

# Methods for improved flexural mechanical properties of 3D-plotted PCL-based scaffolds for heart valve tissue engineering.

Kim Ragaert, Stieven Van de Velde and Ludwig Cardon

CPMT, Associated Faculty of Applied Engineering Sciences, University College Ghent, Belgium

Department of Materials Science and Engineering, Faculty of Engineering and Architecture, Ghent University, Belgium

Filip De Somer

Heart Centre, University Hospital Ghent, Ghent, Belgium.

**ABSTRACT:** While porous PCL scaffolds can be manufactured through 3D plotting with high regularity and reproducibility, it has been a challenge in previous research to mimic the highly flexible behaviour of the natural valve leaflets. In this study, an exploration is made of two separate approaches for the improved flexibility of 3D plotted poly- $\epsilon$ -caprolactone (PCL) scaffolds for heart valve leaflets. Firstly, the scaffold geometry is radically altered towards a very open woven-like structure, by adequately adapting the processing parameters during 3D plotting. Secondly, the base material itself is altered by blending a fraction of low-molecular weight poly-ethylene-oxide (PEO) in the PCL polymer. Scaffolds are 3D plotted for both series and their flexibility is evaluated in an uni-axial indentation experiment. The results are compared to those of the natural valve tissue and it is found that both methodologies result in the desired reduction of the stiffness of the scaffold.

## 1 INTRODUCTION

A growing application of biodegradable polymers is the manufacture of scaffolds for tissue engineering, an advanced multidisciplinary research field meant to meet the growing demand for donor organs and tissues (Langer and Vacanti 1993; Mikos and Temenoff 2000; Isenberg and Wong 2006). According to the tissue engineering principle, biodegradable scaffolds are used as support structures for the culture of the patient's harvested cells in an *in vitro* environment, so as to (re)create healthy tissues meant to replace diseased ones. While this neo-tissue grows, the scaffold slowly degrades into nontoxic components, eventually leaving only the new, functional and healthy tissue behind. This final construct can be implanted into the patient and will not solicit any rejection, because the cells used are the patient's own.

When looking to cardiovascular application and more specifically leaflets for heart valve replacement, the elastic-mechanical properties of the scaffold are just as important as biodegradability and non-toxicity of the material. The leaflet must be strong enough to withstand the blood pressure and at the same time be able to follow the elastic movement of a natural valve. In fact, when researching such valve scaffolds, we do not look for the strongest possible scaffold but for the highest possible compliance with the elastic-mechanical behaviour of natural tissue. Adherence to such compliant behav-

our will provide the correct mechanical stimuli for the differentiation of the seeded cells.

Poly- $\epsilon$ -caprolactone (PCL) is selected as a base material for scaffolds within this research because it:

- is relatively flexible in comparison to other aliphatic polyesters and as such is considered more suitable for use with scaffolds for cardiovascular application (Brody and Pandit 2007);
- displays very good thermal stability. Its degradation temperature is situated in the range of 280 to 330°C (Sivalingam and Madras 2003), which makes it very suitable for use with a melt processing technique like the micro-extrusion for 3D plotting proposed within this work;
- is FDA-approved; it is considered to be compatible with both hard and soft tissues (Guarino, Causa et al. 2008; Eshraghi and Das 2010; Wang, Su et al. 2010) and will degrade slowly in the human body over a period of 24 to 36 months (Stankus, Guan et al. 2004; Nair and Laurencin 2007; Lam, Huttmacher et al. 2009);
- is relatively cheap.

The main disadvantage of PCL is the strongly hydrophobic nature of its surface (Shan, Yuan et al. 2009; Lee and Kim 2010; Yao, Wang et al. 2011), which will lead to non-specific protein adsorption.

