

1 Polyurethane based hybrid ciprofloxacin-releasing wound dressings designed for skin
2 engineering purpose

3
4 Iga Carayon ^{a,*}, Paweł Szarlej ^a, Przemysław Gnatowski ^{a,*}, Edyta Piłat ^a, Maciej Sienkiewicz
5 ^a, Marta Glinka ^b, Jakub Karczewski ^c, Justyna Kucińska-Lipka ^a

6
7 a. Department of Polymers Technology, Faculty of Chemistry, Gdansk University of
8 Technology, Gdansk, Poland

9 b. Department of Analytical Chemistry, Department of Polymers Technology, Faculty of
10 Chemistry, Gdansk University of Technology, Gdansk, Poland

11 c. Institute of Nanotechnology and Materials Engineering, Faculty of Applied Physics and
12 Mathematics, Gdansk University of Technology, Gdansk, Poland

13
14 * **Corresponding author.** Department of Polymers Technology, Faculty of Chemistry, Gdansk
15 University of Technology, Gabriela Narutowicza 11/12, 80-233 Gdansk, Poland.

16 E-mail address: iga.carayon@pg.edu.pl (I. Carayon); przemyslaw.gnatowski@pg.edu.pl (P.
17 Gnatowski)

18
19
20 **Abstract**

21 *Purpose:* Even in the 21st century, chronic wounds still pose a major challenge due to inadequate
22 potential for treatment options, so the latest wound dressings are hybrid systems that enable
23 clinical management, such as hybrid of hydrogels, antibiotics and polymers. These wound
24 dressings are mainly used for chronic and complex wounds, which can easily be infected by
25 bacteria.

26 *Materials and methods:* Six Composite Porous Matrices (CPMs) based on polyurethane (PUR)
27 in alliance with polylactide (PLAs) and poly(vinyl alcohol) (PVA) were prepared and analyzed
28 using optical microscopy. Three different types of hydrogels and their Ciprofloxacin (Cipro)
29 modified variants' ratios were prepared and analyzed using FTIR, SEM and EDX techniques.
30 Six Hybrid Cipro-Releasing Hydrogel Wound Dressings (H-CRWDs) were also prepared and
31 underwent short-term degradation, Cipro release, microbiology and cell viability
32 measurements.

33 *Results:* Average porosity of CPMs was in the range of 69-81%. The pore size of the obtained
34 CPMs was optimal for skin regeneration. Short-term degradation studies revealed degradability

35 in physiological conditions regardless of sample type. A meaningful release was also observed
36 even in short time ($21.76 \pm 0.64 \mu\text{g/mL}$ after 15 min). Microbiological tests showed visible
37 inhibition zones. Cell viability tests proved that the obtained H-CRWDs were biocompatible
38 (over 85% of cells).

39 *Conclusions:* A promising hybrid wound dressing was labeled. Simple and cost-effective
40 methods were used to obtain microbiologically active and biocompatible dressings. The results
41 were of importance for the design and development of acceptable solutions in the management
42 of chronic wounds of high potential for infection,

43

44 *Keywords:* polyurethane; polylactide; composite; ciprofloxacin; skin engineering

45

47 1. Introduction

48 Skin is one of the largest organs in the human body and compromises about 10% of body
49 weight. It acts as a barrier, protecting against the loss of fluids, important for maintaining body
50 homeostasis, and also makes a cover against mechanical and thermal injuries of internal organs.
51 Furthermore, skin acts like an isolation for tissues and organs against harmful external factors
52 (chemical substances, biological pathogens and UV radiation) [1]. Due to its functions, skin is
53 exposed to various types of damage leading to formation of different kinds of wound, like: burn
54 wounds, diabetic ulcers, venous leg ulcers, chronic wounds, etc. [2].

55 Human skin has great potential to regenerate after injury through the hierarchically arranged
56 physiological processes such as: coagulation and hemostasis, inflammation, proliferation and
57 remodeling [3,4]. Usually these steps allow for total regeneration of normal wounds [5].
58 Unfortunately, it is not the case for chronic wounds, which are often defined as wounds that do
59 not regenerate in 90 days [6]. Their healing process is a great therapeutic challenge [7]. Chronic
60 wounds can be caused by many factors, including infection with bacteria or antibiotic-resistant
61 pathogens. Traditional forms of dressings often are dry and do not provide proper wound
62 regeneration environment which can lead to adhesion to the wound leading to difficult and
63 painful removal process [8]. Moreover, traditional dressings have to be replaced often because
64 of the limited possibilities of exudates absorption [9]. Luckily, chronic wounds healing process
65 can be supported by many types of wound dressings and tissue scaffolds, which can be
66 classified as drug delivery systems (DDSs). The main task of DDSs is to deliver the suitable
67 dose of active substance at the right time to a specific place, without causing side effects [6,10].
68 In addition, controlled release systems allow modification of the drugs dose and provide
69 stability of active substance in physiological conditions [11].

