Toxicological aspects of in vitro oral mucosa wound healing models

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Abstract ID: 966
Poster#: P02-41

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Abstract

Wounds in the oral cavity tissues can result from surgical procedures, accidents, or canker sores. Oral wounds present a site for potential infection and increased sensitivity to oral irritants. Untreated wounds can lead to pathogen invasion, chronic pain, poor cosmetic outcomes, and in some cases, extended hospitalization. Thus, damaged oral mucosal tissue needs to be treated and healed as soon as possible. The objective of this project is to develop new in vitro oral wound healing models and determine their toxicological profile using common dentifrice materials. Two tissue models were used in this study (both produced by MatTek Corporation and MatTek IVLSL): a) EpiOral (ORL-200), which consists of normal, human-derived oral epithelial cells cultured to form a highly differentiated model of human (non-cornified) buccal tissue, and b) EpiGingival (GIN-100), a cornified model of the gingival mucosa. On Day 0, tissues were wounded using a 3 mm biopsy punch (representing 11.6% of total tissue area) and wound closure was monitored using brightfield microscopy, histological cross sections, and transepithelial electrical resistance (TEER) measurements. The toxicological profile of the tissues was probed using the common dentifrice additive, sodium dodecyl sulfate (SDS), at 1%. The exposure time which decreased the tissue viability to 50% (ET-50) of wounded tissues (WT) and non-wounded tissues (NWT) was determined. Histological cross sections and brightfield microscopy of the WT showed that the wounds extend down to the underlying inert microporous culture membrane. Within 2 days post-wounding (PW) for the ORL-200 tissue and 4 days PW for the GIN-100 tissue basal cells migrate into the wound to reestablish a continuous monolayer. Immediately following wounding, TEER for WT decreases to <20% of the NWT controls, but TEER increases as wound healing proceeds. Wounding also changes the toxicological profile of the tissues. For the ORL-200 model, the ET-50s for the WT and NWT were 27.8 and 49.1 mins, respectively, which represents a 76% increase in ET-50 for the NWT vs WT. By contrast, in the cornified GIN-100 model, the ET-50s were 54.5 and 124.5 mins, respectively, which is 128% increase in ET-50 for NWT vs WT. These results demonstrate successful development of wound models for tissues of the oral cavity. Wound healing can be monitored with brightfield microscopy, TEER, histology, and sensitivity to a common dentifrice additive. These models will be useful for testing new therapeutic compounds designed to hasten wound closure in the oral cavity and for determining toxicity profiles in barrier-compromised oral cavity tissues. Additionally, wounding has been shown to decrease the ET-50 of ORL-200 and GIN-100 tissues indicating that it could be a useful tool when a more sensitive tissue model is required, such as for testing many in-use tobacco and oral care products that typically give low responses in NWT.

