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Some Irradiation-Influenced Features of Pericardial Tissues Engineered for Biomaterials

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1. Introduction

Engineered xenogeneic tissues are very popular biomaterials used in tissue replacements. For this purpose, various kinds of tissues are exposed to stabilization processes leading to an increase in the biomaterial durability *in vivo* (Ionescu et al., 1982; Khor, 1997; Vesely, 2003). The structure stability is achieved mainly by crosslinking of structural proteins. The main requirements were focused on the reduction of immune response and degradation processes. Such effects have firstly been obtained by the use of formaldehyde (FA) (Paneth & O'Brien, 1966), but resulted crosslinks were reversible. Better results were achieved by the use of glutaraldehyde (GA) being a bifunctional aldehyde. It is widely used from the late 60s of 20th century to reduce the xenografts' antigenicity and proteolytic degradation (Carpentier et al., 1969; Woodroof, 1978). However, the GA cytotoxicity (Gendler et al., 1984; Huang-Lee et al., 1990; Moczar et al., 1994) as well as premature calcification of GA-treated tissues (Golomb et al., 1987; Levy et al., 1991; Thoma & Phillips, 1995) limit the durability of bioprostheses.

These disadvantages of the GA-stabilized biomaterials were a sufficient reason for initializing the tissue stabilization by irradiation being perceived as safer (Mechanic, 1994; Moore el al., 1994). This way of the tissue modification does not cause an incorporation of chemical reagents into the tissue structure, which can be a reason for defects *in vivo* of heart valves stabilized by GA.

Apart from many others, some irradiation methods are proposed as useful for preservation of tissues applied in manufacturing of various biomaterials. The attention has been paid to the tissue stabilization methods based on action of visible (VIS) light or ultraviolet (UV) light, both non-mediated and mediated by a dye (Weadock at al., 1995; Suh et al., 1999; Westaby et al., 1999). The methods using irradiation as modifying or stabilizing factor allow to obtain biomaterials of different durability, both biodegradable and non-biodegradable. The porcine valves or bovine pericardium (BP) irradiation makes it possible to produce non-biodegradable bioprostheses (Westaby et al., 1999; Suh et al., 1999). Irradiation of collagen fibers results in the obtaining biodegradable sponges (Weadock et al., 1996).

2. Photooxidation as a method of tissue stabilization

2.1 Background

It has been well-documented that crosslinking processes in tissues influence their structural modification (Turek et al., 2007), prolongation of durability (Vesely, 2003), increase in biocompatibility (Reardon & O'Brien, 1997) and obtaining sterile products (Stone, 2006).
Photooxidation is one of the methods which make it possible to eliminate the disadvantages of GA (Moore, 1997). Mechanism of photooxidation is still not completely recognized. In the tissues exposed on irradiation, generation of free radicals in the residues of aromatic acids (Fujimori, 1965) and interaction between amino acids residues take place (Mechanic, 1994). One of the hypotheses of crosslinking by photooxidation postulates alteration in the imidazole ring of histidine, leading to the formation of side chains containing aldehyde groups (Weil et al., 1951) or an imidazole peroxide (Au & Madison, 2000).
Dyes may accelerate or facilitate processes of the collagenous materials photomodification (Mechanic, 1994). On the other hand, the presence of dye during photooxidation has a protecting effect on the tissue components against photodegradation (Sionkowska, 2000).
Triplet state catalyst and substrate interact and secondary reactive radicals by electron or hydrogen atom transfer reactions are produced (Spikes & Straight, 1967). In the presence of oxygen electrons and hydrogen atoms the substrates are oxidized. Moreover, dyes, like methylene blue (MB) and riboflavin (RF) generate reactive oxygen species (Halliwell & Gutteridge, 1990; Fernandez et al., 1997). MB, methylene green, rose Bengal, RF and proflavin belong to the group of preferred photocatalysts (Mechanic, 1994).
Photosensitive dyes have been used since 1950s to investigate processes of amino acids oxidation of various proteins (Weil et al., 1952). Following research works indicated that photooxidative process caused modification of methionine (Neumann et al., 1962), tyrosine (Weil et al., 1965), tryptophan (Gurnani et al., 1966) and histidine (Tomita et al., 1969), promoting bonds between the functional groups of the amino acids. One of more attractive and health-safe methods used in biomaterials engineering may be photooxidation of collagenous tissues in the presence of photoactive dyes and VIS light (Mechanic, 1994) or in the presence of UV light (Suh et al., 1999). The photooxidation promotes the forming of covalent bonds between amino acids without cytotoxic linkers.

2.2 Collagen stabilization by photooxidation

Collagens belong to the major proteins group of extracellular matrix which contributes to the structural stability. The name collagen is used for the proteins family forming triple helix of three polypeptide chains (Gelse et al., 2003). In biomaterials engineering, type I collagen plays a key role because of its dominant presence in connective tissues. Collagenous tissues and also isolated collagen as crosslinked or non-crosslinked products are employed in various applications. Native collagen has natural intra- and intermolecular crosslinks which contribute to the stability of collagen fibers. Stabilization processes used in the biomaterials engineering result in introducing additional crosslinks to proteins which influences the decrease in the susceptibility to degradation. The predominant stabilization of xenogeneic collagenous biomaterials may be obtained due to their GA-crosslinking (Jayakrishnan & Jameela, 1996). In the last years attention has been paid to processes using irradiation (Moore, 1997) because of clinical failures of GA-treated tissues (Golomb et al., 1987). It is very important that the collagen photooxidation allows to obtain degradable and non-

degradable collagenous materials. Nowadays both bioprostheses and collagenous sponges being obtained as a result of the collagen crosslinking by photomodification are proposed as stabilized biomaterials (Moore, 1997; Chan & So, 2008). Collagen may be photomodified in various ways. Taking into account that photooxidation may be accompanied by degradation processes, the presence of dye as catalyst and protector seems to be correct for obtaining non-degradable bioprostheses, whereas non dye-mediated photooxidation may be used for obtaining the collagen sponges.

In 1969, Gowri and Thomas showed that soluble collagen could be crosslinked in the presence of MB. It was demonstrated by viscosity increase in photooxidized material. Nearly ten years later, Bernstein and Mechanic (1980) noticed that photooxidation transformed soluble collagen into protein insoluble under the most extreme denaturing conditions and resistant to pepsin digestion.

Additional information has been obtained as a result of investigations carried out by Ramshaw et al. (1994). They have shown that MB sensitized photooxidation of tendon led to its stabilization due to crosslinking of collagen fibrils. After this process, an increase in the thermal stability of the collagen as well as a decrease in the amounts of low-weight molecular fragments obtained after cyanogen bromide (CNBr) cleavage of tendon and simultaneous increase in the formation higher molecular components were demonstrated.

Moore et al. (1994) have demonstrated that during the dye-mediated photooxidation of tissue, the crosslinking of collagen fibrils takes place. This effect was shown in electrophoresis studies of soluble collagen isolated from BP (Moore et al., 1994). The dye-mediated photooxidation caused an even multiple increase effect in molecular weight of various collagen types, indicating the formation of intermolecular crosslinks. The resultant tissue was similar to untreated tissue in texture and elasticity (Moore, 1997). Tissues treated with FA and GA (Cwalina et al., 2002) as well as with tannic acid (TA) (Jastrzebska et al., 2005) indicated greater structural complexity, density and bending stiffness as compared with those resulting from dye-mediated stabilization (Cwalina et al., 2000).

UV irradiation of collagen fibers does not introduce cytotoxic reagents into biomaterials and revealed the mechanical properties comparable to the intact human anterior cruciate ligament (ACL). It was also demonstrated that UV-crosslinked collagen fibers exhibited resistance to nonspecific proteases (Weadock et al., 1996). Moreover, significant enhancement of the bending strength and resistance to enzymatic digestion in UV-treated collagen fibers may be obtained due to glucose synergistic effect (Ohan et al., 2002).

