Bio-IMP: Development of an immunomagnetic separation device for recovery of CD34+ stem cells

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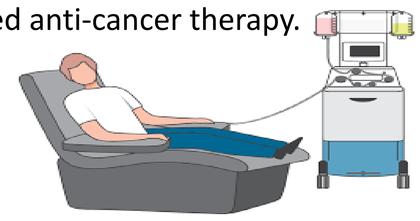
Introduction

Nowadays, the use of stem cells for regenerative medicine and cancer therapy is gradually advancing and holds a promising future for human health. CD34 is an antigen on haematopoietic stem cell discovered in 1984. This particular stem cell is mostly rare (less than 2% in cord blood). Transplantation of **CD34+ stem cells** are clinically used to favour the development of new blood vessels for tissue regeneration after ischemia, or to boost the immune systems renewal after a hyperthermia-based anti-cancer therapy.

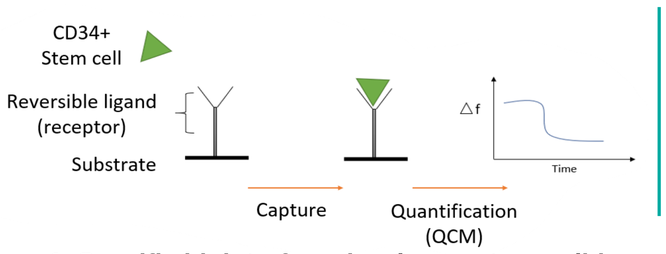


The recovery of these stem cells at the clinical level for transplantation also requires complex methods and equipment. It includes the use of apheresis and expensive drugs such as Granulocyte Colony Stimulating Factor (G-CSF) to immobilize these stem cells. However, with G-CSF alone, only 65% patients are able to mobilize enough CD34+ stem cells. In this project, we propose to develop a **microfluidic device** for the recovery of CD34+ stem cells which can be easily transferred to patients **without the use of immobilizing drugs.**

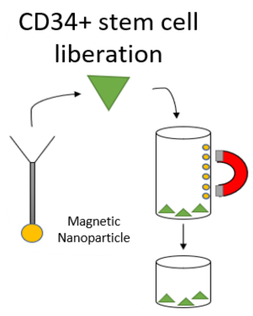
Method

The first phase of this project is to develop a specific biointerface on gold substrate that will allow the grafting of specific receptor (Anti-CD34) to interact positively to the antigen on CD34+ stem cells. As CD34+ stem cells are very expensive and can only be used for 3 days, we start with some preliminary experiments on Human Umbilical Vein Endothelial Cell (HUVEC) which also expresses CD34 marker but on a lower level.

The second phase involves the transposition of the specific biointerfaces onto magnetic nanoparticles. This will be implemented in microfluidic separation device. There will be increased in complexity from pure stem cells to cord blood.

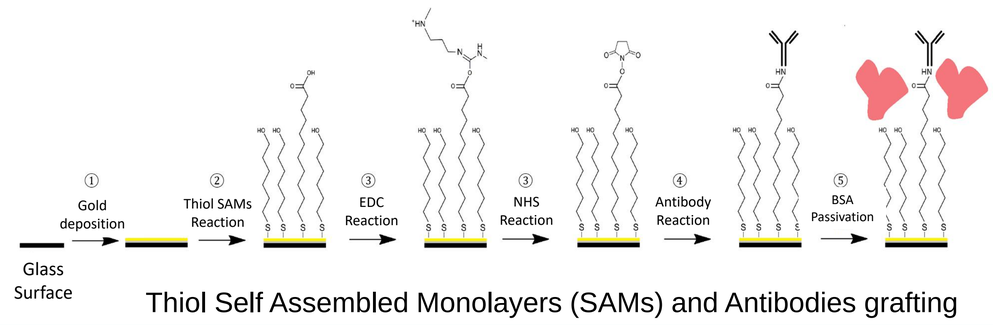


1. Specific biointerface development on solid substrate



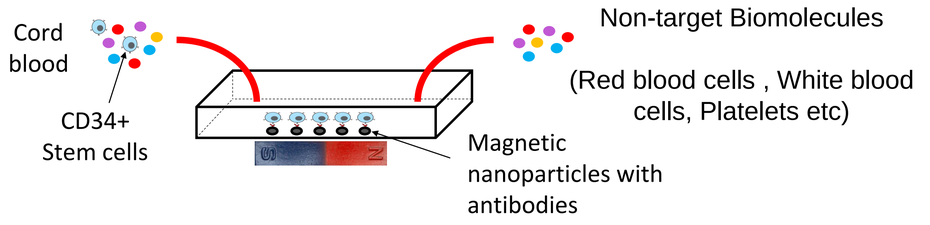
2. Transposition to magnetic nanoparticles

Specific Biointerface Functionalization on Gold



Thiol Self Assembled Monolayers (SAMs) and Antibodies grafting

Implementation in magnetic separation device



Microfluidic Chip

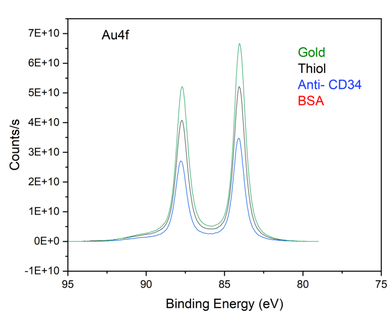
Characterization of biointerfaces

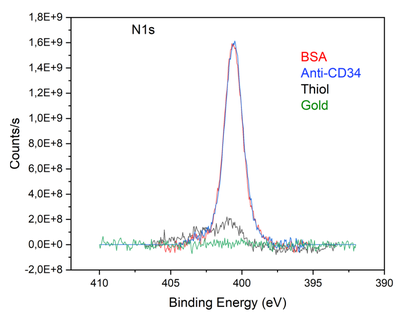
At the beginning of this project, we focus on the biointerface development and its characterization.
At each functionalization step, different surface characterization techniques were used to check the success of each grafting. IR spectroscopy (FTIR – ATR) and X-Ray Photoelectron Spectroscopy (XPS) were used to confirm the successful grafting of antibodies on gold surface. Optical Microscopy was finally used to check the capture of HUVEC cells on the functionalized biointerfaces.

