DEVELOPMENT AND CHARACTERIZATION OF INDOMETHACIN LOADED POLYVINIL ALCOHOL-COLLAGEN SMART HYDROGELS FOR BURNS INJURIES

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Hydrogels play a very important role in burns treatments, alone or in combination with other products, having the capacity to calm and moisturize the wound. Hydrogels are three – dimensional structures with interesting properties, having the ability to induce autolysis debridation, to absorb the exudates and to keep the moist of wound healing. The aim of this study was to develop and characterize some new composite materials based on collagen (COLL), polyvinyl alcohol (PVA) and indomethacin (IND), designed to be used for burns injuries and wound healing. Type I fibrillar collagen gel was extracted from calf hide. Indomethacin was added because of anti-inflammatory effect. Hydrogels with various ratios of collagen, PVA and indomethacin were rheologically tested. The collagenic supports obtained by hydrogels lyophilization were investigated by antimicrobial and cytotoxicity tests. The hydrogels presented a pseudoplastic behavior, and the Herschel-Bulkley model best fitted the rheological data. The biological properties, cytotoxicity and antimicrobial tests revealed the possibility of using them in medical application. Based on the hydrogels performance, we could conclude that the anti-inflammatory spongious matrices based on collagen and PVA are potentially usable for burn injuries and wound healing.

Keywords: collagen hydrogels, PVA, indomethacin, burns

INTRODUCTION

Hydrogels are three-dimensional networks derived from natural or synthetic polymers and are characterized by the ability to retaining large amounts of water or fluids. They have a soft consistency similar to that of human tissue (Ullah *et al*., 2015).

Collagen, the most common protein in the human body, has the ability to take over biological tissues functions. It is widespread in many applications such as vascular grafts or matrices for tissue regeneration (Davidenko *et al*., 2012).

The polyvinyl alcohol is a synthetic polymer that may be used in combination with collagen - a natural polymer in order to avoid glutaraldehyde cross-linking, which at certain concentrations is toxic for the body.

Indomethacin is a non-steroidal and anti-inflammatory drug. It is used to relieve pain and inflammation. It works by blocking cyclooxygenase, a substance present in the human body which is involved in producing chemical irritants as a response to surgery or illness. By blocking the action of this, indomethacin is able to reduce pain and inflammation (Fitzpatrick, 2004; Rasekh *et al*., 2014).

The aim of this study was to prepare and characterize some collagen - indomethacin – PVA hydrogels. In order to obtain a dressing with anti-inflammatory properties,

hydrogels of collagen, polyvinyl alcohol and indomethacin were performed with the purpose of treating and healing burns.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel (COLL) having a concentration of 2.85% (w/w) was extracted from calf hide using technology developed at the Research-Development Textile Leather National Institute Division Leather and Footwear Research Institute – Collagen Department (Albu, 2011). Polyvinyl alcohol (PVA), with molecular weight of 60 000 Da was purchased from Sigma-Aldrich and glutaraldehyde (GA) from Merck (Germany). The indomethacin (IND) was obtained from Fluka.

Preparation of Collagen Hydrogels

The collagen gel with the initial concentration of 2.85% and acid pH was adjusted using 1M sodium hydroxide at pH 7.3 for a better biocompatibility. The final concentration of used collagen gel was 1% (w/v). Thereafter PVA with a concentration of 0.5% and 0.2% indomethacin were dispersed in the collagen gels in different proportions, according to the compositions shown in Table 1. For cross-linking 0.025% glutaraldehyde solution was used.

Table 1.Composition of collagen hydrogels

	COLL, %	PVA, %	IND, %	GA, %
S1	0	100		
S2	100	0		
S3	50	50	0.2	0.025
S4	75	25		
S5	25	75		

The collagen gels, in order to be analyzed, were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer, using a 48 hours lyophilization programme.

Rheological Analysis

The flow properties of the designed hydrogels were determined with a rotational viscometer MultiVisc-Rheometer (Fungi lab) equipped with standard spindle TR 9 and an ultrathermostat ThermoHaake P5. The rheological measurements were carried out at 37°C±0.5°C. The operational conditions were detailed in our previous studies (Paunica-Panea *et al*., 2016; Ghica *et al*., 2012a). The viscosity of the hydrogels was plotted as a function of shear rate and the corresponding curves were obtained. To quantify the hydrogels rheological behavior, the Power law model was applied (eq. 1):

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1)$$

where, m and n are flow parameters correlated with the designed hydrogels composition and determined through the linearization of eq. (1) by double logarithmic method (Ghica *et al*., 2012b).

