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Determination of the elongation forces of oesophageal tissue in uniaxial tensile test

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ABSTRACT

Purpose: The aim of this study was to determine the range of optimal tensile forces for the oesophageal tissue and to characterise the biomechanics of the oesophageal tissue.

Method: The subjects of the study were oesophageal fragments of white Pekin ducks with a length of approximately 50 mm. Mechanical uniaxial tensile tests were carried out on fragments of the oesophagus and the effect of long-term tissue loading on the ability to permanently elongate was investigated. All measurements carried out were performed under laboratory conditions imitating the tissue environment.

Results: Force-elongation characteristics and stress-strain characteristics were determined from the experimental tests. The mean value of the ultimate tensile force in uniaxial tensile test was 45.32 ± 6.67 N. Mean ultimate tensile strain of 0.41 ± 0.02 and mean ultimate tensile stress of 2.04 ± 0.83 MPa were also determined. Tests carried out on the effect of a constant tensile force showed that the average value of permanent strain observed was 32.17 ± 2.26 %.

Summary: The conducted research allowed to characterise the biomechanics of white Pekin duck oesophageal tissue. The analyzed range of the elongation force provides satisfactory permanent tissue strain. Within the force range studied, no effect of the loading force value on the strain capacity of the oesophageal tissue was demonstrated.

Keywords: uniaxial tensile tests, experimental tests, soft tissue, long gap atresia, oesophagus

1. INTRODUCTION

Oesophageal atresia is a relatively common condition affecting approximately 1:3500 newborns [12]. In this condition, a fragment of the oesophagus is not developed, often accompanied by a tracheoesophageal fistula, as it is the case in approximately 86% of recorded cases. It is slightly more common in newborn male children [7]. A special case of oesophageal atresia is long-gap oesophageal atresia (LGEA), representing a major challenge to paediatric surgeons on a global level [15]. The current knowledge does not provide a precise definition of long-gap oesophageal atresia. Some sources state that a distance between the two ends of the oesophagus exceeding 2 cm is classified as LGEA, while the majority of sources inform that long-gap atresia is identified when that distance exceeds 3 cm [8]. This case (type A) accounts for approximately 8% of all reported cases of oesophageal atresia, meaning a frequency of 1:40000 newborns [13]. When the oesophageal structure is not fully formed, this poses a major risk, as affected newborns are unable to feed normally. Consequently, a surgical intervention is required to restore the continuity of the oesophagus.

Over the past 80 years, several innovative treatment procedures for long-gap oesophageal atresia have been developed and introduced into clinical practice. These techniques include the transplantation of a section of the colon or stomach to restore the continuity of the oesophagus, as well as methods involving the elongation of native tissue stumps. The former, although they can be a form of surgical treatment, are associated with many long-term complications, and the majority of surgeons agree that preserving the native oesophageal tissue is a better option than the transplantation [8]. Therefore, many doctors are currently leaning towards traction methods, which involve elongation of the ends of the unformed oesophageal tissue using traction sutures. They enable preserving of native tissue, and this is their definite advantage. Traction techniques include the method established in 1997 and developed by John Foker, a paediatric cardiac surgeon at the University of Minnesota. He noticed that the oesophageal tissue can be elongated by applying appropriate axial load. The Foker process involves placing traction sutures in both oesophageal ends and taking them outside the patient's body, to control traction (Fig. 1.1) [5].

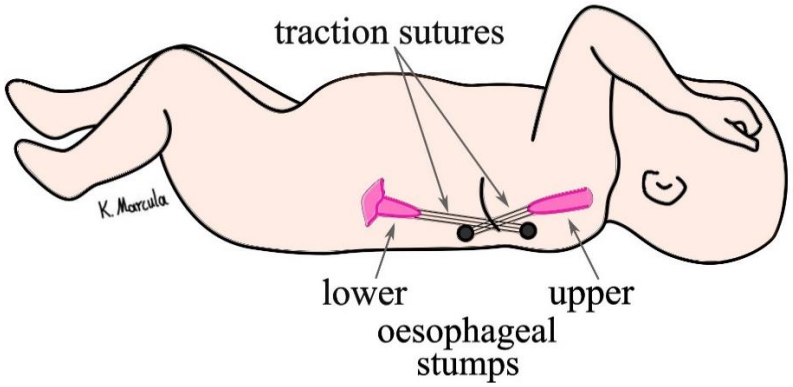

Fig. 1.1 Diagram of the Foker '1' process, with traction sutures placed in oesophageal ends

Despite initial criticism in the academic community, this method was recognised as valuable and offering prospects for further development of surgical treatment for oesophageal atresia in newborns. Traction methods also include the Patkowski method, developed by Professor Dariusz Patkowski, a paediatric surgeon at the Paediatric Surgery and Urology Department in Wrocław. It involves bringing the oesophageal ends together using one or two nonabsorbable traction sutures under moderate tension, with clips applied at the suture entry point. After 5–9 days of applying traction, an attempt is made to suture together both ends of the stumps [2]. Additionally, this method is characterised by the use of internal traction - the sutures are not taken outside the body of the operated patient.

