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Autor	FERNANDA MÜCKLER PEREIRA
Orientador	PATRICIA HELENA LUCAS PRANKE

Bioink production with decellularized spinal cord tissue for 3D bioprinting for neural tissue engineering

Marcelo Garrido dos Santos, João Pedro Prestes, Cristian Teixeira, Luiz Sommer, Fernanda Stapenhorst França, Laura-Elena Sperling, Patricia Pranke

Student: Fernanda Mückler Pereira
Advisor: Patricia Helena Lucas Pranke

Spinal cord injuries have a limited tissue remodeling process, being a highly debilitating trauma. 3D bioprinting has emerged as an alternative to this limitation, by producing 3D scaffolds that mimic an extracellular environment, allowing cell transplantation, and thus, regeneration. The aim of this study is to add a decellularized extracellular matrix to a bioink composition, to increase tissue regeneration, without decreasing the bioink viability. Rat spinal cord tissue was submitted to a 9 hours decellularization process using immersions in 1% sodium dodecyl sulfate (SDS), 1% Triton X-100 and 1X PBS. DNA content was quantified by spectrophotometry, and immunohistochemistry analyses were performed to evaluate the presence of specific neural cell proteins. The decellularized spinal cord tissue (DSCT) was lyophilized and 1,5% DSCT was mixed with 4% alginate, 3% gelatin and PC12 cells to produce the bioink. A disc of $1.5X10^6$ cells/mL was bioprinted. Cytocompatibility of the construct was analyzed using MTT assay. Rheological characterization was performed using a rheometer with Peltier equipment. DNA quantification indicated a 50-fold DNA reduction on the DSCT and the immunohistochemistry analysis showed a decreased cytoskeleton protein expression. The MTT assay indicated that the bioprinted construct presented a tendency towards higher cell viability and adherence than the control after 3 days. The decellularization protocol was effective in removing DNA, providing a matrix without relevant cytotoxicity. Reduced viability was, in fact, observed at the first day after bioprinting. This viability reduction is expected due to shear stress and chemical crosslinking processes. However, the cell death is reversed and presents a cell viability increase of 50% after 3 days. Rheological analysis indicates that the hydrogel has a good printability and preserves structural integrity and high cell viability. DSCT bioink, therefore, represents an easily-available biomaterial for neural tissue engineering via 3D bioprinting.