

Studies on Surface Modification of Silicone Biomaterials

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This paper presents our studies on surface modification of silicone elastomer by collagen grafting/coating and electron beam irradiation. Two types of collagen hydrolysates with different molecular weights were obtained and characterized, which were used in experiments. Both silicone elastomer crosslinking and collagen grafting on the surface of silicone elastomer were achieved by electron beam irradiation.

Keywords: silicone elastomer, collagen, electron beam

Silicone elastomers have been used for over 50 years in medical applications such as surgical implants and catheters [1-3]. This family of materials is highly elastic and transparent; it also has superior compatibility with human tissue and body fluids and is biologically inert; for example, it does not support the growth of bacteria. They also resist common sterilization methods, such as alcohol washing, dry heating, steam autoclaving, ethylene oxide, g-radiation, and electron beam treatments. In addition, they are also important materials that are widely used in microfluidics, microcontact printing, and cell culture studies [2, 4-5]. However, like most polymeric materials, silicone polymers are hydrophobic and do not promote cell adhesion, which is critical in a variety of fundamental development and wound healing processes, such as tissue architecture and function regulation, morphogenesis and angiogenesis [6-7]. Efforts for modifying the surface of silicone polymers have resulted in treating the silicone surfaces with plasma [8], UV-ozone [9-10], laser [11] or accelerated electrons [12]. Despite promoting more hydrophilic and cell-adhesive surfaces, the effects of these treatments are short-lived and deteriorate rapidly over time (hydrophobic recovery) [13-14]. Protein coating is another common treatment for modifying the surface of polymers, including silicone elastomers [15-16]. Cell adhesive proteins, including fibronectin, collagen, and laminin, are often used to promote cell adhesion and work well in many circumstances. However, the use of proteins for surface coating has limitations. For example, after being extracted from other organisms and purified, they may induce undesirable immune responses and increase the risk of infection [17]. In addition, proteins are susceptible to proteolysis and may require additional treatments to function properly. Therefore, protein coating may not be effective for long-term biological applications. Moreover, the physically adsorbed proteins are susceptible to instability due to desorption over time [18] or denaturation due to their stochastic orientation, conformational changes, or unfolding on the surface [19-21].

In this paper, the methods used to modify the surface of the silicone elastomer (PDMS) in order to improve its biocompatibility/hydrophilic characteristics were the following:

Collagen coating and collagen grafting on the surface of biopolymer using ionizing radiation (electron beam); the collagen meets to a great extent the referred to

requirements, being used in the bio-medicinal field with many applications in urology, dermatology, orthopedics, vascular and general surgery [22-24]. In addition, collagen is a good haemostatic, it plays an essential role in the wound healing process, influences the recovery of cartilage and bones.

Crosslinking using electron beam radiation. Furthermore, electron beam irradiation was used instead of thermal crosslinking, to avoid the thermal decomposition of collagen macromolecules. According to some in vivo tests [25], irradiation-assisted crosslinking of silicone elastomer has led to its improved biocompatibility. Crosslinking by electron beam also shows a series of advantages, such as: (1) the resulted products are pure as no peroxide is added; (2) lack of wastes; (3) reduced crosslinking time and power expenditure; (4) the resulted products are sterile, and (5) improved characteristics of the crosslinked products [26-29].

Electron beam irradiation may lead to crosslinking of the silicone elastomer simultaneously with collagen grafting on the surface of silicone elastomer.

Kawamura I et al. [30] have performed experimental studies on an artificial esophagus using a silicone elastomer and collagen extracted from bovine hide. The collagen preparation was then grafted to a silicone, synthetic polymer to form a conjugate. The surface of the silicone was made hydrophilic by subjecting it to a sparking discharge, utilizing high-frequency waves. Collagen was applied and the preparation irradiated with gamma-radiation rays to obtain crosslinking, by which the collagen and the silicone bound tightly. The strength of the material was confirmed by ablation and water immersion tests. The prostheses were prepared using this material, of various lengths and sizes. At both ends of the prosthesis protrusions were made for suturing purposes. A wedge was also made to prevent the tube from becoming disconnected soon after the operation. In conclusion, an attempt was made to develop an artificial esophagus with a conjugate of collagen obtained from living tissues and silicone. Histological examinations indicated that the collagen was not appreciably recognized as a foreign body by the living tissue and the adherence was satisfactory.

