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Understanding the Information in Principal Component Analysis of Raman Spectroscopic Data during Healing of Subcritical Calvarial Defects

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Abstract : Bone healing is a complex and sequential process involving changes at the molecular level. Raman spectroscopy is a promising technique to study bone mineral and matrix environments simultaneously. In this study, subcritical calvarial defects are used to study bone composition during healing without discomposing the fracture. The model allowed to monitor the natural healing of bone avoiding mechanical harm to the callus. Calvarial defects were created using 1mm burr drill in the parietal bones of Sprague-Dawley rats (n=8) that served in vivo defects. After 7 days, their skulls were harvested after euthanizing. One additional defect per sample was created on the opposite parietal bone using same calvarial defect procedure to serve as control defect. Raman spectroscopy (785 nm) was established to investigate bone parameters of three different skull surfaces; in vivo defects, control defects and normal surface. Principal component analysis (PCA) was utilized for the data analysis and interpretation of Raman spectra and helped in the classification of groups. PCA was able to distinguish in vivo defects from normal surface and control defects. PC1 shows that the major variation at 958 cm^{-1}, which corresponds to υ1 phosphate mineral band. PC2 shows the major variation at 1448 cm^{-1} which is the characteristic band of CH2 deformation and corresponds to collagens. Raman parameters, namely, mineral to matrix ratio and crystallinity was found significantly decreased in the in vivo defects compared to surface and controls. Scanning electron microscope and optical microscope images show the formation of newly generated matrix by means of bony bridges of collagens. Optical profiler shows that surface roughness increased by 30% from controls to in vivo defects after 7 days. These results agree with Raman assessment parameters and confirm the new collagen formation during healing.

Keywords : Raman spectroscopy, principal component analysis, calvarial defects, tissue characterization
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