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Macromolecules' Delivery into Human Cells by Osmotic Pressure Changes

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Extended Abstract

The cell membrane, which encases every human cell, acts as a protective barrier that shields cells from the negative effects of the external environment. Consequently, crossing this membrane to introduce a specific molecule into a targeted cell is a challenging task. Meantime, many biological and biomedical studies, such as those investigating the molecular targets of new drugs, protein, and gene functions, nucleic acid delivery, and drug interactions with cellular compartments [1-3], require the introduction of macromolecules that human cells do not readily uptake. Various techniques have been developed to facilitate the delivery of these molecules, one of the most simple and quick being the manipulation of osmotic pressure.

In our research, we investigated osmotic shock-driven intracellular delivery, showing that the key factor for successful molecule introduction *via* osmotic pressure changes is the use of polymers in the entangled regime (where polymer clusters overlap) [4]. This discovery led to the development of an optimized polymeric formulation, offering a fast, simple, and versatile method for intracellular delivery.

Recently, we have been working to refine and expand our technique for broader applicability in high-throughput experiments, particularly those using standard cell biology tools such as flow cytometry. To improve the methodology, we reduced the osmotic pressure gradient while extending the incubation time from seconds to minutes. This adjustment resulted in a fourfold reduction in osmotic shock (from 312 kPa to 78 kPa) while maintaining high cell viability (≥85%) - a significant improvement over traditional methods based on osmolarity modulations.

Using our optimized delivery procedure, we successfully introduced a variety of molecules (polymers, proteins, nucleic acids, nanoparticles) into different cell types, including both cancerous (e.g., lung cancer, triple-negative breast cancer) and normal cells. A pilot study involved measuring fluorescence intensity one hour after delivery. Cells subjected to osmotic shock with a fluorescent probe were compared to a control group - cells exposed to osmotic shock without the probe. For example, when neutral dextran (size of about 4nm in radius) was delivered to HeLa cells, the fluorescence intensity was 60% higher than the control. In contrast, lung cancer cells showed a 36% increase, highlighting the differences in intracellular delivery between cancer cell types.

We believe that our research will significantly advance a variety of biological and medical studies by providing a rapid, universal tool for the intracellular delivery of molecules into human cells, particularly in high-throughput experiments.

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