

Surface modification of biodegradable microcarriers and their characterization by microscopy









C. Rocca¹, C. Sevrin¹, P. Grysan³, S. Krishnamoorthy³, R. Vandeberg¹, D. Toye² and C. Grandfils¹.

1. University of Liège, Centre interfacultaire des biomatériaux (CEIB), Belgium. 2. Chemical engineering Department- Pep's. 3. Luxembourg Institute of Science and Technology (LIST).



Introduction

Regenerative medicine is a recent field allowing to restore or replace tissues, cells, or organs which could be damaged by a variety of factors, such as genetic abnormality, diseases, or trauma. The adoption of human grafts presents several drawbacks, particularly in terms of availability, risks of rejection, infection, weakening of tissue for the donor and the need to add immunosuppressive treatment. Thanks to multipotent characters, stem cells are able to proliferate and differentiate in various cell types and therefore potentially able to rebuild various tissues. Amongst stem cells, mesenchymal cells (MSCs), from umbilical cords or various tissues such as bone marrow or adipose tissue are most specifically interesting for this application.

However, one of the main obstacles to the development of stem cell therapies is linked to the very low number of available stem cells for a human donor and to their poor survival and diffusion ability from their injection site in situ. The adoption of biocompatible and biodegradable microcarriers to amplify in vitro MSCs should solve these challenges. By increasing the cell cultivation surface and providing an injectable support microcarriers are particularly attractive and could avoid the difficult step of cell detachment from non-degradable materials. The optimization of these new microcarriers needs to meet strict criteria of degradation and safety to avoid any risks of allergies, toxicity or of immunological reactions.

Material & Method

Manufacturing

Degradable microcarriers have been manufactured according to an evaporation emulsion procedure, a well-known methodology already adopted in the pharmaceutical industry. These microparticles have been made adopting PLGA: poly(lactic-co-glycolic) acid three different biocompatible aliphatic polyesters : poly(\varepsilon-caprolactone) (PCL), poly(lactide-co-glycolide) acid (PLGA) or poly-I-lactide (PLLA) (Figure 1). To promote cell adhesion and proliferation, the microcarriers have be Ho functionalized on their surface by the deposition of polyelectrolytes using a physical adsorption method. This surface functionalization have been realized with two polycations: chitosan and poly(2-(dimethylamino)ethyl Figure 1: Diagram of the biodegradable microcarriers and polyesters envisaged methacrylate) (PDMAEMA: Figure 2). Due to their very different nature, we should expect to adjust the surface charge density of the microcarriers and the macromolecular mobility of the polycations adsorbed to the surface of the microparticles.

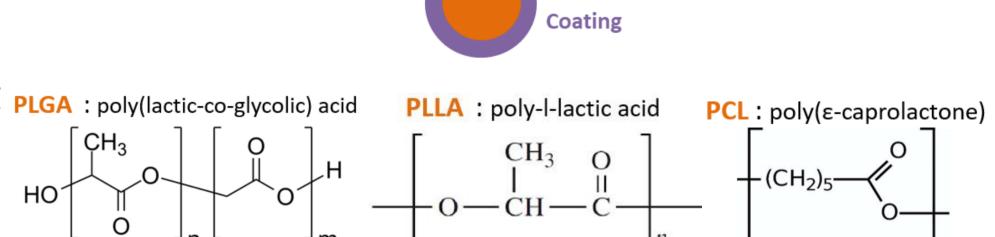
Fluorescent coatings

To highlight polycation depositions on microcarrrier surface, we have adopted two fluorescent labeling approaches. The fluorescein isothiocyanate (FITC) (Figure 3 a)) has been chemically grafted on chitosan thanks to the reactivity of the primary amine groups available on this polysaccharide. This fluorescent probe has given access to a green fluorescent signal at 540 nm under irradiation at 495 nm.

PDMAEMA has been traced by the copolymerization of rhodamine b methacrylate (Figure. 3b). The incorporation of this fluorochrome within this synthetic polycation has given a red fluorescent signal at 600 nm adopting an excitation around 500 nm.

Cellular nuclei labeling

The adhesion efficiency and viability of L929 Fibroblasts on the microcarrier surfaces have been quantified adopting the MTT assay. Cells have been also visualized using the Hoechst dye (Figure 3 c)) which allows to label the nucleus of the cells.



