Microparticle-Incorporated Human Mesenchymal Stem Cell Aggregates for Facilitating Endochondral Ossification

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Statement of Purpose: For critical-sized bone defects, endochondral ossification, the approach of forming bone via a cartilaginous intermediate, may circumvent issues with initially supplying oxygen and nutrients to cells because chondrocytes are equipped to survive in hypoxic conditions. Our lab has previously demonstrated enhanced chondrogenesis induced by TGF-β1 released from gelatin microspheres (GM) within human bone marrow-derived mesenchymal stem cell (hMSC) aggregates (aggs) [1]. Here, a system of hMSC aggregates incorporated with microparticles capable of tailorable growth factor (GF) delivery was developed to partially recapitulate endochondral ossification. We utilized GM to deliver TGF-β1 to first induce cartilage formation and mineral-coated hydroxyapatite (HA) microparticles (MCM) to deliver BMP-2 in a more delayed and sustained manner to facilitate the remodeling of cartilage to bone. BMP-2 has been shown to enhance TGF-β1-induced chondrogenesis, stimulate chondrocyte hypertrophy and induce osteogenesis. Microparticle incorporation for GF delivery may overcome the transport limitations and time/cost inefficiencies of exogenous (exo.) supplementation. This work lays the foundation for an injectable system that can promote bone repair without prior extended *in vitro* culture.

Methods: GM were synthesized using a water-in-oil single emulsion technique and crosslinked with genipin for 2 hours as previously described [1]. MCM were produced by incubating HA microparticles at 37°C for 7 days in modified simulated body fluid (mSBF). GM and MCM were incubated in PBS containing TGF-β1 and BMP-2 at 37°C for 2 and 4 hours, respectively. ^{125}I-BMP-2 release was characterized by measuring radioactivity of supernatant collected during incubation in mSBF at 37°C. hMSCs (2.5E5/agg; P3) were suspended in a basal medium with GM (0.15 mg/agg) with TGF-β1 (400 ng/mg) and/or MCM (0.05 mg/agg) ± BMP-2 (6400 ng/mg). Aliquots were centrifuged in multiwell plates to form aggregates [1]. All aggregates were cultured in basal media (± 10 ng/ml exo. TGF-β1) for 2 weeks followed by osteogenic media (± 100 ng/ml exo. BMP-2) for 3 weeks. Cells-only aggregates receiving exo. GF treatment were examined as controls. At 2 and 5 weeks, aggregates (N=4) were assayed for DNA, glycosaminoglycan (GAG), alkaline phosphatase activity (ALP) and calcium content using Picogreen, dimethylmethylene blue, 4-nitrophenol and o-Cresophthalein complexome assays, respectively. One-way ANOVA with Tukey's *post hoc* tests was used for statistical analysis.

Results: GM within hMSC aggregates mostly degraded after 2 weeks, signifying most loaded TGF-β1 was released [1]. Sustained BMP-2 release from MCM was achieved (~25% in 30 days) with no burst release. Thus, GM is expected to degrade quickly providing early presentation of TGF-β1, and MCM will release BMP-2 in a more sustained manner due to its stronger affinity to BMP-2. GM crosslinking density and MCM coating layer morphology can be modified to tailor GF release [1,2].

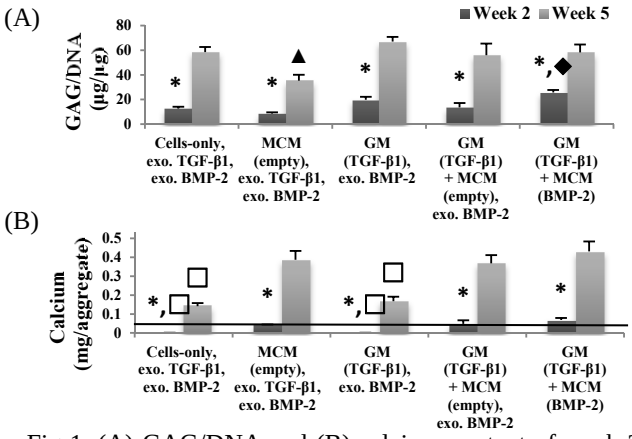

Fig 1. (A) GAG/DNA and (B) calcium content of week 2 and 5 aggregates. Line denotes amount of calcium initially incorporated in each MCM-incorporated aggregate. *Significantly lower than:* ***** *week 5,* ▲ *all other groups and* □ *MCM groups at time point. Significantly higher than:* ♦ *other groups at time point. p<0.05 considered significant.*

At week 2, GAG production was detected in all groups while the only calcium detected was the amount initially incorporated in MCM-treated groups (Fig 1). By week 5, GAG/DNA and calcium content significantly increased in all groups. Groups treated with both MCM and BMP-2 had significantly higher calcium at week 5, indicating these factors may have synergistically promoted mineralization. While aggs treated with only MCM and exo. GF also exhibited high calcium level at week 5, their GAG/DNA was lower than other groups, which may result in less cartilage remodeling into bone at a later time point. Together, cartilage formation occurred prior to mineralization in each group, suggesting endochondral ossification may have transpired. These findings were confirmed histologically and with cells from another donor (data not shown).

Conclusions: Compared to cells-only aggs treated with exo. GF, localized GF delivery resulted in 1) a similar level of GAG production and 2) enhanced mineralization without requiring repeated dosing. Immunohistochemical staining for type I, II, X collagens and osteocalcin is being pursued to confirm the occurrence of endochondral ossification. Future studies may include varying GF release rates and concentrations of microparticles and GF.

References: [1] Solorio LD, et al. Stem Cells Transl Med 2012;1:632-9. [2] Suarez-Gonzalez D, et al. Biomaterials 2012;33:713-21.

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