Yesil-Celiktas Lab

Biomimetic Microsystems

Can microfluidics-enabled platforms expedite the assessment of immune response towards implantable biomaterials for the knee?

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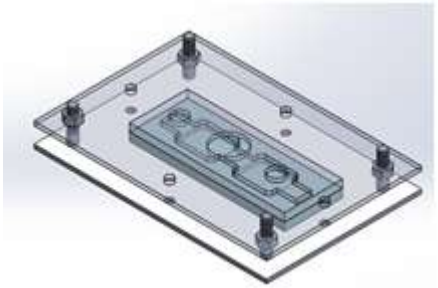
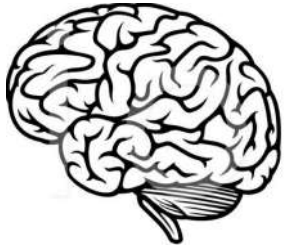
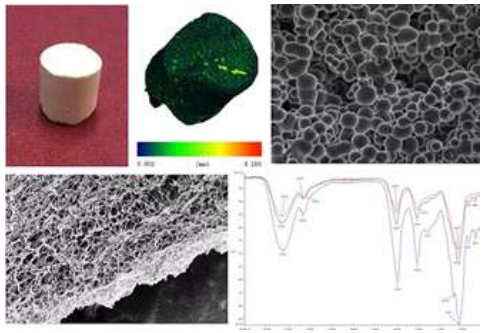
The focus - Biomimetic Microsystems (BioMIC)