This paper presents our experiments on modifying the surface of silicone elastomer by collagen grafting/coating and electron beam irradiation. We focused on obtaining and characterizing two collagen hydrolysates with different

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molecular weights, and testing methods for grafting collagen to the silicone elastomer.

Experimental part

The following materials were used: (1) silicone elastomer Elastosil R 701/70 OH (Wacker-Chemie), (density 1.32 g/cm³, composition - polydimethylsiloxane with vinyl groups and filler, physiologically compatible material; the material is not mutagenic, carcinogenic or teratogenic and is not biologically degradable). (2) two types of collagen hydrolysates with various molecular weights were provided by the National Research & Development Institute for Textiles and Leather.

Plates of silicone elastomer required for tests have been made by compression molding, using an electrically heated hydraulic press, at room temperature, pressure of 150 MPa and time of 5 min. to obtain sheets of dimension 11.5 x 11.5 x 0.2 cm³. Silicone elastomer plates were irradiated with electron beam at 15 Mrad.

The samples of silicone elastomer were packed in a polyethylene film and were irradiated at 15 Mrad irradiation dose in the ALIN-10 electron accelerator under atmospheric conditions and at room temperature. The ALIN 10 is a travelling-wave type, operating at a wavelength of 10 cm and having 164 W maximum output power. The accelerating structure is a disk-loaded tube operating in the $\pi/2$ mode. The optimum values of the EB peak current IEB and EB energy EEB to produce maximum output power PEB for a fixed pulse duration τ_{EB} and repetition frequency f_{EB} are as follows: EEB = 6.23 MeV, IEB = 75 mA, PEB = 164 W (f_{EB} = 100 Hz, τ_{EB} = 3.5 μ s). The EB effects are related to the absorbed dose, D, expressed in Gray (1 Gy = 1 J/kg) and absorbed dose rate, D*, expressed in Gy/s.

Collagen hydrolysates were obtained from semi-processed bovine hide, by chemical-enzymatic processing, following the workflow in figure 1.

The chart in figure 2 presents the two methods of grafting the natural polymer, collagen, on the surface of silicone elastomer, as follows:

- immersing the plate made of silicone elastomer blend in the collagen hydrolysate for 24 h, followed by washing the plate with distilled water, and then crosslinking (the silicone elastomer and collagen) and grafting (the collagen to the silicone elastomer) at the optimal irradiation dose: 15 Mrad;

- crosslinking the silicone elastomer blend with electron beam at the optimal dose of 15 Mrad, followed by immersion of plates in the collagen hydrolysates for 24 h; after immersion, the plates were washed with distilled water.

Mechanical properties of the vulcanisates were measured on a Schopper tensile tester with a nominal rate of the traverse of the moving grip of 460 mm/min. Modulus at 100% strain, tensile strength and elongation at break tests were carried out according to the conditions described in ISO 37/2005, on dumb-bell shaped specimens of Type 2. Tearing strength tests were carried out using angular test

pieces (type II) according to SR EN 12771/2003. Hardness of the vulcanised materials was measured using the Shore A scale with vulcanised samples of 6 mm thickness, by using a hardener tester according to ISO 7619-1/2004. Elasticity was evaluated with a Schoob test machine using 6 mm thick samples, according to ISO 4662/1986. All measurements were taken several times and the resulting values were averaged on 3 to 5 measurements.