The currently used traction techniques are associated with certain problems. The force applied to load the lengthened oesophageal tissue to a great extent depends on the experience and feel of the surgeon performing the procedure. As surgical sutures are made of materials with low deformability, the applied loading force builds up over a short period of time, and this can result in injuries within the tissue structure. Furthermore, the use of traction sutures can cause mechanical damage to the tissue due to the force exerted on the small area of its contact with the sutures. There is also a possibility that the required tissue elongation will not be achieved by a single increase in the suture traction. The aim of the study was to determine the optimum range of force values which can be applied to perform the oesophageal tissue elongation process, and to determine the parameters that could be used in the future to develop a new method based on the use of a dedicated distractor.

2. MATERIAL AND METHOD

2.1. MATERIAL

The subject of the study were fragments of the oesophagus of white Pekin ducks, approximately 50 mm long (Fig. 2.1). Tissues from ducks of both sexes, delivered straight from the abattoir, were immediately excised and separated from the surrounding structures. Only the lower parts of the oesophagus, located close to the stomach, were used in the study, as they were characterised by both flexible walls and a regular shape. Due to the nature of the study, it was necessary to store the tissues under deep-freeze conditions (-20 °C).

Because the study was conducted on post-mortem tissues, authorisation for the study in accordance with Directive 2010/63/EU was not required, as the Directive applies only to experiments involving live animals.

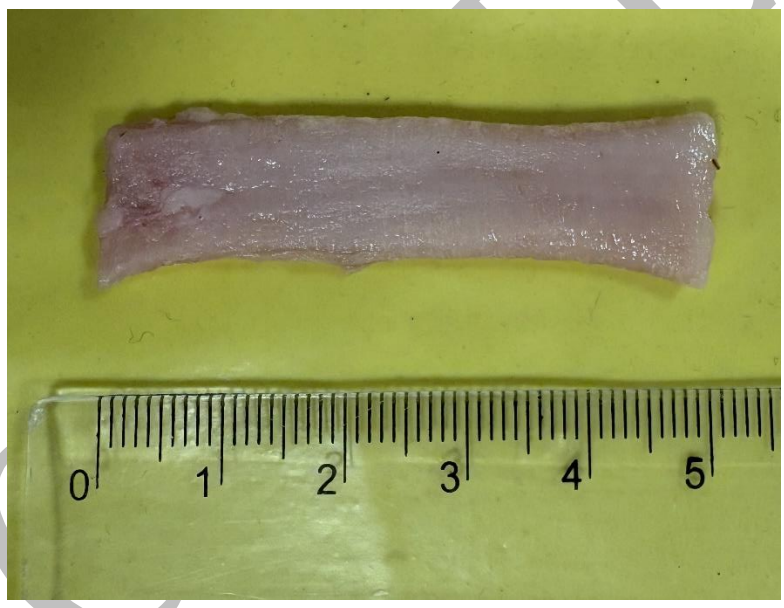

Fig. 2.1 Example of a duck oesophagus sample

2.2. MEASURING SETUP

The experiments were performed on a test bench designed and constructed using incremental technologies, as shown in Fig. 2.2. It included handles, clamps and a climatic chamber (Fig. 2.3). The designed handles ended in a specially shaped mandrel, with bars on its surface to limit sample slippage. Additionally, the mandrel surface was coated with an additional material to increase friction between the oesophagus and the handle. The solutions used ensured that the samples were fixed sufficiently strongly within the range of applied load values. The sample placed on the mandrels was restrained with designed two-piece clamps that ensured

adequate pressure through the threaded connections used, without damaging tissue continuity. During the experiments, the climatic chamber was filled with physiological saline solution (0.9% NaCl) [6]; additionally, the *Lauda E200* recirculating heater was used to maintain a constant liquid temperature of 37 °C. A heating coil made of aluminium alloy and of a shape adapted to the climatic chamber ensured the transfer of heat generated by the heating system to the physiological saline solution. Additionally, a laboratory elevator was used to place the restrained tissues in the filled climatic chamber. The samples with the climatic chamber, were mounted in a high-precision *MTS Tytron 250* electronic testing system of a resolution of 0.001 N and 0.0001 mm (MTS Systems Corporation, USA).

