Decellularized spinal cord-based bioink leads to a better functional outcome after a SCI model in mice

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Keywords: Decellularization; 3D bioprinting; Bioink; Mesenchymal cells; Neural tissue.

Introduction and objective: Three-dimensional (3D) bioprinting combines biomimetic materials and cells to promote functional recovery of tissues and organs. This technique can be applied to overcome challenges in regeneration of tissues with poor intrinsic regeneration capacity, such as the nervous tissue (1). Biomimetic materials can be used to increase the bioink similarity to the native tissue, enhancing functional regeneration (2). In this context, the aim of this study was to produce a bioink using Decellularized Spinal Cord Tissue (DSCT) and mesenchymal cells (MSC) for nervous tissue 3D bioprinting and analyze its effects on a mice T9 contusion SCI model.

Experimental Procedure: The bioink was produced with 1.5% DSCT, 3% gelatin, 4% alginate, 0.1 mg/mL PEDOT:PSS and $1X10^6$ MSC/mL. The material physical properties were evaluated by electrical conductivity measurement, rheological characterization and scanning electron microscopy (SEM). Swelling ratio and degradation were analyzed for 4 weeks. Cell viability was analyzed by live/dead assay. Macrophages previously stressed with LPS were exposed to the bioink and the cell`s phenotype was evaluated quantifying CD206 expression by flow cytometry and IL-1β, IL-6 and IL-10 concentration by ELISA. For the *in vivo* experiments, 65 female mice receive a T9 contusion SCI and were divided in 4 groups: A- Bioink, B- Bioink + MSC nanoengineered vesicles at a concentration of $5 X 10^{10}$/ mL, C- Injury (only PBS) and D- Hydrogel used as base for the bioink. The animals were evaluated by BMS weekly for 6 weeks to access the locomotor recovery.

Results and discussion: PEDOT:PSS addition to the hydrogel slightly increased its electrical conductivity and did not significantly changes the material`s viscoelastic behavior. Since the hydrogel by itself had an adequate conductivity, the following experiments were conducted without PEDOT:PSS. The hydrogel presented shear thinning behavior and low G''/G' ratio, allowing good printability without significantly compromising cell viability (3). SEM images showed a highly porous tri-dimensional structure. Cytokine quantification indicated no difference in IL-6 and IL-1β secretion in the hydrogel group; however, IL-10 production was significantly increased. Macrophage exposure to the hydrogel increased CD206, a classical anti-inflammatory polarization marker, expression (4). CD206 increase was dependent on DSCT presence in the material. The hydrogel reached peak swelling ration at week 1 after printing and lost 24% of its weight at week 2, maintaining a constant weight until week 4. Live/Dead assay showed more than 75% cell viability on week 1. After 6 weeks of the injury and treatment, the animals in group A had a significantly higher BMS score (4.2) when compared to groups B (3) and C (2.9) ($p<0.05$), indicating that the bioink had a positive effect in locomotor recovery.

Conclusions: The bioink presented optimal viscoelastic behavior for 3D bioprinting, induced an anti-inflammatory polarization on macrophages and maintained high cell viability. Those findings are reflected by the *in vivo* experiments, were the bioink promoted locomotor regeneration and increased coordination in the mice. The data mentioned above indicates that the bioink is a promising candidate for SCI repair and regeneration.

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