GelMA-Alginate interpenetrating polymeric network (IPN) for a 3D bioprinted liver model to study plasticizer toxicity

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Conventional 2D cell cultures fail to replicate the complex microarchitecture of the liver. This study aims to develop a 3D bioprinted liver model by synthesizing an interpenetrating polymeric network (IPN) composed of gelatin methacrylate (GelMA) and alginate. By reproducing a porosity gradient and applying dynamic perfusion, the model is designed to mimic the liver lobule microenvironment and assess the toxicological impact of the plasticizer acetyl tributyl citrate (ATBC). A bioink composed of 5% w/v GelMA and 2% w/v alginate was formulated using a dual crosslinking strategy based on ionic crosslinking with $CaCl_2$ followed by UV photocrosslinking in the presence of the LAP photoinitiator. Printability was quantitatively evaluated through comprehensive rheological analyses, while the porosity gradient was achieved by varying UV exposure times (30, 60, and 90 s) across the printed layers. HepG2 cells were encapsulated, and the constructs were bioprinted into hexagonal patterns mimicking the hepatic lobule. Structural stability and liver-specific functions, including albumin and urea secretion, were assessed under both static and dynamic flow conditions using an IvTech bioreactor over a 14-day culture period. Subsequently, the model was exposed to ATBC at concentrations ranging from 1 to 100 μM for toxicological evaluation. The IPN exhibited superior mechanical properties ($G' = 1040$ Pa) compared with single-network hydrogels, reaching stiffness values comparable to those of human liver tissue. In addition, the semi-IPN showed good printability, with a viscosity recovery of 75% following the application of a high shear rate simulating the extrusion process. Morphological analyses confirmed the successful generation of a porosity gradient, with pore diameters ranging from 20 ± 5 μm to 132 ± 45 μm. The bioprinting process preserved high cell viability, while dynamic culture conditions promoted enhanced HepG2 metabolic activity and improved cytoskeletal organization. Toxicological assessment revealed that ATBC induced a dose-dependent decrease in both metabolic activity and liver-specific functions. Overall, the GelMA-alginate IPN hydrogel provided the mechanical stability required for long-term dynamic culture, preventing the structural collapse commonly observed in single-network hydrogels under perfusion. The modulation of UV crosslinking times effectively reproduced the porosity gradient characteristic of the liver lobule, while the integration of dynamic culture conditions further increased the physiological relevance of the model. These findings demonstrate that the proposed bioprinted liver construct represents a reproducible and predictive platform for the safety assessment of contaminants released from food contact materials.