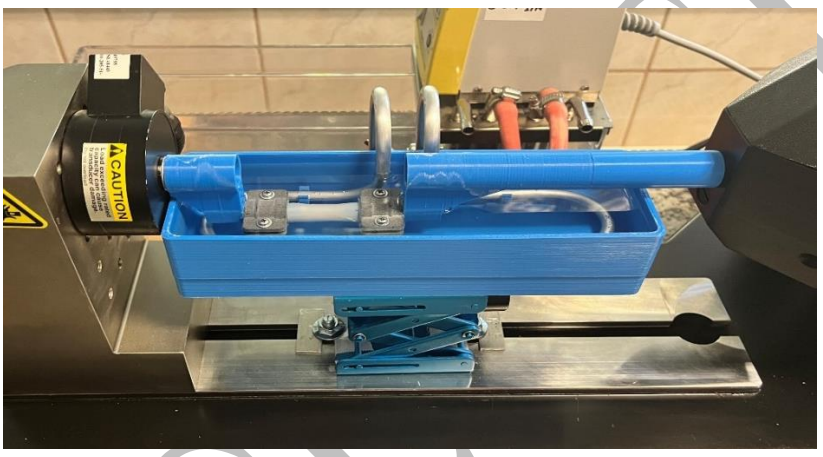

Fig. 2.2 Measuring setup for experimental tests

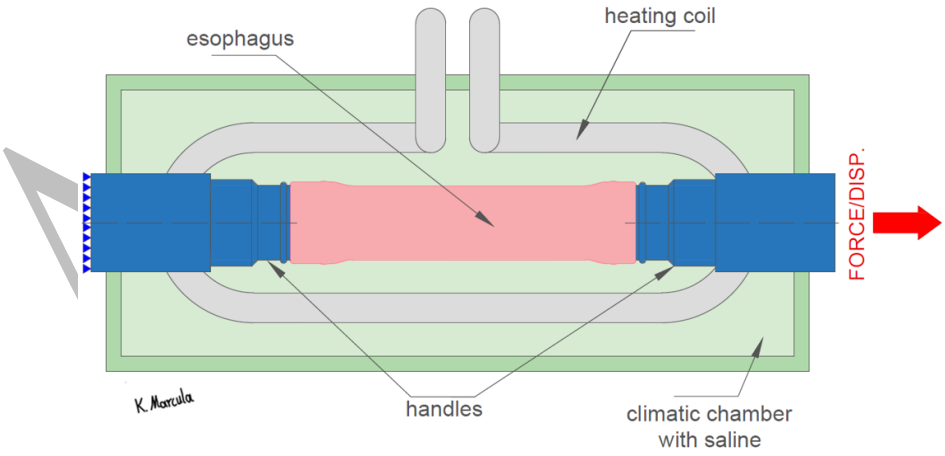

Fig. 2.3 A diagram of the measuring system used, with its specific components highlighted

2.3. QUASI-STATIC UNIAXIAL TENSILE TEST OF OESOPHAGEAL TISSUE

Firstly, the stress-strain characteristics were established for the examined tissue. For this purpose, uniaxial tensile tests were carried out on the duck oesophageal sections. The test group

consisted of 9 samples subjected to axial tension at 10 mm/min [1]. Individual measurements were carried out until a sample broke. From the obtained mechanical characteristics, the values of tensile forces were determined and applied in a further stage of the research.

Prior to measurements, a section was collected from each sample and examined under a microscope to determine the cross-sectional surface area of the oesophageal section. For this purpose, a stereoscopic optical microscope *Stereo Discovery V20* (Zeiss, Oberkochen, Germany) and *AxioVision Rel. 4.8* software were used, with which the outer and inner envelopes of the cross-sectional surface area of the sample were determined, and then the individual surface areas described by the envelopes were subtracted from each other. An example of a measurement is shown in Fig. 2.4.

