Cryoprotective Properties of Completely Synthetic Polyampholytes via Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization

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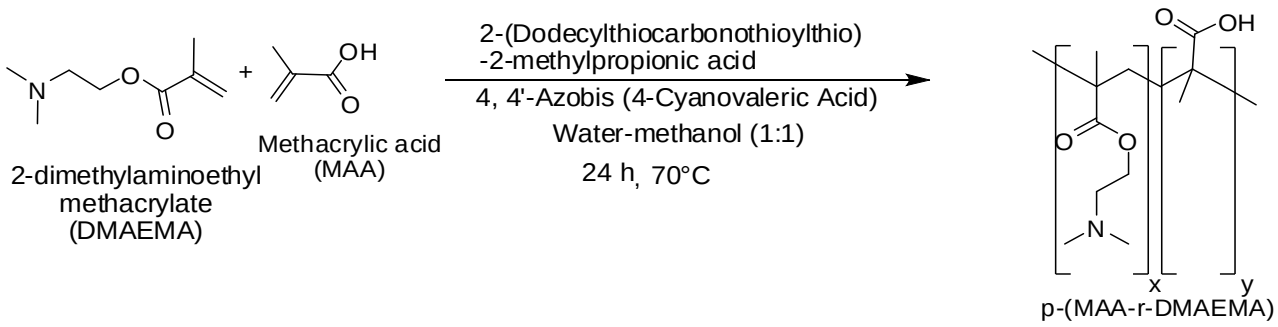
Recently, our group showed that polyampholyte carboxylated poly-L-lysine (COOH-PLL) shows excellent post-thaw survival efficiency and cryoprotective properties against human mesenchymal stem cells while retaining the cells' full differentiation capacity without the addition of any other low-molecular-weight cryoprotectants or proteins. However, the mechanisms through which a non–membrane-penetrating polymer, such as COOH-PLL, could exhibit substantial cryoprotective properties are still not clear. So in this research, a completely synthetic polyampholyte cryoprotectant was developed with cationic and anionic monomers by Reversible Addition Fragmentation Chain Transfer polymerization. The neutralized random polyampholyte, which had an equal composition ratio of monomers, showed high cryoprotective properties in mammalian cells.



Introduction of a small amount of hydrophobic monomer (n-butyl methacrylate and n-octyl methacrylate) enhanced cell viability after cryopreservation (Figure 1), indicating the importance of hydrophobicity. Membrane protective properties of the polyampholyte were found by leakage experiments using liposomes which confirmed that these polyampholytes protected the cell membrane during cryopreservation. Due to low cytotoxicity, this polyampholyte has the potential to replace the convention cryoprotective agent Dimethyl Sulfoxide. The cells proliferated well after cryopreservation with these polyampholytes

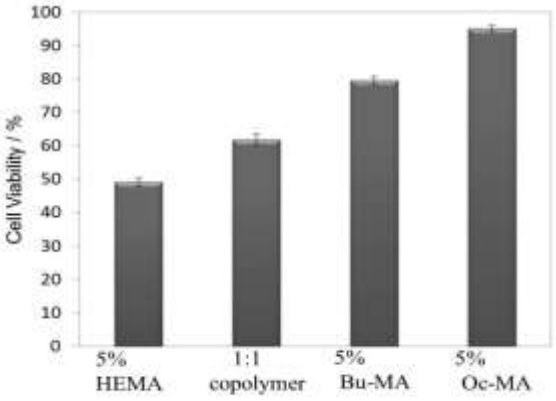


Figure 1. L929 cells were cryopreserved with hydrophobic and hydrophilic (2-hydroxyethyl methacrylate) polymers (10% polymer concentration)

These results suggested that the novel polyampholytes may be useful for cell cryopreservation in research and clinical applications due to its lower cytotoxicity and negligible effects on cell proliferation after thawing.