In Vitro Optimization of Clinical Formulations of Adult Stem Cells with Matrices for Bone Regeneration

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Statement of Purpose:

The use of biomaterial carriers to deliver stem cells to fracture sites is an attractive strategy for regenerating bone. However, there are several challenges in delivering cells to sites of varying size and shape in a way that maintains cell viability and functionality, while offering a flexible and user friendly system for clinicians.

Aastrom Biosciences has pioneered the development of single-pass perfusion (SPP) bioreactor technology for the expansion and enrichment of adult stem and progenitor cells from human bone marrow[1]. The Tissue Repair Cell product, or TRCs, have been transplanted into over 200 clinical patients for a variety of tissue regeneration applications[2]. Currently, TRCs are being tested in Phase II clinical trials to enhance healing of long bone non-union fractures and for maxillary sinus floor augmentation. TRCs are formulated with βTCP matrices and autologous plasma as a binding agent for the surgical delivery of the TRCs to the site of injury.

To optimize the clinical mixing protocol, we developed an *in vitro* culture system for the analysis of the cell product post-mixing. We hypothesized that varying the ratio of the individual components of the final mixture, as well as the method for mixing the cells and biomaterials would impact material handling and/or cell viability and post-mixing functionality. We evaluated both the handing properties of the materials and TRC function after mixing within the cell-matrix constructs. TRC-biomaterial formulations have been optimized for handling and maintenance of TRC viability, metabolic activity, and osteogenic potential.

Methods:

Material handling properties were evaluated by mixing Vitoss micromorsel βTCP matrix particles (1-2mm, Orthovita, PA), Isolyte, and fresh frozen plasma (UM Blood Bank, Ann Arbor, MI) activated with 5% $CaCl_2$ for clotting at varying ratios (1:1:0 to 4:4:1). Clotting time and material properties were evaluated to identify final mixtures with necessary handling properties. TRCs were produced from bone marrow mononuclear cells (Cambrex, MD) via a 12 day ex vivo expansion in a SPP bioreactor[1] using an IMDM base medium with 10 % horse and fetal bovine serum, and antibiotics. Harvested TRCs were washed and suspended in Isolyte with 0.5% human serum albumen at 5 M cells/ml. 5ml of the TRC suspension was then mixed with 5cc of Vitoss micromorsels and 5ml plasma (a 1:1:1 ratio), the plasma was allowed to clot, and 0.1cc of the mixture was scooped into the individual wells of 24 well plates for in vitro analysis. Cell-matrix mixtures were cultured in 1ml of expansion media or DMEM containing 10% FBS and osteogenic supplements for 13 days. Cell viability and total metabolism per well were measured via Live/Dead staining kit (BioVison) and CellTiterBlue kit (Promega). Osteogenic potential was evaluated by total alkaline phosphatase activity per well (Attophos kit, Promega). Data are reported as the mean and standard error, with a sample number of 3 or 4 per time point.

Results / Discussion:

Material Handling: Diluting plasma > 1:1 with the cell suspension buffer resulted in undesirable handling properties, because the plasma did not sufficiently bind the Vitoss matrix. A 1:1:1 ratio of the components provided excellent handling and clotting time was only 2-3 minutes. Poor handling compositions can not be used in a clinical setting and thus were not evaluated *in vitro*.

Cell Viability and Activity: Fluorescent Live/Dead cell images taken at timed intervals during the 13 day cultures routinely showed >90% cell viability in the mixtures, demonstrating that the components and the mixing process had no adverse effects on the TRCs. Cell density per Vitoss particle also noticeably increased qualitatively over time. Cell metabolism per well (normalized to Day 1 readings, Fig 1) increased 6.7 fold +/- 0.3 on Vitoss matrices alone over 13 days, and 7.8 fold +/- 0.3 with Vitoss+Plasma, demonstrating that the addition of plasma either enhances total cell metabolism in the cultures or enhances cell proliferation over time. The negative control demonstrates that the CellTiterBlue signal is not effected by the matrix component of the cultures.

Osteogenic Potential: Alkaline phosphatase activity normalized to metabolic activity per well at 13 days was approximately 50% higher in the TRC+Vitoss+Plasma cultures compared to the TRC+Vitoss cultures (9.1+/-0.1 RFU vs. 6.1+/-0.3 RFU), demonstrating that the TRCs maintained their osteogenic potential after mixing.

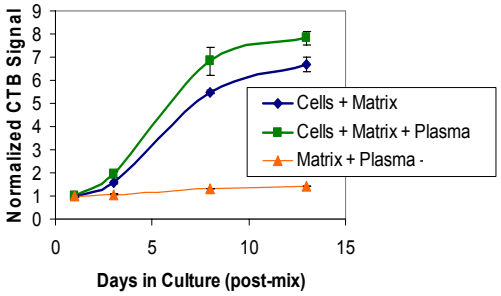

Fig 1. Total cell metabolism per well, normalized to day 1

Conclusions:

TRCs have been formulated with clinically relevant βTCP biomaterials bound with fresh plasma (1:1:1 ratio) while maintaining desirable handling characteristics and cell viability, metabolic activity, and functionality. These materials and formulations will facilitate the clinical testing of TRCs in open surgical bone grafting indications with the potential of ultimately serving as the delivery platform for a commercial cell product.

References:

1) **Mandalam, et al in Ex Vivo Cell Therapy, AP (1999), pg273**
2) **www.aastrom.com**