Localized Mechanical Strain Gradients in Type-I Collagen Biomaterials Around a Sharp Notch Under Tension

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Statement of Purpose: Wounds that penetrate multiple dermal layers in human skin trigger cellular signaling cascades that initiate healing and reconstructive processes. Specifically, in addition to biochemical cues, cells both induce and respond to mechanical signals through a variety of mechanotransduction processes. These mechanical cues may originate directly from the extracellular matrix (ECM) or from neighboring cells within the ECM. Prior studies have correlated tensile stress and strain with a variety of cell responses, including development of scar tissue. However, although the redistribution of strain and stress around sharp notches are known for linear elastic and strain-hardening materials, this information has yet to be established for hyperelastic materials. Such materials include those envisaged as skin replacement scaffolds, specifically collagen and fibrin hydrogels. This knowledge is critical for the design of skin replacement materials for controlled, scarless healing, especially in the vicinity of incisions, sutures and other structural discontinuities. The goal of this investigation was to develop a new protocol for the investigation of mechanical strain gradients in the vicinity of sharp cuts in biomaterial scaffolds, and to apply the protocol to quantitate these mechanical strain gradients in Type-I collagen biomaterials.

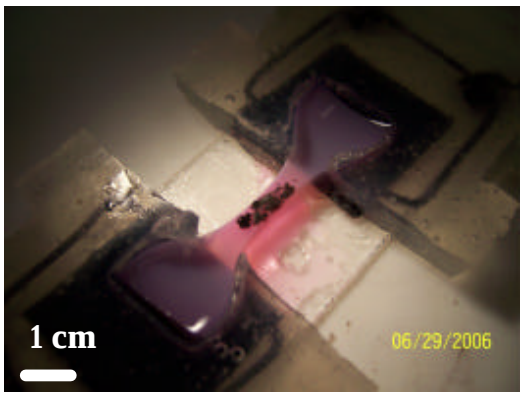

Figure 1. Type-I collagen, notched tensile specimen used to establish a mechanical strain gradient in vitro.

Methods: Vitrogen™ Purified Collagen (3.1 mg/mL) was diluted with 10X phosphate-buffered saline, 0.1M NaOH and Sigma 10X Modified Eagle's medium. Tensile specimens (Fig. 1) were prepared within a novel, two-piece silicone mold containing porous grip membranes at each end. The specimen was mounted directly to the stage on an optical microscope. Side sections of the mold were removed following polymerization of the collagen and the underside of the specimen was decohered from its substrate prior to application of tensile strain. A scalpel was used to give a full-thickness notch through the center of each specimen and inert, image-contrasting markers were applied to the surface of the specimen in the notch tip region. A remote, tensile strain was applied in a step-wise linear fashion and multiple high-resolution images collected at different levels of far-field strain (Fig. 2). Image analysis was then performed using a coordinate system to track movements of individual markers from a series of sequential images. The resultant displacement fields were analyzed to yield the in-plane strain fields, including strain components along trajectories parallel and perpendicular to the crack plane (Fig. 3).

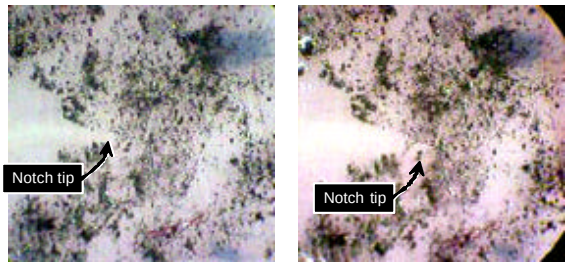

Figure 2. Optical micrographs of the notch tip region at low strain (left) and high strain (right).

Results/Discussion: Significant local strain concentrations were observed near the notch tip of over 180% within a region nearly 1 mm from the notch tip (Fig. 3). Regions of over 20% strain elevation were also observed at distances of up to 2.5 mm from the notch tip (Fig. 3). The magnitudes and spatial dependence of these strain concentrations are then analyzed using known constitutive models for crack-tip strain fields in linear elastic and strain-hardening materials.

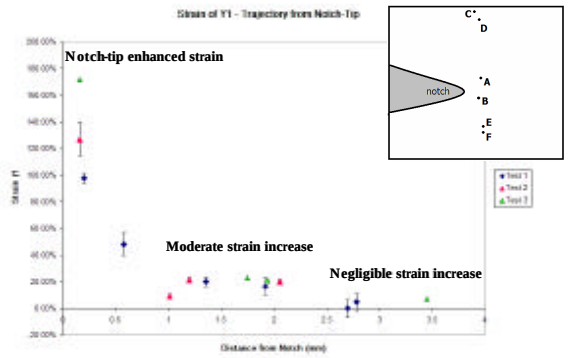

Figure 3. Mechanical strains vs. distance from the notch tip (Fig. 2).

Conclusions: A novel *in situ* tensile strain protocol was developed and commissioned with Type I collagen specimens. Strain concentrations of over 180% were observed within 1 mm of the notch tip. Strain concentrations of ~ 20% were also observed within 3 mm of the notch tip. Increasing remote strain increased local strains for trajectories near the notch tip. These results establish the basis of this approach to correlating mechanical strain gradients to differences in cell function *in vitro*.