Polyurethane-Based Materials Covered with Natural Polymers for Medical Applications

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Two new polymeric materials were prepared by coating of polyurethane (PU) with a collagen (COL) gel and a mixture of collagen-elastin (COL/EL). The bioactivated polymeric materials were examined by scanning electron microscopy (SEM) for pore morphology. They were also comparatively assayed for porosity, mechanical properties and biodegradability in the presence of bacterial collagenase. In vitro biocompatibility studies were performed in a primary culture of human dermal fibroblasts using spectrophotometric and light microscopy methods. These new polymeric materials showed good tensile strength, biodegradability and a high in vitro biocompatibility. They can be used in the field of medical devices where such physical and biological features are necessary.

Key words: polyurethane, natural polymers, collagen, elastin, biocompatibility, biodegradability

Synthetic polymer films are succesfully used in very different fields, such as medicine (medical devices, manufacture), food industry (food packages), chemical industry (polymer synthesis), biology (cell support) due to their mechanical properties extended on a wide area, their ability of being easyly processed in a variety of forms and low costs of production. However, biocompatibility and biodegradability characteristics are often unsatisfactory. Surface properties of the polymers play an important role in cell adhesion and growth [1-3]. The biological polymers used for medical dressing preparation have a high tissue biocompatibility but their mechanical properties are poor, their processability is limited by the necessity to preserve their biological properties and their cost of production is high.

All these reasons explain the recent interest of some research teams to unveil the aspects related to the development of efficient methods to create a class of new polymeric materials with high biocompatibility for multiple applicabilities formed through association of synthetic polymers with natural polymers.

A wide variety of synthetic polymers (polyvinyl chloride, polyethylene, silicone polymers) having chemical inertia and high mechanical strength were tested so far, in the production of medical devices (tubes, drains, catheters, etc) [4]. Because of their nonthrombogeneity, good mechanical properties (elasticity and porosity close to those of sanguine vessels) and degradability, polyurethanes (PU) are recommended for biomedical applications as adhesives, vascular and orthopaedic protheses [5 - 7]. In order to improve the biological characteristics of synthetic polymers, biopolymers like fibrin and macromolecules occuring in extracellular matrix, collagen (COL) and elastin (EL) were used [8]. COL is a protein used to get composites of COL-synthetic polymer type for a variety of medical uses, including dialysis membranes, wound dressings and artificial skin [9, 10]. It is also known as a biopolymer which enables cell adhesiveness, enzymatic degradation and an affinity for autologous growth regulatory molecules [11, 12]. The biocompatibility of synthetic polymers, like polyvinyl chloride and polyethylene terephthalate was increased by mixing or coating with COL [13, 14]. EL is an essential constitutive protein of the extracellular matrix from elastic connective tissues studied lately for its ability of close association with other natural and synthetic polymers [15, 16]. It is known that EL presence might increase fibroblast proliferation, as previously demonstrated in human skin [17, 18]. *In vitro* tests showed that EL peptides improved both the adhesion of the endothelial cells and the strength of the newly produced material. Despite its remarkable mechanical properties and its low thrombogeneity [19], EL has been little used to produce biocompatible composites [20].

The aim of this study is to prepare two new polymeric materials by coating of PU with a COL gel and a mixture of COL/EL, respectively, in order to develop biodegradable and highly biocompatible scaffolds for medical device fabrication.

Experimental part

Polymer preparation. PU was synthesized using a two-step polyaddition reaction, as previously described [21]. There were used diphenylmethane 4,4' diisocyanate (MDI) freshly distilled, from Merck, poly (ethylene glycol) adipate (PEGA, purity 97 %, MW = 2000) and ethylene glycol (EG) (purity 95 %) from Fibrex SA Savinesti-Romania. The molar ratio PEGA:MDI:EG was 1:5:4.

COL type I was prepared in our lab from bovine tendons treated with 1% pepsin (E.C. 3.4.23.1) in 0.5 M acetic acid and precipitation with 2.4 M NaCl, as previously described [22]. COL gel has the following characteristics: 9.35 % hydroxyproline content, 0.8 % hexosamine, 0.5 % dry content by weight and a molecular weight of 308 kDa.

EL was prepared from insoluble elastin powder (calf ligament, Sigma) by stirring in KOH 1M (in ethanol, 1:4, v/v), at 30^{0}C, for 48 h, neutralizing with acetic acid and dyalisys against bidistilled water [23]. The final solution has 1.3 % dry content by weight and 68.7 % protein content.