Other authors have revealed that photochemical crosslinking improves the physicochemical properties of collagenous scaffolds. In the studies collagen gel was modified with laser in the presence of a photosensitizer. This modification significantly reduced the swelling ratio, improved the stress-strain relationship, peak load, ultimate stress, rupture strain, and also tangent modulus of collagen membranes (Chan & So, 2005).

2.3 Photooxidative crosslinking of collagenous tissues

The growing interest in use of photooxidation to stabilize collagen materials for bioprostheses was observed after patenting this method by Mechanic (1994). His invention relates in particular "to a process for photooxidizing collagenous material in the presence of photocatalyst to crosslink and stabilize that material" as well as "to the resulting crosslinked product". The modified collagenous products resulting from the claimed process may be used as biomaterials for vascular grafts, heart valves, pericardial patches, injectable collagen,

or as replacement ligaments. A choice of optimum conditions of photooxidation process, mainly dye concentration, temperature and exposure time, depend both on dye and tissue type as a well as desired application of modified material (Mechanic, 1994).

The BP is a collagenous tissue more often used in studies on dye-mediated photooxidation (Mechanic, 1994; Moore et al., 1994; Bianco et al., 1996; McIlroy et al., 1997). These investigations showed that tissue became stable after treatment with photosensitive dyes. Tissue collagen was resistant to action of chemical and enzymatic agents (Mechanic, 1994; Moore et al., 1994) and to mechanical degradation (Moore et al., 1994). The photooxidized BP maintained the properties of natural tissue, supporting the growth of endothelial cells (Bengtsson et al., 1995), being biocompatible and non-immunogenic (Moore & Phillips, 1997). Moreover, dye-mediated photooxidation of collagen tissues appears to be a feasible way of reducing bioprosthetic heart valves calcification (Mechanic, 1994; Bianco et al., 1996).

The stabilization of pericardium by dye-mediated photooxidation was the main objective in investigations carried out by Moore group (Moore et al., 1994; 1996; McIlroy et al., 1997; Moore & Phillips, 1997). To study the efficiency of collagen modification with this method, various collagenous materials were subjected to photooxidation for varying times from 0 to 91 h (Moore et al., 1994) or even to 168 h (McIlroy et al., 1997). Control samples were dye-treated without lighting or were irradiated without the dye. Samples not exposed to either dye or light were studied as untreated material. It was demonstrated that samples photooxidized in the presence of dye were more resistant concerning protein extraction as compared with the control samples. This resistance was shown to be time-increased, which may reflect the kinetic nature of photooxidation. Dye-mediated photooxidation resulted in an alteration of existing crosslinks and a possible addition of new crosslinks in the tissue (McIlroy et al., 1997). Tissue modified was also more resistant to CNBr and pepsin digestion when compared with control tissue (Moore et al., 1994). CNBr-resistance of photooxidized tissue was partially reversible by 2-mercaptoethanol-pretreatment of tissue. This effect may indicate the possibility of methionine photooxidation, which was confirmed in studies carried out by McIlroy et al. (1997). They also indicated that dye-mediated photooxidation of bovine tissues (pericardium, arteries) resulted in a time-dependent reduction of histidine content. Except methionine and histidine, no other amino acid alteration was detected by amino acid analysis (McIlroy et al., 1997).

Other concept was presented by Suh and co-workers (1999; 2000). In the studies the author modified porcine valves by UV irradiation. A decrease in hydroxyproline content as compared with GA-treated tissue and a decrease in the crimp pattern of collagen fibers for UV-modified tissue were observed (Suh et al., 1999). UV-irradiation of porcine valve influenced the reduction of calcification (Suh et al., 2000). The dye-mediated photooxidation processes were also proposed to stabilize acellular BP (de Visscher et al., 2008).

Photooxidation process of tissues results in their smaller immunogenicity and resistance to degradation. It has been demonstrated that allogeneic osteochondral grafts have many disadvantages, like the possibility of diseases transmission, immunogenic response and less complete graft incorporation. Dye-mediated photooxidation may be used to obtain allogeneic and xenogeneic scaffolds to repair damaged or diseased cartilage (Hetherington et al., 2005; 2007; Kawalec-Carroll et al., 2006). In one of the experiments, human and bovine cartilages were photooxidized in the presence of MB and then the grafts were implanted to mice. The photooxidized bovine grafts, native cartilage and photooxidized human grafts did not induce a significant response. Microscopic studies revealed some degree of fibrous

encapsulation and inflammatory infiltration in all studied tissue samples. Nowadays, a dye-mediated photooxidation is tested as a method of obtaining xenogeneic osteochondral grafts (Kawalec-Carroll et al., 2006).

2.4 Properties of photooxidized collagenous tissue

Photooxidation processes belong to the alternative tissue stabilization methods. Comparison of the tissues properties resulted from the dye-mediated photooxidation or non dye-mediated photooxidation give grounds to expect correct long term results.

Moore's team (Moore et al., 1994) carried out *in vivo* investigations in which dye-mediated photooxidized collagenous tissues were subcutaneously implanted in rats. It has been shown that modified material indicated higher stability as compared with control samples. After tissues explanation it has been demonstrated that photooxidized material underwent calcification, but this effect was minimal as compared with tissues modified using GA.

Dye-mediated photooxidation has not influence on the sterility of collagenous material. Non-aldehyde method using iodide-based sterilization for dye-mediated photooxidized tissues was proposed by Moore and co-workers (1997). This way of sterilization allows to obtain sterile biomaterial without the changes of collagen structure. Moreover, biocompatibility tests showed that photooxidized and afterwards iodine-sterilized (Moore et al., 1997) BP and porcine pericardium (PP) tissues were non-cytotoxic, non-hemolytic and non-mutagenic (Moore & Phillips, 1997). In contrast, tissues treated with GA were found to be cytotoxic (Nimni et al., 1987; Huang-Lee et al., 1990).

Moreover, no negative symptoms have been observed after dye-mediated photooxidized collagenous material implantation in rabbits. Histopathological studies have not indicated significant macroscopic reaction, but only slight microscopic response (Moore, 1997; Moore & Phillips, 1997). Besides, a lack of the photooxidized collagenous material toxicity has also been demonstrated through tests for intracutaneous toxicity (irritation) and acute systemic toxicity (Moore & Phillips, 1997).

Moore and Phillips (1997) also investigated immunogenicity of BP and PP tissues that were modified using GA or dye-mediated photooxidation and subsequent sterilization in iodine-based solution. Tissues were homogenized and suspensions were injected into rabbits at three-week intervals. Antibody response was determined using a radioimmunoassay. These investigations demonstrated that both controls and modified tissues (photooxidized or treated with GA) showed low antibody levels.

Dye-mediated photooxidation of pericardial tissues was found to be a process which favors the growth of endothelial cells (Bengtsson et al., 1995). Investigations were carried out using BP or PP specimens, on which saphenous vein endothelial cells were seeded and incubated for 1 week. Cultured endothelial cells were grown as a confluent lining similar to native endothelium. This effect was demonstrated by scanning electron microscopy. Results obtained by Bengtsson et al. (1995) may be very important for a long-term durability of implants, manufactured using photooxidized tissues. Dye-mediated photooxidized pericardium did not exhibit any significant increase in thermal stability reflected by shrinkage temperature, but a rise in ultimate tensile strength was indicated (Moore et al., 1996). It was because of the modification and crosslink formation of existing matrix components in pericardial tissue (McIlroy et al., 1997). However, studies in the sheep model of bioprosthetic heart valves manufactured from dye-mediated photooxidized BP have

revealed the damages as the result of tissue abrasion on the cloth covering the stent (Butterfield & Fisher, 2000).
Photomodification by UV-irradiation of porcine valves resulted in their resistance to collagenase action and significantly smaller susceptibility to calcification as compared with GA-treated tissue (Suh et al., 1999; 2000).
Dye-mediated photooxidation of allogeneic and xenogeneic ostechondral grafts stabilized the cartilage surface (Kawalec-Carroll et al., 2006). It has been demonstrated that xenogeneic grafts revealed a regeneration effect in the defects but no fusion between the graft and host cartilage took place (Hetherington et al., 2007).