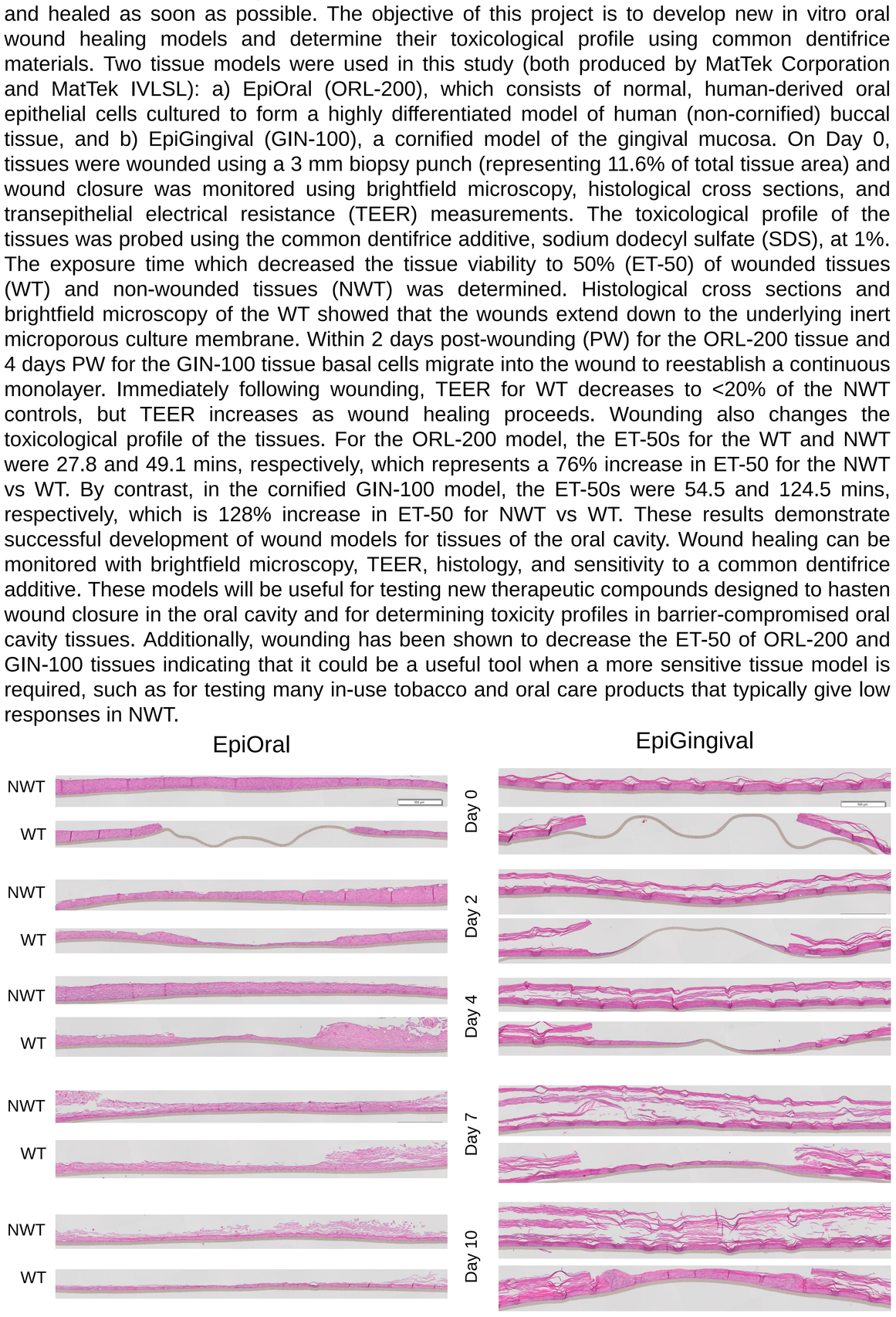

Figure 1: H&E-stained histological cross-sections of non-wounded tissue (NWT) and wounded tissues (WT) in vitro reconstructed tissue models. Tissues were wounded with a 3 mm biopsy punch on Day 0. Wound closure was followed over a 10-day period.

Methods

Tissue Preparation: EpiOral (ORL-200) and EpiGingival (GIN-100) tissues consist of normal, human-derived oral epithelial cells. The cells have been cultured on specially prepared cell culture inserts (0.6 cm^2 surface area) for up to 7 days at the air-liquid interface in serum-free medium to form multilayered (8-11 cell layers), highly differentiated 3D models of the human buccal and gingival phenotype. The tissue models exhibit in vivo-like morphological and growth characteristics which are uniform and highly reproducible (**Figure 1**). Following differentiation, circular wounds were produced in the center of ORL and GIN tissues using a 3mm biopsy punch.

Histology: Wounded and non-wounded ORL and GIN tissues cultures were fixed in 10% formalin (overnight, room temperature) on Days 0, 2, 4, 7, and 10. Fixed tissues were paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E) as per standard procedures (**Figure 1**).

Brightfield Microscopy: Wound closure was visualized by brightfield imaging using an inverted microscope (ECHO Rebel, San Diego, CA) with a 4x objective through the transparent culture membrane **(Figure 3)**. Wounded tissues were placed into individual wells of a 24-well plate containing TEER Buffer (PBS w/ $CaCl_2$ and $MgCl_2$) for imaging.

TEER Measurement: Transepithelial electrical resistance (TEER) was measured throughout the experiment using an EVOM2 volt-ohmmeter and EndOhm-12 chamber (World Precision Instruments, Sarasota, FL). For wounded tissues, relative tissue barrier at each time point was calculated using the following equation: *% TEER = TEER (WT) / TEER (NWT) x 100*

ET-50 Determination: Wounded and non-wounded tissues were apically exposed to 100 µL of 1% SDS for 5, 20, and 80 minutes (ORL-200) or 20, 80, and 240 minutes (GIN-100). Untreated wounded and non-wounded tissues served as negative controls (NC). Following treatment with SDS, tissue viability was determined using an MTT assay. Percent viability was determined using the equation: *% viability = OD (X)/OD (NC)*100*. The time to reduce the tissue viability to 50% following exposure to 1% SDS was interpolated mathematically for the wounded and non-wounded tissues **(Table 1).**

