Controlled temperature responsive particles production by Membrane Emulsification for Cell Culture and Release

Vamanu M. P., Holdich R. G.
Centre for Biological Engineering, Loughborough University, UK (M.P.Vamanu@lboro.ac.uk)

INTRODUCTION AND OBJECTIVES

One of the current issues for therapeutic applications is developing a large scale system for culturing stem cells. Ideally, this would be a system in which cells are produced in a three dimensional suspension culture using compatible microcarriers. Existing microcarriers are efficient at encouraging cell attachment and proliferation; however, recovery of the cells requires the use of proteolytic enzymes which are damaging to critical cell adhesion proteins (Baumann H., 1979). From this point of view, temperature responsive polymers appear to be a valid option for achieving the goal of this project.

The current innovative study is to engineer microcarriers in terms of particle size and surface coatings, as well as temperature responsiveness for cell release. All these benefits are based on particle production by Membrane Emulsification. 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 (Holdich R.G., 2010).

The polymer of choice is 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 (Canavan H.E., 2005). These characteristics make pNIPAM a very attractive material for Tissue Engineering applications.

MATERIALS AND METHODS

All chemicals used in this work were acquired from Sigma Aldrich (UK), unless otherwise stated. Our Stirred Cell device equipped with a hydrophobic disc shaped metallic membrane with uniform 20 µm pores (Figure 1) provided by MicroPore Technologies Ltd. (UK) was used to produce pNIPAM microspheres in a controlled and reproducible manner. The device uses a motor driven paddle-blade stirrer placed above the membrane which provides the shear at the membrane surface. Stirrer speed settings ranged from 2 V to 10 V and were expressed as maximum shear stress at the transitional radius. The dispersed phase was injected through the membrane by using a peristaltic pump (Watson Marlow 101U) with rates ranging between 200 and 1200 litres of dispersed phase per square meter of membrane surface area per hour. The continuous phase volume used was 100 cm^3 and for each experiment 10 cm^3 of dispersed phase was injected.

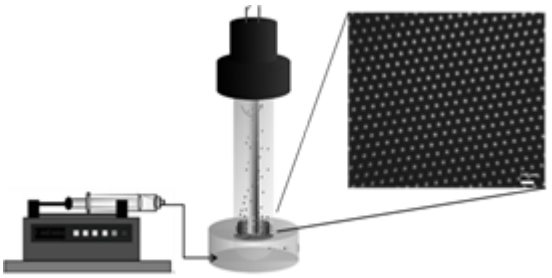

Figure 1: Schematic of the Stirred Cell equipped with a hydrophobic 20 µm pores nickel membrane

Monomer droplets were thus generated. By using the free radical polymerization chemistry employing Ammonium Persulfate and TEMED, solid pNIPAM particles were produced. The produced pNIPAM particles were characterised by laser diffraction (Malvern Instruments Mastersizer 2000) to assess size and size distribution expressed as span. Photographs of the obtained particles were taken with an optical microscope (Leitz Ergolux). Particle surface was studied by SEM operated at 10 kV (Cambridge Instruments StereoScan 360). Temperature responsive behaviour of the produced particles was studied by recording the refractive index variation with temperature increase by using an automated refractometer (Rudolph Research Analytical J357). Generated particles were further used for cell culture of ATCC 3T3 fibroblastic cells. Single cells were seeded on pNIPAM particles and cell viability was assessed by Live/Dead staining assay (Invitrogen, UK). Viable cells expressed green fluorescence, while dead cells expressed red fluorescence.

RESULTS AND DISCUSSION

The Stirred Cell platform allows a precise control over process parameters. By varying the shear stress generated at the membrane surface and the injection rate of the dispersed phase, particle size can be modified in a controlled manner. We have produced temperature responsive particles with sizes ranging between 70 µm and 190 µm and span values ranging from 0.499 (monodispersed) to 1.2 (Figure 2).

As shown in Figure 2, the median particle diameter generally increases when injection rate increases and decreases with the increase in shear stress applied. This behaviour can be explained by a shorter droplet formation time due to an increased shear stress, thus

forming smaller droplets and particles. The lowest span value obtained was 0.499 suggesting monosized pNIPAM particles as shown in Figure 3. Phase contrast photographs were taken and SEM was used to confirm that the generated particles have a solid core (Figure 3).

