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Degradation of the Mechanical Properties of Poly-Lactide-Caprolactone-Collagen Composite for Pulmonary Artery Banding after Implantation into a Rat’s Peritoneum

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Abstract: Pulmonary artery banding is a surgical procedure performed when there is a shunt between the left and right ventricle. Its aim is to constrict the lumen of the pulmonary artery by using a band to reduce blood flow to the lungs. In this study, we report the results of investigating the mechanical properties of a composite composed of poly(L-lactide-co-ε-caprolactone) layers and a collagen matrix (PLCL-COLL). PLCL layers were obtained by electrospinning, impregnated with collagen solution, and finally cross-linked to increase the stiffness of the material. Bands of PLCL-COLL were implanted into a rat peritoneum and explanted after 1, 3, and 6 months *in vivo*. The mechanical properties of the material before and after implantation were determined using uniaxial tensile tests. The same was done with samples of strips prepared from GORE-TEX material. By comparing the results of tensile tests before implantation and after explantation, it was found that PLCL-COLL degrades in the rat’s body and that it exhibits a mechanical response showing of elastic modulus values that correspond well to arterial biomechanics (elastic modulus measured in the initial linear region of the deformation was found to be: 4.14 MPa ± 1.11 MPa, 2.34 MPa ± 1.02 MPa, 1.11 MPa ± 0.77 MPa, and 0.88 MPa ± 0.60 MPa before implantation, and 1, 3, and 6 months after implantation respectively). Similar to the elastic modulus, the strength of the PLCL-COLL composite decreased during *in vivo* exposure (1.32 ± 0.32 MPa, 0.60 ± 0.26 MPa, 0.44 ± 0.11 MPa, and 0.46 ± 0.28 MPa before implantation, and 1, 3, and 6 months after implantation respectively). In our experiments, PLCL-COLL material was always more compliant than GORE-TEX (elastic modulus 34.7 MPa ± 2.06 MPa before implantation, and 9.35 MPa ± 6.80 MPa after implantation). The results suggest that PLCL-COLL could be a suitable candidate for the development of artery banding tapes, and also for further use in cardiovascular surgery.

Keywords: Bioresorbable material; Collagen; Degradation; Elasticity; ε-caprolactone; In vivo; L-lactide; Pulmonary artery banding.

Highlights:

Poly(L-lactide-co-ε-caprolactone)-collagen composite was developed.

The absorbable composite material is designed for use in cardiovascular surgery.

Uniaxial tensile tests have shown it to be more compliant than ePTFE materials.

Tensile tests showed that the mechanical properties of the composite degrade in vivo.

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1. Introduction

Pulmonary artery banding (PAB) was introduced in 1952 as a surgical procedure to reduce the blood flow rate in pulmonary circulation [1]. It entails the constriction of the pulmonary artery (PA) lumen using a band tightened around the vessel [1-6]. Figure 1 shows a sketch of PAB. One typical application is a ventricular septal defect (VSD), through which, within heart muscle contraction, blood can flow from the left to the right ventricle, which subsequently causes excessive inlet of blood to the lungs. The aim of PAB is therefore to prevent blood from overfilling lungs that would otherwise develop pulmonary hypertension and lead to undesirable remodeling of the blood vessels in pulmonary circulation [2-6]. Although spontaneous closure of VSD may occur after PAB, it is usually a palliative stage that needs to be followed by an intervention focusing directly on VSD treatment. Other conditions in which PAB might be applied, for example, are a complex defect the reconstruction of which involves physiological training of the ventricle prior to achieving its full pumping function and hypoplastic left heart malformations [6-10].

PABs are created with a help of commercially-available polymer bands that can be prepared from various materials, such as expanded PTFE, nylon, Dacron, and polydioxanone [2-5,11]. In the past, there was even a device that enabled controlled narrowing of the pulmonary artery cross-section [12-14] (FloWatch, EndoArt S.A, Switzerland), but nowadays, according to the NICE report [15], this seems to be commercially unavailable.

One of the disadvantages of PAB is the need for the debanding. The debanding procedure in its essence means that once the restriction of the cross-section is no longer necessary, PAB needs to be loosened or its stitches cut to allow onward free growth of PA. This can be done either surgically or by dilation of the artery with the help of a balloon catheter [16-19]. To minimize the number of interventions, a PAB band can be made using bioabsorbable material which makes it possible to omit the debanding procedure. The application of polydioxanone tapes has been reported in literature for the creation of resorbable PAB [20-22].

