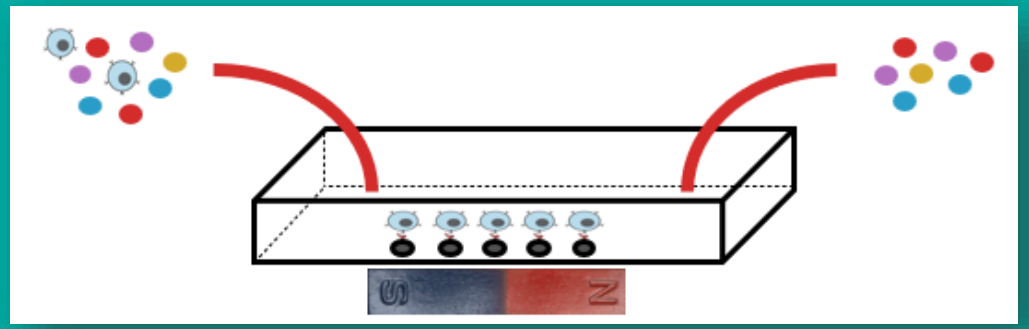
ECASIA Conference

Development of a microfluidic separation device for recovery of CD34+ Stem cells



Authors:

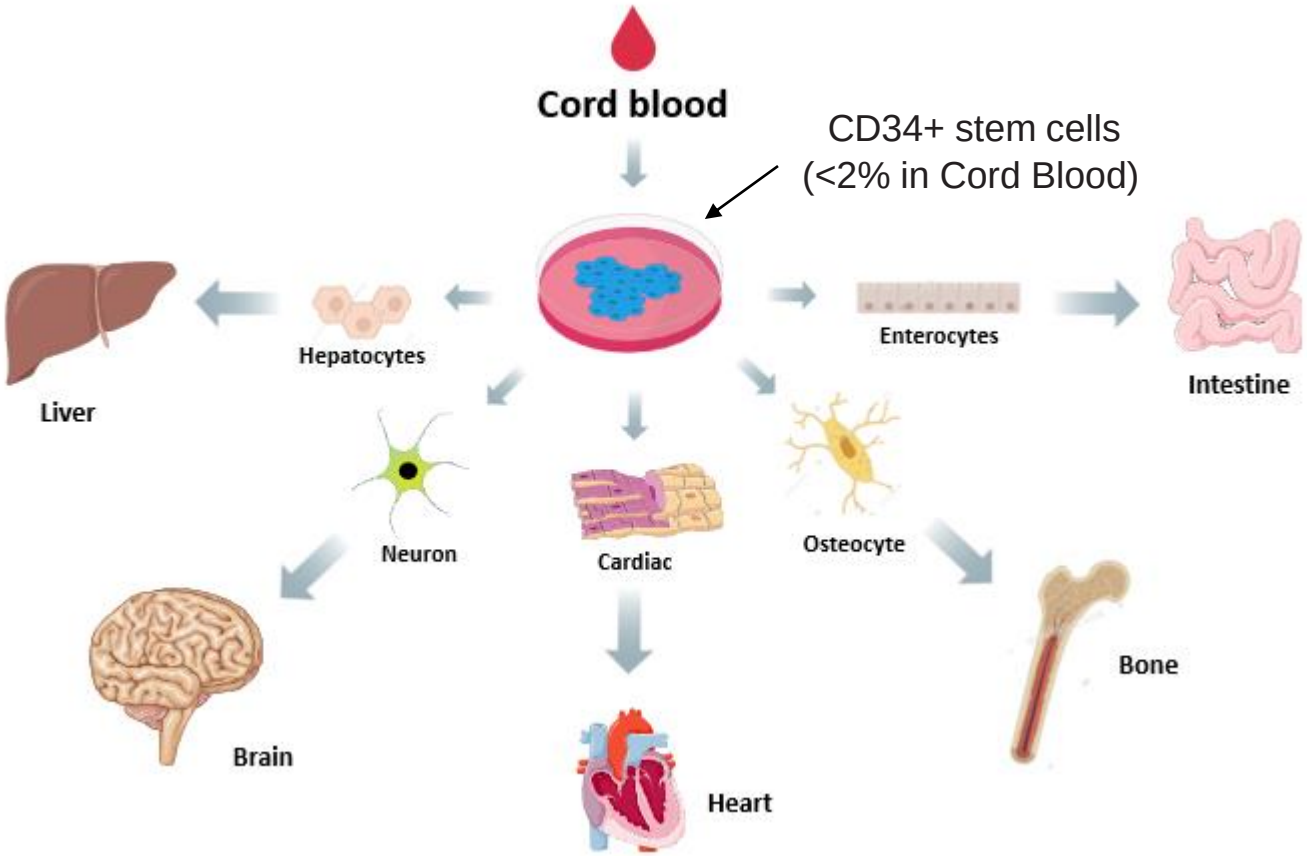
Paul Sarpong, Agathe Figarol, Thérèse Leblois, Vincent Humblot

FEMTO-ST Institute, UMLP, CNRS, Besançon - France

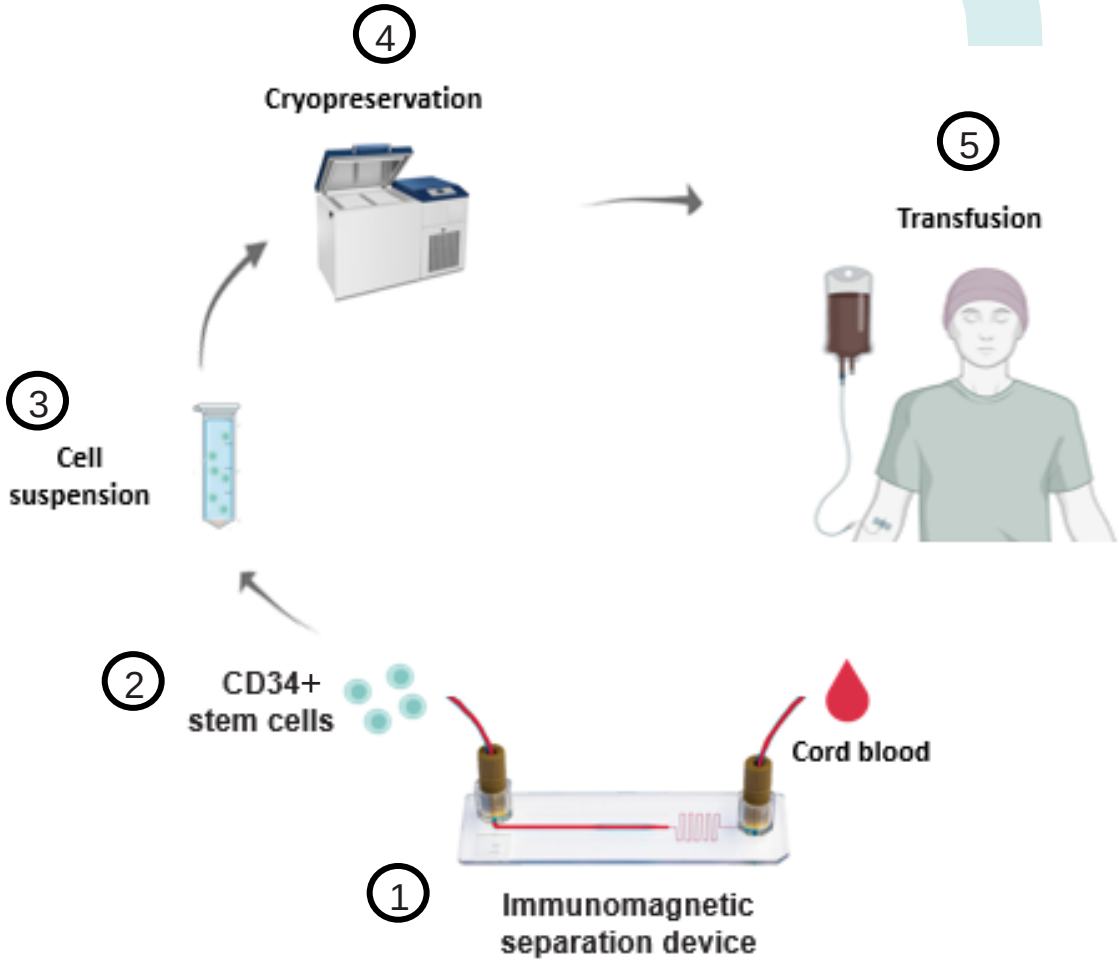
18th September 2026

BioIMP project (FEDER 2024 – 2028)

