Morphological Characterization of Osteoblast-derived Histoids

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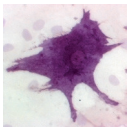
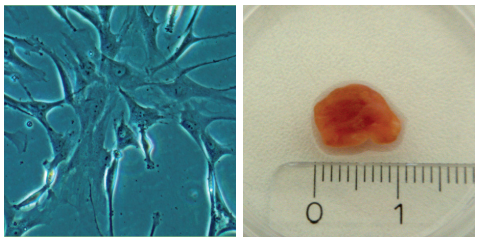
INTRODUCTION



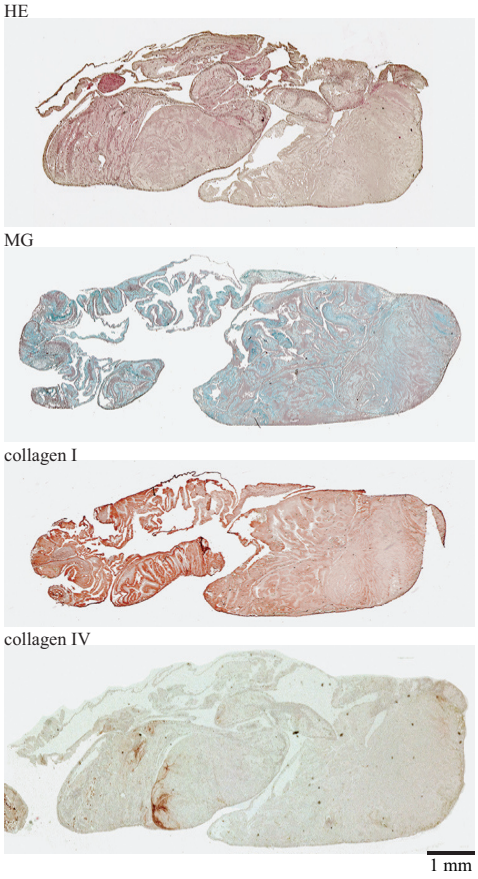
In vitro studies are usually performed in 2D cell cultures, which are not fully representative for in vivo conditions. In vivo, cells are 3D organised in tissues. Natural tissues display complex specific histoarchitecture composed of multiple cell types. 2D cultures cannot provide these conditions and thus cannot reproduce or simulate many biological processes. The 3D culturing concept has been introduced to overcome these restrictions. Such cultures are in principal based on cultivation of cells on scaffolds. Recently there has been an increasing number of reports about successful scaffold-free 3D cultivation of various cell types, including osteoblasts. Such histoids, however, have not been extensively investigated in long-term cultures. Here, we study the morphology of osteoblast-derived histoids using histo- and immunohistochemistry as well as synchotron radiation-based micro computed tomography (SRμCT).

CELL CULTURE

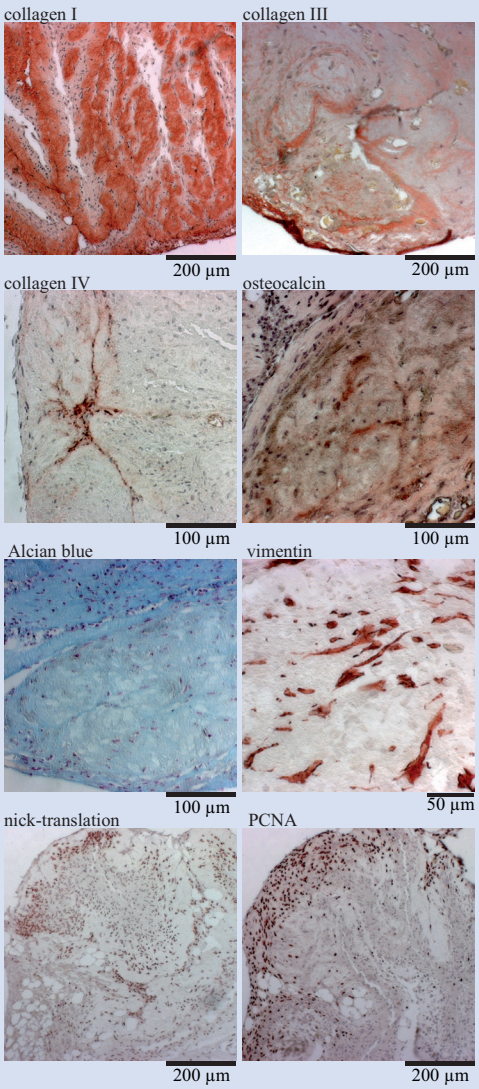


Primary human osteoblasts, isolated from femur neck spongy bone, were expanded as 2D culture in non-mineralizing osteogenic medium. A thick cellular membrane with abundant matrix that lost partially surface contact formed. This was intentionally released and randomly folded. The histoid grew in non-mineralizing osteogenic medium during 80 weeks.