2.5 Possibility of application of photooxidized collagenous products

Photooxidized products based on the collagenous components may found many commercial applications for biomedical uses. Dye-mediated photooxidized pericardial tissue was proposed to manufacture bioprosthetic heart valves. As yet, such valves were implanted into juvenile sheep because of their susceptibility to tissues calcification (Bianco et al., 1996). Since calcification processes represent a major percentage of clinical complications appearing during bioprostheses use, this animal model has been perceived as the most appropriate. Using of them, it was possible to verify early obtained results and to indicate many positive features of dye-mediated photooxidized tissues as compared with GA-stabilized ones. The first and at the same time the most important advantage of dye-mediated photooxidized tissue is that it did not undergo significant calcification. Besides, this tissue indicated high stability when implanted into organism. After explantation, such tissue was flexible and demonstrated collagen structure similar to that of unimplanted tissue (Moore, 1997). Contrary effects have been obtained as a result of investigations carried out on sheep with implanted valves prepared using GA-treated tissues. The valves after explantation showed calcification causing death of animals (Bianco et al., 1996).
Extra advantage of dye-mediated photooxidized pericardial tissues is that such materials (as valve or aorta' section) implanted into organism of juvenile sheep was overspread with layer of flat cells morphologically similar to endothelial cells. Their endothelial character was confirmed by positive staining using von Willebrand's method (Moore & Phillips, 1997). This test showed biocompatibility between cells crept on the implant and endothelial cells. Westaby et al. (1999) have shown in the juvenile sheep model that photomodified valves prepared using dye-mediated photooxidation (PhotoFix™) revealed minimal calcification. The sheep model study started by Carbomedics Inc. was the next important contribution to photooxidized valves investigations. However, clinical failures caused by abrasion of the inflow surface of the leaflets against the cloth-covered inner face of the outer valve frame were revealed (Butterfield & Fisher, 2000). These problems may result from processes of endothelialization which are much more rapid in sheep than in humans (Vesely, 2003). At presence, Carbomedics Inc. offers to sell CardioFix Pericardium obtained by PhotoFix Technology for intra-cardiac repair, great vessels repair, suture lines buttressing and pericardial closure. According to the producer, CardioFix Pericardium exhibits handling and suturability characteristics of autologous tissue.
The most important feature of xenobioprostheses is the initiation of recellularization of the tissues. The concept of a hybrid valve manufactured from a decellularized dye-mediated BP and allogeneic cells may be promising (de Visscher et al., 2008). As such collagenous products are biocompatible they influence a rapid recellularization of hybrid materials both

in vivo and *in vitro*. Implantation of recellularized dye-mediated BP can result in a reduction of failures noted by Butterfield and Fisher (2000).
Pericardium has more homogenous structure than valve tissues. Photostabilization of pericardium tissues seems to be simpler. However, Suh and co-workers proposed porcine valve modified with UV-irradiation as bioprostheses (Suh et al., 1999; 2000).
Other possible application of photooxidized products includes biodegradable products like sponges or scaffolds. Weadock et al. (1995) have shown that collagen fibers crosslinked by UV irradiation may be used for ACL reconstruction because of mechanical properties, enzymatic resistance and biocompatibility (Weadock et al., 1995; 1996).
Chan and co-workers have demonstrated that collagen crosslinked with rose Bengal under laser light can be used to produce encapsulated structures with proteins for controlled protein release. This method may be useful for the production of collagenous scaffolds (Chan & So, 2005; 2008; Chan et al., 2008a; 2008b).

2.6 Concluding remarks

Summarizing information concerning dye-mediated photooxidation of collagen and collagenous tissues, it may be stated that: (i) photooxidized collagenous tissues do not undergo excessive calcification being the main reason for clinical complications taking place in case of using implants stabilized with GA; (ii) thanks to formation of transversal bonds in the photooxidized collagenous tissues, their strong crosslinking takes place; modified tissue becomes stable, i.e. does not undergo degradation by chemical agents or enzymatic digestion and demonstrate higher mechanical endurance; (iii) photooxidized collagenous tissues are non-toxic, non-immunogenic, biocompatible and maintain the growth of endothelial cells.
Such features of collagen and collagenous tissues photooxidized either in the presence of or without the photoactive dyes confirm the possibility of using these materials in long-term medical implants.

3. Investigated tissues and methods of their photomodification

3.1 Tissues preparation

PP or BP were obtained from the local abattoir directly after animal slaughtering. Before transportation the tissues were rinsed in cooled solution (4°C) of phosphate-buffered saline (PBS; pH 6.5). During transport tissues were placed in the containers with PBS solution in cooler box (4°C). Fibrous part of pericardium was mechanically separated from the pericardial sac. Before photomodification, tissular fat, heavy vasculatures and ligaments were removed.

3.2 Methylene blue-mediated photooxidation of bovine pericardium

The photooxidation process was carried out for 5, 15, 30, 45, 60, 90 and 120 min, using VIS light (12 W light bulb) and 0.05% solution of MB in PBS (pH 6.5), at 23°C. Samples of BP were incubated in 50% solution of saccharose in PBS for 1 h. In the next step, tissues were incubated in the presence of MB and cleaned air. Finally, the tissues were VIS-irradiated in the presence of dye and cleaned air. The distance between the light source and the dye solution level was 15 cm.

3.3 Methylene blue-mediated photooxidation of porcine pericardium

The photooxidation process was carried out for 8 and 24 h. As a control, native tissue and tissue incubated for 24 h with MB without irradiation were used. This photooxidation process took placed in three stages. In the first one PP was incubated (1 h) in 50% solution of saccharose in PBS to improve the efficiency of dye penetration. The second stage was the incubation of tissue with MB (0.05% solution in PBS; pH 6.5) in the presence of cleaned air. In the last stage the tissue was VIS-irradiated (50 W light bulb) for 8 or 24 h in aerated MB-PBS solution, at 15˚C.

3.4 Short term riboflavin-mediated photooxidation of porcine pericardium

The samples were exposed to VIS-irradiation (50 W light bulb) for 1, 2, 3 h under aeration in the presence of 0.1% solution of RF in PBS (pH 6.5). Before modification, tissue samples were soaked for 1 h in 50% solution of sacchorose. The photooxidation process was carried out at 15°C. The distance between the light source and the dye solution level was 15 cm.

3.5 Photomodification of porcine pericardium with visible or ultraviolet light

The PP samples were UV or VIS-irradiated (50 W light bulb) for 1, 2 and 3 h, at 15°C. During irradiation the tissue samples were soaked in PBS (pH 6.5; 5 mm solution layer). The distance between the light source and the PBS solution level was 15 cm (Fig. 1).

3.6 Ultraviolet-modification of tannic acid crosslinked porcine pericardium

The samples of PP were stabilized by crosslinking with 2% solution of TA in PBS (pH 6.5; 4°C) for 4, 24 and 48 h. TA-crosslinked tissues were UV-irradiated for 1 h, at 15°C, under 5 mm layer of PBS solution. The distance between the light source and the PBS solution level was 15 cm (Fig. 1).

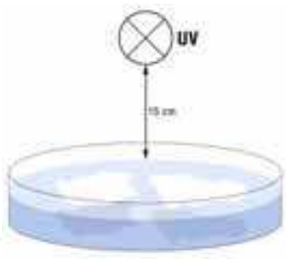


Fig. 1. Photomodification by UV-irradiation of PP.