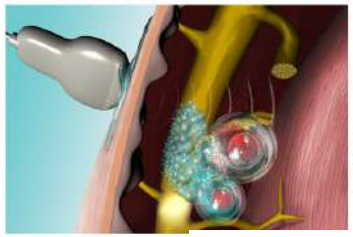
Microphysiological systems

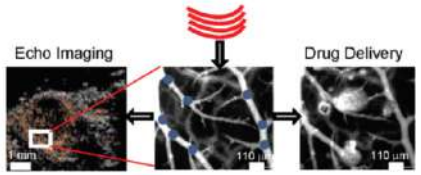


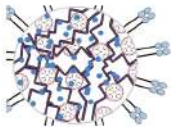
Biomaterials

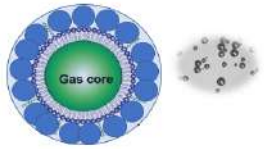


Responsive drug delivery







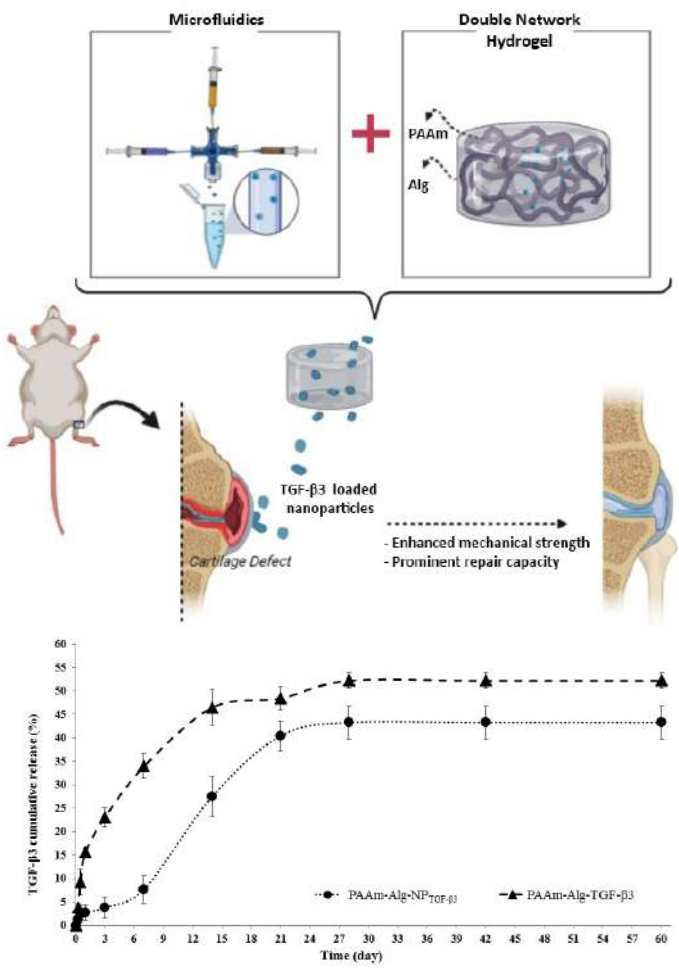


Implantable devices, biomaterials

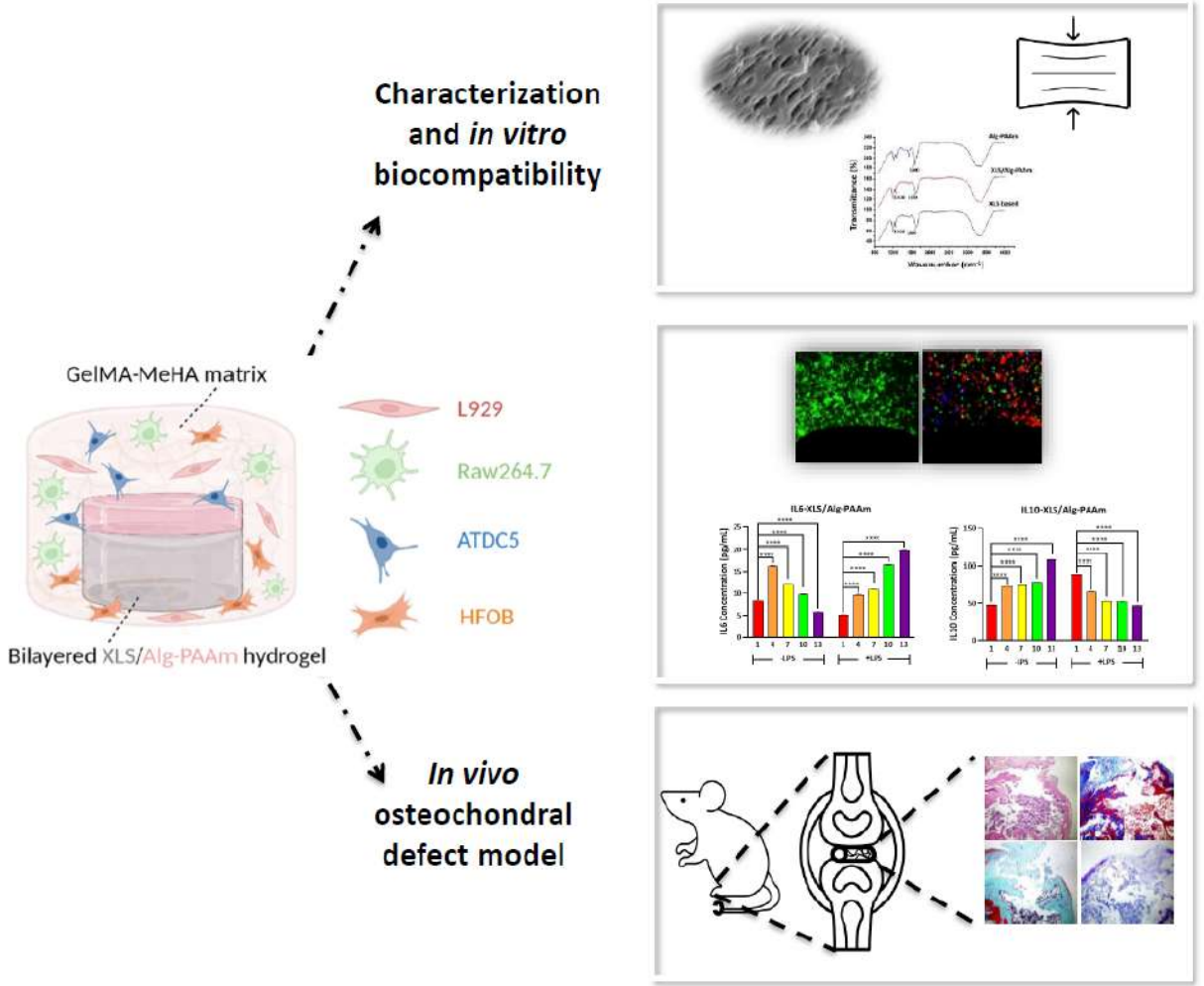
emerged as critical solutions for various healthcare problems and their use in either therapeutics or for preventive healthcare is well established.

However, adverse immune reactions in the host body is often a major barrier to their success

Implantable devices, biomaterials



Saygili et al. (2021) An alginate-poly(acrylamide) hydrogel with TGF-β3 loaded nanoparticles for cartilage repair: biodegradability, biocompatibility and protein adsorption. ***International Journal of Biological Macromolec*ules**, 172, 381-393



Saygili et al. (2022) Bilayered laponite/alginate-poly(acrylamide) composite hydrogel for osteochondral injuries enhances macrophage polarization: an in vivo study. ***Biomaterials Advances***, 2022, 134, 112721.

