Spheroid Pellet Culture of Human Bone Marrow Mesenchymal Stem Cells for Endochondral Based Bone Formation

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INTRODUCTION: Endochondral bone ossification is the most common process used by bone to repair fractures and is advantageous over intramembranous ossification where vascularization is low in the injury site [1,2]. This process involves the creation of a cartilage template which slowly ossifies through the changing in growth factor expression and the introduction of calcification promoting molecules. Taking advantage of the endochondral bone formation has been of interest to tissue engineering for bone repair. Current methods have primarily explored the use of scaffold-based constructs, each with varying levels of success [2,3]. Alternatively, this study aims to create scaffold-free bone spheroid pellet cultures using human bone marrow mesenchymal stem cells and the transition from chondrogenesis to osteogenesis to mimic the endochondral bone formation.

METHODS: Human Bone Marrow Mesenchymal Cells (P2; RoosterBio MSC-001) were placed in spheroid promoting 96-well plates (Sarstedt BIOFLOAT 83.3925.400) at $2.5x10^5$ cells per well with 200 µL of media. Three after seeding (approximate time pellets were formed), pellets were split into 5 groups with varying exposure ratios between chondrogenic media (CH) and osteogenic media (OS) over a 42-day feeding period: (i) 100% CH, (ii) 75% CH - 25% OS, (iii) 50% CH - 50% OS, (iv) 25% CH - 75% OS, and (v) 100% OS. CH media contained TGF-β1 to induce chondrogenesis while the OS media contained serum and β-Glycerophosphate to induced osteogenesis. Media was replaced every 2-3 days and switched to OS media at respective time intervals depending on the group. Photos were taken at intervals during the feeding period of the pellets to observe any morphologic changes. After the 42-day feeding period pellets were harvested and photographed.

RESULTS: Photos throughout the feeding period indicated that the pellets fed 100% CH media were the most delicate and tended to lose their structure. However, upon harvesting fully formed pellets were obtained, despite the fractured appearance under the microscope. Pellets subjected to the CH media for longer durations (75% CH - 25% OS and 100% CH) maintained their structure but also displayed the presence of 'satellites' (smaller masses attached to the pellet; Figure 1). After the 42-day culture period, pellets were between 500– 800 um with translucency proportional to the exposure to CH media (Figure 2). Similarly, pellet size was proportional to the degree of OS media exposure with 100% OS pellets having the largest diameters. In addition, upon handling, it was noticed that pellet harness was also associated with the extent of exposure to OS media with the 100% OS pellets displaying the greatest hardness.

DISCUSSION: This study demonstrates the capability of producing bone pellets through the endochondral pathway through the transition between chondrogenic and osteogenic media. All pellets exhibited the presence of satellites, which was most commonly associated with the 100% CH pellets. It is speculated that the lack of mineralization of the chondrogenic pellets promoted the creation of satellites. Satellite structures have been observed in previous cell pellet studies using MSCs [4]. This could also explain the smaller diameters of the pellets more exposed to the CH media. Pellet hardness increased with the extent of osteogenic media suggesting the presence of mineralization. Future work will focus on the biochemical properties (collagen and mineral content) and histological appearance of the generated pellets to determine the best strategy for developing scaffold-free tissue engineered bone constructs.

SIGNIFICANCE: Development of methods to create scaffold-free tissue engineered bone through endochondral ossification have the potential to improve bone repair by creating autologous tissue that can be implanted into fracture or defect site.

REFERENCES: [1] Bueno *et al.* (2011) *Biological Foundations*2] Sheehy *et al.* (2019) *Materials Today Bio 3* 100009;[3] Freeman *et al.* (2017) *Tissue Eng Part B* Rev 23(2):128-141;[4] Allon *et al.* (2009) *SAS J* 3(2):41-49

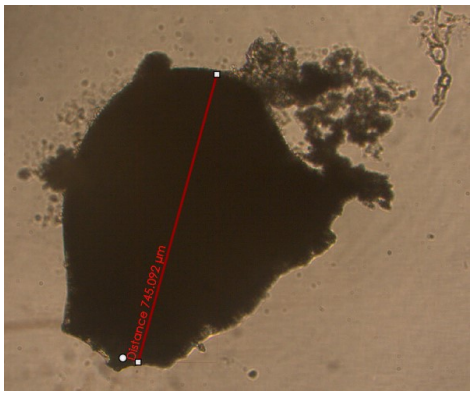

Figure 1: Spheroid pellet created with a 25% CH media 75% OS media feeding schedule with noticeable satellites attached to larger mass.

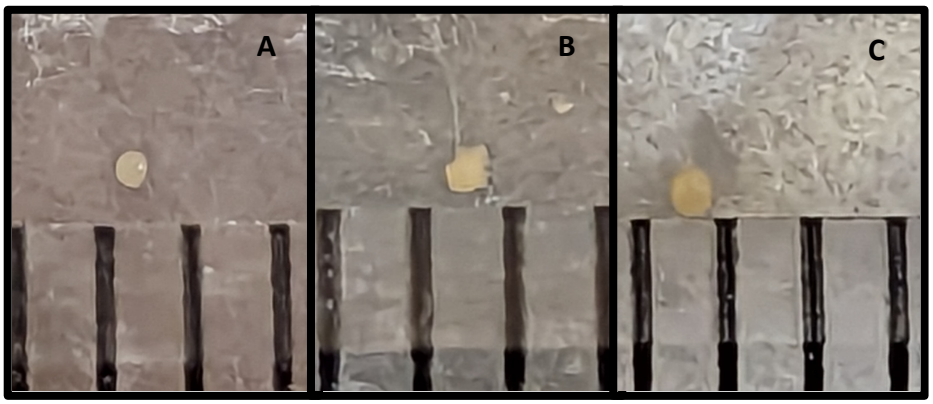

Figure 2: (A) 100% CH pellet, (B) 50% CH 50% OS pellet with noticeable attached satellite, (C) 100% OS pellet. From left to right the translucency of the pellets decrease.