In previous research, different series of porous 4-layer PCL scaffolds were manufactured through 3D-plotting, with individual filament sizes from 127 to

410  $\mu\text{m}$  and spacing between filaments 1.8 times the filament size. It was found that while the scaffolds with the thinnest filaments best approximated the uni-axial mechanical properties of natural valve cusp tissue, they were generally too stiff to be viable as valve leaflet scaffolds (Ragaert, Cardon et al. 2010; Ragaert, De Somer et al. 2011). Therefore, the current research explores two methodologies to further reduce the stiffness of the PCL scaffolds.

Firstly, it is considered to further modify the scaffold geometry towards a more flexible structure. The porosity of the scaffolds is increased, the number of layers is reduced to three and plotting speed is adjusted so that the polymer filaments are placed similar to a woven structure. These scaffolds are named the woven-like geometry for the purpose of this manuscript.

In a second instance, we have opted to modify the PCL material itself. PCL is blended with low-molecular weight poly-ethylene-oxide (PEO) in order to reduce the polymer stiffness. These scaffolds are named the PCL-PEO scaffolds for the purpose of this manuscript.

Scaffolds are 3D plotted for both series and are tested for their mechanical properties in an uni-axial indentation test. The resulting values are compared to those of the natural valve cusp tissue, which have been previously determined with the same experimental setup (Ragaert, De Somer et al. 2012).

## 2 MATERIALS AND METHODS

### 2.1 Materials

For the scaffolds with woven-like geometry, PCL CAPA 6500 from Perstorp (UK) is used. The manufacturer reports a weight-averaged molecular weight of 84500 Da, a polydispersity of 1.78 and a maximum crystalline fraction of 56%.

For the PCL-PEO blends, three solutions were prepared with each a different concentration of PEO: one, five and ten weight-percent (wt%) PCL (Sigma Aldrich-Belgium,  $M_n=80000$  Da) and PEO (Sigma Aldrich-Belgium,  $M_n=2000$  Da), were first dissolved in chloroform, after which a precipitation reaction in cooled diethylether was performed. The different blends are hereafter referred to as PCL-PEO1, PCL-PEO5 and PCL-PEO10, for their respective PEO contents. The blends were manufactured by the PBM group at Ghent University, who also conducted the necessary characterization to confirm the concentrations of PEO in the respective blends (Ragaert 2011).

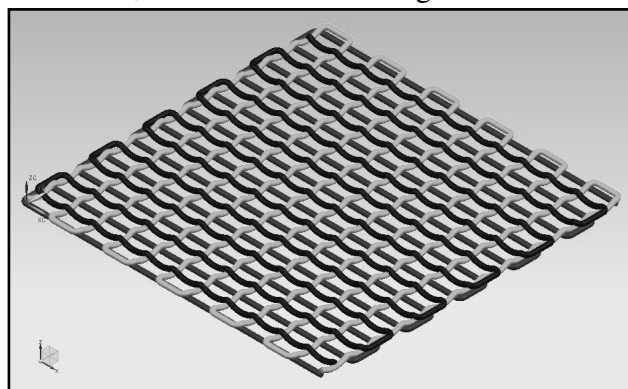
### 2.2 3D plotting of woven-like scaffolds

The woven-like scaffolds were manufactured with the BioScaffolder (SysEng, Germany), a 3D plotting device available at the CPMT group. Workings of

the apparatus have been previously detailed (Ragaert, Cardon et al. 2010). In short, the machine melts the thermoplastic polymer in a mobile dispense head and extrudes thin filaments which are deposited on a plotting table. The final product is built up layer-by-layer.

Concerning the porous geometry, the mounted extrusion needle had a diameter of 127  $\mu\text{m}$  and a strand distance (the center-to-center distance between two adjacent filaments) of 900  $\mu\text{m}$  was set. The most important processing parameters include a processing temperature of 125°C, plotting speed of 85 mm/min and an extrusion screw speed of 11 rpm.

From earlier research (Ragaert 2011), it had become apparent that as a rule-of-thumb, the strand distance should remain below twice the filament diameter for the different layers to provide sufficient support for the subsequent filaments to remain taut. By increasing the strand distance well above this practical limit, the filaments will sag into the underlying pores. Additionally, the used plotting speed is in fact too slow in comparison to the extrusion rate provided by the screw, allowing for sufficient material deposition to plot the sagging filaments without thinning or breaking. Together, these altered processing conditions realize the woven-like structure of the scaffold, which is shown in Figure 1.



