Supporting Information

Stimulation of wound healing by electroactive, antibacterial and antioxidant polyurethane/siloxane dressing membranes: in-vitro and in-vivo evaluations

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S1. Spectroscopic, mechanical and dynamic-mechanical analysis

NCO content of polyurethane prepolymer was determined according to the procedure reported in ASTM D-2572. The OH number of CO and RM was measured by a method reported in ASTM D-4294. FTIR spectra were obtained using a Bruker IFS 48 instruments. All spectra were obtained under air as a function of time with 16 scans at a resolution of 4 cm^{-1} and a spectral range of 500–4000 cm^{-1}. The UV–visible spectra were monitored on a UV–Vis spectrophotometer (Bel photonics, LGS 53). Dynamic mechanical thermal analysis (DMTA) was performed using a Triton instrument (model Tritec 2000, England) within a temperature range of -100 to 200 ºC, a heating rate of 3ºC/min at a frequency of 1 Hz in tensile mode. Sample dimensions were 30×10×1 mm^3. The values of storage modulus, loss modulus, and tan δ versus temperature were recorded for each sample. The maximum temperature of tan δ curves was considered as glass transition temperature of the samples. The measurement of tensile strength, elongation at break, and modulus of samples at dry and hydrated states were carried out using a tensile tester (Instron 6025) with a cross head speed 50 mm/min. Samples were cut into bar model with 50 mm length and 5 mm width. The test was performed in room temperature. For each sample five specimens were tested.

S2. Conductivity and electroactivity analysis

The electrical conductivity (σ, $S.cm^{-1}$) of the samples, at both doped and undoped states, was measured at room temperature using a home-made four-point probe instrument, utilizing the following equation:

$$\sigma = \frac{I\ Ln2}{V\ \pi\ d_n}$$

, where V, I and d_n represented potential in volt, current in ampere and thickness of the sample in cm, respectively. To assess the electrochemical properties of the samples, cyclic voltammetry (CV) experiments were performed in a three-electrode electrochemical cell using Autolabpotentiostat/galvanostat instrument (PGSTAT30). Samples for cyclic voltammetry were prepared by formation of thin films on platinum sheets as working electrode and Ag/AgCl as reference electrode. The cyclic voltammogram was recorded by scanning potential between 0 and 1 V at a scan rate of 50 mV s^{-1} in 1.0 M HCl.

S3. Equilibrium water absorption (EWA) and water vapor transmission rate (WVTR) of membranes

The completely dried sample was accurately weighed and soaked in PBS at room temperature until the equilibrium swelling was attained (about 48 h). The weight of swelled membrane was determined after being gently wiped with filter paper to remove the surface liquid. EWA% was determined using the following equations.

$$\mathbf{EWA\%} = \frac{\mathbf{W_s - W_d}}{\mathbf{W_d}}$$

W_d and W_s designate the weights of dry and swelled membrane, respectively.

The moisture permeability of the membranes was determined by the measurement of WVTR across the material as expressed by ASTM E96/E96M. The WVTR is calculated using the following equation:

$$\mathbf{WVTR} = \frac{W_i - W_t}{A \times t}$$

WVTR is expressed in g m^{-2} day. A, W_i and W_t designate the area of cup mouth (m^2), the weight of water containing cup before and after placing in the oven at 37 ^oC and 35% humidity and t shows the duration of measurement.

S4. Gel content and surface hydrophilicity

Gel content of thermally cured membranes was evaluated. For this purpose, the membranes were dried under vacuum for 24 h at room temperature and weighed. Then, the samples were extracted by THF in a Soxhlet extractor for 24 h. The insoluble part was dried at 50^oC and weighed. The gel content was defined as follows:

$$\mathbf{Gel\ content}\ \% = \frac{W_d}{W_i} \times 100$$

,whereW_d and W_i designated the weight of dried membrane after extraction and the initial weight of the membrane, respectively. Static water contact angle measurements were performed to analyze surface hydrophilicity of the as-prepared membrane using an optical video contact angle system (OCA-15-plus, Dataphysics, Germany). The contact angle was

determined by running Image J 1.44p software on digital pictures taken from interfaces of membranes and droplets. All measurements were done in triplicate for both doped and undoped samples.