70 Nowadays, the therapy of the various types of wounds uses novel, specially designed dressings
71 based, in example, on hydrogels [12], polyurethane fibers [13] or foams [14], alginates [15],
72 collagens [16], hydrofibers [17] and hydrocolloids [18,19]. The choice of the proper type of
73 dressing depends mainly on the type of wound, the healing phase of dressing application and
74 also dressing desired function (absorption of exudation, hydration of wound, occlusive function
75 or antibacterial effect) [20–23]. Materials most commonly applied as a wounds treatment
76 exhibit healing properties according to the TIME concept (Tissue, Infection, Moisture, Edge),
77 which means that they provide a moist healing environment, protection of wound edges,
78 resistance to contaminations, allow gas exchange and control of infection [23,24]

79 Recent trends in wound dressings design is focused on application of synthetic polymer
80 compositions containing polyurethanes (PURs). PURs are considered as one of the most
81 biocompatible and hemocompatible synthetic polymers available on the market these days [25].
82 PURs are commonly used biomaterials in medical applications and devices (catheters, aortic
83 balloons, implants, etc.). Their broad application spectrum is related to their unique structure
84 and properties like significant flexibility, good biocompatibility *in vitro*, non-toxic degradation
85 products [26]. Moreover, through different modifications, PURs biological activity and
86 attractiveness for cells proliferation can be adjusted [27]. Among other synthetic polymers
87 polylactide (PLAs) and poly(vinyl alcohol) (PVA) are also great candidates for usage in the
88 skin engineering field. Both PLA and PVA represents good biocompatibility and
89 biodegradability – features needed in the field of tissue engineering of skin.

90 To provide suitable antibacterial protection, fluoroquinolones are applied as a factor that
91 enhances the regeneration of infected wounds. Fluoroquinolones show wide antibacterial
92 activity and they are effective against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S.*
93 *aureus*), which are the most common bacteria responsible for wound infections [28,29]. For
94 example, Sripriya et al. [30] produced collagen bilayered dressings with ciprofloxacin (Cipro)
95 for infected wounds. *In vitro* assays proved their effectiveness against *S. aureus* and *P.*
96 *aeruginosa*. Furthermore, *in vivo* tests on rats showed increased regeneration process in the
97 group treated with collagen bilayered dressing containing Cipro. It was observed that sustained
98 release of Cipro from obtained dressings eliminates the bacteria occurring in wounds.
99 Additionally, Choipang et al. [31] in 2018 obtained PVA hydrogel dressings containing
100 poly(D,L-lactide-*co*-glycolide) (PLGA) and Cipro hydrochloride nanoparticles. Their
101 effectiveness was confirmed against *E. coli* and *S. aureus*.

102 In our team, we had been working on synthesis and fabrication of antibacterial scaffold obtained
103 with the use of PUR/PLA blends and modified with Cipro. Kucińska-Lipka et al. has described
104 design, synthesis and characterization of PURs crosslinked with PVA as a new proposition for
105 regenerative medicine. Performed characterization showed that tensile strength of the materials
106 was in the range of 41–52 MPa and contact angle of their surface was in the range of 38–47°.
107 The obtained PVA-crosslinked PURs did not show significant progress of degradation after 3
108 months of incubation in a phosphate-buffered saline (PBS). Thus, obtained materials may act
109 as a slowly-degradable material, which can provide long-term physical support in tissue
110 engineering purpose. A performed short-term hemocompatibility study showed that obtained
111 PVA-crosslinked PURs do not significantly influence blood components and a cytotoxicity test,
112 performed with the use of MG 63 cell line, revealed the great cytocompatibility of the obtained



113 materials [32]. Carayon et al. fabricated antibacterial and degradable scaffolds that may be used
114 in the field of skin regeneration. Degradable PURs were obtained by using amorphous α,ω -
115 dihydroxy(ethylene-butylene adipate) macrodiol (PEBA). PURs were processed with PLA (5
116 or 10 wt%). To meet the antibacterial requirement, the previously obtained hybrid PUR-PLA
117 scaffolds (HPPS) were modified with Cipro (2 or 5 wt%). Performed studies showed that Cipro-
118 modified HPPS, obtained by using 5 wt% of PLA, possess suitable mechanical characteristics,
119 morphology, degradation rates, and demanded antimicrobial properties to be further developed
120 as potential scaffolds for skin tissue engineering [33].

