Aseptic Processing of Human Allogeneic Dermis* Provides a Natural Scaffold with a Biocompatible Environment Conducive of Cell Attachment and Infiltration

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INTRODUCTION

Allogeneic dermal grafts are being used as wound coverings for burn and various wound applications. Acellular versions of these grafts can be used as scaffolds that allow for host cell infiltration, revascularization and tissue in-growth. During preparation, these grafts undergo various chemical processes and/or sterilization to remove cellular components thus minimizing the risk of graft rejection as well as providing safe implants. Depending on the methods used, these processes can alter the natural characteristics of the grafts, and negatively affect the graft's handling properties as well as its interactions with host cells.

The objectives of this study were to evaluate potential effects of processing on the graft features and in vitro interaction with cells.

MATERIALS AND METHODS

The dermal tissue underwent decellularization and chemical disinfection and was aseptically processed (no terminal sterilization was performed). Matrix structure and collagen architecture at the final stage of processing were evaluated using transmission electron microscopy, gel electrophoresis, enzymatic degradation and histology and were compared to unprocessed human dermis (control). Cell interactions with the scaffold were also investigated in vitro with a fibroblast cell attachment assay.

Histology (H & E staining). Tissue sections were fixed in 10% neutral buffered formalin prior to paraffin embedding, sectioned and stained via hematoxillin and eosin (H & E). All histological processing was done at Premier Laboratory (Longmont, CO).

Transmission electron microscopy (TEM): Tissue sections were fixed in buffered glutaraldehyde, subsequently dehydrated, sectioned and imaged. All processing and imaging was performed at Structure Probe, Inc. (Westchester, PA).

Gel electrophoresis: Tissue samples were digested enzymatically, homogenized and subsequently analyzed via Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) to separate proteins based on their electrophoretic motility, a representation of mainly collagen, and other ECM protein survival throughout the processing of dermis, when compared to native tissue and purified collagen.

Enzymatic degradation: Tissue samples were digested with a collagenase and thermolysine solution and the released soluble peptides and amino acids were quantified via spectrophotometery upon their reaction with ninhydrin and hydrindantin added into the digested sample. A greater concentration of peptides is suggestive of a matrix that has undergone processing-based degradation.

In vitro fibroblast attachment. Tissue samples were prepared and seeded with neonatal human foreskin fibroblasts (ATCC Manassa, VA) on both sides (dermal and epidermal). After 30 minutes, the tissue sections were washed to remove any non-adherent cells and incubated at 37°C for 1 hour in complete growth medium. Attached cells were quantified using CyQuant Cell Proliferation Assay (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Non-cell seeded controls were measured for all samples.

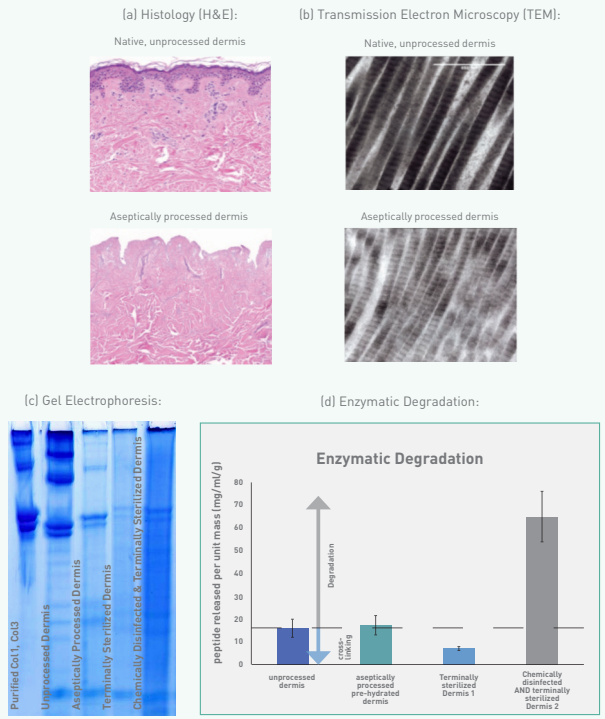

Figure 1: Matrix protein structure and integrity is maintained in dermal tissues after aseptic processing as verified by (a) Histology (H&E), (b) Transmission Electron Microscopy (TEM), (c) SDS-PAGE (gel electrophoresis) and (d) enzymatic degradation assay compared to untreated, native tissue

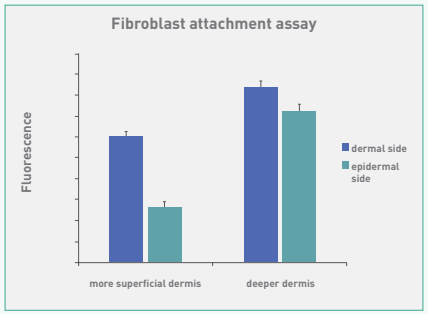

Figure 2: Fibroblast attachment is enhanced and more uniform in deeper layers of dermis

RESULTS

Histologically, it is shown that aseptic processing can successfully provide a decellularized dermal matrix retaining a structural architecture closely resembling that of unprocessed, native dermis. Further, TEM shows the retention of the striated nature when concerned with the dermal proteins, both before and after aseptic processing.

SDS-PAGE and Enzyme Degradation aimed to investigate the molecular integrities of scaffolds processed aseptically as well as via terminal sterilization. Upon electrophoretic molecular separation via SDS-PAGE, it can be shown that the molecular banding in the aseptically processed scaffold, most closely resembles those seen in native tissue and in the purified collagen controls.

The enzyme degradation assay measures the ability of a collagenous material to withstand enzymatic attack. A higher peptide/amino acid concentration relative to native dermal tissue is indicative of a matrix that has undergone increased degradation, most probably due to a greater percentage of processing-based denaturation. Conversely, peptide/amino acid concentration significantly below that of native tissue indicates a more resistant matrix, which may be due to a highly cross-linked matrix.

Processing with harsh chemicals such as sodium hydroxide and acetone and terminally sterilizing with gamma irradiation may cause severe degradation of the matrix. Other types of terminal sterilization may cross-link the matrix. Aseptic processing and disinfection with appropriate chemicals can achieve matrix decontamination while preserving the matrix structure and integrity both mechanically and biochemically to more closely resemble native tissue.

Architecturally, the nature of dermis changes with depth, that is, the matrix fibrils become increasingly porous when approaching the hypodermis, which presumably would allow for enhanced cellular in-growth. Upon investigation, it was shown that an aseptically processed dermis cut with a closer proximity to the hypodermis allows for enhanced cell attachment than a more superficial layer and approximately equal fibroblastic infiltration regardless of seeding location (top or bottom of scaffold); contrastingly, it was shown that the near-epidermal matrix is less conducive to cellular in-growth, suggesting a deeper cut into the dermis may be superior for cell incorporation.

CONCLUSION

Aseptic processing of dermal grafts preserves the natural tissue architecture (as evidenced by histology, gel electrophoresis and TEM) and integrity (verified by enzymatic degradation) of the tissues and provides a graft that promotes cell attachment and infiltration. Porosity of the matrix also plays a role in the amount of cells that attach to the surface of the graft, with higher porosity encouraging increased cell attachment. Processing with harsh chemicals and terminal sterilization may cause severe alterations to the native matrix.

REFERENCES

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