Fourier transform infrared (FTIR) spectroscopy. Changes in the chemical structure of natural rubber samples with/without collagen were determined by FTIR spectroscopy. Investigations were made at room temperature (25°C) using a JASCO FT/IR 4200 mono fascicle spectrophotometer in the ATR mode, using a diamond accessory. The results are the averages of thirty scans, made in the absorption mode, between 4000 - 560 cm⁻¹.

Chromatography. For qualitative and quantitative determination of amino acid content in the collagen hydrolysates, the high-performance liquid chromatography (HPLC) technique was used. The experimentally obtained collagen hydrolysates from semi-processed hide pieces were analyzed using a Thermo Electron – Finnigan Surveyor chromatograph with DAD (Diode Array Detector).

To determine ash, calcium, total nitrogen, amine nitrogen, molecular weight, dermal substance, gravimetric and volumetric methods of analysis were used, and the pH was potentiometrically determined.

Results and discussions

Physical-mechanical properties of the silicone elastomer crosslinked with electron beam at 15 Mrad are presented in table 1. The optimal EB dose necessary to crosslink the silicone elastomer was established in our previous determinations [1, 31, 32] and is of 15 Mrad.

Collagen hydrolysates, having the chemical characteristics presented in table 2, were obtained from semi-processed bovine hide, by chemical-enzymatic processing, according to the workflow presented in fig. 1.

Figures 3 and 4 illustrate the FT/IR spectra for experimentally obtained collagen hydrolysate solutions, by alkaline-enzymatic and alkaline hydrolyses. These spectra highlight the presence of bands of stretching vibrations ν and deformation vibrations δ , at wavelengths characteristic to chemical bonds found in polypeptides, peptides and amino acids. Table 3 details the identified spectral

Table 1
CHARACTERISTICS OF BLENDS BASED ON SILICONE ELASTOMER CROSSLINKED WITH EB

Characteristics	Value
Hardness, °ShA,	84
Elasticity, %,	45
Tensile strength, N/mm ²	2.6
Elongation at break, %	20
Elongation set, %	3
Tear strength, N/mm	4.5

Collagen hydrolysate solution	Characteristics							pH
	S.U. %	Total ash %	Calcium, expressed as CaO %	Total nitrogen %	Dermal substance %	Amine nitrogen %	Average molecular weight, Da	
I*	4.16	15.38	1.60	13.94	78.37	0.92	14900	7.86
II**	4.21	10.21	1.19	15.20	85.51	0.83	16300	10.26

* Obtained by alkaline-enzymatic hydrolysis

** Obtained by alkaline hydrolysis

Table 2
CHEMICAL CHARACTERISTICS OF COLLAGEN HYDROLYSATES

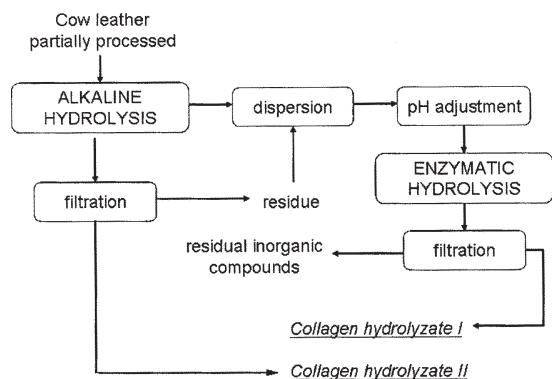


Fig. 1. Obtaining collagen hydrolysates

Fig. 2. Flow diagram of preparation procedure for samples of silicone elastomer (PMMS) coated with collagen