**Figure 1: The woven-like structure of the first series of modified PCL scaffolds.**

Scaffolds were plotted to a square geometry of 15x15 mm<sup>2</sup>, with three layers stacked according to a 0-90 pattern.

### 2.3 3D plotting of PCL-PEO scaffolds

PCL-PEO scaffolds were produced on the same BioScaffolder apparatus. Only limited amounts of material were available, about four to five grams per blend type. As an optimization of the parameter set had to be done using up as little material as possible, the larger needle section of 200  $\mu\text{m}$  was chosen to avoid possible material flow problems. The strand distance was set at 360  $\mu\text{m}$  and the extrusion temperature at 115°C. Processing parameters were further fine-tuned for each blend. In general, plotting speed and extruder screw ratio were reduced for higher PEO contents; which displayed a more vis-

cous behaviour. The dispense head was disassembled and cleaned for every material changeover.

Scaffolds were plotted to a square geometry of  $15 \times 15 \text{ mm}^2$ , with four layers stacked according to a 0-90 pattern. Some scaffolds were also manufactured in pure PCL to obtain a reference value for the mechanical properties of the unblended PCL.

## 2.4 Mechanical properties

The experimental setup and the principle of the flexural indentation test are shown in Figure 2. The tissue is placed over a round hole, where it is held in place by a half-sunk rubber ring and fixed in place by a clamping plate. The tissue is then indented by a descending ball probe, to which the central hole has been aligned. Three tissue properties are derived from the test:

- *Extension at break* [mm] (EXT): the depth of indentation by the probe at the moment of rupture (defined as a sudden decrease in load with 50%);
- *Maximum load* [N] (ML): the maximum load which can be applied to the cusp prior to rupture;
- *Stiffness parameter* [N/mm] (ST): the slope of the linear section of the indentation-load curve. This is a measure for the stiffness of the tissue.

ML and EXT describe the ultimate properties of the cusps. ST on the other hand describes the flexural response of the leaflet under an applied load and provides the most valuable information when considering the physiological functionality of the tissue.

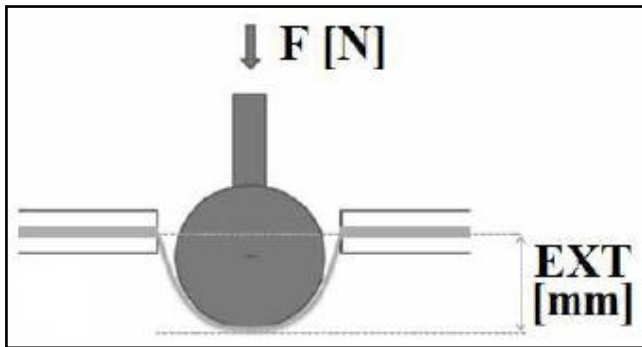


Figure 2: Principles of the uni-axial indentation test.

All tests were performed on a LF Plus Universal material tester (Lloyd Instruments, UK), with a load cell of 1 kN, a ball probe of 4.45 mm in diameter and an indentation speed of 25 mm/min. The ball probe approached the centre of the hole till perforation, which was defined as a sudden decrease in load with 50%. A lower force limit of 0.1 N was set as the zero-indentation point for the scaffold. Force and displacement of the ball probe were recorded and used for calculation of ML, EXT and ST. Per series, three scaffolds were tested and their results averaged. All results are represented as mean  $\pm$  standard deviation.

The results are compared to the respective values for natural valve cusp tissue, which are:  $\text{EXT} = 3.26 \pm 0.64 \text{ mm}$ ,  $\text{ML} = 13.05 \pm 4.48 \text{ N}$  and  $\text{ST} = 5.97 \pm 1.69 \text{ N/mm}$  (Ragaert, De Somer et al. 2012).