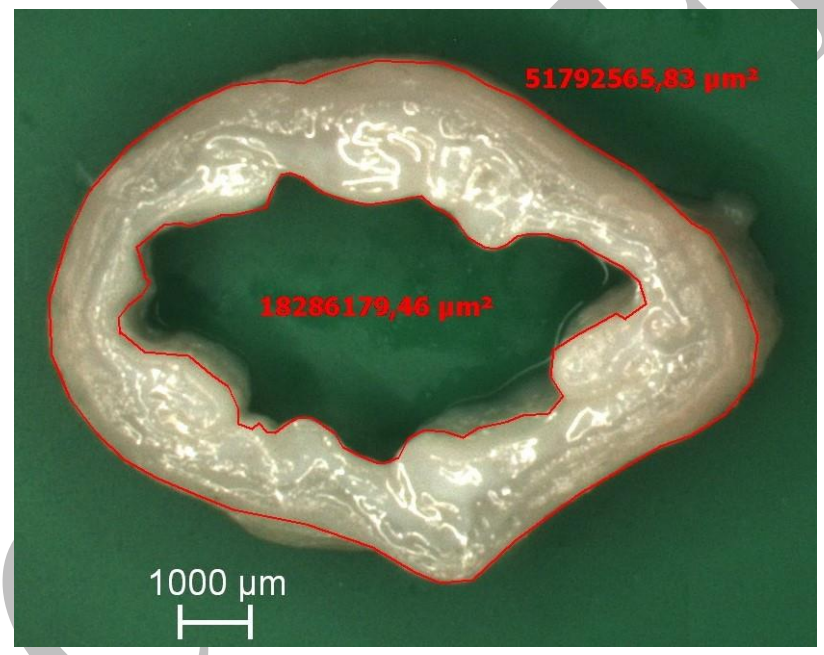

Fig. 2.4 Example of a cross-sectional surface area measurement for a specimen of the duck oesophagus

2.4. THE EFFECT OF LONG-TERM LOADING ON PERMANENT STRAIN OF THE OESOPHAGUS

At the next stage of the study, the effect of applying a constant force on the oesophageal tissue strain was verified. For this purpose, tests were carried out on 16 samples, in groups of 4 samples for each force load of 5, 10, 15 and 20 N. The measurement procedure (Fig. 2.5) involved reaching the set tensile force in 5 minutes and maintaining its constant value for a further 4 h. The tensile force was then reduced to 0.5 N [16] at a speed corresponding to the force build-up rate, and then the force was maintained at 0.5 N for 20 min [3]. This time allowed the tissue to relax completely. At the last stage of the experiment, the permanent strain of the tissues was recorded.

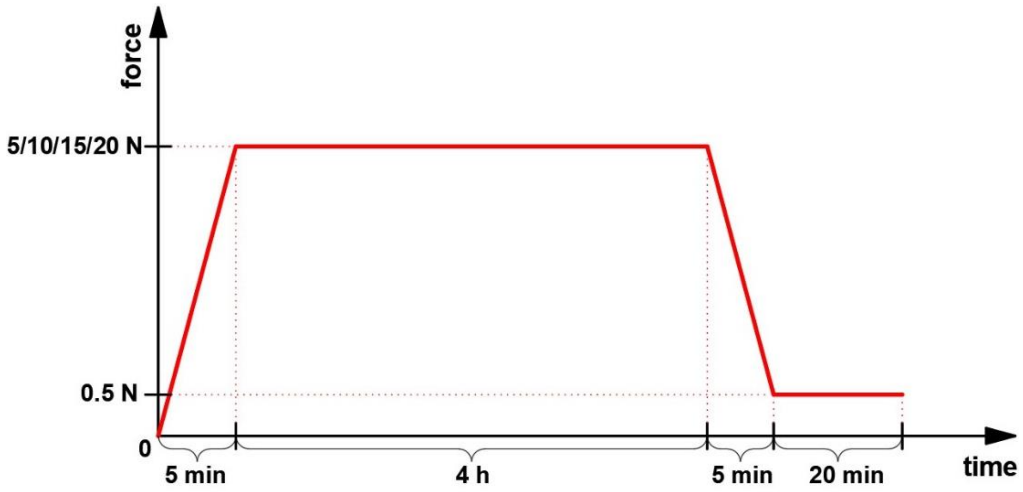

Fig. 2.5 Chart presenting the measurement procedure used to check the effect of loading with a constant force on the sample strain

3. RESULTS

The obtained results were used to plot charts showing the course of force-elongation characteristics for the oesophageal tissues examined (Fig. 3.1). It can be seen that the elongation resulting in the sample breaking is within the range of 15 to 20 mm. The mean ultimate tensile force for the conducted tests was 45.32 ± 6.67 N. It can also be seen that the pattern is analogous for all oesophageal samples examined.