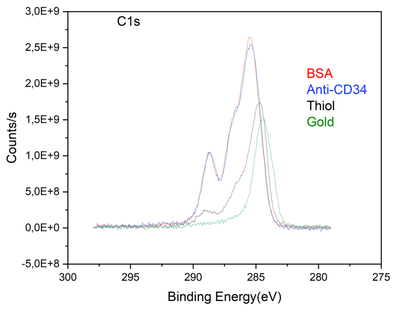
XPS

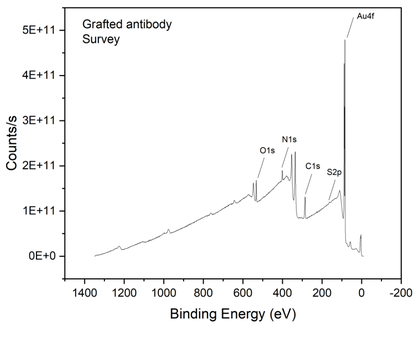
The survey spectra shows C, O, N and S on top of Au.
Au4f signal decreases as organic layer thickness increases.
The N1s and C1s signals at 400 eV and 289 eV are assigned to antibodies.
Bovine Serum Albumin (BSA) signal not increasing suggests already packed antibody layer.

Successful grafting of antibodies on top of biointerface/Au



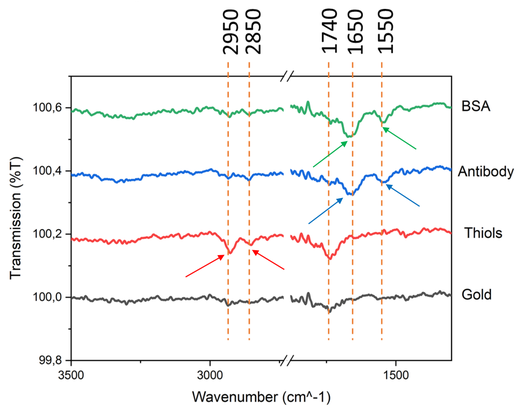






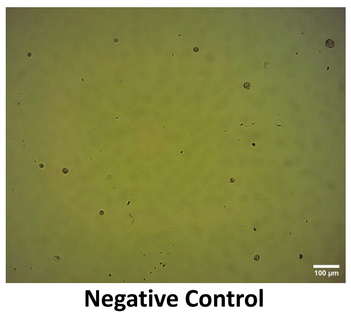
FTIR - ATR

Peaks at 2950 cm^{-1} ,2850 cm^{-1} and 1740 cm^{-1} confirm thiols grafting.
Presence of Amide I and Amide II bands at 1650 cm^{-1} and 1550 cm^{-1} indicates the grafting of antibodies

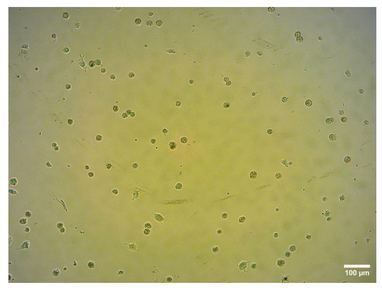


Successful grafting of antibodies on top of biointerface/Au

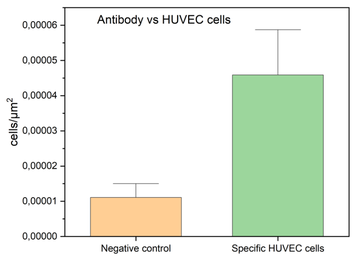
Static Capture test



Negative Control



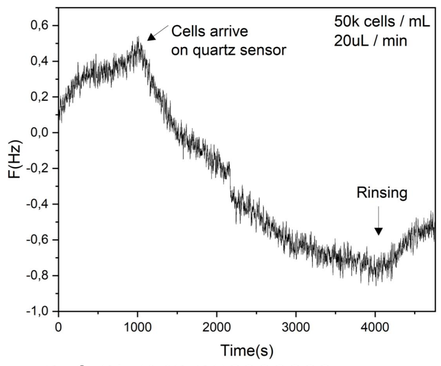
HUVEC cells



Specificity

A specificity of detection of 76% was achieved during capture of HUVEC cells

Dynamic Capture test (QCM)



50k cells injected in QCM device at a flow rate of 20µL/min.

Frequency drop > 1 Hz = ~ 11k cells.

Capture of 1/5 of the cells by the biointerface in flow

Conclusions

XPS and FTIR-ATR were used to validate grafting of antibodies on gold substrate during biointerface development. Static capture shows a specificity detection of 76% despite low level of CD34+ markers on HUVEC cells. A dynamic capture test using QCM shows 1/5 cells captured out of 50k cells injected.

Perspectives

The next step will consist of static and dynamic capture test using CD34+ stem cells. We expect higher capture efficiency during this step. There will also be transposition of the specific biointerfaces onto magnetic gold nanoparticles and implementation in a microfluidic separation device.

Acknowledgements

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