## 3 RESULTS AND DISCUSSION

### 3.1 Properties of the woven-like scaffolds

The results for the woven-like scaffolds are shown in Figure 3, in which slanted S-shaped curves may be discerned for all scaffolds. After an initial low-force response, the scaffolds stiffen into a linear flexural behaviour, which is topped of by the final plateau, representative of the rupture of the scaffold by the ball probe. The deduced mechanical properties for the woven-like scaffolds are:  $\text{EXT} = 2.23 \pm 0.10 \text{ mm}$ ,  $\text{ML} = 5.51 \pm 0.07 \text{ N}$  and  $\text{ST} = 3.79 \pm 0.17 \text{ N/mm}$ .

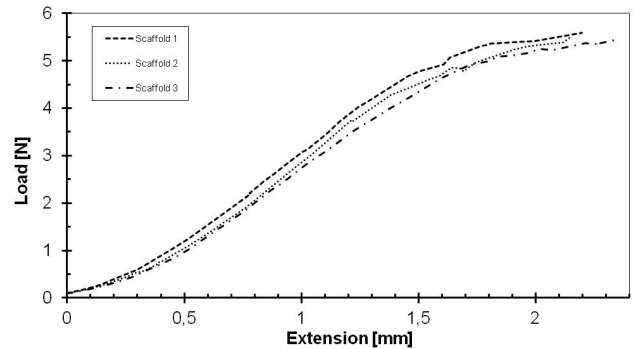


Figure 3: Results for the woven-like scaffolds.

As described earlier, the stiffness parameter ST is considered to best reflect the flexural behaviour of the leaflet. The ST value is significantly lower than that of the natural cusp tissue. This is, however, considered a positive evolution and an important one at that. In previous research (Ragaert, De Somer et al. 2011), the lowest value achieved (4-layered PCL scaffolds with the same filament size) was 11 N/mm, well above the range of the natural tissue. It was also found that with the 3D plotted scaffolds, the stiffness of the construct is easily increased by lowering the strand distance, or by using thicker individual filaments. The limitation has always been that the stiffness could not be sufficiently reduced. By creating the open woven-like structure, a lower ST value is realized for the first time. It is expected that said value can easily be elevated again by modifying the geometry towards a more dense structure.

### 3.2 Properties of the PCL-PEO blended scaffolds

The PCL-PEO scaffolds display a similar type of flexural behaviour, but are clearly resilient to higher levels of load. The load-displacement curves of PCL-PEO10 are displayed in Figure 4 by means of example.

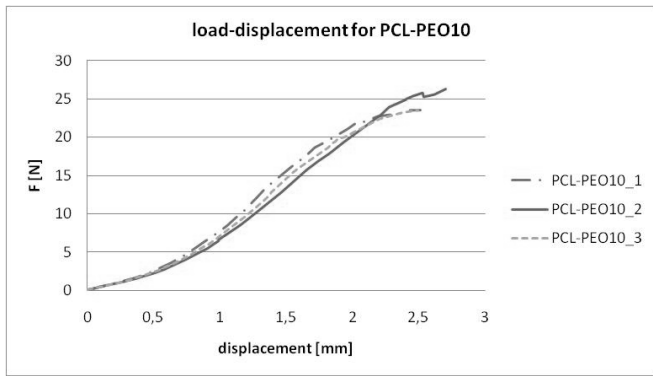


Figure 4: Results for the PCL-PEO10 scaffolds.

The calculated values of EXT, ML and ST are listed in Table 1 per blend type. All three properties can be seen to decrease with an increasing percentage PEO mixed in to the blend.

