fig. 2). Fourteen-week experiments are in progress. HA/TCP transplants without hBMSCs (Fig. 2f) also failed to demonstrate bone formation, demonstrating that hBMSCs did not migrate between the wells in the combi-cassettes.

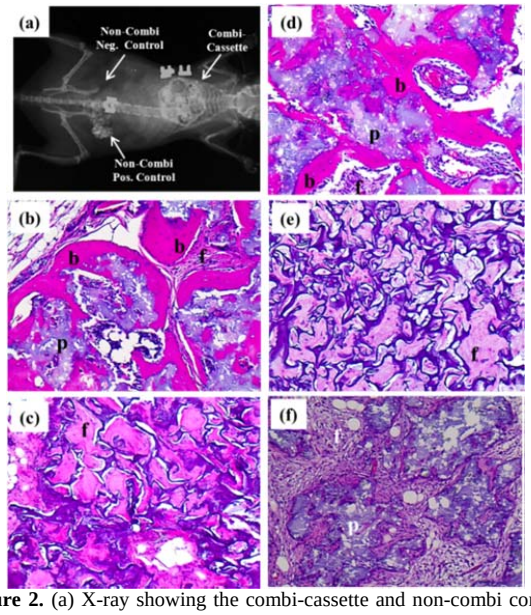

Figure 2. (a) X-ray showing the combi-cassette and non-combi control transplants after 8-week implantation. Histological sections from 8-weeks for (b) non-combi HA/TCP-hBMSCs positive control, (c) non-combi Gelfoam-hBMSCs negative control, (d) combi-cassette HA/TCP-hBMSCs positive control, (e) combi-cassette Gelfoam-hBMSCs negative control, (f) combi-cassette HA/TCP without hBMSCs negative control. [20× magnification, hematoxylin & eosin staining, paraffin embedding, demineralized, b = bone, p = HA/TCP particle, f = fibrous tissue]

Conclusions: We have developed a combinatorial platform for high-throughput *in vivo* screening of osteogenesis by hBMSCs on various scaffold formulations. Typically, a mouse can receive 4 subcutaneous implantations. The combi-cassette increases this to 7 implants, increasing efficiency and reducing animal to animal variability. In addition, each well in the combi-cassette uses 66% less material (cells and scaffold) than the traditional "non-combi" transplants. The combi-cassette also makes for efficient sample handling since the whole cassette can be fixed, embedded and stained at once and all 7 transplants appear on one slide. This approach also has the ethical benefit of reducing the number of animals required for each test.

References: [1] Krebsback, et al., Transplantation 63, 1059, 1997. [2] Mankani, et al., Tissue Eng. 14, 1949, 2008.