EXAMINATION AND COMPARISONS OF EVPOME/ ALLODERM® COMPOSITIONS WITH HUMAN MUCOSAL TISSUES USING SCANNING ACOUSTIC MICROSCOPY

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Statement of Purpose: Our objectives are to compare both natural and engineered tissue morphologies using scanning acoustic microscopy (SAM); this will lead into testing similarities in elastic properties between the two tissues. The principal advantages to using SAM over conventional optical microscopy include non-destructive testing: no staining is required to enhance contrast (the cells and tissues are not killed; hence the physical properties of the tissues can be examined more accurately). Additionally, ultrasound microscopy will allow speckle tracking to measure the elastic properties of the engineered and natural tissues. Future examinations will involve imaging and elasticity testing of related soft tissues, including the vaginal mucosa and ureter tissues. Similarities in the engineered oral mucosal tissues' composition and elasticity may provide potential for transplantation of these tissues to other anatomical areas to repair/replace tissues due to damage or disease.

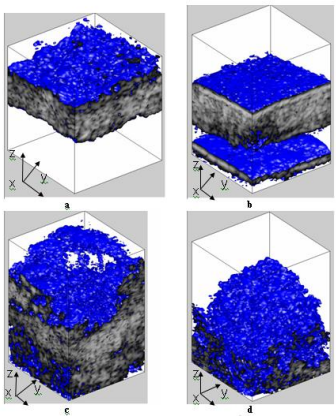
Methods: Tissue preparation – *ex vivo* oral mucosal equivalents (EVPOME): The protocol for harvesting human oral mucosal tissue was approved by a University of Michigan Internal Review Board. All individuals signed informed consent before the tissue samples were procured. A keratinized oral mucosa sample was taken from an out-patient at the University of Michigan Oral and Maxillofacial Surgery Clinic. Oral mucosa keratinocytes were enzymatically dissociated from the tissue sample, and a primary cell culture was established and propagated in a chemically-defined, serum- and xenogeneic products- free culture medium, with calcium concentration of 0.06mM. The AlloDerm® (acellular human cadaver dermis) was soaked in 5μg/cm^2 human type IV collagen overnight prior to seeding cells to assist the adherence of cells, then approximately 1.5 x 10^5 cells/cm^2 of oral keratinocytes were seeded onto the type IV collagen pre-soaked AlloDerm®. The composites of the keratinocytes and the AlloDerm® were then cultured, in the submerged condition, for 4 days to form a continuous epithelial monolayer. The calcium concentration was raised to 1.2mM at this moment. After 4 days, the equivalents were raised to an air-liquid interface to encourage epithelial stratification and cultured for another 7 days, resulting in a fully-differentiated, well-stratified epithelial layer on the AlloDerm®

SAM apparatus and imaging: AlloDerm®, EVPOME, and natural mucosal tissues were immersed in deionized water and imaged with a single element transducer, producing ultrasonic B-scans. We scanned the surfaces of the EVPOME, AlloDerm®, and keratinized oral mucosa (palate) and non-keratinized oral mucosa (buccal mucosa), showing the acoustic signal between the interface of the sample and water on the sample's apical side.

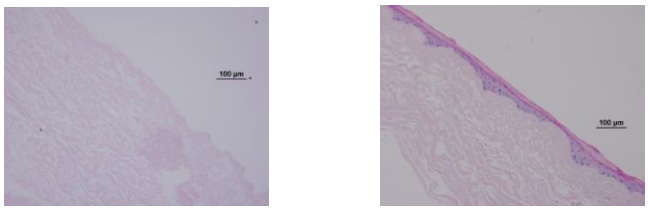
Histology: AlloDerm® and EVPOME were fixed with 10% formalin, embedded in paraffin, cut in 5 μm sections, and stained with hematoxylin and eosin.

Results: The blue images were produced by creating a threshold of the acoustic signal; everything below the threshold is made transparent, everything above it is in grayscale. Every point at the threshold is part of the tissue surface. Some of the speckle signal dropped below this threshold, which was artificially rendered as blue surfaces inside the tissue. True surfaces from the specular reflections (such as in Figures 1a – d) are rendered at the threshold value, not at the peak; there is no second boundary between the tissue interface and the lower tissues. The undulated surface of the AlloDerm® results in higher presence of grayscale (Figure 1a) within the surface as compared to the EVPOME image (Figure 1b). The human mucosal samples for both the palate and buccal mucosa showed irregularities on their surfaces, indicative of the higher transparency and grayscale levels on their surfaces (Figures 1c and 1d).

Conclusions: For the EVPOME, the reduction in grayscale on the surface is indicative of the uniformity of the superficial keratinized layer of stratified epithelium formed by seeded oral mucosa keratinocytes – verified by the differences in the histology images between the AlloDerm® (Figure 2a) and EVPOME (Figure 2b). Although the human tissues show surface irregularities, the scanned areas are only 1% of the total samples and may not accurately represent the actual tissues' compositions.



Figures 1 a-d 3-dimensional SAM micrographs of the AlloDerm®(a); EVPOME (with mounting clay visible on the underside) (b); human palate (c); human buccal mucosa (d) tissues. Samples a-c are 1mm^2 in the X-Y directions (surface area) and 1.5mm in the Z-direction (depth). Sample d is 1mm^2 in the X-Y directions and 1.2mm in the Z-direction.



a **b**

Figures 2a-b. Histology of the AlloDerm® (a) and EVPOME (b), The surface of AlloDerm on which oral mucosa keratinocytes are seeded is undulated (Fig 2a), resulting in "irregularity". In contrast, the superficial layer (a keratinized layer overlying basal and parabasal layer) of the EVPOME showed the uniformity (Fig 2b).

References:

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