Human microphysiological systems

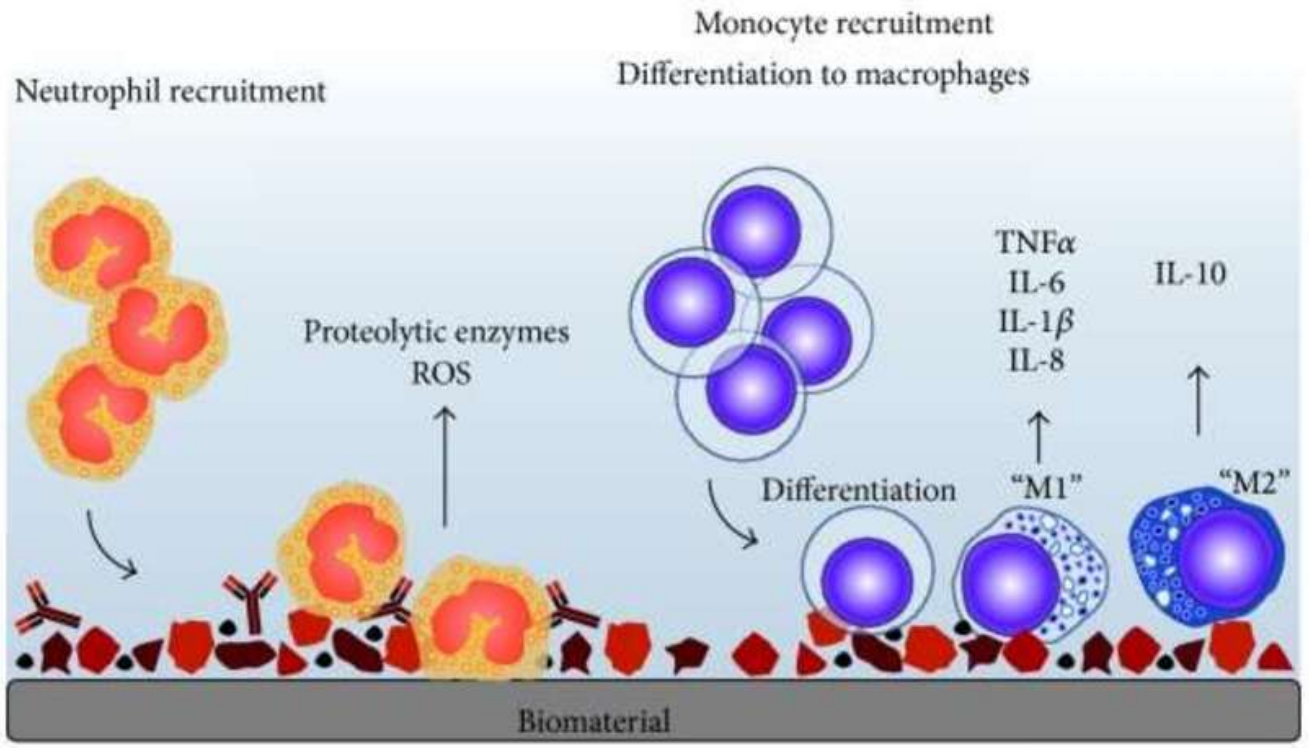
MPS consists of the platform (chip, device), the tissue construct, and the tissue culture media

These clusters of "assembled cells" have functional significance and can mimic *in vivo* organ structure.

Foreign body response-on-a-chip

The aim was to develop a microfluidic platform modeling the cascade of events during immune cell response to implants

This concept is adapted to study FBR in a physiologically relevant *in vitro* setting, through the development of a multilayered FBROC system to reproduce the Innate immune cell interactions with implants