4. Experiments description

4.1 Density investigation of bovine pericardium modified by methylene blue-mediated photooxidation

Crosslinking reactions in tissues result in an increase in concentrations of additional inter- and intramolecular covalent bonds and also other interactions as compared with native tissue. The modified pericardium tissues indicate the increased strength and simultaneously the decreased chemical degradability, including lower susceptibility to proteolytic enzymes digestion. The aim of the present study was to evaluate (using radioisotopic investigations)

the fixation effects in BP tissues modified by the MB-mediated photooxidation or the GA-treatment. One of the stabilization effects, i.e. density increase in the photooxidized tissue due to crosslinking processes was studied by the determination of the $^{60}Co^{2+}$ accumulation in the tissue samples and their permeability to cobalt ions. Photooxidized tissues were compared with both native and GA-treated (0.2% in PBS, 30 min) tissues.
Radioactivity of the tissue samples and the filtrates penetrating through samples were examined using Packard spectrophotometer. The tissue samples were MB-photooxidized or GA-treated, then ^{60}Co (in $^{60}CoCl_2$ solution) accumulation in the tissue samples as well as their permeability to cobalt ions were examined.
Each experiment included three main stages: (i) selection of samples (all of 20 mm diameter) indicating identical permeability to PBS-solution from the tissue pieces of equal masses (0.14+0.01g, 0.21+0.02g or 0.29+0.03g); (ii) crosslinking of tissue samples indicating equal mass and permeability to PBS-solution; (iii) radioisotopic assays for testing binding capacity and permeability to $^{60}Co^{2+}$ of pericardial tissues (native, GA-treated and photooxidized).

4.2 Mechanical properties of porcine pericardium modified by methylene blue-mediated photooxidation

All fibrous proteins determine the mechanical properties of connective tissues. During their modification, important changes occur in interactions between the tissue components. These changes may result in the tissues' better integrity or failures. The aim of present study was the evaluation of the impact of MB-mediated photooxidation on mechanical properties of PP tissues. The results of these measurements were expected to be very useful in the evaluation of conformational changes effects in tissues after irradiation.
The strength tests were performed employing force-meter AFG 25N (Advance Force Gauge; Mecmesin) that was situated on a movable arm of an electromechanical stand.
The tissue was cut up along fibers into stripes. Each tissue stripe was 35 mm long and 10 mm wide. The breaking force (F_b) was performed as a function of time (10 measuring cycles per second). The streeps were subjected to stretching at the speed of 0.3 mm/s. The tissue samples were placed between two handles maintaining the streeps' initial length of 25 mm. During measurements, samples were fully immersed in isotonic NaCl solution to protect the tissue against drying up. These tests were carried out at the room temperature (21±2°C).
Statistical analyses were carried out using the computer program Statgraphic Plus, version 2.1. The normality of analysed variables distribution was examined using the Shapiro-Wilk test. The significance of differences between average values calculated for the modified samples and those not modified was estimated using the t-Student test.

4.3 Stability of porcine pericardium after riboflavin-mediated photooxidation

The purpose of the present work was to investigate the influence of RF and VIS light in the presence of atmospheric oxygen on the structure of PP. Changes in the stability of tissue structure were evaluated on the basis of SDS-PAGE electrophoresis and histological investigations. Impeded extraction of proteins from the tissue was found to be an indicator of its higher stability. It was shown that relations between the proteins extraction and tissues' morphology prove tissue stability (Moore et al., 1996; Cwalina et al., 2002; 2005; Turek et al., 2007). Before electrophoresis, native and irradiated samples (1 g) were homogenised in 50 ml of water (Polytron PT 2100 - Kinematica AG). Next, aliquots of 1.5 ml

of tissue homogenates were collected and concentrated by centrifugation (14000 x g) for 10 min. to obtain samples of 0.5 ml volume.
Electrophoretic studies were performed according to SDS/NaCl Laemmli method (1970). Electrophoresis was carried out in 10% separating gel with 4% stacking gel, using voltage 140 V. Separated proteins were visualized in the gel using 0.05% Coomassie Brillant Blue R 250 (CBB) dissolved in solution of methanol : acetic acid : water (25:10:65). For destaining, gels were incubated in the same solution without dye (Cwalina et al., 2005). The qualitative analyses of the electrophoregrams were performed using Biotec Fischer System.
Histological studies were carried out under the Polyvar 2 – Leica light microscope, under magnification 200×. Tissue samples were dehydrated in absolute ethanol, and then embedded in paraffin wax. Six micron samples were stained routinely with Harris hematoxylin and erythrosine. Procedure of preparation-documentation was performed using the Quantament 500 Plus System.

4.4 Stability of porcine pericardium after visible and ultraviolet light irradiation

The aim of the present work was to evaluate the influence of the VIS- and UV-irradiation on the PP structure. Changes in the tissue structure stability were evaluated on the basis of SDS-PAGE electrophoresis and histological investigations (as described in section 4.3).

4.5 Stability of UV-irradiated tannic acid-crosslinked porcine pericardium

The aim of present study was to evaluate the TA-modified PP stability after the tissue UV-irradiation. Changes in the stability of tissue structure were evaluated on the basis of SDS-PAGE electrophoresis and histological investigations (as described in section 4.3). However, two methods were used for staining gels: the first with 0.05% CBB and the second with silver.
Investigated tissue samples were subjected to the SDS/NaCl extraction and to enzymatic digestion in solution containing 1.5 g of pancreatin (P) (5000 U of amylase, 30 U of lipase, 3.7 U of proteases/0.15 g of P) in 100 ml of PBS (pH 6.5), for 3 h (Cwalina et al., 2005).

5. Results

5.1 The influence of tissue modification on its permeability to cobalt ions

Changes in density of native BP and tissues modified by MB-mediated photooxidation or GA-crosslinking were revealed. The efficiency of crosslinking processes was evaluated based on the $^{60}Co^{2+}$ accumulation in the tissue samples and on their permeability to cobalt ions. Decreases in radioactivity (reported as counts per minute; cpm) of the tissue samples of various masses (i.e. thickness) after their photooxidation (Fig. 2A) as well as filtrates penetrating the same samples (Fig. 2B) seem to confirm the tissue crosslinking effect.
The permeability to $^{60}Co^{2+}$ and these ions accumulation in the photooxidized tissues were inversely proportional to the samples' thickness (Figs 2A and 2B). Similar dependence was observed in case of filtrates penetrating GA-treated tissues (Fig. 3), although $^{60}Co^{2+}$ accumulation in tissue samples remained at the same level. The GA-treated tissue samples indicated lower binding capacities as compared with the photooxidized samples of equal mass (thickness), pointing to lower crosslinking efficiency of the photooxidation used.

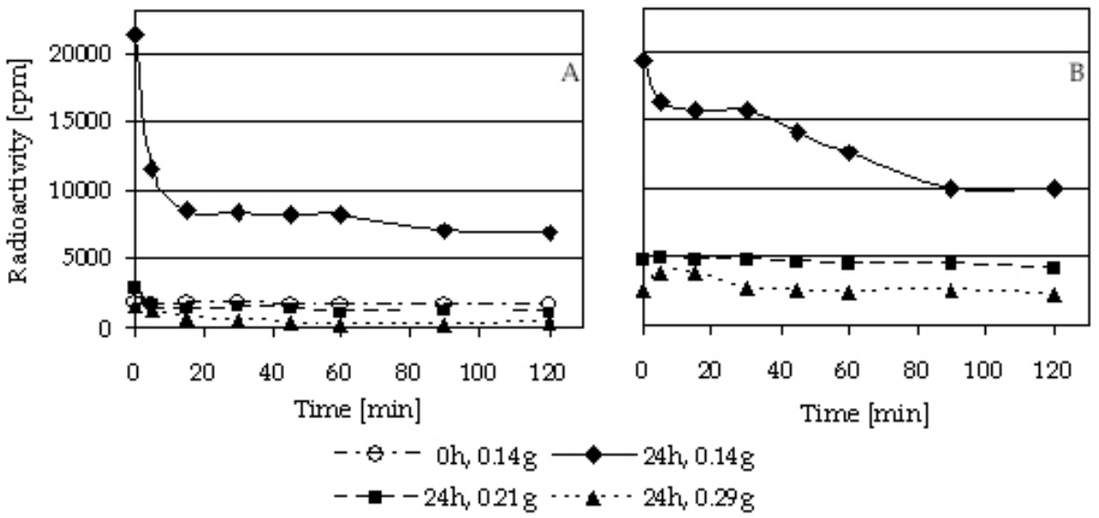


Fig. 2. The influence of photooxidation time on BP density, evaluated by radioactivity of the samples of various weights (0.14, 0.21, 0.29 g) (A); filtrates penetrating these samples (B).

