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Internal structures of scaffold-free 3D cell cultures visualized by synchrotron radiation-based tomography

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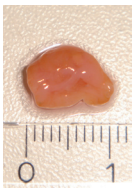
INTRODUCTION



Cell cultures are established in vitro models for studying cellular processes. For many decades cells have been cultivated in 2D. More recently the importance of the third dimension in cell biology has been better understood. The growing knowledge has attracted further attention to 3D culturing. The characterization of 3D cultures includes methods, adapted from the ones established for 2D cultures. These methods, however, encounter certain restrictions, especially when it comes to the evaluation of the 3D spatial organization. Histological sectioning allows studying this aspect. Unfortunately, the extracted information is restricted to the rather arbitrarily selected slices. In addition, because of the destructive nature of the sectioning, further studies are often impossible. Hence non-destructive methods for the 3D characterization are highly desirable. Some pioneering studies have successfully applied synchrotron radiation-based micro computed tomography (SRμCT) for the visualization and even quantification of biological meso- and microstructures, including scaffolds for tissue engineering. In this study we explored the potential of SRμCT for the characterization of scaffold-free, human osteoblast-derived histoids.

CELL CULTURE

Human osteoblasts were isolated from femur neck cancellous bone from donors undergoing reconstructive orthopaedic surgery according to a previously described protocol [Thorwarth G et al., Plasma Proc Polym 2007]. Long-term histotypic cultures of millimeter size, which could be regarded as pre-state of bony tissue, were grown under static culturing conditions. Histoids A, B and D were grown only in non-mineralizing osteogenic medium (NMOM: DMEM + 15% FCS + 10 nM dexamethasone + 50 μg/ml ascorbic acid). Histoid C received initially NMOM and then for the rest of culturing time minerilizing osteogenic medium (MOM: DMEM + 15% FCS + 10 nM dexamethasone + 50 μg/ml ascorbic acid + 10 mM ß-glycerophosphate). The table below summarizes the cultivation scheme of the histoids.

	NMOM	MOM
Histoid A	56 weeks	-
Histoid B	80 weeks	-
Histoid C	28 weeks	128 weeks
Histoid D	136 weeks	-

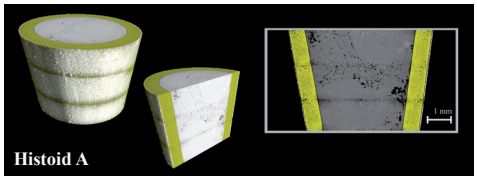
SRμCT

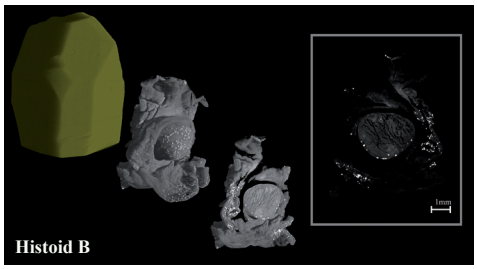
Prior to tomographical examination the Histoids B, C and D were fixated with gluteraldehyde, stained either with osmium tetraoxide or osmiumammine-B according to earlier protocols [Müller B et al, Microsc Microanal 2006] and then embedded either in paraffin or methylmethacrylate. Histoid A was examined without staining and embedding, immersed in a 4% formaldehyde solution. The table below summarizes the staining and embedding scheme of the histoids.

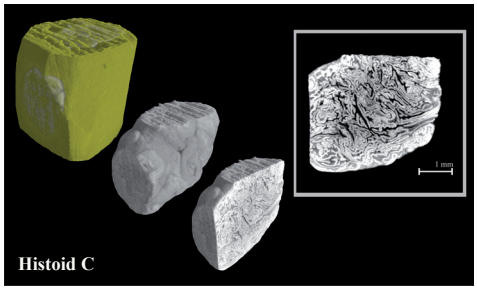
	STAINING	EMBEDDING
Histoid A	-	-
Histoid B	osmium tetraoxide	paraffin
Histoid C	osmium ammine-B	MMA
Histoid D	osmium ammine-B	paraffin

