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MECHANICAL PROPERTIES OF ARTERY-ARTERY CONNECTION BASED UPON TRANSGLUTAMINASE CROSS-LINKED GELATIN

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Original scientific paper

The possibility of surgical repairing of blood vessels by biocompatible adhesive represents an alternative to the conventional sewing techniques. The aim of our study was to evaluate mechanical properties of arteries glued by a cross linked gelatin. Series of quasistatic uniaxial tensile tests of two overlapping arterial strips bonded by the two component glue (gelatin linked by the enzyme transglutaminase) was carried out. The 3D digital image correlation system gave local deformations, and thus the mutual slipping of the bonded strips could be evaluated. The effect of TGA and gelatin concentrations were estimated on the basis of observed data. Recorded maximum stresses were rather small (only tens of kPa). However, cross-linking activity on contact surfaces was proved histologically.

Key words: *Biocompatible glue, transglutaminase, tensile tests, 3D correlation, image analysis*

INTRODUCTION

Transglutaminases (TGases) are enzymes (EC 2.3.2.13) that catalyze formation of extensively cross-linked, generally insoluble protein polymers with high resistance to proteolytic degradation. These biological polymers are indispensable for the organism in order to create barriers and stable structures and their accumulation is found in a number of tissues and processes where such properties are important, including skin, hair, blood clotting and wound healing. TGase is involved in numerous human diseases and its role in human health was reviewed recently in [1,2].

Industrial transglutaminase is extracted from animal blood or produced in commercial quantities by fermentation, see [3]. It can be used as a binding agent to improve the texture of protein-rich foods such as milk products, surimi or ham [4]. Ajinomoto Co of Japan was the first to develop the microbial TGase for food applications under the trade name ActivaTM. This TGase is a single polypeptide chain with a molecular weight of about 38.000, consisting of 331 amino acids, [5].

The ability of TGases to crosslink different proteins and to form their irreversible hydrogels provides grounds for its use in tissue engineering. Jürgensen, et al. tested the capacity of tissue TGase to increase the adhesive strength at a cartilage-cartilage interface [6]. The ability of the microbial TGase and the mushroom tyrosinase to catalyze formation of strong and permanent gels from gelatin and chitosan solutions was examined by Chen [7]. Chitosan was not required for the TGase-catalyzed gel formation, although the gel formation was faster, and the resulting gels were stronger if reactions were performed in the presence of this polysaccharide. The microbial TGase was also already investigated as an alternative biomimetic adhesive based on enzymatic gelatin crosslinking; the TGase-gelatin adhesive bound the opposing porcine tissues together with adhesive strengths which were significantly higher than the strength observed for commercial fibrin sealants [8]. Hu and Messersmith also found that the shear adhesive strength of TGase cross-linked hydrogels of selected peptide substrates equals or is better than the fibrin sealant for tissue and collagen surfaces [9]. Another study suggests that the microbial TGase-crosslinked gelatin may provide a simple, safe, and effective adhesive for ophthalmic applications [10]. Recently, the microbial TGase-gelatine adhesive was successfully tested also as a simple, safe, and cost-effective surgical haemostatic sealant [11].

The aim of this study was to evaluate the possibility to use the commercial microbial TGase as an adhesive for vascular tissues. TGases can offer alternative to the traditional chemical crosslinking, which has seriuos drawback due to the toxicity of the chemical reagent. Previously published results suggest that adhesives based on TGase – protein crosslinking may afford the benefits of fibrin sealants without the need for blood products.

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EXPERIMENTAL WORK

The experiments were carried out with strips of vascular tissues (typical size 70 x 8 x 1 mm) excised from a human thoracic aorta (TA), abdominal aorta (AA) and carotide (CA), shown in Fig.1.

