Surface Analysis of New Microcarriers Tailored for Cell Therapy.

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Abstract

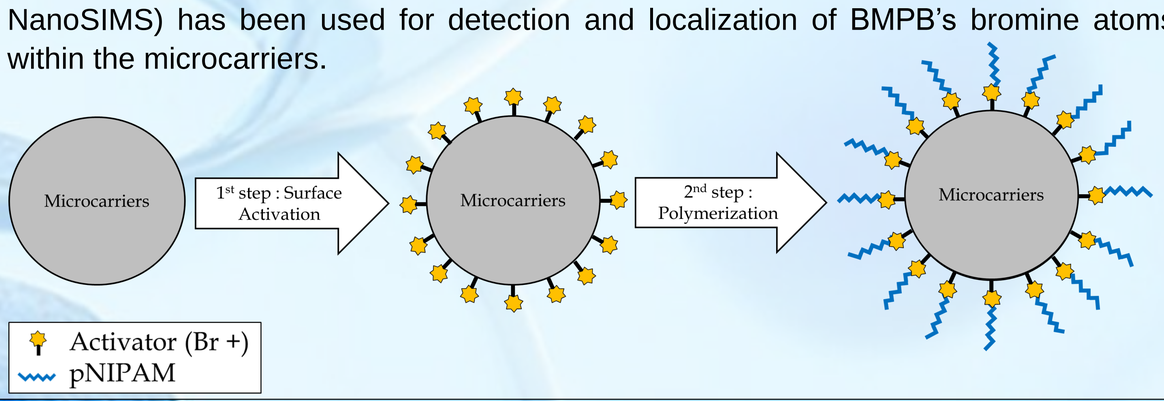
Several clinical studies have reported the benefit of the administration of Mesenchymal stromal cells (MSC) in various cell therapies. However, these studies have also highlighted that their routine applications need urgently new cell substrates to amplify MSC *in vitro* in GMP conditions. Indeed, MSC are scarce in the human body compared to the total dose requested in cell therapy. It is therefore critical to amplify MSC *in vitro* to achieve clinically relevant cell doses. If microcarriers are attractive substrates for this purpose, MSC cultivation on the microcarriers currently available on the market has been demonstrated unsuccessful, mostly due to the difficulties to detach the cells efficiently.

The main aim of our research relies upon the optimization of the surface properties of microcarriers promoting MSC culture *in vitro*. To achieve this purpose, the outer surface of microcarriers has been functionalized by grafting a thin polymer layer composed of a “smart macromolecule”, mainly composed of poly N-isopropylacrylamide (pNIPAM). The composition of this shell has been tailored to promote the adhesion and spreading of MSC with the ability to control their fast and efficient detachment following a small change in temperature.

Due to the shape of microcarriers, specific microscopy technique must be adapted to analyse the efficiency of grafting reaction. At this stage, we have demonstrated some reliable characterization methods based on Time-of-Flight and Nanoscale Secondary Ion Mass Spectrometry (ToF and NanoSIMS), Scanning Electron Microscopy (SEM), Atomic Force Microscopy *(*AFM) and Fluorescent Microscopy for two types of microcarriers: Dextran and Polystyrene (PS) based microcarriers.

Methods

- **Activation steps** using 2-Bromo-2-methylpropionyl bromide (BMPB) has been used as the initiator of the pNIPAM synthesis.
- **ATRP** has been adopted for the synthesis of the pNIPAM layer. The major advantage of this polymerization technique is to involve radical species whose reactivity is reduced using a reversible transfer mechanism to ensure precise control of propagation, limiting transfer and termination reactions.
- **Scanning Electron Microscopy (SEM)** associated with **Atomic Force Microscopy (AFM**) were used to verify the morphological change of the microcarriers after synthesis.
- **Fluorescence-labeled proteins** with Fluorescein-isothiocyanate (FITC) have been adopted to verify the thermoreactive properties of pNIPAM grafted on microcarriers.
- **Time-of-Flight** and **Nanoscale Secondary Ion Mass Spectrometry** (i.e. ToF and NanoSIMS) has been used for detection and localization of BMPB’s bromine atoms within the microcarriers.