CONTEXT



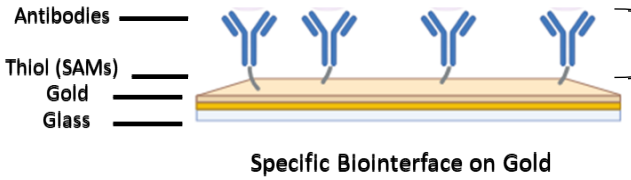
CD34+ Stem cell differentiation

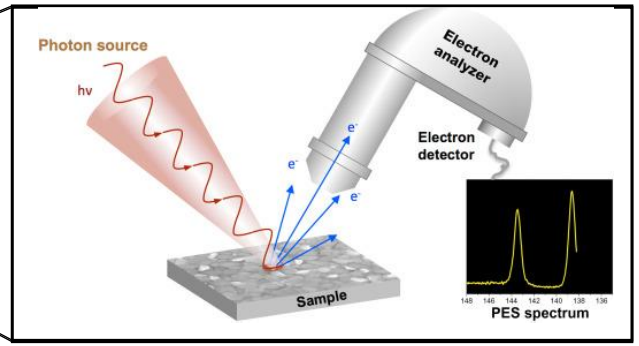


Regenerative medicine

STRATEGY

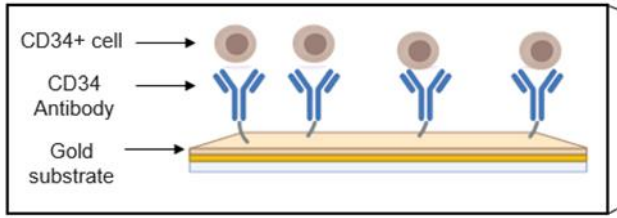
① Specific Biointerface Development





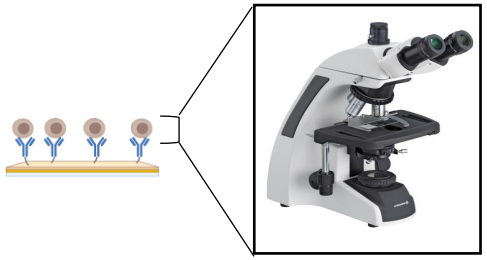
XPS characterization of Specific Biointerface

