

# Pluripotent stem cell recovery from polymer microcarriers produced by Membrane Emulsification.

Vamanu M.P. and Holdich R.G\*.

Centre for Biological Engineering, Loughborough University, Loughborough, United Kingdom (M.P.Vamanu@lboro.ac.uk)



## INTRODUCTION AND OBJECTIVES

Embryonic stem cells (hESCs) hold a great potential for therapeutic applications. However, their use is hindered by the need for a large-scale culture system, as laboratory scale systems are inefficient, insufficient and labour intensive (Xu C., 2001). Such a system has to be capable of mass producing cells and preserving their capacities.

Our solution is developing a system in which cells are produced in a three dimensional culture by using microcarriers. Commercially available microcarriers are good at encouraging cell attachment and proliferation, but cell detachment is problematic as it requires the use of proteolytic enzymes which are damaging to critical cell adhesion proteins (Baumann H., 1979).

The current innovative study is to engineer the microcarriers in terms of particle size, surface coating and properties, as well as triggered shrinkage or thermo-responsiveness for cell release. All these benefits are based on particle production by Membrane Emulsification (Holdich R.G., 2010). Membrane Emulsification is a relatively novel technique that has been first introduced in 1986 by Nakashima and Shimizu for the production of emulsions by employing a glass membrane (Nakashima T., 1986). Membrane emulsification has many advantages over conventional emulsification methods as it offers the possibility of producing very fine emulsions of controlled droplet sizes and with a narrow size distribution; allows the use of shear-sensitive compounds, such as proteins and has a simple design (Holdich R.G., 2010).

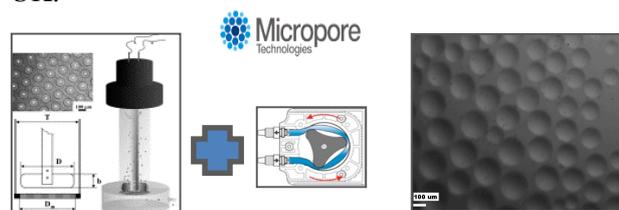
The polymers of choice are alginate and chitosan because of their biocompatibility and biodegradability and poly N-isopropylacrylamide (pNIPAM) because of the sharpness of its phase transition, biocompatibility and transition temperature at about 32°C, close to the physiological value. This paper will only focus on the work carried out with pNIPAM.

pNIPAM structure consists of repetitive units of hydrophilic (amide) and hydrophobic (isopropyl) groups. pNIPAM in water demonstrated remarkable hydration-dehydration changes in response to changes in temperature. These characteristics make pNIPAM a very attractive material for Tissue Engineering applications. Cells are cultured on the hydrophobic

surface at 37°C, above transition temperature and can be readily detached from the surface by lowering the temperature. The hydration and expansion of the polymer chains on the surface as a function of the temperature reduction are responsible for the cell detachment without the need for proteolytic enzymes (Canavan H.E., 2005).

## MATERIALS AND METHODS

All chemicals used for this work were acquired from Sigma Aldrich, UK unless otherwise stated. Particle production was performed in two stages: first, droplet production by Membrane Emulsification using the Dispersion Cell presented in Figure 1 and second, polymer cross-linking by free radical polymerization. The membranes used for this work are Nickel membranes with uniform pore sizes and inter-pore distances, also provided by MicroPore Technologies, UK.



**Figure 1: Set-up for pNIPAM droplet production by membrane emulsification**

The transition temperature of pNIPAM was measured as a turbidity variation with temperature increase at 500 nm by using a microplate reader (BMG Labtech). The hESC line, H9 (WiCell Research Institute, USA) was cultured in feeder-free conditions on hESC-qualified Matrigel™ (BD Biosciences, UK) in fully defined medium, mTeSR1 (StemCell Technologies, France). The cells were dissociated using Accutase (Invitrogen, UK) and seeded onto the produced carriers and on a commercially available thermo-responsive surface provided by NUNC UpCell (Germany).

Prior to 3D culture, the hESCs were assessed for pluripotency by employing Flow Cytometry and ImmunoCytoChemistry (ICC). For ICC, the cells were cultured on glass coverslips until 70% confluent, after which they were fixed, permeabilized and stained with DAPI (Invitrogen, UK), phalloidin (Sigma Aldrich, UK) and pluripotency markers (SSEA-1, SSEA-4, OCT 4) (R&D Systems, UK).

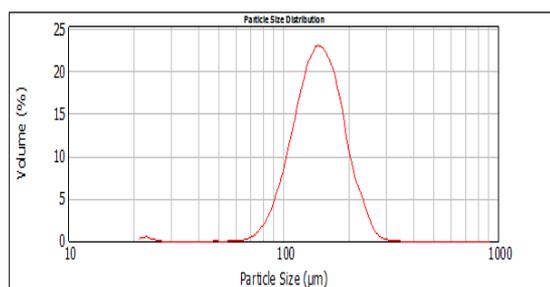
The 3T3 fibroblasts (ATCC, USA) were cultured using a high glucose DMEM supplemented with 10% foetal calf serum. Fibroblast dissociation was done by using TryPLE Select (Invitrogen, UK). Single cells were seeded on produced microcarriers and on the NUNC UpCell (Germany).