Hydrogels Antimicrobial Activity

For testing the antimicrobial activity, Nutrient Agar culture medium was used sterilized at 121°C for 15 minutes and poured into Petri dishes. After solidification, the Petri dishes were inoculated with 100 mL of cell suspension of *Staphylococcus aureus* ATCC 25923, all over nutrient agar. In each case were carried out 2 holes (with a diameter of about 6 mm) with the help of sterile glass tubes and through these holes were inserted 40 µL of each sample. The test plates were thermostatted for 18 hours at 37°C, after which they were analyzed. For a preliminary assessment of the possible cytotoxic effects, human osteosarcoma MG 63 cells were cultured in the same well with tested samples. First, the samples were cut to obtain samples with the same size and then were sterilized by maintaining for 24 hours in 70% (v/v) ethanol. Subsequent, the samples were washed with sterile distilled water and further maintained for 24 hours in specific cell culture conditions: 37°C, 5% CO_2, relative humidity < 95%, pH 7.2-7.4, in DMEM medium with 1‰ glucose, supplemented with 10 % heat-inactivated fetal bovine serum and antibiotics: penicillin 300 UI/mL, streptomycin 300 µg/mL and neomycin 150 µg/mL. After these steps the samples were seeded with a 25.000 cells/mL cell suspension and placed in culture condition. Phase contrast microscopy images were taken at day 2 and day 4 of the culture, in order to evaluate the culture state surrounding tested samples. For comparison it was used MG 63 cells cultured only in culture medium.

RESULTS AND DISCUSSION

After lyophilization the 3D porous matrices based on collagen-polyvinyl alcohol - indomethacin were obtained, with the appearance presented in Figure 1.

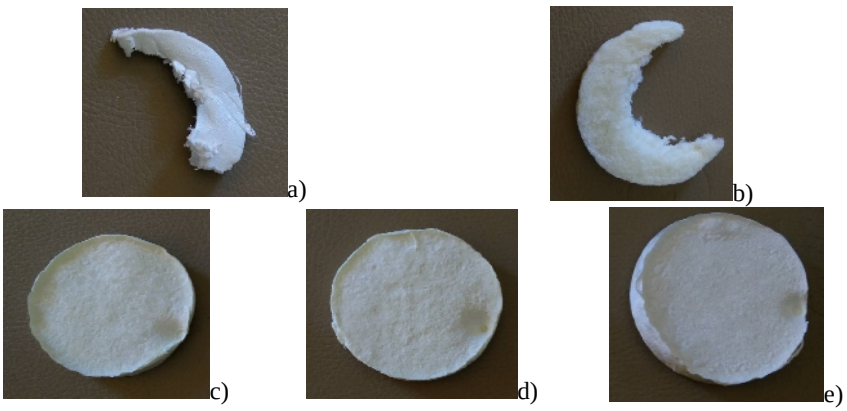

Figure 1. Collagen spongious forms: a) S1; b) S2; c) S3; d) S4; e) S5.

The influence of formulation factors, namely the ratio PVA: COLL on the rheological behavior of the tested hydrogels, plotted as viscosity versus shear rate, is shown in Figure 2.

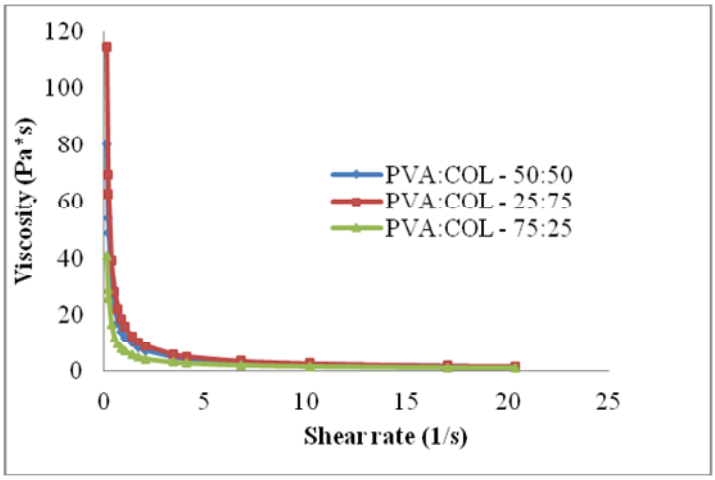

Figure 2. Plots of viscosity as a function of shear rate for the collagen hydrogels evaluated at 37°C

As can been seen in Figure 2 similar flow profiles were recorded for the designed hydrogels, the viscosity decreasing with shear stress increase. This behavior conducts to pseudoplastic properties of the samples which facilitate the formulations flow and consequently their suitable manipulation (Ghica *et al*., 2015).