121 To meet the requirements of skin engineered substitutes we proposed a fabrication of hybrid
122 ciprofloxacin-releasing wound dressing (H-CRWD). H-CRWD is a combination of solid
123 composite porous matrix (CPM) covered with Cipro-loaded biocompatible hydrogels (CLHs).
124 CPMs were obtained with the use solvent casting/particulate leaching technique (SC/PL) and
125 consist of composition of PUR/PLA or PUR/PVA at different concentrations (10, 20 or 30
126 wt%). CPM is coated with hydrogel, which is loaded with Cipro (CLHs) to provide immediate
127 antibacterial action after wound dressing application on the burned, wounded or traumatized
128 skin. Produced H-CRWDs represents suitable physicochemical properties, favorable
129 morphology, degradation rate, and satisfactory biological performance when CPMs are
130 composed of PUR/30PVA. Such hybrid wound dressings, currently fabricated in a laboratory
131 scale, may be the future simple and cost-effective solutions to treat chronic and complex
132 wounds, which are one of the biggest medical challenge of 21st century.

133

134 2. Materials and methods

135 2.1. Materials fabrication

136 2.1.1. Composite Porous Matrices (CPMs) Fabrication

137 CPMs were fabricated by simple and cost effective solvent casting/particulate leaching (SC/PL)
138 technique. In the first step 100g of polymer mix containing PUR (Epaline Epaflex 380 A 10
139 25), PLA (Ingeo 7032D) or PVA (Mowiol 4-88 Mw 31,000) (10, 20 or 30% w/w) was prepared.
140 Then PUR/PVA mixes were dissolved in DMSO and the PUR/PLA mixes were dissolved in
141 THF/DMSO mixture (1:6 w/w). Solutions were stirred at reflux at 90°C until dissolution of
142 polymers. Weight concentration of PUR/PLA and PUR/PVA polymer mixtures in DMSO and
143 THF/DMSO solutions were equal to 20%. In the next step sodium chloride (diameter fraction
144 of 50 – 200 μm) was added as a porogen to 10 grams of obtained homogeneous solutions under
145 rigorous mixing. Sodium chloride was being added to the mixture until a paste-like consistency

146 was obtained (40 g of NaCl). The mixture was then transferred to glass, round molds (10 cm
147 diameter and 0.5 cm height) and placed in freezer for 48h at -20 °C. The obtained thin matrices
148 were submerged in distilled water for 7 days, and then dried for 1 day at 60°C. Symbols and
149 description of obtained matrices were listed in the **Table 1**.

150

151 **Table 1.** Symbols of obtained Composite Porous Matrices (CPMs) with their brief description.

152

Symbol	Description
PUR/10PLA	Matrices made of PUR and 10 wt% of PLA
PUR/10PVA	Matrices made of PUR and 10 wt% of PVA
PUR/20PLA	Matrices made of PUR and 20 wt% of PLA
PUR/20PVA	Matrices made of PUR and 20 wt% of PVA
PUR/30PLA	Matrices made of PUR and 30 wt% of PLA
PUR/30PVA	Matrices made of PUR and 30 wt% of PVA

153

154 2.1.2. Cipro-Loaded Hydrogels (CLHs) Fabrication

155 Unmodified hydrogels were obtained as follows: solution of PVA (4 wt%) and Borax (2 wt%)
156 were prepared in distilled water (70°C/16h, magnetic stirring). Solution of PVA and Borax were
157 mixed together to obtain 100 grams of solution at 1:3, 1:2 and 1:1 ratio at 90°C and stirred for
158 2h. After that time homogenous solutions were obtained.

159 To load ciprofloxacin hydrochloride (Cipro) within hydrogel's net, following steps were
160 undertaken: Cipro (1.5 wt%), L-ascorbic acid (10 wt%, LAA) and PVA (4 wt%) were mixed
161 together at 90°C for 2h. After that time, Borax solution (2 wt%) was added to the homogenous
162 solution of PVA containing Cipro and LAA at three different ratios: 3:1, 2:1 or 1:1 to obtain
163 100 grams of solution. LAA was used to reduce pH to acidic values and prevent precipitation
164 of Cipro. Due to this step, obtained PVA-Cipro-LAA solutions were homogenous while mixing
165 with Borax solution. Symbols and descriptions of both unmodified (Table 2. H1-H3) and Cipro-
166 modified hydrogels (Table 2. H4-H6) were listed in **Table 2**. Solutions at all ratios were
167 transferred into round molds and dried for 24h at 20°C.

168

169 **Table 2.** Symbols of obtained CRHs with their brief description.