Results

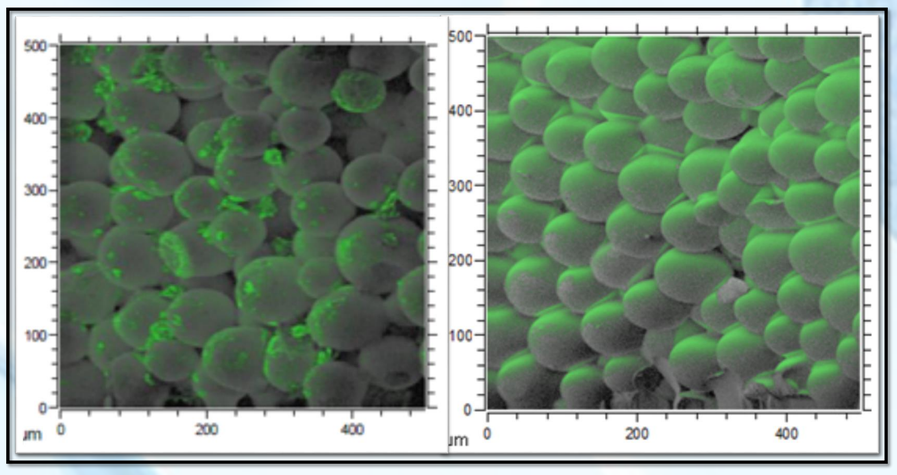


Figure 1: *Mapping of Br elemental detection of activated Cytodex I microcarriers observed under ToF-SIMS. Synthesis solvent affect bromine content repartition homogeneity on the surface of the microcarriers.*
Left: *Cytodex I activated in dimethylsulfoxyde.*
Right: *Cytodex I activated in acetonitrile.*

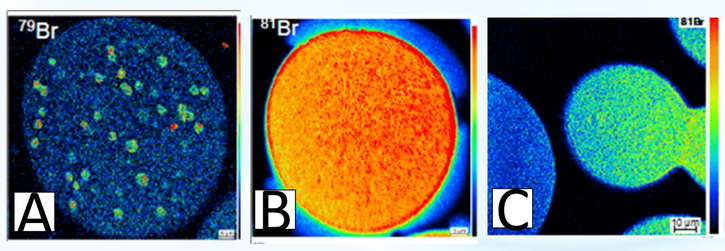


Figure 2: *Mapping of Br elemental detection of activated Cytodex I microtome slices, observed under NanoSIMS. Synthesis solvent also affect bromine content inside the microcarrier.*
A: *Cytodex I control,*
B: *Cytodex I activated in dimethylsulfoxyde,*
C: *Cytodex I activated in acetonitrile.*

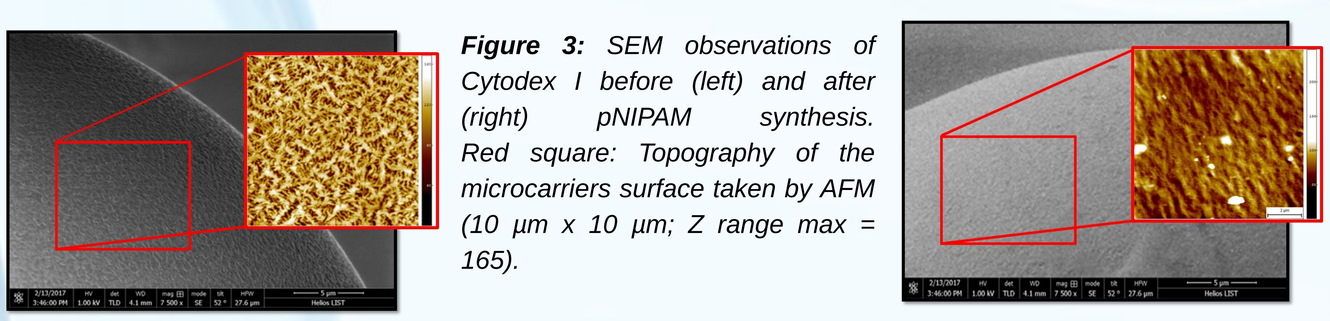


Figure 3: *SEM observations of Cytodex I before (left) and after (right) pNIPAM synthesis. Red square: Topography of the microcarriers surface taken by AFM (10 µm x 10 µm; Z range max = 165).*

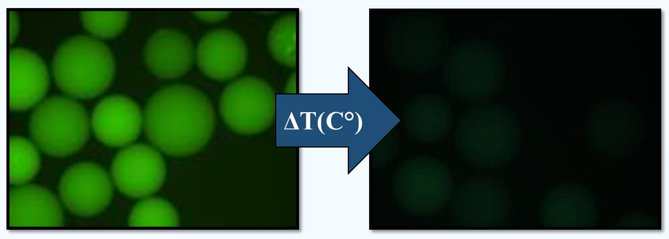


Figure 4: *Thermal release of FITC-proteins from pNIPAM microcarriers, observations taken under fluorescent microscope mounted with green filter.*