The Power law equation applied to rheological data conducted to flow parameters, m and n, mentioned to Materials and Methods section, and listed in Table 2. The determination coefficients values, ranging between 0.9988 and 0.9993 indicate that this rheological model fitted very well the experimental data.

Table 2. Fitting parameters of the Power law rheological model for designed collagen hydrogels

Hydrogel	m	n	R^2
Gel PVA:COLL - 50:50 (S3)	13.037	0.794	0.9989
Gel PVA:COLL - 25:75 (S4)	15.718	0.857	0.9993
Gel PVA:COLL - 75:25 (S5)	7.432	0.748	0.9988

From Table 2 it is observed that the ratio PVA: COLL markedly influence the parameter m, (associated with the viscosity recorded for the shear rate of $1 \cdot s^{-1}$). Thus, the highest value of this parameter was recorded for a ratio PVA: COLL of 25:75 (S4), while a ratio PVA: COLL 50:50 (S3) causes a decrease in the value of m to about 1.20 times. The increase of PVA concentration in the formulation at a ratio PVA: COLL 75:25 (S5) leads to the lowest values of the parameter m, recordings decrease of approximately 43% compared it with the gel S3, and respectively 53% compared to the gel S4.

The results of antimicrobial activity of tested hydrogels are presented in Figure 3.

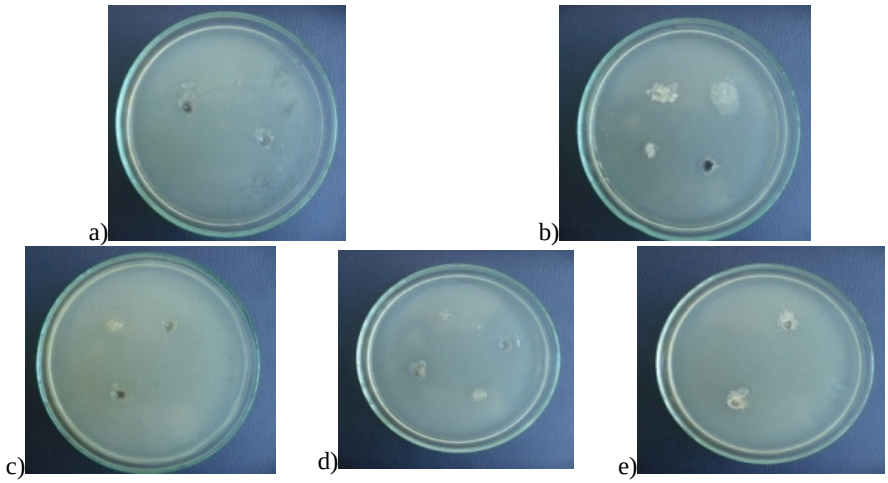

Figure 3. Results for antimicrobial activity: a) S1; b) S2; c) S3; d) S4; e) S5

Following the analysis of antimicrobial activity, no zones of inhibition were observed around any samples that increased bacterial strain evenly across the entire culture medium. Therefore, no evidence of antibacterial activity against strain used as indicator was shown. This was expected because there was used not any antimicrobial agent. Is recommended that lyophilized samples to be sterilized by γ radiation or to obtain hydrogels under sterile conditions.

In the Figure 4 is presented the phase contrast images of MG 63 osteosarcoma cells after 2 and 4 days of culture of the matrices S1 – S5.

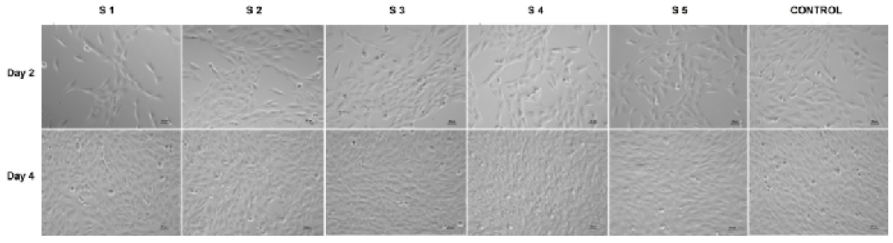

Figure 4. Phase contrast images of MG 63 osteosarcoma cells after 2 and 4 days of culture in presence of samples S1-S5. As control MG 63 cells cultured only in culture medium were used. Scale bar 20μm.