② Static Capture



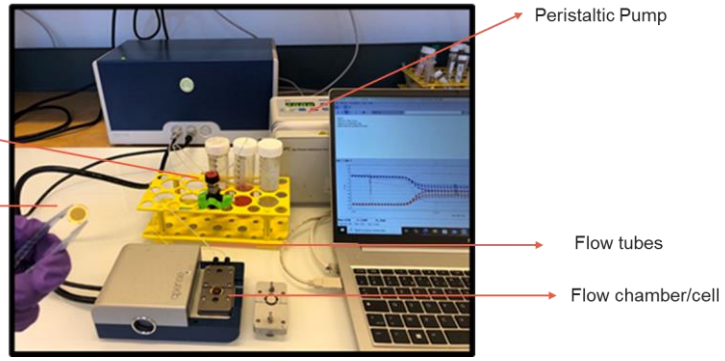


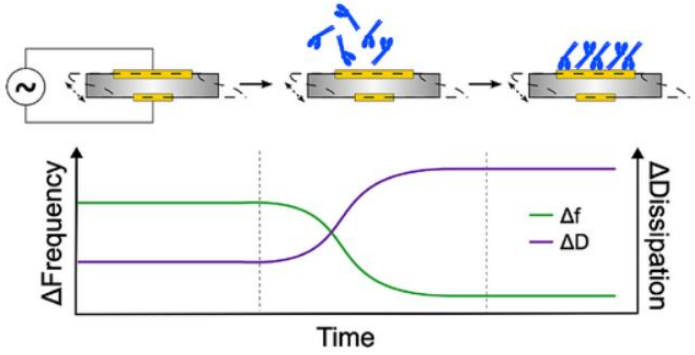
Cell capture in a 12-well culture plate



Observation of captured cells on specific Biointerface

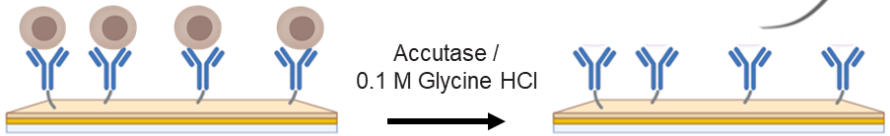
③ Dynamic Capture

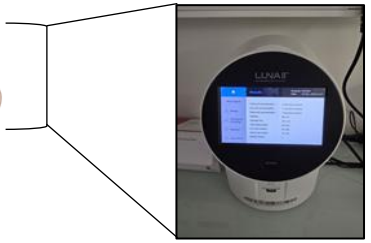




Quantification of captured cells using QCM

④ Recovery and Viability of cells



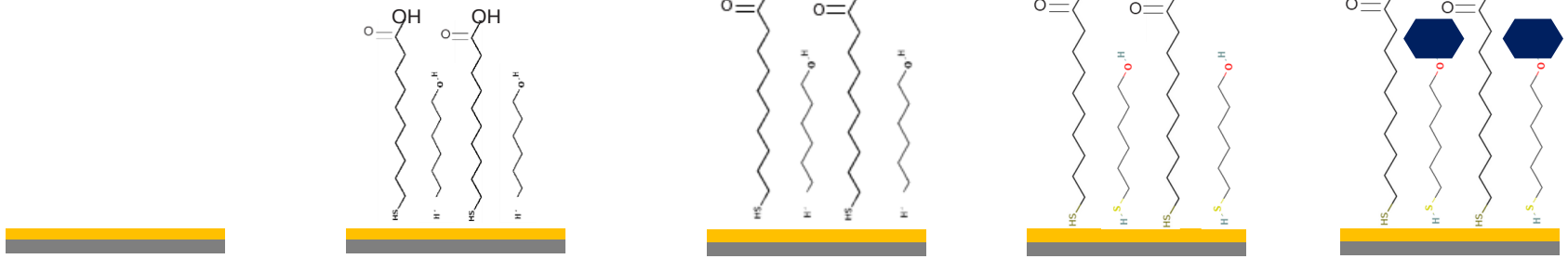


Luna Device

Cell viability of detached cells using Luna Device

SPECIFIC BIOINTERFACE DEVELOPMENT

1. THIOL MUA - EDC/NHS – ANTIBODY (TENA) BIOINTERFACE



Gold surface ①

Thiol MUA SAMs ②

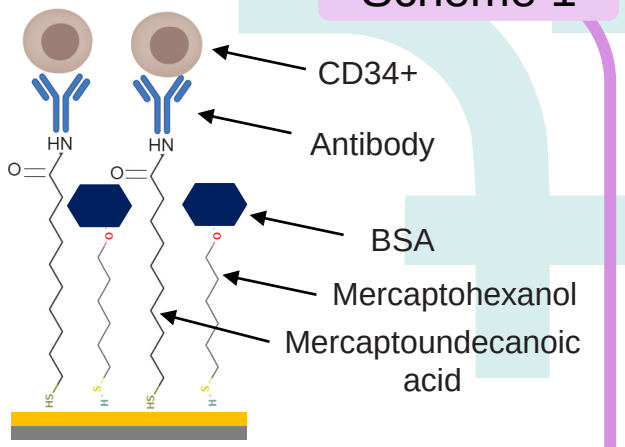
EDC / NHS Activation ③

Antibody Grafting ④

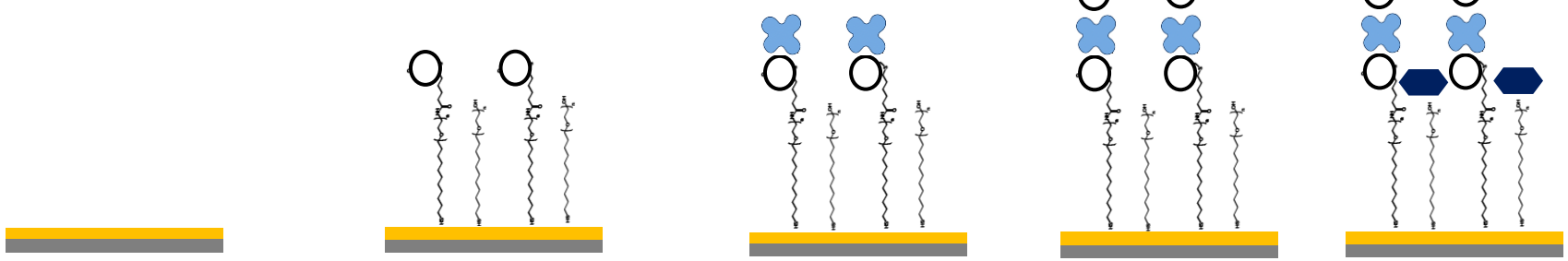
BSA Passivation ⑤

Cell Capture ⑥

Scheme 1



2. THIOL BIOTIN - STREPTAVIDIN – BIOTINYLATED – ANTIBODY (TSBA) BIOINTERFACE



Gold surface ①

Thiol-biotin ②

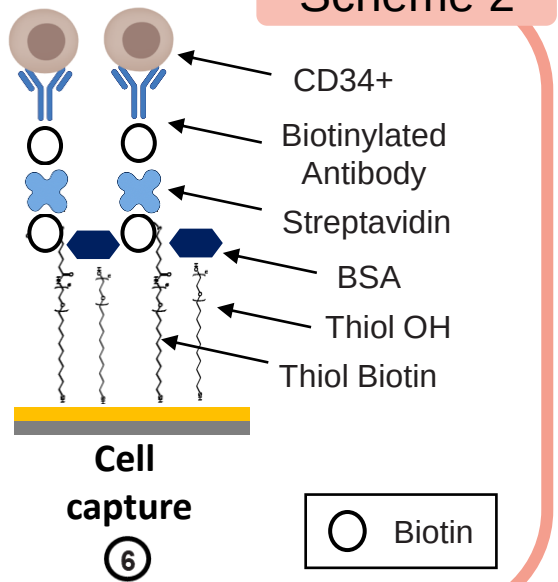
Streptavidin ③

Biotinylated Antibody ④

BSA Passivation ⑤

Cell capture ⑥

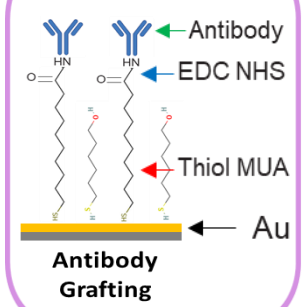
Scheme 2



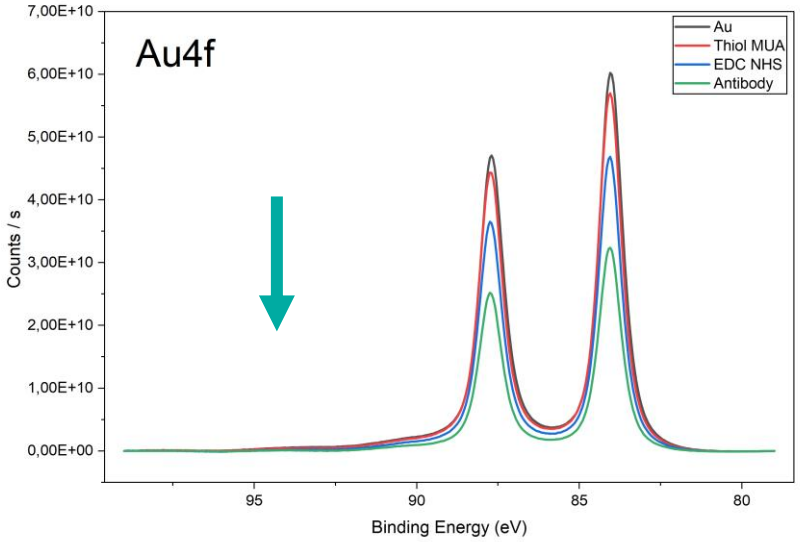
XPS CHARACTERIZATION

1. THIOL MUA - EDC/NHS – ANTIBODY (TENA) BIOINTERFACE

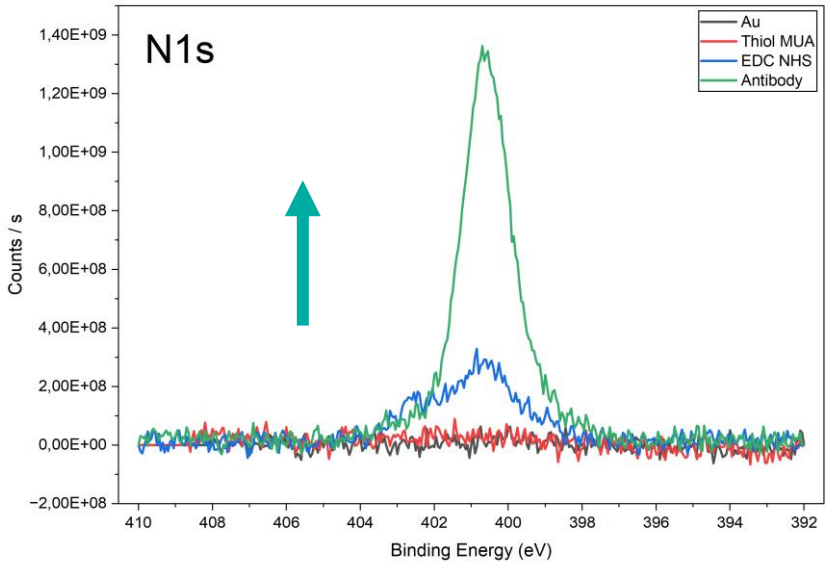
Scheme 1



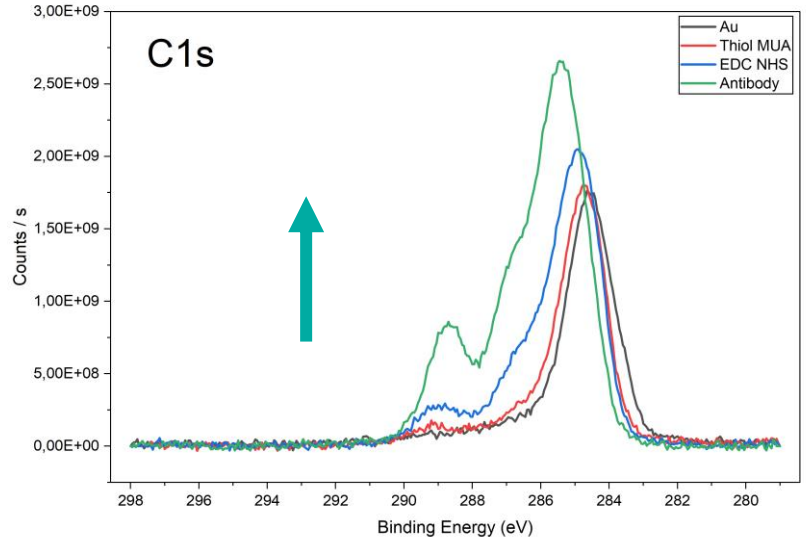
High resolution XPS elemental analysis of specific biointerface (Thiol MUA)



- Au4f spectrum shows decrease of gold signal as organic layer thickness increases



- High intensity of N1s (401 eV) showing grafting of antibody



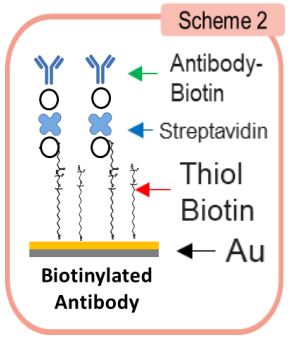
- High intensity of C1s (289 eV) of the C=O of the amide bond, and (287 eV) of the C – N amine bond showing antibody grafting

Conclusion:

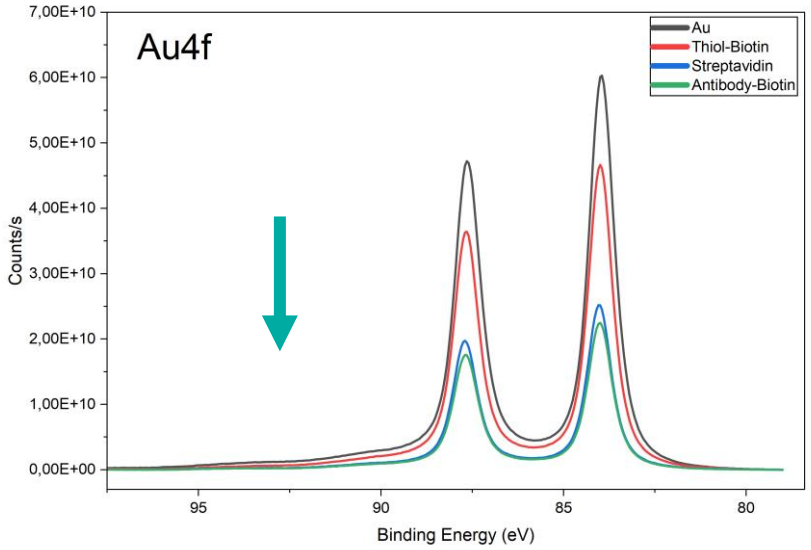
Successful grafting of antibodies on biointerface

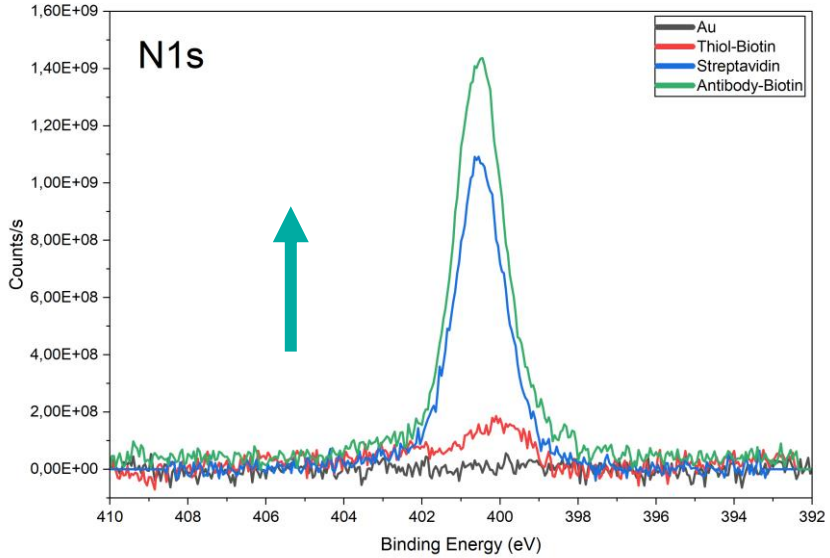
XPS CHARACTERIZATION

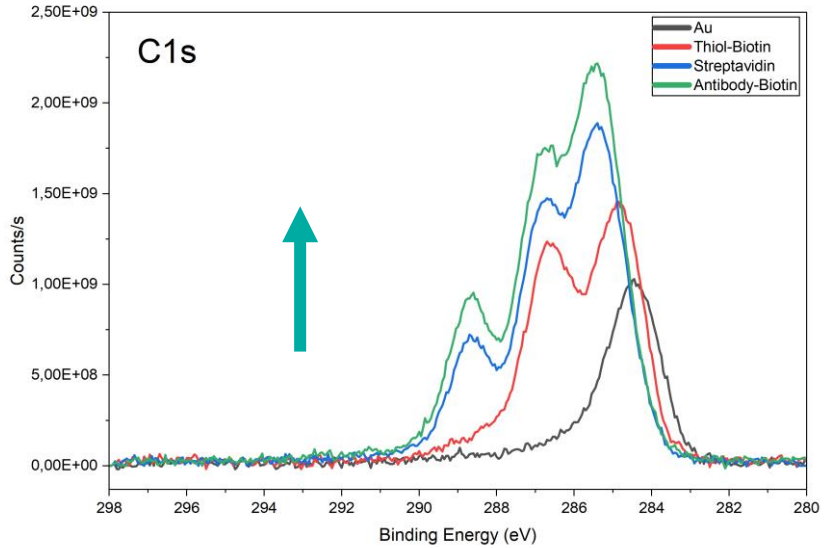
2. THIOL BIOTIN - STREPTAVIDIN – BIOTINYLATED – ANTIBODY (TSBA) BIOINTERFACE



High resolution XPS elemental analysis of specific biointerface (Thiol Biotin)







