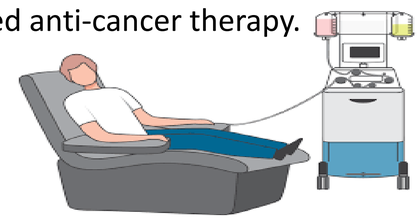
# Bio-IMP: Development of an immunomagnetic separation device for recovery of CD34 stem cells

Paul Sarpong, Agathe Figarol, Thérèse Leblois, Vincent Humblot
Université Marie et Louis Pasteur, CNRS
Institut FEMTO-ST
F-25000 Besançon, France

COFINANCÉ PAR L'UNION EUROPÉENNE
RÉGION BOURGOGNE FRANCHE COMTE

## 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.

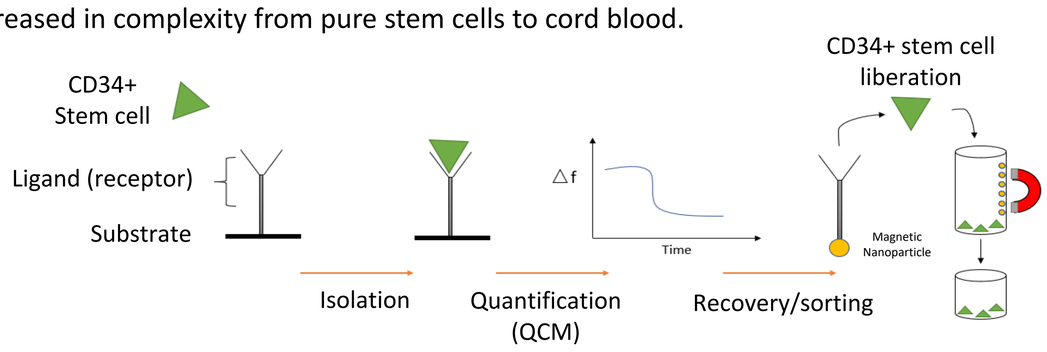


The recovery of these stem cells at the clinical level for transplantation also requires complex methods and equipment. It includes the use of apheresis and expensive drugs such as Granulocyte Colony Stimulating Factor (G-CSF) to immobilize these stem cells. However, with G-CSF alone, only 65% patients 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.**

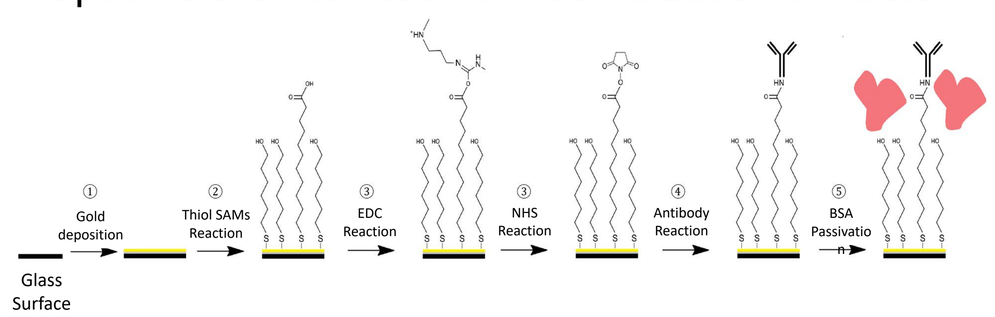
## Method

**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 (5000 €/ 1 million cells) and can only be cultured in a week, we start with some preliminary experiments on Human Umbilical Vein Endothelial Cell (HUVEC) which also expresses CD34 marker but on a lower level.

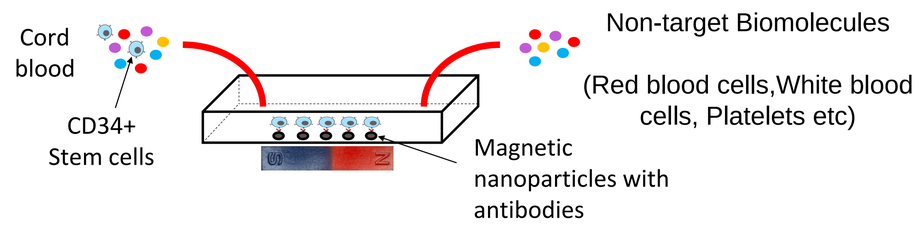
**The second phase** involves the transposition of the specific biointerfaces onto magnetic nanoparticles. This will be implemented in microfluidic separation device. There will be increased in complexity from pure stem cells to cord blood.




