3D BIOPRINTING OF A BIOINK CONTAINING DECELLULARIZED SPINAL CORD TISSUE

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SUMMARY

Over the last years, 3D bioprinting has emerged as a promising approach in the field of regenerative medicine. This technique allows for the production of three-dimensional scaffolds to support cell transplantation due to its ability to mimic the extracellular environment, a key feature for improving tissue regeneration. The biomaterial structure as well as its components drive cell fate and dictate the ability to regenerate damaged tissue. One alternative for enhancing cell adhesion, survival and proliferation is the use of decellularized extracellular

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matrix as a bioink component. The aim of this study has been to produce a bioink using lyophilized Decellularized Spinal Cord Tissue (DSCT) of rats for

3D bioprinting. The spinal cord tissue of the rats was collected (CEUA 35781), cut in 1 cm length segments and submitted to the decellularization process with 1% sodium dodecyl sulfate (SDS), 1% Triton X-100 and PBS in a 9 hour protocol. To assess the decellularization efficacy, the genomic DNA content was quantified and histological sections of the samples were stained with DAPI or with hematoxylin and eosin. The collagen content was measured by spectrophotometry. MTT cytotoxicity assay was used to analyze DSCT cytocompatibility. The bioink was produced using 1,5% lyophilized DSCT, 4% alginic acid, 3% gelatin and PC12 with cell density of $1.5X10^6$ cells per mL. This bioink was then used to print a disc with an Octopus bioprinter (3DBS). The cytocompatibility of the bioprinted material was analyzed both by MTT and Live/Dead assay. After the decellularization, the spinal cord tissue showed a 50-fold reduction of genomic DNA content when compared to the control spinal cord tissue, and the histological staining indicated only a few cells. The DSCT presented a slight decrease of collagen content when compared to the control spinal cord. MTT assay indicated that the DSCT did not alter the cell viability. The bioink was used to bioprint a disc-like structure with 0.3 mm height and 10 mm diameter with a total volume of 50 µL. The MTT test indicated that the bioprinted material presented a tendency towards higher cell viability and adherence in comparison with the control after 3 and 7 days. The Live/Dead and DAPI staining showed a 3D cell distribution on the printed material. In conclusion, a bioink was produced combining alginic acid, gelatin, PC12 cells and the lyophilized decellularized tissue. The bioprinted material was not only able to maintain cell viability but also stimulated cell growth on a 3D structure. Therefore, this bioink may be an easily-available biomaterial for Central Nervous System tissue engineering.

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