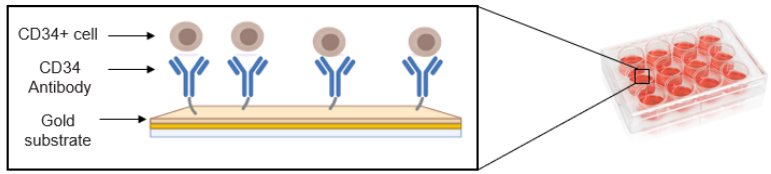
- Decrease in Au4f signal as a function of the organic layer thickness.
- High intensity of N1s at 401 eV indicating grafting of antibodies
- Increase in C1s at 289 eV, corresponding to the C=O of the amide bond, and 287 eV, corresponding to the C – N of the amine bond

Conclusion:

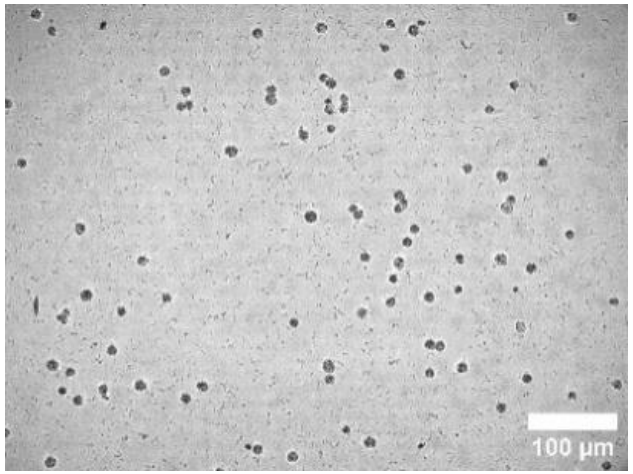
Successful grafting of antibodies on biointerface

STATIC CELL CAPTURE

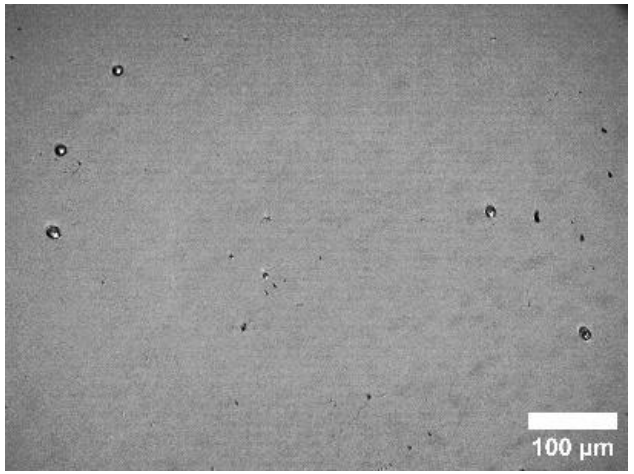
1. THIOL MUA - EDC/NHS – ANTIBODY (TENA) BIOINTERFACE



Static cell capture in a 12 - well culture plate

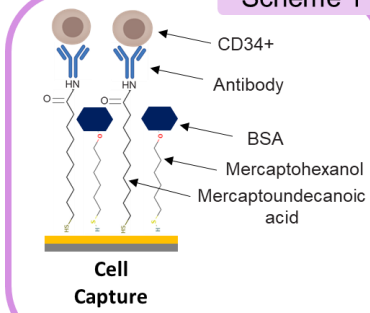


CD34+

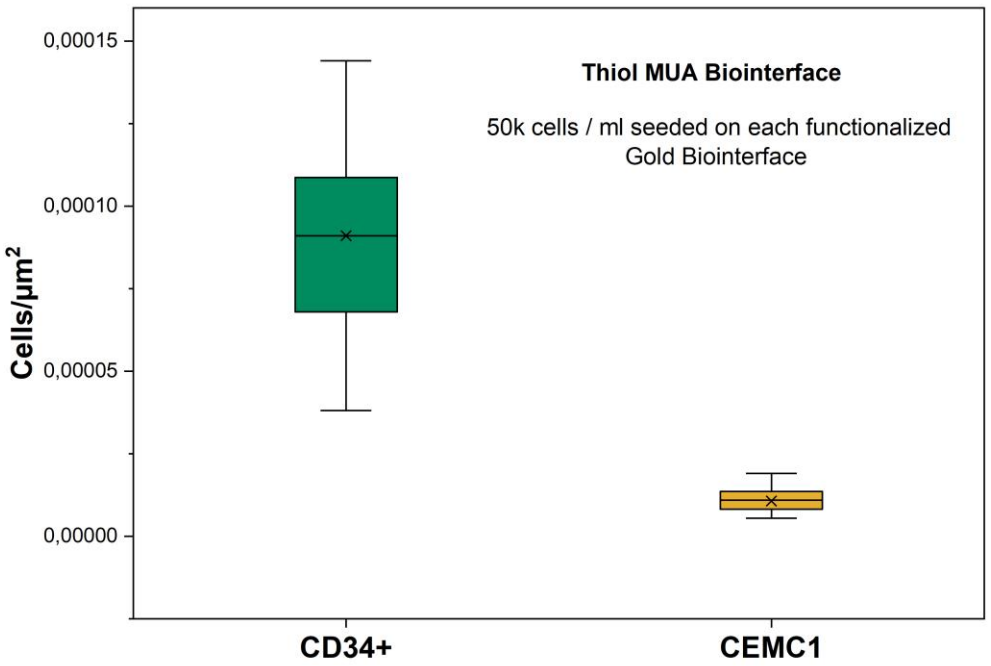


CEMC1

Scheme 1







Repetitions
(N = 3 , n = 9)

45 images

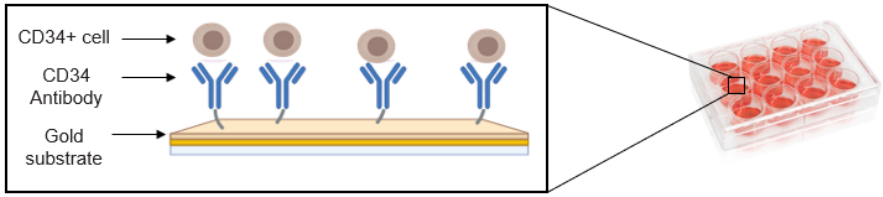
$$\% \text{ Specificity} = \frac{(CD34+)-(CEMC1)}{(CD34+)} x\ 100$$

Conclusion:

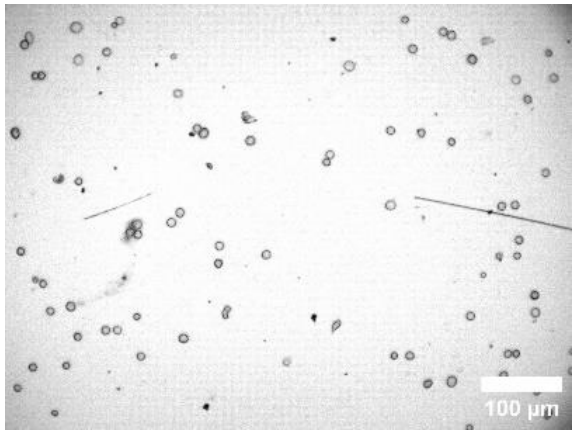
It shows up to **80% specificity for CD34+** cells compared to CEMC1 negative control

