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# Examination of the Impact of Polymer Composition and the Test Direction on Tensile Properties of Bilayer Vascular Grafts Using a Multilevel Full Factorial Design Approach

**Suzan Ozdemir[1*], Janset Oztemur[2], Hande Sezgin[3], Ipek Yalcin Enis[4]**
[1, 2, 3, 4] Textile Engineering Department, Istanbul Technical University, Istanbul, Turkey

**Abstract**
Currently, there are no effective commercially available synthetic small-diameter vascular grafts (<6 mm) for bypassing coronary or carotid arteries. This has encouraged ongoing research efforts to create a vascular graft that combines adequate mechanical characteristics with sufficient biological performance. The mechanical properties of the scaffold, such as tensile strength, burst pressure, compliance, and blood permeability, are heavily influenced by its design and composition. Herein, electrospun bilayer vascular grafts are fabricated by using polycaprolactone (PCL), polylactic acid (PLA), and poly(L-lactide-co-ε-caprolactone) (PLCL) polymers. As the inner and outer layers are constructed by implementing different fiber orientations and polymer compositions, the effect of polymer selection within the inner layer and the test direction on the tensile strength and strain of the vascular prosthesis is assessed via a multilevel full factorial design approach. The results indicate that both the polymer type and the test direction, as well as their interactions, have statistically significant influences on the tensile strength and strain values.

**Keywords**: fiber orientation, full factorial design, tensile strength, vascular grafts.

## 1. Introduction

Cardiovascular disease stands as the leading worldwide cause of mortality, with an anticipated annual death toll projected to hit 23.3 million by the year 2030 [1]. There is an immediate clinical need for vascular grafts that possess strong mechanical durability to address cardiovascular diseases. Although autologous or synthetic vascular grafts are currently used in clinical practice, their application and effectiveness are hindered due to the unavailability of suitable native blood vessels from patients or the potential risks of thrombosis and infection associated with synthetic prostheses [2]. Tissue-engineered vascular grafts are innovative substitutes designed to replicate the attributes of native vessels, serving as promising replacements for damaged blood vessels [3]. In order to closely replicate the mechanical and structural traits of autografts, it is essential to have scaffolds that possess properties similar to those found in the native arteries [4]. Within native blood vessels, endothelial cells play a crucial role by releasing soluble substances that uphold hemostasis and preserve the vessel walls' anti-thrombotic characteristics; whereas the *media* and *adventitia* layers consist of smooth muscle cells and fibroblasts, oriented circumferentially to provide the mechanical resilience required to withstand the hemodynamic pressure [5]. Hence, in order to replicate this complex multilayered structure, recent research studies have indicated the potential utility of bilayer vascular grafts in various vascular tissue applications [6]. Through the utilization of the electrospinning method, it becomes possible to create a multilayered nanofibrous scaffold with distinct properties in each layer, effectively mimicking the characteristics of vascular tissue [7]. Taking into account the morphology of native blood vessels, it is essential to incorporate highly aligned structures to promote the formation of not only a single endothelial cell layer along the direction of blood flow but also a substantially thicker smooth muscle layer with circumferential orientation [8]. Implementing fiber orientation and designing grafts with multiple layers are essential to meet the demanding requirements of vascular grafts, encompassing both mechanical properties like tensile strength and burst strength, as well as biological factors such as cell infiltration and orientation [9]. Material

selection is another critical factor that influences mechanical response, degradation time, and surface properties, all of which must align with the specific requirements for tissue regeneration [10]. Among synthetic biopolymers, PCL, PLA, and PLCL are widely chosen in vascular tissue engineering applications for their beneficial properties [11, 12]. PCL is flexible and degrades slowly, whereas PLA is strong, biocompatible, and can degrade at adjustable rates [13]. As a result, numerous studies have employed the blending or combination of these polymers as a promising strategy [14, 15].

In this study, bilayered vascular grafts, composed of PCL, PLA, and PLCL polymers, are manufactured using the electrospinning method. The impact of polymer selection for the inner scaffold layer, as well as the testing direction, on the materials' tensile strength values, is examined and validated through a multilevel full factorial design.

