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RESEARCH ARTICLE

Advancing Intra-Diffusion of Peptide Colloids in Cell-Clustered Spheroids for Enhanced Cryo-Recovery

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ABSTRACT

3D cellular models have been increasingly integrated into the drug development process, but maintaining their functional integrity over extended periods remains a major barrier to clinical translation and commercialization. Cryopreservation represents a key strategy for enabling long-term storage of intact 3D cellular systems; however, the limited diffusion of conventional agents into densely organized structures often compromises post-thaw recovery. We introduce a colloidal solution containing nanoscale peptide-colloid hybrids designed to preferentially interact with the extracellular matrix. This solution infiltrated spheroids and distributed homogenously through 3D spheroidal space, which outperformed conventional cryoprotectants in preserving post-thaw structural integrity and functional activity. At sub-picomolar colloidal concentration, the solution leverages a synergetic Gibbs effect to generate an ice-melting thermodynamic efficiency comparable to high-concentration colligative dimethyl sulfoxide (DMSO). Remarkably, the colloid nanoparticles can be readily removed from the spheroids with a single step of post-thaw rinsing, mitigating the risks associated with residual cytotoxicity.

1 | Introduction

In vitro assay models, including advanced 3D cultures such as spheroids, organoids, and microphysiological systems (MPSs), have been widely applied in drug screening and disease modeling [1–8]. These 3D cellular models have gained significant attention because they recapitulate tissue environments, enabling physiologically relevant cell-cell and cell-extracellular matrix (ECM) interactions [9–15]. Recent policies such as the Food and Drug Administration (FDA) Modernization Act 2.0 have further accelerated the adoption of non-animal alternatives in drug development, thereby accelerating the demand for advanced 3D cellular models [1, 16, 17] Despite their increasing importance, major barriers still hinder the clinical translation and commercialization of these models. Long-term storage often results in structural and functional deterioration [18–23]. Moreover, building such models in-house poses substantial technical challenges, as establishing complex 3D constructs requires considerable expertise, and the associated protocols are often difficult to reproduce [24–26]. Cryopreservation has been widely used to preserve biological samples structurally and functionally for prolonged periods at low temperature by adding cryoprotective agents [27–34]. However, this approach faces critical limitations when applied to complex 3D cellular models. Their architectural complexity, compared to single cells, restricts the diffusion of agents into the inner regions [16, 35, 36], resulting in nonuniform distribution that ultimately causes damage during cryopreservation [37, 38]. To compensate for this limited penetration, high concentrations of agents are typically required, which can induce osmotic imbalance and result in osmotic shock [39–41]. After