Both on the second and fourth day after initiating the cell culture no cytotoxic effects were observed by phase contrast microscopy. Cell morphology seems to be normal indicating that no cytotoxic compound was released from tested samples into the medium to change the cell morphology. The number of rounded cells was relatively equal for all samples. For a more precisely characterization of samples effects on osteosarcoma cells other assay are needed for evaluating proliferation level, oxido-reductase enzymes activity, alkaline phosphatase activity, gene transcription level etc.

CONCLUSIONS

Collagen - polyvinyl alcohol - indomethacin (COLL-PVA-IND) hydrogels with different ratios of COLL: PVA were obtained and characterized in order to be used as burn wound dressing. The increase of PVA concentration in the formulation at a ratio PVA: COLL 75:25 (S5) leads to the lowest values of the parameter m, recording a decrease of approximately 43% compared it with the gel S3, and respectively 53% compared to the gel S4.The hydrogels were rheologically tested, the ratio PVA: COLL strongly influencing their flow properties. The collagenic supports obtained by hydrogels lyophilization were investigated by antimicrobial and cytotoxicity tests. The biological properties, cytotoxicity and antimicrobial tests revealed the possibility of using them in medical application. Based on the hydrogels performance, we could conclude that the anti-inflammatory spongious matrices based on collagen and PVA are potentially usable for burn injuries and wound healing.

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REFERENCES

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Davidenko, N., Gibb, T., Schuster, C., Best, S.M., Campbell, J.J., Watson, C.J. and Cameron, R.E. (2012), "Biomimetic Collagen Scaffolds with Anisotropic Pore Architecture", *Acta Biomaterialia*, 8, 667-676.

Fitzpatrick, F.A. (2014), "Cyclooxygenase Enzymes: Regulation and Function", *Current Pharmaceutical Design*, 10, 577-588.

Ghica, M.V., Albu, M.G., Dinu-Pîrvu, C. and Moisescu, Şt. (2012a), "*In vitro* Kinetic Release and Flow Behavior of some Collagen-Minocycline Topical Hydrogels", *Revista de Chimie*, 63(9), 929-935.

Ghica, M.V., Albu, M.G., Coară, Gh. and Dinu-Pîrvu, C. (2012b), "The Influence of Crosslinking Agent on Kinetic Release and Rheological Behaviour of some Collagen-Niflumic acid Hydrogels", *Proceedings of the 4th International Conference on Advanced Materials and Systems (ICAMS)*, Bucharest, Romania, 267-272.

Ghica, M.V., Albu, M.G., Dinu-Pîrvu, C. and Moisescu, Şt. (2012), "*In vitro* kinetic release and flow behavior of some collagen-minocycline topical hydrogels", *Revista de Chimie*, 63(9), 929-935.

Ghica, M.V., Ficai, A., Marin, Ş., Marin, M., Ene, A.M. and Pătraşcu, J.M. (2015), "Collagen / Bioactive Glass Ceramic / Doxycycline Composites for Bone Defects", *Romanian Journal of Materials*, 45, 307-314.

Lu, J., Lin, X., Jiang, B., Li, X., Chen, J. and Zhang, X. (2005), "Preparation and Characterization of Collagen by Hydrogel Formation Method", *Key Engineering Materials*, 288-289, 377-380.

Paunica-Panea, G., Ficai, A., Marin, M.M., Marin, S., Albu, M.G., Constantin, V.D., Dinu-Pîrvu, C., Vuluga, Z., Corobea, M.C. and Ghica, M.V. (2016), "New Collagen-Dextran-Zinc Oxide Composites for Wound Dressing", *Journal of Nanomaterials*, 2016, 7 pages.

Rasekh, M., Karavasili, C., Soong, Y.L., Bouropoulos, N., Morris, M., Armitage, D., Li, X., Fatoutous, D.G. and Ahmad, Z. (2014), "Electrospun PVP-Indomethacin Constituents for Transdermal Dressings and Drug Delivery Devices", *International Journal of Pharmaceutics*, 473, 95-104.

Ullah, F., Othman, M.B.H., Javed F., Ahmada, Z., Akil, H.Md. (2015), "Classification, Processing and Application of Hydrogels: A Review", *Materials Science and Engineering C*, 57, 414-433.