FBR-on-a-chip platform

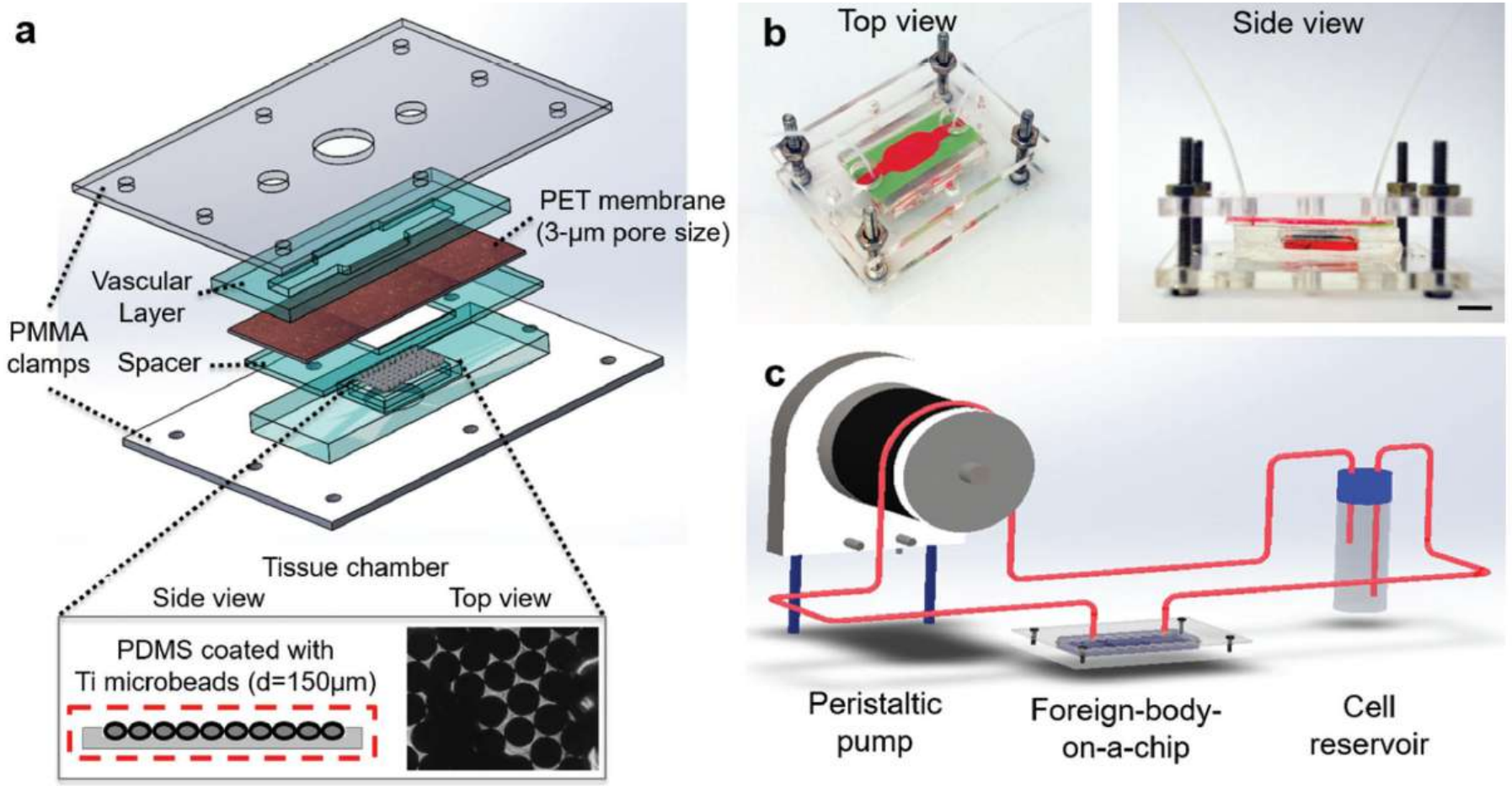
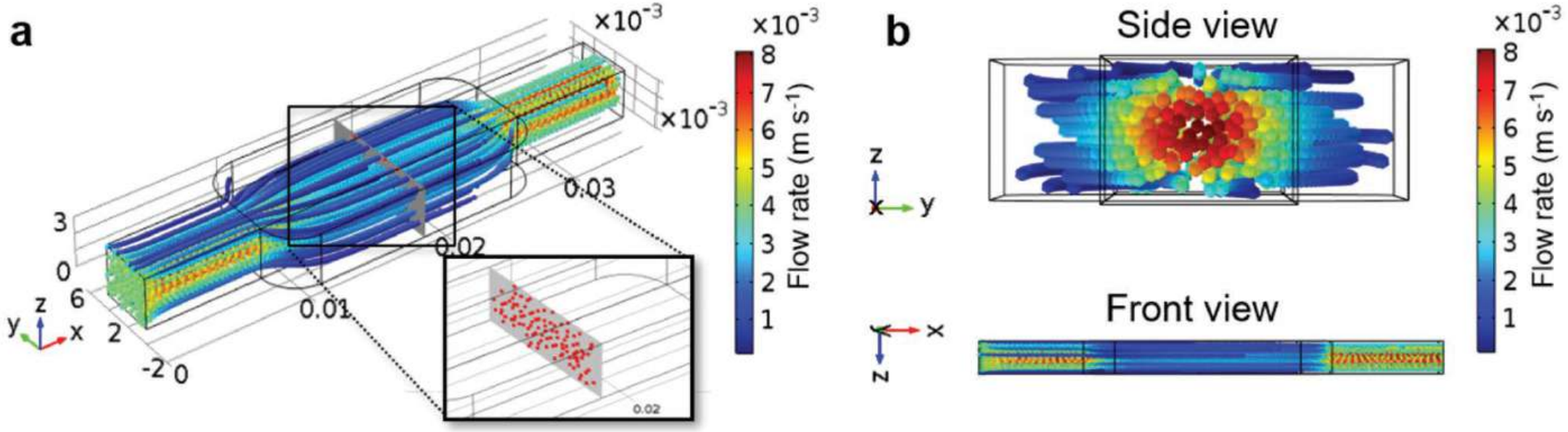


Figure 1. Design of the FBROC device. a) Exploded schematic diagram showing the multilayer structure of the bioreactor, where an endothelialized porous membrane is sandwiched in between a vascular channel on top and a tissue chamber at the bottom, the latter of which implant of Ti microbeads was placed. b) Perspective- and side-view photographs showing the bioreactor in the multilayer configuration. c) Schematic diagram showing the operation of the FBROC device, where immune cells are circulated from the top vascular channel of the bioreactor to probe their interactions with the Ti microbeads in the bottom tissue chamber through the endothalial barrier.