170

	Symbol	Description
Unmodified hydrogels	H1	Hydrogel made of PVA and borax solutions in ratio 1:1 (w/w)
	H2	Hydrogel made of PVA and borax solutions in ratio 2:1 (w/w)

	H3	Hydrogel made of PVA and borax solutions in ratio 3:1 (w/w)
Modified hydrogels	H4	Hydrogel made of 4% PVA, 1.5% Cipro and 10% AA solution and mixed with a borax solution in ratio 1:1 (w/w)
	H5	Hydrogel made of 4% PVA, 1.5% Cipro and 10% AA solution and mixed with a borax solution in ratio 2:1 (w/w)
	H6	Hydrogel made of 4% PVA, 1.5% Cipro and 10% AA solution and mixed with a borax solution in ratio 3:1 (w/w)

171

172 2.1.3. Hybrid Cipro-Releasing Hydrogel Wound Dressing (H-CRWD) Fabrication

173 An immersion technique was used to fabricate H-CRWDs. Obtained CPMs (Table 1) were
 174 immersed in hydrogels solutions (Table 2) for 1h at 90°C (to prevent premature gelation
 175 process). After that, obtained H-CRWDs were transferred to the drier and dried for 24h at 40°C.
 176 **Table 3** summarizes content of all H-CRWDs. **Fig. 1** visualizes the step-by-step process of H-
 177 CRWDs fabrication.

178

179 **Table 3.** Symbols of obtained Cipro-Releasing Hydrogel Wound Dressings (CRWDs) with
 180 their brief description.

181

Symbol	Description
D1	Dressing made of PUR/30PLA matrix covered with H4
D2	Dressing made of PUR/30PLA matrix covered with H5
D3	Dressing made of PUR/30PLA matrix covered with H6
D4	Dressing made of PUR/30PVA matrix covered with H4
D5	Dressing made of PUR/30PVA matrix covered with H5
D6	Dressing made of PUR/30PVA matrix covered with H6

182

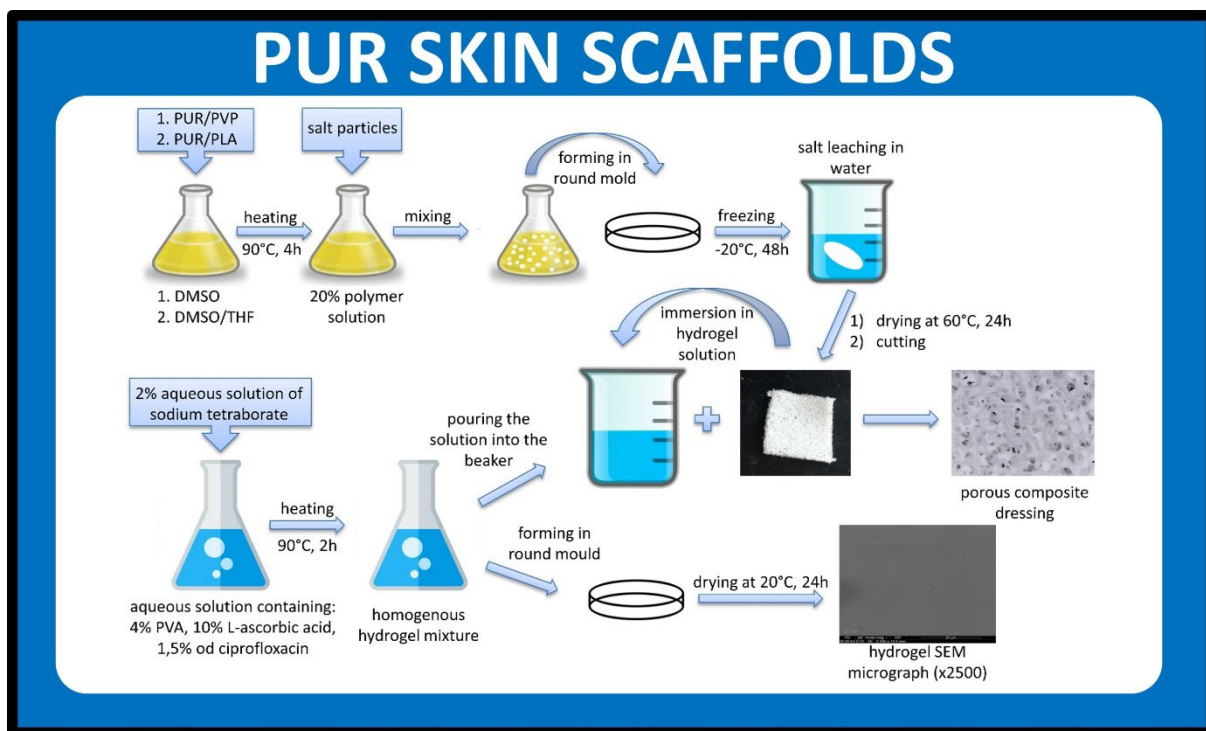


Figure 1. Scheme of hybrid composite porous matrices development.

