8^{th} European Medical and Biological Engineering Conference (EMBEC 2020)

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ABSTRACT BOOK

Tomaž Jarm, Samo Mahnič-Kalamiza, Aleksandra Cvetkoska,
Damijan Miklavčič (Editors)

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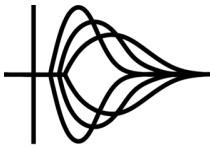

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Ethylene glycol improves cryopreservation of cell-seeded electrospun scaffolds in cryobags

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Cryopreservation of functional three-dimensional tissue-engineered constructs (TECs) is an inevitable step in ensuring their on-demand availability for clinical applications. Currently used protocols for TECs cryopreservation would greatly benefit from implementing efficient technological steps assuring improved heat and mass transfer, low toxicity and high cell cryoprotection. Therefore, we developed and validated an integral approach based on "in air" freezing in cryobags, optimization of thawing procedure and freezing solutions for efficient cryopreservation of cell-seeded electrospun scaffolds. Porous scaffolds were produced from polycarpolactone and polylactic acid (ratio 2:1) using blend electrospinning under previously optimized process parameters. The scaffolds (diameter 16 mm, thickness 100 um) were UV sterilized and seeded with human primary osteogenic sarcoma cell line SAOS-2 according to a modified protocol yielding homogeneous cell distribution. On day 3 of cultivation, the scaffolds were loaded for 10 min either using 10% (v/v) dimethyl sulfoxide (DMSO) or ethylene glycol (EG), and their combination with 0.3 M sucrose and frozen in polypropylene/polyethylene in-house made cryobags. For sucrose containing CPA cocktails, cell pretreatment with 0.1 M sucrose 24 h before freezing was also evaluated. Cell-seeded scaffolds were frozen using 1 K/min cooling rate down to -100°C in a Planer programmable freezer. The samples were stored at -140°C for 7 days and thawed in two steps using water bath and pre-warmed culture medium. Heat transfer during thawing was simulated using ANSYS software. On day 1, 3 and 7 before freezing and after thawing, functionality testing was performed using live-dead as well as metabolic activity assays, respectively. In addition, mechanical properties of cell-free scaffolds were analyzed using an Instron machine. Preservation of cell-cell and cell-biomaterial interactions was evaluated using scanning electron and confocal laser scanning microscopy. Mechanical testing revealed that our cryopreservation approach does not affect the properties of unseeded scaffolds. Both microscopic techniques showed the preservation of cellular integrity, cell-scaffold and cell-cell interaction post-thaw independently of the CPAs used. Quantification of the viability data demonstrated significantly higher cryoprotective effect of EG as compared to DMSO in combination with sucrose in the freezing solution. The results on metabolic activity of cells after thawing correlated with viability data. In summary, our work suggests that using controlled cryopreservation steps and EG as a non-toxic CPA, efficient cryopreservation of TECs in cryobags becomes feasible.