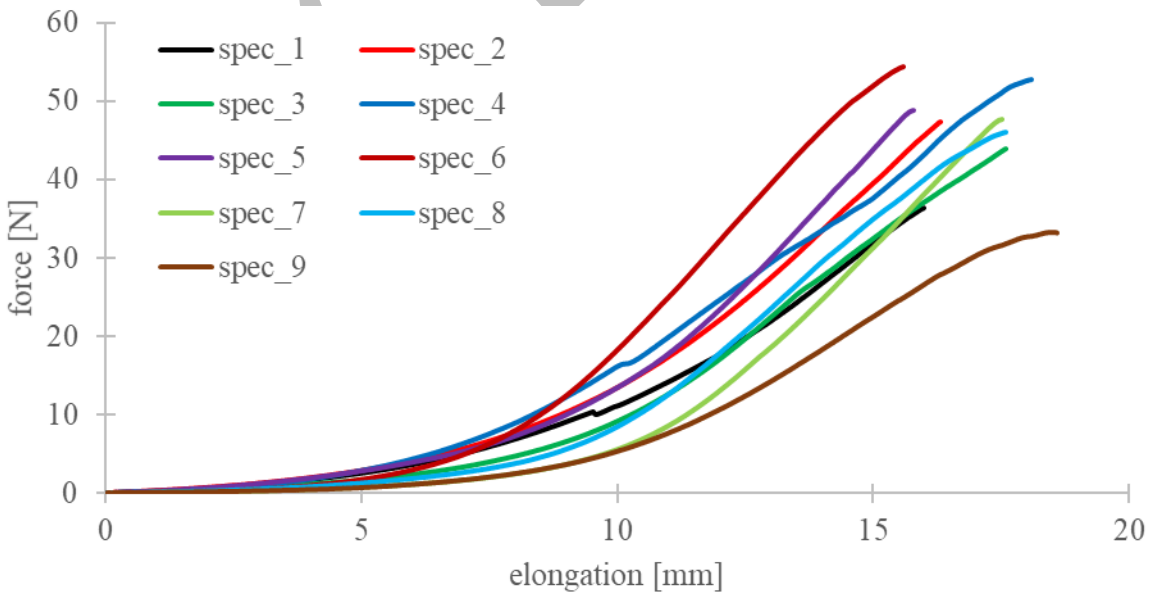

Fig. 3.1 Summary of the force-displacement relationship for the tested samples

The obtained force-displacement characteristics, the determined initial sample cross-sectional surface areas, A_0, and the initial sample lengths, L_0, were used to calculate the values of the true stress and strain. For this purpose, the initial sample volume, V_0, was determined:

$$V_0 = A_0 \cdot L_0 \quad (1)$$

It was assumed that the sample volume, V_0, did not change during stretching, but only its length ΔL and cross-sectional surface area changed. The current cross-sectional surface area [14], A_{cu} was described by the following formula:

$$A_{cu} = \frac{V_0}{\Delta L + L_0} \quad (2)$$

Therefore, the true stress was determined using the formula:

$$\sigma = \frac{F}{A_{cu}} \quad (3)$$

where F was the current loading force applied to the sample.

The formula was used to determine the true strain:

$$e = \ln\left(\frac{\Delta L + L_0}{L_0}\right) \quad (4)$$

A graph showing the stress and strain characteristics of the samples was plotted from the calculated stress and strain values. The obtained mean ultimate tensile strain value was 0.41 ± 0.02 and the mean value for the ultimate tensile stress was 2.04 ± 0.83 MPa. A clear increase in stress values in all cases occurred when strains equal to 0.20 were reached. Additionally, averaged stress and strain characteristics were determined, based on the previously determined courses (Fig. 3.2).

