**Hyaluronan-collagen bioink for the printing of intervertebral disc models**

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Introduction: The intervertebral disc (IVD) is a vital structure of our spine, essential for movement. Aging and trauma eventually lead to its degeneration, which results in debilitating pain for the patient and loss of mobility. Despite the high prevalence of this condition and its burden on the population and healthcare system, disc degeneration is still poorly understood and treated. This is in part due to the lack of suitable models, which could be used for further research and development of novel treatments. Current models are either oversimplistic, failing to adequately represent the disc's heterogeneous composition, structure, and mechanical function, or based on animal tissue, which is often poorly representative of humans. Continuous advances in 3D bioprinting over the past years have given rise to a technique which allows fabrication of complex structures with precise and reproducible control over the cell microenvironment. As a first step towards developing a better, reproducible, and representative model of the intervertebral disc, we have formulated a material suitable for the 3D bioprinting of structures resembling the gel-like nucleus pulposus (NP) of the IVD.

Methods: Type I collagen and hyaluronan, both essential components of our tissues, have been combined at concentrations approaching those of the NP. Due to inherent properties of collagen and a chemical modification of hyaluronan with tyramine, the resulting composite material can form an elastic gel via changes in pH, enzymatically induced crosslinking in the presence of horseradish peroxidase (HRP) and hydrogen peroxide ($H_2O_2$), or exposure to green light. By initially employing pH increase together with enzymatic crosslinking, the composite is turned into a soft gel which can be extruded through a printer's nozzle. The printed structures can then be further strengthened via exposure to green light. We studied the material rheologically by monitoring its gelation and transitions between liquid and elastic state during extrusion-like conditions, evaluated its response to compressive loads, seeded the material with bovine NP cells to demonstrate its biocompatibility, and printed simple lattice-based 3D structures.

Results: We observed good material extrudability. Under high strains (such as those in the printer nozzle) the material flowed, followed by a recovery of its shape and elasticity when the strains were decreased (as they would after printing). Shear storage modulus after exposure to light reached 4.6 kPa, whereas under compression the light crosslinked gels exhibited a 5.3 kPa equilibrium modulus. Both the shear and compressive properties are in the range of those previously reported for healthy human NP. Embedded NP cells demonstrated good viability and proliferation after 5 days of culture.

Conclusions: We present a bioink with rheological and compressive properties within the range of healthy human NP. To our knowledge, this is also the first bioink simultaneously composed of biochemically suitable components representative of native NP, and approaching the high concentrations observed in tissue. This work brings us a step closer to better, reproducible, and representative 3D printed IVD models, and the promise of new insights into the treatment of disc degeneration.

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