Maintenance of Extracellular Matrix Components in a Decellularized Human Dermis Product

Anna LeMoine, BSc, MSc, PhD; Vassilis Vayianos, BSc, MSc, PhD; Patti Gary RN, CWCN

This study assessed efficiency of DNA removal, preservation of extracellular matrix (ECM) structural features, and preservation of biomolecular composition following decellularization of human dermis.

This study demonstrated the following:

EFFICIENCY OF DNA REMOVAL

METHOD:
- Double stranded residual DNA was quantified using the Quant-iT™ PicoGreen® kit

RESULTS:

> 99% reduction in DNA content

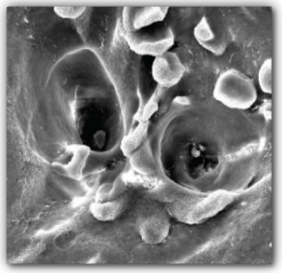
PRESERVATION OF ECM STRUCTURE

METHOD:
- Histological H&E, DAPI, and Verhoeff van Gieson staining
- Immunohistochemical staining for key biomolecules

RESULTS:
- ***Maintenance of native structural features.***

METHOD:
- Scanning electron microscopy on a FEI Quanta 400 (ESEM)

RESULTS:
- ***Tracts present in the papillary and reticular dermis consistent with vascular channels.***
- ***Organization of collagen around the tract.***



PRESERVATION OF BIOMOLECULES

METHOD:
- Liquid Chromatography / Mass Spectrometry (LC-MS) testing to identify extracellular matrix biomolecules

RESULTS:
- ***>800 extracellular matrix components identified: proteins, glycosaminoglycans, and growth factors.***

Relevant Extracellular Matrix Biomolecules Present
Hyaluronic Acid
Transforming Growth Factor β
Growth Factor Receptors
19 Different Collagens

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Tissue Regenix Group Plc

INTRODUCTION

Using a patented process, a decellularized human dermis product (DCD) has been developed[1,2] and successfully used in clinic to treat patients with non-healing wounds[3,4]. The terminally sterilized DCD is produced using a validated process and is intended to provide a scaffold which supports host cellular in-growth and revascularization when used to replace or repair integumental tissue. As such it is key that essential extracellular matrix (ECM) components are maintained within the scaffold. Several techniques were therefore used to characterize the ECM of DCD.

METHODS

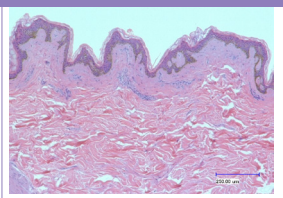
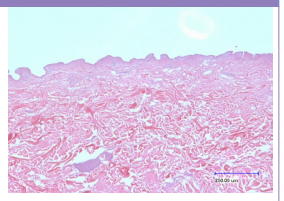
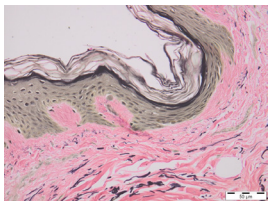
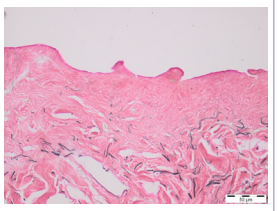
- DCD samples (n=3) and an unprocessed control (n=1) were prepared into formalin fixed paraffin embedded blocks before sectioning and:
  - Histological staining for H&E, DAPI and Verhoeff van Gieson
  - Immunohistochemical (IHC) staining for Collagens I, III, IV, Fibronectin and Laminin
- DCD was prepared for scanning electron microscopy using fixation, dehydration, critical point drying and sputter coating prior to imaging on a FEI Quanta 400 (ESEM)
- Double stranded DNA was quantified using the Quant-iT ™ PicoGreen® kit on freeze dried and ground DCD* samples (n=4) following DNA extraction and purification (Qiagen Dneasy kit)
- Extracellular matrix biomolecules within DCD samples (n=4) were identified following freeze drying, grinding, in-gel digestion and Liquid Chromatography/Mass Spectrometry (LC-MS) testing. Comparison to the UniProt database was used for identification

DNA RESULTS

Sample #	DNA content in DCD (ng/mg dry weight)	% DNA reduction from unprocessed human skin
1	< LOD	< LOD could not calculate; essentially ≥ 99%
2	< LOD	
3	5	99.8
4	6	99.7

