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# EFFECT OF STERILIZATION ON MECHANICAL PROPERTIES OF BIOLOGICAL COMPOSITE

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**Abstract.** *In this study, composite tubes were manufactured from biological collagenous matrix and reinforcing polyester mesh. The effect of sterilization on mechanical properties of this structure was evaluated using inflation-extension tests. Samples were exposed to two types of sterilization (ethylene oxide and irradiation). The control (non-sterilized) samples were also tested. It was found that the process of sterilization (especially irradiation) dramatically affects the final mechanical properties of the material. These findings should be taken into account when such collagenous material is assumed to be used in tissue engineering.*

## 1 INTRODUCTION

Composite materials are heterogeneous mixtures of two or more homogeneous components, which have been bonded together. In composites, properties or set of properties can be attained which could not have been obtained separately. Many in nature occurring materials can be regarded as composite e.g. bones, blood vessels, woods and others. Man-made composites are used since thousands of years, e.g. straw and natural fibers in bricks, laminated woods, etc. [1].

Over the past few decades, tissue engineering has been focused on development of biological substitutes to restore, maintain, or improve tissue functions. Collagen is the most abundant biological material used for tissue engineering. It is the basic constituent of skin, bones, ligaments and connective tissues. Collagen-based biomaterials have been studied extensively for a variety of biomedical applications, including dialysis membranes, wound dressings and artificial skin. Although native collagen possesses high tensile strength, the chemical treatment necessary for isolation makes the reconstituted collagen very poor in mechanical properties [2, 3]. A possible means to circumvent the problem is to reinforce natural polymer matrix by synthetic fibers or structures. Moreover, the properties of such composite could be modulated through composition of constituents in the material.

Routinely used sterilization process for medical products, e.g. high pressure steam (autoclaving) and dry heat cannot be considered for heat- and watersensitive biomaterials like collagen structures [4]. Currently the most widely utilized methods for collagenous materialsterilization are ethylene oxide (EO) gas infiltration and gamma irradiation. Earlier investigations focused on physical or chemical alterations after these two methods [5]. EO was claimed to alter the mechanical and physical properties of collagen slightly, but with a high risk of toxic residues [5]. Gamma irradiation, once introduced as the simplest and most effective way of sterilization without toxic substances, breaks chemical bonds, affects tensile strength and modulus, thus affecting the exposed material fundamentally [6,7].

In our study, composite tubes were manufactured from biological collagenous matrix and reinforcing polyester mesh. The effect of sterilization on mechanical properties of this structure was evaluated using inflation-extension tests.

## 2 MATERIAL AND METHODS

Tubular samples of hybrid composite were manufactured using extrusion while the polyester mesh was integrated into biological collagenous matrix (Fig 1). Two samples were sterilized using ethylene oxide, two samples by gamma irradiation and two control specimens were left unsterilized.

Samples were placed in physiological solution for 24 hours before testing. Prior to the mechanical tests, two rings were cut out from the specimen at both ends, and the mean reference dimensions of the samples (external radius, thickness) were determined by means of image analysis of digital photographs, Table 1.

### 2.1 Inflation-extension test

Each specimen was mounted in the experimental setup (Fig. 1) and marked with liquid eye-liner. The samples were pressurized until destruction using a motorized syringe (Standa Ltd, Vilnius, Lithuania). The intraluminal pressure was monitored by pressure transducer (Cressto s.r.o, Czech Republic). The experiments were performed at room temperature (22°C). The deformed geometry was recorded by a CCD camera (Dantec Dynamics, Skovlunde, Denmark). In the data post processing, the radius of the sample and length be-

tween marks during pressurization was evaluated by the edge detection algorithm in Matlab (MathWorks, MA, USA).

