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Na^+-Complexed Dendritic Polyglycerols for Recovery of Frozen Cells and Their Network in Media

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In this study, a novel phenomenon is identified where precise control of topology and generation of polyglycerol induce the retention of Na^+ ions in biological buffer systems, effectively inhibiting ice crystal growth during cryopreservation. Unlike linear and hyperbranched counterparts, densely-packed hydroxyl and ether groups in 4th-generation dendritic polyglycerol interact with the ions, activating the formation of hydrogen bonding at the ice interface. By inhibiting both intra- and extracellular ice growth and recrystallization, this biocompatible dendritic polyglycerol proves highly effective as a cryoprotectant; hence, achieving the cell recovery rates of ≈134–147%, relative to those of 10% dimethyl sulfoxide, which is a conventional cryoprotectant for human tongue squamous carcinoma (HSC-3) cell line and human umbilical vein endothelial (HUVEC) cells. Further, it successfully recovers the network-forming capabilities of HUVEC cells to ≈89% in tube formation after thawing. The Na^+ ion retention-driven ice-growth inhibition activity in biological media highlights the unique properties of dendritic polyglycerol and introduces a new topological concept for cell-cryoprotectant development.

1. Introduction

Cryopreservation, being vastly involved in coldchain logistics of biological products operating under sub-zero temperatures, is crucial for basic research and various applications in biomedical engineering and clinical practice.[1–5] However, ice crystallization during freezing and thawing can cause significant damage to biological samples. To mitigate this, cryoprotectants (CPAs) such as dimethyl sulfoxide (DMSO)[6] and glycerol[7] are widely used, typically added in large quantities to enable deferred freezing or vitrification. While these low-molecular weight CPAs are effective to a certain degree, they face challenges in preserving complex biological systems such as human embryonic stem cells, cell monolayers, and multicellular systems.[8–10] Current strategies involve developing materials that mimic antifreeze proteins (AFPs) found in nature.[11] It is well-established that AFPs bind to ice through specific amino acid sequences inducing antifreeze activity via the Kelvin effect.[12–14] Subsequent studies have designed various materials, including polymers,[15–18] polypeptides,[19,20] and nanoparticles,[21–27] to inhibit ice crystal growth through strong hydrogen bond formation with the ice crystal face.[28] However, it should be noted that their effectiveness can be diminished in biological media containing high salt concentrations because the design of these materials mainly relies on their binding affinity through hydrogen bonding to the ice interface.[18]

Polyglycerol (PG) features a polyether backbone with hydroxyl groups, offering exceptional structural stability, hydrophilicity, and flexibility, making it ideal for hydrogen bonding with water. Its excellent biocompatibility makes it valuable for applications in biology, cosmetics, pharmaceuticals, and food.[29,30] Linear polyglycerol (*lin*PG), with its flexible structure capable of high internal flexibility compared to hyperbranched polyglycerol (*hb*PG), has proven useful for molecular recognition and interactions with cell surfaces.[31] The distinct branching patterns of polyglycerol significantly influence its properties and applications. Dendritic polyglycerol (*d*PG) features a fully branched architecture with no linear units, corresponding to a degree of branching (DB) of 1.0. In contrast, *hb*PG possesses a partially branched structure, comprising both dendritic and linear units. The diverse topologies of polyglycerol allow for controlled hydrogen bonding with water through its unique 3D structure, demonstrating significant potential across various fields.[32,33] For example, we reported, by precisely controlling the degree of branching (DB) of PG, even a single type of *hb*PG can either promote or inhibit ice growth and recrystallization as its concentration varies and influences the extent of hydrogen-bonding formation with pure water molecules.[34,35] The design of polyglycerol species needs to be developed better in order to extend their effectiveness in ice-growth

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