STATIC CELL CAPTURE

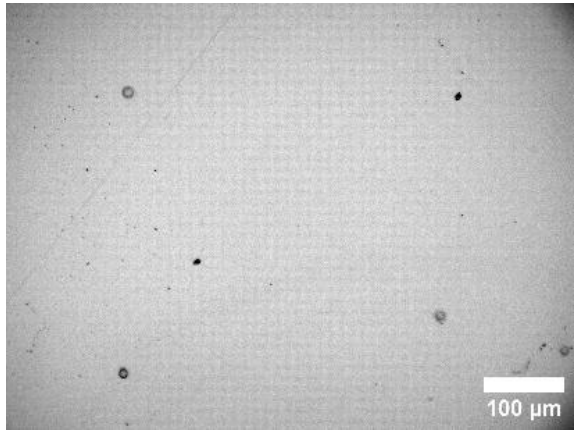
2. THIOL BIOTIN - STREPTAVIDIN – BIOTINYLATED – ANTIBODY (TSBA) BIOINTERFACE



Static cell capture in a 12 - well culture plate

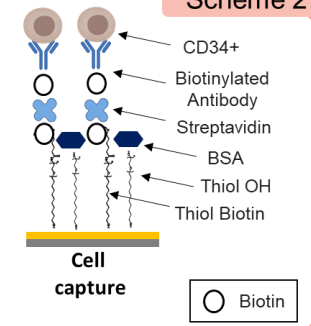


CD34+

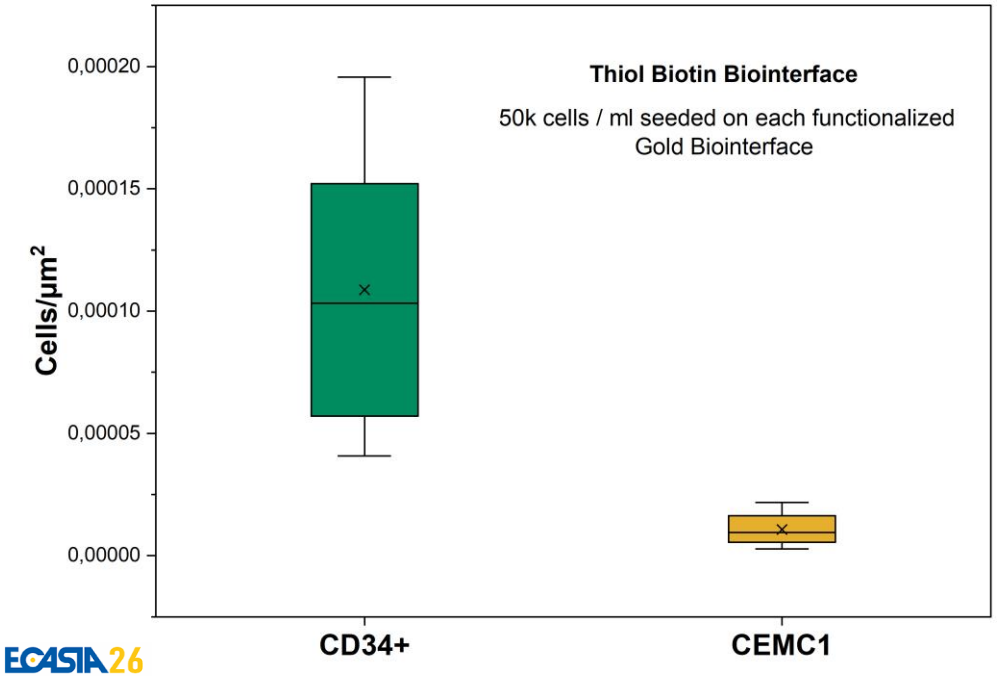


CEMC1

Scheme 2







Repetitions
(N = 3 , n = 9)

45 images

$$\% \text{ Specificity} = \frac{(CD34+)-(CEMC1)}{(CD34+)} x\ 100$$

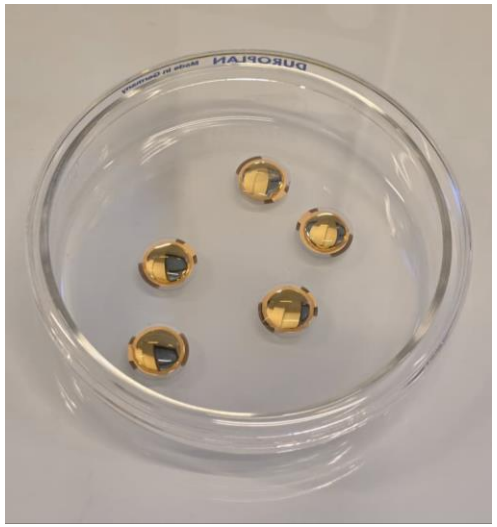
Conclusion:

- Shows up to **85% Specificity for CD34+** cells as compared to CEMC1 negative control
- **Improvement on specificity** on this biointerface

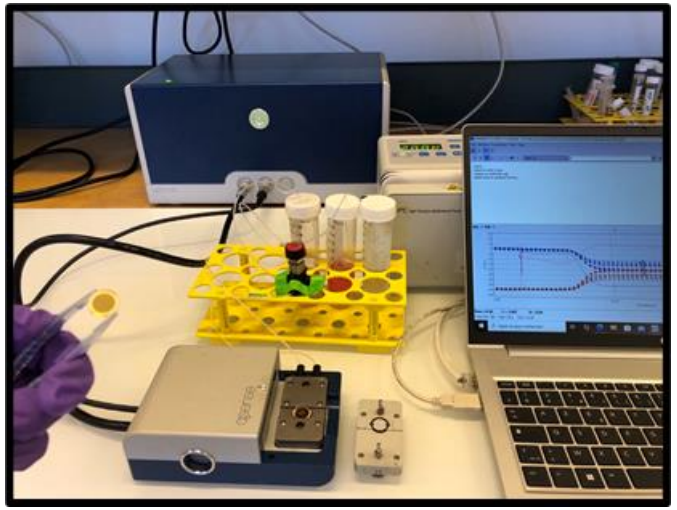
EX-SITU VS IN-SITU LIGAND GRAFTING

Disadvantages of **ex-situ** ligand grafting:

- Poor Signal Stabilization
- Poor repeatability
- Time consuming



Ex-situ ligand grafting



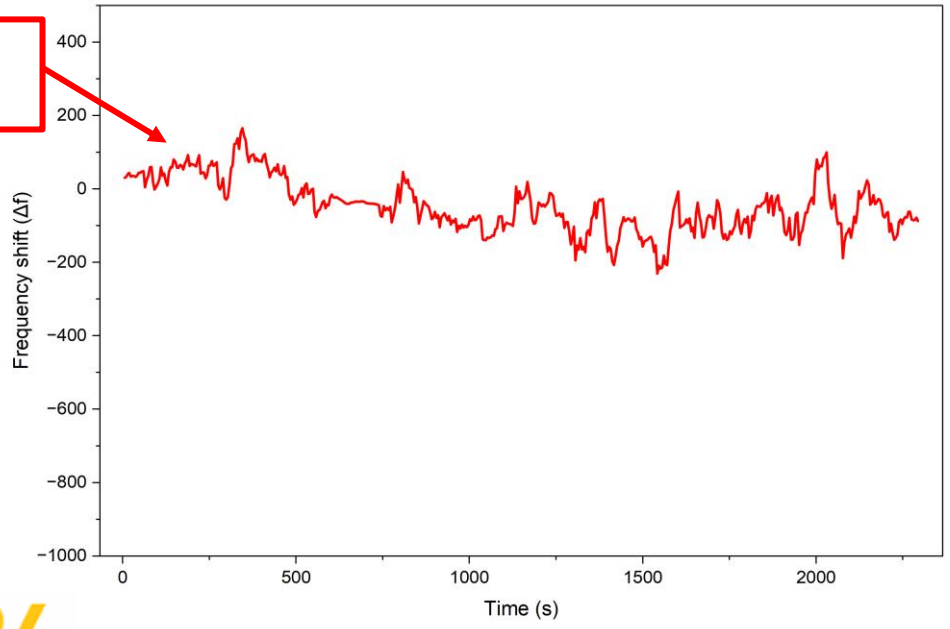
In-situ ligand grafting with QCM

Advantages of **in-situ** ligand grafting:

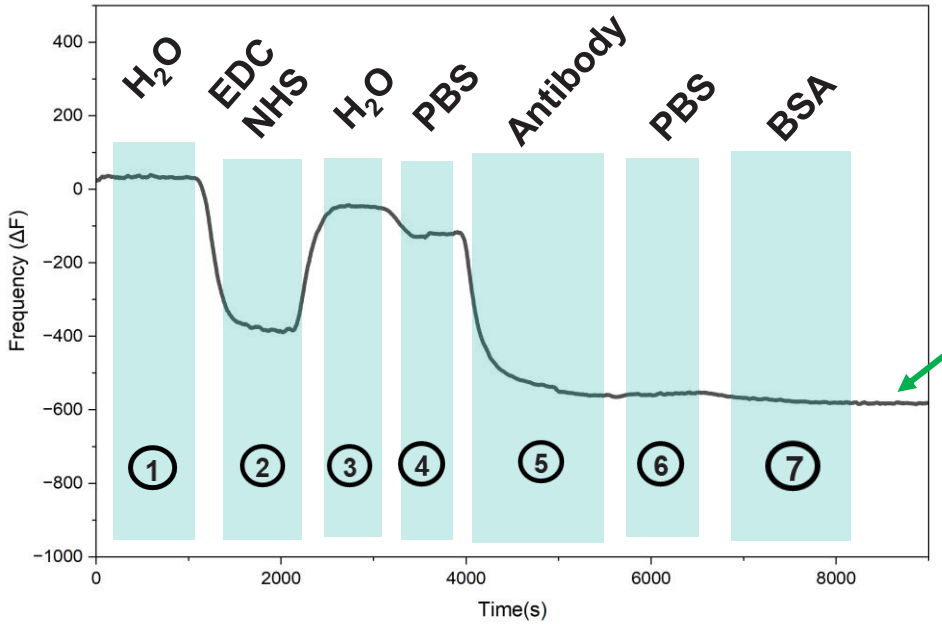
- Good Signal Stabilization
- Good repeatability
- Doesn't require XPS characterization

QCM Signal after ex-situ ligand grafting:

