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## Development of electrospun composite as substitutive diaphragm membrane

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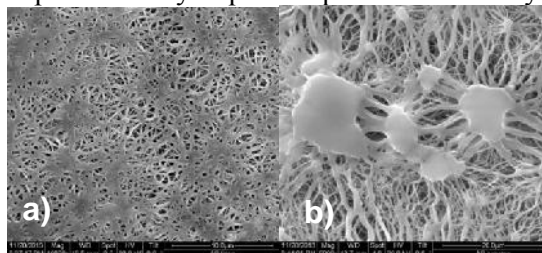
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**Abstract.** The diaphragm is the most important muscle for respiration with a bi-domed structure, which is separating thoracic cavity from abdominal cavity. Partial formation of diaphragm, with unknown reason during fetal development caused a birth defect called congenital diaphragmatic hernia (CDH). It allows the abdomen contents to go up into the chest cavity resulting in pulmonary hypoplasia, which is the major cause of the mortality. There are several types of membrane, which can be used as prostheses to close the existing hole. In this project, we study the tensile properties of electrospun PA-6 nanowebs and electrospun PA-6/B composite as substitution membrane by a comparison with the tensile properties of tendon part of pig's diaphragm.

### 1. Introduction

The diaphragm is a layer of muscle and tendon that serves as the main part of respiration and plays a vital role in the breathing process. Diaphragmatic hernia that occurs in about 1 in 5,000 live births, is defined as congenital or acquired defect [1]. As the treatment, if the opening in the diaphragm is small then it can be sewn. Otherwise, if it has a large size then the surgeon will require implanting an additional tissue or membrane. Currently, Gore-Tex® (PTFE) as a synthetic biocompatible material is used to patch this opening in University Hospital in Strasbourg/France (figure 1).

The problem is that Gore-Tex® implantable membrane is not elastic enough to follow the natural growth of the child, so this patch usually requires replacement later by the child growth [2].



**Figure 1.** SEM images of double-faced current implanted Gore-Tex; a) Smooth surface b) Rough surface



Main objective of our study is to design a substitution membrane by electrospun nanofibers web. Mechanical properties of substitutive membrane have to be close to the human diaphragm that have been represented by pig's diaphragm in this study.

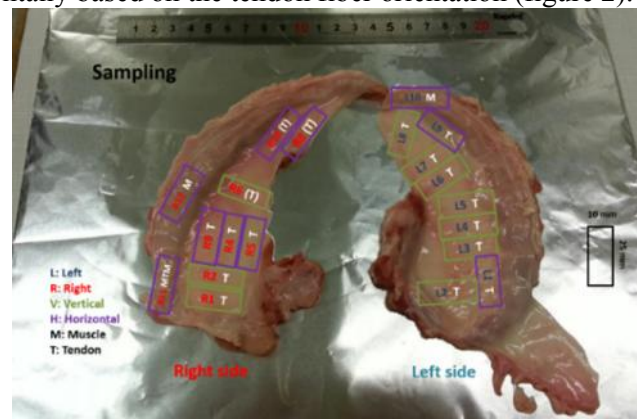
## 2. Materials and Methods

### 2.1. Uniaxial Tensile Test on: Pig's Diaphragm, electrospun PA-6 nanofibers and electrospun PA-6/B composite

The insufficiency of data on mechanical behaviour of human soft tissue is firstly because of the difficulty of obtaining human tissues for testing and secondly because of the lack of the standard testing technics for mechanical testing of soft tissues [3]. For this reason, we performed some mechanical tests on pig's diaphragm (withdrawn from University Hospital, Strasbourg/France), which is the most feasible, and the nearest case to simulate the human one.

Diaphragm is contained of dense collagen fibres, arranged diagonally from lateral to medial direction.

Therefore pig's samples were excised from different parts of diaphragm from the left and right side, vertically and horizontally based on the tendon fiber orientation (figure 2).



**Figure 2.** Sampling from the fresh pig's diaphragm, left and right part

Soft tissue and electrospun samples have been placed between special frames using pneumatic grips before being tested. A velocity of 10 mm/min and grips distance of 15 mm are set on MTS machine for all the tested samples by following ISO 527-3 standard. A temperature controller chamber (Instron 3119.005) was mounted to the MTS tensile machine to keep the temperature stable at 37°C (human body temperature). To reduce the slippage of the samples between the grips, which was challenging during the tensile test one-side sandpaper frame with waterproof backing which the samples were stitched to them was used.