## 2. Experimental part

### A. Materials

PCL (Mn 80,000), PLA (Mn 230,000), and PLCL (70:30 Resomer® LC 703 S) are employed as polymers. The solvents used in the system consist of chloroform, acetic acid, and ethanol. All the chemicals are sourced from Sigma Aldrich.

### B. Methods

**Electrospinning of bilayer scaffolds**

The solutions for electrospinning are prepared by dissolving PCL, PLA, and PLCL polymers in a chloroform/ethanol/acetic acid solvent system (8/1/1 wt%). Polymer concentrations are set at 8% for neat PCL and also its blend with PLA, whereas 10% for PLCL. Two types of bilayer vascular grafts (PCL_PLCL and PCLPLA_PLCL) are produced by using neat PCL and PCL/PLA blend with a blending ratio of 80/20 for the inner layer, while PLCL is used in its neat form for the outer layer. The fabrication of the bilayer vascular grafts with a 5 mm diameter occurs using a custom-made electrospinning unit supplied by Inovenso, Turkey (Nanospinner, Ne100+) in two stages. In the initial step, the inner layer is electrospun over the tubular collector for 20 minutes with a rotational speed of 200 rpm to create randomly distributed fibers. Subsequently, the outer layer is electrospun on top of the inner layer for 50 minutes, utilizing a rotational speed of 10,000 rpm to produce radially oriented fibers and thicker layers. The solutions are delivered at a rate of 3 ± 0.5 ml/h to a needle tip with a 0.6 mm diameter and subjected to a voltage of 11 ± 1 kV. Electrospinning is performed at a temperature of 30 ± 3 °C and a relative humidity of 70 ± 10%.

**Wall thickness**

The wall thicknesses of the vascular grafts are measured with the Standard Gage Electronic External Micrometer (Hexagon Metrology, Turkey)

**Tensile testing**

The evaluation of tensile strength and elongation of the vascular grafts are performed with a Zwick-Roell Z005 universal testing machine, which is equipped with a 200N load cell (Zwick-Roell, Germany). Testing is conducted in multiple configurations: longitudinal (0) and circumferential (90) orientations in planar form, as well as in the longitudinal direction in tubular form (T0) to mimic the position of the scaffold *in vivo*. The planar samples are prepared into dimensions of 10x15 mm (width x length), whereas tubular samples, with a length of 1.5 cm, are prepared for testing. Tensile testing is executed at a cross-head speed of 10 mm/min, maintaining a gauge distance of 5 mm.

**Statistical analysis**

Statistical assessment is performed using the Minitab 16 statistical software program. The impact of polymer type and test direction on the tensile strength and elongation values of vascular scaffolds is evaluated by utilizing the general full factorial design approach. Factors are determined as polymer with 2 levels and direction with 3 levels, whereas the responses are tensile strength and elongation. The ANOVA analysis is done to determine the significance of the parameters in the designed experimental study. The multilevel full factorial design mentioned here involves six experimental runs. In this investigation, each combination of factors and levels undergoes testing three times. As a consequence, there are a total of 18 runs in the experimental design layout (3x2x3). To assess whether the model is compatible with the experimental results or not, the $R^2$ and $R^2$-adjusted values provided by the ANOVA analysis are utilized [16]. Also, the *p*-values below 0.05 signify statistical significance of the factors [17].

## 3. Results and Discussion

**Wall thickness**

The thickness values of the graft's walls are presented in Table 1. These results are sufficient for

both tensile testing and vascular graft applications, as the wall thickness of tissue-engineered blood vessels typically falls within the range of 200 μm to 600 μm [18]. Wall thickness is a crucial parameter that should not exceed certain limits, as it can influence factors such as degradation time and mechanical properties, including strength and compliance [19]. Therefore, if necessary for subsequent stages, it can be adjusted by altering the electrospinning duration.

