Mechanical Response of Explanted Collagen-nanofiber Composites Intended for Pulmonary Artery Banding

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Abstract: Pulmonary artery banding (PAB) is an intervention used in neonates suffering from certain congenital heart diseases to reduce pulmonary over-circulation. Our project deals with the research and development of a new banding material based on collagen-polycaprolactone (COLL-PCL) and collagen-poly[L-lactide/caprolactone] (COLL-PLCL) composites. The composites are biodegradable and give the advantage of PAB as a one-step procedure. However, the rate of degradation of the composites in a real body environment is unknown but can be determined by means of biomechanical analysis. COLL-PCL and COLL-PLCL materials were prepared by electrospinning, followed by the impregnation of the layers with collagen (type I) dispersion and implanted into the abdominal walls of adult Wistar rats. The composites were explanted after 4 weeks and their mechanical properties were evaluated by means of the uniaxial tensile test. Both COLL-PCL and COLL-PLCL were found to maintain their integrity up to explantation, and the results of the tensile experiments confirmed sufficient ultimate stress (0.51 ± 0.13 MPa).

Keywords: caprolactone; L-lactide; nanofiber composite; pulmonary artery banding; tensile test.

1 Introduction

There are several procedures in the field of cardiovascular surgery which are based on the mechanical interaction of the arterial wall and the external support. A typical example is Pulmonary Artery Banding (PAB) in infant patients [1–3]. PAB is a palliative procedure that reduces pulmonary over-circulation in neonates suffering from certain congenital heart diseases and constitutes the first stage of intervention prior to the complete repair of cardiac defects. The principle of PAB consists in the implantation of a band around the pulmonary artery, which results in the reduction of blood flow.

Our project deals with the research and development of a new banding material based on collagen-nanofiber composites, which are biodegradable and give the advantage of PAB as a one-step procedure. The rate of degradation of the composites in a real body environment is unknown. However, PAB materials have to maintain their integrity for a sufficiently long time to be capable of fulfilling their hemodynamic function. Thus the present study is designed to determine whether PAB materials based on biodegradable collagen-nanofiber composites will preserve their integrity after a 4-week exposition to *in vivo* conditions.

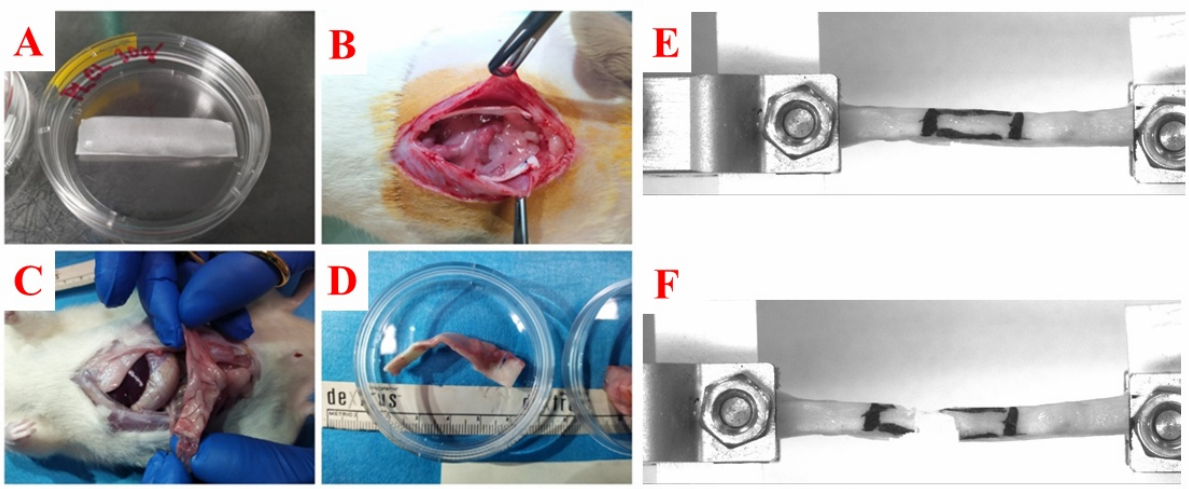

Fig. 1: Representative example of PAB composite. (A) Banding before implantation moistened for 1 hour by physiological solution; (B) Two bandings implanted and fixed by suture on the abdominal wall; (C) Banding 4 weeks after implantation; (D) Explanted banding; (E) A sample mounted in the testing machine; (F) The resulting state after tensile test.

