New method for encapsulating single cells within tunable microgels could boost efficacy of cell-based therapies

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Alginate hydrogels - which are derived from the polysaccharide found in brown seaweed - have emerged as an effective material for manipulating cells and tissues due to their biocompatibility and the ability to tune their mechanical and biochemical properties to match physiological conditions found inside the body.

Already they have been demonstrated to influence the differentiation of stem cells, incite immune attacks on cancer cells, and weaken tumors' resistance to chemotherapy, but as of yet, hydrogels have mostly been useful for controlling groups of cells at large rather than individual cells. For example, alginate capsules filled with hundreds of pancreatic islet cells can be implanted in diabetic patients. However, these capsules are millimeters in size and eventually become surrounded by thick scar tissue that blocks the biological signals of islet cells and renders the implant ineffective.

Now, thanks to the joint efforts of a team from the Wyss Institute for Biologically Inspired Engineering at Harvard University and the Harvard John A. Paulson School of Engineering and Applied Sciences (SEAS), a new and highly effective microfluidic method for encapsulating single cells in microscale hydrogels sets the stage for a dramatic increase in the specificity of control that can be exerted upon cells and their ability to survive implantation. The research was reported October 31 in the scientific journal *Nature Materials*.

"There's been a tremendous amount of work to try and understand how biomaterials can determine cell function and fate, but the majority of that work has been done in populations of cells," said David Mooney, Ph.D., a Wyss Core Faculty member and the Robert P. Pinkas Family Professor of Bioengineering at SEAS, who is the corresponding author on the new study. "With this work, we take everything we have learned and take it down to the single cell level, enabling us to influence cell behavior on a whole different scale."

Mooney teamed with fellow Wyss Core Faculty member David Weitz, Ph.D., who is the Mallingkrodt Professor of Physics and Applied Sciences at Harvard University and SEAS and who is co-author on the study, to achieve the novel microfluidic-based method for encapsulating single cells within microgel capsules. The co-first authors on the study are Angelo Mao, a graduate researcher at Wyss and SEAS, and Jae-Won Shin, Ph.D., who was formerly a Wyss Institute Postdoctoral Fellow and is currently Assistant Professor of Pharmacology and Bioengineering at University of Illinois at Chicago.

"This is an exciting and important extension of cell-based biomaterials to the level of single cells, which can then serve both as a precise building block for larger cell structures and as a means of investigating the behavior at the level of single cells, providing unprecedented insight into cell function and properties," said Weitz.

Pre-existing single cell encapsulation methods result in relatively large gel capsules - consisting of a very thick hydrogel layer around encapsulated cells - in proportion to the size of the cell captured inside. On average, encapsulated cells take up a mere four percent of the volume of these larger capsules, meaning there is an extremely excessive hydrogel layer. And these pre-existing methods often fail to capture cells at all, resulting in many, many empty capsules and therefore an inefficient process.

In contrast, the microfluidic-based method described by the Mooney and Weitz team achieves a much thinner hydrogel layer around encapsulated cells. These aptly-called "microgels" have a volume, on average, that consists 40 percent of a single cell and 60 percent hydrogel layer, resulting in a much smaller capsule size. What's more, the method results in formation of far fewer, if any at all, empty capsules.

At that small size, microgel-encapsulated cells can be delivered intravenously, opening new pathways for therapeutic interventions to treat cancers, tissue injuries, and a wide variety of immune disorders. With a thinner hydrogel layer between encapsulated cells and the body's environment, cell therapies can exert influence on the body faster, kicking their disease-fighting effects into action sooner.

Microgel-encapsulated cells also stand a better chance of thriving inside the body after injection; currently stem cell therapeutics are challenged by how quickly the body clears cells that are injected 'naked'. Yet microgels infused with growth and anti-inflammatory factors could act as life-sustaining rafts for injected cells, ensuring their survival and ability to carry out their therapeutic purpose.

As done in pre-existing techniques, the team first coated cells in calcium carbonate nanoparticles, a step that facilitates cell encapsulation when mixed with an alginate polymer solution. But for the first time, before mixing with a polymer solution, the team washed away the nanoparticles that had not adhered to cells using a water and oil emulsion inside a microfluidic device. What remained were predominantly microgel-encapsulated single cells.

"Even though each cell is encapsulated in its own individual thin hydrogel layer, the process is extremely fast and can encapsulate one thousand cells per second inside one microfluidic channel," said Mao.

The researchers envision that their method can improve cell-based therapies, help explore heterogeneity between cell populations that underlie tumors and other abnormalities, and even enable a paradigm shift in precision tissue engineering.

"Mini tissues could plausibly be formed from meticulous cell-by-cell construction, giving us scrupulous control over the composition of engineered tissues that has not been yet been possible," said Shin.

The promising development would not have happened without collaboration between Mooney, who is an expert in tissue engineering and biocompatible hydrogels, and Weitz, who is an expert in using 'designer' emulsions inside microfluidic devices to encapsulate active materials drop by drop.

"It's really a great example of what can happen at the Wyss when people can ally with colleagues of different expertise and really rally around a shared goal," Mooney said.

"Enabling microgel encapsulation of single cells should allow much better integration and vascularization of implanted cellular therapies, for example in treatment of diabetes or Parkinson's disease, and provide new ways to study and control behavior of individual cells both inside and outside our bodies," said Wyss Institute Founding Director Donald Ingber, M.D., Ph.D., who is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and the Vascular Biology Program at Boston Children's Hospital, as well as Professor of Bioengineering at Harvard SEAS.

More information: Deterministic encapsulation of single cells in thin tunable microgels for niche modelling and therapeutic delivery, *Nature Materials* (2016) DOI: 10.1038/nmat4781

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