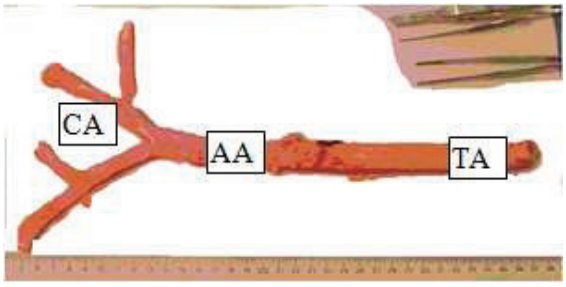

Figure 1. **Excised aorta**

The aortal trunk was harvested within autopsy of 59–year–old male donor, stored in the saline solution at temperature 4°C for 48-hours after death, sectioned and tested at room temperature. The whole procedure was approved by the Ethics Committee of the Faculty Hospital Kralovske Vinohrady (Prague).

The two component adhesive was prepared from gelatine – partially hydrolyzed collagen (10% w/w gelatine dissolved in water) maintained at 50°C that was mixed with the microbial TGase just before bonding (mass fraction of the TGase enzyme in the glue varied from 0 to 3%). Adhesive layer was formed between the intimal layers of partially overlapping strips (connected surface 10 x 8 mm); thickness of the adhesive layer is estimated to 0.5 mm or less, based upon the weighted mass of glue. Dimensions of strips were identified by image analysis of photograps using the ImageJ freeware software. Special cutting, assembly and pressing jigs were used for the strips cutting and bonding, see Fig.2, with the aim to preserve uniform geometry and compressive forces during subsequent curing, that proceeded at a constant temperature 37°C for 60 minutes.

Figure 2. **Bonding and pressing of vascular tissue strips by clips**

Tensile tests started immediately after the clips were removed from the cured samples, using the 1D extensiometer MTS 858.2 Mini Bionix (MTS Inc.), at a constant rate of elangation 0.5 mm/s (L_0=40mm corresponding deformation rate is 0.0125 s^{-1}) up to the connection failure. The whole process was recorded not only by a force transducer, but also by a pair of high speed CCD cameras of the 3D digital image correlation system (DIC) Q-450 + Istra 4D 4.2.1 software (Dantec Dynamics Inc.), [12], see Fig.3.

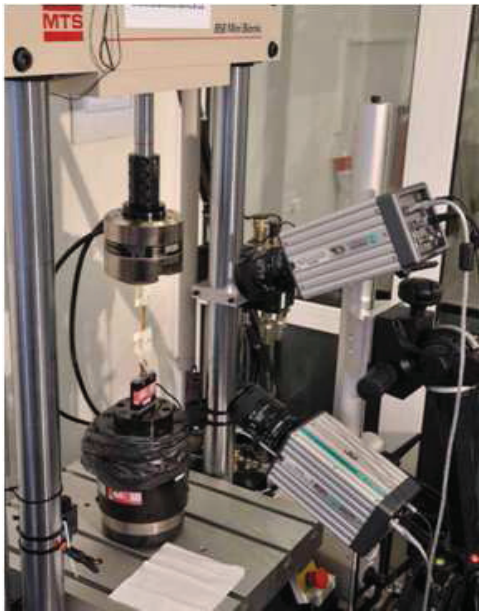

Figure 3. **MTS Mini Bionix extensiometer and Q450 digital image correlation system**

The DIC system requires optically non-uniform surface of measured samples so that the correlation of two images could resolve *x,y,z* coordinates of individual surface points (a matching pattern of randomly distributed speckles seen by both cameras must be identified). In this case the fine pepper, characterized by a non uniform distribution of particle size and colour, was sprayed upon the surface of tissue. The pepper speckles proved to be a good marker giving acceptable compromise between the resolution and the robustness of DIC. Comparison between unloaded and loaded configurations under stereoscopic view enables correlation algorithm to evaluate local displacements and deformations. This procedure was applied at focused surfaces of the samples in order to analyse their displacements. Bearing in mind that samples were created by two overlapped strips, three region of interest were considered in each sample. The entire situation is depicted in Fig. 4. There are terminal segments 1,3 formed by a

single strip and the central region 2 where the overlapping strips are bonded.