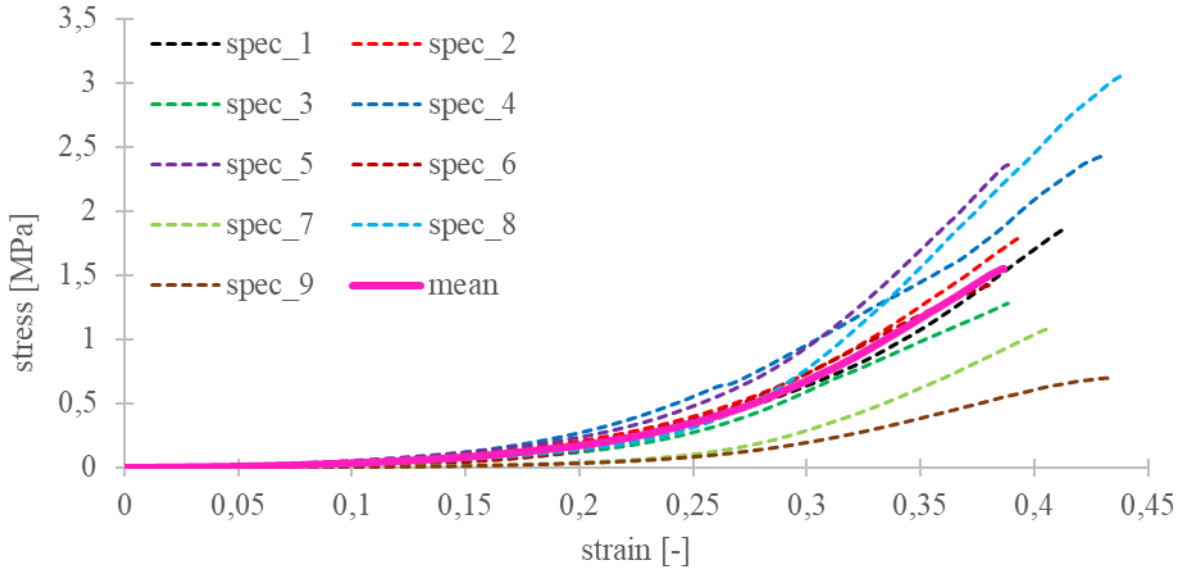

Fig. 3.2 Averaged stress and strain characteristics

Tests involving long-term loading of the oesophagus fragments were used to determine the permanent longitudinal strain of the tissues. An example of an experiment for a 10 N load is shown in Fig. 3.3. It can be seen that the largest increase in strain values occurred while force was building up to reach the set value, and in the case presented it was approximately 32%. Then, at the stage of maintaining the constant force for 4 h, that value increased to approximately 40%. A permanent strain at the level of 34.1% was observed after load ceased to be applied to the tissue.

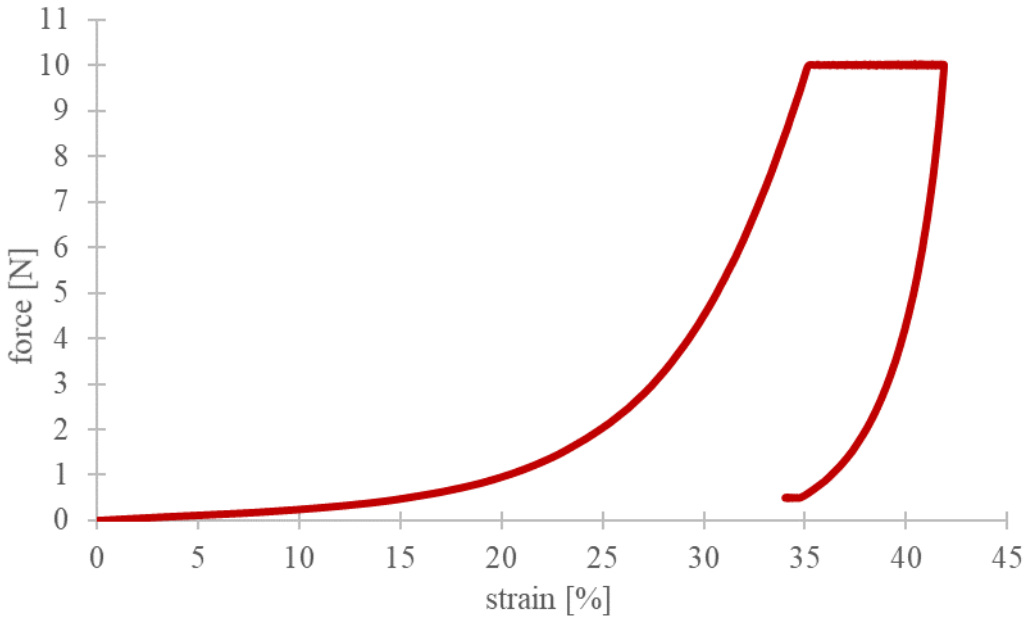

Fig. 3.3 Example of permanent strain measurement for a force load of 10 N

From the point of view of the analysed research problem, the most important parameter of the conducted test is the permanent strain value recorded after the load ceased to be applied to the samples. Fig. 3.4 shows a summary of the obtained values of the permanent strain for all conducted measurements; additionally, Fig. 3.5 includes statistics for the obtained results. It can be seen that the permanent strain values obtained are similar to each other, regardless of the value of the loading force. Furthermore, the mean values determined do not show a clear trend. It can be concluded that in the range of loading forces analysed, no increase in permanent tissue deformation was observed with increasing force. This could be caused by the fact that the loading forces tested were sufficient to elongate the elastin fibres contained in the tissue microstructure. Additionally, repeatability can be seen within the study groups.