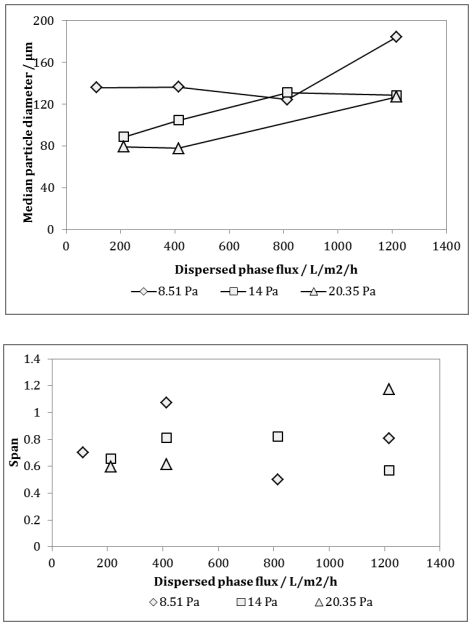

Figure 2. Effect of dispersed phase flux and shear stress on the median particle diameter

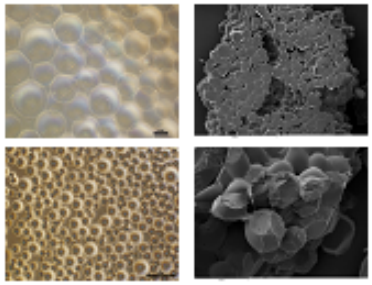

Figure 3. Microscope photographs of particles and SEM analysis confirming particles with solid core

Phase transition temperature of produced pNIPAM particles was measured by recording the refractive index variation with temperature increase (Figure 4). The 50% value of the slope is associated to the polymer's phase transition temperature. The recorded values are within the range of 31 to 33 degrees Celsius which is consistent with previous literature reports. When comparing different formulations, the harder crosslinked pNIPAM hydrogels appear to have a faster response with temperature increase.

The generated pNIPAM particles were further used for cell culture with ATCC 3T3 fibroblast cells. Live/Dead staining was used to assess cell viability of the attached cells compared to Cytodex 1 (GE Healthcare) (Figure 5). The results are comparable to Cytodex 1 which is a demonstrated platform for cell attachment and proliferation, suggesting that the generated pNIPAM particles also have a promising applicability.

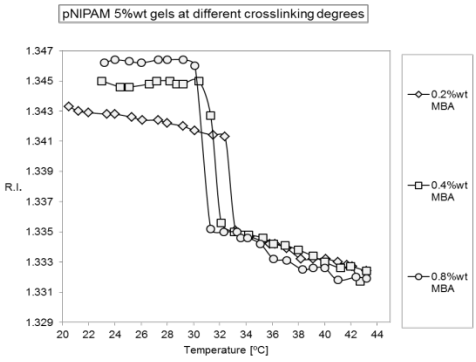

Figure 4. Phase transition temperature of different crosslinked pNIPAM hydrogels

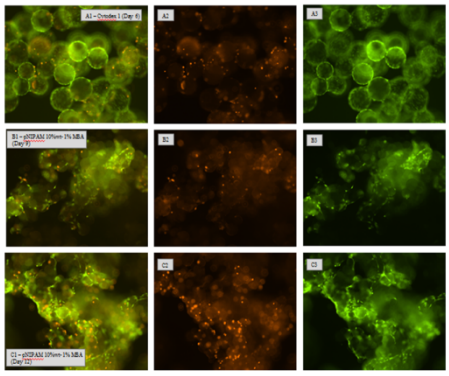

Figure 5. 3T3 cells at passage 30 on: (A) Cytodex 1 Day 6 in culture; (B) and (C) pNIPAM Day 9 and Day 12 in culture. (1) Merged images (2) Dead cells. (3) Live cells.

CONCLUSIONS

We have successfully shown that our method offers an improved control over process parameters, thus allowing a controlled production of monosized particles. We have shown that the particles exhibit a temperature responsive behaviour and that cell attachment and proliferation is possible.

REFERENCES

- 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.
- 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.
- Holdich R.G. et al. (2010) *Membrane emulsification with oscillating and stationary membranes.* Ind. Eng. Chem. Res. 49:3810-3817.