

## Coagulation induced by biomaterials

Over the last several years, a lot of advances in cell biology have opened the door to regenerative medicine, both at tissue and organ level. Promoting the culture of these tissues is troublesome, and it usually requires a complex setup in 3D. To facilitate the three-dimensional tissue formation, an **scaffold** is often employed. It consists of a support (biological or synthetic) that allows cell attachment and growth in an organized way<sup>1</sup>.

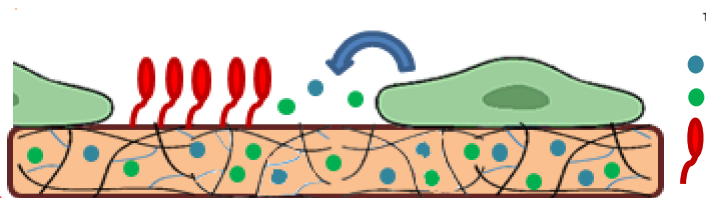
There are a lot of different biomaterials with diverse characteristics that can be used as scaffold, depending on the tissue we want to reproduce. Nowadays, one of the most employed biomaterials are **hydrogels**, due their mechanical and physical properties, their stability at physiological pH and temperature and because it has been proved that they are not cytotoxic to the surrounding tissue<sup>2</sup>.

Unfortunately, the contact of the blood directly with the scaffold surface promotes unspecific protein adsorption, which entails blood cells adhesion, like platelet or erythrocytes and finishes in **intrinsic coagulation pathway activation**. This coagulation ends creating a thrombus, which can obstruct the blood flow through the circulatory system, causing hypoxia and the subsequent organ or **tissue atrophy**, or even an embolism if the thrombus becomes detached<sup>3</sup>. **Preventing thrombosis is currently one of the major challenges for biomaterials.**

## Objective

To determine if the designed biomaterial can prevent the blood coagulation at the same time that it acts as a scaffold, testing it *in vitro*.

## Designed biomaterial



- Hydrogel (Poly(ethylene glycol))
- VEGF (Growth factor)
- PDGF-BB (Growth factor)
- Heparin

**Fig 2.** The designed biomaterial consists of a hydrogel (PEG) scaffold with encapsulated growth factors as VEGF and PDGF-BB, coating with heparin (an antithrombotic enzyme) and endothelial cells cultivated over it.

## Methodology

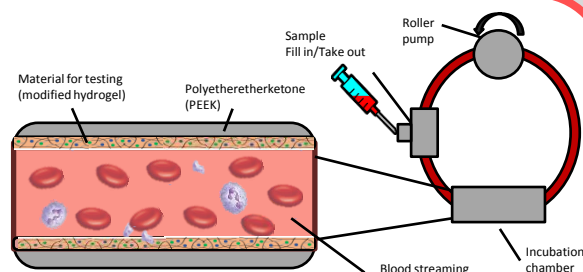
In order to validate the thrombogenicity of the designed material several analyses will be performed. The studies will be done on perfusion systems, where blood is kept in movement at a given velocity.

Blood will be obtained from healthy donors, and treated in advance with a small heparin dose (1U/ml) to prevent coagulation until the start of the experiment. The blood will be recirculated through the circuit showed in **Fig 3**.

The blood will be analysed before and after the incubation step in the circuit. Cell number will be measured using a Coulter Counter. ELISA assays will be performed to quantify the presence of components characteristic of coagulation (such as thrombin-antithrombin), immune response (C5a, C3a), platelet activation (platelet factor 4) and leukocyte activation (Elastase).

Hemolysis will be tested via free hemoglobin quantification in plasma.

Cell adhesion to the material's surface and any morphology changes will be examined using Scanning Electron Microscopy (SEM) and by Confocal Microscopy.



**Fig 3.** Blood will be recirculated through a circuit consisted of 3mm diameter silicone tubes, a section with valves that allow the affluence and exit of blood from a reservoir, a roller pump to regulate the blood flow and the incubator chamber. In order to maintain the pH value, CO<sub>2</sub> and temperature constant, all the experiments will take place in an incubator<sup>4</sup>.

## Conclusions

We have proposed a method to test the hemocompatibility of a new modified hydrogel-based biomaterial has been proposed. If the results are promising, it could bring the chance of using a new biomaterial capable of avoiding coagulation while maintaining its mechanical and chemical properties.

By coating the hydrogel with heparin, the likelihood of gaps in direct contact with the blood would be bypassed, while encapsulating growth factors inside would allow inducing a controlled attachment of endothelial cells.

This hypothetical new biomaterial could have a great potential for tissue-engineering, but more assays should be done before determining their full functionality.

**Project duration:**  
4 years  
**Estimated cost:**  
89.600€

## References

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- 3.Gorbet, M.B., and M.V. Sefton. "Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes". *Biomaterials* 25(26), 2004:5681–5703.
- 4.U. Streller, C. Sperling et al. Design and Evaluation of Novel Blood Incubation Systems for *In Vitro* Hemocompatibility Assessment of Planar Solid Surfaces. *J Biomed Mater Res B Appl Biomater*, Jul 15 2003; 66(1):379-90