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# CRYOPRESERVATION OF POLY (LACTIC-CO-GLYCOLIC ACID) NANOFIBERS PREPARED BY ELECTROSPINNING FOR TISSUE ENGINEERING

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Introduction: Tissue Engineering (TE) involves the knowledge of a number of areas in order to promote the generation and/or regeneration of damaged tissue. To do so, it employs electrospinning, forming biomaterials in the form of nanostructured scaffolds which mimic the extracellular matrix (ECM), providing adhesion and cell proliferation. The biomaterial in this study was developed using poly (lactic-co-glycolic acid, PLGA), a synthetic polymeric compound with high biodegradation potential within a short period of time. With the aim of clinical application, cryopreservation has been studied in PLGA scaffolds for the purpose of preserving biomaterials for extended periods for future use in tissue transplantation. Aims: This study aims to cryopreserve nanofibers of poly (lactide-co-glycolic acid) obtained by electrospinning for applications in tissue engineering. Methodology: A protocol was established to produce scaffolds with 18% PLGA (Purasorb®) in acetone:hexafluoro (9:1). This process was carried out using the electrospinning method in accordance with the following parameters: power supply with voltage of +18kV and -5kV, 23G needle, 1.2mL, polymer solution, distance of 15cm between the nozzle needle and the collector plate, average flow 1,74mL.h-1 and temperature of 20°C. After their production, the scaffolds were cut with edges of 2.3 x 2.3cm and transferred to cryovials. Following this, each scaffold was treated with the addition of the following cryoprotectants in fetal bovine serum (FBS): DMSO, ethylene

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glycol and glycerol at concentrations of 5 and 10%. The cryotubes were then cryopreserved in nitrogen and after 3 days were thawed in water at room temperature, washed with saline and dehydrated with ethanol. They were then analyzed macroscopically and by scanning electron microscopy (SEM). Results: Macroscopic evaluation showed no variation between the treatments. All the experimental groups showed wrinkling of the biomaterial, even without the addition of cryoprotectants and without exposure of the biomaterial to nitrogen, resulting in about a 1cm size reduction. Through SEM analysis, cracks were observed in the treated nanofibers with 5% ethylene glycol and 5% glycerol. Diameter evaluation of the nanomaterials showed that treatment with 10% DMSO caused a variation in the biomaterial with increased diameter of the nanofibers. Scaffolds frozen and treated with SFB alone showed average diameter of 1.45μm; with 5% DMSO, 1.46μm ($p$=0.458); with 10% DMSO, 1.52μm ($p$=0.009); with 5% ethylene glycol, 1.38μm ($p$=0.882); with 10% ethylene glycol, 1.49μm ($p$=0.712); with 5% glycerol, 1.41μm ($p$=0.998 and with 10% glycerol, the average diameter was 1.37μm ($p$=0.818) (n=30 per treatment). Conclusion: It is concluded that cryopreservation of biomaterials with 10% DMSO, the gold standard for cryopreservation of cells, causes a variation in the nanostructure of PLGA scaffolds, which may cause variation in the growth of cells for use in regenerative medicine and tissue engineering. Financial support: CNPq, CAPES, FAPERGS and Stem Cell Research Institute.

Keywords: Cryopreservation. Biomaterials. Polymers. Poly lactide-co-glycolic acid. Tissue Engineering.