A Novel *Ex Vivo* Model of Peristomal Skin Damage for Development of Ostomy Adhesives

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Wound Healing Society Annual Meeting

May 2024

Abstract

Ostomy surgery is often the only effective treatment for diseases affecting normal digestive or urinary function[1]. The resulting stoma, or opening, in the abdomen is affixed with a Class I waste collection device (ostomy pouch) that is secured to the surrounding skin with a hydrocolloid adhesive. Repeated removal of adhesives and exposure to stool and urine from the stoma are the primary causes of peristomal skin damage, which in turn can lead to complications and reduced quality of life[2]. Therefore, ostomy pouches must not only adhere to the skin and protect it from stool or urine, but also be amenable to repeated removal and re-application for the duration of their use. Our goal is to develop novel adhesive formulations that can a) adhere for extended periods of time; b) be removed with minimal skin trauma; and c) minimize the impact of stoma leakage. Human clinical modeling of peristomal skin irritation is difficult, costly and inherently low throughput, which reduces their utility as screening tools for formulation development. Therefore, to achieve our goal we developed an *ex vivo* model of peristomal skin using discarded abdominoplasty tissue to identify novel adhesive formulations for further development. Our current objectives are to understand the effects of repeated tape-stripping with six different hydrocolloid adhesive formulations and the damage caused to skin by a mixture of digestive enzymes. Morphological analysis of hematoxylin and eosin (H&E) stained cross sections of tape-stripped tissue (applied and removed five times) revealed distinct differences in the extent of damage to the stratum corneum, ranging from no damage to complete loss with exposure of the stratum granulosum. Additionally, transepidermal water loss (TEWL) was measured immediately after tape-stripping to assess whether a correlate to the extent of epidermal damage exists. To visualize the extent of skin damage resulting from exposure to stoma-relevant digestive enzymes, we topically applied a mixture of trypsin, chymotrypsin, and elastase for 1 h to skin tissue. Exposure to these enzymes caused significant decompaction of the stratum corneum and ablation of the stratum granulosum, as determined by H&E staining. Moreover, the enzyme mixture significantly reduced the immunofluorescent expression of epidermal barrier proteins filaggrin and loricrin. In contrast, trypsin alone did not induce changes in the epidermis. Taken together, our results show that *ex vivo* abdominal skin is responsive to both mechanical and enzymatic modes of damage and thereby may serve as a surrogate to peristomal skin damage experienced by ostomates. Moving forward, we will combine tape-stripping and enzymatic exposure to model the complex peristomal environment and characterize adhesive formulations with superior skin protective capabilities.

Introduction

Skin irritation owing to leakage and other factors during ostomy device usage is a major concern amongst ostomates. Some studies report greater than 70% of people with an ostomy experience peristomal skin irritation [2]. While products and technologies can be designed to help minimize leakage or protect the skin barrier function, there is a need to develop better pre-clinical models that can predict the effectiveness of such technologies.

Ex vivo skin models have been widely used for studying skin barrier injury and repair [3]. The effects of tape stripping and chemically-induced damage and repair have been characterized in such models using transepidermal water loss (TEWL) and morphological analyses [4,5]. Such model systems could be effective tools for understanding the causative modalities of skin complications arising from peristomal etiologies such as medical adhesive-related skin injury (MARSI) and skin irritation resulting from direct contact with stool. Stomal output is known to be neutral to slightly alkaline in pH [6], which favors the activity of digestive enzymes know to be present in stool such as trypsin, chymotrypsin and elastase [7]; therefore it is important to understand the impact of digestive enzymes on peristomal skin structure and function.

Materials and Methods

- *Ex vivo* human skin* organ culture
  - Culture at the air-liquid interface with 10% DMEM + 10% fetal bovine serum
  - **De-identified, discarded tissue is obtained with IRB approval (Northwestern University)*
- Modeling the ostomy environment
  - Topical application of digestive enzymes
  - Repeated application and removal of adhesives (“tape-stripping”)
- Hematoxylin and eosin (H&E) histology on 4 µm tissue sections for morphological and morphometric analysis of formalin-fixed, paraffin-embedded (FFPE) specimens
- Indirect immunofluorescence on frozen tissue sections to detect barrier proteins

Skin Organ Culture Workflow

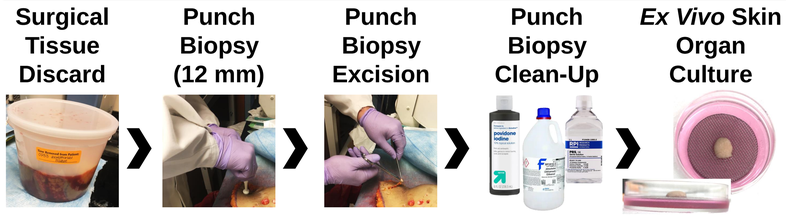

Figure 1. De-identified, discarded abdominal tissue is excised with a 12 mm punch biopsy for enzyme exposure studies or through excision of a larger piece for tape-stripping studies. Samples are cleaned of visceral fat and are disinfected with 10% povidone-iodine solution and 70% ethanol then rinsed in phosphate buffered saline. Skin organ cultures are placed at the air-liquid interface with media prior to stripping or enzyme treatment.

