Comparison of blood vessel decellularization and preconditioning with whole blood

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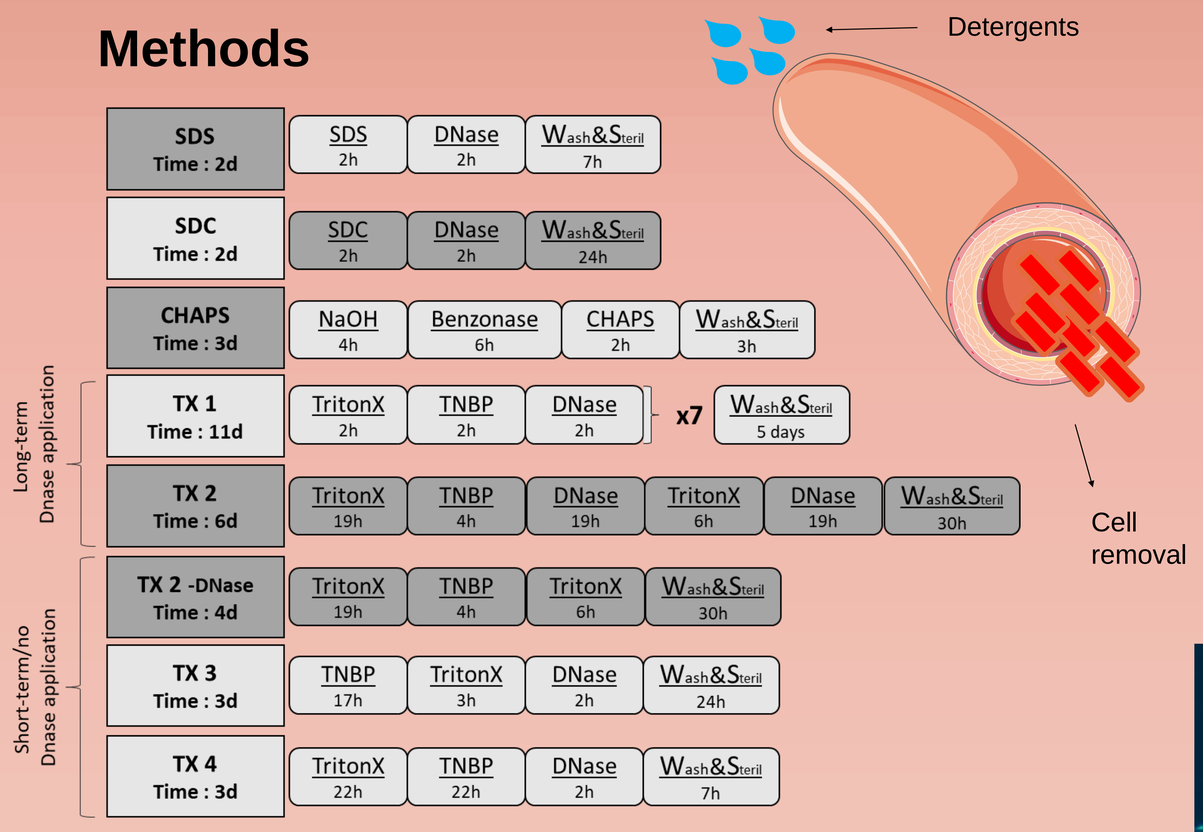
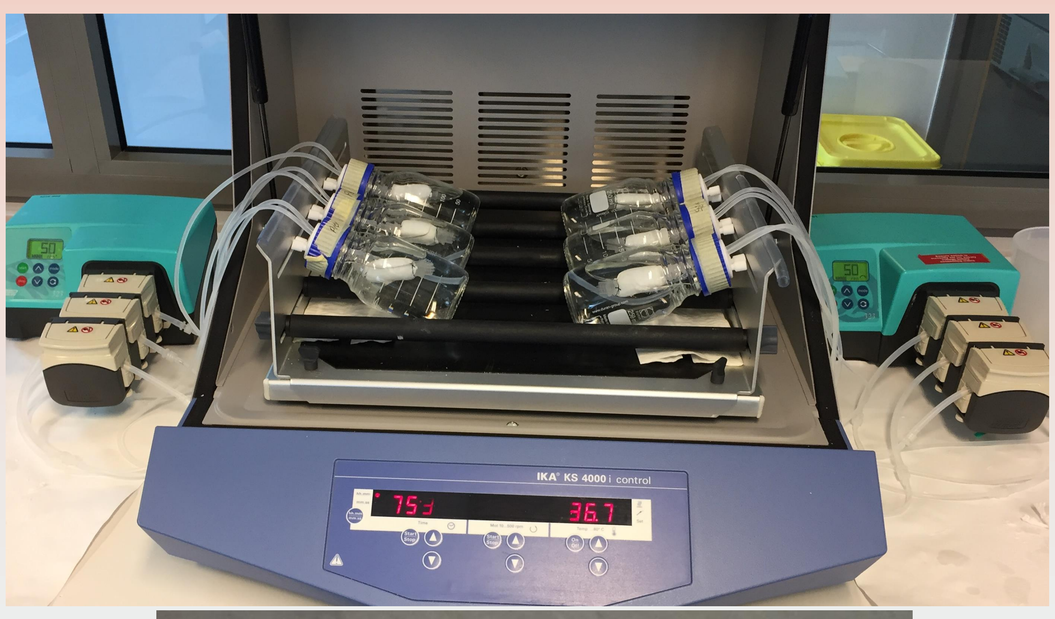
Background

Vascular diseases often require transplantation of biomaterials at the target site. The Extracellular Matrix (ECM) of blood vessels can be used as a vascular graft, since it is an ideal (stem) cell niche due to the presence of growth factors and cytokines (1). It can be decellularized and used as a scaffold for recellularization with the patients' own cells, which help preventing thrombosis. There are multiple protocols for the decellularization of blood vessels, but varying experimental conditions make comparison difficult. In this study we compared commonly used detergents for decellularization and subsequent recellularization with the patients' whole blood.

