

Comparative Analysis of Acellular Dermal Matrices as a Vehicle for Stem Cell Therapy Delivery

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Introduction

BACKGROUND

Full-thickness wounds, present many surgeons with complex reconstructive considerations, often requiring serial returns to the operating room, and high morbidity burden for patients. One fundamental reason for decreased regenerative potential in full-thickness wounds is the loss of local stem cells. LGR+ epithelial stem cells express leucine-rich repeat-containing G-protein coupled receptors and are located at the follicular bulge.¹ They are of particular interest because they can produce all cellular lineages of the skin and have great potential in augmenting tissue regeneration.¹

One challenge with stem cell therapy is the ability to provide targeted delivery of stem cells into a wound. Acellular dermal matrices (ADMs), however, provide a promising scaffold for stem cell delivery.²⁻⁵ ADMs have been generated for wound healing applications by mimicking the damaged extracellular matrix.⁵⁻⁸ This damaged extracellular matrix would otherwise be rich in structural and molecular components needed for tissue regeneration.⁵⁻⁸ They undergo a decellularization process to decrease immunogenicity and vary in degree of processing and origin.⁵⁻⁸

PURPOSE

This study aims to compare the potential of four ADMs made from different origins, processing methodologies, and structures as scaffolds for stem cell therapy to augment wound healing.

Methods

ADMs seeded with LGR+ epithelial stem cells *in vitro*

ToxiLight assays – cytotoxicity (MTT) assay – cell proliferation

Four commercially available ADMs were seeded with rodent LGR+ epithelial stem stem cells.

1. ADM discs (4mm ± 0.1mm) were placed in 96-well plates prior to being aseptically seeded with LGR+ stem cells (1.0 x 10⁵ cells/well).
2. ToxiLight assays were measured at 1, 3, and 5 days, and MTT assays were measured at 1, 3, 6, and 9 days after seeding in accordance with existing literature.