2 Methods

COLL-PCL and COLL-PLCL composite bandings were prepared from nanofibrous layers based on L-lactide/caprolactone copolymer (PLCL; 70/30 mol/mol; PLC 7015, Corbion, The Netherlands) or polycaprolactone (PCL; Merc, USA). The PLCL nanofibrous layers were prepared by needleless electrospinning (NS 1WS500U, Elmarco, Czech Republic) under a direct current from a 10 wt% solution of chloroform and ethanol (Penta, Czech Republic) in 8:2 weight ratio. PLCL layers were prepared with a surface density of 15 (PLCL 15) and 30 (PLCL 30) g/m^2, both with an average fiber diameter of 50–1,000 nm. PCL nanofibrous layers were prepared by needleless electrospinning (NS 1WS500U, Elmarco, Czech Republic) under a direct current from a 16 wt% solution of chloroform and ethanol (Penta, Czech Republic) in 8:2 weight ratio. PCL layers were prepared with the surface density of 30 (PCL 30) g/m^2, with an average fiber diameter of 50–700 nm. The matrix of the composite was composed of type I collagen (COLL, calf skin, VUP Medical, Czech Republic). The volume fraction of all fibrous reinforcement in the composite was 70 $\pm$ 10 vol%. The composite bandings were prepared using the following procedure. An aqueous collagen dispersion (5 wt%) was prepared by means of the swelling of collagen in deionized water (37 °C for 12 hours), double homogenized using a disintegrator (5.000–15.000 rpm, 1–20 min with a delay of 20 minutes). Following homogenization, five separate layers of nanofibrous reinforcement were impregnated with this dispersion, placed in form and left for 36 hours at room temperature. After achieving the constant weight of composites, the stability of the collagen component was enhanced by cross-linking with a 95 wt% ethanol solution containing EDC (N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride) and NHS (N-hydroxysuccinimide) (4/1 w/w, Sigma Aldrich, USA). Following a reaction period of 2 hours at 37 °C, the composites were washed in 0.1 M Na_2HPO_4 (for at least 2$\times$ 20 min), followed by rinsing with deionized water (for at least 2$\times$ 20 min) and left in a form for 36 hours at room temperature until the constant weight was reached. The final stage involved cutting the samples into 40 mm $\times$ 6 mm strips and sterilization by gamma irradiation (25 kGy, BIOSTER, a.s., Czech Republic). Fig. 1A documents the final composite in the hydrated state ready to be implanted into the experimental animal.

Experimental implantation was approved by the Animal Welfare Advisory Committee of the Ministry of Education, Youth and Sports of the Czech Republic (approval ID MSMT-19760-2020-3). Six male Wistar rats aged 41 $\pm$ 2 weeks (Masaryk University Brno, Czech Republic) were divided into 3 groups; a group with implanted COLL-PLCL 15g, COLL-PLCL 30g and COLL-PCL 30g. The surgical procedure was performed under general anesthesia. The anesthetized animals were placed on a tempered operating table and continually supplied with oxygen through a face mask and monitored by pulse oximetry. Then an approximately 30–40 mm incision was made on the *linea alba* and on the abdominal wall, where 2 composite bands were inserted into the peritoneum (that is $n = 4$ for COLL-PLCL 15g group, $n = 4$ for COLL-PLCL 30g and COLL-PCL 30g groups). Bandings were fixed at the implantation site with a non-absorbable suture. The wound was closed in 2 layers