PA band materials, such as the above-mentioned ePTFE, nylon, Dacron, and polydioxanone, are in most cases significantly stiffer in comparison with arterial walls. When the mechanical response of these materials is expressed within the framework of the linear elasticity, the Young modulus is frequently determined in the range of several tens of MPa to several GPa [23-27,49], whereas a linearized description of the mechanical behavior of arteries yields the Young modulus of elasticity in the range of tens of kPa to several MPa [27-30]. This discrepancy led us to the idea of preparing PA bands which would be more compliant, but at the same time resorbable in order to preserve the advantage of one-step PAB procedure.

Over the past decade, our research team has gained considerable experience in using the electrostatic spinning method to prepare new collagen-based materials for biomedical applications [31-33]. At present, electrospinning is proving to be a promising method for the development of materials used in cardiovascular surgery such as vascular replacements [34-36]. With a help of ε-caprolactone, it is possible to achieve mechanical properties which are relatively close to the biomechanics of vascular walls, and at the same time resorbable, which can be particularly advantageous in palliative or temporary interventions [25,34-39]. E-caprolactone is often combined with L-lactide so that the former imparts compliance to the final material and the latter imparts strength and higher degradability [25,34,38,40].

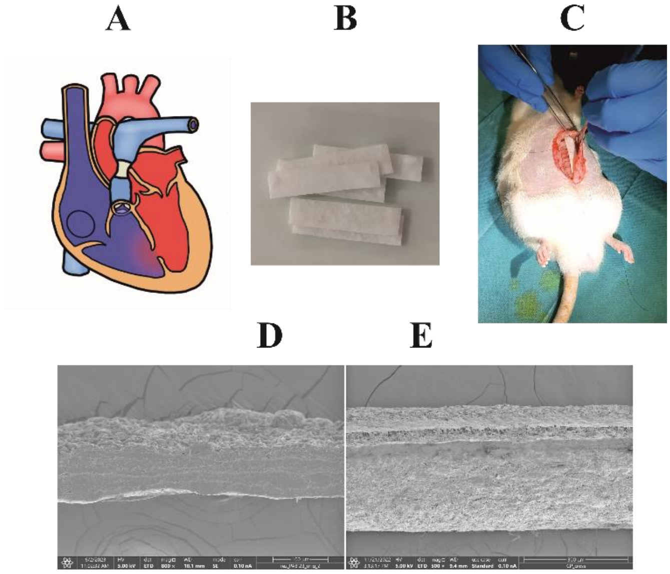

Figure 1. A - scheme of the heart with banding on a common section of the pulmonary artery. B - PLCL-COLL strips in dry state. C - implantation of PLCL-COLL bands into a rat peritoneum. D - Scanning electron microscope image of PLCL-COLL band (mag. 800x, bar 100 μm). E - Scanning electron microscope image of the GORE band (Cardiovascular Patch; mag. 500x, bar 300 μm). Note that the images in the panels are not at the same scale.

In this paper we report the results of the study on the development of a resorbable material for arterial banding. The composite material used for PAB consists of poly(L-lactide-co-ε-caprolactone) layers prepared by electrospinning, subsequently embedded in a collagen matrix (PLCL-COLL). The PLCL-COLL strips were implanted into a rat peritoneum to verify their degradability in a living environment. The mechanical properties of PLCL-COLL prior to implantation and after explantation were determined using uniaxial tensile tests. Comparison with bands made of commercially-available PAB material bought at W. L. GORE & Associates, Inc., suggested that PLCL-COLL is more compliant under all circumstances than the GORE material used, and its mechanical properties are gradually degraded in the rat peritoneum.

2. Materials and Methods

2.1 PLCL-COLL fabrication and tested samples

PLCL-COLL composite sheets were fabricated from nanofibrous layers consisting of poly(L-lactide-co-ε-caprolactone) copolymer (PLCL; 70/30 mol/mol; Purasorb PLC 7015, Corbion, Netherlands). The layers were obtained by way of needleless electrospinning (NS 1S500U production line with precisely-controlled NSAC150 air-conditioning unit, Elmarco, Czech Republic) under direct current from 10 wt% PLCL solution in chloroform and ethanol (in 8:2 weight ratio; Penta, Czech Republic). The layers were electrospun to a surface density of 30 g/m^2. The fiber diameter in electrospun PLCL ranged from 50 to 700 nm.