PU/COL and PU/COL/EL material fabrication. First, the PU sheets were immersed in 1 % (w/w) poly(methyl methacrylate) (PMMA) ethanolic solution and then dried at 80^{0}C, for 10 min. in an air-ventilated drying stove.

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Solutions of 12 % COL and COL/EL (9:1, w/w) in a mixture of ethanol:distilled water in a ratio of 1:2 (w/w) were prepared and heated at max. 60^{0}C. Surface modified PU sheets were immersed in the natural polymer solutions for 15 min. and cooled at 5 ^{0}C, for 10 min. The composite materials were cross-linked in a 0.5 % (w/w) glutaraldehyde solution, at 50^{0}C, for 3 h, followed by thoroughly washing in bidistilled water, for 5 min. and then were immersed in 20 % glycerine (w/w), for 10 min. Materials were finally air-dried at 80^{0}C.

Material characterization. Material surfaces and cross-sections were examined by SEM. The samples were processed in the low vaccum mode and visualized using an ESEM, Quanta 400, FEI, Philips (Holland). Pore sizes were determined using ImageJ and ImageAnalyser soft for digital image processing. The porosity (ε) and density (*d*) of the polymeric materials were determined by the method described by Zhang et al. [24]. The following equations were used:

$$\varepsilon = (v1-v3) / (v2-v3) \cdot 100\ \% \quad (1) \text{ and } \quad d = w1 / (v2-v3) \quad (2),$$

where:

w1 is the sample weight;

v1 is the volume of water;

v2 is the total volume of water plus the impregnated material;

v3 is the residual water volume after the water-impregnated material was removed.

Polymer mechanical properties were measured on a dynamometer Zwick FP 10 (Germany), according to SR ISO 527-200.

Material degradation. Samples of polymeric material (0.5 . 0.5 cm) were weighed and immersed in 3 mL Tris-HCl buffer, *p*H 7.4 containing 2U/mL type I collagenase (Sigma). The degradation was conducted at 37^{0}C in a water bath, for 5 days. The extent of material degradation was determined by measuring the amount of protein in solution using Bradford assay.

Human dermal fibroblast culture. A primary culture of human dermal fibroblasts was obtained from human dermis explants and were used at passage 4. Materials were cut to 10-mm discs and sterilized by exposure to the ultraviolet light source in an UV sterilization cabinet (Scie-Plas, England) for 4 h. They were fited into the bottom of a 24-well tissue culture polystyrene plate and 50 μL of cells in Dulbecco's Modified Eagle's Medium (DMEM) with 10 % fetal bovine serum (Sigma) were added at a density of 1.2 . 10^6 cells/mL. The plate was kept in an incubator for 3h. Then, another 450 μL culture medium (DMEM) were added to each well. Cell viability was assayed by measuring the mitochondrial dehydrogenase activity using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test, which is based on the conversion of soluble tetrazolium salts to formazan by NAD and NADH dehydrogenases from the viable cells [25]. To analyze cell morphology, cell/scaffold constructs were stained with Hematoxylin and visualized at a stereomicroscope Kruss (Germany).

Statistics. Data are expressed as mean ± standard deviation (S.D.) Statistical analysis was performed using paired Student's test. Differences were considered significant at $p<0.05$.

Results and discussion

Morphology of the PU/COL and PU/COL/EL materials

The electron micrographs showed that PU sheets presented a microporous morphology (fig. 1A, B). After immersion in natural gels, it was observed the presence of a collagenic surface with open and interconnected pores for PU/COL material (fig. 1C) and a compact coating of PU surface with COL-EL gel (fig. 1E). The analysis of materials' cross-sections demonstrated that PU/COL scaffold had oval shaped pores, with uniform sizes, as it can be observed in the detail (fig. 1D). The PU/COL/EL material presented two distinct zones having different morphologies (fig. 1F). In some zones, pores of aprox. 20 μm can be found and in other zones the pores were smaller than 1 μm (table 1).

