Microencapsulation of embryonic and mesenchymal stem cells for scalable bioprocessing

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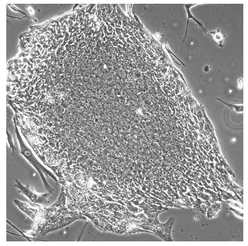
STEM CELL BIOMANUFACTURING

What are stem cells?



Stem cells have the potential to develop into many different types of cells in the body. They serve as a repair system for the body. Under the right conditions in the body or laboratory, stem cells divide to form daughter cells. These daughter cells either become new stem cells (**self-renewal**) or become specialized cells (**differentiation**) with a more specific function. There are several different types of stem cells, including:

- **Embryonic stem cells (ESCs)** – able to differentiate into almost all cell types; highly proliferative
- **Mesenchymal stem cells (MSCs)** – able to differentiate into a closely related family of cells; not as proliferative as ESCs

Why is stem cell research important?

Given their unique **regenerative** abilities, stem cells offer the potential to treat many diseases. This could be achieved through cell transplantation as a therapy or through use of the cells to develop new drug therapies. The insights uniquely gained from stem cell research can be used to combat a wide range of congenital and genetic diseases, traumatic tissue injuries, and degenerative ailments.

Why are biomanufacturing methods necessary?

There are many challenges to overcome before stem cell-derived therapies can be broadly available, including the lack of processes to manufacture viable and homogeneous cell populations. While advances in stem cell biology continue, there is a need for concurrent engineering of the bioprocessing that is integral to stem cell biomanufacturing. The biomanufacturing industry is expanding, with commercial development of stem cell products projected to be $10 billion within the next 6-8 years.

What are the challenges?

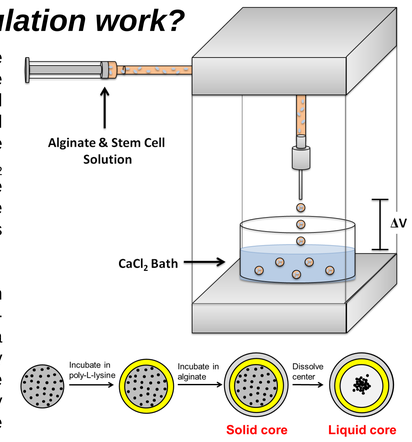
Before stem cells can be translated clinically, must determine ways to produce high cell numbers using efficient and scalable approaches

Traditional bioprocessing occurs in large scale **bioreactors**. However, bioreactors impart **hydrodynamic forces** which can damage or influence the differentiation of stem cells. In addition, stem cells cannot be cultured as a single cell suspension. **Microencapsulation** offers a potential solution through the protection from hydrodynamic shear forces and through prevention of aggregate **agglomeration**.

How does microencapsulation work?

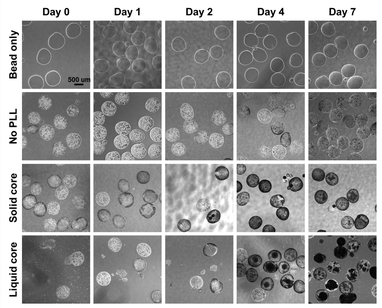
Single stem cells or stem cell aggregates are suspended in an alginate solution. A syringe pump is used to push the alginate-cell solution through a nozzle to form small diameter beads. Additionally, a voltage gradient between the nozzle and a $CaCl_2$ bath pulls the negatively charged alginate solution towards the crosslinking bath. Once the beads drop into the bath, Ca^{2+} ions crosslink the alginate beads.

Alginate capsules with a liquefied core can be created by first coating with a poly-L-lysine (PLL) followed by a coating with a secondary alginate layer. The positively charged PLL stabilizes an alginate shell. The core of the bead can then be liquefied by treating with sodium citrate to chelate the stabilizing Ca^{2+} ions in the inner core.

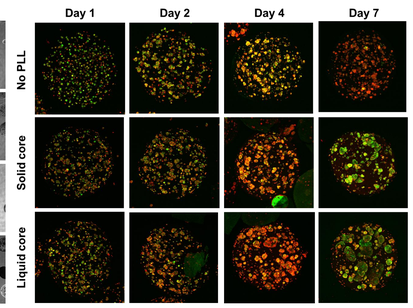


SINGLE CELL ENCAPSULATION

Encapsulated single ESCs were cultured for seven days in three different formats: alginate, PLL-coated alginate, and PLL-coated alginate with a liquefied center. The highest viability and proliferation occurred in the liquid core beads, as ESCs began to proliferate and form small aggregates.