2.2. Characterization techniques

2.2.1. Fourier transform-infrared spectroscopy (FTIR)

Examination of samples using the FTIR spectrometer allows the determination of characteristic vibrations for functional groups occurring in the material. To demonstrate the presence of characteristic bonds in the obtained hydrogels, samples were tested with a Nicolet 8700 spectrometer (Thermo Electron Corporation, Wilmington, DE, USA) equipped with a Specac's Golden Gate and a single reflection diamond ATR unit. The study was conducted at a resolution of 4 cm^{-1} . Each sample was tested using 256 scans per sample in the spectral range from 4000 to 500 cm^{-1} .

2.2.2. Scanning electron microscopy (SEM) with Energy-dispersive X-ray spectroscopy (EDX)

The hydrogels morphology was examined using an FEI QUANTA 250 FEG SEM microscope (Thermo Fisher Scientific, Waltham, MA, USA) at an accelerating voltage of 20 kV and magnification of 1000x and 2500x. To determine the atomic composition of the produced hydrogel dressings, an energy-dispersive X-ray spectrometer was used. The scanning time of measurement was 200 s per sample. Before analysis hydrogels were cut into cylindrical samples (diameter = 0.8 cm, area = 0.5 cm^2) and covered with gold using a sputter Quorum 150T E

202 (Quorum Technologies, Laughton, East Sussex, UK). EDX analysis was performed 3 times for
203 each sample type.

204 2.2.3. Optical microscopy, pore sizes and porosity

205 Morphology assessment of obtained porous matrices before and after degradation was done
206 using the Digital Microscope, model avp028f8 certified by FC & CE (Flood Control and
207 Coastal Emergency) and RoHS (Restriction of Hazardous Substances). The images were
208 observed using magnifications of 40x and 800x. To record microscopic images, a desktop
209 computer with VidCap software was used. Obtained images allowed also to determine the size
210 and type of pores and additionally evaluated the interconnection of pores. ImageJ Software
211 (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA,
212 <https://imagej.nih.gov/ij/>, 1997-2021) was used to analyze microscopic images. The porosity
213 was measured using the following equation (1):

$$214 \quad P = 1 - \left(\frac{V_1 - V_2}{V_2 - V_3} \right) \times 100 \% \quad (1)$$

215 Where:

216 V_1 - volume of ethanol [cm^3];

217 V_2 - volume of ethanol after immersion of matrix [cm^3];

218 V_3 - volume of ethanol after removal of matrix [cm^3].

219 2.2.4. Short-time degradation (STD) study

220 Studies of the degradation were carried out in 5M NaOH, 2M HCl and 0.01 PBS (Phosphate-
221 buffered saline) aqueous solutions. Round samples (diameter = 0.7 cm, area = 0.38 cm^2 ,
222 thickness = 0.2 cm) were cut out from obtained porous matrices. Three samples were taken
223 from each type of matrix and were stored in the 1.5 ml solution of selected medium. The
224 degradation process was performed at 37 °C for 7 and 14 days (5M NaOH and 2M HCl) or for
225 1, 7, 14, 28 and 56 days (0.01M PBS). Before the studies, samples were dried and weighted
226 using Thermobalance (RADWAG MAX50/SX) set at 60 °C. After the degradation process the
227 samples were rinsed with distilled water, dried at 60 °C for 24 h and weighted again. The mass
228 loss of the samples was measured by following formula (2).

$$229 \quad M = \left(\frac{m_i - m_0}{m_0} \right) \times 100 \% \quad (2)$$

230 Where:

231 M - mass loss [%];

232 m_0 - initial mass [g];

233 m_i - mass after degradation process [g].

234

235 2.2.5. High Performance Liquid Chromatography (HPLC)

236 The release of active substances from produced composite dressings (D1-D6) was performed
237 using a Agilent 1200 LC system consisting of ALS autosampler, binary pump, degasser,
238 thermostated column compartment and DAD detector ($\lambda = 277$ nm). The chromatographic
239 separation an active substance (Cipro) was carried out using ZORBAX Eclipse XDB-C8
240 (Agilent) LC column (150 x 4.6 mm, 5 μ m) using isocratic conditions with mixture of 0.02 M
241 KH_2PO_4 solution (acidified to pH = 2.7) and acetonitrile (80:20 v/v). Flow rate of 0.8 mL/min
242 was used and injection volume was established as 20 μ l. Temperature of column was maintained
243 at 35 °C. Cipro calibration was realized using external calibration method. Working standard
244 solutions (0.5, 1, 5, 10, 50, 100 μ g/mL) were prepared daily by dilution of stock solution with
245 distilled water and analyzed in triplicate (n = 3). Real-world samples (D1-D6) were cut into
246 pieces with diameter = 0.7 cm, and thickness = 0.2 cm). In following step, prepared samples
247 were immersed in deionized water (5 mL) and incubated at 37°C. After specified time of
248 incubation, for each sample the water phase was analyzed. Samples were prepared and analyzed
249 in triplicate.