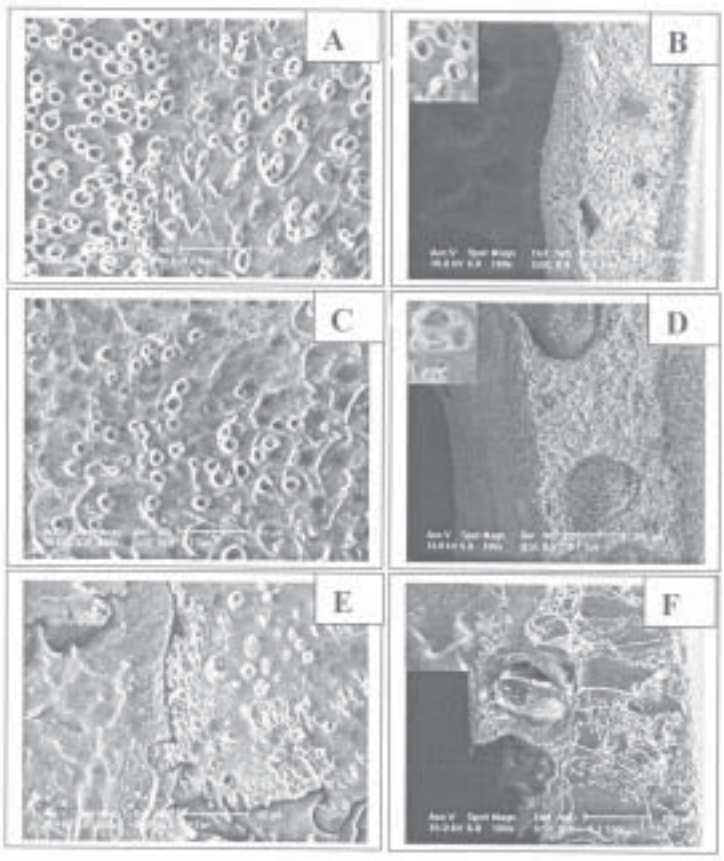

Fig. 1. Scanning electron micrographs of PU (A, B), PU/COL (C, D) and PU/COL/EL (E, F) scaffold surfaces (A, C, E) and cross-sections (B, D, F). The scaffolds were prepared using 1 % PMMA and glutaraldehyde cross-linking

Table 1
MORPHOLOGICAL AND MECHANICAL PARAMETERS OF THE MATERIAL VARIANTS

Polymer	Pore size (μm)	Porosity (%)	Density (g/cm^3)	Tensile strength (dN/cm^2)	Elongation at break (%)
PU	6.06±0.36	90.76	0.011	84	220
PU/COL	12.23±0.61	80.20	0.032	79	238
PU/COL/EL	2.03±0.10	75.70	0.036	70	240

The porosity decreased after coating with COL gel and COL/EL mixture (table 1), thus PU sheet had the highest porosity (90.76 %). Density varied in an inverse proportion. These results indicated that coating of PU with natural polymers lead to both a decreasing of scaffold porosity and decreasing of pore size. This effect was also reported for PU/COL porous scaffolds and might be due to interactions between the amide bonds of COL and urethane/urea bonds and also to the additional mass added to PU from COL and COL/EL, respectively [26].

Mechanical properties of PU material variants

The mechanical properties of the polymeric variants are presented as average tensile strengths and elongations at break in table 1. Tensile strengths values varied according to the coating layer composition. Several parameters like molecular weight, composition and structure influence the tensile strength and elongation at break of PU-based materials [27]. The results of the present study showed that, after coating, composite materials presented little modification of their mechanical parameters and can be processed similarly to PU sheets.

Biodegradation of PU material variants

In vitro degradation of the PU-based composite materials was assessed in buffer with collagenase, over a 5-days period. Materials with COL/EL coating layer released a significantly smaller protein quantity than those with COL alone ($p<0.02$) (fig. 2). Both polymeric composites containing COL degraded significantly faster than PU sheets when treated with collagenase ($p<0.025$).

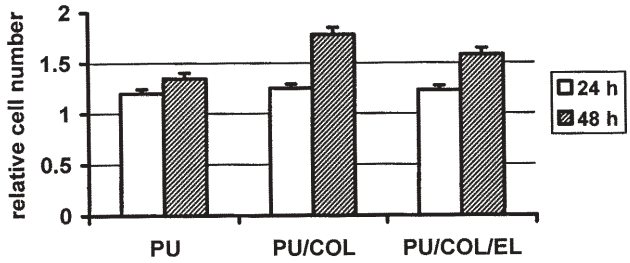

Fig. 2. Degradation of PU material variants in the presence of collagenase

These results showed that COL and especially COL/EL mixture improved the biodegradability of PU material variants. PU, like other synthetic polymers, presented a lack of collagenase sensitivity. Other teams studied the degradation of a PU/COL porous scaffold and observed a mass loss in collagenase presence, at 56 days, bigger than in buffer [26]. This might be explained by considering that the carboxylic acid groups of the water-soluble COL degradation products act to accelerate polyester degradation [28, 29].