Cell viability and cytotoxicity was assessed by using Alamar Blue assay (Invitrogen, UK) and cell counts were done by Trypan Blue exclusion method using an automated system.

## RESULTS AND DISCUSSION

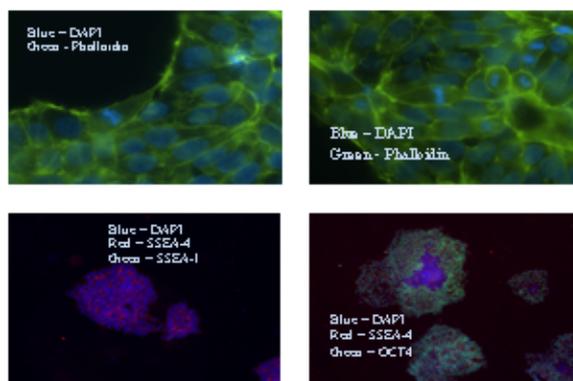
The Dispersion Cell platform technique allows a precise control over process parameters. By varying the shear stress provided by employing a mechanical stirrer and the injection rate of the dispersed phase, the particle size can be modified in a controlled manner.

The size and size distribution of the produced particles was measured by Malvern Instruments Mastersizer. We have produced pNIPAM particles with sizes between 100 and 200  $\mu\text{m}$  with narrow size distribution (Figure 2).



**Figure 2: Size distribution for produced pNIPAM particles**

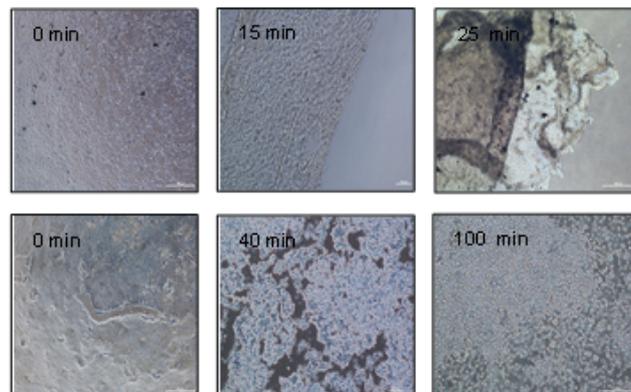
Following characterization, the produced particles were sterilized by autoclaving and used with hESCs, H9 cell line and 3T3s fibroblasts. Prior to microcarrier culture, the H9 cells were assessed for pluripotency by Flow Cytometry and ICC (Figure 3).



**Figure 3: Immunocytochemistry images for H9 cells cultured on tissue culture plastic**

Both cell types were also cultured on the UpCell

surface for 48 h prior to the detachment study consisting of lowering the temperature from 37°C to room temperature. Phase contrast pictures were taken at different time intervals and necessary time for complete cell detachment was recorded (Figure 4).



**Figure 4: Cell detachment study performed on the NUNC UpCell. The top pictures represent 3T3 fibroblast cells, while the bottom pictures the H9 hES cells.**

## CONCLUSIONS

We have successfully produced pNIPAM microcarriers with sizes within 100-200  $\mu\text{m}$  with narrow size distribution by using the Dispersion Cell as a platform technology. The produced microcarriers were characterized and further used for 3T3 and hESCs expansion. The cell release properties of the prepared beads were compared to a commercially available product (NUNC UpCell).

## REFERENCES

- Xu C. et al. (2001) *Feeder-free growth of undifferentiated human embryonic stem cells*. Nature Biotechn. 19: 971-4.
- Baumann H. et al. (1979) *Effect of trypsin on the cell surface proteins of hepatoma tissue culture cells*. J. Biol. Chem. 254(10):3935-3946.
- Holdich R.G. et al. (2010) *Membrane emulsification with oscillating and stationary membranes*. Ind. Eng. Chem. Res. 49:3810-3817.
- Nakashima, T.; and Shimizu M. (1986). *Porous glass from calcium alumino boro-silicate glass*. Ceramics 21: 408.
- Canavan H.E. et al. (2005) *Cell sheet detachment affects the extracellular matrix: a surface science study comparing thermal liftoff, enzymatic and mechanical methods*. J. Biomed. Mat. Res. 75A:1-13.

## ACKNOWLEDGMENTS

I would like to thank EPSRC for funding, my supervisor for support and Rob J. Thomas and Juan Guijarro-Leach for providing me with the two cell lines, as well as the expertise and advice.