The resulting PLCL-COLL sheets comprised three PLCL layers. The matrix of the PLCL-COLL composite was composed of type I collagen (COLL; calf skin, VUP Medical, Czech Republic). Composites were prepared by means of impregnation of PLCL sheets with 5 wt% aqueous dispersion of collagen. After impregnation, the PLCL-COLL sheets were placed into a form and left in a laminar flow box at a room temperature for up to 36 hours (until the constant weight was reached after evaporation of water). The cross-linking of the collagen matrix was carried out as the final step in PLCL-COLL sheet fabrication. This was done by soaking the PLCL-COLL sheets in 95 wt% solution of EDC (N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride) and NHS (N-hydroxysuccinimide) (EDC/NHS = 4/1, wt/wt, Sigma Aldrich, USA) in ethanol. After a reaction time of 2 hours at 37 °C, the composites were washed in 0.1 M Na_2HPO_4 (for at least 2 x 20 min), subsequently rinsed in deionized water (for at least 2 x 20 min), and left to dry (until the constant weight was achieved). The volume fraction of PLCL reinforcement in the final composite was 70 ± 10 vol%. The fabrication procedure yielded square PLCL-COLL sheets of an approximate size 100 x 100 mm^2. Finally, the sheets were packed and sealed in indicator bags and exposed to sterilization at a nominal dose of 25 kGy (BIOSTER, Inc., Veverská Bítýška, Czech Republic). In the last preparation step, the sterilized PLCL-COLL sheets were cut into 42 x 6 mm rectangular samples.

In order to compare the properties of the PLCL-COLL material developed and the PAB materials used in clinics at present, samples of surgical material used in PAB at the Department of Surgery of University Hospital Pilsen were purchased from W. L. Gore & Associates, Inc., (USA; hereinafter referred to as GORE). These were bands cut from GORE Preclude Pericardial Membrane and GORE Cardiovascular Patch. Details of these products can be found at https://www.goremedical.com/products.

A total of 65 strips were prepared for the experiments. 9 PLCL-COLL strips were designated for measuring mechanical properties before implantation, 3 x 12 PLCL-COLL strips were designated for assessment of the mechanical response after explantation, 6 GORE strips were designated for determining mechanical properties before implantation, and 14 GORE strips were designated for verifying the effect of implantation into a rat peritoneum.

2.2 *In vivo* experiment

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-33799/2021-4). Twenty five male Wistar rats (> 480 g, Velaz, Prague, Czech Republic) were implanted by PLCL-COLL or GORE strips applying the randomization of a strip type, a side of implantation and a time of explantation. The animals were subjected to light inhalation anesthesia induced by isoflurane in oxygen using an anesthetic machine (Vetnar 1100, Grimed, Czech Republic) connected to an inhalation chamber. Following the elevation of the abdominal wall of the restrained animal, the anesthetic mixture was carefully injected into the peritoneal cavity so as not to affect the intestine. The anesthetic mixture was prepared prior to each experiment in a syringe via the mixing of propofol (100 mg/kg; Propofol 2%, Fresenius Kabi, Germany), medetomidine (0.1 mg/kg; NarcoStart®, Produlab Pharma B.V., Netherlands), and nalbuphine (0.1 mg/kg; Nalbuphin Orpha, Orpha-Devel Handels und Vertriebs GmbH, Austria). The anesthetized animals were placed on a tempered operating table and continually supplied with oxygen through a face mask and monitored by pulse oximetry. An incision of approximately 30 – 40 mm was then made on the linea alba and on the abdominal wall, where 2 bands were inserted into the peritoneum. Bands were fixed at the implantation site with a non-absorbable suture. The wound was closed in 2 layers with an absorbable suture and treated with a liquid bandage. Following surgery, the animals were provided with analgesia for 3 days, and 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 euthanized after 1, 3, and 6 months and the bands were explanted, immersed in physiological solution, and transported for biomechanical analysis.

2.3 Mechanical testing

The mechanical behavior of all materials was determined using uniaxial tensile testing, which was carried out using a Zwick/Roell universal testing machine (Zwick/Roell, Germany) designed for testing soft tissues (blood vessels) and elastomer materials. The testing machine is equipped with electromechanical actuators that work within load range ± 200 N (compression-tension mode), HBM U9C (Hottinger Brüel & Kjaer GmbH, Germany) ± 25N load cell, and electromagnetic position sensor with working range 0 – 75 mm (position resolution 1 μm). Although the system has a built-in video-extensometer (5 MPx uEye 3.0 CMOS camera), the displacement achieved by the machine crosshead in loading was used within final data processing to determine sample deformation due to the highly-irregular and non-uniform geometry of the explanted samples. The applied loading rate in the tensile tests was 0.5 mm/s. All data were stored on a control PC with 20 Hz sampling rate for further post-processing. The mechanical properties of COLL-PLCL were determined in hydrated state. Non-implanted samples of PLCL-COLL were kept 2 hours in a phosphate buffer solution before the experiment, and explanted samples were stored in a physiological solution until the experiment. Tensile tests were carried out in air immediately after removal from the hydrating environment (the average time to test was 3 minutes and every test was completed within 1 minute).