In vitro biocompatibility of PU material variants

Human dermal fibroblasts were cultured on the PU composite materials for 24 h and 48 h. After each period of time, the number of viable cells was determined by mitochondrial activity assay (fig. 3). Results represent mean of 3 determinations ± S.D.

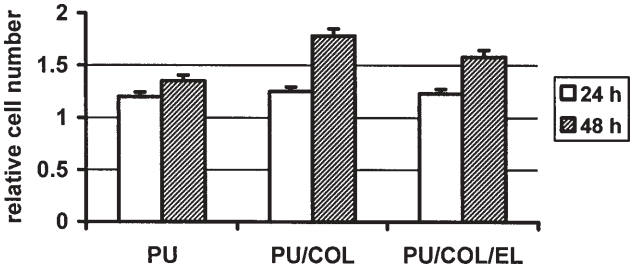

Fig. 3. Human dermal fibroblast proliferation on PU variants after 24 h and 48 h of cultivation. The relative cell number is based upon MTT absorption and reflects mitochondrial activity in viable cells

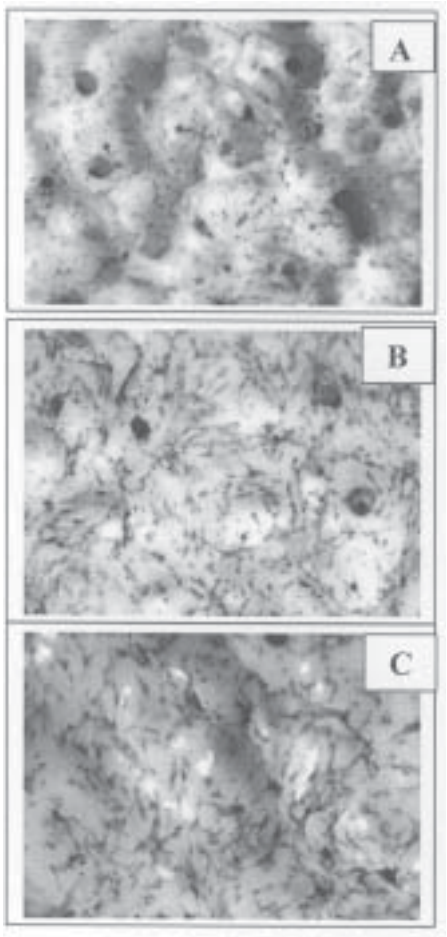

Fig. 4. Bright-field stereomicroscope images of human dermal fibroblasts cultured on PU (a), PU / COL (b) and PU/COL/EL (c) scaffolds for 48 h (x 45, Hematoxylin staining)

No significant differences were found at 24 h of cell cultivation on polymeric variants. Both materials supported cell proliferation, with fibroblast growing more extensively on the PU/COL and PU/COL/EL variants than on PU sheet ($p<0.05$), at 48 h after cell seeding. The human dermal fibroblast number continued to increase during the culture period. The benefit of coating the PU sheets with COL and COL/EL, respectively, was demonstrated, in our study, by higher fibroblast proliferation values, assessed as mitochondrial activity, after 48 h of cell culture, than for PU alone.

Several studies demonstrated the ability of cells to modify their shape in response to substrate structure and composition. Our light microscopy observations showed that, after 48 h, the cells adhered to the polymeric surfaces, retaining their normal, elongated morphology (fig. 4). Moreover, cells migrated into the scaffold matrix.

Conclusions

Two new polymeric biomaterials prepared by coating of PU sheets with natural polymers (COL gel or COL/EL mixture) were characterized as possessing good mechanical properties, morphological characteristics, biodegradability and a high biocompatibility. They are recommended as materials for medical device fabrication where such physical and biological features are necessary.

