Rat Adipose derived stem cell (rASC) adhesion and viability on non-cross-linked porcine acellular dermal matrix (ncl-PADM) in vitro

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Statement of Purpose:

Previous work in our lab with non-cross-linked porcine acellular dermal matrix (ncl-PADM) demonstrates its excellent clinical utility in reconstructive surgery[1, 2]. Additionally, we recently showed that adipose tissue derived stem cells (ASCs) increased cellular infiltration, revascularization, and remodeling of ncl-PADM ventral hernia repairs *in vivo* [3]. In this study, we report the viability, adhesion, and proliferation of ASCs onto ncl-PADM *in vitro*.

Methods:

Subcutaneous fat tissue was harvested from syngeneic Brown Norway rats. ASCs were cultured in polystyrene flasks with α-minimum essential medium containing 20% fetal bovine serum (FBS), 2mM L-glutamine, 100µg /ml penicillin and 100 µg/mL streptomycin and incubated at 37°C, 5% CO_2, and 90% humidity. To determine the fraction of stem cell population in the cultured ASC's, cells were labeled with antibodies including CD 29, CD 44, CD 90, CD 31, CD 45 and P4HB (prolyl 4-hydroxylase) and analyzed with fluorescence activated cell sorting (FACS). FACS data analysis was completed with FlowJo software.

ASCs (P4-P7) were seeded onto ncl-PADM and cell viability was obtained using Live/Dead Viability/Cytotoxicity Kit. Cell adhesion on ncl-PADM was assessed using MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)-assay for 40 minutes to 4 hour. Cell attachment and morphology was examined using SEM at 1 h, 4 h and 24 h after plating. ASC proliferation was also analyzed using MTT assay for 1-14 days. To analyze & optimize the effect of ASCs seeding density on ncl-PADM, cell adhesions on ncl-PADM seeded at 3 initial seeding densities including 25,000, 50,000 and 100,000 cells/cm^2 was compared using MTT-assay.

Results:

Flow cytometry results revealed that 99% of cultured cells were CD 29$^+$, CD 44$^+$ and CD 90$^+$ (Fig 1). ASCs cultured on ncl-PADM for 2 hours stained 99% positive for Calcein live cell staining. SEM pictures demonstrated initial stages of cellular attachment with round morphology (Fig 2) 1 h after plating where as the cell attachment was well established with flat morphology after 4 h incubation. MTT data for initial cell adhesion from 40 min to 4 hours at all initial cell seeding densities including 25,000, 50,000 and 100,000 cells/cm^2 showed a maximum adhesion of 8400 cells/cm^2 (±3400), 13900 cells/cm^2 (±15500) and 14353 cells/cm^2 (±7849) respectively cells proliferated from 1 day to 14 days on ncl-PADM. A comparison of MTT data for 1 day ASCs culture on ncl-PADM with 25,000 cells/cm^2 and 50,000 cells/cm^2 showed only 6500 cells/cm^2 (±750) and 9300 cells/cm^2 (±1500). MTT data showed no significant Proliferation of ASC on ncl-PADM within 1 week with a maximum of 7000 cells/cm^2.

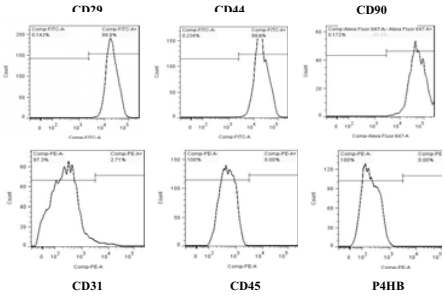
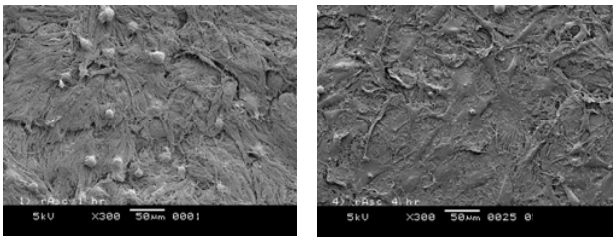


Fig 1: FACS analysis of rASC shows 99.8% positive for stem cell adhesion markers CD 29 CD 44, stromal cell marker CD 90, negative for hematopoietic cell markers CD 31, CD 45 and fibroblast marker P4HB.



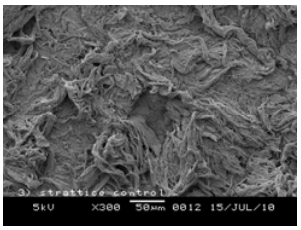


Fig 2: SEM images of ASCs seeded onto ncl-PADM at 1 h (top left), 4 h (top right) and ncl-PADM structure without cells (bottom).

Conclusions:

Ncl-PADM provides a suitable scaffold for ASCs to adhere and may be useful for clinical reconstructive applications. The optimum seeding density for cell study would be 10,000 cells/cm^2. In future projects we will evaluate the outcomes of ncl-PADM/ASC constructs for ventral hernia repairs. Mechanical properties, ASC differentiation pattern, growth factor stimulation and histological appearance will be compared to control ncl-PADM repairs to establish potential benefits of ASC-based bioprosthetic mesh for reconstructive surgical applications.

References:

1. Burns NK et al; Plast Reconst Surg 2010; 125(1):167-176
2. Butler CE et al; J. Amer. Col. Surgeons 2010; 211(3):368-376
3. Altman AM et al; Plast Reconst Surg 2010; 126(3): 845-854

Disclosure:

Dr. Butler serves as a consultant for LifeCell.