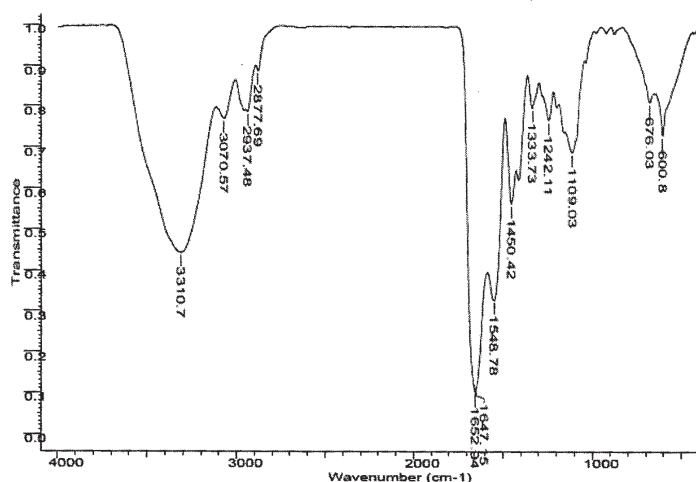
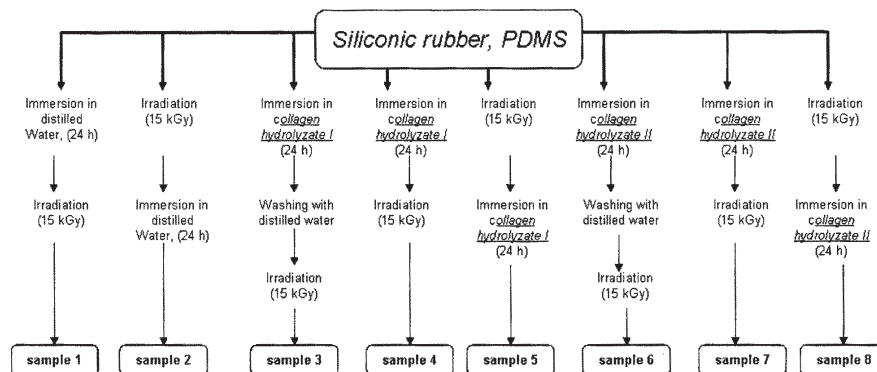


Fig. 3. IR spectral analysis of collagen hydrolysate (I) resulting from the alkaline-enzymatic hydrolysis of semi-processed hide

assignments and the potential compounds in which those vibrations may occur.

All essential amino acids in the composition of collagen hydrolysate obtained by alkaline-enzymatic hydrolysis (I) and which are not found in the composition of collagen hydrolysate obtained by alkaline hydrolysis (II), are non-polar amino acids, which contain the CH_3 group. These are the compounds likely to form free radicals that graft to the silicone matrix.

For qualitative and quantitative determination of amino acid content in the collagen hydrolysates, the high-performance liquid chromatography (HPLC) technique was used, with the results presented in table 4.

Amino acid compositions of collagen hydrolysates are in accordance with IR spectra in figures 3 and 4. Quantification of certain amino acids in collagen hydrolysates confirms the presence of compounds suggested by the types of vibrations identified according to the characteristic spectral bands. It should be noted that certain essential amino acids such as threonine, valine, leucine, isoleucine only occur in the composition of hydrolysates obtained by alkaline-enzymatic hydrolysis. Of these essential amino acids, threonine is found in the

highest amount (more than double the totalled amounts of valine, leucine and isoleucine).

Biomaterials based on silicone elastomer and collagen obtained by grafting the natural polymer, collagen, to the surface of silicone elastomer by means of electron beam using the two methods mentioned in the previous section, were characterized by FTIR. Analyzing the FT/IR - ATR spectra presented in figure 5 (table 5), of experimental samples studied, we can notice the differentiated effect of collagen hydrolysate I, which contains non-polar essential amino acids, compared to that of collagen hydrolysate II, lacking essential amino acids. This aspect is easily noticed, especially in the $1100\text{--}1660\text{ cm}^{-1}$ spectral range, where obvious differences in transmittance occur for samples 3, 4 and 5, treated with collagen hydrolysate I, containing essential amino acids, compared to transmittances for control samples 1 and 2 and those for samples 6, 7 and 8, treated with collagen hydrolysate II, lacking essential amino acids. As a result, significant samples are 3, 4 and particularly sample 5, which reflects a polydispersion of the protein material grafted on the surface of silicon elastomer. For example, stretching vibration $\nu_{\text{C-OH}}$ in the secondary alcohol, specific to the band at $1100\text{--}1120\text{ cm}^{-1}$, could be attributed to threonine, the deformation vibration δ_{CH_2} specific to the band at $1430\text{--}1470\text{ cm}^{-1}$, could be attributed to proline, as the vibration δ_{NH} of the band at $1550\text{--}1610\text{ cm}^{-1}$ is specific to amide II and vibration $\nu_{\text{C=O}}$ of the band at $1630\text{--}1660\text{ cm}^{-1}$ is specific to amide I. The fact that these compounds in the protein

