MicroangioCT of the microvasculature of murine teeth and bones

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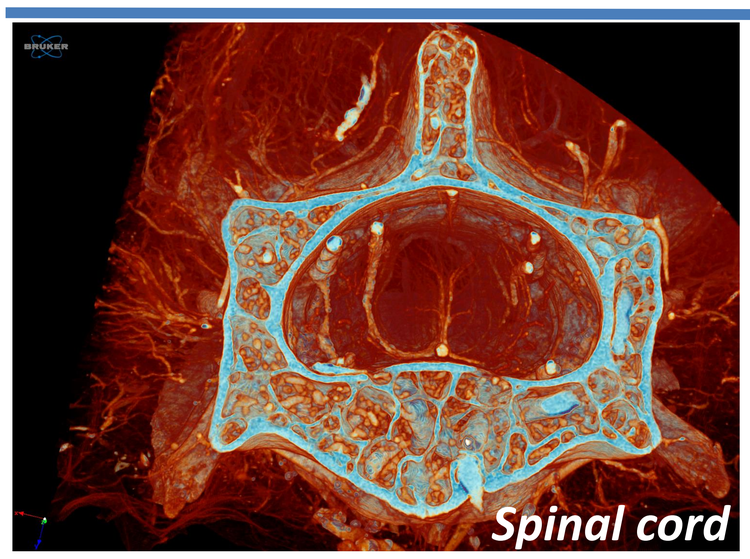

Background/Rationale: In the bone biology research the 3D-imaging of the vascularization within the bone tissue without the tissue destruction remains a major challenge. Application of microCT-technique with appropriate contrast agents could help resolve the situation.
Aims: To test whether the polymer-based contrast agent µAngiofil® is suitable for the visualization of the microvasculature inside the bony structures or even within the bone tissue. The secondary aim was to develop decalcifying protocols that would not interfere with solidified µAngiofil® and enable the subsequent correlative imaging or scan of the same sample with preserved intravascular contrast agent.

Methods/Results

To achieve a better perfusion of the vessels within the bone, the perfusion time has been significantly prolonged (the visual control of the success of the perfusion as we do it in sift tissues was not possible). The good perfusion of the neighboring soft tissues is only an indirect marker of the perfusion within the bone. With the improved perfusion protocol, we achieved good and reproducible results in the murine teeth (Fig. 1).