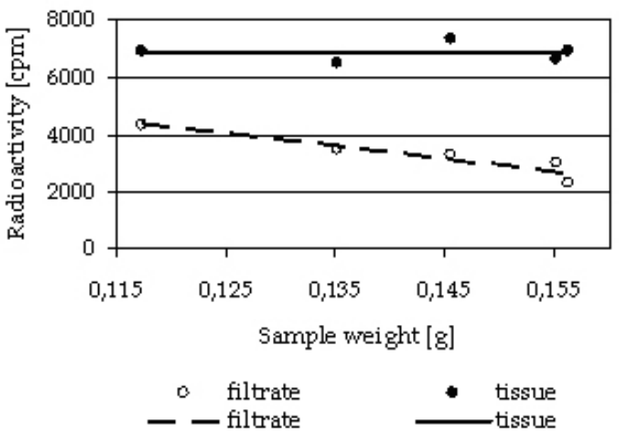


Fig. 3. Radioactivity of the GA-treated BP samples and filtrates penetrating these samples.

Sample weight (mg)	Specific radioactivity [cpm/mg]					
	Tissue samples			Filtrate the tissue samples		
	Native	Photooxidized	GA-treated	Native	Photooxidized	GA-treated
120	-*	-*	57	-*	-*	36
140	139	72	49	152	49	24
160	-*	-*	42	-*	-*	17
210	23	20	-*	14	5	-*
290	9	8	-*	5	1	-*

Table 1. Specific radioactivity of BP samples and filtrates penetrating these samples (* - not measured).

It seemed to be worth recalculating data concerning the tissue samples' permeability to $^{60}Co^{2+}$ and the ions' binding in the tissues in reference to the samples mass. Thus, the values of investigated samples specific radioactivity have been obtained (Table 1).
Almost directly proportional dependence between ^{60}Co-specific activities in crosslinked BP samples (indicative of bound ions) and filtrates penetrating these tissues (indicative of free ions) has been presented in Fig. 4.

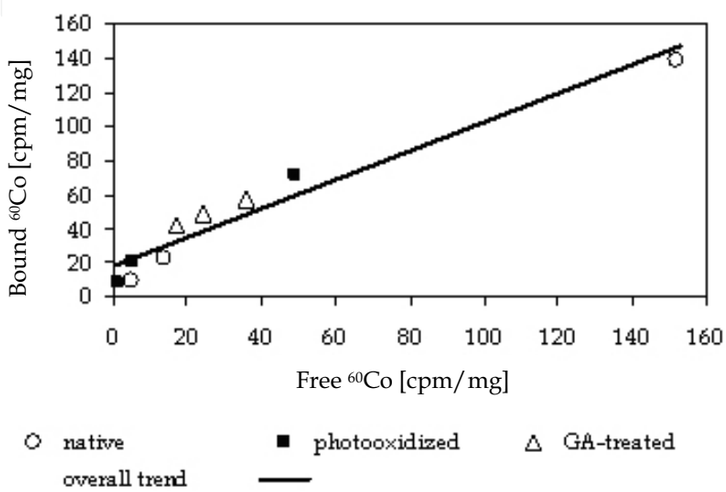


Fig. 4. Dependence between ^{60}Co-specific activities in crosslinked BP samples (bound ions) and filtrates penetrating these tissue samples (free ions).

5.2 The influence of methylene blue-mediated photooxidation on mechanical properties of porcine pericardium

MB-mediated photooxidation leads to significant changes in mechanical properties of modified PP in comparison with native tissue. They are shown in Figure 5 as F_b changes during the PP samples testing, where the most characteristic pictures selected from each series of samples are presented. All F_b–time curves are non-linear and their function graphs are asymmetrical. In case of the modified tissues, wider peaks in the curves were observed. Besides, higher differentiation between graphs representing individual samples in the group of the modified materials was observed than between graphs representing samples of native tissue.
Statistical calculations of F_b have been shown in Table 2. Arithmetic mean and standard deviation of F_b values obtained for six native tissue samples were 1.1±0.13 kG, pointing to their moderate variability (V=11.8%). About three times higher coefficient of variability (V=29.7%) has been calculated for the group of six samples MB-treated without irradiation, where arithmetic mean and standard deviation were 1.18±0.35 kG. The Measured F_b values ranged from 0.6 to 1.7 kG.
The difference between the group of these samples and the group of native tissue samples was not statistically significant. In case of nine samples exposed to MB-action combined with irradiation for 8 h, the mean value of F_b was 0.88±0.16 kG, with coefficient of variability V=18.2%. Prolonged irradiation (24 h) led to the inconsiderable decrease of F_b mean value (0.75±0.19 kG) calculated for eight samples, with coefficient of variability V=25.3%. Results

obtained for both groups of irradiated samples treated with MB were statistically different from the group of native tissue samples.

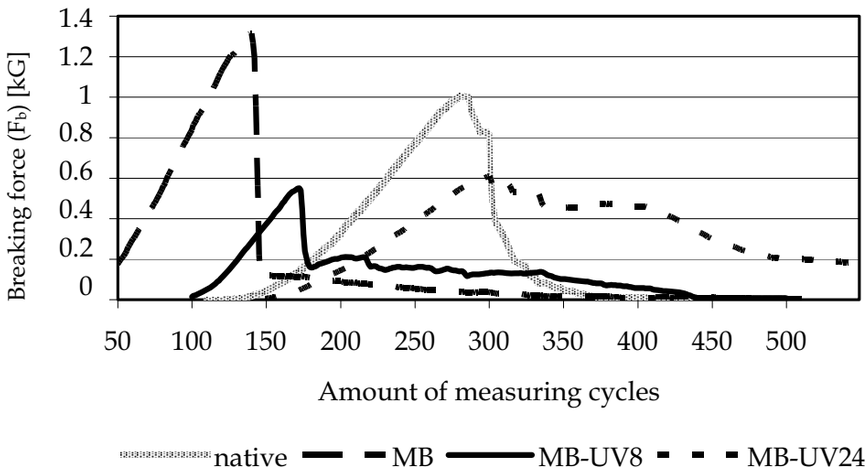


Fig. 5. Breaking force (F_b) measured for tissue samples: native (N); exposed to MB without irradiation (MB); and photooxidized for 8 h (MB-VIS 8) or 24 h (MB-VIS 24).

Sample No.	Breaking force F_b (Kg)			
	N	MB	MB-VIS 8	MB-VIS 24
1	1.0	1.1	0.9	0.8*
2	1.2	1.7	0.7	0.5
3	1.0	0.6	0.9	0.8
4	1.1	1.2*	0.9	0.9
5	1.3	1.2	1.1	0.9
6	1.0*	1.3	1.1	1.0
7			0.8	0.5
8			0.9	0.6
9			0.6*	
X	**1.10**	**1.18**	**0.88**	**0.75**
SD	**0.13**	**0.35**	**0.16**	**0.19**
V(%)	**11.8**	**29.7**	**18.2**	**25.3**
t-Student test (α=0.05)		**SNS**	**SS**	**SS**

Table 2. Breaking force (F_b) measured for pericardial tissues: native (N); exposed to MB without irradiation (MB), and photooxidized for 8 h (MB-VIS 8) or 24 h (MB-VIS 24); X – arithmetic mean; SD – standard deviation; V – coefficient of variability; SS – statistically significant; SNS – statistically not significant; * – values presented in the Figure 5.

