Development of a Protein-coated Hydrogel Microcarrier for Stem Cell Expansion

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The methods for controlling stem cell cultures in drug discovery or tissue engineering have been steadily progressing. However, cost-effective strategies to expand a large number of stem cells while retaining their unique properties are needed. Within a project partly funded by a Eurostars grant [1]. CSEM is developing a hydrogel microcarrier that can be used as a stem cell culture support in a stirred bioreactor. In this report the results of the first step of this project, the material selection, are presented.

For the expansion of stem cells, stirred bioreactors have shown several advantages over conventional stationary cultures. Firstly, the cell yield is higher and secondly the environment surrounding the cell is more homogeneous, reducing oxygen and metabolite concentration gradients to a minimum. In addition, the culture in a stirred bioreactor can be almost completely automated. This is not true for stationary 2D culture, with application limited to cell production. Still, there is currently no established method to culture a large population of cells using bioreactors. In this regard, CSEM together with the Swedish company Biolamina AB are developing a hydrogel microcarrier coated with a special stem cell supporting protein, LN-521, that should allow homogeneous 2D culture of pluripotent stem cells in stirred bioreactors. Biolamina develops and sells the recombinant LN-521. Within this project they will improve the production of the LN-521 to ensure enough supply coating of the microcarrier for example. CSEM is responsible for the development of the microcarrier, which consists of the following main parts:

- Material selection
- Microcarrier development
- Bioreactor culture protocol optimisation

In the first part of the project, different hydrogel materials were molded into 1 x 5 mm circular pads. The protein LN-521 was then linked to these materials by different strategies: covalently by binding to a specific functional group on the protein or non-specific via adsorption or absorption by immersing the dry gel in a protein solution. The quantification of the protein linkage by biochemical methods and the evaluation of the suitability of the material as a stem cell culture support determined if a material was suitable for the intended application. In this project, variations of eight chemically different materials were examined. For the optimization of the physical and biochemical properties of the material the following factors were varied: polymer concentration, crosslinking method and protein-binding method.

The hydrogel microcarrier formation was performed in a microfluidic flow focusing chip with two inlets for the aqueous phase and one for the oil phase.

Of all materials tested, CSEM identified one acrylamide-based candidate that can support the stem cell culture. Many of the tested materials did not support stem cell culture at all. For the selected material, the protein was covalently linked to the material. The material has been tested extensively with pluripotent stem cells. The cells attach, grow and maintain their very motile phenotype when they are grown on this material (see Figure 1).

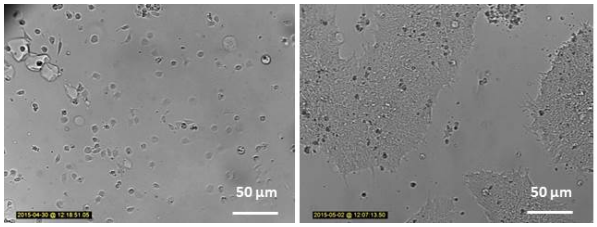

Figure 1: Pluripotent stem cells attach well to the laminin-functionalized acrylamide material; images at 1 and 48h after seeding.

Preliminary results showed that it is possible to make microcarriers out of this material in a relevant size range for cell culture (see Figure 2). The polymerization can be initiated by UV or by the addition of a chemical compound. Depending of the polymerization method the initiation is inside or outside of the chip.

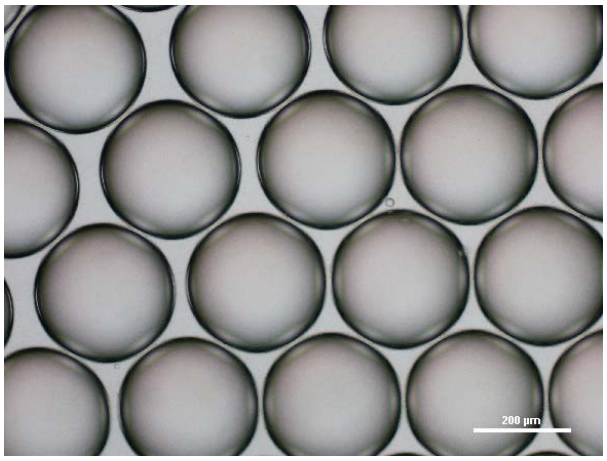

Figure 2: Acrylamide droplets with a very low size distribution produced by the microfluidic flow focusing setup at CSEM.

In the next step, the team at CSEM will set the conditions to produce larger amounts of polymerized acrylamide microcarriers. Next year, the consortia hope to deliver the first results on how well the LN-521 coated microcarriers support stem cell culture in stirred bioreactors.

[1] Eurostars project Production of LAMinin-521™ coated MICrocarriers for stem cell expansion (LAMMIC), No. 8 972