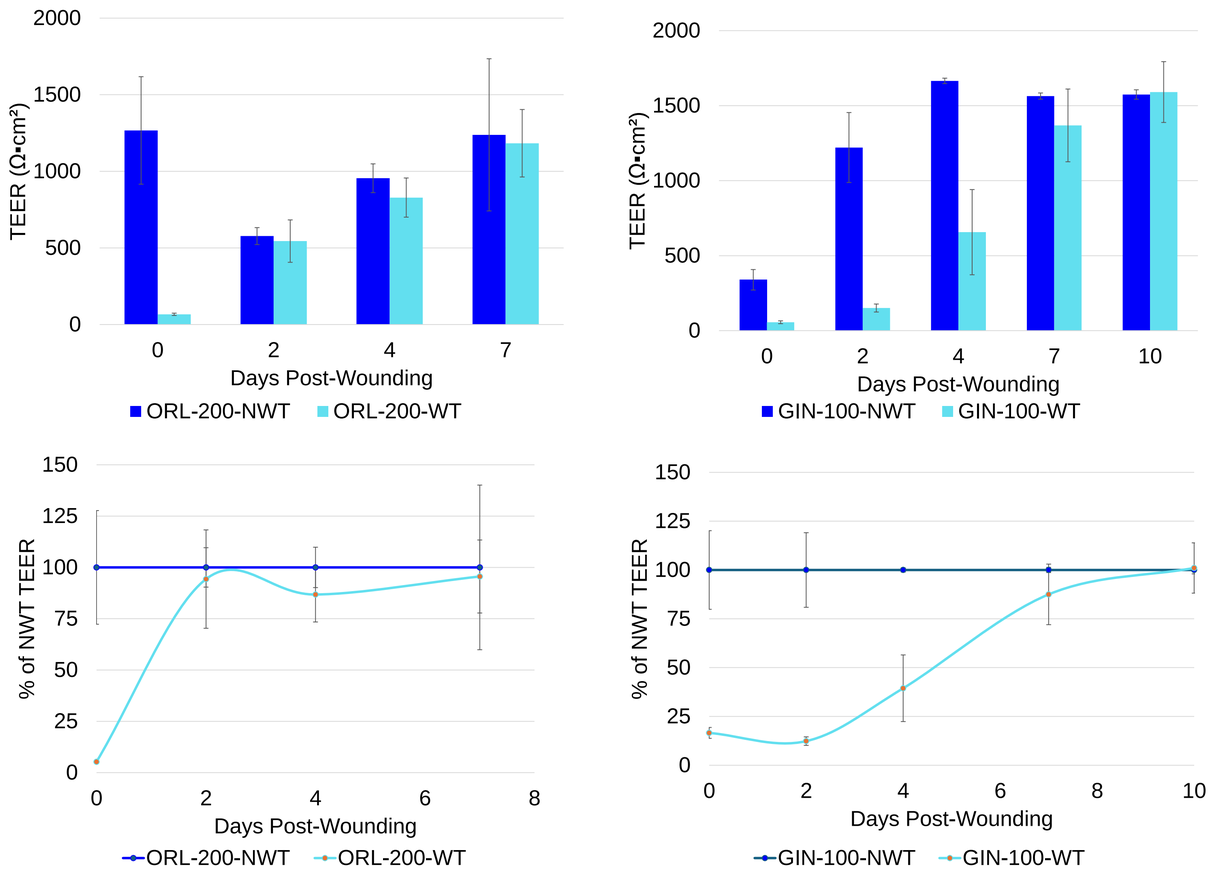

Figure 2: Comparison of TEER values for wounded EpiOral and EpiGingival tissues (ORL-200-WT and GIN-100-WT, respectively) vs. non-wounded tissues (ORL-200-NWT and GIN-100-NWT) for 7- and 10-days post-wounding.