S5. In vitro cytocompatibility assays

To check possible cytotoxicity of materials may leach out from the dressing membranes, the freshly synthesized samples were immersed in the culture medium for 3 days at 37 ºC and then MTT assay was performed on extracted leachates using L929 fibroblast cells. The percentage of relative cell viability was calculated according to following equation:

$$\text{cell viability}\% = \frac{OD_{sample} - OD_{positive\ control}}{OD_{negative\ control}}$$

Where OD designates the optical density

S6. Evaluation of cell adhesion and proliferation

Cell attachment and proliferation on prepared membranes quantitatively assessed using MTT assay. At first, the sterilized membranes were cut in a size to completely cover the bottom of each well of a 96-well culture plate. Then the culture medium contained 5×10^3 L929 fibroblast cells were placed on the membrane and incubated at 37ºC for 45 min to enable the cells to attach onto the membrane surface. Then, the membrane/cell construct were provided with more culture medium for 1, 3, and 5 days, respectively. The seeded membranes were then washed with PBS to remove unattached cells and transferred to the new culture plates. Cells were harvested after 3 min incubation with 0.25% trypsin and 1 mM EDTA at 37 ºC

and the quantification was made by the MTT assay according to the procedure described for cytocompatibility assessment.

The spatial distribution and morphology of the L929 cells attached onto the surface of the membranes were assessed after staining of cells by a fluorescent dye. For this purpose, the above mentioned procedure was followed. After seeding of cells on the samples surface and their incubation for specifically determined times of 1, 3, and 5 days, the samples were washed three times with PBS. The adhered cells were fixed with 2.5% glutaraldehyde at room temperature for 8 min. The cells were dyed with DMSO containing 2% of curcumin for 8 min; followed by washing the samples with PBS several times. The stained cells were observed by an inverted microscope.

S7. Antibacterial activity of membranes

Antibacterial activity of the prepared membranes was studied using "colony forming count" method according to the procedure reported in ASTM E 2180-07. Bacteria at inoculated concentration of 2×10^8 CFU/ml, and membranes with the dimensions of 1 cm×1 cm were used throughout the tests. 18 h cultures prepared from three microorganisms including S. aureus (ATCC 6538), P. aeruginosa (ATCC 15449) and C. albicans (ATCC 10231) in tryptic soy broth. Microbial broth cultures were adjusted to 3×10^8 cells/ml with a spectrophotometer. 1 ml of these cultures was inoculated into Erlenmeyer flasks containing 100 ml molten agar slurry (0.3 g Agar-agar, 0.85 g NaCl, 100 ml water). A thin layer of the inoculated agar slurry (100 µl) is pipetted onto the test and untreated control materials (triplicate samples). After the specified contact time (24 h commonly used), surviving microorganisms are recovered via elution of the agar slurry inoculum from the test substrate into neutralizing broth and extracted via vortexing that provide complete removal of the inoculum from the test

polymers. Serial dilutions were made, and then spread plates were made of each dilution. Agar plates and dilution broths are incubated for 48±2 h at 37 ºC as optimal temperature for test organisms. Microbial colonies from each dilution series were counted and recorded. The percentage of microorganism reduction was calculated according to the following equation:

$$\text{Geometric mean} = \frac{\log_{10}X1 + \log_{10}X2 + \log_{10}X3}{3}$$

$$\text{Reduction}\,\% = \frac{(a-b)}{a} \times 100$$

, where x designates number of organisms recovered from the incubation period control or incubation period treated samples, a designates the antilog of the geometric mean of organisms recovered from the incubation period control samples and b designates the antilog of the geometric mean of organisms recovered from the incubation period treated samples**.**

S8. Antioxidant efficiency of membranes

The antioxidant efficiency of specimens were tested by measuring their capacity to scavenge the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, using the method reported by Serpen with minor modification.[1] The samples in doped state were powdered by crashing in liquid nitrogen. DPPH (3.0 ml, 100 μM) and dispersion of samples (1 mg) in methanol was stirred and incubated in a dark place for 15 min. Then, wavelength scanning was performed using a UV–vis spectrophotometer. The DPPH degradation was calculated using the following equation:

$$\text{DPPH scavenged } (\%) = \frac{A_B - A_S}{A_B} \times 100$$

, where A_B being the absorption of the blank (DPPH + methanol) and A_S the absorption of the sample (DPPH +methanol +sample).