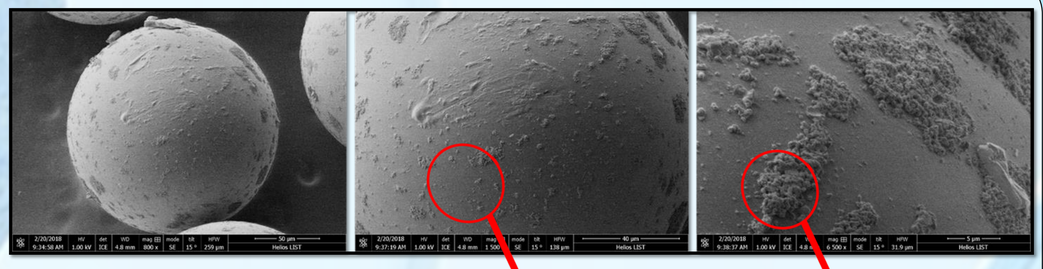
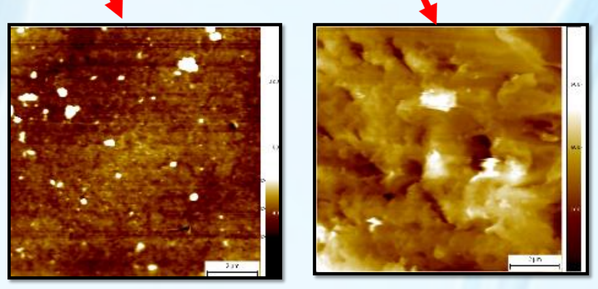


Figure 5 & 6: *SEM observations of PS beads after functionalization. Unexpected roughness was observed by SEM.*
Red circle: Topography of the microcarriers surface taken by AFM (10 µm x 10 µm; Z range max = 150 nm (left) and 600 nm (right).



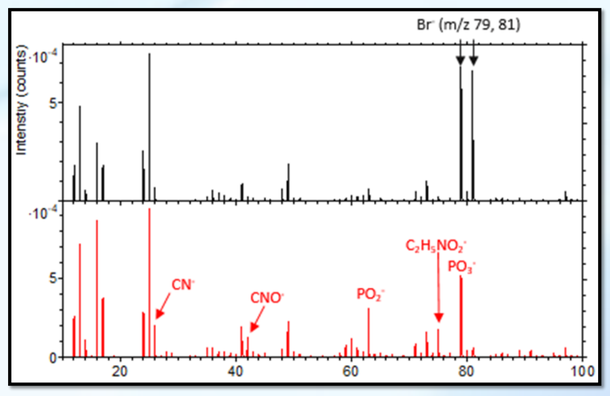


Figure 7: *Elemental detection of activated PS microcarriers observed under ToF-SIMS. The roughness detected by SEM was assigned to hydroxyapatite depot and not to pNIPAM.*
Red spectra: PS beads control
Black spectra: PS beads activated

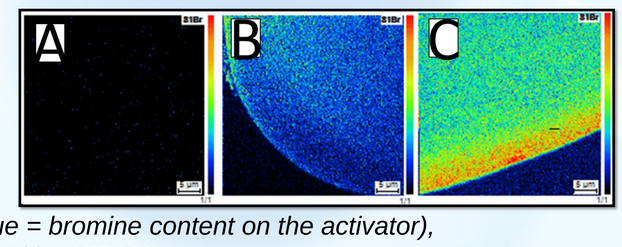


Figure 8: *Mapping of Br elemental detection of functionalized PS microtome slices, observed under NanoSIMS.*
A: *PS microcarriers control,*
B: *PS microcarriers activated (Blue = bromine content on the activator),*
C: *PS microcarriers polymerized with pNIPAM.*

Discussion & Perspectives

Microcarriers offer major advantages to amplify MSC in comparison to classical flat 2D culture surface. The use of pNIPAM grafted on different surfaces has been reported as a new substrate to facilitate cell detachment without using conventional treatment with enzymes, preserving intact tight junctions between cells and proteins of the extracellular matrix. Our current study has allowed to develop several complementary techniques to characterize BMPB activated and thermo-reactive pNIPAM functionalized microcarriers. Also, these techniques highlight several issues like the presence of hydroxyapatite depot on the surface of PS microcarriers available on the market.

As a follow-up of these results, the functionalized microcarriers behavior should be investigated with Mesenchymal Stem Cell (MSC).

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