Simulations of monocyte distribution inside the vascular chamber

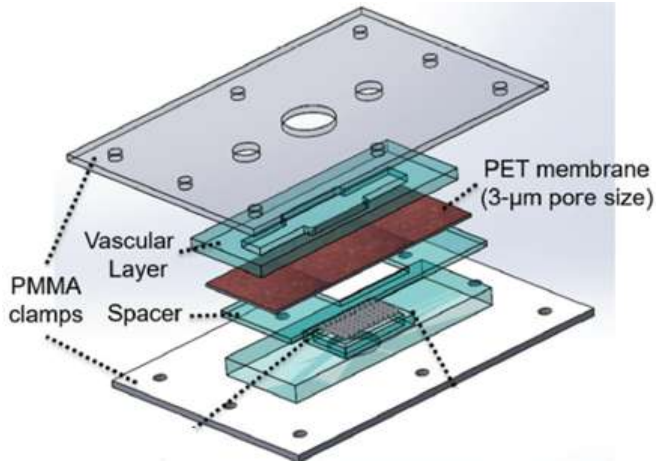
Monocytes at density of 1×10^6 cells mL^{-1} at the optimized flow rate of 400 µL min^{-1}

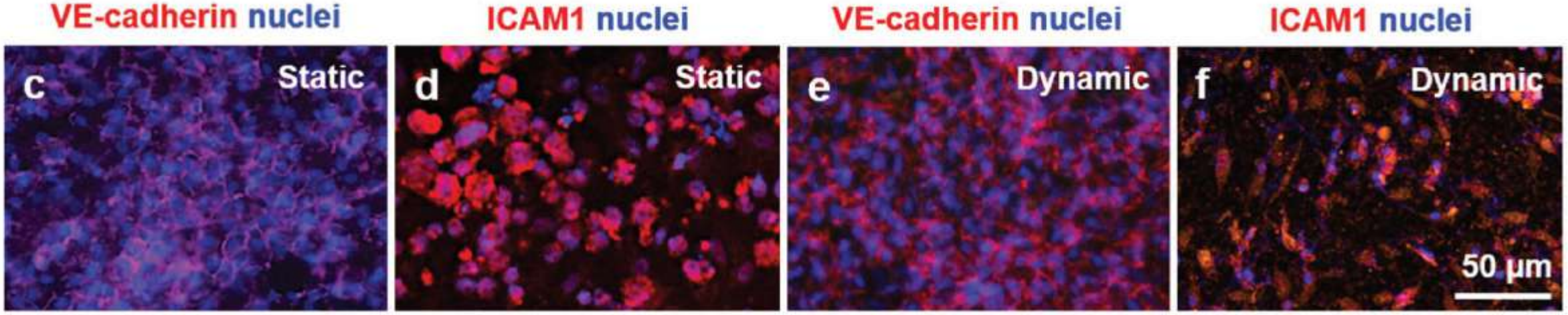
The cells could be uniformly distributed across the chamber emulating their homogeneous distribution



The functional endothelial barrier between the vascular and the tissue chambers

HUVECs were seeded on the PET membrane at a density of 7.5×10^4 cells cm^{-2}. Strong expression of endothelial biomarkers, indicating that the flow rate used did not have adverse effects on the function of the endothelial cells

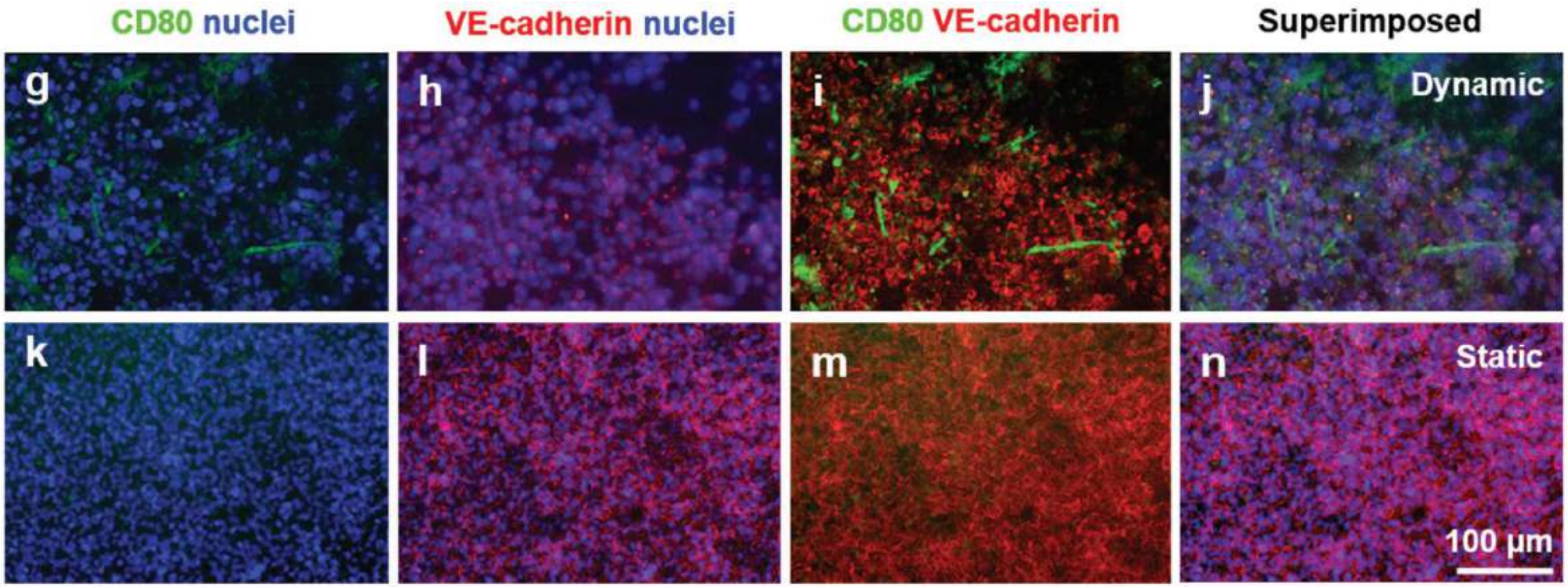




Immunostaining of VE-cadherin and ICAM for confluent HUVECs cultured under static and dynamic conditions on the porous PET membrane