2.4 Experiment post-processing and statistics

The mechanical response of the measured specimens was expressed by nominal stress $\sigma = F/S_0$, where F is the recorded loading force and S_0 is the reference cross-sectional area of the specimen. The cross-sectional area of the specimen was determined as the product of the average width (determined from a photograph of the specimen) and the average thickness (determined by repeated measurement using a micrometer with clamping force of 0.5 N). Deformation was expressed as engineering strain $\varepsilon = u/L$, where L is the length of a sample determined from a photograph taken after mounting into the machine and u is the displacement of the crosshead in the experiment. Initial Young modulus of elasticity E_{ini} was determined as the slope of the regression line in a $\varepsilon - \sigma$ graph restricted to the linear response of the samples, $0.01 \leq \varepsilon \leq 0.04$. The ultimate tensile strength, σ_{max}, was determined as the maximum (nominal) stress that the material could withstand. The Kruskal-Wallis test (*KW-test*) and Dunn test (*D-test*) were used to detect inter-group differences and to identify pairs of significantly-different groups. A significance level of 0.05 was used in all cases.

3. Results

The number of samples originally intended for mechanical tests and the number of samples with which these tests were successfully carried out differ slightly. This is a consequence of the interaction of the samples with the living environment during implantation and led to significant loss of regularity in the geometry of the samples, which even made tensile testing impossible in several cases. This is documented in Figure 2, which shows a PLCL-COLL band that has not been implanted (panel A, uniform geometry) and a band that was tested after 3 months in the living environment (panel B, irregular shape caused by biological interaction). In two cases, the specimens degraded in the rat's body to the extent that they delaminated during handling prior to the tensile test. A total of 61 successful tensile tests were performed, with the following distribution among groups: 9 PLCL-COLL samples were measured before implantation, 10 PLCL-COLL samples 1 month after implantation, 11 PLCL-COLL samples at 3 months after implantation, and 10 PLCL-COLL samples at 6 months after implantation. As far as the GORE PAB strips are concerned, 6 samples were measured before implantation and 13 samples were tested after implantation, of which 2 samples were subjected to *in vivo* conditions for 2 months, 6 samples for 3 months, and 5 samples were tested at 6 months after their implantation.

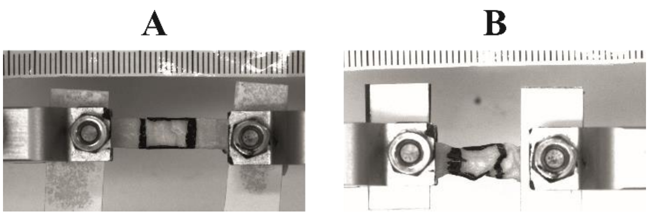

Figure 2. PLCL-COLL specimens clamped in a tensile testing machine. A - sample that has not been implanted, B - sample tested after 3 months in the rat peritoneum.

3.1 PLCL-COLL and GORE before implantation

The mechanical response obtained in tensile tests of the hydrated PLCL-COLL composite is shown in Figure 3 (brown lines). Panel A puts the PLCL-COLL response within the context of PAB bands created from GORE materials, whereas panel B is restricted to the stress and strain experienced by PLCL-COLL. The stress-strain curves of PLCL-COLL end at the maximum stress that the material can carry (ultimate tensile stress, σ_{max}). This was found to be σ_{max} = 1.32 MPa ± 0.32 MPa (mean ± SD). The deformation measured at σ_{max} was ε_{max} = 0.72 ± 0.15. Figure 3 also shows the responses obtained with 3 strips of GORE Preclude Pericardial Membrane (blue lines) and 3 responses of GORE Cardiovascular Patch (red lines). GORE bands were not tested to failure because it is well known that, as elastomers, they can be stretched to several hundreds of percent of their original length, which is far beyond the sensible limit of their application in PAB. As for the initial Young's modulus of elasticity of PLCL-COLL strips, this was E_{ini} = 4.14 MPa ± 1.11 MPa, whereas Preclude Pericardial Membrane exhibited E_{ini} = 35.7 MPa ± 2.12 MPa, and Cardiovascular Patch E_{ini} = 33.7 MPa ± 1.76 MPa.