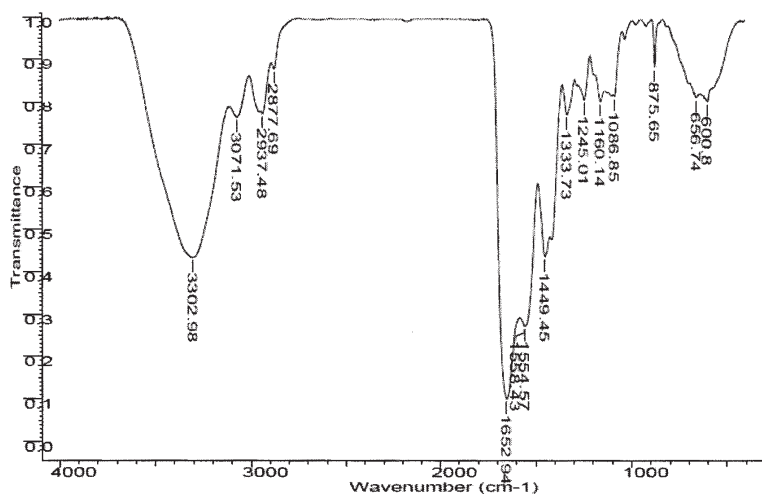


Fig. 4. IR spectral analysis of collagen hydrolysate (II) resulting from the alkaline hydrolysis of semi-processed hide

Table 3

MAIN SPECTRAL ATTRIBUTIONS IN COLLAGEN HYDROLYSATES

Figure	Identified spectral band, cm^{-1}	Spectral attribution	
		Band, cm^{-1}	Type of vibration
3	3310.70	3300-3500	ν_{OH}
4	3302.98	3300-3400	ν_{NH}
3	3070.57	3030-3130	ν_{NH}
4	3071.53	3030-3130	ν_{NH}
3	2937.48		ν_{CH} of $\text{C}=\text{C}$
3	2877.69	2720-3060	ν_{CH}
4	2937.48	2727-2950	ν_{CH_2}
4	2877.69		ν_{CH_2}
3	1652.94		$\nu_{\text{C}=\text{O}}$
4	1647.15	1630-1660	$\nu_{\text{C}=\text{O}}$
4	1652.94		$\nu_{\text{C}=\text{O}}$
4	1554.57	1550-1610	δ_{NH}
4	1558.43	1550-1610	δ_{NH}
3	1450.42	1430-1470	δ_{CH_2}
4	1449.45	1430-1470	δ_{CH_2}
4	1160.14	1140-1230	$\nu_{\text{C}-\text{OH}}$ (phenol)
3	1109.03	1100-1120	$\nu_{\text{C}-\text{OH}}$ (secondary alcohol)
4	1036.85	1010-1075	$\nu_{\text{C}-\text{OH}}$ (primary alcohol)
3	676.03		δ_{CH}
4	656.74	500-1000	δ_{CH}
4	875.65	500-1000	δ_{CH}

Table 4

AMINO ACID CONTENT OF COLLAGEN HYDROLYSATES

Amino acids	Content, mg/100ml hydrolysate	
	I	II
Aspartic acid	10.10	1.35
Glutamic acid	11.17	1.49
Serine	5.64	1.97
Histidine	20.17	0.78
Glycine	1.37	11.11
Threonine	15.12	-
Alanine	7.39	-
Tyrosine	-	8.26
Valine	7.45	-
Isoleucine	1.24	-
Leucine	4.49	-
Proline	0.21	61.14
Total	84.35	86.10