250 2.3. Statistical analysis

251 The statistical analysis was performed with the use of the Origin Pro 8.5 software (OriginLab
252 Corporation, Northampton, MA, USA). To determine the statistical differences, one-way
253 ANOVA ($\alpha = 0,05$), two-way ANOVA ($\alpha = 0,05$) and post-hoc Tukey test ($\alpha = 0,05$) (n=3)
254 were used.

255 2.4. Biological Performance

256 2.4.1. Microbiology tests

257 Microbiological tests were performed with a use of H-CRWDs based on PUR/30PLA and
258 PUR/30PVA systems modified with hydrogels (H4-H6, Table 2) containing 1.5% of Cipro.
259 Selection of H-CRWDs containing 30 wt% of PLA or PVA in a PUR/PLA or PUR/PVA
260 composition was dictated by their porosity, which was the highest for those compositions. For
261 comparison, hybrid wound dressings based on PUR/PLA or PUR/PVA were fabricated in
262 exactly same way as it was in case of H-CRWDs, with a step exclusion related to their
263 modification with Cipro. Neat hybrid wound dressings served as a control.

264 Methodology of microbiological tests was similar to the one previously published [33].
265 *Staphylococcus aureus* (Gram positive). Selected bacterial strain was cultivated in 20 ml of
266 fresh and sterile Luria broth (LB) medium. The LB medium contained: casein peptone 10.0
267 g/L; yeast extract 5.0 g/L; NaCl 10.0 g/L dissolved in deionized water. Cultivations were carried
268 out in 200 ml sterile Erlenmeyer flasks on a rotary shaker at 170 rpm at 37 °C for 18–24 h.
269 After the incubation time, 100 µL of each bacterial strain culture was transferred into 10 mL of
270 sterile LB medium in 100 mL sterile Erlenmeyer flasks. Next, bacterial strains cultivations were
271 carried out on a rotary shaker at 170 rpm at 37 °C to get the log phase of bacterial growth
272 (OD600 values 0.4–0.7). For determination of antibacterial activities, 100 µL of each bacterial
273 strain suspensions in the log phase of growth were placed on sterile LA medium with a sterile
274 glass rod. The LA medium contained: casein peptone 10.0 g/L; yeast extract 5.0 g/L; NaCl 10.0
275 g/L; agar 15.0 g/L dissolved in deionized water. Prior to the examination, unmodified and
276 Cipro-modified HPPS were sterilized by the exposition to UV radiation for 30 min and placed
277 on plates with sterile tweezers. Sterile samples of unmodified and Cipro-modified HPPS
278 scaffolds were placed on bacterial cultures on LA plates and incubated at 37 °C for 24 h. After
279 the incubation, the diameter of the presence or absence of growth inhibition zones around
280 samples of unmodified and Cipro-modified HPPS was measured. All analyses were done in
281 triplicate.

282 2.4.2. Cytotoxicity Assay

283 Cytotoxicity of obtained H-CRWD obtained with the use of PUR/30PVA was studied
284 according to the ISO 10993-5 standard in indirect cytotoxicity test using MTT assay. This
285 selection was dictated by the favorable properties of these samples reached in short-term
286 degradation test and drug release study.

287 Samples were sterilized 30 min each side under UV radiation. Then, an extract of studied
288 samples was prepared in culturing medium DMEM/F-12 supplemented with FBS; 5 µg/ml
289 penicillin with streptomycin; 5 µg/ml amphotericin B (Corning). Prepared extracts were
290 incubated for 24 h at 37 °C and 5% CO₂. After that time, extracts were filtrated and placed in
291 the culturing wells with CC1163 cells, which were cultured as follows: cells were seeded on 24-
292 well culture plates with a density of 19 000 cells per 1 cm² (ThermoFisher) and cultured for 24
293 h at 37 °C and 5% CO₂ in the supplemented DMEM/F-12 culturing medium (Corning). After
294 24 h of cells incubation with extract, the MTT assay was performed. The absorbance of
295 prepared solutions was studied by using Spectrophotometer set at $\lambda = 570$ nm (ThermoFisher).
296 The results were were showed in the graph as cells viability towards control (100% of viability).