Monocyte-endothelium interactions

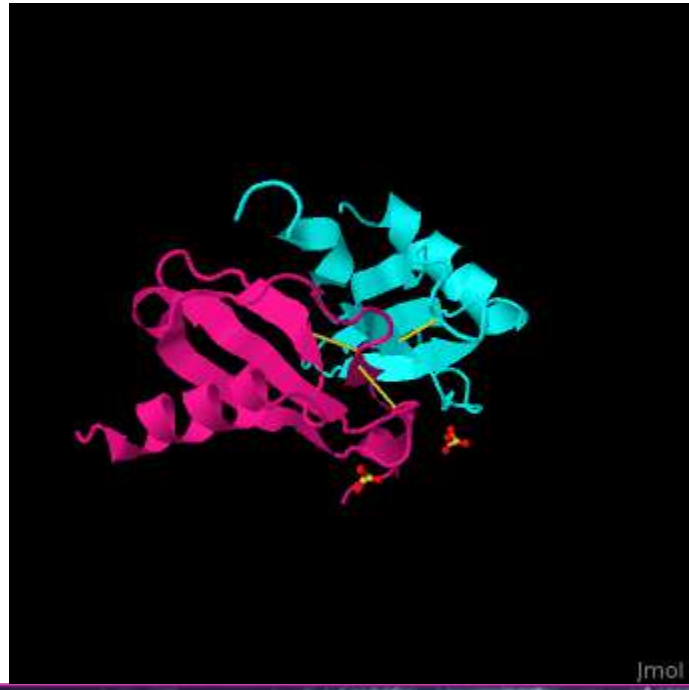
Immunostaining of THP-1 monocytes with CD80 antibody after 4 d of dynamic culture revealed the expression of CD80, suggesting the activation and interaction of THP-1 monocytes with the HUVECs through attachment and spreading on the endothelial barrier under fluid flow, possibly induced by shear stress. Under static culture condition, CD80 expression was minimal indicating that the TPH-1 cells became barely activated with limited interactions of these cells with the endothelium.



The migration of THP-1 monocytes through the endothelial barrier

Specifically, we compared the monocyte migration under static and dynamic conditions, as well as in the absence and presence of MCP-1.

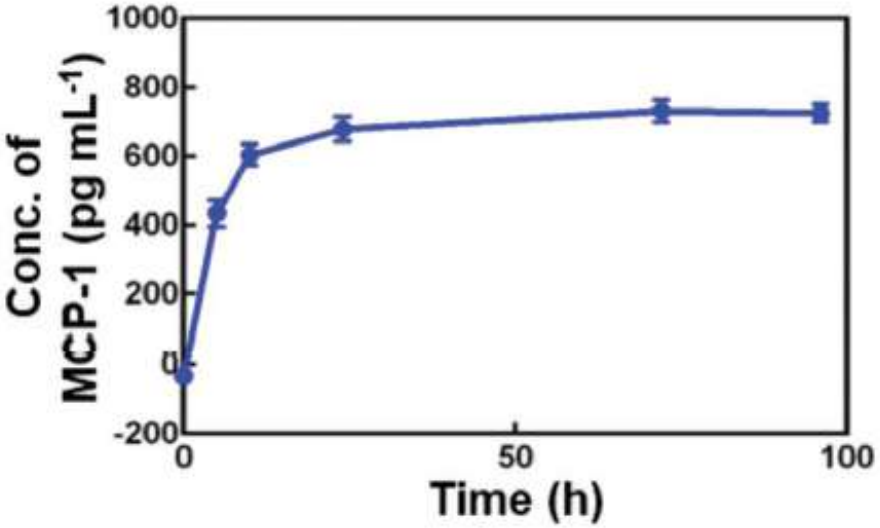
MCP-1 is a key chemokine regulating the migration/infiltration of immune cells such as monocytes and macrophages, and is released by the stromal tissues upon implantation of foreign materials during FBR.



The migration of THP-1 monocytes through the endothelial barrier

To mimic such a process, we encapsulated MCP-1 in a ring of GelMA hydrogel at the bottom chamber of the device, surrounding the tissue chamber coated with Ti microbeads.