S9. In vivo studies

The wound healing performance of the wound dressings were evaluated using rat model. Thirty six male Wistar rats weighing about 200 g per rat were used for the experiments. All animal experiments were performed in compliance with guidelines approved by the Animal Use and Care Administrative Advisory Committee at the University of Tehran, Tehran, Iran. Animals were acclimatized under standard animal laboratory conditions for 7 days prior to the experiments.

Animals were anaesthetized by intramuscular injection of ketamine at a dosage of 20 mg kg^{-1} body weight. The skin of dorsal region of rats was shaved and disinfected with 70% ethanol and then a full thickness wound of about 1.5 $cm^2$was created by cutting off the skin using a sterile surgical scissor. The rats were randomly divided into three groups. For group 1, wounds just covered with sterile cotton gauze without any other intervention (control group). For groups 2 and 3 of rats, the nonelectroactive (NESiPU4) and electroactive (EASiPU2) dressings were applied, respectively. Then, they were covered with sterile gauze cloth and fixed with elastic adhesive bandage to prevent dislodging or peeling off the dressing by rat. All the animals were kept in separate cages in an air conditioned room and they were fed with commercial rat food and water until they were sacrificed. At day 3, the dressings of all three groups were changed with new ones. After various postoperative days, macroscopic

photographs of the wounds were taken, and the wound area was measured. The percentage of wound reduction was calculated according to the following formula:

$$\text{Wound closure rate}(\%) = \frac{A_0 - A_t}{A_0} \times 100$$

, where A_0 and A_t are designated to initial wound area and wound area at designated time, respectively.

For histological analyses, the animals were sacrificed periodically on the 14th and 20th day of experiments and tissues from the wound site of individual animals were removed. The tissues specimens separately fixed in 10% formalin dehydrated through graded alcohol series and embedded in paraffin wax. Serial sections of 5 μm thickness were cut and stained with Hematoxylin & Eosin (H&E) and Masson's Trichrome. These stained sections were observed under a microscope and photomicrographs were captured to understand the histology of the wounds.