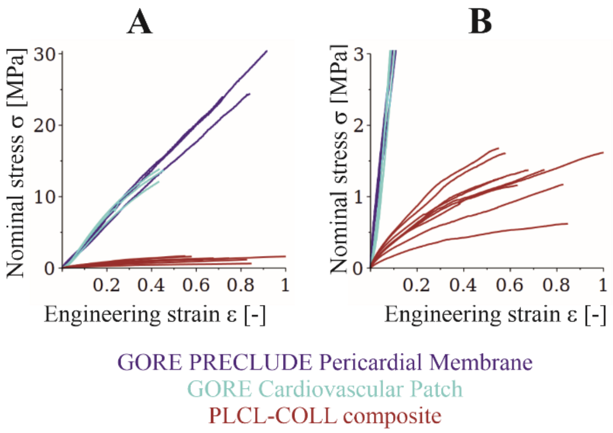

Figure 3. Mechanical response of materials before implantation. A - overall test record, B - detail for stress values transmitted by PLCL-COLL composite. All materials show more-or-less linear behavior (at a scale in line with the scale of the experiment).

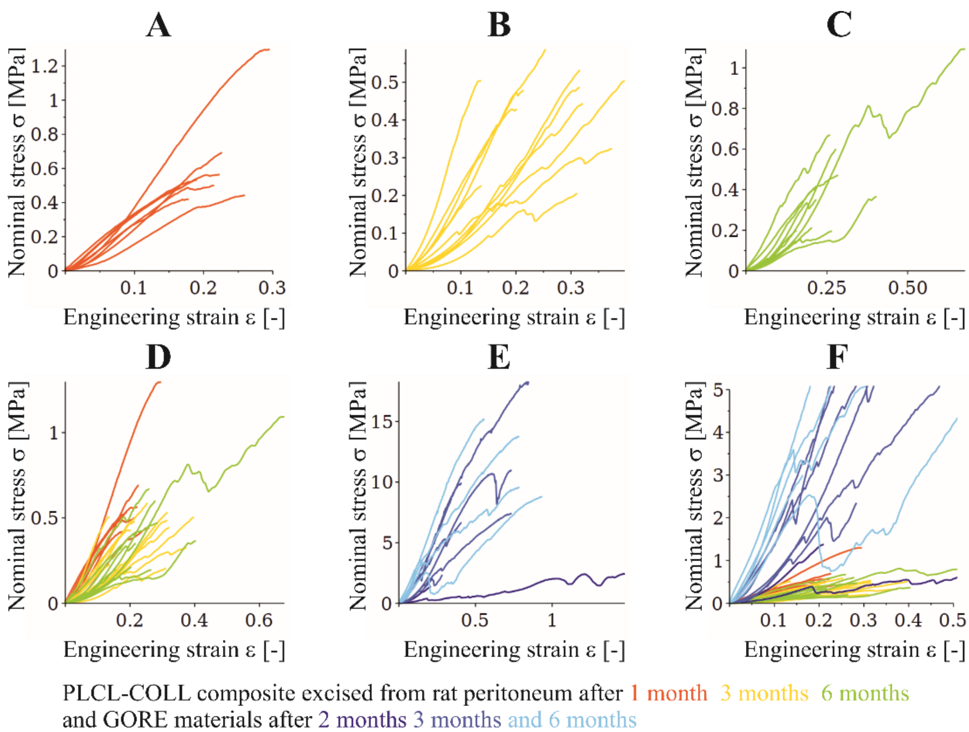

Figure 4. Uniaxial tensile tests of explanted samples. Panel A – PLCL-COLL after 1 month in the rat peritoneum, B – PLCL-COLL after 3 months, C – PLCL-COLL after 6 months, D – all PLCL-COLL samples subjected to *in vivo* interaction, E – GORE materials after explantation from the rat peritoneum, F – all mechanical responses of the samples subjected to *in vivo* conditions.

3.2 Mechanical response of explanted materials

The stress-strain curves of the samples that were subjected to *in vivo* conditions are summarized in Figure 4. The upper row shows PLCL-COLL mechanical responses after 1, 3, and 6 months of interaction in the rat peritoneum (panel A – C), whereas the lower row of Figure 4 contains panels depicting mutual comparisons of the responses. In particular, panel D shows the responses of

PLCL-COLL after biological interaction at unified scale, panel E highlights the effect of the length of time of *in vivo* conditions on GORE materials, and panel F displays all responses of explanted materials under a scale of axes restricted to PLCL-COLL behavior.

