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COMPARATIVE EFFICIENCY OF DETERGENT-BASED DECELLULARIZATION PROCEDURES IN VASCULAR TISSUE ENGINEERING

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Introduction

Atherosclerosis is the leading cause of mortality and morbidity across the world. Conventional therapies available today offer good clinical results, however, the *„gold standard” treatment* is considered *surgical bypass grafting*. It assumes blood flow improvement by reconnecting blood vessels with *specific vascular conduits*, *biological or synthetic* ones.

Studies concerning the evaluation of different grafts' patency rates have been conducted already. They have shown satisfactory results for replacement of large- and medium-diameter arteries. Nevertheless, *the optimal vascular substitutes appliable to small-diameter vessels are still in the research and development*.

Considering these limitations, high attention has been focused on manufacturing vascular grafts by tissue engineering techniques (TEVGs). A number of different approaches have been taken in this research field. They can be broadly categorized into *scaffold-based methods using synthetic or natural materials and decellularized natural matrix techniques* (Fig. 1).

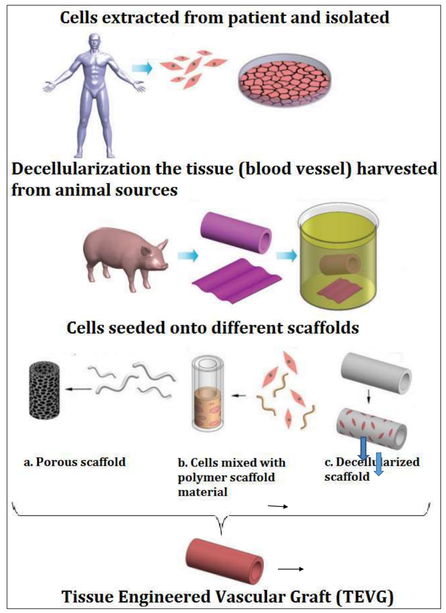


Fig. 1 **TEVGs manufacture using decellularized/polymeric matrices**

Keywords

Tissue engineering, vascular graft, extracellular matrix, decellularization

Purpose

To evaluate the effect of the detergents widely used in tissue decellularization on histology of blood vessels and to understand their potential impact on functional changes.

Material and methods

Fresh porcine aortas (PAs: lenght 70–170 mm, lumen diameter 14–25mm, wall thickness 2–3mm) were obtained from a local meat . The samples were cleaned off excess connective tissue and fats and rinsed in ddH_2O for 24h (Fig. 2).

The research protocol included 5 experimental and one control group (Tab. 1).

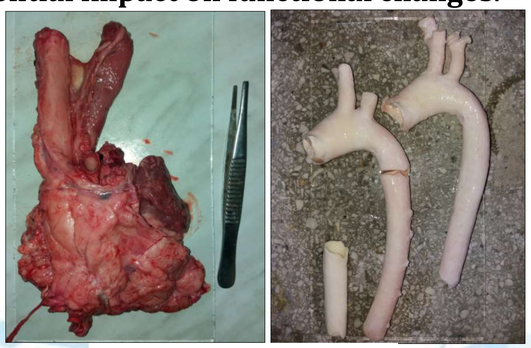


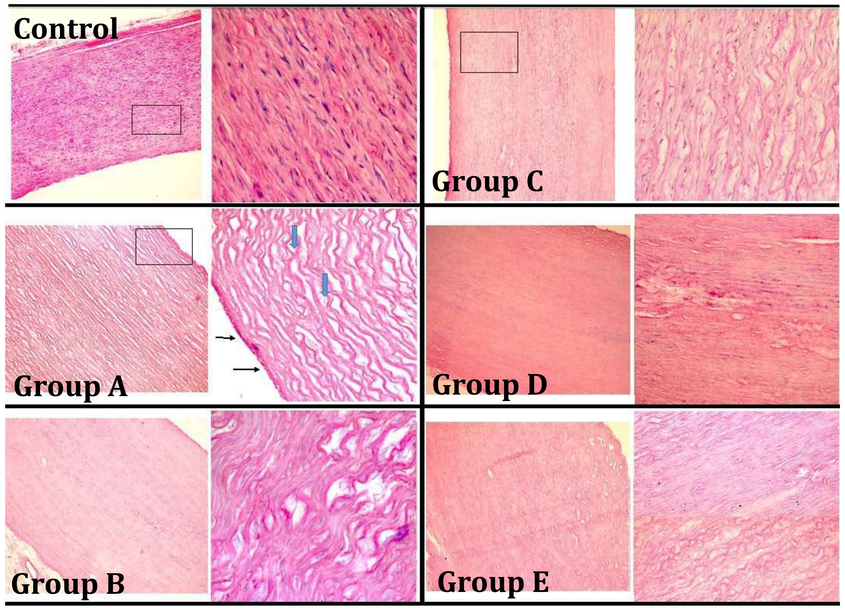
Fig. 2 **Fresh porcine aortas**

Material and methods

Continuous shaking (150 rpm) at 37C for 48hrs					
A.	B.	C.	D.	F.	CONTROL
1%SDS	**1%SDC**	**1%TX-100**	**0,5(SDC+SDC)%**	**0,5(SDS+SDC+TX)%**	**PBS**
Washing step in PBS solution for 24hrs					

For statistical calculations, SPSS software was used. Data were expressed as DNA mean in ng per milligram (ng/mg) ± SD. Statistical significance was obtained by one-way ANOVA in conjunction with Turkey post-hoc procedure. The significance level was set up at p=0,05.

Results



Hematoxylin / eosin staining of native and decellularized PA (magnification, ^{X}10 / ^{X}40)

Native tissue: normal aorta with normal tunica media and tunica intima in adult pig

Group A: complete decellularization with few endothelial cells remaining; mostly of collagen fibers preserved

Group B: decellularization quality >60%; elastic fibers structure significantly altered

Group C: remnant cells visible over all the layers

Group D: decellularization quality >60%

Group E: almost no nuclear material visible (>80%)

DNA quantification

DNA quantification (ng/mg) $\text{Nucleic Acid Concentration} = \frac{OD\ 260 - ODblank}{Pathlength} * \text{Standard Coefficient} * \text{Sample Dilution}$					
A.	B.	C.	D.	F.	CONTROL
60,71±5,60	54,24±15,63	29,97±12,05	59,82±6,18	54,91±6,21	88,39±12,44

All DC groups had significantly lower DNA content (p<0,05) compared to native tissue.

Conclusions

Detergent-based decellularization technique were found to be more effectively in cellular components elimination. However, biocompatibility and mechanical properties assessments should be carried out in future studies.