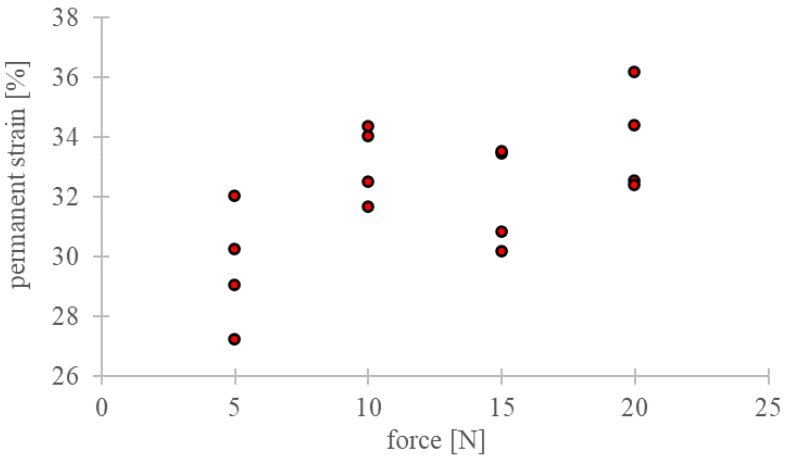

Fig. 3.4 Summary of the permanent strain values obtained for loading forces of 5, 10, 15 and 20 N

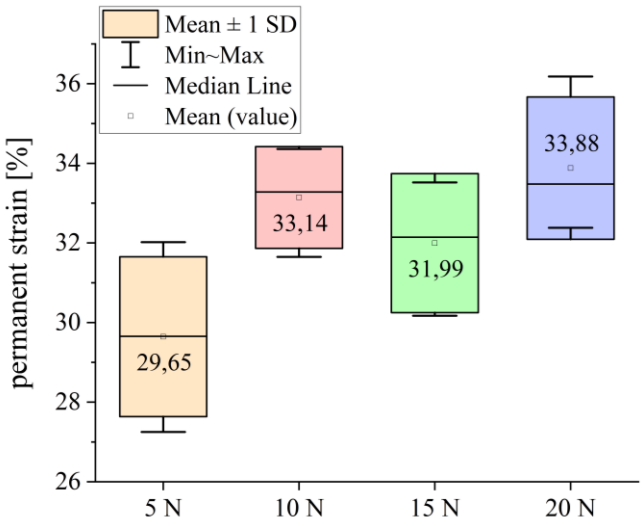

Fig. 3.5 Average results of permanent strain for loading forces of 5, 10, 15 and 20 N

As the results obtained within the study groups did not show a clear upward trend, an analysis was made comparing all the tested samples, regardless of the loading force (Fig. 3.6). It can be seen that the mean value of the permanent strain was 32.17 ± 2.26 %.