**Tensile strength and elongation**

When the tensile testing results are assessed, it becomes evident that the circumferential orientation within the outer layer significantly contributes to the tensile strength values of the vascular grafts tested in the same direction, as the fibers better withstand the applied stress in this alignment [20]. Conversely, employing a PCL/PLA blend for the inner layer is less favorable for both tensile strength and elongation values when compared with the PCL layer in the longitudinal direction due to the immiscibility of the polymers, leading to phase separations within the structure and resulting in mechanical failure [21]. Similarly, the elongation value of PCL_PLCL in the circumferential direction is lower than that in the longitudinal direction due to the pre-existing fiber stretch, which restricts their ability to elongate [22]. However, the opposite is observed in the PCLPLA_PLCL sample. It is hypothesized that since the inner layer primarily influences the mechanical response in the longitudinal direction, while the outer layer mainly dictates the mechanical behavior in the radial direction due to its orientation, blending negatively impacts elongation results in the longitudinal direction, leading to lower elongation values compared to the oriented PLCL layer.

**Table 1 Tensile strength, elongation and wall thickness values of scaffolds**

| Sample code | Wall thickness (μm) | Test direction | Tensile strength (MPa) | Elongation (%) |
|---|---|---|---|---|
| PCL_ PLCL | 283±70 | 0 | 4.64±0.82 | 1043.77±106.61 |
| | | 90 | 9.87±0.74 | 696.16±102.22 |
| | | T0 | 5.42±1.29 | 1274.43±216.84 |
| PCLPLA_PLCL | 291±46 | 0 | 2.72±0.61 | 457.81±143.57 |
| | | 90 | 10.21±0.86 | 711.98±125.60 |
| | | T0 | 3.05±0.36 | 588.56±154.14 |

**Statistical analysis**

The ANOVA analysis outcomes pertaining to tensile strength and elongation, generated through the Minitab software program, can be found in Table 2 and Table 3. According to these findings, the sum of squares (SS) and *p*-values indicate that both the polymer factor *(p*=0.000 for both tensile strength and elongation) and the test direction factor (*p*=0.000 for tensile strength and *p*=0.003 for elongation) exert a statistically significant influence on the results of the tensile testing. Additionally, the interaction between these two factors also demonstrates a significant impact (*p*=0.003 for tensile strength and *p*=0.000 for elongation). Upon scrutinizing the Sequential Sum of Squares (Seq SS) values, it becomes evident that the effect of test direction on tensile strength surpasses that of the polymer factor and the interaction between these factors. Conversely, the type of polymer has a more pronounced effect on elongation values compared to the test direction and their interaction.

**Table 2 The ANOVA response table of tensile strength**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **Polymer** | 1 | 8.977 | 8.977 | 8.977 | 25.54 | 0.000 |
| **Direction** | 2 | 152.548 | 152.548 | 76.274 | 217.01 | 0.000 |
| **Polymer*Direction** | 2 | 6.965 | 6.965 | 3.483 | 9.91 | 0.003 |
| **Error** | 12 | 4.218 | 4.218 | 0.351 | | |
| **Total** | 17 | 172.708 | | | | |

