Adult-Derived Adipose Stem Cells for Bone Regeneration

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Statement of Purpose: An adult stem cell (ASC) is an undifferentiated cell found among differentiated cells in a tissue or organ. It can renew itself and differentiate to yield the major specialized cell types of the tissue or organ. The primary roles of ASCs in a living organism are to maintain and repair the tissue in which they are found. Given the right conditions, some adult stem cells have the ability to differentiate into a number of different cell types. Adult tissues reported to contain stem cells include brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, liver and adipose tissue. Adipose is an attractive source of ASC because it is abundantly available, easily accessible, and routinely discarded in medical procedures. In vitro, adult-derived adipose stem (ADAS) cells have been induced to form adipose, muscle, cartilage, and bone. Due to their availability and plasticity, many consider that autologous ADAS cells will become the basis of therapies for many serious and common diseases. The present study demonstrates the use of ADAS cells to form bone tissue.

Methods: *Cell culture:* Human adult adipose tissue from a fresh surgical specimen was obtained; ADAS cells were isolated and expanded using a hydrogel cell-culture system. Cells were cultured in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) and gentamicin. *Cell identification:* ADAS cell verification was completed using fluorescence-activated cell sorting (FACS) analysis. The population of ADAS cells in culture was identified by the presence of surface markers – cellular differentiation (CD) antigens. *Osteogenic differentiation in vitro:* ADAS cell cultures were induced towards osteogenic differentiation using osteogenic media (DMEM with 1% FBS, dexamethasone, beta-glycerol phosphate, ascorbic acid) or by exposure to demineralized bone matrix (DBM). Osteogenic differentiation was determined by alkaline phosphatase (ALP) activity. As controls, C2C12 cells were cultured and tested similarly. *Bone formation in vivo:* Cultured ADAS cells ($1x10^8$/mL) and/or DBM (4 mg/mL) were encapsulated within the hydrogel culture system and injected into subcutaneous site of athymic nude mice. The injected mixtures were harvested at 1, 2, and 4 weeks, and were analyzed to determine osteogenic differentiation using histological methods.

Results / Discussion: *Cell culture:* The fibrin-based hydrogel cell-culture system showed excellent biocompatibility in terms of allowing cellular proliferation and migration. *Cell identification:* FACS analysis revealed that the cell population isolated was positive for the following cell surface antigens: $CD44^+$ and $CD90^+$. The isolated cells were negative for the following surface antigens: $CD14^-$, $CD31^-$, and $CD34^-$. These results are consistent with established ADAS cell lines. *Osteogenic differentiation in vitro*: The ALP activity was elevated more than 1,000-fold in ADAS cells than C2C12 cells (Figure 1).

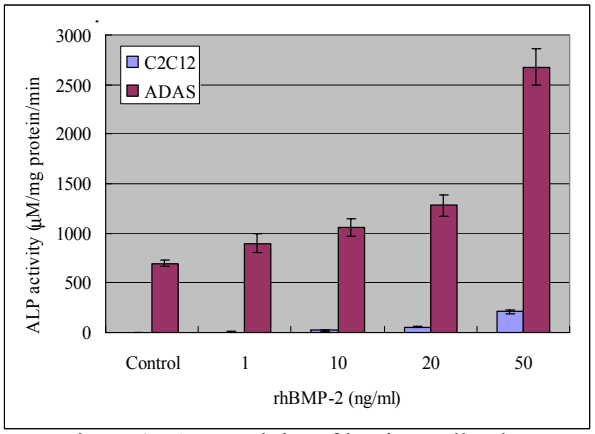

Figure 1: ALP activity of in vitro cell cultures

Bone formation in vivo: At 1, 2, and 4 weeks post-implantation, greater vascularization was visually evident for the ADAS cells/DBM/hydrogel implant than the DBM/hydrogel. Furthermore, histologic examination of the 4 week implants revealed a higher degree of new bone formation in the ADAS cells/DBM/hydrogel mixture than the DBM/hydrogel mixture (Figure 2).

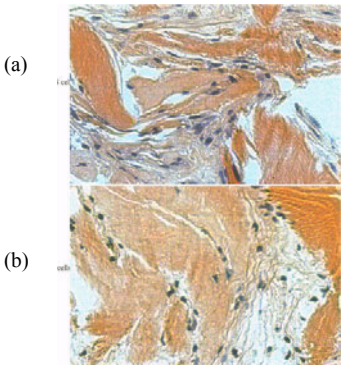

Figure 2: Hemotoxylin and Eosin (H&E) stain of (a) DBM/hydrogel and (b) ADAS cell/DBM/hydrogel implants after 4 week implantation.

Conclusions: This study illustrates the potential clinical use of ADAS cells. Human adipose tissue was harvest and ADAS cells were isolated and successfully expanded in culture using a novel cell-culture system. The cells were induced towards osteogenic differentiation *in vitro*, and used to form bone *in vivo*. The use of adult derived autologous stem cells eliminates the potential of rejection, chronic inflammation and disease transmission when using cellular therapies for the treatment of disease or the generation of tissues.