

Human Adipose-Derived Stem Cells Adhere to Acellular Dermal Matrix

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Human acellular dermal matrix (ADM) is commonly used in the surgical closure of soft tissue defects not amenable to primary repair. Adipose-derived stem cell (ASC) therapy in the context of soft tissue loss may enhance local microvascular density and wound healing [3]. The current study aimed to determine whether ASCs adhere to ADM *in vitro* and whether stem cell-seeded ADM is a viable approach for surgical application.

Materials and Methods

In accordance with the Tulane University Institutional Review Board, ASCs were isolated from discarded adipose tissue obtained at body-contouring procedures as described [5]. The ASCs were characterized by fluorescence-activated cell sorting as previously described [1].

Acellular dermal matrix 0.4 to 0.8 mm in thickness was obtained (AlloDerm; LifeCell, Branchburg, NJ, USA, and FlexHD; MTF, Edison, NJ, USA). Passage 1 to 5 cell suspensions in growth medium were seeded into multiwell plates covered with ADM, papillary dermis side facing up, at a density of 5×10^4 cells/cm² for histology, 7.5×10^4 cells/cm² for quantitative studies, and 1.0×10^6 cells/cm² for scanning electron microscopy (SEM).

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Adipose-derived stem cells were transfected with green fluorescent protein as described [4]. Adherence was quantified by fluorescent cell counts at 15, 30, 60, and 120 min. Specimens for histology and SEM were seeded and incubated under standard culture conditions for 24 h. Specimens were hematoxylin and eosin (H&E) stained. Adaptations of established methods were used to perform SEM [2].

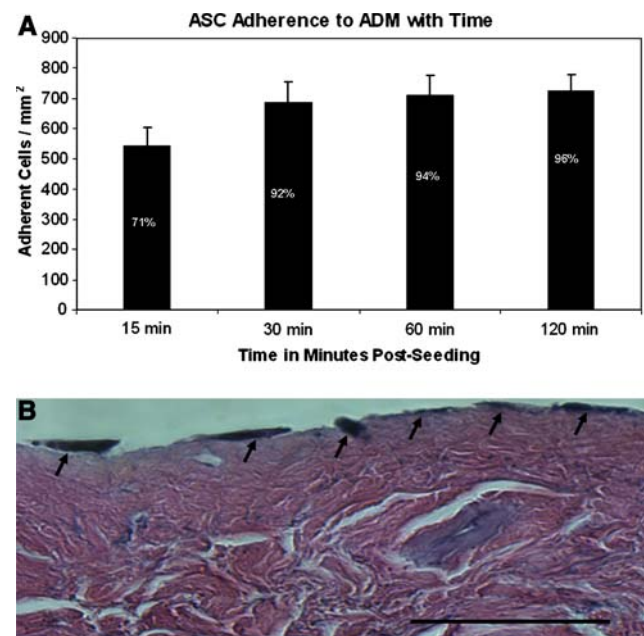


Fig. 1 Adhesion of adipose-derived stem cells (ASCs) to human acellular dermal matrix (ADM) with time and histology of stem cell-seeded ADM. **(A)** Quantitative adhesion. Values are expressed as mean \pm standard deviation. Cells were seeded at a density of 7.5×10^4 cells/cm². **(B)** Histology: Hematoxylin and eosin-stained specimen of acellular dermal matrix papillary surface seeded with ASCs. Arrows indicate adherent ASCs spreading along the ADM surface. Scale bar represents 50 μ m

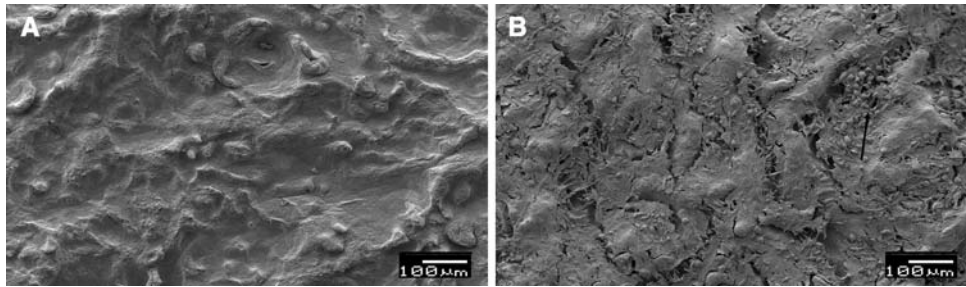
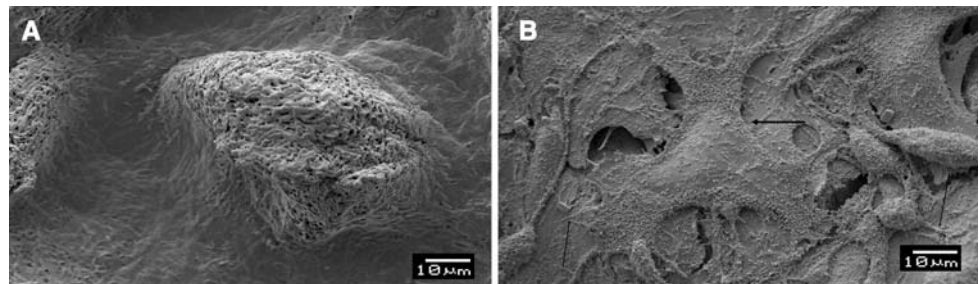


Fig. 2 Low-power scanning electron microscopy (SEM). (A) Unseeded control, $\times 100$. Note the relatively smooth undulating topography of the acellular dermal papillae. (B) Acellular dermal

matrix (ADM) seeded with 1×10^6 cells/cm² for 24 h, $\times 100$. Arrow indicates a subregion of relatively dense adherence. A generalized blanketing effect of seeded stem cells is noted

Fig. 3 High-power scanning electron microscopy (SEM). (A) Unseeded control, $\times 1,000$. (B) Seeded acellular dermal matrix (ADM), $\times 1,000$. Center: Large arrow indicates spreading adherent stem cell. Small arrows indicate fibrillar projections anchoring cells to dermal matrix substratum



Results

Adipose-derived stem cells were negative for the leukocyte markers CD45 and CD11b and positive for the intermediate filament nestin, CD44, CD90, and CD105. Quantitative adhesion experiments showed 543 ± 62 adherent cells/mm² at 15 min (71%), 688 ± 69 cells/mm² at 30 min (92%), 713 ± 63 cells at 60 min (94%), and 727 ± 54 cells/mm² at 120 min (96%). Both H&E (Fig. 1) and SEM analyses (Figs. 2 and 3) confirmed stem cell adhesion to ADM.

Discussion

The ultimate goal of the described technique is to isolate ASCs, seed ADM, and use the product in one operative procedure. Thus, the observation that ASCs adhere to ADM at high rates within a 2-h time frame is significant. Furthermore, SEM identified active attachment of stem cells to ADM, with the extension of microvilli and lamellipodia acting as focal anchorage points.

Conclusion

The findings show that ASC-seeded ADM is a pliable material with potential dual function as a mechanical reconstructive agent and as a biolayer of stem cells for targeted delivery.

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