- Difficult to reach stability



About 250 Hz peak to peak noise



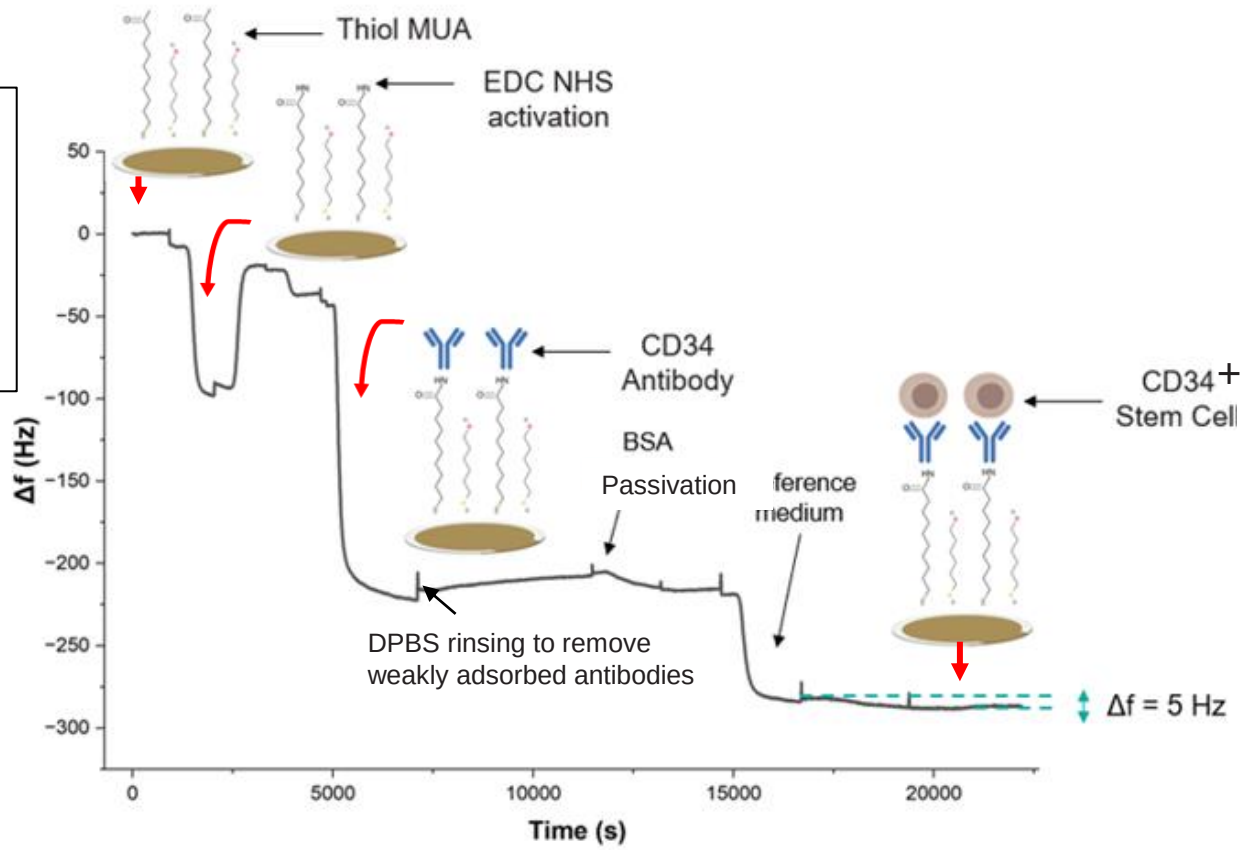
- Stable signal

About 2 Hz peak to peak noise

Thiol MUA - EDC/NHS – CD34 Antibody biointerface

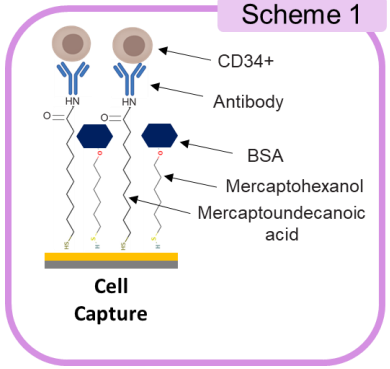
- Real-time in-situ functionalization and cell capture on 10 MHz quartz sensor

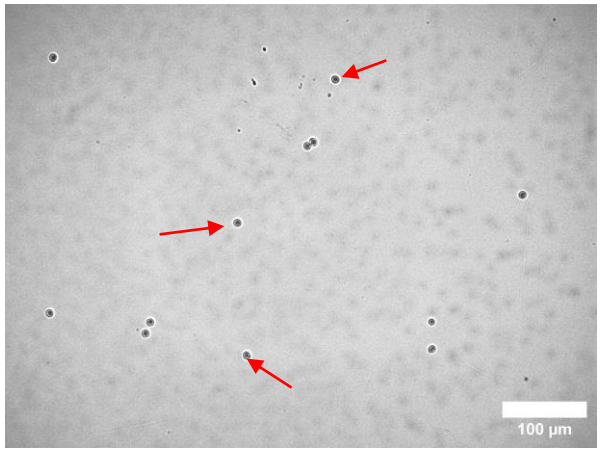
Reference Thiol MUA already grafted on Quartz surface overnight before experiment



Dynamic capture of CD34+ cells in QCM

Scheme 1





Microscopy image of quartz surfaces with captured cells

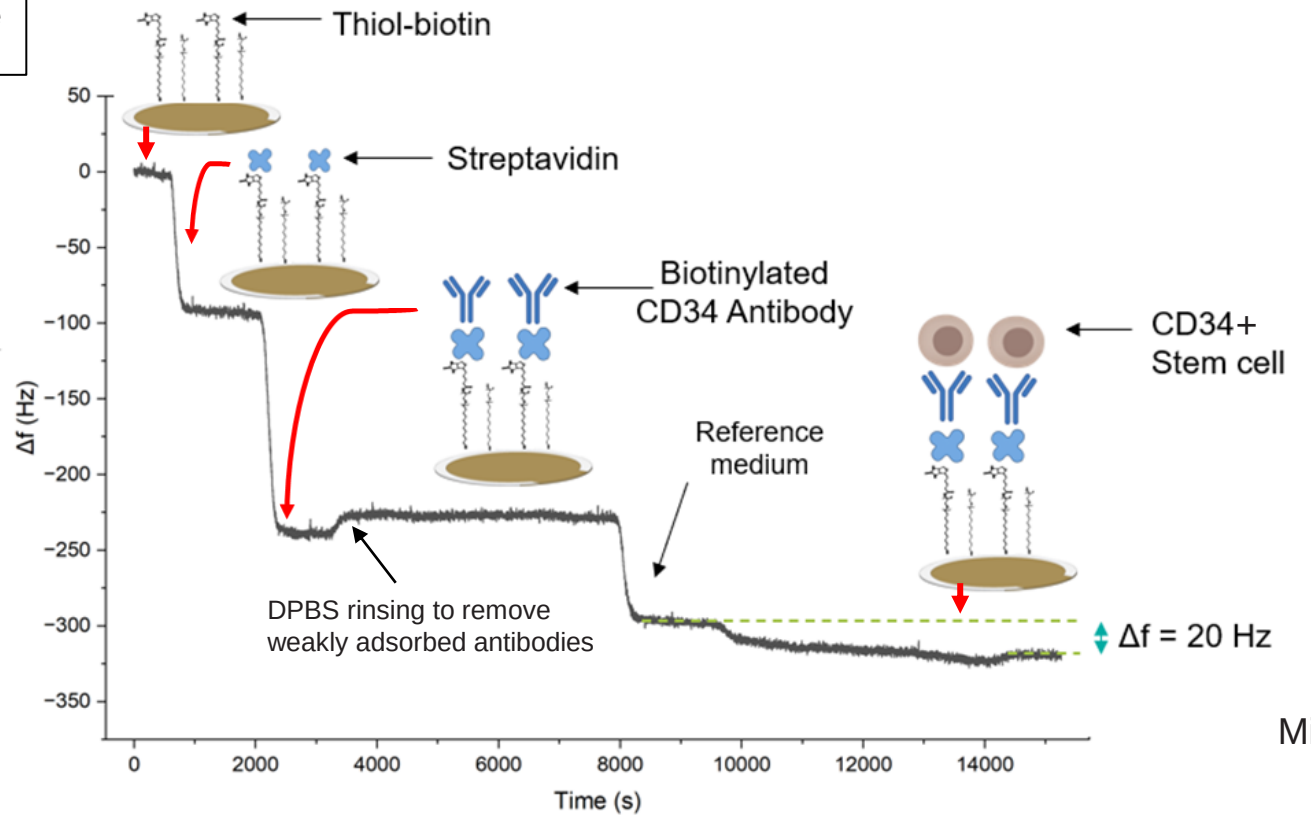
Quantification analysis:

- A frequency drop of **5 Hz** corresponding to 1100 cells captured out of 300k cells passed
- Single passage, concentration: 700k cells/ml
- **Efficiency of capture up to 0.4%**

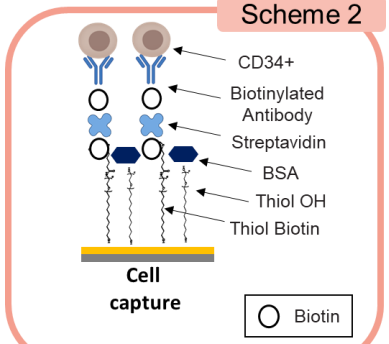
Reference Thiol Biotin already grafted on Quartz surface overnight before experiment

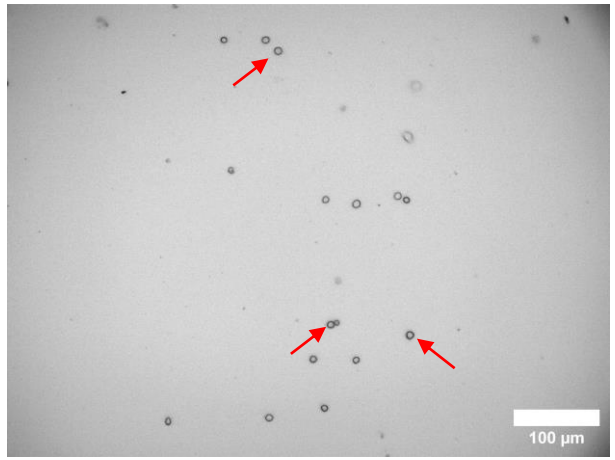
Thiol Biotin - Streptavidin – CD34 biotinylated Antibody Biointerface