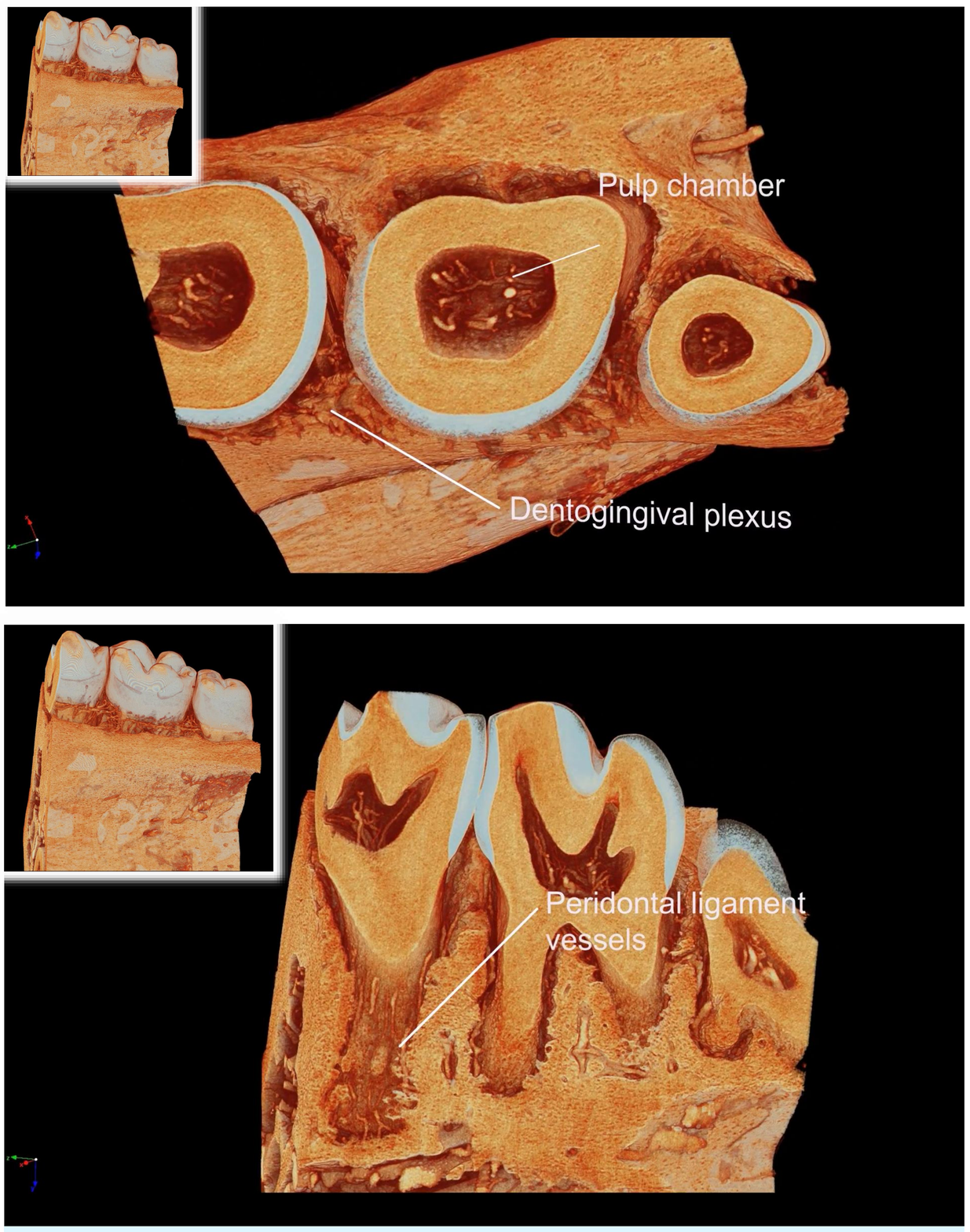

Fig 1. Microangio-CT of the vasculature of murine teeth with the use of µAngiofil. The microvasculature of the mandibular and the teeth (including the pulp chamber – see the upper image) can be clearly visualized even without the decalcification.
Scanning parameters: Skyscan 1172; Accelerating voltage 80 kV, voxel size side 1.0µm, filter Al 0.5mm, rotation step 0.1, frame averaging 4, 360 degrees.

References:
1. Schaad L, Hlushchuk R, Barré S, Gianni-Barrera R, Haberthür D, Banfi A, Djonov V. "Correlative Imaging of the Murine Hind Limb Vasculature and Muscle Tissue by MicroCT and Light Microscopy." Scientific Reports.7:41842. (2017).

The established decalcifying protocols enabled scanning of the murine hindlimb with less artefacts around the bone and usually at significantly lower acceleration voltage (Fig. 2). The subsequent histological evaluation of the sample has been also greatly facilitated (1).

