

ASSESSMENT OF BURN WOUND TISSUE IN SITU BY MULTIPHOTON MICROSCOPY EMPLOYING FLUORESCENCE AND SECOND HARMONIC GENERATION CONTRASTS IN LIVE ANIMALS

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Current assessment of burn wound depth and progression of healing for proper choice of treatment is based on time-consuming and invasive techniques that may interfere with the healing process. One way to overcome these problems is to use noninvasive techniques. We use multiphoton microscopy (MPM) that employs fluorescence and second harmonic generation (SHG) contrasts to noninvasively follow the burn healing process in situ as healing progresses in live animals. Healing progression was followed in a partial thickness burn wound made on the dorsum of anesthetized Sprague-Dawley rats with a 2.8 cm diameter brass cylinder heated at 80°C for 60 seconds and pressed against the shaved rat skin for 6 seconds. During the first five days, burn healing was characterized by changes in the organization of collagen into a compact, mat-like assembly, suggesting progressive degradation of collagen within the injury site. We also detected increased follicular cell damage and a strong auto-fluorescence signal from cellular debris. Deposition of new collagen was seen after the scab fell off and gradually increased thereafter as detected with an increase in the SHG signal intensity. A great increase in the density of cells was observed as well. From day 21 on, a network of capillaries with blood flow was observed. The epidermal cell layer and the blood vessel network became progressively more organized. At day 29, fibrillar collagen had SHG signal levels and morphology of the nearly preburn state. These findings were corroborated by histology/histochemistry. In conclusion, MPM technology that employs fluorescence and SHG contrasts is instrumental in following the healing process, in particular during the early stages of healing. The degree and rate with which these events occur early after burning could help clinicians make treatment decisions. The outcomes of healing at later times would be indicative of the effectiveness of the treatment applied.