In contrast to the mechanical responses gained from intact materials, the explanted materials in Figure 4 show rather non-linear behavior. Another phenomenon present in the stress-strain curves in Figure 4 is their irregularity (they are not monotonous and smooth), which is a consequence of damage caused by *in vivo* interaction. However, despite the damage caused by the biological environment (physical, chemical, and cellular effects on the PAB material), it is clear that the σ–ε curves for PLCL-COLL are roughly ordered by the length of exposure to the *in vivo* environment: the red curves are the steepest (1 month *in vivo*), whereas the green curves are the most gradual (6 months *in vivo*); see panel D in Figure 4. In other words, after 6 months of *in vivo* conditions PLCL-COLL exhibits stronger degradation of mechanical properties (is more compliant) than after 1 month. Similarly, panel F shows that the GORE materials remain stiffer than PLCL-COLL after *in vivo* exposure.

To express this fact quantitatively, we employed initial elastic modulus determined within the deformation interval, where responses show negligible deviation from linearity. Figure 5 shows the experimental stress-strain curves in this linear region. The following values of E_{ini} were determined: 2.34 MPa ± 1.02 MPa for PLCL-COLL after 1 month, 1.11 MPa ± 0.77 MPa after 3 months, and 0.88 MPa ± 0.60 MPa after 6 months of *in vivo* interaction. A *KW-test* suggested that significant differences exist between these groups ($p < 0.01$), and a *D-test* revealed that E_{ini} (1 month) is significantly different from E_{ini} (3 months) and from E_{ini} (6 months); $p < 0.01$ in both cases. The whole situation is depicted in a box-plot in Figure 6 (panel A). The same result is obtained when the intact PLCL-COLL group is added to the comparison. Figure 6 clearly shows that PLCL-COLL strips before implantation are significantly stiffer than the strips explanted from the rat peritoneum (*D-test* showed $p < 0.01$ in all cases).

As for the mechanical behavior of GORE materials, when non-implanted GORE are merged as one group because of the negligible differences between them, the comparison of stiffnesses in Figure 6 (B) shows that the elastic modulus of GORE before implantation is the highest among all measured materials, and that the explanted GORE strips are stiffer than PLCL-COLL. This finding is documented in panel F of Figure 4, 5, and 6. For the sake of completeness, we add that the values of E_{ini} for GORE were determined as follows (Preclude Pericardial Membrane and Cardiovascular Patch merged together): 34.7 MPa ± 2.06 MPa before implantation, 9.35 MPa ± 6.80 MPa after implantation. Somewhat surprisingly, when GORE materials were arranged in groups with respect to the length of time *in vivo*, they showed 1.74 MPa ± 1.2 MPa, 7.05 MPa ± 5.07 MPa, and 15.15 MPa ± 5.21 MPa for 2, 3, and 6 months of *in vivo* conditions respectively. It should be noted at this point that the implantation time for the GORE materials was scheduled the same as for PLCL-COLL. However, due to difficulties with the laboratory equipment, there was a delay and instead of one month, the GORE samples were explanted after two months.

Regarding the strength that was determined for the PLCL-COLL material after implantation, panel D in Figure 4 shows a comparison of the tensile curves. The ultimate stresses are given by the positions of the maxima. The mean strength values for *in vivo* exposure at 1 month, 3 months, and 6 months respectively were: 0.60 ± 0.26 MPa, 0.44 ± 0.11 MPa, and 0.46 ± 0.28 MPa (mean ± SD). Figure 6 (panel C) shows a graphical comparison using a box-plot. The decrease in ultimate strength when comparing pre-implantation and post-implantation samples is statistically significant. The *KW-test* for the group medians reached $p < 0.01$, and the *D-test* for the paired differences between PLCL-COLL strength pre-implantation and post-implantation (1, 3 and 6 months) gave $p < 0.01$ in all cases. The differences in strength between 1, 3 and 6 months *in vivo* were not statistically significant (*KW-test*, $p = 0.17$). Pairwise comparisons were as follows: p(1-month, 3-months) = 0.17, p(3-month, 6-months) = 0.65, and p(1-month, 6-months) = 0.07.

