**Bioprinting De Novo Cartilage with ECM-based Bioink**

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**Introduction:** Bioprinting is an emerging technology for fabricating complex tissue structures and whole organs. The majority of the bioinks used in bioprinting consists of one or two polymers and are therefore insufficient to mimic the complexity of the native extra cellular matrix (ECM) containing a vast array of morphogens, growth factors and matrix molecules. The potential of ECM bioinks have been shown recently [1], however these required the support of a thermoplastic scaffold. Our goal was to develop a bioink that could be incorporated with ECM particles from multiple sources to facilitate 3D bioprinting without the need of structural support materials. The self-supporting scaffolds printed using the ECM bioink demonstrated high precision and overhanging structures. This bioink could be used with ECM particles from many different tissue types to produce complex shapes required for multi-tissue organ printing.

**Methods:** Particulated cartilage BioCartilage® (Arthrex, USA) was mixed with a gellan and hyaluronan polymer blend to form a bioink which was a liquid paste at 37ºC. Extrusion printing using the bioprinter Biofactory® (RegenHu, Switzerland) was carried out at room temperature with a 300 micron needle and a printing speed of 300mm/min. A thermo-reversible pluronic-based support hydrogel was co-printed as a temporary sacrificial structure also serving as a CaCl2 reservoir to ionically crosslink gellan. After printing, the support material was eluted in 4ºC 102mM CaCl2 solution. The young’s modulus of the bioink was tested with a texture analyzer TA.XT Plus (Texture Technologies, USA) using an 10 mm in diameter indenter along with a 5 N loading cell at a rate of 0.01 mm•sec⁻¹. Young’s modulus was calculated from the linear part of the stress-strain curve with sample size n=4.

**Results:** The ECM Bioink presented here allows the reconstitution of cartilage particles into new structures with compositional and functional characteristics of the original tissue. ECM hydrogel cubes up to 6.6 mm in height composed of 22 layers were printed with high precision and overhanging structures. As seen in figure 1 the conversion of the computational model to 3D cartilage structure was conducted precisely and the overhanging structures were stabile after the support elution. Bioprinted structure in figure 2 illustrates the structural integrity after the support elution. The bioink scaffolds had a young’s modulus of 5.9 ± 0.45 kPa which is comparable to covalently crosslinked UV irradiated hydrogels [2]. Furthermore, all three components of the cartilage ECM Bioink are already in clinical use, thus avoiding the potential toxicity and regulatory hurdles of the UV crosslinking and meth/acrylation approach of many Bioinks.
Discussion: The de novo cartilage structures illustrated the versatility and the integrity of the ECM bioink. The young’s modulus of 5.9 ± 0.45 kPa is inferior to native cartilage young’s modulus of 0.48-0.64 MPa [3] but similar to nucleus pulposus 5.39 ± 2.56 kPa [4]. However, in tissue engineering the goal is not to implant scaffold reproducing the mechanical properties of native tissue, but to trigger and support the natural healing response. Therefore, balancing the mechanical stiffness requirements against the need for a permeable open network is necessary. Furthermore, bioprinting enables tailoring of the construct architecture to the loading patterns, thus further stabilizing the structure. This recently developed bioink and printing method could be used to facilitate bioprinting of complex shapes without a need of permanent supporting structures, thereby significantly expanding the range of bioprinting possibilities.

Significance: The bioink approach presented here makes use of clinically approved materials which should lead to faster regulatory acceptance compared to other bioinks. This bioink can be augmented with ECM particles from multiple tissue types, thus taking us a step closer to true multi-tissue organ printing.

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