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EVALUATION OF THE EFFECT OF CRYOPRESERVATION OVER THE FUNCTIONALITY OF BONE-GENERATING CELL/TISSUE CONSTRUCTS

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KEYWORDS

Cryopreservation, Goat Bone Marrow Stem Cells, SPCL

ABSTRACT

The aim of this work was to study the effect of cryopreservation over the functionality of both 2D and 3D tissue engineered constructs and to compare the cellular survival and metabolic activity of cells seeded, cultured and cryopreserved on them. Apart from evaluating the effect of cryopreservation on the cells cultured onto polymeric scaffolds, this work also intended to evaluate its effect over the scaffold/material itself.
For this, several 3D porous scaffolds and 2D nonporous discs made of starch and poly(caprolactone) were seeded with goat bone marrow stem cells and cryopreserved for 7 days in liquid nitrogen. After this period, the samples were analyzed and compared to non-cryopreserved samples.
The obtained results suggest that it is possible to maintain cell viability and scaffolds properties upon cryopreservation of tissue engineered constructs based on starch scaffolds and goat bone marrow mesenchymal cells using standard cryopreservation methods. Also, this study tends to suggest that the greater porosity and interconnectivity of scaffolds favour the survival of construct's cellular content during cryopreservation processes. These findings indicate that it might be possible to prepare off-the-shelf engineered tissue substitutes and preserve them in order to be immediately available upon patient's need.

INTRODUCTION

Cell-scaffold constructs are expected to find a growing number of applications in the regeneration of human tissues. Facing a growing demand for cultured cells and tissues, the tissue engineering community is becoming increasingly concerned with the issue of preserving and storing "living" biomaterials. Cryopreservation seems to be the more tenable option for solving this problem since it is based on the principle that chemical, biological and physical processes are effectively "suspended" at cryogenic temperatures (Karlsson and Toner 1996). Being cryopreservation mostly applied to cell suspensions it becomes necessary to assess if common cryopreservation methods are as well applicable to three-dimensional cell-scaffold constructs.

MATERIALS AND METHODS

In this work, goat bone marrow cells (GBMC´s) were used. These cells were expanded at 37ºC, in a 5% CO_2 wet atmosphere, in DMEM basal medium.
Multiple 8 mm diameter and 3 mm thickness SPCL fiber mesh scaffolds produced by fiber bonding and 8mm diameter and 3mm thickness SPCL discs obtained by injection moulding were seeded with $1x10^6$ cells each and cultured in osteogenic medium for 7 days. After this culturing period, half of the constructs (both discs and scaffolds) were collected for analysis and the other half were cryopreserved in liquid nitrogen for 7 days immersed in a 10% DMSO cryopreservative solution. Experimental controls, consisting of GBMC cell suspensions and unseeded scaffolds and discs were kept in the same conditions during the experiment. After this step, the remaining constructs were thawed, cultured for an extra 4 days recovery period and then prepared for

characterization, namely by MTS and DNA Quantification, Scanning Electron Microscopy (SEM) analysis and Atomic Force Microscopy (AFM).

RESULTS

By examining the results obtained from the MTS and DNA analysis (figure 1), it was possible to determine that the cell viability/survival in the 3D scaffolds was greater than in discs. These results tend to show that 3D porous structures are more favorable for cell survival after cryopreservation than 2D nonporous discs. By being more able to attach and grow in the interior of pores, and consequently in the interior of the scaffold's architecture, cells can be more protected from injurious cryopreservation effects. These results show that the porosity and pore interconnectivity of scaffolds can in fact be important factors in the survival of cells seeded on scaffolds exposed to cryopreservation processes.

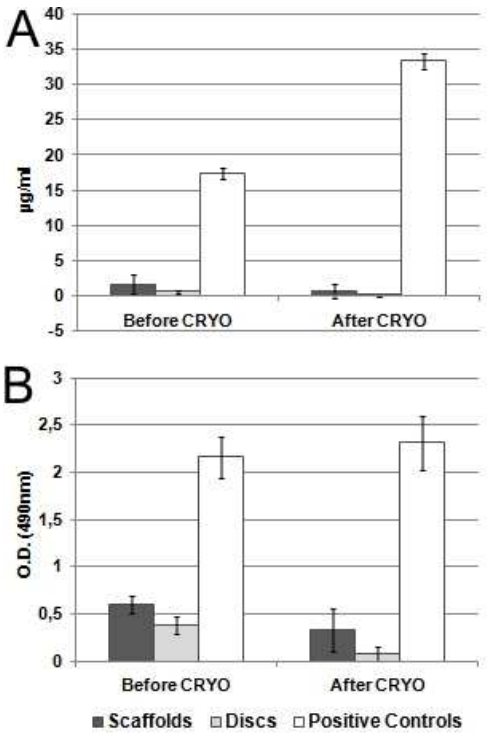

Figure 1- Quantification of cell survival by DNA quantification and cellular viability by MTS quantification B).

Additionally, through the SEM analysis it was possible to observe that the cellular morphology was not affected by the cryopreservation process and that cells remained attached to the material's surfaces and continued proliferating after thawing. These results suggest that the number of seeded cells was accurate and the proportion of the cryoprotective agent (DMSO) was adequate to keep cell viability. It was also possible to observe by SEM that the cryopreservation process did not interfere in the material surface morphology. This was more accurately confirmed by AFM analysis.

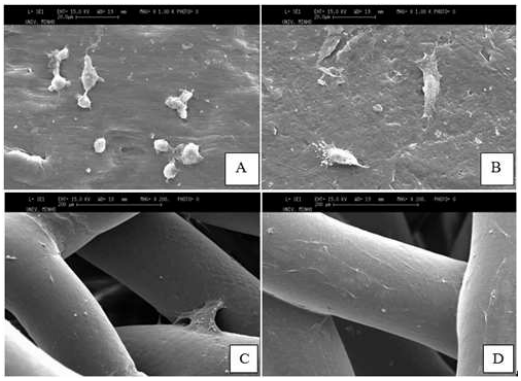

Figure 2- SEM pictures of SPCL discs and scaffolds before and after cryopreservation at magnification 1000x. GBMC-seeded SPCL discs before A) and after B) cryopreservation (magnification 1000x); GBMC-seeded SPCL scaffolds before C) and after D) cryopreservation.

DISCUSSION AND CONCLUSIONS

The obtained results suggest that it is possible to maintain cell viability and scaffolds properties upon cryopreservation of tissue engineered constructs based on starch scaffolds and goat bone marrow mesenchymal cells using standard cryopreservation methods. Also, this study tends to suggest that the greater porosity and interconnectivity of scaffolds favors the survival of construct's cellular content during cryopreservation processes. These findings indicate that it might be possible to prepare off-the-shelf engineered tissue substitutes and preserve them in order to be immediately available upon patient's need.

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