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3D printed, artificial extracellular matrix for potential neuroregeneration

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ABSTRACT

Traumatic Brain Injury (TBI) is a complex, heterogeneous injury ranging in severity and clinical outcomes. TBI is characterised by various stages of brain damage. The primary injury causes mechanical injury to the brain whereas the secondary injury results in further damage from molecular cascade responses. Despite great strides in knowledge and research, no significant improvement in TBI treatments has occurred to date. As this injury affects a significant portion of the population, it is prudent for research to be dedicated towards the development of an effective treatment. Artificial Extracellular Matrix (aECM) hydrogel scaffolds are gaining in popularity due to their inherent ability to provide a microenvironment similar to the *in vivo* microenvironment. Since the Extracellular Matrix (ECM) is directly implicated in scar formation, the fabrication of an aECM scaffold to prevent early glial scar formation in TBI may provide the much-needed therapeutic strategy in potentially regenerating affected neurons. It is postulated that if intervention can occur soon after injury, ECM manipulation of the injury site can occur, which may promote native functioning and lead to functional recovery. With the drive towards patient customisable treatments, 3D printing is becoming an indispensable fabrication tool. Despite the advancements in 3D bioprinting, 3D biomaterial development for neural tissue engineering is hindered due to the numerous material requirements which need to be satisfied. This study aimed to investigate and design the proposed concept of a 3D printable hydrogel peptide aECM as a novel strategy for the treatment of TBI. An initial study into material selection for hydrogel 3D printing identified Gelatin Methacryloyl (GelMA) and a combination of collagen and elastin, as the material candidate for biomaterial development. Further optimizations saw the inclusion of Genipin into the biomaterial and resulted in the realization of the potential formation of a semi and full Interpenetrating Polymer Network (IPN). Thermodynamic results confirmed the formation of the two IPN biomaterials since the endothermic peak of the semi IPN and full IPN occurred at 140.59 °C and 168.18 °C, respectively indicating the increased thermal stability of the full IPN system. Thermogravimetric Analysis (TGA) indicated that the main loss of weight occurred between approximately 175 ˚C - 475 ˚C and approximately 200 ˚C - 500 ˚C for the semi IPN (64% weight loss) and full IPN (67% weight loss), respectively. Molecular vibrations analysis indicated that two minutes of Ultra Violet (UV) crosslinking did not affect the Amide peaks of the biomaterial IPN systems as the Amide bands are still intact with similar transmittance intensities. Therefore, no alteration to the peptide secondary structure was noted. Both IPN biomaterials were further shown to be amorphous in nature with a high Full Width at Half Maximum (FWHM) value of 7.82 and 7.67 for the full IPN and semi IPN, respectively. The IPN biomaterials have a stiffness of around 600 Pa and are suitable for softer tissue engineering applications – i.e. the brain. Furthermore, viscoelastic studies for all samples indicate that G'>G'' and demonstrates that the hydrogels are more "solid" in nature. Furthermore, the semi IPN displayed the highest elasticity followed by the GelMA polymer and then the full IPN. In addition, both IPN biomaterial scaffolds displayed a "shape-memory" feature in artificial cerebral spinal fluid (aCSF). Scanning Electron Micrographs indicated that the IPN biomaterials had a morphological structure with a significant resemblance to the native rat cortex. Both IPN scaffolds exhibited an increased biodegradation rate of 8 and 6 days, for the semi IPN and full IPN, respectively. The full IPN exhibits 1.5 times greater fluid uptake ability than the semi IPN and after 8 hours, the semi IPN and full IPN had a fluid uptake of 483.0 % and 725.6 %, respectively, in comparison to their initial dry masses. Both biomaterial scaffolds were shown to support the growth of PC12 cells over a 72-hour period. Furthermore, the increased nuclear eccentricity and nuclear area was shown to support the postulation that the IPN biomaterials maintain the cells in a healthy state encouraging cellular mitosis and proliferation. In addition, through fluorescent cell tracking, cellular migration was observed throughout the IPN biomaterial scaffolds. The Genipin component of the full IPN was further shown to exhibit antimicrobial properties in microdilution assays followed by the determination of the minimum bactericidal concentration. This suggests that Genipin can prevent the growth of pathogens associated with post-surgical brain infections. This research therefore contributes to the collection of potential biomaterials for TBI applications. Lastly, 3D printing coupled with the two novel biomaterials can assist in the progression of neural treatments towards patient specific scaffolds through the development of Custom-Neural-Scaffolds (C-N-S) as no two brain injuries are the same. In conclusion, the two biomaterial scaffolds developed in this research demonstrate potential for neuroregeneration in new TBI cases.