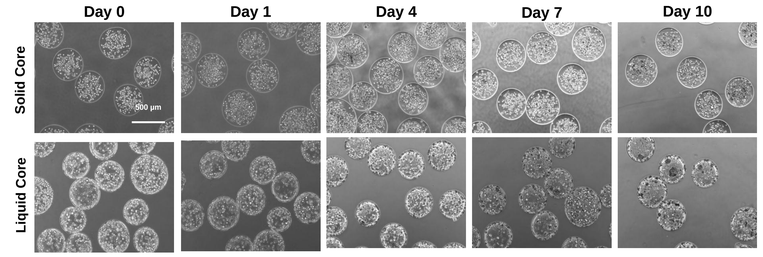


Phase images of single ESCs cultured in different encapsulation formats



Live (green) and dead (red) staining of single ESCs using confocal microscopy

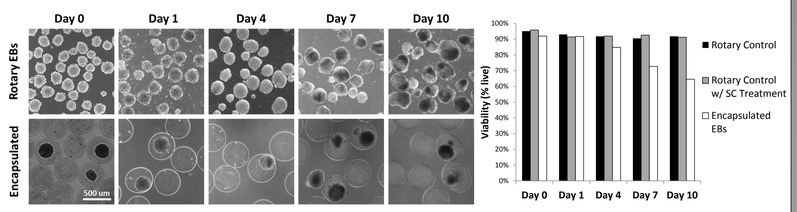
Single MSCs were encapsulated and cultured for ten days in PLL-coated alginate and PLL-coated alginate with a liquefied center. Proliferation and formation of small MSC aggregates was observed in liquid core beads at a much slower rate compared to ESCs.



Phase images of single MSCs cultured in solid and liquid core alginate capsules

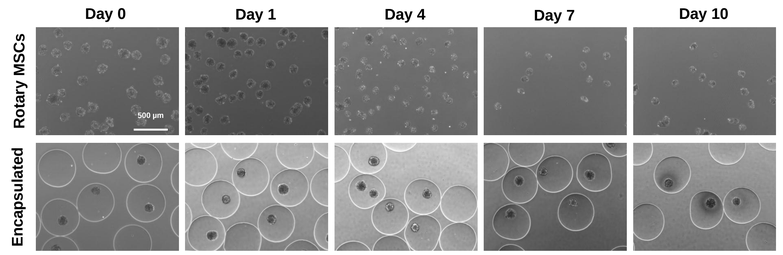
AGGREGATE ENCAPSULATION

Encapsulated ESC aggregates were cultured without PLL coating. Using a low density during encapsulation, single aggregates made up 89.8% ± 9.2% of the cell-laden beads, as opposed to beads containing two or more aggregates. The system therefore allows for isolation of individual aggregates, which may improve micro-environmental control and enable studies of sequestered cell populations.



AGGREGATE ENCAPSULATION

MSC aggregates (500 cells per aggregate) were encapsulated and cultured for 10 days in non-coated alginate beads. MSCs do not appear to proliferate within this encapsulated aggregate format. However, this system prevents agglomeration of MSC aggregates by isolating individual aggregates and enables the study of MSCs in a scaffold-free three dimensional microenvironment.



Phase images of encapsulated 500 cell MSC spheroids without PLL coating

CONCLUSIONS

- Alginate encapsulation of single stem cells in liquid core alginate beads enhances proliferation and viability compared with other formats.
- Encapsulation of stem cell aggregates maintains cell viability and prevents agglomeration from occurring.

FUTURE DIRECTIONS

- Examine the mechanics of alginate beads under varying encapsulation conditions (e.g. ratio of guluronic acid to mannuronic acid, poly-L-lysine coated, presence of single cells or aggregates) using micro-scale compression system
- Determine the impact of encapsulation on stem cell secreted factors
- Assess the culture of encapsulated aggregates in stirred suspension culture

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