HISTOLOGY



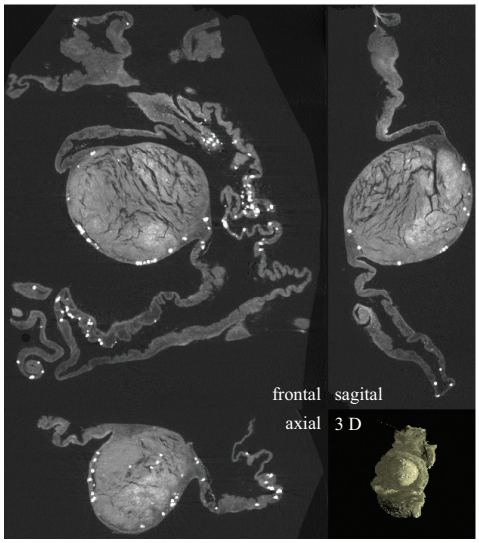
4 μm thin sections of a formalin fixated and paraffin embedded histoid were characterized by histochemistry, immunohistochemistry and nick-translation.

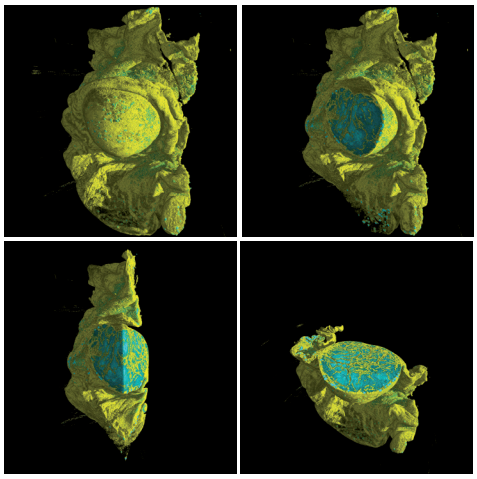


Cell-rich regions with loose ECM, regions with dense ECM and low cell number, and a collagen-rich membranous outer shell containing multilayered cells comprise the complex histoarchitecture of the histoid. Collagen I and muccopolisaccharides are expressed in abundance. Collagens III and IV, and osteocalcin expression is more restricted. Osteocalcin is predominantly expressed in the dense ECM. However, it is found also in conjunction with singular cells. Cells with varying sizes (20-100μm) and morphology are present. Polygonal/dendritic cells are predominantly located in the core and cuboidal/spheroidal cells at the periphery. Adypocytes are found either in clusters at the periphery or isolated in the core. Cell viability is preserved in the core of the histoid. Proliferative activity is higher at, but not restricted to the periphery.

SRμCT

The SRμCT measurements in the standard absorption contrast mode were performed at the beamline BW2, HASYLAB at DESY, Hamburg, Germany, using a photon energy of 24 keV. To investigate the sample five tomographical scans were performed and combined after reconstruction. The reconstructed 3D volume consists of 1535 x 1535 x 2037 voxels, representing a volume of 8.2 x 8.2 x 10.9 mm³. The measured spatial resolution corresponds to about 9.1 μm at a voxel length of 5.34 μm.





In the figure at the top 2D slices through the reconstructed volume and a 3D representation of the histoid are given. The white spots here are accumulation of the used contrast agent OsO_4. In the colored figures below the reconstructed data set was segmented to emphasize the 3D spatial organisation of the histoid. Different virtual cuts are shown to visualize the complex internal histoarchitecture.

CONCLUSION AND ACKNOWLEDGEMENT

The histomorphology of the histoid displays similarities to natural osteoid. The applied culture system sustains the proliferation and differentiation potential of the cells even after a prolongated cultivation time. Conventional histological methods allow analyzing ECM-composition, cell morphology and type as well as cellular processes such as viability, proliferation and death. The obtained information, however, is restricted to the examined slice and is 2D. The SRμCT yields details about the 3D spatial organization of the histoids. But features on the cellular and subcellular level can not be precisely examined. The SRμCT is an useful tool for the identification and selection of areas of interest prior to histological characterization.

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