Promoting the Clinical Relevance of 3D Bioprinting

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer offentligen försvaras i sal Arvid Carlsson, Medicinaregatan 3, fredagen 25 februari 2022 kl 13.00

av

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Fakultetsopponent:

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Avhandlingen baseras på följande arbeten:

I. **Apelgren, P.**, Amoroso, M., Lindahl, A., Brantsing, C., Rotter, N., Gatenholm, P., Kölby, L. *Chondrocytes and Stem Cells in 3D-bioprinted Structures Create Human Cartilage in vivo*. PLOS ONE 2017; 12(12): e0189428

II. **Apelgren, P.**, Amoroso, M., Säljö, K., Lindahl, A., Brantsing, C., Stridh Orrhult, L., Markstedt, K., Gatenholm, P., Kölby, L. *Long-term in vivo integrity and safety of 3D-bioprinted cartilaginous constructs.* Journal of Biomedical Research 2020; 1-9

III. **Apelgren, P.**, Amoroso, M., Säljö, K., Montelius, M., Lindahl, A., Stridh Orrhult, L., Gatenholm, P., Kölby, L. *Vascularization of tissue engineered cartilage – sequential in vivo MRI display functional blood circulation*. Biomaterials **276**, 121002 (2021).

IV. **Apelgren, P.**, Sämfors, S., Säljö, K., Mölne, J., Gatenholm, P., Troedsson, C., Thomson, E.M., Kölby, L. *Biomaterial and biocompatibility evaluation of tunicate nanocellulose for tissue engineering and 3D bioprinting applications*. Submitted manuscript.

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Abstract

This thesis focuses on the development of methodologies enabling the reconstruction of autologous, functional, and long-term-stable cartilage-like tissue using 3D bioprinting technology and animal experiments. The stability, resilience, and *in vivo* viability of the printed cells and tissue vascularization, as well as the observed immunogenicity and safety, represent the main issues evaluated and discussed in this thesis. Furthermore, the mechanical properties of the applied biomaterials are evaluated in detail.

Study I
Background: This study quantitatively assessed the proliferative capacity of chondrocytes in the presence and absence of stem cells in the 3D bioprinting setting.
Results: We observed significant increases in the number of chondrocytes and cluster formations during the study period. Compared with pure human nasal chondrocyte (hNC) group, we identified a significant additional proliferative effect in the group containing both hNCs and stem cells, and histologic analysis confirmed the expected production of collagen type II in the extracellular matrix, as well as the distribution of glycosaminoglycans in the cartilage-like tissue. Additionally, fluorescence *in situ* hybridization analysis confirmed that the chondrocytes were of human origin, and their male phenotype verified the male chondrocyte-donor source.

Study II
Background: In this study, we evaluated the results of subcutaneous implantation of 3D-bioprinted constructs mixed with human chondrocytes and stem cells over the course of 10 months.
Results: We observed no signs of necrosis, tumors, ossification, or other adverse effects. Moreover, the constructs remained well-preserved, and histologic analyses showed thriving, proliferating chondrocytes in cartilage-like formations.

Study III
Background: This study mapped the vascularization of gridded 3D-bioprinted constructs.
Results: Perfusion data from magnetic resonance imaging revealed progressive vascularization inside of grid holes that were confirmed as being filled with blood vessels connected to host circulation according to histologic analysis. Additionally, immunohistochemical analysis of endothelial cells confirmed the vascular arrangement, with collagen II production further indicating chondrocyte proliferation and cartilage formation.

Study IV
Background: In this study, we evaluated the biocompatibility (according to ISO standards) and mechanical properties of tunicate-derived nanocellulose (TNC) as a novel biomaterial.
Results: We determined TNC biocompatibility as equivalent to that of expanded polytetrafluoroethylene while also exhibiting excellent mechanical properties.

Keywords
3D bioprinting, cartilage, chondrocytes, stem cells, tissue engineering, nanocellulose, hydrogel, bioink, vascularization, biocompatibility

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