5.3 Biochemical and morphological changes in porcine pericardium after riboflavin-mediated photooxidation

The influence of RF-mediated photooxidation on biochemical and morphological features reflecting stability of PP structure has been investigated. Changes in structure stability of the collagenous tissue can be reflected by changes in number of polypeptides of various molecular weight, which are released from photomodified tissues as compared with the native tissue. The electrophoretic profiles of polypeptides extracted from native pericardium and tissues treated with the RF in the presence of VIS light have been shown in Figure 6. Electrophoretic profiles of peptides extracted from all samples indicate similar patterns in range of the molecular weights of 15-160 kDa. Polypeptides of the highest molecular weights (above 200 kDa) were released from native tissue (Fig. 6, line 2) and RF-treated tissues irradiated for 1 h (Fig. 6, line 3). When PP was photooxidized for increasing periods, there was an increase in quantity of polypeptides extracted from the tissues. The peptide bands did not change in quality, although their intensities were increased with longer irradiation time (Fig. 6, lines 2; 4; 5).

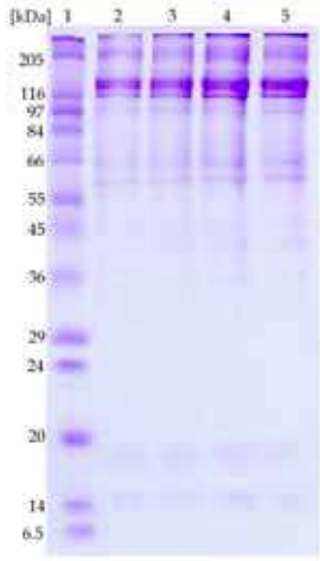


Fig. 6. Electrophoretic profiles of polypeptides extracted from PP samples. Lines: 1 – molecular weight standard; 2 – native tissue; 3; 4; 5 – tissues treated with RF and photooxidized during 1, 2 or 3 h, respectively.

Histological images of the investigated pericardium have been shown in Figures 7-10. Native tissue indicates tight structure with small slits in extracellular matrix. Correct aggregations of fiber bundles of various size and fibroblast nuclei are visible (Fig. 7). The structure of native tissue (Fig. 7) is considerably different from tissue samples treated with RF and VIS-irradiated samples for 1, 2 and 3 h (Fig. 8-10, respectively). Gradual evanishment of some morphological features in the tissues modified by RF-mediated photooxidation was observed as a result of the irradiation period prolongation. After irradiation during 1 h, homogeneous structure of tissue was observed. Moreover, degradation of fibrous structure of pericardium tissue and the disintegration of fibroblast nuclei was noted (Fig. 8). Additionally, after longer RF-mediated photomodification of the tissues a decrease in their cellularity was observed as a result of cell nuclei progressive loss (Fig. 9). After 3 h

modification, looser extracellular matrix with evident slits in the tissue structure was visible. Moreover, a lack of fibroblast nuclei as well as the matrix perforation was observed (Fig. 10).

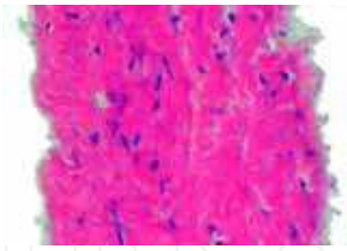


Fig. 7. Native tissue

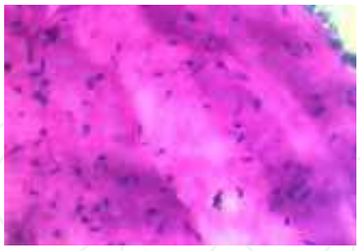


Fig. 8. Tissue treated with riboflavin and light during 1 h.



Fig. 9. Tissue treated with riboflavin and light during 2 h.

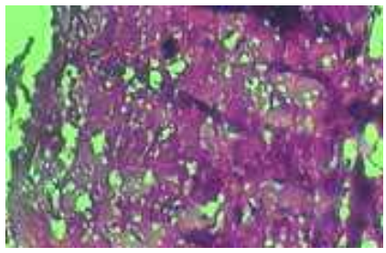


Fig. 10. Tissue treated with riboflavin and light during 3 h.

5.4 Biochemical and morphological changes in porcine pericardium irradiated by visible or ultraviolet light

The influence of the PP irradiation with UV or VIS light on electrophoretic profiles of polypeptides extracted from tissues has been shown in the Figure 11. An electrophoretic pattern representing native tissue (Fig. 11, line 2) comprises of polypeptides with molecular weights of 16-213 kDa. Non significant qualitative and quantitative changes were observed after SDS/NaCl extraction between electrophoretic profiles of tissues: native (Fig. 11, line 2) and irradiated (Fig. 11, lines 3-8).

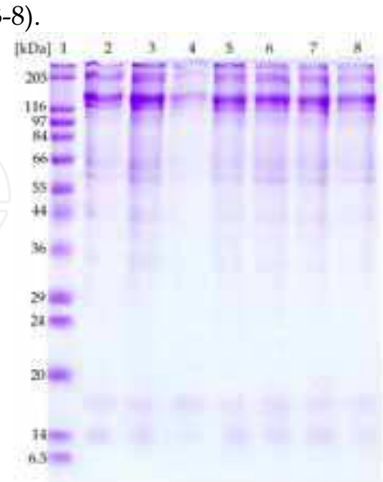


Fig. 11. Electrophoretic profiles of polypeptides extracted from the pericardium samples. Lines: 1 – molecular weight standard; 2 - native tissue; 3; 4; 5 - UV irradiated samples, during 1, 2 or 3 h, respectively; 6; 7; 8 – VIS irradiated samples, during 1, 2 or 3 h, respectively.

Moreover, changes in electrophoretic patterns of samples irradiated with UV and VIS light were also non-significant.

Significant differences were revealed in morphology of tissues irradiated by UV and VIS light. Particularly, it is worth noting the total evanishment of morphological features in the UV-irradiated tissues. Independently of UV-irradiation period, the degradation of PP-morphological components was shown. However, single fragments of connective tissue fibers may be identified. The lack of fibroblast nuclei and the intensive basophilia of extracellular matrix were observed (Fig. 12-14).



Fig. 12. Tissue irradiated with ultraviolet light during 1 h.



Fig. 13. Tissue irradiated with ultraviolet light during 2 h.

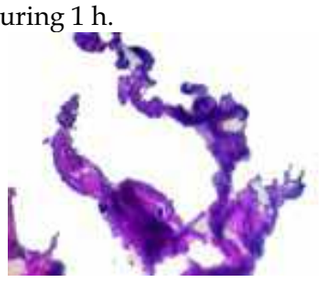


Fig. 14. Tissue irradiated with ultraviolet light during 3 h.

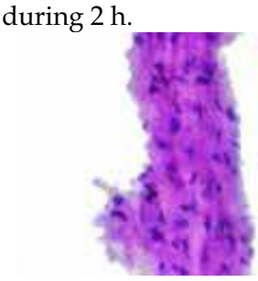


Fig. 15. Tissue irradiated with visible light during 1 h.



Fig. 16. Tissue irradiated with visible light during 2 h.

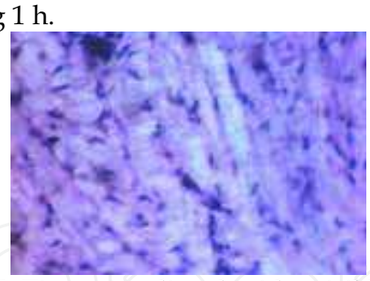


Fig. 17. Tissue irradiated with visible light during 3 h.

More favorable action to the tissue structure by VIS-irradiation was revealed. Irradiation during 1 h makes it possible to maintain fibroblast nuclei and partly fibrous structure (Fig. 15). The prolongation of irradiation period to 2 h and 3 h influences the nuclei disintegration and the appearance of significant swelling of connective tissue fibers (Fig. 16, 17). Diameter of single fibers in this tissue sample is increased as compared with the fibers of native tissue (Fig. 17).