- Real-time in-situ functionalization and cell capture on 10 MHz quartz sensor



Scheme 2





Microscopy images of quartz surfaces with captured cells

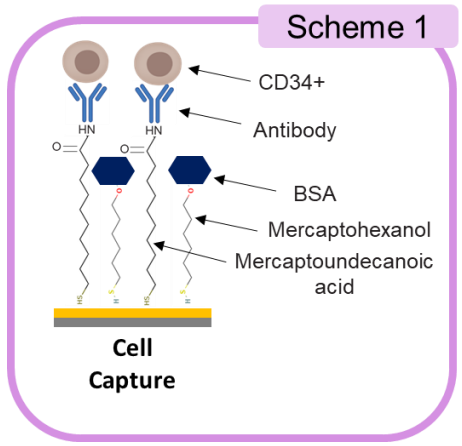
Quantification analysis:

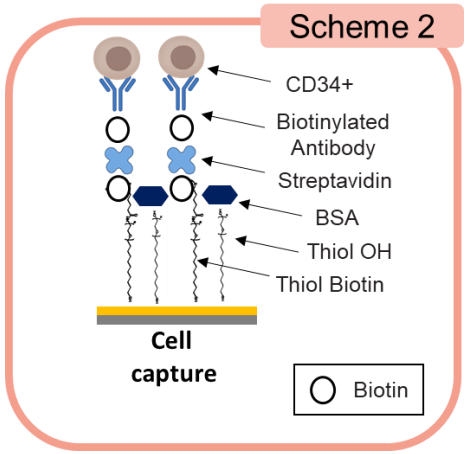
- A drop of **20 Hz** corresponding to 4400 cells captured out of 300k cells passed
- Single passage, concentration: 700k cells/ml
- **Efficiency of capture up to 1.5%**

Conclusion:

- Improved capture efficiency of up to **1.5%**
- Probably better antibody orientation

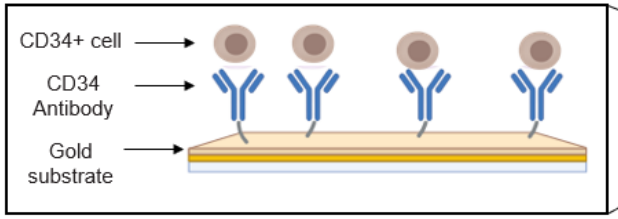
BIOINTERFACE AND CELL CAPTURE

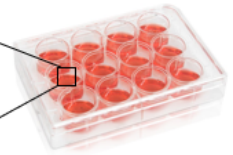




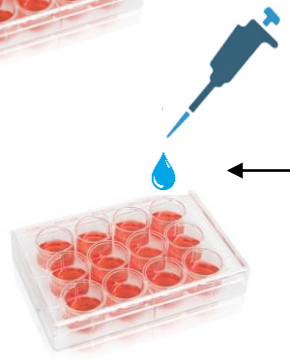
			Ex-situ	In-situ
TENA	XPS	**№ of Antibodies (ab/μm²)**	147	151
	Microscopy	**Capture (Cells/μm²)**	9.0 x 10 $^{-4}$	9.7 x 10 $^{-6}$
	Antibody orientation	**Specificity (%)**	80	-
TSBA	XPS	**№ of Antibodies (ab/μm²)**	224	**342**
	Microscopy	**Capture (Cells/μm²)**	10.1 x 10 $^{-4}$	**39,4** x 10 $^{-6}$
	Antibody orientation	**Specificity (%)**	85	-

REVERSIBLE EX-SITU CELL CAPTURE





Static CD34+ stem cell capture

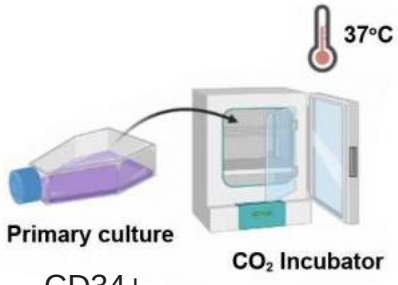


Addition of Accutase or 0.1 M Glycine – HCl to detach captured CD34+ stem cells



Observation of gold substrate surface after cell detachment

Check viability of detached cells using LUNA device



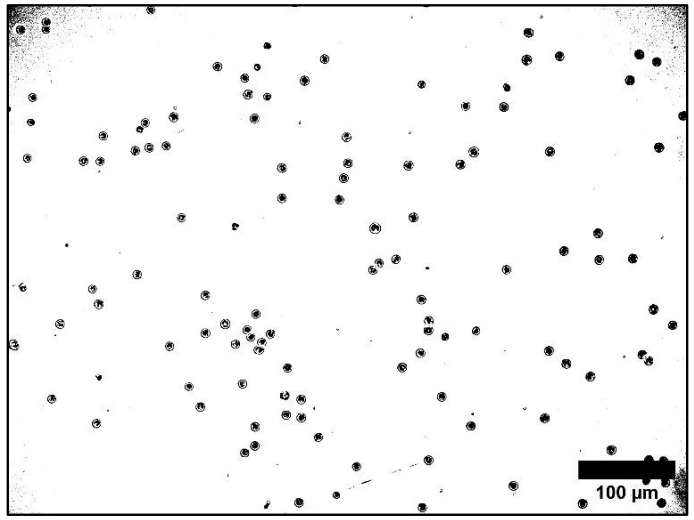
CD34+ stem cells

Culture of CD34+ stem cells after detachment to validate their viability and expansion ability

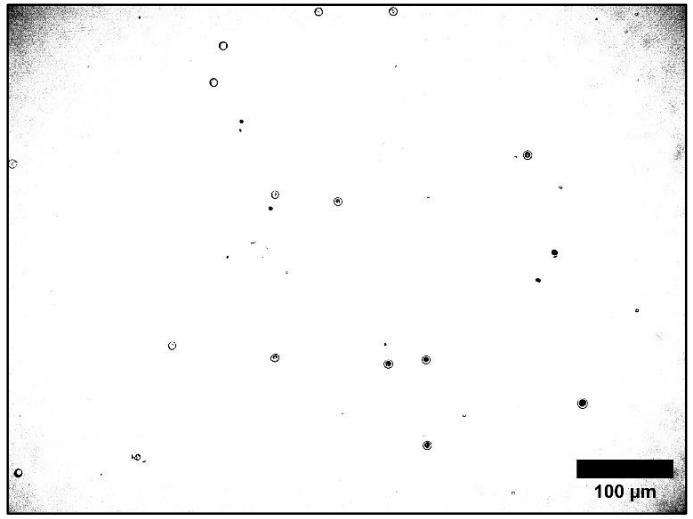
EUROSTIA 26

REVERSIBLE CELL CAPTURE

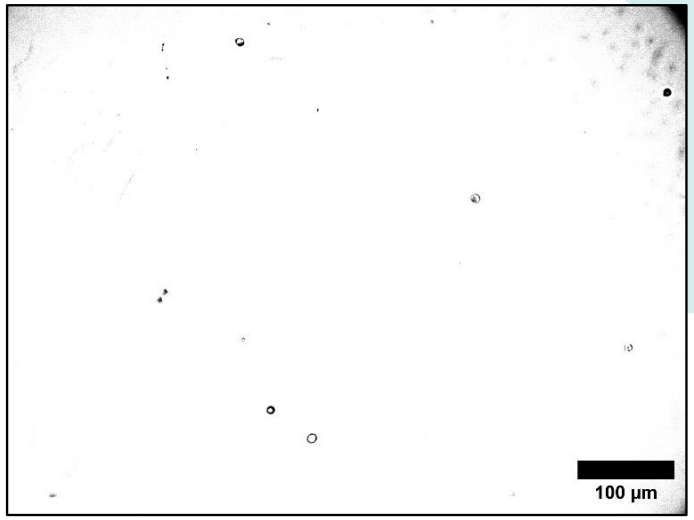
Positive Control



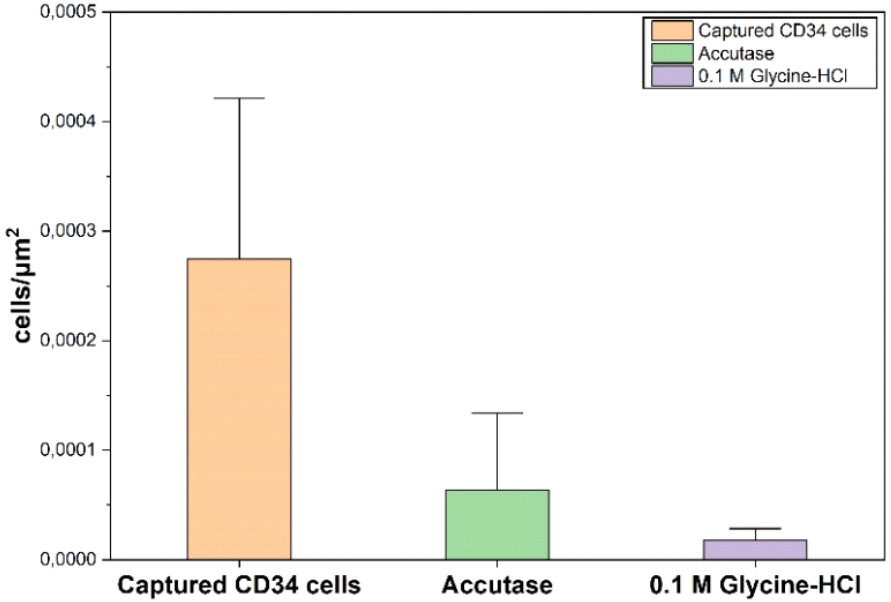
Captured $CD34^+$ cells on gold substrate