Results: Enzyme Exposure

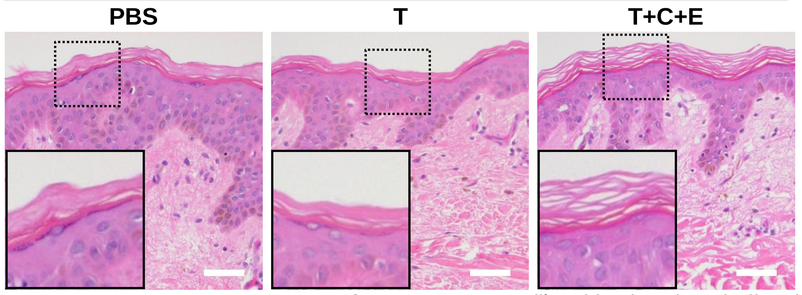

Figure 2. Enzymes diluted in phosphate buffered saline (PBS, pH 7.4) consisting of 0.6% trypsin (T) or a combination of trypsin, chymotrypsin, and elastase (T+C+E, 0.6% each), were applied to the surface of skin organ cultures for 1 h. H&E micrographs were analyzed using Zeiss software to measure the thickness of the stratum corneum. **, $P = 0.006$ vs. PBS and #, $P = 0.012$ vs. Trypsin by one-way ANOVA and Tukey’s post-hoc test. Scale bars = 50 µm.

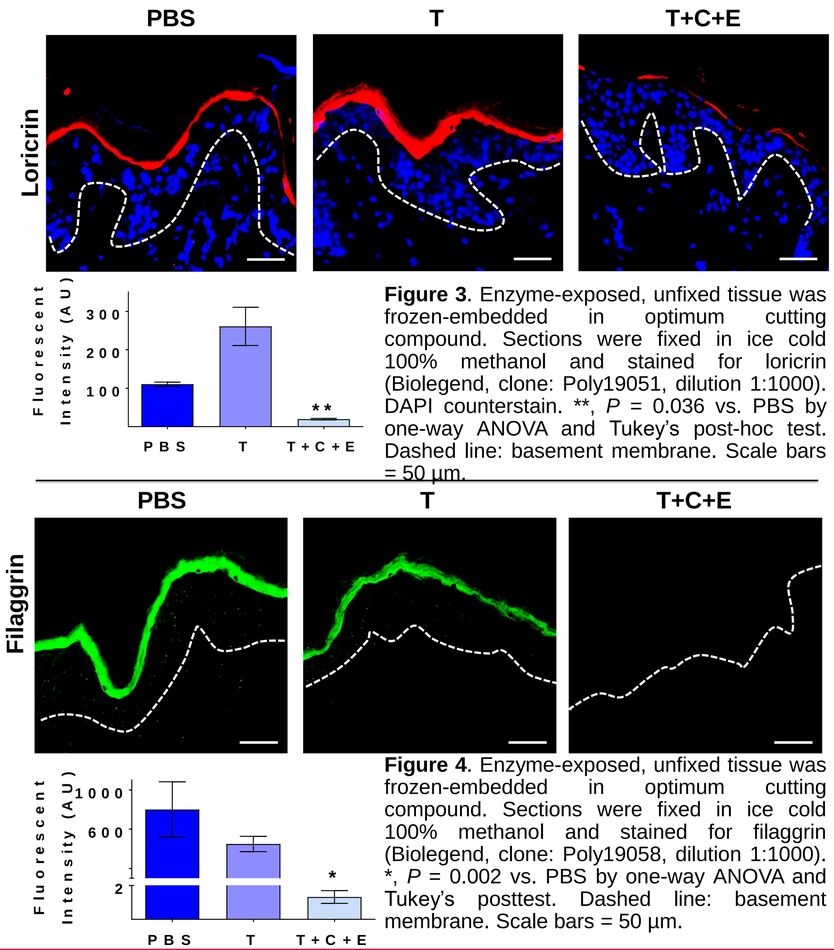

Figure 3. Enzyme-exposed, unfixed tissue was frozen-embedded in optimum cutting compound. Sections were fixed in ice cold 100% methanol and stained for loricrin (Biolegend, clone: Poly19051, dilution 1:1000). DAPI counterstain. **, $P = 0.036$ vs. PBS by one-way ANOVA and Tukey’s post-hoc test. Dashed line: basement membrane. Scale bars = 50 µm.