Methods

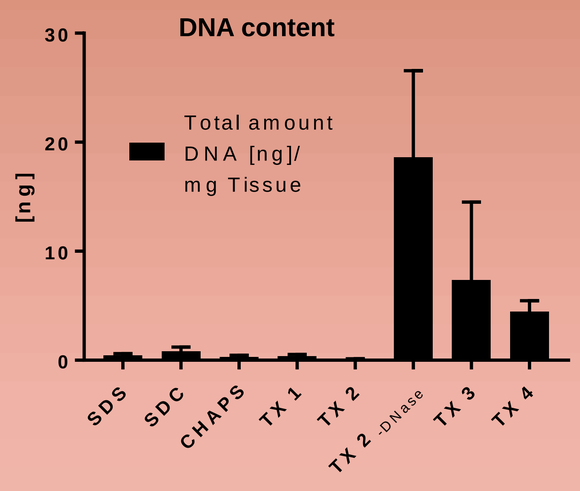


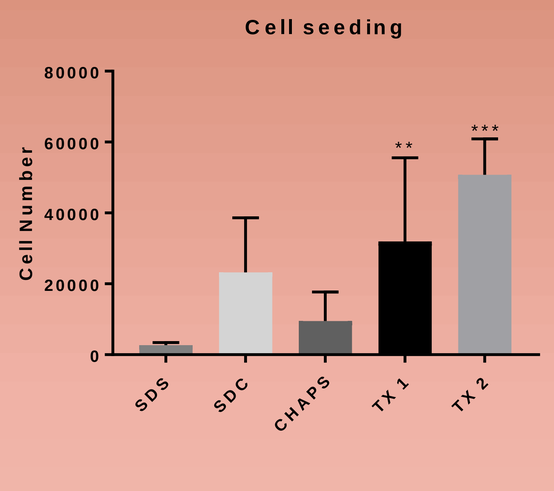
The commonly used detergents SDS, SDC (sodium deoxycholate), CHAPS and multiple protocols for TritonX were compared for their cell removal efficacy in a perfusion bioreactor setup on porcine vena cava. Parameters compared were DNA content, histology, ECM composition, biodegradability, mechanical properties, collagen denaturation, cytotoxicity and recellularization with HUVEC cells and whole peripheral blood.



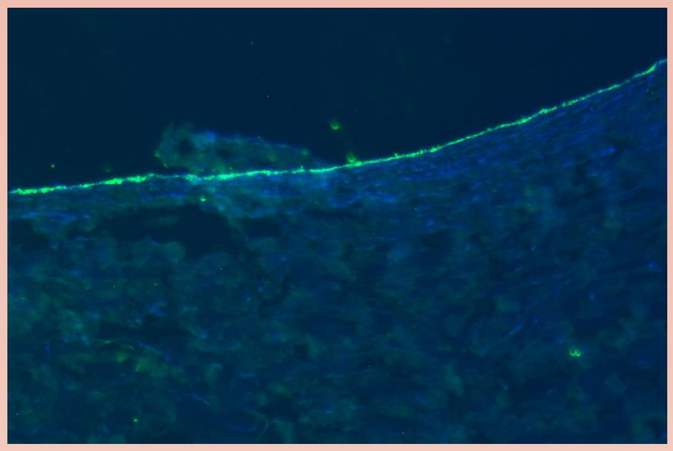
Results

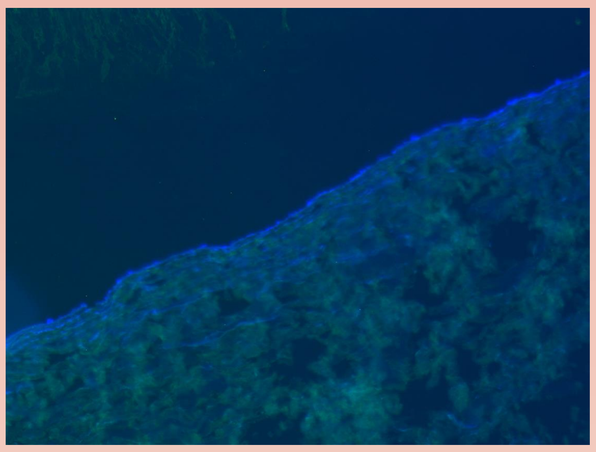
Our results show that the detergents SDS, SDC, CHAPS and Triton X (protocol 1 and 2, utilizing long term Dnase treatment) could efficiently remove cells from the ECM. Methods utilizing short term DNase application were not successful. Also, mechanical properties were similar to the native blood vessel, and morphology, collagen denaturation and biodegradability were not majorly altered. Cell seeding with HUVECs showed best attachment to blood vessels decellularized with TritonX.





Furthermore, we tested the recellularization with 25 mL whole peripheral blood for 1 week to investigate attachment of endothelial (progenitor) cells from the blood on the surface of the vessel (2). We observed attachment of CD31+ cells at parts of the vessels, while other parts did not show viable cells.





Conclusion

Blood vessel decellularization and subsequent recellularization ist most efficiently achived with TritonX protocols in combination with Dnase. Recellularization with peripheral blood shows potential as an application in regenerative medicine but currently yields inhomogenous results

Novel findings

- Blood vessel decellularization with TritonX requires extensive DNase treatment
- Long term incubation in a perfusion bioreactor increases stiffness of the ECM
- Intensive DNase treatment increases collagen denaturation

References

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