BIOPRINTING OF VASCULARIZED BONE TISSUE EQUIVALENTS

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Bone tissue is one of the most frequently transplanted tissues. Since procedures like the transplantation of autologous bone bear risks, though, regenerative medicine and tissue engineering reach to face those problems by engineering bone substitutes by using suitable materials and living cells. A crucial factor is the vascularization of the constructed tissue to ensure supply of the included cells with nutrients and oxygen. For the fabrication of such bone tissue equivalents, evolving manufacturing techniques like bioprinting can be used to construct geometrically defined three-dimensional structures.

Hydrogels based on methacrylated gelatin, hyaluronic acid and hydroxyapatite particles or methacrylated and acetylated gelatin were developed for the encapsulation of human adipose-derived stem cells (ASCs) or human microvascular endothelial cells (ECs), respectively [1,2]. ASC-laden bioinks were printed in a grid geometry (Fig. 1A) and in a cylindrical geometry (Fig. 1B), and the resulting hydrogels were cultured under static or under dynamic conditions in a perfusion reactor, respectively, for 28 days. Additionally, combination hydrogels containing both ECs and ASCs were build up via microextrusion printing and cultured under co-culture conditions. Bone matrix formation as well as the development of capillary-like structures were assessed via rheological measurements and staining of matrix components, as well as staining of EC markers and basal membrane.
We developed bioinks on that can - by further addition of hydroxyapatite - either support the osteogenic differentiation of ASCs and formation of a bone matrix, or the formation of vascular structures by ECs (Fig. 1C). The bioinks were used to build up geometries like 3D grids, cylindrical structures and combination hydrogels of bone and vascularization hydrogels via a microextrusion-based printing system, which were afterwards cultured for up to four weeks under static or dynamic culture conditions.
Evaluation of the hydrogels by mechanical analysis and staining of bone specific proteins like collagen type I (Fig. 1D), alkaline phosphatase (Fig. 1E) and osteopontin showed formation of a bone matrix. This was not only observable in hydrogels cultured under osteogenic conditions, but also after culture under control conditions without the addition of osteogenic supplements, indicating osteoinductive properties of the hydroxyapatite. Co-culture of ASCs and ECs in a suitable hydrogel environment resulted in improved formation of bone matrix and capillary structures compared to the respective monocultures. Additionally, the perfusion culture in a bioreactor allowed the build-up and successful culture of cell-laden hydrogel constructs with a volume of >1 cm2.

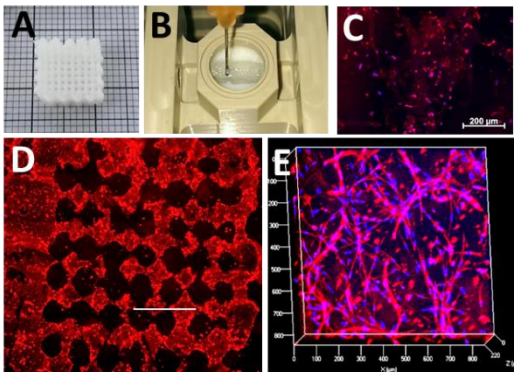

Figure 1: (A) Printed 3D grid structure made of ASC-laden bone bioink. (B) Printing of bone cylinder in bioreactor. (C) ALP-staining (red) in the perfusion cultured bone cylinder. Scale: 200 µm. (D) Section of the osteogenically cultured grid structure, stained for collagen type 1 (red). Scale: 2000 µm. (E) Formation of capillary network in vascularization hydrogels (red: PECAM-1, blue: DNA)

In conclusion, we were able to develop bioinks and a printing process which allow the successful build-up of bone tissue equivalents whose bioreactor culture enables the set-up of relevant geometries and sizes.

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