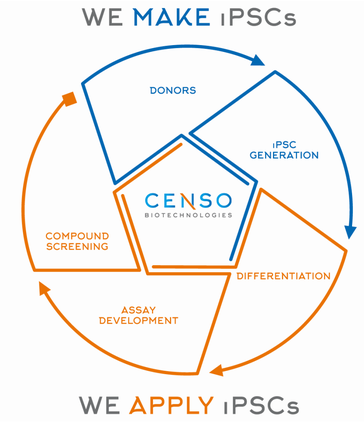
Encapsulation for the Room Temperature Preservation of iPSC-Derived Microglia Using Novel Hydrogel Technology



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Introduction

Storage and transportation of viable cells is limited to cryopreservation or transportation of live cells already in flasks/plates which requires temperature control and the risk of peeling for adherent cells. In addition, both methods risk loss of viable cells and activation of cells upon thaw. Atelerix Ltd. have developed a novel method of room temperature storage and transportation utilising hydrogel encapsulation. Here we provide proof of concept for the encapsulation of iPSC-derived macrophage precursors with minimal loss of viable cells. Upon recovery, cells retained classic cell surface markers with no difference to cells which were kept in culture in parallel.

Differentiation of iPSCs

A robust differentiation methodology has been derived by Censo Biotechnologies to generated microglia like cells from iPSCs which have been extensively characterised[1]. This protocol has been used to differentiate over 10 hES and hiPSC control and disease lines and was found to be robust and consistent in the production of microglia-like cells. Hemangioblasts are formed during this process and release macrophage progenitors which can be matured to microglia.

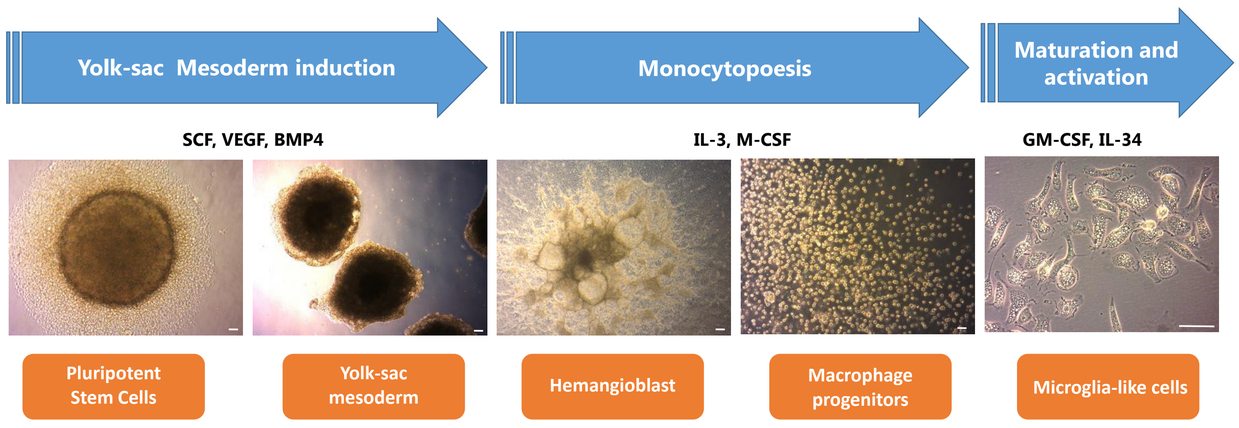

Figure 1. iPSC differentiation to Microglia via Mesoderm. The multistep process of deriving microglia from human iPSC. Scale bar 100μm.

Hydrogel Encapsulation

Technology developed by Atelerix allows for the encapsulation of cells, both in suspension and adherent cells. Here, this method was tested for the encapsulation of iPSC-derived macrophage progenitors and hemangioblasts. Cells were recovered using a gentle non-toxic buffer, so that cells can be used immediately with no alterations to cell phenotype and function.