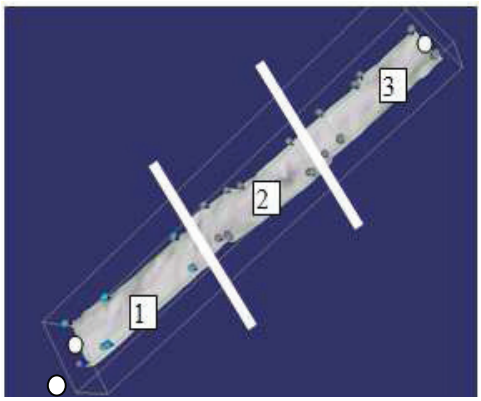

Figure 4. **DIC image of a loaded sample**

While 18 samples were tested by loading in the MTS extensiometer, one control sample was intended for histological analysis with the aim to assess the structure of the cross-linked tissue. Formaline-fixated sample was cut into thin slices (3 μm thick), and stained by eosin and hematoxylin.

ACHIEVED RESULTS AND THEIR ANALYSIS

Tensile tests carried out with the constant concentration of gelatine (10% w/w) and increasing concentration of TGase enzyme (0 to 3%), exhibit significant scatter of the recorded stress – strain relationships. Slight stress stiffening and the enzyme concentration effect are shown for thoracic aorta in Fig.5. Deformations $\varepsilon = \Delta L / L_0$ and normal stresses $\sigma = F / A_0$ are related to the initial configuration and to the cross-section of a single strip.

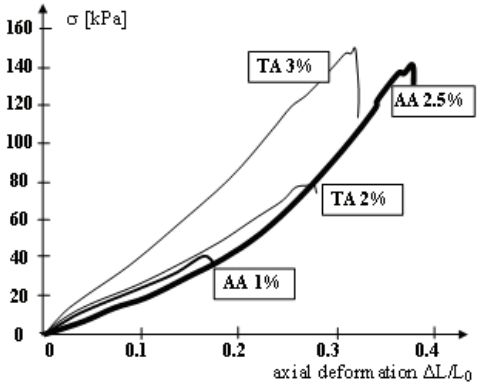

Figure 5. **Stress-strain relationship for the AA and TA samples (% refers to the mass fraction of TGase)**

The recorded maximum shear stresses before failure are presented in Fig.6 for all samples (shear stresses are related to the initial contact area). A slight trend of increasing maximum stresses with the TGase concentration is apparent (the ultimate stresses are increased about 3 times).

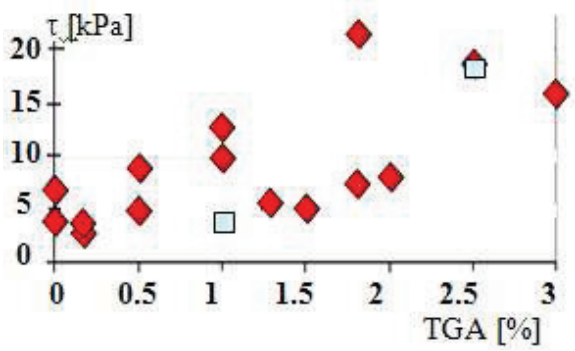

Figure 6. **Maximum shear stresses (squares represent samples with 8% gelatine)**

Results of DIC technique were compared with the displacements obtained from the MTS extensometer. The mean deformations, evaluated from displacement of the correlated end-points (see the circles in the Fig.4 located at the ends of the digital camera viewport) were about 5% less than the MTS values. This difference can be attributed to boundary effects and of course to a measurement error. On the other hand when the deformations in the segments 1,2,3 were evaluated separately (either by averaging local deformations determined by the Istra software, or by evaluation of displacements of selected points located inside these segments, see Fig.4), the resulting overall displacement

$$\Delta L = L_1\varepsilon_1 + L_2\varepsilon_2 + L_3\varepsilon_3$$

was much lower, by about 25%. As soon as the phenomenon was observed in all tested samples the difference cannot be ascribed only to experimental errors, and should be interpreted as a mutual strip-slip.