5.5. Effect of tannic acid and UV-irradiation interactions on the biochemical features of porcine pericardium

The electrophoretic profiles of polypeptides stained with CBB or silver, extracted from native and TA-stabilized tissues before and after their irradiation with UV and digestion with P were shown in Figure 18 A and B.

Electrophoretic profiles representing tissues modified with TA and UV-irradiation (Fig. 18. A and B, lines 5, 6, 7) or only UV-irradiated (Fig. 18 A and B, line 3) revealed no significant quantitative changes as compared with native tissues, although some different polypeptides are visualized as the additional bands whereas the other bands are missing in particular lines representing adequate samples in the electrophoregrams obtained using two different staining methods (with CBB or silver). However, significant differences in tissues' structure were revealed in electrophoretic profiles of samples digested with P (Fig. 18 A and B, lines 4 and 8). Higher resistance to enzymatic digestion was shown for the sample modified by TA-crosslinking and UV-irradiation (Fig. 18 A and B, line 8). UV-irradiation and P-digestion of tissue resulted in its destroying and easier removing polypeptides of molecular weights lower than 66 kDa (Fig. 18 A and B, line 4).

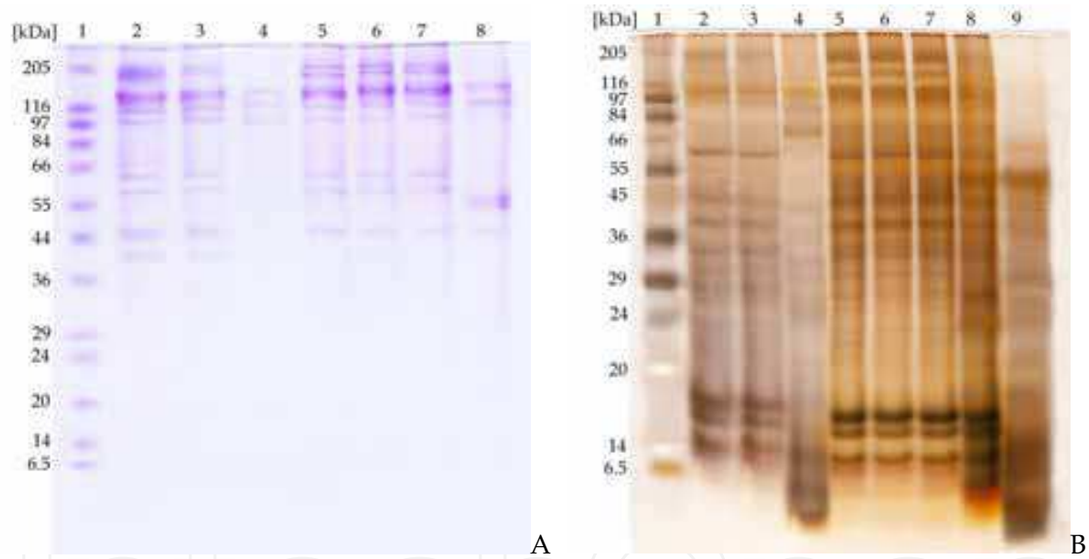


Fig. 18. Electrophoretic profiles of peptides extracted from porcine pericardium samples; A – polypeptides stained with CBB; B – polypeptides stained with silver. Lanes: 1 – molecular weight standard; 2 – native tissue; 3 – UV-irradiated tissue; 4 – UV-irradiated tissue, digested with P; 5 – tissue crosslinked with TA for 4 h and UV-irradiated; 6 – tissue crosslinked with TA for 24 h and UV-irradiated; 7 – tissue crosslinked with TA for 48 h and UV-irradiated; 8 – tissue crosslinked with TA for 4 h and UV-irradiated, digested with P; B – lane 9 – pancreatin.

6. Discussion

6.1. Influence of photomodification on pericardium density

Collagen is responsible for structural integration of collagenous tissues. In the tissue structure, collagen is organized with other proteins and other elements as fine-mesh sieve.

Collagen type I is the main component of pericardium. Density of this tissue is dependent on the crosslinking degree of collagen.
In this study, the BP stability after the MB-mediated photooxidation or GA-treatment was evaluated on the basis of the $^{60}Co^{2+}$ (in $^{60}CoCl_2$ solution) accumulation in the tissue samples as well as on the tissue samples permeability to $^{60}Co^{2+}$. It was shown that both of these characteristics may be useful to confirm the increase of tissue density, which is a result of crosslinking processes and may indicate the tissue fixation effects.
The reduced $^{60}Co^{2+}$-binding capacity in the photooxidized tissues (Fig. 2A) may be the evidence for the decrease in number of free bonding sites due to effective formation of intra- and intermolecular crosslinks between the protein particles in the tissue structure.
On the other hand, the decrease in the photooxidized tissue samples permeability to $^{60}Co^{2+}$ (Fig. 2B) may point to the modified tissue acting as a "molecular sieve" of higher density, in comparison with the native tissue density. The tissues lower binding ability and permeability to $^{60}Co^{2+}$ were attributed both to their higher compactness and thickness.
The ^{60}Co radioactivity in filtrates penetrating the GA-treated tissue samples were also mass-dependent, whereas the cobalt ions accumulation in these tissues was not (Fig. 3).
Changes in the samples' specific activities (Table 1) confirm the mass-dependent increase of the crosslinked tissues compactness as well as their decrease in binding capacities. The specific radioactivity values calculated for tissue-bound and free $^{60}Co^{2+}$ were almost directly proportional regardless of the crosslinking process or the lack of it (Fig. 4).
Concluding, it may be stated that the fixation effects in photomodified pericardium depend on the tissue thickness and time of its exposition to the light and dye. The exposition time is of special importance in case of the thin tissues photooxidation.

6.2. Assessment of mechanical properties of modified pericardium

Mechanical properties of collagenous connective tissues are related to their hierarchical structure, in which type I collagen plays one of the most important role. Pericardium is the tissue consisting mostly of type I collagen. The tensile strength of collagen fibers is the result of the presence of covalent crosslinks. Crosslinking changes the mechanical properties of collagenous materials (Kato & Silver, 1990; Olde Damink et al., 1996; Caruso & Dunn, 2004). It was shown that crosslinking of collagen causes an increase of the elastic modulus and the failure stress of this protein (van der Rijt, 2004).
In our study, the photooxidation of pericardium in the presence of MB resulted in significant changes of mechanical properties after 8 and 24 h modification (Fig. 5; Table 2). Incubation with dye (without irradiation) did not cause significant changes. F_b measured for the photooxidized pericardium was lower. Other authors showed that the breaking stress of individual collagen fibrils increased to 30% after crosslinking by carbodiimide with the N-hydroxysuccinimide and 22% after crosslinking by GA (Yang et al., 2008). However, physical processes and chemical agents influence the mechanical properties in various ways. Moreover different effects after modification of isolated collagen fibers and collagenous tissues may be obtained.
In the studies of Butterfield and Fisher (2000), the failures of heart valves made of photooxidized BP were attributed to this material increased abrasiveness. In our studies, lower F_b measured for MB-mediated tissues as compared with native tissues may correspond to these results. However, Suh et al. (1998) demonstrated that UV-iradiation of

the collagen in porcine heart valves led to improvement of their mechanical properties and that this effect was the most advantageous after 24 h UV-exposition.
Generally, the dye-mediated photooxidation is the stabilization method which bases on catalysis of the processes of additional crosslinks formation in all proteins. In case of connective tissues irradiation, border between photostabilization and photodegradation effects may be fluid and it depends on reaction conditions. Undoubtedly, during dye-mediated photooxidation new crosslinks are formed. However, native crosslinks may be influenced by photolysis.

