Enrichment of mesenchymal stem cell population modulates cell differentiation

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Statement of Purpose: An understanding of substrate-based control of cell function is critical to the design of biomaterials for regenerating, repairing or replacing diseased tissues. We previously demonstrated that modification of an organic substrate via self assembly of mineral can modify the cell contact distribution and motility of mesenchymal bone marrow cells [1]. Cellular contacts are the major organelles responsible for transferring information between intracellular and extracellular environments, and are major players in cell differentiation. Bone marrow however is a complex mixture of cells. Two major types of cells reside in bone marrow: hematopoietic and stromal cells, including differentiated, progenitor, and stem cells. Extremely low numbers of stem cells in the bone marrow and damaging methods of cell separation also make the study of stem cells complicated. To enrich the mesenchymal bone marrow with stem-like cells an assay was developed administered 5-fluorouracil (5-FU) in vivo [2]. 5FU is chemotherapy drug that blocking DNA synthesis by targeting thymidylate syntase and kills actively proliferating nucleated cells. Stem cells proliferate very slowly and surviving 5FU treatment increasing the percent of stem cells in the population. We propose that mineralization of a substrate seeded with an enriched population of stem-like mesenchymal cells can direct osteogenic differentiation of bone marrow cells.

Methods: Glass coverslips were coated with PLGA (lactic/glycolic acid ratio 85:15) by dipping in 3.5% solution of polymer in chloroform. Half of the PLGA coated coverslips were mineralized in modified simulated body fluid for five days [1]. C57/BL6 mice (4-6 wks old) were used as marrow donors. Animals were injected with 5FU (150 ng/kg injection in the tail vain of mice) after five days bone marrow cells were collected. Sca-1^+, CD-117^+ were used as progenitor cell markers to monitor the level of enrichment of cells. A portion of 5FU and control cells were stained and marked cells were counted using Flow Cytometry ($1x10^6$ cells for each staining). Two groups of cells, 5FU treated and control cells were seeded on PLGA or mineralized PLGA different substrates at 35000/cm^2. Cells were grown for 8 days to the monolayer in MesenCult Basal Medium and Mesenchymal Stem Cell Stimulatory Supplements (StemCell Technology Inc., BC, Canada) designed for stem cell proliferation. After 8 days the medium was changed to the osteogenic medium and was changed every three days. Three coverslips was used per each time point, coverslips were fixed and medium collected at 7, 14 and 21 days after start of differentiation, two coverslips per time point with PLGA and mineral were left without cells as a control. Differentiation was estimated by measuring the level of osteocalcin, and mineralization (von Kossa staining).

Results/Discussion: The number of cells expressing Sca-1^+ and CD-117^+ was enriched up to 40 and 20X, respectively, compared to cells from non-5FU treated mice. Osteogenic differentiation increased on both substrates after 14 days (Fig. 1,2). Cells seeded on mineralized substrates express significantly greater levels of osteocalcin. The enriched cell population demonstrates higher expression of osteogenic markers.

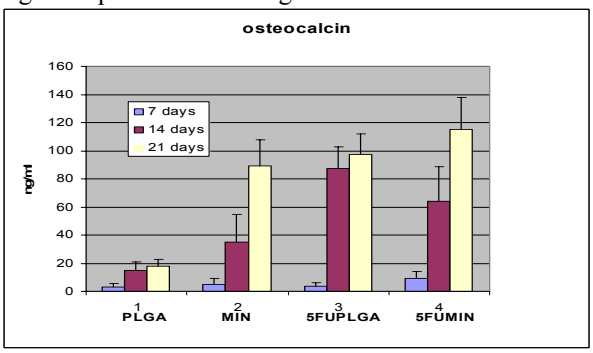

Fig. 1. Osteocalcin expression in control and 5FU treated cells differentiated on PLGA and mineralized PLGA. Osteocalcin expression increased in the cell population treated with 5FU compared to control cells at 14, 21 days ($p<0.05$), and increased on mineralized substrates compared to PLGA ($p<0.005$), by 2 way ANOVA.

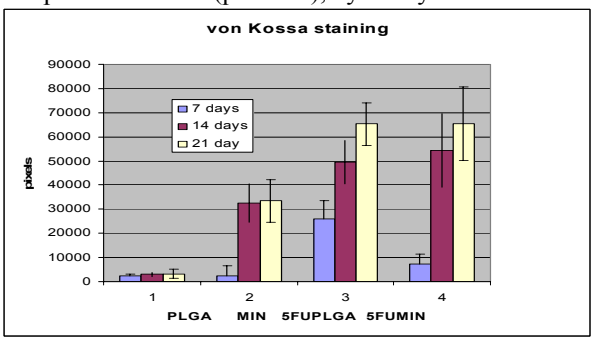

Fig. 2. von Kossa staining of coverslips with differentiated cells. Images of coverslips were taken, Scion program was used to measure the amount of dark pixels, subtracting the amount of background pixels determined from coverslips without cells,. Significant difference between 5FU treated cells and control cells ($p<0.005$), 2 way ANOVA.

Conclusions: Mineralization of a substrate can result in an enhancement of differentiaton. Similarly, enrichment of the mesenchymal stem cell population in bone marrow can improve cell differentiation. Effects of biomaterial surface modification are lessened when an enriched population of cells is used. .

References:

[1] Leonova, et al., JBMR A. 2006; 79: 263-270.

[2] Wang, et al., Stem Cell 2006; 24: 1573-1582.

Acknowledgments:

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