**S = 0.592858 R-Sq = 97.56% R-Sq(adj) = 96.54%**

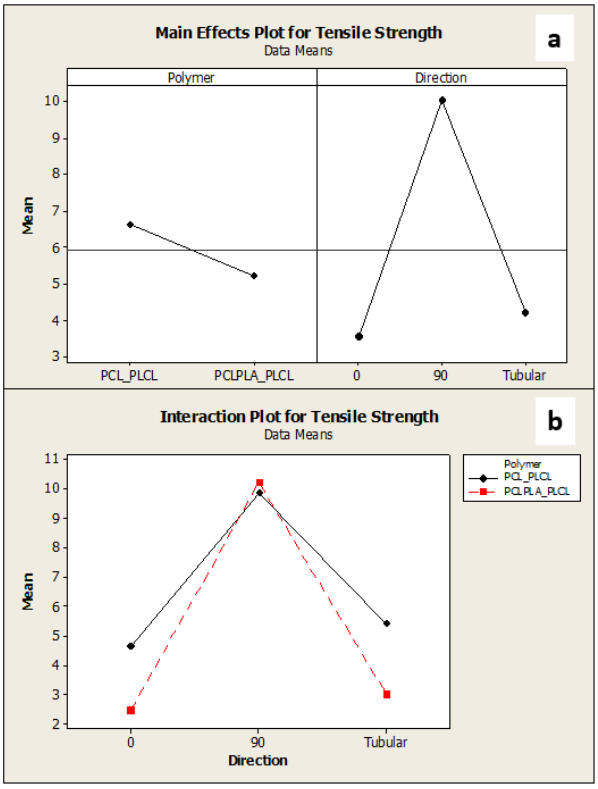


**Figure 1 a. Main effects plots and b. interaction plot for the tensile strength of vascular grafts**

If the testing direction is kept the same, the selection of polymer material proves to significantly impact both tensile strength and elongation values. For instance, when subjecting bilayer tubular scaffolds to tensile testing in axial direction (0), those featuring a PCL inner layer exhibit notably higher values for tensile strength and elongation, measuring at 4.64 MPa and 1043%, respectively, compared to scaffolds with an inner layer of PCLPLA, which record values of 2.72 MPa and 457%, respectively. Conversely, when the polymer type remains constant, variations in tensile strength and elongation values are observed based on the testing direction. This effect is visually depicted in Figure 1(a) for tensile strength and Figure 2(a) for elongation, providing a clear representation of their respective influences on the outcomes of the tensile testing.

Figures 1(b) and 2(b) illustrate the intersection of the lines representing two factors. The fact that these lines are not parallel indicates that there is an interaction occurring between these factors, which in turn affects both the tensile strength and elongation [23].

**Table 3 The ANOVA response table of elongation**

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| **Polymer** | 1 | 881627 | 881627 | 881627 | 99.04 | 0.000 |
| **Direction** | 2 | 168921 | 168921 | 84460 | 9.49 | 0.003 |
| **Polymer*Direction** | 2 | 475948 | 475948 | 237974 | 26.73 | 0.000 |
| **Error** | 12 | 106824 | 106824 | 8902 | | |
| **Total** | 17 | 1633319 | | | | |

**S = 94.3502 R-Sq = 93.46% R-Sq(adj) = 90.73%**

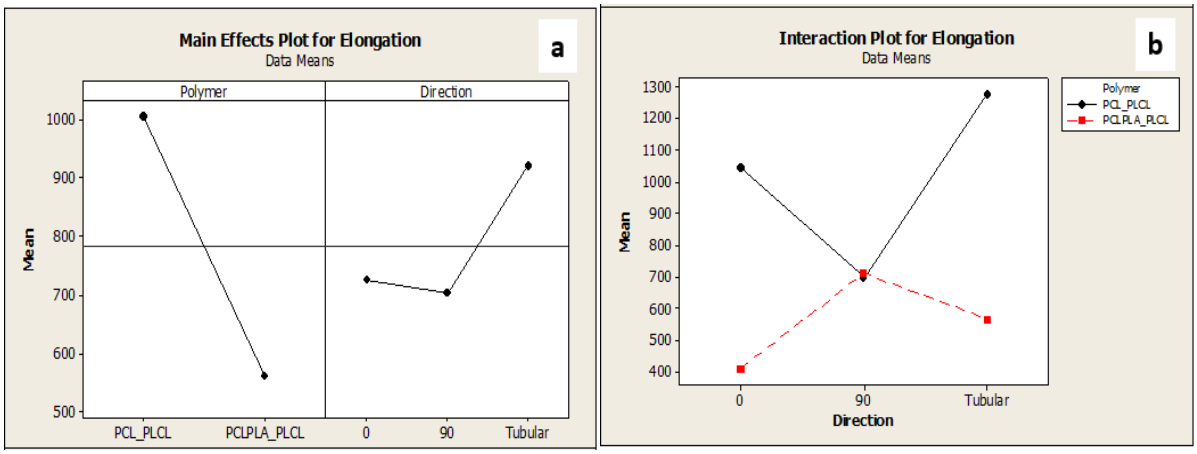


**Figure 2 a. Main effects plots and b. interaction plot for the elongation of vascular grafts**

## 4. Conclusion

In the study, bilayer vascular grafts are fabricated using PCL and PCLPLA as inner layers, along with a PLCL outer layer with a radial fiber orientation. The study assesses the effects of polymer selection in the inner layer and the testing direction. The results show that using PCL for the inner layer is advantageous in terms of elongation and mechanical strength, especially in the axial direction. On the other hand, since the outer layer of the material has fiber orientation in the circumferential direction, the testing direction also affects the tensile testing values. It is also observed that the interaction between these two factors is as important as the individual effects of both parameters. The statistical analysis supports the significance of both factors and their interactions.

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