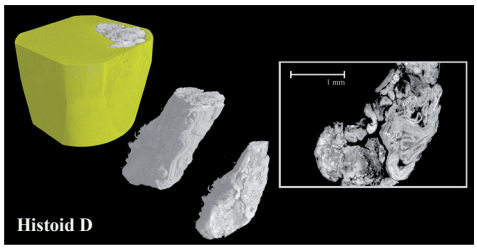
The tomograms were acquired at the beamlines BW 2 (Histoids A and D) and W 2 (Histoids B and C) in absorption contrast mode. The tomography experiment at HASYLAB at DESY has been operated by the GKSS Research Center [Beckmann F et al, Proc SPIE 2006]. The photon energies were adapted to the total absorption of the specimens, namely 10 keV for Histoid A, 26 keV for Histoid B and Histoid C, and 16 keV for Histoid D. The spatial resolution was determined by means of the modulated transfer function of a metal edge [Müller B et al, Proc SPIE 2002]. For the Histoids A, B, C, and D these values corresponded to 6.75 μm at the pixel size of 5.22 μm, 4.19 μm at the pixel size of 2.62 μm, 4.98 μm at the pixel size of 3.30 μm, and 4.04 μm at the pixel size of 2.26 μm, respectively. In general, the tomograms were combined from individual scans with voxel resolution. Consequently, the data were large and reduced applying appropriate binning factors.

VISUALIZATION



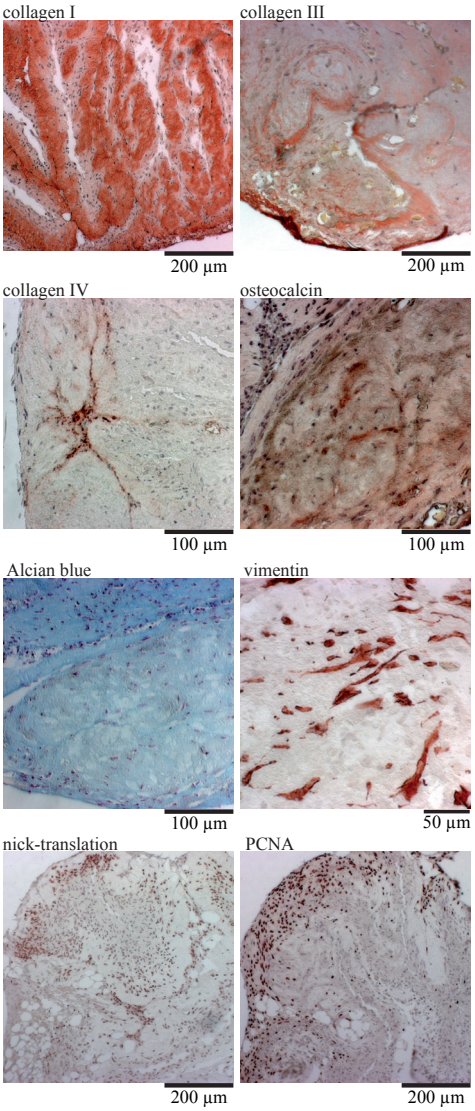






The figures above show 3D visualization of the acquired data (VG Studio Max, Volume Graphics, Heidelberg, Germany).
It was impossible to obtain images with sufficient contrast without precursory staining (Histoid A) at the applied SRμCT parameters, whereas examination protocols including staining with osmium tetroxide and osmium ammine-B yielded distinctly higher contrast, allowing the evaluation of the acquired images. The histoids exhibited similar spheroidal/ovoidal outer shape. The SRμCT examination revealed complex internal structures, principally combining membraneous and nodular components in various configurations. Variations in the 3D spatial organization of the different histoids, presumably due to the different origins (i.e. donors) and/or cultivation schemes, could be clearly recognized.

HISTOLOGY



The figure above shows results of the histological examination of Histiod A. The composition of the ECM is complex, with collagen I and mucopolisaccharides expressed in abundance, and collagens III and IV, and osteocalcin more restricted. Cells with varying sizes (20-100 μm) and morphology are present. Polygonal/dendritic cells are predominantly located in the core and cuboidal/spheroidal cells at the periphery. Adypocytes are easily recognized in clusters at the periphery and isolated in the core. Cell viability is preserved also in the core. The cellular turnover is relatively higher at the periphery as seen by the increased proliferative activity (PCNA) and apoptosis (nick-translation).

CONCLUSION & ACKNOWLEDGEMENT

The SRμCT yields details about the 3D spatial organization of the histoids. Information about ECM-composition, cell morphology and type as well as cellular processes such as viability, proliferation and death, however, have still to be provided by histological methods. The SRμCT together with the advanced visualization techniques is a non destructive approach for the identification and selection of areas of interest in 3D space prior to histological sectioning. Our study of the millimeter-sized, scaffold-free histoids demonstrates that microarchitecture and the features on the cellular level can be made threedimensionally visible and quantified with high precision, combining SRμCT data with sophisticated computer vision tools. Further studies are needed to improve the efficiency of the combined tomographical-histological examination protocol.

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