### Specific Biointerface Functionalization on Gold




### Implementation in magnetic separation device



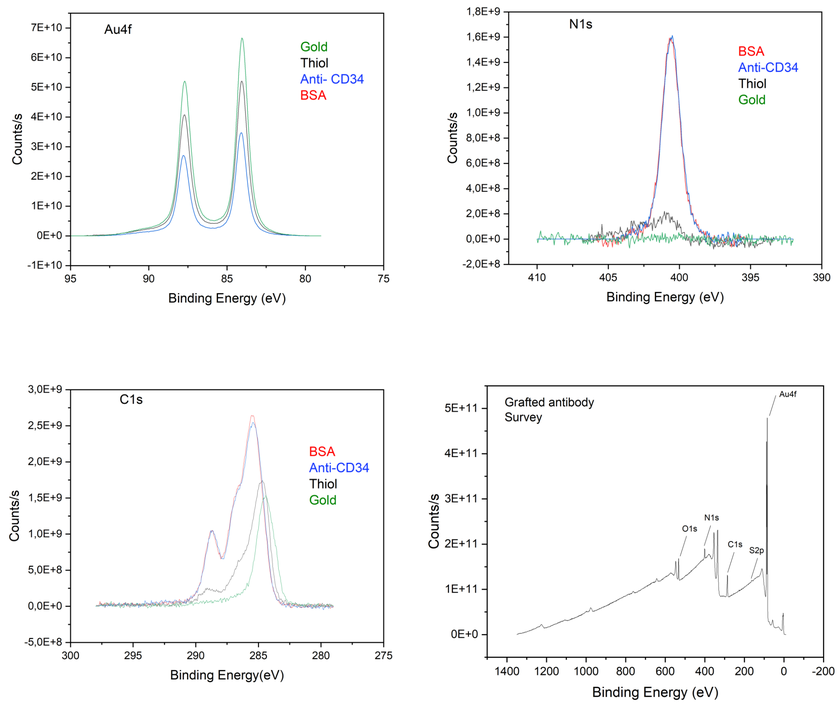

## Characterization of biointerfaces

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

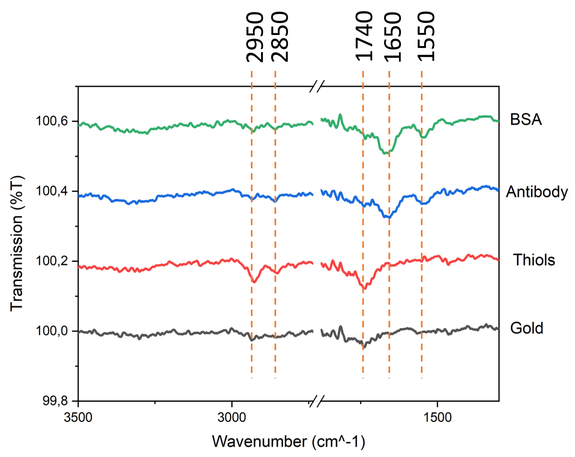
The survey spectra shows the presence of C, O, N and S on top of Au, as expected from the functionalization strategy, On the Au4f signal, we notice the decrease of the gold signal as a function of increasing organic layer thickness.
The high intensity of N1s together with the increase of the C1s contribution @ 289 eV validate the successful grafting of antibodies.
Bovine Serum Albumin (BSA) passivation does not show any signal increase suggesting an already packed/saturated antibodies layer.

### High resolution elemental analysis of specific biointerface on Gold

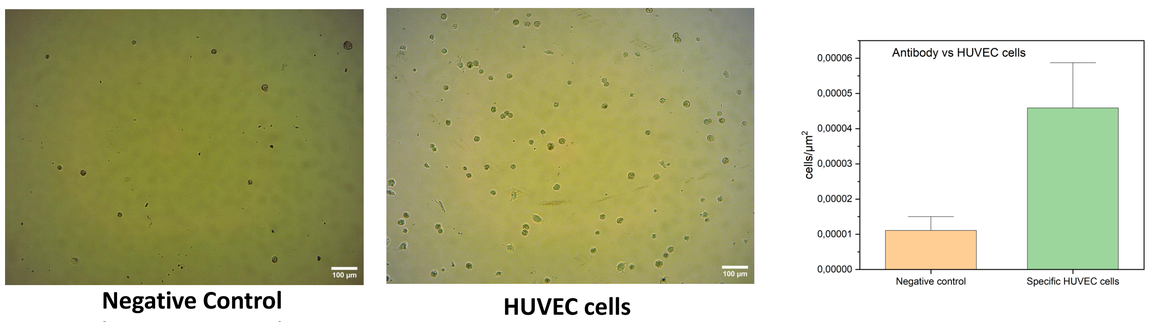



### FTIR - ATR

The peaks at 2950 $cm^{-1}$ and 2850 $cm^{-1}$ shows the presence of $CH_2$ and $CH_3$ carbon chains after the grafting of thiol. The peaks at 1650 $cm^{-1}$ and 1550 $cm^{-1}$, respectively assigned to Amide I and Amide II IR features confirm XPS data with successful grafting of antibodies.




### Antibody to Cell Interaction




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

## Conclusion

We succeeded to functionalize gold surfaces using surface chemistry to graft antibodies via thiols SAMs. The higher number of cells per area on the positive surface indicate successful cells capture by antibodies. This validates the static capture of cells using our specific biointerfaces. Despite the low level of CD34+ markers on HUVEC cells, a specificity detection of 76% was achieved.

## Perspectives

The next step will consist of quantification of cell capture to be performed using QCM in a dynamic detection condition.
There will also be transposition of the specific biointerfaces onto magnetic gold nanoparticles and implementation in a microfluidic separation device.
Finally, there will be liberation of captured stem cells from grafted antibodies whilst maintaining their viability.

## Acknowledgement

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**Contacts**
Paul Sarpong
paulkofimensah.sarp@femto-st.fr

**Besançon**
www.femto-st.fr