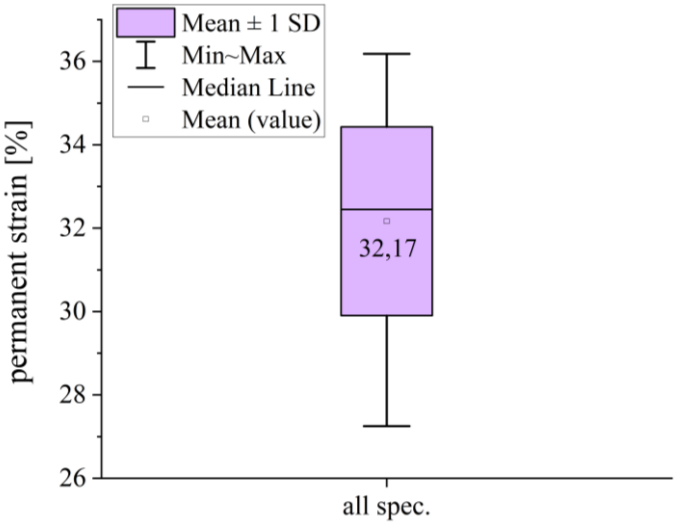

Fig. 3.6 Summary of averaged results of permanent strain measurements

4. DISCUSSION

The tensile tests carried out on fragments of the duck oesophagus enabled determining the ultimate tensile force and ultimate tensile stress values for individual samples. The study showed an analogous pattern for all tissues subjected to uniaxial tensile testing. Therefore, repeatability was found within the study group. The mean value of the ultimate tensile force was 45.32 ± 6.67 N and is consistent with the current state of knowledge. In the study by Oetzmann von Sochaczewski et al. [10], analysing tensile forces for traction with traction sutures, mean values of 25.65 and 25.19 N were observed, depending on the place to which the load was applied, while the maximum recorded forces exceeded 30 N. Although the study material was different (those researchers used porcine oesophagus), a similarity can be observed in the results. The differences in the recorded values resulted from the fact that Oetzmann von Sochaczewski et al. used traction sutures, which caused mechanical damage to the tissue due to the smaller contact area between the tissue and the sutures. In our experiments, the force was distributed evenly over the entire cross-section. Toczewski et al. [15] also conducted a study to determine tensile forces using traction sutures, and the subject of their study was the oesophagus of white Pekin ducks. The mean value of ultimate tensile forces obtained ranged from approximately 4 to 8.5 N, depending on the suture placement technique. Such a large discrepancy in the results was probably caused by a different traction technique, which inflicted

mechanical damage to the tissue under the pressure exerted by the sutures. When analysing the obtained stress and strain curves, significant differences can be seen, compared to the current state of knowledge. In a study conducted by Ngwangwa et al. [9], who analysed the biomechanics of lamb oesophagus in a biaxial tensile test, it can be seen that the averaged stress value is 82.87 ± 30.36 kPa in the circumferential direction of the tissue, and 41.42 ± 32.02 kPa in the longitudinal direction. The load was applied to the samples until the strain of 50% was reached, which did not necessarily result in breaking of the test tissues. Similar ranges of stress values were also obtained by Durcan et al. [4] in their study investigating the biomechanics of the human oesophageal layer. The stress values they recorded did not exceed ca. 140 kPa for the longitudinal direction and ca. 110 kPa for the circumferential direction. In the case of the conducted tests, the samples broke at a mean rupture strain of 0.41 ± 0.02, and the recorded mean rupture stress values for the measurement group were 2.04 ± 0.83 MPa.

The mean values of permanent strain for the samples were determined on the basis of long-term loading of duck oesophageal tissues by applying a constant force. By analysing the graph shown in Fig. 3.5, it can be seen that the values obtained differed slightly, depending on the applied loading force. No clear increasing trend was observed to indicate a relationship between the permanent strain value and the loading force. It was therefore concluded that the range of forces tested allowed for obtaining satisfactory values and an analysis of the results was conducted comparing all loaded samples, irrespective of the load value. On this basis, a permanent strain value of 32.17 ± 2.26% was found. A. K. Saxena et al. [11] conducted an analysis of permanent strain values after cyclic loading of lamb oesophageal tissues. They showed that the value of permanent tissue strain is influenced by the value of cyclic strain, with which the specimens were loaded over 5 cycles. The maximum permanent strain recorded by them was 23.5%. Thus, it can be concluded that permanent changes in the strain already occur with cyclic loading, while when the load is maintained for longer, the obtained results are more satisfactory. This is also confirmed by the example graph shown in Fig. 3.3, where it can be seen that the strain value increased while maintaining a constant loading force. Additionally, the forces analysed by A. K. Saxen et al. were approximately 2, 6 and 16 N, and corresponded to the range of forces examined by us.

The conducted research has some limitations due to the research methods used. Experimental studies were performed under laboratory conditions imitating the tissue environment, while the samples used were post-mortem tissues. Therefore, any healing processes, the presence of which may affect the results obtained, were eliminated. At this stage of the research, no

histological analysis was conducted to assess the damage to the tissue microstructure. Therefore, the effects of the tensile forces applied to the test samples cannot be clearly determined. In the further stages of the development of the ongoing research, it is planned to assess the tissue microstructure after the process of elongating the duck oesophagus in order to avoid tissue damage that could lead to disruption of the normal biomechanics of the oesophagus. The samples tested, due to the nature of the conducted research, had to be subjected to deep-freezing, which may have affected the results obtained. Additionally, the test material used (fragments of oesophageal tissues from white Pekin ducks), despite similarities at the histological level, was also characterised by a deviation, as it did not contain striated cells in the muscle layers. At further stages of the research, it is also planned to increase the size of the study groups.

5. Conclusions

The results obtained lead to the following conclusions:

- with the conducted strength tests it was possible to characterise the oesophageal tissue and understand its biomechanics,
- the range of analysed elongation forces ensures a satisfactory permanent strain in the tissues and, in addition, no effect of the loading force on the tissue strain capacity was demonstrated within this range.

The research conducted to date may have a significant impact on the development of surgical techniques for oesophageal elongation in neonates affected by long-gap atresia. Further research plans include focusing on development of a dedicated distractor that will be able to ensure sufficient oesophageal elongation while avoiding current problems and reducing the invasive nature of the oesophageal elongating procedure.

6. Acknowledgements

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