### 2.2. Electrospinning of PA-6 electrospun nanofibers and PA-6/B composite

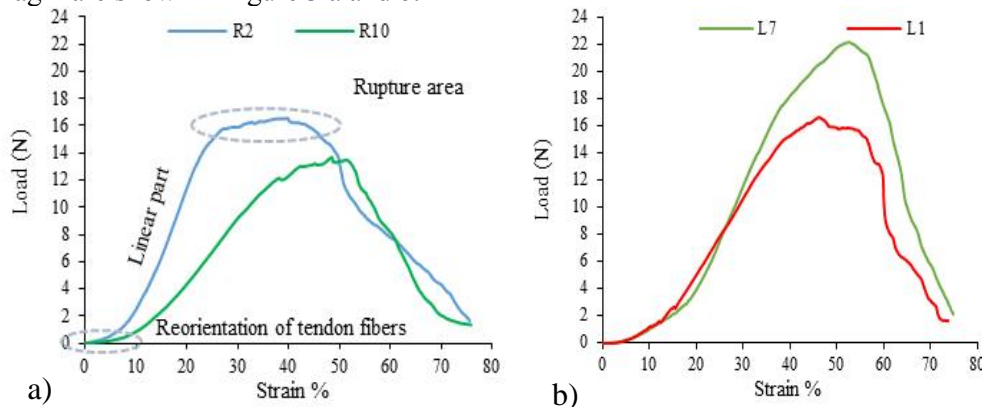
PA-6 was chosen as a polymer to produce electrospun nanoweb because of its biocompatibility and expected adequate mechanical properties. Different electrospinning conditions have been tested by using a vertical homemade electrospinning booth to obtain optimal conditions of producing PA-6 nanofibers. To improve the strain properties of electrospun PA-6 nanofibers, a thin layer of elastomer material (material B) was used while electrospinning of PA-6 solution. Electrospinning time was varied: 15, 30, 60, 90 minutes. Electrospun nanofibers were observed via SEM micrographs and diameters were measured thanks to ImageJ software.

## 3. Results and discussion

Mechanically, tendon works as a force transmitter during rapid muscle contraction. A basic similarity is considered in the structure and function of most tendons. Tendon shows a non-linear behaviour in

uniaxial tension. Once a load is applied (toe region of load- strain curve), the fibres start stretching parallel to the load direction. As the load further increases, the collagen fibres reorient in order to carry a greater proportion of load. By continuing to increase the load, a transition from low to high stiffness occurs and is known as the strain stiffening effect where the fibres will be over stretched and begin to rupture until the failure happens [4].

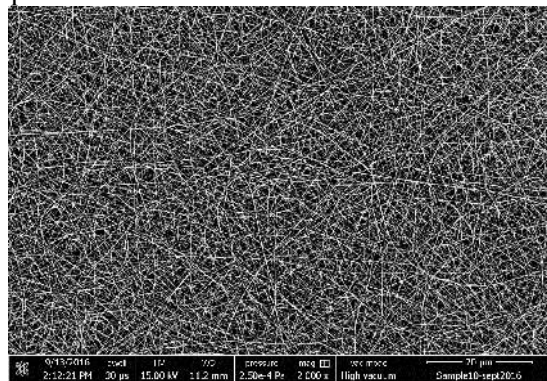
The specimens, slipped from any of the grippes, were not included in the results. The tensile curves of two tendon samples of right (R10: cut vertically and the R2: cut according to the tendon direction) and left (L7: cut vertically and L1: cut according to the tendon direction) side of the diaphragm are shown in figure 3 a and b.



**Figure 3.** Load-strain % curves of right side (a) and left side (b) of pig's diaphragm

The interval of maximum load between 7 and 18 N as well as the interval of strain between 27 and 50 percent are obtained by testing different numbers of samples from right side of diaphragm. Load-Strain % curves of the left side samples revealed the interval of maximum load between 8 and 23 N and strain between 15 and 53 percent.

To produce electrospun PA-6 nanofibers, 20 wt. % solution of PA-6/Formic acid with 0.1 mL/h feed rate was injected to the needle by applying 30 kV of voltage between needle and collector with a distance of 15 cm. In figure 4, the SEM micrograph of electrospun PA6 nanofibers after 15 minutes electrospinning is presented.



**Figure 4.** SEM image of 15 minutes electrospun PA-6 nanofibers

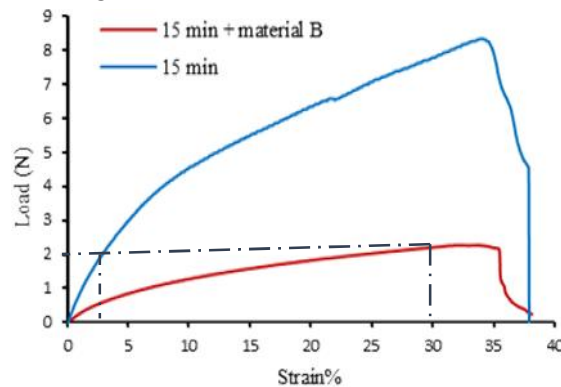
30 single nanofibers diameter were randomly measured by ImageJ software. The SEM micrograph displays the nanofibers with an average diameter of 184 nm.

Tensile test results on shorter time of spinning of electrospun PA-6 nanofibers shows improved mechanical properties. By increasing electrospinning duration on material B (after 30 minutes of electrospinning), delamination has been observed between two layers leading us to conclude that shorter time of electrospinning on material B is more favourable (figure 5).



**Figure 5.** Delamination between material B and Electrospun PA-6 nanofibers

A comparison between tensile results of 15 minutes electrospun PA-6 and electrospun PA-6/B composite is demonstrated in figure 6.



**Figure 6.** Comparison load-strain % curves of 15 minutes electrospun PA-6 and electrospun PA-6/B composite. Electrospun PA-6/B composite displays more deformation for example for the load of 2 N compared to electrospun PA-6 nanofibers web with the same time of electrospinning.

#### 4. Conclusion

The mechanical tests confirmed that regardless of the sampling region, the pork's diaphragm presented isotropic properties. For the optimum electrospinning conditions, an average diameter of 184 nm without defects was obtained. It was also observed that by increasing the time of electrospinning, the rupture force of the sample increased but the strain decreased. Moreover, an important increase of the electrospinning time, led to a total loss of the elasticity of elastomer material and delamination was occurred.

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