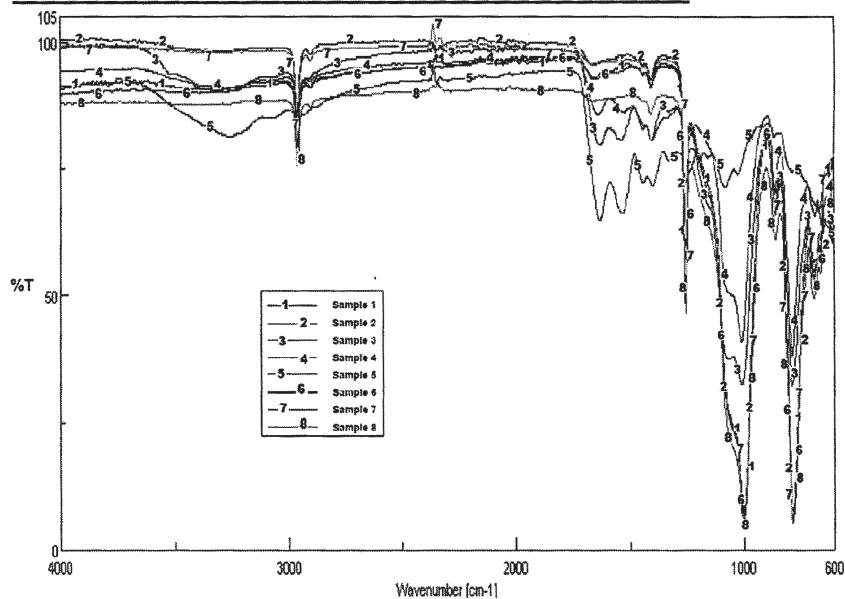


Fig. 5. IR spectral analysis of samples based on silicone elastomer and collagen

material are found on the surface of silicone elastomer even after washing, proves once more that electron beam irradiation of the silicone elastomer created the conditions

for collagen grafting, with strong bonds, at least within the limits of experimental conditions. Bands between 3000 and 3600 cm^{-1} which occur in samples 3, 4 and particularly

Table 5
MAIN SPECTRAL ATTRIBUTIONS IN SAMPLES BASED ON SILICONE ELASTOMER AND COLLAGEN

Sample	Spectral attribution	
	Band, cm ⁻¹	Type of vibration
3, 4, 5	3000-3600	$\nu_{\text{Si-O}}$
1-8	2800-3060	$\nu_{\text{CH}}, \nu_{\text{CH}}, \nu_{\text{CH}_2}$
3,4,5	1630-1660	$\nu_{\text{C=O}}$
3, 4, 5	1550-1610	δ_{NH}
3, 4, 5	1430-1470	δ_{CH_2}
1-8	1250-1265	δ
1-8	1000-1200	$\nu_{\text{C-OH}}$
1-8	500-1000	δ_{CH}

in sample 5 and which do not occur in the other samples are due to the formation of Si-O bonds.

Conclusions

This paper presents our experiments on modifying the surface of silicone elastomer by collagen grafting/coating and electron beam irradiation. We focused on obtaining and characterizing two collagen hydrolysates with different molecular weights, and testing methods for grafting collagen to the silicone elastomer. By using FT/IR - ATR spectroscopy, we noticed the differentiated effect of collagen hydrolysate I, which contains non-polar essential amino acids, compared to that of collagen hydrolysate II, lacking essential amino acids. The fact that these compounds in the protein material are found on the surface of silicone elastomer even after washing, proves once more that electron beam irradiation of the silicone elastomer created the conditions for collagen grafting, with strong bonds, at least within the limits of experimental conditions.

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