The longitudinal ($\lambda_z$) and circumferential ($\lambda_\theta$) stretch ratios were computed according Eq. (1). Here $L$ and $l$ is the length between marks in reference and deformed configuration, respectively. $R$ and $r$ is the unloaded and loaded middle radius of the sample, respectively.

$$\lambda_z = \frac{l}{L} \qquad \lambda_\theta = \frac{r}{R} \tag{1}$$

The influence of the sterilization was evaluated through circumferential stress, computed according thin-walled tube approximation. The circumferential stress ($\sigma_\theta$) induced by an internal pressure is computed using the Young–Laplace equation (2). In (2) $P$ is intraluminal pressure, $r$ is deformed middle radius and $h$ is deformed thickness of the sample, respectively.

$$\sigma_\theta = \frac{Pr}{h} \tag{2}$$

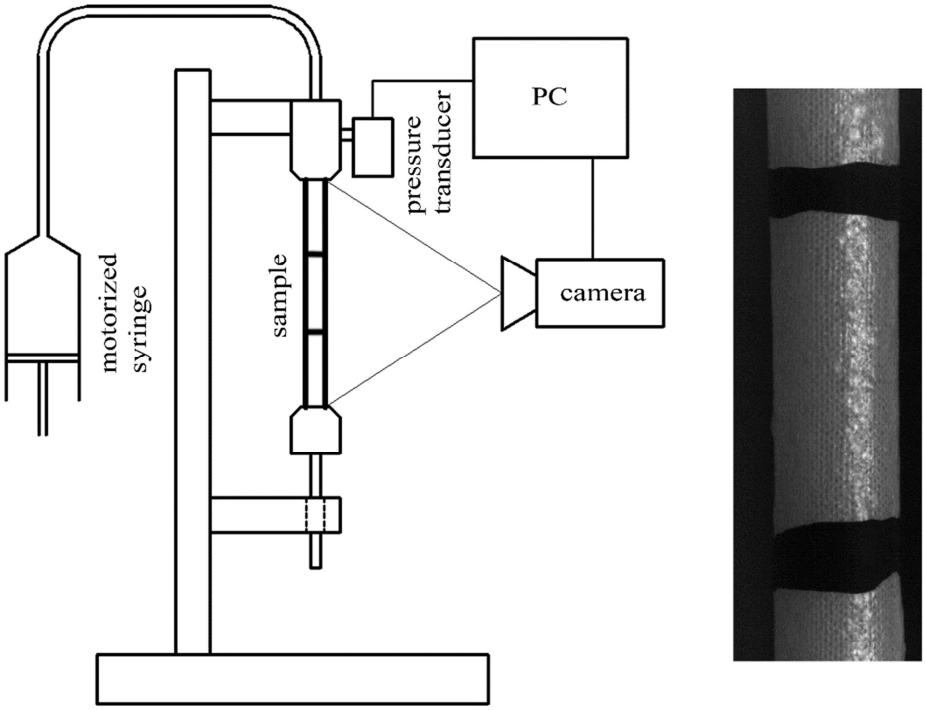


Figure 1: The experimental inflation-extension set-up (left panel) and the picture of the sample from CCD camera (right panel).

## 3 RESULTS

The reference dimensions of samples are listed in Table 1. Final circumferential stress – stretch curves are plotted in Fig. 2.

| Sample | $R_o$ [mm] (mean ± SD) | $H$ [mm] (mean |
|---|---|---|
| **Non-sterilized 1** | 3.80 ± 0.02 | 0.75 ± 0.17 |
| **Non-sterilized 2** | 4.26 ± 0.12 | 0.99 ± 0.28 |
| **Ethylene oxide 1** | 3.87 ± 0.09 | 0.62 ± 0.13 |
| **Ethylene oxide 2** | 3.65 ± 0.02 | 0.87 ± 0.25 |
| **Radiation 1** | 3.80 ± 0.06 | 0.68 ± 0.20 |
| **Radiation 2** | 3.45 ± 0.10 | 0.55 ± 0.07 |

Table 1: The reference dimensions of tubes. Here $R_o$ is the reference outer radius, $H$ is the reference overall thickness, respectively.

