Autologously Enriched Human Bone Marrow Aspirate for Bone Tissue Engineering

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Statement of Purpose: Treatment of large segmental bone defects is still a major challenge in orthopedic surgery. In addition to an adequately porous and mechanically stable scaffold, a clinically relevant cell source is essential for effective bone regeneration. Bone marrow aspirate (BMA) is readily available and is an excellent source for autologous progenitor cells and growth factors. However, the volumetric amount of BMA required to fill in a large defect does not contain adequate numbers of the progenitor cell population, thus leading to limited bone formation[1]. Here we propose concentrating BMA (cBMA) using an FDA approved and automated system, and the cBMA direct use for bone tissue engineering. By demonstrating significantly enhanced mineralization, the cBMA in combination with a scaffold system could potentially be used to develop effective tissue engineering strategies for large segmental bone defect repair and regeneration.

Methods: Fresh human bone marrow aspirate (BMA) was purchased (Lonza, Walkersville MD); 35ml of BMA was enriched to a final volume of 5ml using an automated cell separator, *Magellan*® (Arteriocyte Medical Systems, Hopkinton MA). CFU-*f* assay was carried out by plating 100µl of cBMA and BMA into 150mm culture plates. After incubation for 14 days at 37°C in 5% humidified CO_2, cells were washed with PBS and then stained with 0.5% crystal violet for 10 minutes. Stained colonies were analyzed using ImageJ software. Optimally porous and biomechanically compatible poly(lactide-*co*-glycolide) (PLGA) scaffolds were fabricated using thermal sintering followed by porogen leaching method[2,3]. Osteoblastic potential of cBMA was examined by directly placing 50ul of BMA or cBMA onto tissue culture well plates and PLGA scaffolds. BMA seeded wells or scaffolds were subsequently cultured in osteogenic media for 21 days, and then stained and quantified for mineralization using Alizarin Red staining and quantification assay[3].

Results: While many different BMA enrichment techniques are available, there is a clinical need to employ completely automated systems that can be used for rapid, beside-processing of BMA. We utilized the *Magellan*® system (Figure 1), as it is completely automated and FDA approved for cell enrichment.

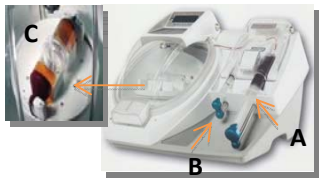

Figure1: *The Magellan® System (Arteriocyte). Automated syringe pumps for (A) BMA delivery and (B) collection syringe. (C) Proprietary disposable separation chamber.*

To examine cBMA enrichment, a colony forming unit (CFU-*f*) assay was performed. While both BMA and cBMA displayed similar number of colonies (Figure 2C), cBMA showed greater uniformity and a significantly larger colony area than BMA cells. Larger CFU-*f* area indicates significantly higher number of cells/colony for the cBMA than BMA.

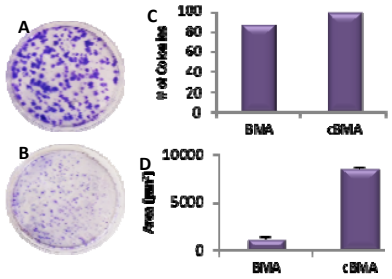

Figure 2: *CFU-f at day 10. A: Concentrated; B: non-concentrated; C: number of colonies; D: average area of colonies.*

Next we investigated the osteogenic potential of both BMA and cBMA, both in monolayer and 3-D porous scaffolds. cBMA cells demonstrate a significantly enhanced mineralization over the BMA seeded groups (Figure 3A). Furthermore, Alizarin Red staining, shown in Figure B &C display uniform mineralization throughout the well or the seeded three-dimensional scaffold, confirming the superiority of the enriched cell population for bone tissue engineering.

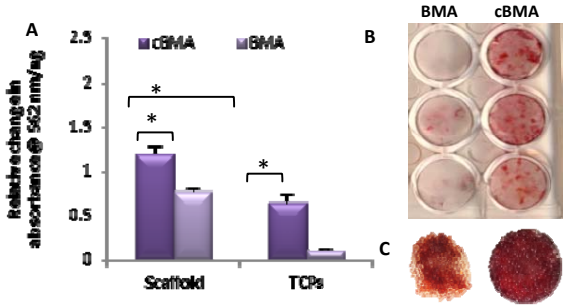

Figure 3: *Alizarin Red Staining of hBMSCs cultured in monolayer and 3-D scaffolds. cBMA displayed significantly higher mineralization than BMA. A: Alizarin Red quantification of cBMA and BMA; B: BMA and cBMA in monolayer culture; C: BMA and cBMA on 3-D scaffold.*

Conclusions: Autologous bone marrow aspirate is a great source of progenitor cell population is a great cell source for bone regeneration. The challenge arises from the use of large volumes of BMA in order to obtain enough osteo-progenitor cells which become inefficient method for bone regeneration. Here, we demonstrated the feasibility of concentrating BMA using a rapid, bedside, fully automated system and its subsequent direct differentiation into osteoblasts. Also in this study, we demonstrated the feasibility of bone formation of cBMA onto mechanically stable and porous scaffolds. The use of cBMA could lead to the development of intra-operative and clinically applicable tissue engineering strategies for segmental bone regeneration.

References: 1. Amini et al. Crit Rev Biomed Eng. 2012;40:363-408. 2. Igwe et al. J Tissue Eng Regen Med. 2012 (Ahead of print). 3. Amini et al. Tissue Eng: Part A. 2012;18:13, 1376-1388.

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