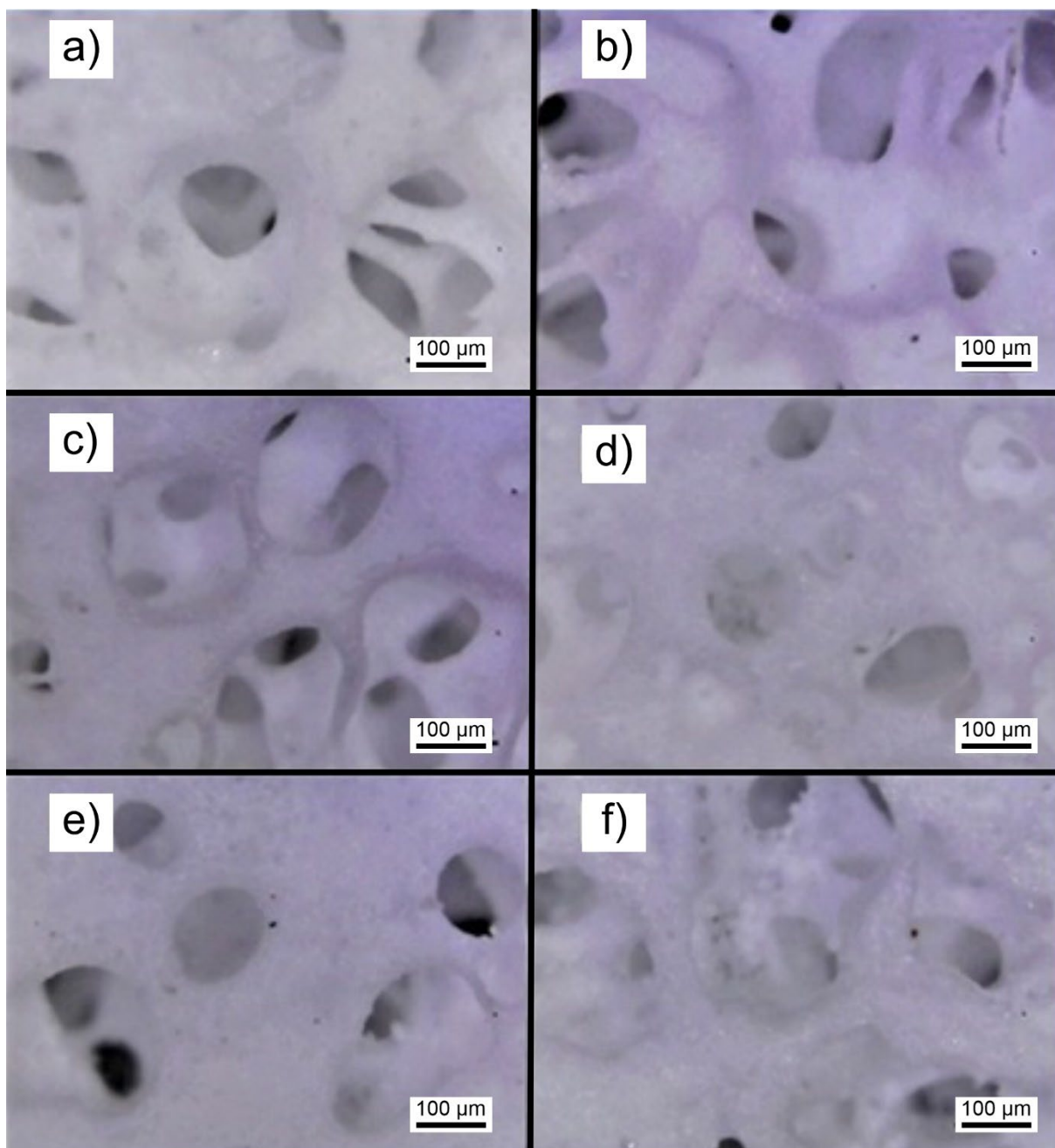
297 The statistical analysis was performed with the use of the Origin Pro 8.5. Statistical differences
298 were evaluated by the one-way ANOVA ($\alpha = 0.05$) and post hock Tukey test ($\alpha = 0.05$).
299

300 3. Results

301 3.1. Composite Porous Matrices (CPMs) Characterization

302 3.1.1. Optical microscopy and porosity calculation

303 Microscopic images of CPMs were shown in **Fig. 2**. Obtained CPMs were characterized by
304 average porosity ranging from 69 to 81 %. Detailly, for PUR/10PLA, PUR/20PLA and
305 PUR/30PLA CPMs porosity was determined as follows: $69.22 \pm 2.01\%$, $73.06 \pm 2.33\%$ and
306 $72.03 \pm 2.04\%$, respectively (**Figure 3**). One-way ANOVA test confirmed that those values
307 were not statistically different ($\alpha = 0.05$).



308
309
310

Figure 2. Optical microscopy of CPMs: a) PUR/10PVA, b) PUR/20PVA, c) PUR/30PVA, d) PUR/10PLA, e) PUR/20PLA, f) PUR/30PLA. Magnification of 800x.