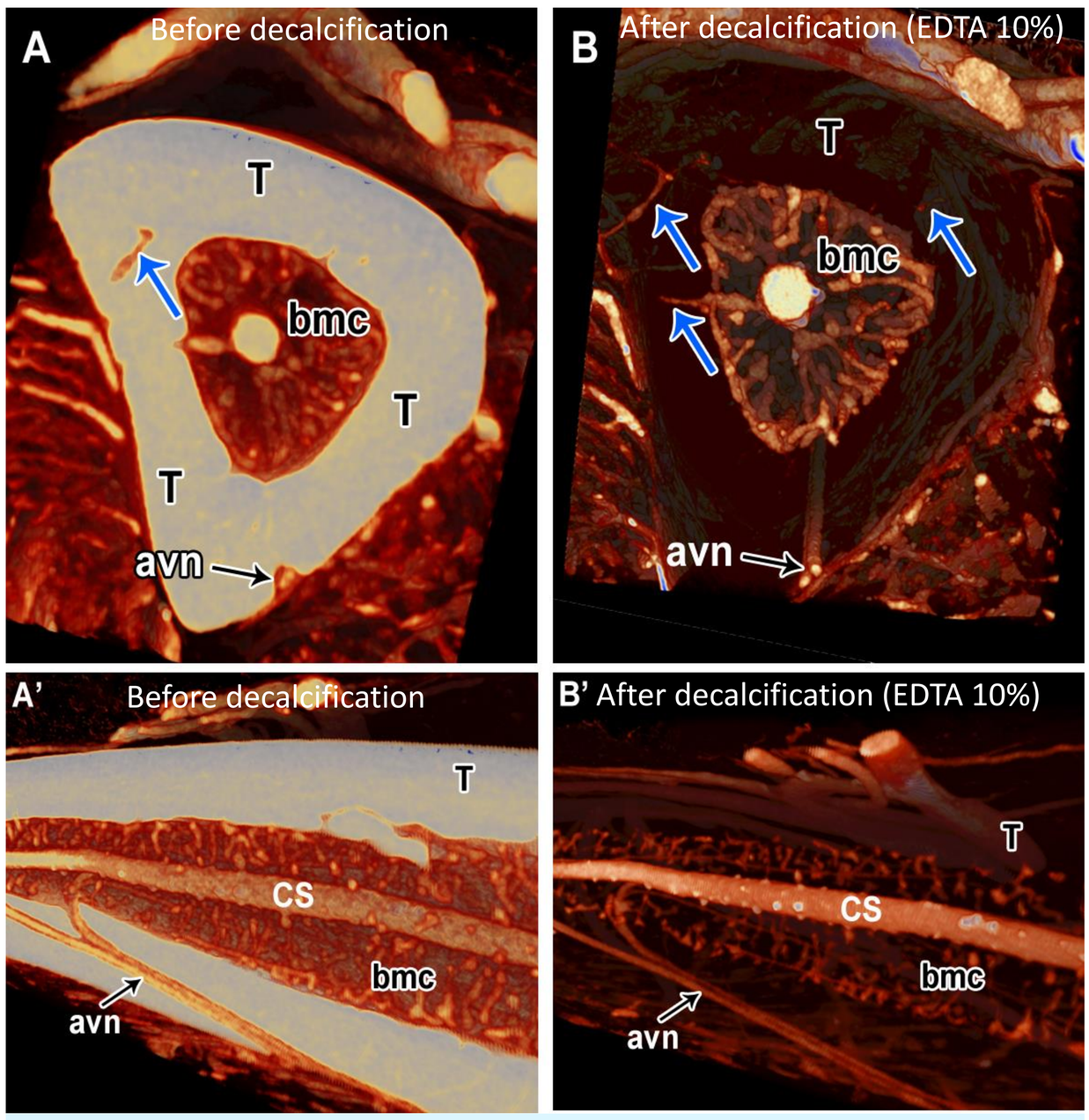

Fig 2. Microangio-CT visualization of the murine tibia bone before (A & A') and after decalcification with EDTA 10% (B & B'). Due to higher X-ray absorption the tibia bone is lighter in A & A'. In B & B' it is transparent due to low X-ray absorption after the decalcification. Therefore, the connecting vessels between the periostal vessels and the vessels of bone marrow cavity (=bmc) are easily detectable (blue arrows in B & B'). The visualization of the vessels within the medullar cavity (CS=cenral sinus) is also improved. At external surface of tibia the supplying arteries are visible (avn=*arteria et vena nutricia*).

Conclusion

The µAngiofil® is suitable for the visualization of the microvasculature within the bone tissue. The developed decalcifying protocols enable the subsequent correlative imaging or scan of the same sample with preserved intravascular contrast agent.