Cutaneous Wound Healing

Overview:
Wound healing is a coordinated measure of immunity and thus a comprehensive immune response (Epstein et al., 1999, Kiecolt-Glaser et al., 1995). Wound healing is integrative, involving multiple phases. The first phase, the inflammatory response, is known to be stress-sensitive (Padgett et al., 1998; Rojas et al., 2002). The other phases require the mobilization and/or proliferation of platelets, granulocytes, cytokines, chemokines, fibroblasts, and keratinocytes (Martin, 1997; Padgett et al., 1998). It is both biologically and ecologically relevant to most animals. It can be performed without specific reagents or antibodies, and so can be performed in more non-traditional systems and even in the field.

Materials & Equipment:
- Surgical, sterile biopsy punches (Miltex Instrument Company, York, PA)
- Anesthetic (animal appropriate, e.g., lidocaine)
- Surgical fine tissue scissors and forceps
- High resolution digital camera
- Restraint stage with size reference for animal
- Image analysis software (e.g., Image-Pro Plus, Media Cybernetics, Silver Springs, MD; or Image J, http://rsbweb.nih.gov/ij)

Procedure:
1. Small animals should be anesthetized prior to procedure, while larger animals can receive a subcutaneous injection of local anesthetic (0.02-0.05 ml of 1% lidocaine; efficacy of injection should be determined by response to a stimulus of the injection site) prior to biopsy.

2. Cutaneous biopsies should be performed on an area of the animal that will not interfere with the animal’s movements or be irritated by the animal’s normal activities. For example Small Lizards receive biopsies on the dorsal surface of their pelvis.

3. Biopsies are made using small sterile punches (2-3.5mm; Miltex Instrument Company, York, PA), and the size of the biopsy punch should be appropriate for the study animal. The punch is lightly twisted to create a uniform circular cut through the skin. The resulting circle of skin is then removed using forceps creating a controlled-size cutaneous wound. The controlled-size and depth of the biopsies lead to little overall impact on the animal, and this same procedure has been used in lizards as small as 4g (French et al. 2005).

4. The wound should be digitally photographed immediately after biopsy. In order to photograph the wounds, animals need to be secured in a uniform fashion with a scale reference (e.g., metric
ruler) for size measurements. Digital images can be taken either with a digital camera (in the field) or a camera attached to a light microscope (in the laboratory). Handling and photographing times should be kept to a minimum to minimize stress to the animal, as wound healing is a stress sensitive response.

5. Wounds should then be re-photographed and re-measured after a given period of time. The length of healing time should be based on initial pilot studies that document the comprehensive healing profile for the study animal (French et al., 2006, Padgett et al., 1998, Rojas et al., 2000, Rojas et al., 2002).

6. At the end of the study, the digital photographs are randomized and then analyzed to assess wound size (i.e., area) using image analysis software (e.g., Image-Pro Plus, Media Cybernetics, Silver Springs, MD; or Image J, http://rsbweb.nih.gov/ij), such that the investigator is blind to the treatment of the animal. Area is the best measure to assess wound size. Other measurements, such as circumference and diameter, are less informative due to most wound not healing in a uniform manner.