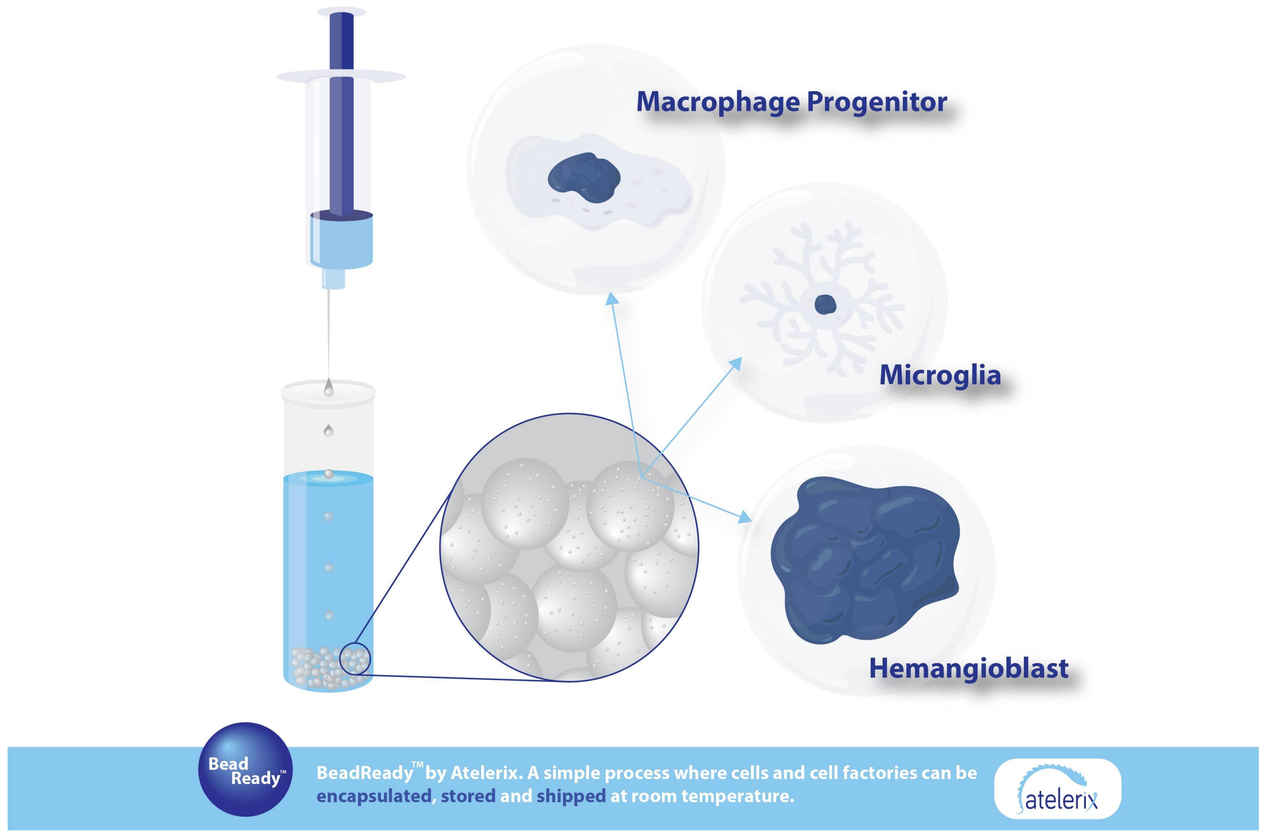

Figure 2. Encapsulation process for iPSC-derived macrophage progenitors and hemangioblasts. Cells and hemangioblasts are prepared in encapsulation buffer and dropped using a syringe or Pasteur pipette to form spheres. These can then be stored in cryovials or other vessels and maintained or transported at room temperature. Cells are released without causing damage or alterations to the cells.

Hemangioblast Encapsulation

Hemangioblasts are capable of producing macrophage which can then be matured to microglia-like cells. Hemangioblasts were encapsulated and transported at room temperature for 5 days and upon release placed back in culture. Macrophage progenitors produced were analysed and compared to cells produced from non-encapsulated controls.

Figure 3 Hemangioblasts following recovery from encapsulation. Production of macrophage progenitors started 4 days following recovery, and continued least 20 days. Scale bar 100μm