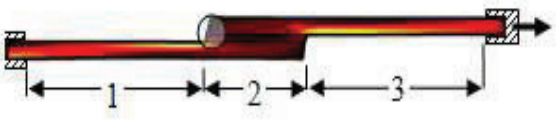

Figure 7. **Elastic deformation of connected strips**

This apparent slip appears since the very beginning of loading, see Fig.8 for thoracic and abdominal aortas

(compare these values with the overall elongation in Fig.5). Results indicate that the increase TGAse concentration slightly decreases the slip.

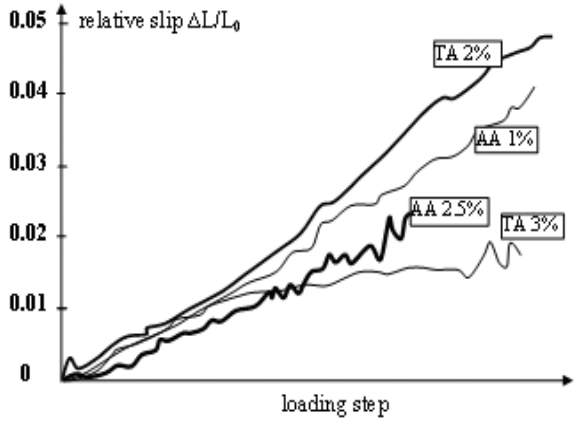

Figure 8. **Relative strip-slip for the AA and TA samples in Fig.5**

This is not quite clear whether this phenomenon is reversible or not, because all experiments proceeded until the breakaway. The apparent slip could be possibly a manifestation of a purely elastic shear deformation, see schematic illustration of FE analysis in Fig.7. Despite the low values of recorded ultimate stresses, histological analysis revealed a cross-linking activity of TGase between contact surfaces, as is shown in Fig. 9. However, in such a way created bonds do not seem to be plentiful. This may be affected by a procedure of formalin fixation and by subsequent manipulations during preparation of histological sections.

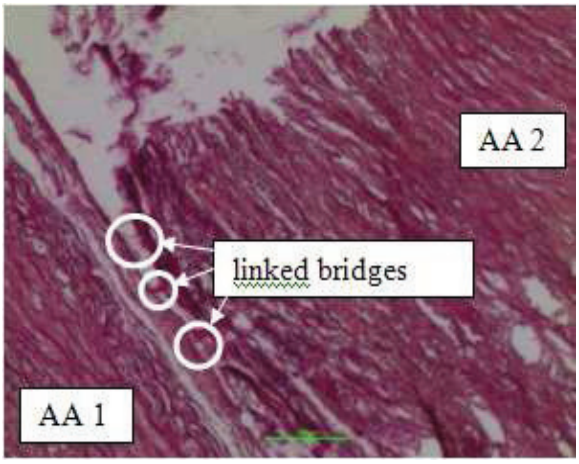

Figure 9. **Histology of AA connection - detail of the region enclosed by circle in Fig.**7

CONCLUSIONS

The paper analyses strength of biohadvesive bonds applied to vascular tissues. The glue was formed by gelatine cross-linked by the transglutaminase enzyme. Approximately the 3-times increase of the maximum stresses was achieved at maximum concentration of TGA (3%). The breakaway of all tested samples occurs at the connected surface, leaving surface of the connected tissues almost untouched. It seems that the glue is only partially cross-linked with the surface layer of tissues and relative weakness of these links suggests mutual slip of bonded strips upon extension test. Kinematical analysis performed using digital image correlation proved presence of differences in local deformations which may be interpreted as an apparent slip.

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