Figure 4. Enzyme-exposed, unfixed tissue was frozen-embedded in optimum cutting compound. Sections were fixed in ice cold 100% methanol and stained for filaggrin (Biolegend, clone: Poly19058, dilution 1:1000). *, $P = 0.002$ vs. PBS by one-way ANOVA and Tukey’s posttest. Dashed line: basement membrane. Scale bars = 50 µm.

Results: Tape-Stripping

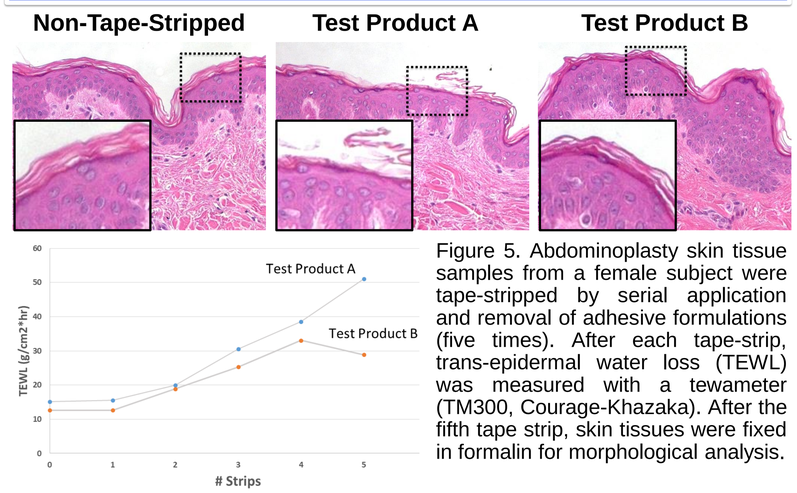

Figure 5. Abdominoplasty skin tissue samples from a female subject were tape-stripped by serial application and removal of adhesive formulations (five times). After each tape-strip, trans-epidermal water loss (TEWL) was measured with a tewameter (TM300, Courage-Khazaka). After the fifth tape strip, skin tissues were fixed in formalin for morphological analysis.

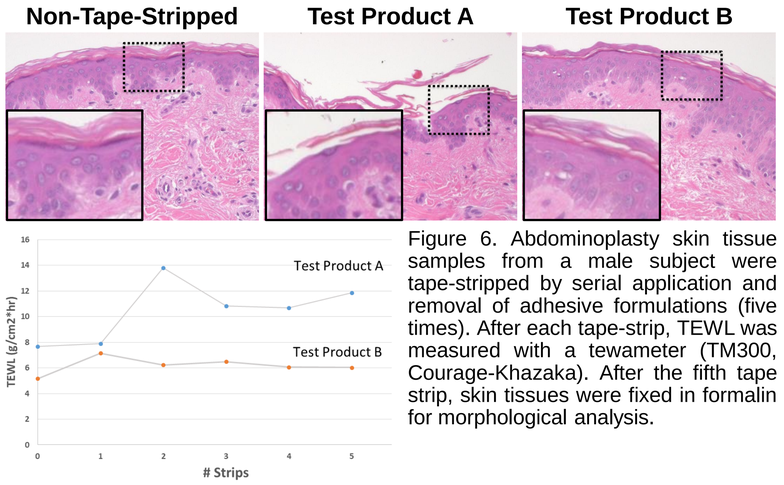

Figure 6. Abdominoplasty skin tissue samples from a male subject were tape-stripped by serial application and removal of adhesive formulations (five times). After each tape-strip, TEWL was measured with a tewameter (TM300, Courage-Khazaka). After the fifth tape strip, skin tissues were fixed in formalin for morphological analysis.

Conclusions

- A combination of topically-applied (1 h) digestive enzymes (trypsin + chymotrypsin + elastase), but not trypsin alone caused loss of keratohyalin granules and perturbed the stratum corneum (Figure 2).
- The combination of enzymes significantly reduced the expression of barrier proteins filaggrin and loricrin (Figures 3 and 4).

Skin barrier protein aggregates (keratohyalin granules) and specific barrier proteins (loricrin and filaggrin) are substrates for digestive enzymes. This suggests that an ostomy adhesive should be engineered to inhibit activity of digestive enzymes.

- The extent of damage to the stratum corneum and stratum granulosum caused by tape-stripping is dependent on the adhesive formulation (Figures 5 and 6).

The degree of skin barrier damage determined by morphological analysis of tissue cross section micrographs correlates with increased trans-epidermal water loss across different adhesive formulations.

- *Further development of preclinical ostomy models combining enzyme exposure and tape-stripping will be useful to quantify the effectiveness of skin health technologies and lead to faster, more effective product development for ostomy care.*

References

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