Table 1: Mechanical properties for the PCL-PEO scaffolds.

| Blend     | EXT [mm]    | ML [N]       | ST [N/mm]    |
|-----------|-------------|--------------|--------------|
| PCL-PEO1  | 2.72 ± 0.10 | 28.26 ± 0.50 | 11.65 ± 0.20 |
| PCL-PEO5  | 2.60 ± 0.08 | 24.88 ± 1.32 | 11.12 ± 0.02 |
| PCL-PEO10 | 2.57 ± 0.12 | 24.34 ± 1.58 | 10.42 ± 0.49 |

The decrease in ST, the parameter which is most relevant for the flexural behaviour under operation conditions, appears to be directly related to the amount of PEO in the blend. This is illustrated in Figure 5. The linear fit of the ST - % PEO relationship can be deduced as:

$$ST = 11.793 - 0.1368 * (\text{wt\% PEO}) \text{ [N/mm]} \quad (1)$$

This would imply that for the unblended PCL, the ST value would be 11.79 N/mm. This value matches the ST value found for the unblended PCL scaffolds, which was  $11.64 \pm 0.20$  N/mm.

From these results, it is apparent that the addition of a low-molecular weight PEO fraction leads to a proportional reduction of the compound's – and therefore the scaffold's – stiffness. It may be possible to use this plasticizing effect of PEO in order to obtain mechanical properties more like those of the natural tissue. It must be remarked that PEO is soluble in water and will dissolve within 24 hours if submerged (Kim, Park et al. 2007). This means that the PEO component should not be counted upon for structural integrity once the scaffold had been placed within a medium for cell culturing. Disintegration of the PEO fraction will result in a porous PCL scaffold which must remain structurally intact and fully functional. As such, PEO concentrations can never be too high within the blend. It would be advisable for further research to include a study of the effect of the PEO removal from the scaffold on the structure and the mechanical properties.

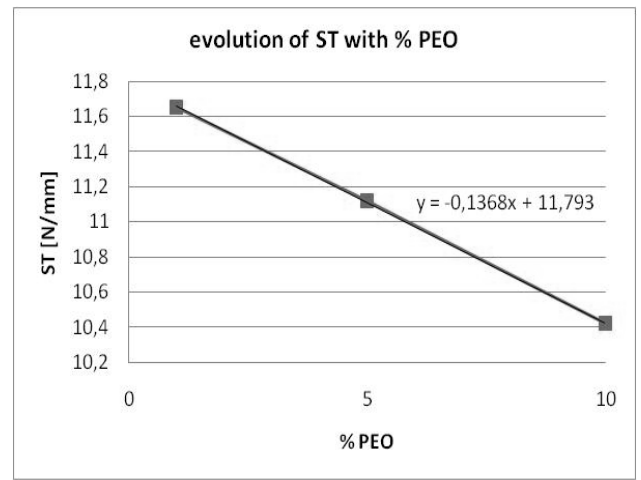


Figure 5: Relation between ST and wt% PEO.

### 3.3 Prospects

The two approaches of adapting the geometry as well as the base material, have been successful in improving the flexibility of the PCL-based leaflet scaffolds. By creating the extremely open woven-like structure it was even possible to realize mechanical properties lower than those of the natural tissue, a feat which had not been accomplished for 3D-plotted PCL-based scaffolds prior in this research.

Tried separately, it was evident that addition of the low-molecular weight PEO fraction into the polymer blend also resulted in the reduction of the high ST value of the scaffolds of more conventional and coarser geometry.

As such, both venues have been shown valid for further investigation and the combination of the two approaches should allow for a broad range of flexible-mechanical properties to be realized.

However, some relevant expected effects of the PEO blending have not been investigated, such as the leaching out of the PEO fraction in the physiological environment. This would result in an additional *in vitro/in vivo* weakening of the construct and a more micro-porous scaffold surface, the latter of which may actually be beneficial to cell attachment.

## 4 CONCLUSIONS

It has been found in this exploratory study that the stiffness of PCL-based scaffolds can be reduced by (i) adapting the scaffold geometry to an open woven-like structure, as well as (ii) altering the base material by blending in lower-strength polymer fractions. Combining these aspects of geometry and material will allow for the more exact mimicking of the natural tissue's flexible behaviour by leaflet scaffolds.

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