3-D Reconstruction Of Pulpal Blood Vessels By Using Confocal Microscopy

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Objectives: The objective of this study was the three-dimensional reconstruction of pulpal vascular plexus, that was achieved by using Cy5-conjugated anti-CD34 and immunofluorescent confocal microscopy.

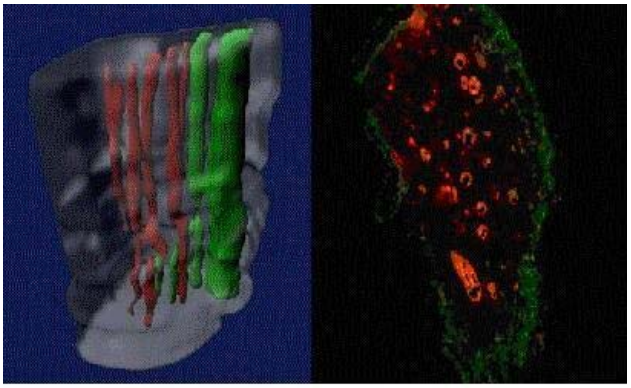
Methods: The endothelial cell marker (CD34) mouse monoclonal antibody (clone QBEnd/10) was purchased from Novocastra (Newcastle, UK). CyTM5-conjugated goat anti-mouse antibody was purchased from Jackson (West Grove, PA). Serial sections of a moderate thickness (10mm) were taken and after appropriate staining they were observed and digitized by using a confocal microscope (Leica TCS SP2 AOBS).

Two different methods a surface-based and a volumetric one, were applied in order to receive our final reconstructions.

The ELLIPSE 3-D software package has been used for the surface reconstruction. Contours that delineate the boundaries of the structures of interest were manually traced in each section and the triangular mesh that represents the external surface was constructed.

The second method utilized volumetric representation with the use of transparency. The EIKONA 3-D software was used for this purpose. Contrary to the previous case, a semi-automatic procedure was followed for the reconstructions. The user interactively manipulated the transfer functions that define the transparency-density, transparency-gradient and transparency-color (for all three basic colors) relations in order to obtain good semi-transparent color visualization of the structures of interest.

Results: Immunofluoresence using Cy5-conjugated anti-CD34 in human dental pulp demonstrated strong and homogenous positive staining pattern of pulpal vessels and capillaries while odontoblasts and nerve bundles were negative. Comparing the two 3D reconstruction methods, the first has given better visual results (Figure 1.a), but is more time-consuming since it requires manual delineation of structures and cannot provide information about the interior of the structures. The second method is far less time consuming while also preserving information for the volume (Figure 1.b).



(a) (b)

Conclusion: Both methods seem to be useful tools for the reconstruction of dental pulp vasculature.