6.3. Assessment of the stability of pericardium photooxidized in the presence of riboflavin

This assessment of the tissue stability was evaluated by the measurement of quantity of polypeptides extracted with SDS/NaCl from PP using the Laemmli method (1970). The quantity of the proteins is inversely proportional to the extent of the tissue stability (McIlroy et al., 1997).
In electrophoretic profiles presented in Figure 6, the time dependent increase in content of peptides indicating almost the same molecular weights in all the tissues tested (both native and modified) has been observed. Surprisingly, the obtained results suggest that modified tissues did not possess the stable structure; the pericardium treatment with RF in the presence of VIS light and atmospheric oxygen resulted in swelling of the tissue structure. This effect was visible as early as after 2 h of the tissue photomodification. It may be due to the aeration of tissues during their treatment. The inhibitory effect of dissolved oxygen on the modification of collagen was also observed by other authors (Kato et al., 1994).
Microscopic observations show disappearances of fibrous structure as well as gradual broadening of extracellular matrix and decrease in cellularity of the tissues modified for 1 and 2 h (Fig. 8 and 9), as compared with the native material (Fig. 7). After 3 h of the tissue treatment, very loose extracellular matrixes as well as evident slits in tissue structure were observed (Fig. 10).
A reason for the cells damage may be the dynamic formation of reactive oxygen species such as superoxide anion, hydrogen peroxide, and the hydroxyl radical in the reaction mixture (Akiba et al., 1994; Sarkar et al., 1997). An electron transfer from the sensitizer triplet state to molecular oxygen is the usual pathway of superoxide anion formation in oxygenated aqueous solutions (Fernadez et al., 1997). On the other hand, it has been shown that UV irradiation of the collagen solution causes the loss of the protein ability to form natural fibrils (Fujimori, 1965). It is possible, that RF-mediated photooxidaton in the presence of VIS light causes the damage of collagen fibrils which build the tissue structure, leading to the effect observed in the Figures 6, 8-10.
Obtained results suggest that tissues modified by RF-mediated photooxidation may be used as biodegradable materials.

6.4. Assessment of the stability of pericardium modified by visible and ultraviolet light

The crosslinking processes catalysed by VIS or UV light do not introduce the exogenous chemical reagents into the structure of proteins (mainly of collagen) and tissular biomaterials, enabling elimination of the disadvantages resulted from the GA-treatment. However, during UV-irradiation both crosslinking and fragmentation of collagen helixes

take place. The domination of one of these effects results from process conditions, including the exposure period and distance between light source and collagenous material. The collagen form is also significant in the processing of materials containing this protein (Kaminska & Sionkowska, 1996; Cwalina et al., 2003). Different irradiation effects may be obtained by photomodification of freeze-dried collagen, hydrated collagen and collagenous tissues.

In our studies, the same proteins were extracted from all investigated tissue samples, native and VIS- or UV-treated. Non significant changes in electrophoretic patterns between samples irradiated with UV and VIS light were observed. Thus, electrophoretic studies did not reveal biochemical changes (Fig. 11). However, histological images of the UV-irradiated samples showed the disappearance of tissue structure and the intensive basophilia (Fig. 12-14). Similar effects were observed in case of the VIS-treated samples. Morphological changes point to the processes of the tissue photodegradation during its irradiation without the protective action of dye (Fig. 15-17).

These results indicate that the collagen photomodification in the presence of VIS or UV light may be suitable for obtaining collagenous sponges.

6.5. Influence of ultraviolet irradiation on the stability of tannic acid-crosslinked pericardium

UV-irradiation causes increase in the durability of collagenous materials. However, this method is not as effective as GA treatment at reducing biodegradation. The structure of UV-modified collagenous materials may be strengthened by synergistic interaction of UV-irradiation with TA. This synergistic effect of physical (UV-irradiation) and chemical (TA-treatment) stabilization may be reached by two mechanisms. Firstly, TA belongs to chemical crosslinking reagents. In comparison with GA, TA is less cytotoxic (Insenburg et al, 2004) and does not accelerate tissue calcification (Insenburg et al., 2006). Moreover, TA-modification leads to increase of tissues resistance to enzymatic degradation (Cwalina et al., 2005) and is effective as sterilization method (Latte & Kolodziej, 2000; Akiyama et al., 2001). Secondly, TA is composed of gallic acid residues and glucose molecules. It was demonstrated that the generation of free radicals in the residues of aromatic acids plays a key role in photomodification of collagenous materials (Cooper & Davidson, 1965; Fujimori, 1965). The introduction of additional aromatic residues of TA into the tissue may influence the increased efficiency of its modification. Collagen crosslinking by glucose is also taken into consideration. Moreover, Ohan et al. (2002) have showed that interactions during glucose-treatment and UV-irradiation give positive results in collagen modification.

In this work, the TA-treated PP was influenced by UV-irradiation. Taking into account that some combined treatments of collagenous tissues are effective in their structure stabilization, we expected beneficial effects due to proposed modification procedure including the TA-stabilization followed by UV-irradiation.

Comparison of the electrophoretic patterns (obtained after staining polypeptides with CBB and silver) of the native tissue, the UV-irradiated tissue, the same tissue after digestion with P for 3 h as well as the tissues treated with TA for 4, 24 or 48 h and then UV-irradiated did not confirm our expectations (Fig. 18 A and B). The CBB-stained gel (Fig. 18 A) seemed to indicate the TA-treated tissues crosslinking effect and their structure stability, which was reflected by the increase in number of polypeptides of higher molecular weights (Fig. 18 A). However, the silver-stained polypeptides patterns showed that the TA-crosslinked particles

of high molecular weight were more hydrolyzed after UV-irradiation and digestion with P as compared with the native tissue (Fig. 18 B). Simultaneously, a concomitant increase in number of small polypeptides has been observed. Besides, the higher biochemical affinity of P to the TA-treated tissue structural components has been observed (Fig. 18 B). The results also suggest that crosslinked proteins separated from the TA-stabilized PP samples after their UV-irradiation were less tolerant to P-digestion than the native tissue samples.
Obtained results suggest that the TA may be used to attain the prolongation of biodegradation period in the UV-crosslinked collagenous sponges. Besides, release of TA during the sponge biodegradation may in additional way support the biomaterial healing effect on wounds.

6.6. Summary

Irradiation is one from amongst several physical treatments used for collagenous materials stabilization before their utilization in biomaterials. For valve prostheses production, BP and PP may be used. These tissues fixation by chemical substances alone or by their combinations with some physical methods is attributed to the crosslinks formation in proteins, mainly in the collagen. The tissues stabilized due to their crosslinking may act as molecular sieves of higher density in comparison with the native tissue. Increase in the tissues compactness is accompanied by the decrease in number of free bonding sites in structure of crosslinked tissue proteins. In this work it was shown that photooxidation of BP caused decrease in the tissue permeability to $^{60}Co^{2+}$ ions pointing to the tissue higher density. The fixation effects in the pericardium tissues after dye-mediated photooxidation depend on their thickness and time of exposition to the light and dye. The exposition time is of special importance in case of the thin tissues photooxidation. The mechanical properties of photomodified PP were statistically lower as compared with the native tissue. Lower F_b measured for photomodified tissues may result from co-occurrence of crosslinking and photodegradation processes. Both UV- and VIS-irradiation of PP, alone or in the presence of RF resulted in significant changes of the tissue morphological and biochemical features. Especially interesting results have been obtained after the PP treatment with TA and UV light. Such modified tissues were more stable to SDS/NaCl extraction and enzymatic digestion as compared with native (fresh) and UV-treated non-modified tissues.

7. Conclusions

In conclusion, photooxidation permit obtaining bioprostheses being non biodegradable as well as biodegradable biomaterials like collagen sponges. The TA may be used to attain the prolongation of biodegradation period in the UV-crosslinked collagenous sponges. Besides, release of TA during the sponge biodegradation may in additional way support the biomaterial healing effect on wounds.

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