Gold substrate after cell detachment with Accutase



Gold substrate after cell detachment with 0.1 M Glycine - HCl



Surface density of cells after cell detachment

Repetitions
(N = 3 , n = 9)

45 images

Efficiency of cells detachment

$$\left|\frac{(Accutase\ )-(CD34+)}{(CD34+)}\right| x\ 100 = 76\%$$

$$\left|\frac{(Glycine\ \ HCl)-(CD34+)}{(CD34+)}\right| x\ 100 = 87\%$$

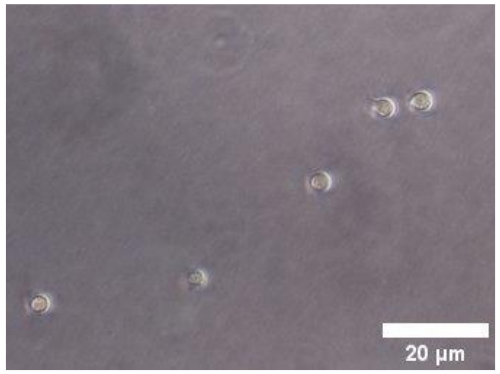
Efficiency of cells detachment :

Accutase – 76%
0.1 M Glycine HCl – 87%

Conclusion:
Glycine-HCl more efficient than Accutase.
What about viability of detached cells?

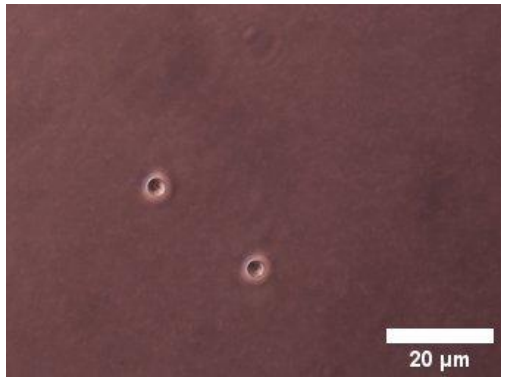
VIABILITY AND EXPANSION

Viability after reversible capture



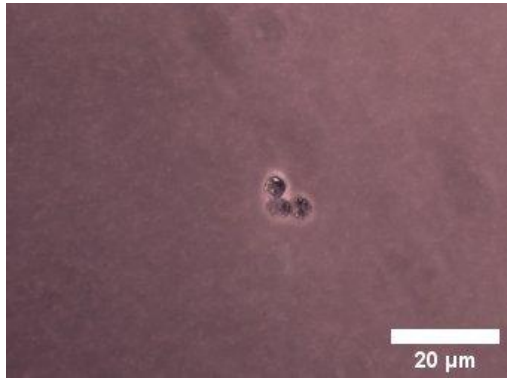
(a) Freshly thawed CD34+ cells

86% viability



(b) CD34+ Cells after detachment with Accutase

78% viability



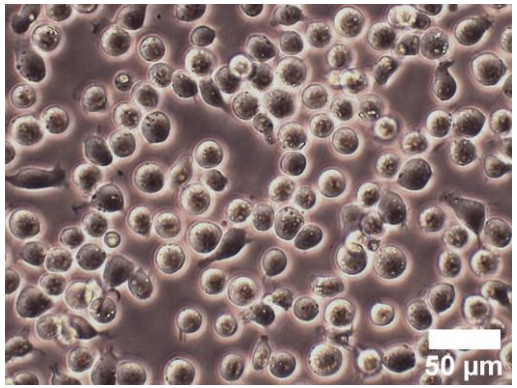
(c) CD34+ Cells after detachment with 0.1M Glycine-HCl

0% viability

Conclusion:

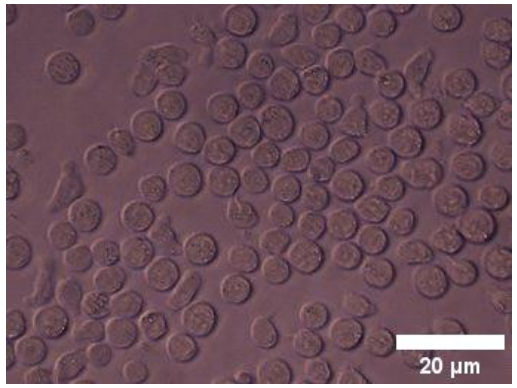
- The use of 0.1M Glycine-HCl indeed kills the cells
- Accutase keeps cell viability and makes it much preferrable for application in regenerative medicine.

Expansion of detached cells



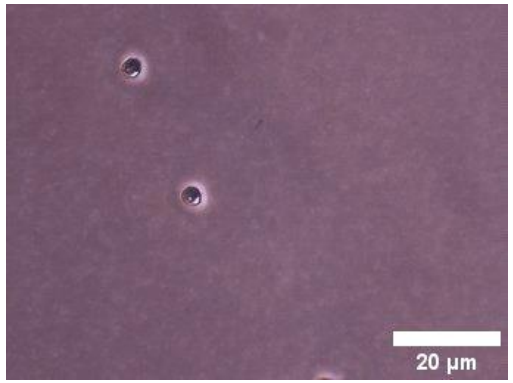
(a) Re-culture of CD34+ cells after 3 days

Cells expand after culture



(b) Re-culture of CD34+ cells detached with Accutase after 3 days

Cells expand after culture



(c) Re-culture of CD34+ cells detached with 0.1 M Glycine-HCl cells after 3 days.

Cells cannot expand after culture

Successful expansion of detached cells using Accutase

GENERAL CONCLUSION

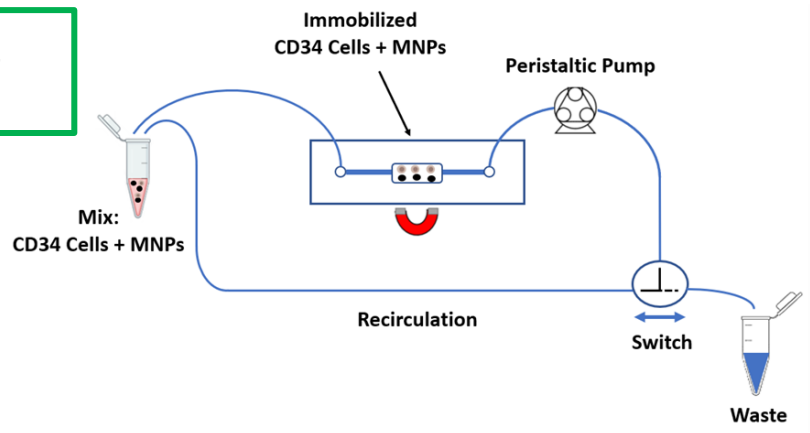
- **Successful** grafting of two **biointerfaces**
- **Thiol-biotin biointerface** shows improved **static cell capture Specificity** of up to 85% of CD34+ stem cells compared to CEMC1 cells
- It shows a higher **dynamic capture** with a frequency drop of 20 Hz but only **1.5% of the cells**
- **Successful** reversible capture with **Accutase** showing up to **78% cell viability**

PERSPECTIVES

Implementation in a microfluidic chip

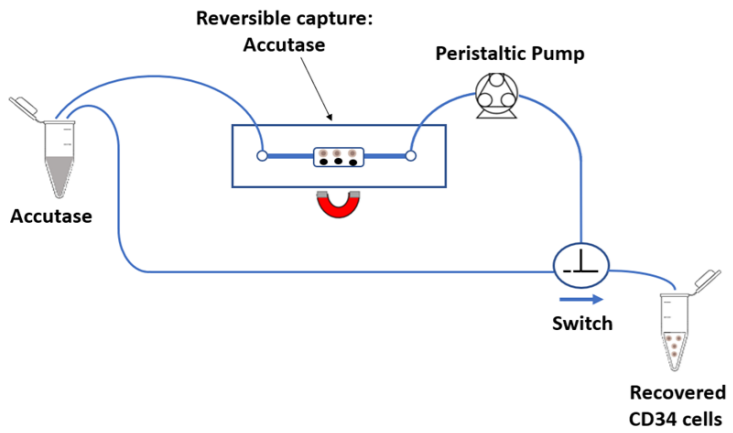
Dual capture

Capture

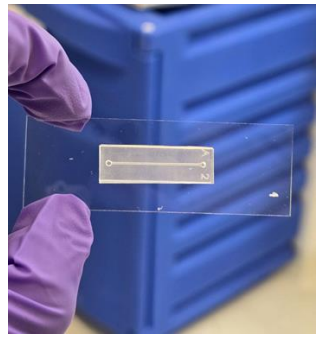


(a) Immobilization of CD34+ cells on MNPs

Release



(b) Recovery of CD34+ cells using Accutase



Microfluidic chip device fabrication

THANK YOU!

ACKNOWLEDGEMENT:

ECASIA 26

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Femto-st , BIND team

Questions?