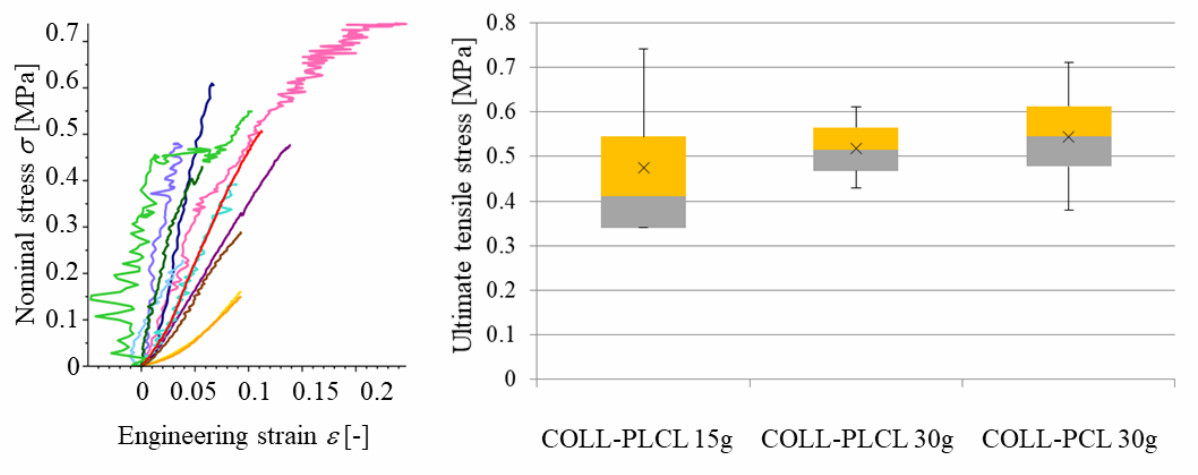

Fig. 2: Results of the uniaxial testing. Mechanical response in the first loading cycle (left). Box-plot of the nominal strength for all investigated groups (n = 4 in each group). There is not a statistically significant difference amongst the medians at the 95% confidence level.

with an absorbable suture and treated with a liquid bandage. Following surgery, the animals were provided with analgesia for 3 days and the animals were kept under conventional conditions (12/12 dark/light cycle) for the duration of the experiment according to EU directive 2010/63/EU in sterile polycarbonate microisolators (Bioscape, Castrop-Rauxel, Germany) with bedding (Lignocel Select fine, JRS, Rosenberg, Germany) and free access to water and pelletized feed (Sniff, Germany). The animals were sacrificed after 4 weeks, the bandings were explanted, immersed in physiological solution, and transported for the biomechanical analysis. Panels B–D of the Fig. 1 document above described phases of the experiment.

The mechanical integrity of explanted PAB materials was evaluated by means of a uniaxial tensile test. Mechanical testing was carried out with a multipurpose electromechanical tensile testing machine Zwick/Roell with built-in videoextensometer and U9C HBM load cells (±25N). Tensile tests were carried out either as a simple monotonous loading up to failure, or as a cyclic loading-unloading procedure, where the 6^{th} cycle was conducted up to the failure. The reference dimensions of the samples were determined by means of an image analysis of photographs of the samples taken before testing. Quantities determined during the experiment were the ultimate tensile stress σ_{ult}, the strain at failure ε_{ult}, and the initial Young modulus E_{ini}. Ultimate tensile stress was defined as the ratio of the force at failure to the reference cross-section area. The strain at failure was calculated from the distance of marks made on the surface of the sample, which was measured by the videoextensometr, by employing $\varepsilon = \Delta L/L$, where L is the reference distance between the marks. Finally, E_{ini} was determined as the slope of the tangent made to the stress-strain curve in the first loading cycle considering $\varepsilon \in [0, 0.02]$.

3 Results and discussion

It was found that both the COLL-PCL and COLL-PLCL materials kept their integrity until explantation and the samples were capable of withstanding normal manipulation (see Fig. 1D,E). Prior to the tensile testing, explanted samples were halved in their width, thus the number of conducted experiments increased from 6 to 12, and the average dimensions of a sample were 40 mm × 3 mm. Seven samples were subjected to a simple monotonous tensile test to a failure, and five were tested in the cyclic procedure ending with ultimate loading.