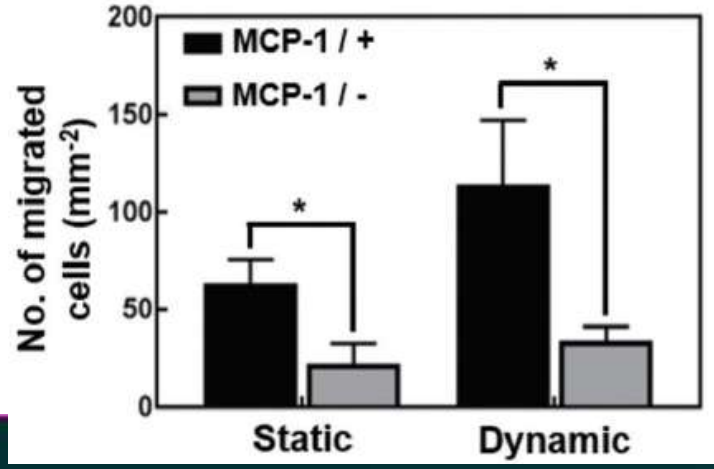
The release of MCP-1 from the GelMA ring was quantified by collecting the medium outflow from the top vascular chamber under the same perfusion condition in the presence of the endothelial barrier, which showed a sustained release profile over the 4-d period with an initial burst release in the first day.

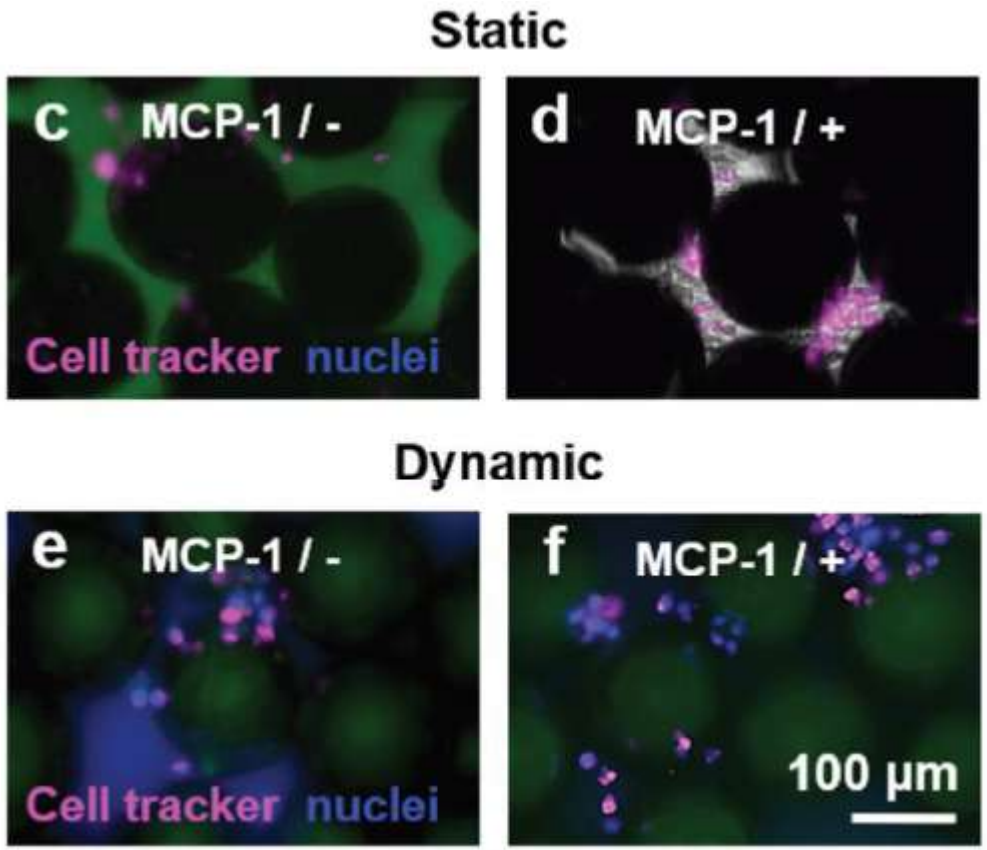


The migration of THP-1 monocytes through the endothelial barrier

THP-1 monocytes were cultured on the vascular channel on the top of the membrane with HUVECs under the **static condition** and **in the absence of MCP-1**, only very few cells could transmigrate through the endothelium to reach Ti microbeads at day 4. Increased number of THP-1 monocytes transmigrated when **MCP-1 was slowly released** from the GelMA ring for the same period of time.

Under the **dynamic flow condition**, migration was higher both in the presence and absence of MCP-1 compared to static conditions





