Frontiers of Biomaterial Cryopreservation for Banking

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Abstract

Effectively improved of tissue banking methods for natural and engineered tissues, complex allotransplants and organs are desperately needed for transplantation. Banking of living cellular tissues using current tissue banking practices employing conventional cryopreservation by freezing isn't feasible thanks to the well documented damage caused by ice formation. An alternative ice-free cryopreservation approach is vitrification. The formation of ice can be prevented by the presence of high concentrations of cryoprotectants with preservation of extracellular matrix components and optional preservation of cells. Ice-free vitrification works for a spread of natural and engineered tissues, employing a formulation consisting of DMSO, formamide and propanediol, referred to as VS55, but are unsuccessful at sample volumes over a few mLs. The major constraints for scale-up of cryopreservation by ice-free vitrification have been avoidance of ice nucleation during warming and mechanical forces generated by glasses at low temperatures. In this presentation I will focus on strategies for avoidance of ice nucleation. Our first successful strategy for giant tissue samples was an 83% formulation based upon an equivalent cryoprotectants, referred to as VS83. This formulation can beused to retain viable chondrocytes in large osteochondral grafts or for non-viable cardiovascular grafts with retention of extracellular matrix integrity, depending upon the way in which the formulation is added and removed before and after vitrification. Non-viable cardiovascular grafts with intact matrix have been a major research focus for the last 10 years and both in vitro and in vivo results demonstrated significantly reduced immunogenicity in heart valves (Figure), including reduced memory T-cell proliferation and most recently modulation of TGF-β1 from latent to active form among other statistically significant effects. We have recently been successful in scaling up the viable preservation of large tissue samples using either nano warming, inductive heating of iron nanoparticles, or convection warming using improved ice-free vitrification formulations. Cryopreservation is that the use of very coldness to preserve living cells and tissues during a quiescent status for an extended period, without losing their viability, activity, and performance. Several works have been devoted to the cryopreservation of engineered biological substitutes, but there is still the need for information on the cryopreservation of bio fabricated osteoblast constructs, a paramount goal addressed in this work. Pioneer studies demonstrate that post-thawing osteoblast viability was better maintained (40–50%) when cryopreserved in dimethyl sulfoxide (DMSO) than in other cryoprotectant additives. Moreover, it was reported that post-thawing cell viability was reduced for attached osteoblasts and increased with osteoblast density [27]. Unlike those early studies concentrated in the osteoblast post-thawing viability due to the use of different cryoprotectant additives under traditional two-dimensional (2D) cell culture, this work aims to evaluate the recovery of the metabolic activity and function of 3D-bioprinted osteoblast-containing structures and hydrogel-encapsulated osteoblasts infiltrated in beta-tricalcium phosphate (β-TCP) scaffolds; both freshly bio fabricated structures were named osteoblast constructs for the sake of simplicity. Among the different alternatives, a hydrogel mixture composed of gelatine with alginate was selected for the encapsulation and 3D-bioprinting of osteoblasts. This hybrid hydrogel is definitely processed into 3D structures, shows short-term stability after ionic crosslinking, supports cell proliferation in vitro, promotes better cell metabolic activity than pure alginate and supports bone healing in vivo. Besides, the rationale of the utilization of β-TCP scaffolds was to mimic the chemical and mechanical microenvironment in bone tissue and, therefore, increase the structural properties of the constructs for better long-term in vitro manipulation. Variables such as the osteoblast maturity phenotype and the pre-cryopreservation culture period were studied for the first time and compared with the results of non-preserved counterparts. The short-term cryopreservation at −80 °C was studied as the proof of concept, discovering important aspects for the successful cryopreservation of osteoblast constructs. Cell viability after bioprinting was determined by a live/dead viability kit. Briefly, the samples were rinsed in PBS and incubated for 15 min with 2 µM Calcein-AM and 1.5 µM ethidium homodimer-1 solution in PBS and finally rinsed twice in PBS before imaging with a fluorescent microscope coupled to a CCD camera, using appropriate filter sets. Cell viability was measured via counting live and dead cells using ImageJ software. The cell metabolic activity was evaluated by XTT assay and the osteoblastic phenotype was evaluated by the alkaline phosphatase (ALP) activity and Alizarin red S staining. 3D-bioprinted osteoblast constructs and β-TCP scaffolds infiltrated with encapsulated osteoblasts were subject to the cryopreservation protocol after 3 and 7 days of culture. Such points were selected to allow the recovery of the cell viability after biofabrication and promote cell–cell interactions within the constructs. Briefly, after being washed two times with PBS at 37 °C, the samples were transferred to a new 24-well cell culture plate and covered with 500 µL of 20% DMSO at 4 °C diluted in cell culture medium. Immediately the plate containing the samples was frozen at a cooling rate of 1 °C/min up to −50 °C and finally stored in a freezer at −80. For the evaluation of the cell metabolic activity and the ALP activity after cryopreservation the samples were thawed by adding 500 µL of cell culture medium at 37 °C. Immediately after samples defrosted the medium was removed; the samples were washed two times with cell culture medium and continued their culture up to 14 days at 37 °C in 500 µL of medium. The biological assays referred were performed immediately after thawing and after 7 and 14 days of effective culture, i.e., without considering the period of cryopreservation. Results were compared with the outcomes of the counterparts cultured for the same periods but non-

cryopreserved. The biological responses studied include the osteoblast post-thawing metabolic activity and the recovery of the osteoblastic function of 3D-bioprinted osteoblastic structures and beta tricalcium phosphate scaffolds infiltrated with osteoblasts encapsulated in a hydrogel. The obtained structures were cryopreserved at −80 °C for 7 days using dimethyl sulfoxide as cryoprotectant additive. After thawing the structures were cultured up to 14 days. The results revealed fundamental biological aspects for the successful cryopreservation of osteoblast constructs. In summary, immature osteoblasts take longer to recover than mature osteoblasts.