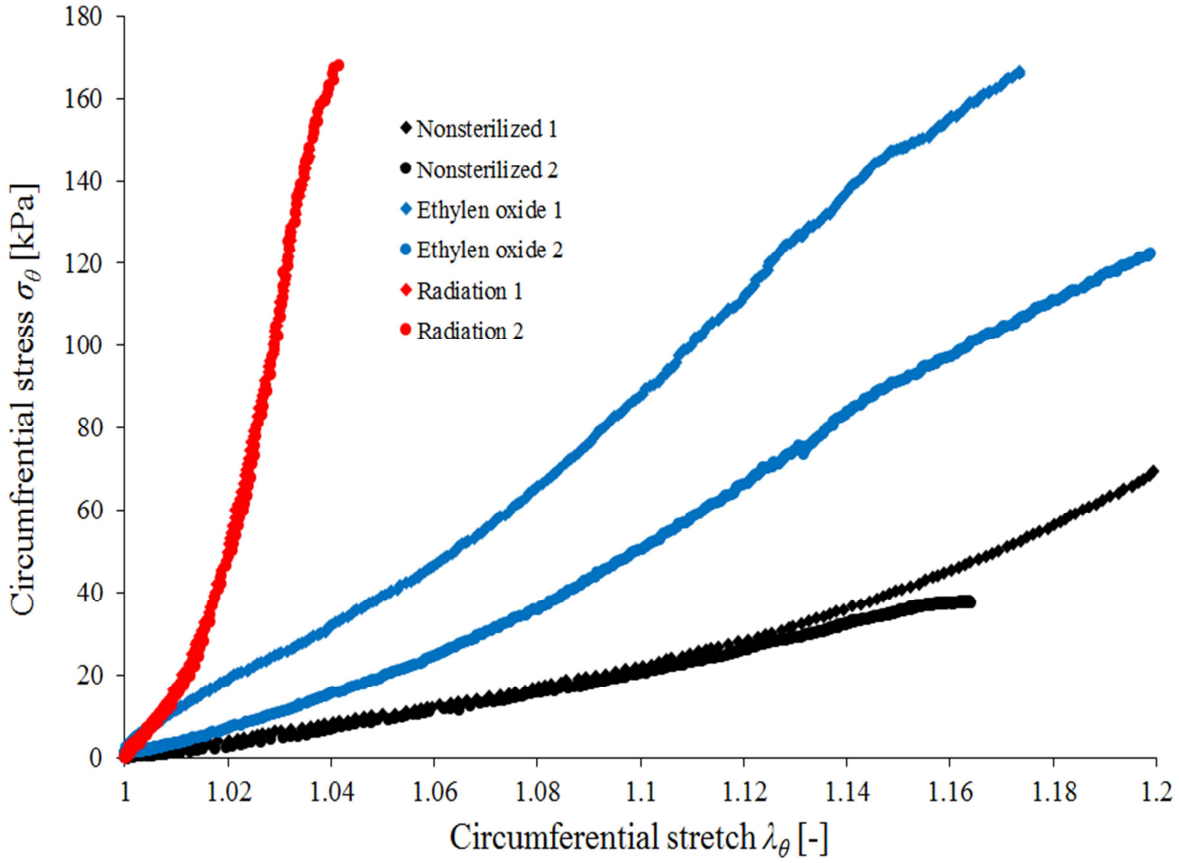


Figure 2: Stress – stretch curves for control/non-sterilized (black), ethylene oxide (blue) and irradiation (red) sterilized samples, respectively.

## 4 DISCUSSION AND CONCLUSIONS

In our study, biological composite samples were manufactured from collagenous matrix and reinforcing polyester mesh. The effect of sterilization (ethylene oxide and irradiation) on mechanical properties of this structure was examined using inflation-extension tests. This influence was evaluated through circumferential stress computed according thin-walled tube approximation. This is one of the limitations of the study, where the ratio of radius and thickness of the samples suggests using thick-walled tube model. However, authors wanted to compare the response of samples and the thin-walled model is simple and sufficient for this purpose.

Fig. 2 shows that the final mechanical properties are dramatically affected by process of the sterilization. Control samples showed the most compliant behavior and low breaking strength. Specimens sterilized using ethylene oxide are stiffer but the deformation at the destruction point is similar as for control samples, while the irradiation changed behavior of the material completely. Here the breaking strength is close to the samples sterilized by ethylene oxide, but the stiffness is very high. These observations should be interpreted through cross-linking process. During the sterilization, additional bonds are created between individual polymer (collagen) chains [8]. The more additional bonds are formed, the stiffer response is observed in the stress-stretch plot.

It was concluded, that the sterilization processes significantly change mechanical response of collagenous material. These findings should be taken into account when such biological material is assumed to be used in tissue engineering.

## ACKNOWLEDGEMENT

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