The differentiation of THP-1 monocytes into M1 or M2 phenotype

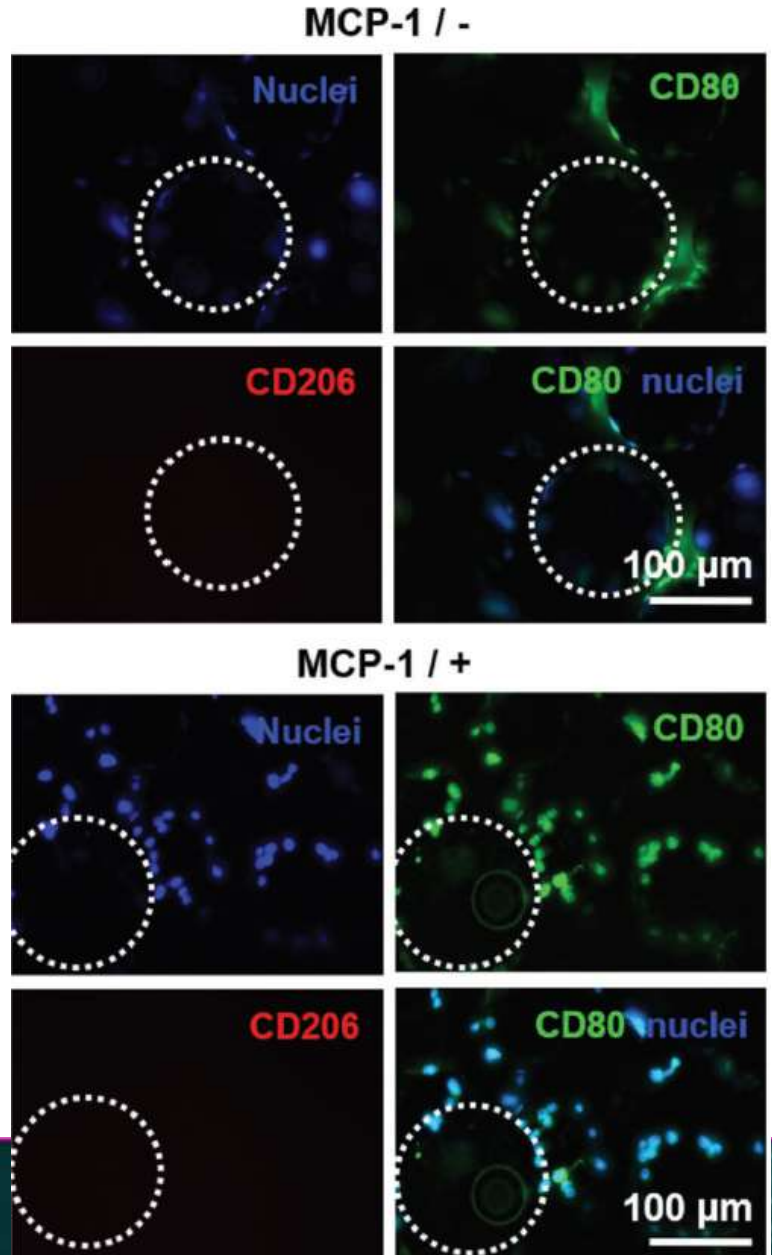
Evaluated by immunostaining of the cells with CD80 or CD206 antibodies, respectively, after they have migrated through the endothelial barrier and attached onto the Ti microbeads, under dynamic flow condition at day 4.

CD80 is M1-specific phenotype marker and thus overexpressed in pro-inflammatory (M1) subtype cell population, whereas CD206 is M2 phenotype marker whose expression is increased as the cells differentiate into anti-inflammatory alternatively activated (M2) subtype cell population

The differentiation of THP-1 monocytes into M1 or M2 phenotype

Increased number of transendothelial-migrated THP-1 cells were differentiated into CD80-positive cells, primarily located in the tissue chamber containing Ti microbeads, both in the absence and presence of MCP-1,

- indicating that the differentiation of THP-1 was towards the pro-inflammatory M1 phenotype
- confirming the recognition of the Ti microbeads as foreign body by the THP-1 cells



Concluding remarks

The design and fabrication of multilayered FBROC system to mimic the immune cell-foreign body interactions

Expanded to study personalized FBR on vast categories of subjects including but not limited to, implants reported in the current work as well as biologically active materials and engineered tissues

High throughput screening

A roadmap for potential standardization issues relevant for organ on chip

CEN CENELEC EUROPEAN STANDARDIZATION GET INVOLVED

Organ On Chip

In the short to medium term, more efforts are anticipated in engineering advanced microfluidic systems to develop organ-on-chip platforms for predictive translation of preclinical findings into clinical studies

In June 2021, the Technical Boards of CEN and CENELEC have set up a Focus Group on Organ on chip. The secretariat of the Focus Group is held by NEN. The Focus Group will hold its first kick-off meeting virtually on 02 March 2021.

The CEN-CENELEC Focus Group on Organ on chip (hereinafter 'FGOOC') shall ensure interaction between all relevant European stakeholders interested in potential standardization in the field of organ on chip, map ongoing activities, define priority needs and opportunities and recommend further action to ensure that standards support the deployment of organ on chip in industry and help to ensure its regulatory acceptance.

The FGOOC has been created following the outcome of the 'Organs on chip: building a roadmap towards standardization' workshop organized in 2021 together with the **Joint Research Centre (JRC),** which identified initial needs for standardization in the field of 'Organs on chip and set the basis for an active dialogue and cooperation between the communities of researchers and standardizers.

WG1: terminology, ecosystem, interdependencies

WG2: biosciences

WG3: engineering

WG4: experimental design and data Management

WG5: user perspective and regulatory Legal and ethical aspects

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