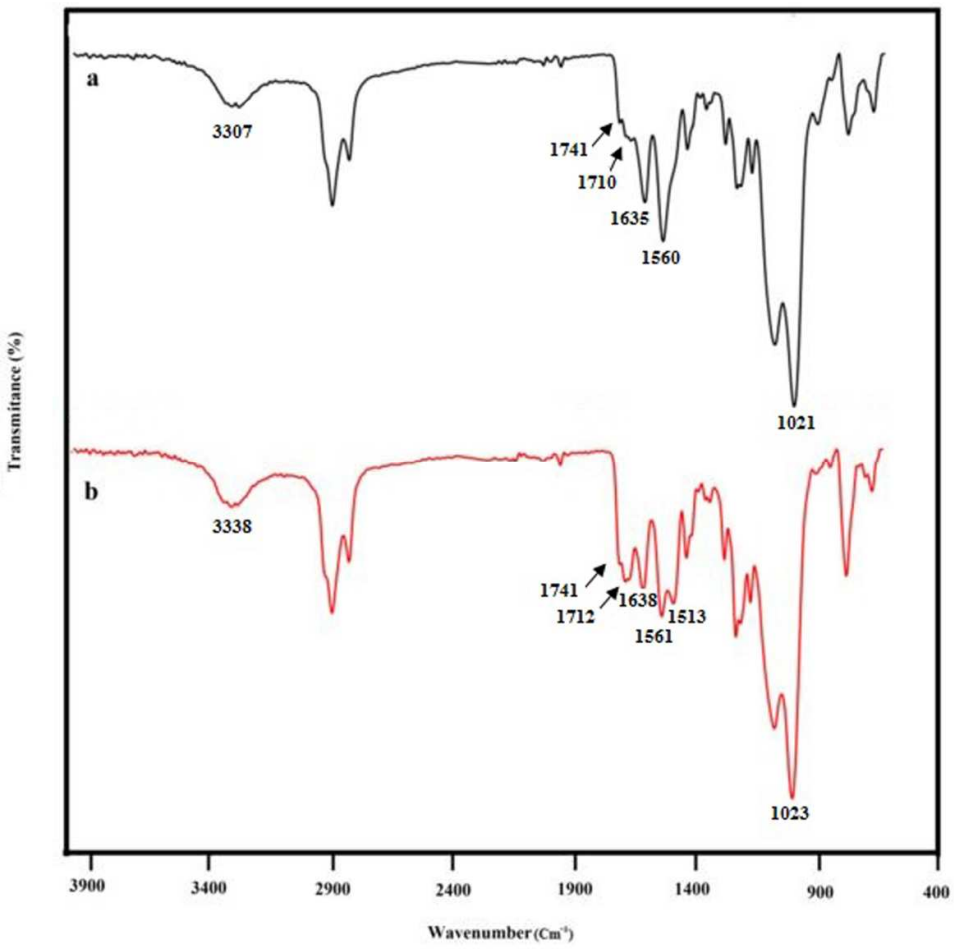

Figure S1: ATR-FTIR spectra of a) NESiPU4 and b) EASiPU2

Table S1:Tensile properties of membranes [x)]

Sample	Tensile strength (Mpa)		Initial modulus (Mpa)		Elongation at break (%)	
	Dry	Wet	Dry	Wet	Dry	Wet
NESiPU4	4.2±0.2[a]	3.1±0.1[a]	0.125±0.04[a]	0.112±0.01[a]	38.4± 2.2[a]	24.3±1.5[a]
EASiPU1	6.2±0.2[b]	4.2±0.1[b]	0.254±0.06[b]	0.191 ±0.02[b]	26.2±0.9[b]	20.5±0.6[b]
EASiPU2	7.5±0.4[c]	4.7±0.2[c]	0.385±0.03[c]	0.256 ±0.05[c]	20.2±0.5[c]	17.6±0.8[c]
EASiPU3	7.9±0.3[c]	4.9±0.1[c]	0.562±0.05[d]	0.345±0.06[d]	16.1±0.7[d]	15.5±0.6[c]

[x)] According to analysis of variances ($p \geq 0.05$) the difference between quantities with similar superscripts (a-d) is not significant for data of each column.

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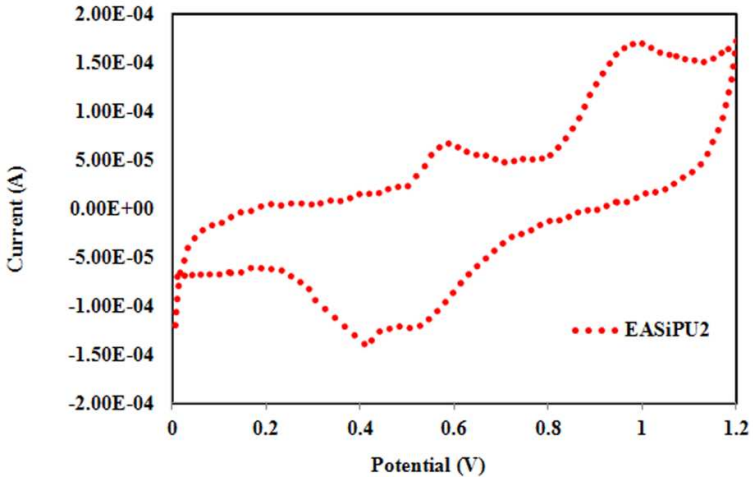

Figure S2: Cyclic voltammogram of EASiPU2 membrane.

Table S2: Bacterial reduction percent of the prepared membranes

Sample	*S. aureus*	*P. aeruginosa*	*C. albicans*
NESiPU4	0	0	0
Doped EASiU1	87.2	89.3	88.7
Doped EASiPU2	99.1	98.5	98.3
Doped EASiPU3	100	100	100

(1) Serpen, A.; Capuano, E.; Fogliano, V.; Gökmen, V. A New Procedure to Measure the Antioxidant Activity of Insoluble Food Components. *J. Agric. Food Chem.* **2007**, *55*, 7676–7681.