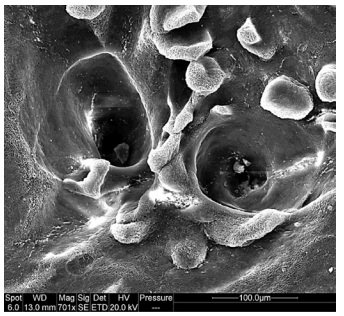
HISTOLOGY AND IMMUNOHISTOCHEMISTRY

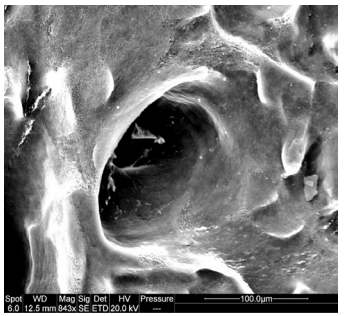
All stains and labels showed maintenance of structural features and/or ECM components investigated, a summary of which can be found below.

Stain/Label	Unprocessed (control) human skin	DCD*
H&E		
Verhoeff van Gieson		
DAPI	Positive nuclear staining seen in epidermis and dermis	No nuclear staining detected in dermis
Collagen I	Localization: Throughout dermis but absent from epidermis Intensity: ++	Localization: Throughout dermis Intensity: +
Collagen III	Localization: Throughout dermis but absent from epidermis Intensity: ++	Localization: Throughout dermis Intensity: ++
Collagen IV	Localization: Basement membrane underlying epidermis and dermal blood vessels Intensity: ++	Localization: Basement membrane underlying epidermis and dermal blood vessels Intensity: ++
Laminin	Localization: Basement membrane underlying epidermis and dermal blood vessels Intensity: +	Localization: Basement membrane underlying epidermis and dermal blood vessels Intensity: ++
Fibronectin	Localization: Diffuse labelling within connective tissue of superficial dermis, dermal papillae Intensity: ++	Localization: Diffuse labelling within connective tissue of superficial dermis, dermal papillae, discrete bundles Intensity (superficial dermis): + Intensity (dermis bundles): ++

SCANNING ELECTRON MICROSCOPY

Tracts consistent with vascular channels were found, highlighted by organization of collagen around the tract. Vessel sizes reminiscent of capillaries in the papillary dermis and larger venules/arterioles in the reticular dermis.





LIQUID CHROMATOGRAPHY / MASS SPECTOMETRY

>800 extracellular matrix biomolecules, including proteins, GAGs, and growth factors, were identified in DCD. Presented below are those most relevant.

Fibrous Structural Proteins		Proteoglycans	Glycoproteins	Glycos-aminoglycans
Collagen I	Collagen X	Decorin	Fibronectin	Hyaluronic Acid
Collagen II	Collagen XI	Heparin	Fibrillin	Chondroitin SO[4]
Collagen III	Collagen XII	Lumican	Vitronectin	
Collagen IV	Collagen XIII	Versican	Tenascin	Growth Factors
Collagen V	Collagen XIV	Biglycan	Fibrinogen	TGF-β
Collagen VI	Keratin	Mimecan	Integrin α	GF Receptors
Collagen VIII	Elastin	Perlecan	Integrin β	
Collagen IX	Laminin			

CONCLUSIONS

- DCD is successfully decellularized, with a 99% reduction in DNA content.
- DCD maintains its native structure and many of its ECM components.
- DCD retains vascular like tracts which may aid angiogenesis.
- DCD has the potential to be an effective tissue scaffold for the repair and replacement of integumental tissue.

References
1. Hogg, P. et al., 2013. Development of a decellularised dermis. Cell and Tissue Banking, 14(3), pp.465–474.
2. Hogg, P. et al., 2015. Development of a terminally sterilised decellularised dermis. Cell and Tissue Banking, 16(3), pp.351–359.
3. Greaves, N.S. et al., 2013. Single-stage application of a novel decellularized dermis for treatment-resistant lower limb ulcers: Positive outcomes assessed by SIAscopy, laser perfusion, and 3D imaging, with sequential timed histological analysis. Wound Repair and Regeneration, 21(6), pp.813–822.
4. Greaves, N.S. et al., 2015. Acute cutaneous wounds treated with human decellularised dermis show enhanced angiogenesis during healing. Plos One, 10(1), p.e0113209

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