Results

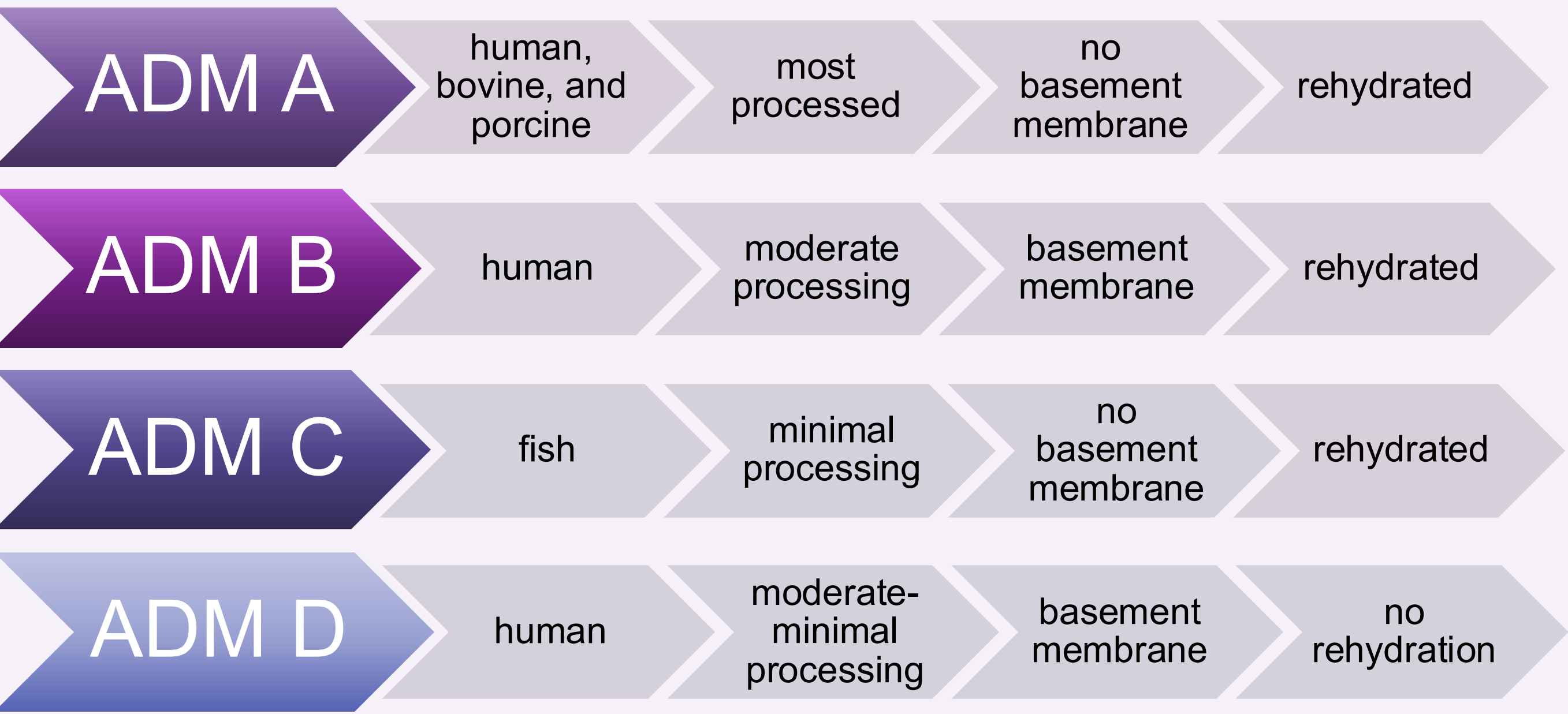
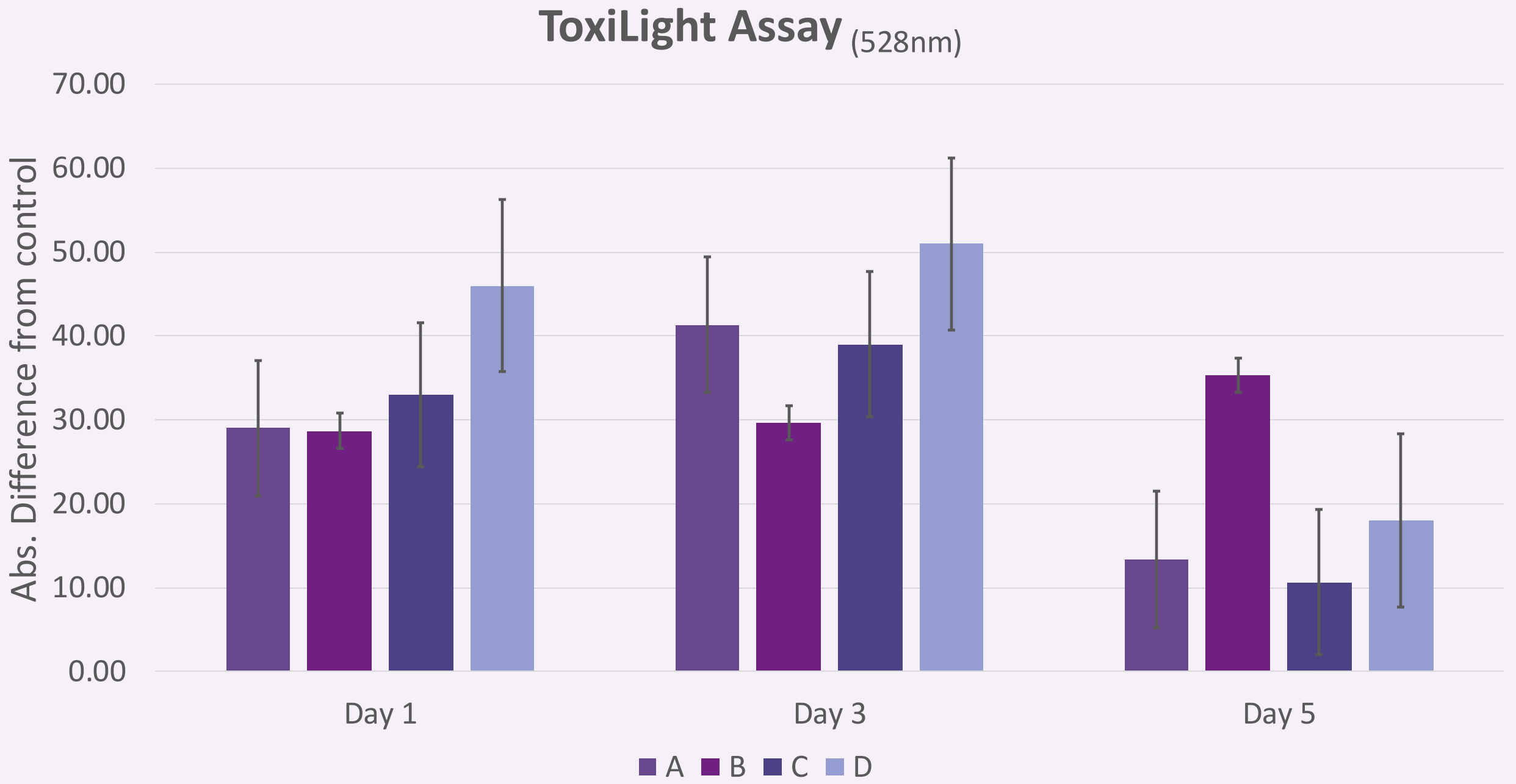


Figure 1. Acellular Dermal Matrix Characteristics

ADM A was the most processed, did not have a basement membrane, and derived from components of human, bovine, and porcine dermis. ADM B and D had moderate processing, did have a basement membrane, and were derived from human dermis. ADM C had little processing, no basement membrane, and was derived from fish dermis. All ADMs required rehydration, except ADM D.

Figure 2. ToxiLight Assay

Toxilight™ assay results showing adenylate kinase (AK) release as a marker of cytotoxicity. Luminescence is proportional to membrane damage and cell death. Therefore,, high values indicate increased cell death burden. As such, when comparing wells with the same number of cells, wells with lower signals, may be an indicator for increased cell viability.



ADM A and C demonstrated the least cytotoxicity, followed by ADM D then C.