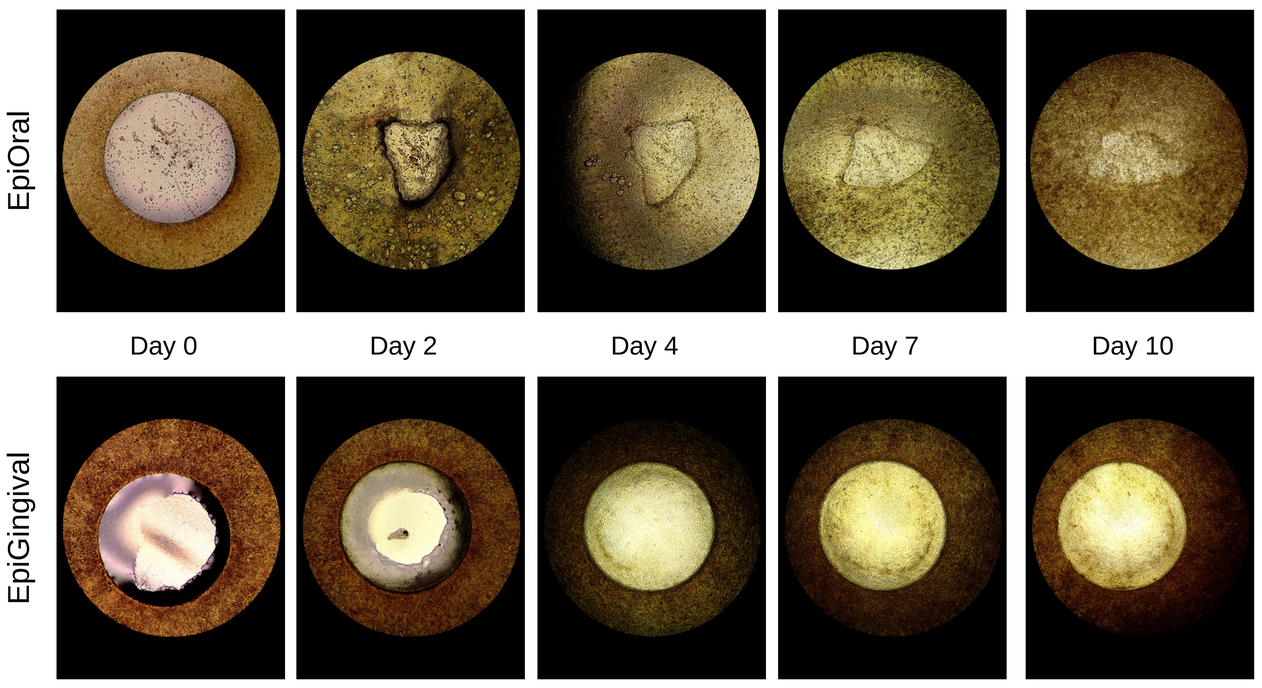

Figure 3: Brightfield photos showing wound closure for the EpiOral and EpiGingival tissues. Tissues were wounded with a 3 mm biopsy punch on Day 0 and then observed over a 10-day period post-wounding.

Tissue	ET-50 NWT (min)	ET-50 WT (min)	% Decrease (WT / NWT)
ORL-200	**49.1**	**27.8**	**43.4**
GIN-100	**124.5**	**54.5**	**56.2**

Table 1: Exposure time for 1% SDS which reduced tissue viability to 50% (ET-50) for wounded (WT) and non-wounded (NWT) EpiOral (ORL-200) and EpiGingival (GIN-100) tissues.

Conclusions

- Histological cross sections and brightfield microscopy of the WT showed that the wounds extend down to the underlying inert microporous culture membrane (**Figure 1 & 3**).
- Within 2 days post-wounding (PW) for the ORL-200 tissue and 4 days PW for the GIN-100 tissue, basal cells migrate into the wound to reestablish a continuous monolayer (**Figure 1**).
- Immediately following wounding, TEER for WT decreases to <20% of the NWT controls, but TEER increases as wound healing proceeds (**Figure 2**).
- For the ORL-200 model, the ET-50s for the WT and NWT were 27.8 and 49.1 mins, respectively, which represents a 43.4% decrease in ET-50 for the WT vs NWT. In the cornified GIN-100 model, the ET-50s were 54.5 and 124.5 mins, respectively, which is 56.2% decrease in ET-50 for WT vs NWT (**Table 1**).
- The wounded models will be useful for testing new therapeutic compounds designed to hasten wound closure in the oral cavity and for determining toxicity profiles in barrier-compromised oral cavity tissues.
- The wounded tissues are more susceptible to toxic insult, as evidenced by decreased ET-50s for WT vs NWT. We anticipate that the wounded models will be useful tools for testing in-use tobacco and oral care products that typically give low responses in NWT.