Generation of Adipose Tissue from Adipose Derived Stem Cells in HyStem Hydrogels

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Introduction . Currently there are no permanent solutions for the repair of soft tissue contour defects due to tumor resection, congenital defects, and trauma. While a few inert materials (i.e. plastics) have been proposed for implantation to fill a subcutaneous defect, none can fully restore the lost adipose tissue[1]. In this work we propose to use the stromal vascular fraction (SVF) and the inherent adipose derived stem cells (ADSC) in combination with HyStem-C, a hyaluronic acid based matrix, to address soft tissue contour defects and eventually as a means to replace lost adipose tissue. SVF is obtained from human lipoaspirates that are routinely discarded. Hystem-C is a semi-synthetic extracellular matrix (ECM) hydrogel that can be used for either 2D or 3D cell cultures or as a minimally invasive delivery matrix for cellular therapies[2]. In this work we will (1) evaluate HyStem-C's functionality as a matrix for ADSC culture (both 2D and 3D), (2) evaluate the effects of seeding density on cellular growth, (3) the ability to differentiate ADSC to mature adipocytes i*n vitro* on and in HyStem-C, and (4) the *in vivo* response of human SVF injected in HyStem-C in a nude mouse model.

Materials and Methods. Adipose derived stem (ADSC) cells were isolated from human lipoasperates as previously described[3, 4]. The stromal vascular fraction (SVF), which contains the ADSCs, was also isolated. ADSCs or SVF were added to pre-gel HyStem-C solutions to obtain 3D cell cultures. For 2D culture cells were plated on top of gelled HyStem-C. Various cell seeding densities and the effects of SVF were examined. Cultured were allowed proliferative to approximately 80% confluency at which point StemPro adipogenesis differentiation media was added to the culture. After 14 days, cultures were fixed and stained using H&E and Oil Red O to identify mature adipocytes. Differences in culture conditions were identified by quantitative image analysis. For *in vivo* studies, 12 nude mice received 100 uL dorsal subcutaneous injections of HyStem-C, HyStem-C + SVF, SVF in saline, and saline.

A cell density of 500,000 cells/mL was used for both Hystem-C + SVF and SVF in saline experimental groups. Animals were sacrificed at 4, 8, and 12 weeks and tissue was cryosectioned and stained for H&E, Massons Trichrome, Oil Red, and IHC human markers[5].

Results. Metabolic cellular activity was observed in both 2D and 3D cell cultures as measured by Alamar Blue assays. 2D cultures reached confluency near day 8 and cell activity corresponded. The growth rate of purified ADSC was not statistically different from heterogenous plating of SVF and it's resulting adherent population, Figure 1. ADSC's cultured in 2D and then differentiated showed positive staining with Oil Red O. Cultures grown on tissue culture plastic were comparable with those grown on HyStem-C.

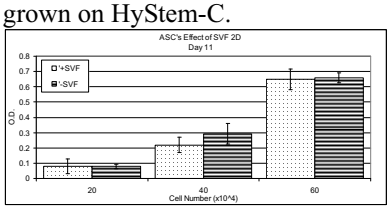

Figure 1. SVF and purified ADSC proliferation rate at various seeding densities

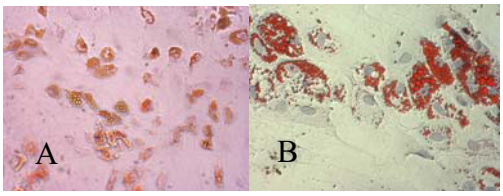

Figure 2. Oil Red O staining in of SVF cultured on (A) HyStem-C and (B) TCPS.

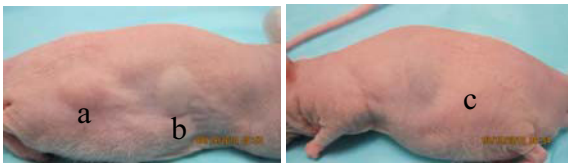

Figure 3. Subcutaneous injections of (a) ADSC in HyStem, (b) HyStem, and (c) ADSC in saline

Discussions and Conclusions. With over 200,000 liposuction procedures each year, subcutaneous adipose tissue is an abundant and readily accessible source for ADSC[6]. The stromal vascular fraction (SVF) which contains ADSC as well as circulating blod cells, fibroblasts, pericytes, endothelial cells and preadipocytes can be easily obtained from lipoaspirate samples. In this work, we determined that the minimally manipulated SVF results in comparable tissue genesis as the more purified ADSC. This is significant in that the regulatory hurdles are reduced for a minimally manipulated point-of-care cell therapy. Future studies will explore injection volume on adipose tissue generation and any diffusion limiting effects. Additionally, biocompatibility and safety/toxicity evaluations in accordance with ISO-1099 will be performed.

References

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