MTT Assay (570 nm - 620 nm)

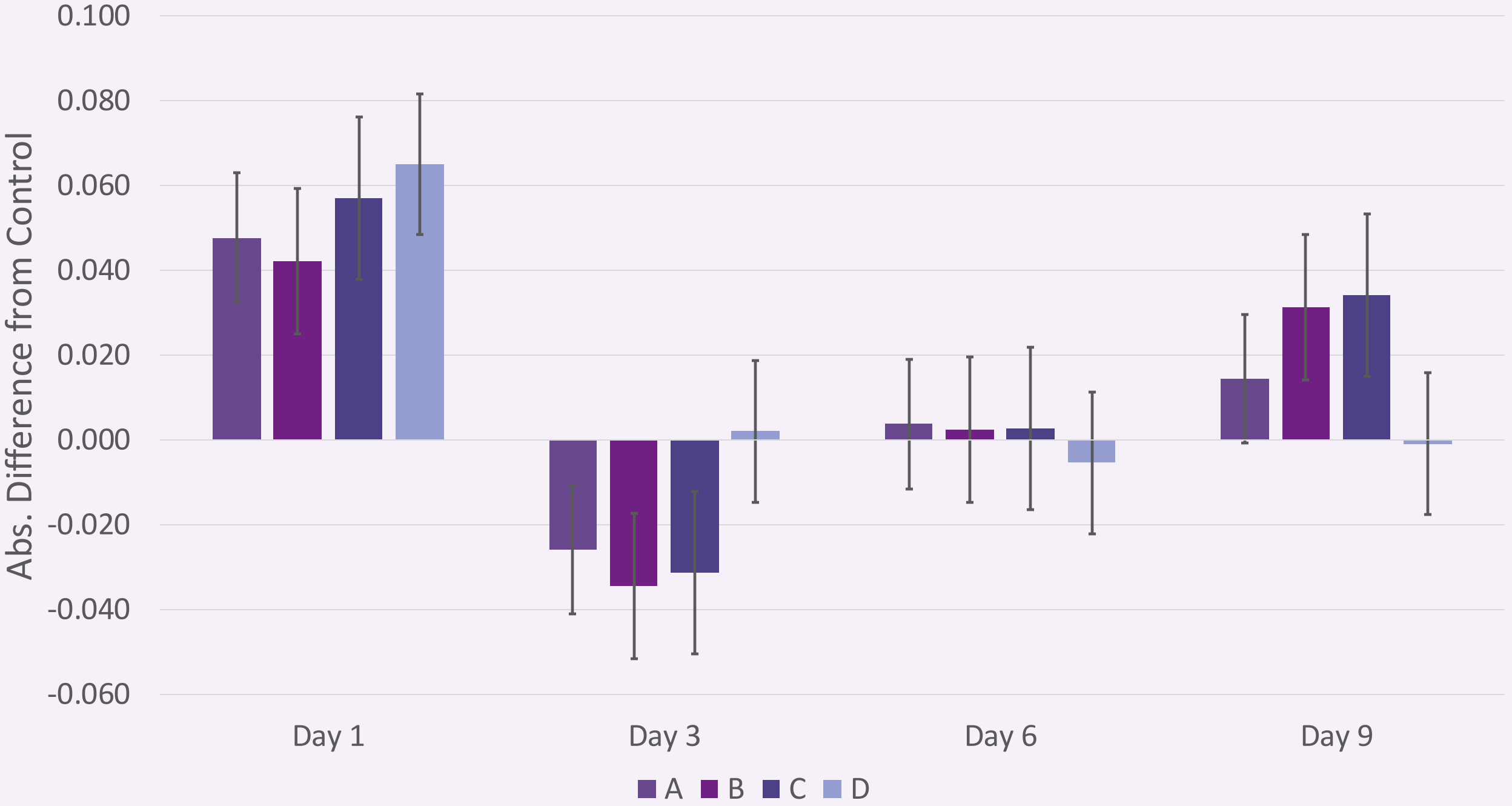


Figure 3. MTT Assay
MTT is a sensitive and reliable indicator of active cellular metabolic activity. It is used as a measurement of cell proliferation and viability.

MTT assays demonstrated increased cell proliferation on day nine after seeding for all ADMs, except ADM D.

ADM B and C exemplified the most proliferation by day 9.

Discussion

Overall, the findings support existing literature that ADMs are a promising option as a scaffold for stem cell delivery. In addition, the study underscores the importance of carefully selecting ADMs to optimize their role as stem cell therapy delivery platforms in wound healing applications. The study also highlights the novelty of using this specific subset of LGR+ epithelial stem cells.

There may be improved stem cell viability in ADMs that

- are less processed
- included xenograft components
- required rehydrating prior to stem cell seeding

Stem cell viability was not affected by

- presence of basement membrane

LIMITATIONS

- Timing of assays compared to media changes
- Shelf-life variance between the different ADMs used
- *In vitro* nature of study

FUTURE DIRECTIONS

- DAPI Cell staining for degree of stem cell integration within ADMs
- *In vivo* studies, with eventual clinical practice
- Stem cell differentiation and utility in other tissue types

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