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# Artificial Cells Capable of Communication by Using Polymer Hydrogel

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**Abstract :** The first artificial cell was produced by Thomas Chang in the 1950s when he was trying to make a mimic of red blood cells. Since then, many different types of artificial cells have been constructed from one of the two approaches: a so-called bottom-up approach, which aims to create a cell from scratch, and a top-down approach, in which genes are sequentially knocked out from organisms until only the minimal genome required for sustaining life remains. In this project, bottom-up approach was used to build a new cell-free expression system which mimics artificial cell that capable of protein expression and communicate with each other. The artificial cells constructed from the bottom-up approach are usually lipid vesicles, polymersomes, hydrogels or aqueous droplets containing the nucleic acids and transcription-translation machinery. However, lipid vesicles based artificial cells capable of communication present several issues in the cell communication research: (1) The lipid vesicles normally lose the important functions such as protein expression within a few hours. (2) The lipid membrane allows the permeation of only small molecules and limits the types of molecules that can be sensed and released to the surrounding environment for chemical communication; (3) The lipid vesicles are prone to rupture due to the imbalance of the osmotic pressure. To address these issues, the hydrogel-based artificial cells were constructed in this work. To construct the artificial cell, polyacrylamide hydrogel was functionalized with Acrylate PEG Succinimidyl Carboxymethyl Ester (ACLT-PEG2000-SCM) moiety on the polymer backbone. The proteinaceous factors can then be immobilized on the polymer backbone by the reaction between primary amines of proteins and N-hydroxysuccinimide esters (NHS esters) of ACLT-PEG2000-SCM, the plasmid template and ribosome were encapsulated inside the hydrogel particles. Because the artificial cell could continuously express protein with the supply of nutrients and energy, the artificial cell-artificial cell communication and artificial cell-natural cell communication could be achieved by combining the artificial cell vector with designed plasmids. The plasmids were designed referring to the quorum sensing (QS) system of bacteria, which largely relied on cognate acyl-homoserine lactone (AHL) / transcription pairs. In one communication pair, “sender” is the artificial cell or natural cell that can produce AHL signal molecule by synthesizing the corresponding signal synthase that catalyzed the conversion of S-adenosyl-L-methionine (SAM) into AHL, while the “receiver” is the artificial cell or natural cell that can sense the quorum sensing signaling molecule form “sender” and in turn express the gene of interest. In the experiment, GFP was first immobilized inside the hydrogel particle to prove that the functionalized hydrogel particles could be used for protein binding. After that, the successful communication between artificial cell-artificial cell and artificial cell-natural cell was demonstrated, the successful signal between artificial cell-artificial cell or artificial cell-natural cell could be observed by recording the fluorescence signal increase. The hydrogel-based artificial cell designed in this work can help to study the complex communication system in bacteria, it can also be further developed for therapeutic applications.

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