Fig. 2 displays the stress-strain relationships recorded in the first loading cycle of all tested samples (left panel). Experimental curves exhibit a certain degree of longitudinal oscillations which is an experimental artefact induced by light reflections which disturb the auto-tracking function of the videoextensometr. The imperfect geometrical shape of the marks, which were made manually on the surfaces of the samples, also contributes to these artefacts (see Fig. 1E, F). It is worth noting that the explanted samples exhibit a rather irregular geometry. This is a result of an interaction with the biological environment (see Fig. 1A, D, E).

The specific values of the ultimate tensile stress, the strain at failure, and the initial Young modulus determined in the first cycle are presented in Tab. 1. The strength of materials is also presented in the form of the

Tab. 1: Results of the uniaxial tensile tests. Each group consisted of four samples.

	COLL-PLCL 15g				COLL-PLCL 30g				COLL-PCL 30g			
σ_{ult} [MPa]	0.48	0.74	0.34	0.34	0.61	0.48	0.43	0.55	0.58	0.71	0.51	0.38
mean $\pm$ SD	0.48 ± 0.19				0.52 ± 0.079				0.55 ± 0.14			
ε_{ult} [-]	0.14	0.23	0.28	0.25	0.066	0.043	0.057	0.11	0.26	0.16	0.11	0.13
mean $\pm$ SD	0.23 ± 0.060				0.069 ± 0.029				0.17 ± 0.067			
E_{ini} [MPa]	2.9	6.4	2.5	2.0	2.5	6.5	9.9	7.0	5.0	3.3	3.4	4.0
mean $\pm$ SD	3.5 ± 2.0				6.5 ± 3.0				3.9 ± 0.78			

box-plot in Fig. 2 (right panel). The data shows that all materials retained a certain elasticity during four weeks of *in vivo* degradation. Statistical comparison based on the Kruskal-Wallis test suggests that the composites do not differ from each other with respect to the ultimate stress (p = 0.63), however, one should not overrate this result based on a small number of observations. On the other hand, Tab. 1 shows that significant differences could exist in the deformability (here represented by the strain at failure), which is clearly higher for COLL-PLCL 15g and COLL-PCL 30g compared to COLL-PLCL 30g. An inverse result is obtained with regard to the Young modulus, which is a clear consequence of its definition. Thus, from the strength point of view, all composites seem to be interchangeable. However, when deformability is taken into account, COLL-PCL 30g could represent a suitable candidate for a PAB material. Nevertheless, this is a preliminary analysis which will be followed by a more detailed study, involving a larger number of samples and a longer period of *in vivo* degradation.

4 Conclusions

Tensile experiments confirmed a sufficient strength of all variants tested (0.51 ± 0.13 MPa, total pooling). Thus, these preliminary results suggest that bands based on COLL-PCL and COLL-PLCL should preserve their mechanical function under *in vivo* conditions for at least one month. When both strength and deformability are taken into account, COLL-PCL 30g could be a suitable candidate for a PAB material. However, the small number of experiments prevents us from reaching a final conclusion. Further study will also involve other methods of biomedical research such as microscopy, histological investigation, and FTIR.

Acknowledgement

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References

[1] E. Buratto, et al., Long-Term Outcome After Pulmonary Artery Banding in Children With Atrioventricular Septal Defects, Annals of Thoracic Surgery 106 (2018) 138–144, doi: 10.1016/j.athoracsur.2018.02.086.

[2] B. Cools, et al., Pulmonary artery banding as ’open end’ palliation of systemic right ventricles: An interim analysis, European Journal of Cardio-Thoracic Surgery 41 (2012) 913–918, doi: 10.1093/ejcts/ezr078.

[3] J. Kobr, et al., Right Ventricular Pressure Overload and Pathophysiology of Growing Porcine Biomodel, Pediatric Cardiology 37 (2016) 1498–1506, doi: 10.1007/s00246-016-1463-y.