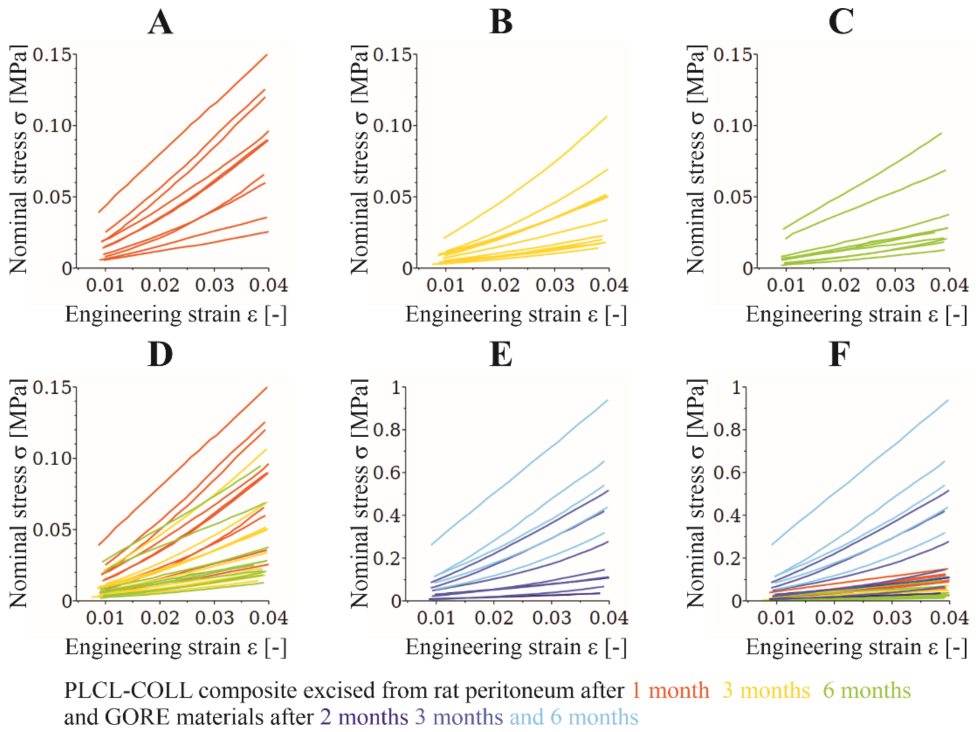

Figure 5. Stress-strain curves restricted to the interval where E_{ini} was determined. Panels are arranged in the same way as in Figure 4. In particular, panel D shows that the longer the exposure to *in vivo* condition, the weaker the mechanical response of the PLCL-COLL composite, while panel F shows that explanted PLCL-COLL bands are more compliant than GORE bands.

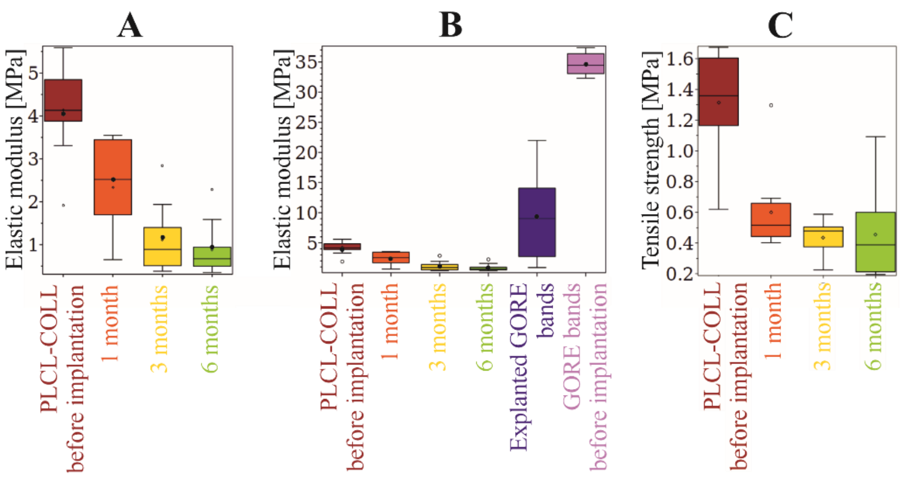

Figure 6. Box-plots of the initial elastic modulus. Panel A details the stiffness of PLCL-COLL, panel B provides a comparison of all materials together, and panel C shows ultimate tensile strengths.

4. Discussion

This paper presents the results of a study focusing on the mechanical properties of a new biodegradable material for PAB made from PLCL-COLL composite. The composite was obtained by impregnating PLCL layers in a collagen solution, with subsequent cross-linking of the collagenous matrix. The PLCL layers themselves were fabricated by way of electrospinning using commercially-available PLCL. Uniaxial tensile tests of PLCL-COLL bands showed a systematic decrease in the initial elastic modulus, which is interpreted as being a consequence of material degradation in the rat peritoneum (average E_{ini} = 4.14, 2.34, 1.11, and 0.88 MPa before, and after 1, 3, and 6 months of implantation respectively).

