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Angaben zur Veröffentlichung / Publication details:

Pautke, C., T. Tischer, Stephan Vogt, C. Haczek, A. Neff, H. Deppe, M. Schieker, and A. Kolk. 2005. "Seven-colour fluorochrome labeling of bone growth: enhancing the number of fluorochromes by spectral image analysis [Abstract]." International Journal of Oral and Maxillofacial Surgery 34 (Supplement 1): 74. https://doi.org/10.1016/s0901-5027(05)81166-x.

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O39.5 SEVEN-COLOUR FLUOROCHROME LABELING OF BONE GROWTH: ENHANCING THE NUMBER OF FLUOROCHROMES BY SPECTRAL IMAGE ANALYSIS

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Bone formation and remodeling in vivo can be assessed by polychrome labeling using calcium-binding fluorescent dyes. The number of fluorochromes, however, limits this technique due to the fact that with increasing number, fluorescent spectra inevitably overlap, which makes discrimination more difficult. Therefore, bone formation can only be investigated in a limited number of time intervals. In order to enhance discrimination and to increase the number of the intervals we used eight fluorochromes and performed spectral image analysis. Non-critical size defects of the mandibular angle and the femur of male Wistar rats served as a model for bone formation. Eight different fluorochromes were administered subcutaneously every third day starting at day four after surgery. Bone specimen were embedded in methylmethacrylate and analyzed by spectral image acquisition using a Sagnac type interferometer (ASI, Israel). Seven of the eight applied fluorochromes could be resolved using spectral image examination. With BAPTA we present a new fluorochrome useful for bone labeling. Spectral decomposition and subsequent linear unmixing makes possible depiction of each individual fluorochrome without interference of any other enabling a reliable morphometric analysis of labelled regions. Furthermore, due to the superior sensitivity of the spectral camera, the thickness of the bone sections can be reduced so that much thinner sections could be analyzed. To our knowledge, this is the first presentation of a seven-colour bone labelling. Expanding the number of fluorochromes offers a more detailed sequential analysis of bone formation and remodelling. Moreover, a sophisticated morphometric analysis of each single fluorochrome is possible even in regions with overlapping fluorochrome signals.