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References

1. AGRAWAL, C.M., RAY, R.B., J. Biomed. Mater. Res., **55**, 2001, p. 141
2. DEMIR, M.M., YILGOR, I., YILGOR, E., ERMAN, B., Polymer, **43**, 2002, p. 3303
3. SHEARER, H., ELLIS, M.J., PERERA, S.P., CHANDHURI, J.B., Tiss. Eng., **12**, 2006, p. 2717
4. YIANNI, J., Med. Dev. Technol., **20**, 1995, p. 165
5. DESAI, S., THAKORE, I.M., SARAWADE, B.D., DEWI, S., Europ. Polym. J., **36**, 2000, p. 711
6. COOPER, S., LAMBA, N.M.K., WOODHOUSE, K.A., Polyurethanes in biomedical applications, CRC Press, New York, 1997
7. LUPU, M., MACOCINSCHI, D., IOANID, G., BUTNARU, M., IOAN, S., Polymer Int., **56**, 2007, p. 389
8. BONZON, N., CARRAT, X., DEMINIERE, C., DACULSI, G., LEFEBVRE, F., RABAUD, M., Biomaterials, **16**, 1995, p. 881
9. AUGER, F.A., LOPEZ VALLE, C.A., GUIGNARD, R., In vitro Cell Dev. Biol., **31**, 1995, p. 432
10. PIEPER, J.S., KRAAN, P.M. van der, HAFMANS, T., KAMP, J., BUMA, P., SUSANTE, J.L.C. van, BERG, W.B. van den, VEERKAMP, J.H., KUPPEVELT T.H. van, Biomaterials, **23**, 2002, p. 3183
11. DOLDER, J. van den, JANSEN, J.A., J. Biomed. Mater. Res., **83**, 2007, p. 712
12. MOLDOVAN, L., BUZGARIU, W., CRACIUNESCU, O., OPRITA, E.I., ZARNESCU, O., Rom. Biol. Sci., **I**, nr. 3-4, 2004, p.3
13. LUNGU, M., POGONARIU, A., PRECUP, M., MOLDOVAN, L., OANCEA, A., ZARNESCU, O., Roum. Biotechnol. Lett., **2**, nr. 2, 1997, p. 153
14. DUTOYA, S., LEFEBVRE, F., DEMINIERES, C., ROUAIS, F., VERNA, A., KOZLUCA, A., LE BUGLE, A., RABAUD, M., Biomaterials, **19**, 1998, p.147
15. DUTOYA S, VERNA A, LEFEBVRE F, RABAUD M, Biomaterials, **21**, 2000, p. 1521
16. CRACIUNESCU, O., MOLDOVAN, L., BUZGARIU, W., ZARNESCU, O., BOJIN, D., RADU, G.L., Roum. Biotechnol. Lett., **9**, 2004, p. 1785
17. GROULT, V., HORNEBECK, W., FERRARI, P., TIXIER, J.M., ROBERT, L., JACOB, M.P., Cell Biochem. Funct., **9**, 1991, p. 171
18. GHUYSEN-ITARD, A.F., ROBERT, L., JACOB, M.P., C.R. Acad. Sci. Paris, **315**, 1992, p. 473
19. LIU, S.Q., TIESCHE, C., ALKEMA, P.K., Biomaterials, **25**, 2004, p. 101
20. DAAMEN, W.F., NILLESEN, S.T., HAFMANS, T., VEERKAMP, J.H., van LUYN, M.J., van KUPPEVELT, T.H., Biomaterials, **26**, 2005, p. 81
21. LUPU, M., MACOCINSCHI, D., EPURE, V., IOANID, A., GRIGORAS, C.V., IOAN, S., J. Macromol. Sci. B Physics, **45**, 2006, p. 395
22. OPRITA, E.I., MOLDOVAN, L., CRACIUNESCU, O., BUZGARIU, W., TARDEI, C., ZARNESCU, O., Central Eur. J. Biol., **1**, 2006, p. 61
23. RABAUD, M., LEFEBVRE, F., DUCASSOU, D., Biomaterials, **12**, 1991, p. 313
24. ZHANG, S.M., CUI, F.Z., LIAO, S.S., ZHU, Y., HAN, L., J. Mat. Sci.: Mat. Med., **14**, 2003, p. 641
25. MOSMANN, T., J. Immunol. Methods, **65**, 1983, p. 55
26. GUAN, J., STANKUS, J.J., WAGNER, W.R., Cell Transplantation, **15**, 2006, p. S17
27. OPREA, S., VLAD, S., Mat. Plast., **44**, nr. 2007, p. 26
28. GUAN, J.J., SACKS, M.S., BECKMAN, E.J., WAGNER, W.R., J. Biomed. Mater. Res., **61**, 2002, p. 493
29. OH, H.J., KIM, W.Y., LWW, D.S., LEE, Y.S., J. Ind. Eng. Chem., **6**, 2000, p.425

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