Vibrational microscopic characterization of healing processes in a human skin wound

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Vibrational microscopy and imaging are now poised to address important biomedical and pharmacological issues, including the diagnosis of pathological states. The current presentation describes the first attempt at vibrational imaging of a healing wound.

Proper wound healing entails a complex series of events, both in space and time. Confocal Raman and IR microscopic imaging provide evidence for the temporal expression and spatial location of a variety of collagen and keratin species that appear during the healing process. Our goal is to correlate the spatial distribution of the phenotypes we observe with the particular sequence of activated genes that characterize the healing.

Three characteristic phases of wound repair (inflammation, proliferation including re-epithelialization, and remodeling) overlap in time and space. We have utilized a human skin wound healing model to correlate changes in genotype and phenotype with IR and Raman microspectroscopic images during re-epithelialization. IR or Raman images were collected at 0, 2, 4 and 6 days following wound generation. The experimental protocols, validated as IR images, clearly delineate the keratin-rich migrating epithelial tongue (MET) from the collagen-rich wound bed. Factor analysis of IR imaging data sets acquired 6 days post wounding reveal subtle spectral differences that map to distinct spatial distributions, which are correlated with immunofluorescent staining patters of different keratin types. The temporal sequence of events is explored through a comparison of gene array analysis with vibrational microscopy images [1].

In addition to studies of the keratin species as noted above, analysis of lipid IR spectral properties revealed the presence of lipid class(es) with disordered chains in the vicinity of the MET. The presence of lipid ester C=O bands colocalized with the disordered chains provided evidence for the presence of carbonyl- containing lipid species, consistent with a shotgun lipidomics study [2]. Gene array data complemented the biophysical studies and provided a biological rationale for the generation of the disordered chain species. Enhancement of fatty acid desaturases 1 and 3, and stearoyl-CoA desaturase, which regulate the unsaturation of fatty acids, was observed. These data suggest a mechanism for increasing the conformational disorder of the lipid chains. Finally, the lipid vibrations tracked in the current study provide significant supporting evidence for prior biochemical studies implicating the involvement of lipid rafts of unusual composition during wound healing. These rafts have been suggested as a control mechanism for keratinocyte activation and migration. Overall, these experiments demonstrate the feasibility of acquiring detailed molecular structure information from proteins and lipids involved in wound healing events.

References

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