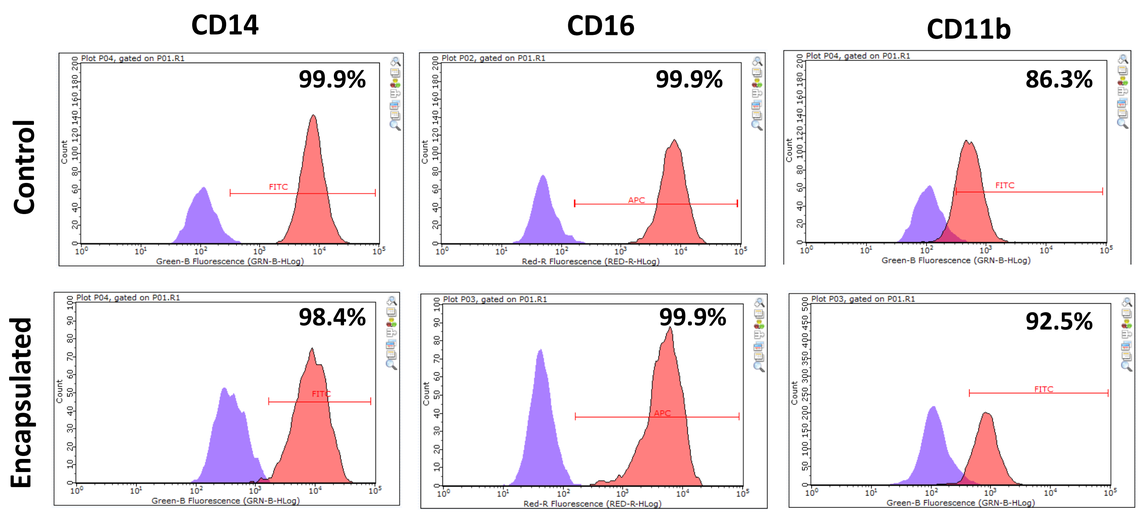

Figure 4. Characterisation of macrophage progenitor cells produced by hemangioblasts following recovery. 8 days after recovery, macrophage progenitors cells were collected and cell surface receptor expression assessed using flow cytometry. Here we show no difference in expression when compared to control cells produced by hemangioblasts which were maintained in culture in parallel to those encapsulated.

Macrophage Progenitor Cell Encapsulation

Macrophage progenitor cells were encapsulated for 5 days and transported at room temperature. Upon recovery, cells showed 85% viability, measured by trypan blue staining. Cells were placed back in culture, and phenotype and function assessed. Encapsulated cells retained expression of classic macrophage/microglia markers and were also shown to engulf β-amyloid at similar level as control cells (non-encapsulated).

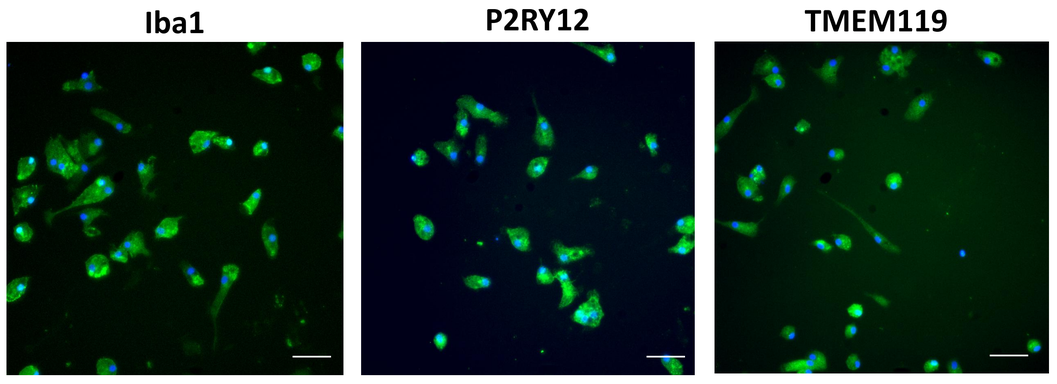

Figure 5. Staining of microglia markers following encapsulation. Following recovery, cells were placed in culture for 24h prior to fixing and staining for microglia markers. Blue: Hoechst, Green: Iba1/P2RY12/TMEM119. Scale bar 50μm

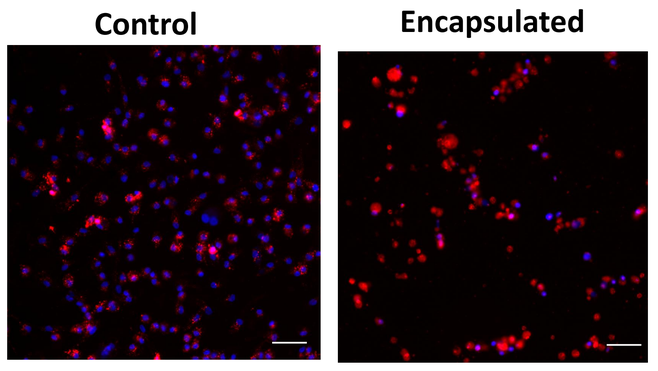

Figure 6.. Phagocytosis of β-Amyloid by microglia after encapsulation. Cells were incubated with β-Amyloid for 2h prior to fixing and imaging. Both encapsulated and control cells show similar levels of uptake demonstrating no loss of function. Blue: Hoechst, Red: β-Amyloid. Scale bar 100μm

Conclusion

The novel hydrogel encapsulation technology developed by Atelerix was shown to be effective in the storage and transportation of iPSC-derived cells and hemangioblasts with no effect on cell phenotype or function with minimal cell death.

This technology offers a simple alternative to cryopreservation and transportation of live cell cultures which require extreme care and temperature control. In addition, we have demonstrated minimal loss of cells with no activation, change in cell phenotype or function.

Acknowledgments and References

1. Haenselet *et al* (2016) Stem Cell Reports