Microcarriers

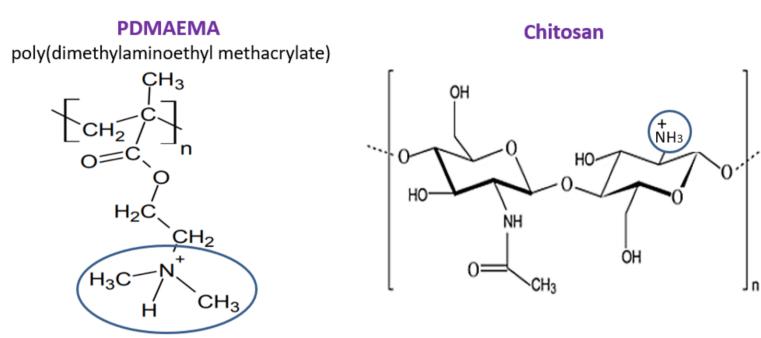


Figure 2: Polyelectrolytes studied: Chitosan and PDMAEMA

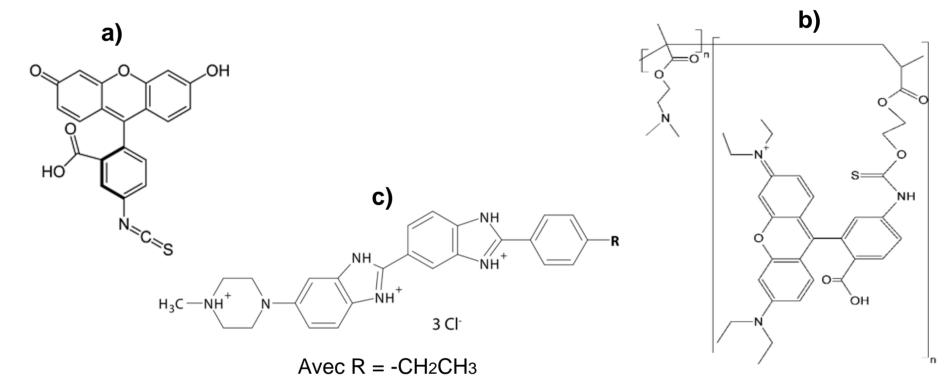


Figure 3: a) FITC; b) PDMAEMA-Rhodamine; c) Hoechst Reagent

Results

Demonstrations of the coatings on microcarriers.

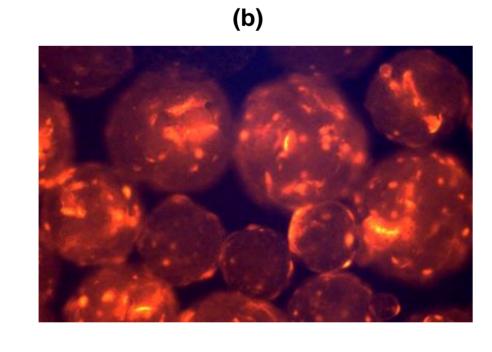


Figure 4: Observation under fluorescent microscope of PLLA microcarriers after coating with chitosan- FITC (a) and Rhodamine b-co-PDMAEMA (b)

Cellular nuclei labeling

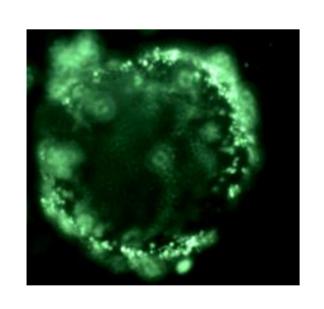


Figure 5: Fluorescent labeling using Hoechst reagent of L929 fibroblasts cultured on PLLA microcarrier coated with PDMAEMA Mw 20,000

SEM scanning microscopy

Visualization of the morphology, surface roughness and the impact of the coating on the topography of the polyester microcarriers adopting SEM (Figure 6).

PCL with chitosan PCL without coating PCL with PDMAEMA 90kDa PCL with PDMAEMA 20kDa PLLA without coating PLLA with chitosan PLLA with PDMAEMA 90kDa PLLA with PDMAEMA 20kDa PLGA without coating PLGA with chitosan PLGA with PDMAEMA 90kDa PLGA with PDMAEMA 20kDa

Figure 6: SEM images of microcarriers made from PCL (a), PLLA (b) and PLGA (c) before and after coating

Conclusions

- Our fluorescent labeling approach has successfully demonstrated the adsorption of our two polycations. At this stage, this data remains qualitative both in terms of polymer surface density and thickness and characteristics of the polymer layer deposed on the surface of the microbeads. The presence of heterogeneity in the fluorescence intensity detected for our two markers suggests a certain heterogeneity of the polycation layers. The presence of a microscopic relief on the surface of the microcarriers before coating could be a source of heterogeneity of the fluorescent signal (Figure 4).
- After two days of incubation, the L929 fibroblasts adhered efficiently to the microcarrier surface as visualized with the Hoechst reagent (Figure 5).
- The PCL and PLGA microcarriers have a smooth topography unlike PLLA microcarriers which highlight a roughness at a micron scale level.
- The polymer coatings have no significant impact on the size and morphology of polyester microcarriers, whatever the nature of the polyesters.

These encouraging results therefore support our strategy to adopt these new microcarriers to amplify stem cells for tissue engineering.