The values of the elastic modulus found correspond well to the stiffness known for arteries [27-30, 49]. Although arteries may exhibit tensile curves with tangential modulus of up to around 10 MPa, this would involve very large stretches which are not, however, the loading mode that a band is subjected to within PAB procedure. One also should bear in mind that PAB is a procedure usually performed on neonates or infants, whose arteries are much more compliant than those of adults, from which most of the data reported in literature have been taken [41,43]. Therefore, the average initial elastic modulus 0.88 MPa $\leq E_{ini} \leq$ 3.78 MPa of the PLCL-COLL determined in our study is much closer to the values of elastic modulus known from arterial biomechanics than the values known for GORE materials from literature [41, 42, 49].

In terms of the strength and extensibility required to manipulate and to form PAB, the hydrated PLCL-COLL composite showed values that are sufficient for PAB (tens of percent of extension and an average strength of 1.32 MPa, which converted to manipulation force gives 4.62 N for a specimen width of 10 mm and an average thickness of 0.35 mm). The conclusion regarding the degradation of PLCL-COLL *in vivo* based on elastic modulus is in agreement with the strength measurements, which showed that the average strength before implantation was 1.32 MPa, whereas after 1 month, 3 months, and 6 months in vivo, the strength decreased to 0.60, 0.44, and 0.46 MPa, respectively. Moreover, these differences in strength before implantation and after exposure to the in vivo environment are statistically significant.

In order to obtain a direct comparison between the behavior of PLCL-COLL composite and the materials currently used for PAB, after consultation with colleagues from University Hospital Pilsen we purchased GORE materials such as Preclude Pericardial Membrane and Cardiovascular Patch, which were used as control samples. The GORE materials proved to be much stiffer than PLCL-COLL, both before implantation (by about one order of magnitude, average E_{ini} = 34.7 MPa) and after explantation (average E_{ini} = 9.35 MPa, Figure 3, 4F, 5F, and 6B). Somewhat surprising was the finding that even GORE strips significantly degraded in the rat peritoneum – at least in terms of elastic modulus measured at $0.01 \leq \varepsilon \leq 0.04$. Expanded PTFE is not usually reported in literature as a material that would undergo substantial deterioration of its mechanical properties *in vivo* [44, 45]. It is even possible to find studies which, by contrast, show an increase in stiffness in *in vivo* experiments with GORE materials [46-48]. On the other hand, when we analyzed the cross-section area of GORE explanted strips, it was found that it increased significantly. In this case, it is possible that the stress decrease observed in tensile tests of GORE materials is actually due to the increased cross-section area (at the same loading force) and not any degradation of the material. The increase in the cross-section area could be a natural consequence of biological interaction accompanied by cell migration, colonization, and proteosynthesis, leading to an implanted material being surrounded by new tissue. This was likely not sufficiently removed during the preparation of explanted samples for tensile tests. However, we were not able to confirm this hypothesis during the present study, as histological examinations of GORE strips were unavailable to us.

Our results do have some limitations, which we should mention. These are mainly the effects of measurement uncertainties, which can be linked to the noise that accompanies tensile tests, or rather the force and displacement recorded within an experiment. This is tied to undulations in the stress-strain curves in Figure 4. The explanted materials that were subjected to biological interaction were degraded as a result of the action of body enzymes, hydrolysis, and even possible cell adhesion. This not only affected the shape of the samples (they lost their original technically-regular geometry, mentioned before), but also their internal structure. Moreover, this biological degradation is not uniform. *In vivo* interaction resulted in samples that are far from the ideal specimens envisaged by, for example, ASTM standards for testing technical materials in industry. When we compare the stress-strain curves presented in Figure 3 (before implantation) and the curves in Figure 4 (after explantation), it is clear that PLCL-COLL responses were significantly disturbed by biological interaction, and that this is the true cause of the scatter in the measured values for stress and strain (Figure 4). Note that Figure 3, i.e., the stress-strain curves recorded in the pre-implantation state, does not contain such disturbances (the stress-strain relationships are almost ideal lines).

Despite this aforementioned noise, it remains clear that the PLCL-COLL mechanical response became more compliant after implantation, which we believe to be a consequence of *in vivo* degradation. Moreover, the PLCL-COLL material was always more compliant than the GORE material. Thus, in terms of mechanical properties, we consider PLCL-COLL to be a suitable candidate for the onward development of a bioresorbable material for pulmonary artery banding. We will continue our work in the field presented, and in an upcoming study would like to include histological, cytological, and chemo-structural investigations so as to be able to evaluate the consequences of *in vivo* interaction in more detail, and accurately quantify the changes in the PLCL-COLL composite that occur due to implantation.

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