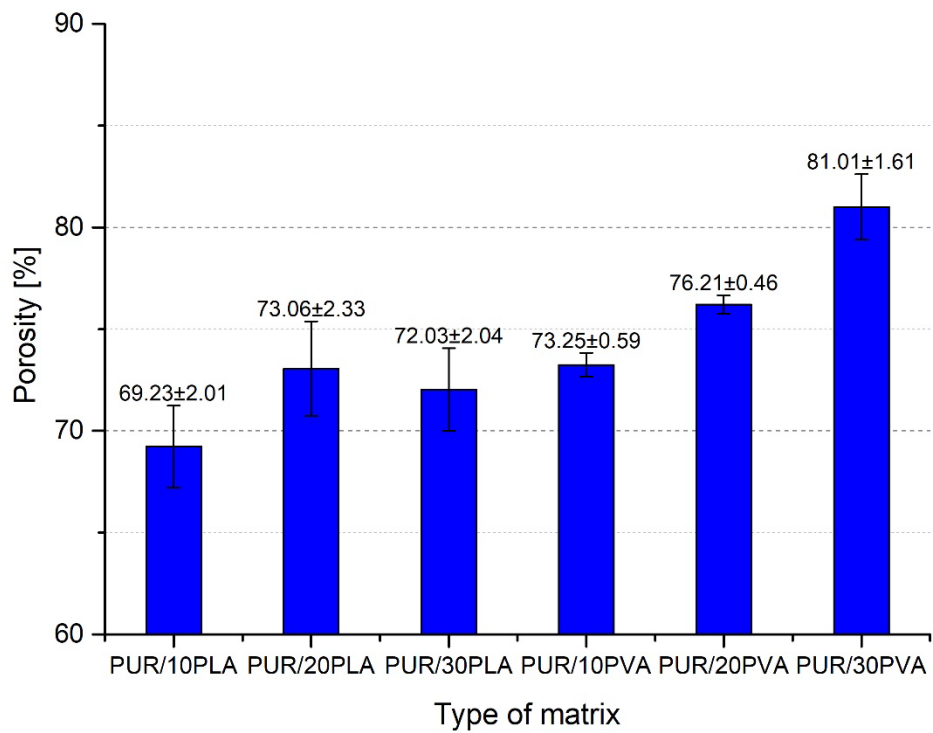
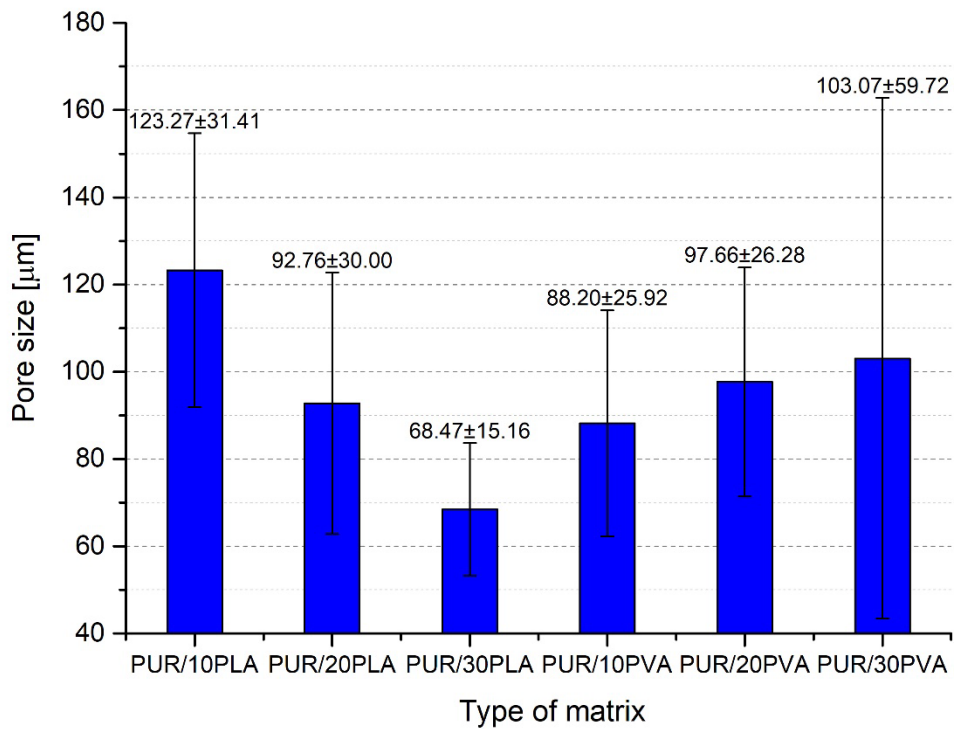


Figure 3. Average porosity of obtained CPMs.



311
312
313

314

315 **Figure 4.** Average pore sizes of obtained CPMs.

316

317 However, for PUR/10PVA, PUR/20PVA and PUR/30PVA samples identified porosity was
318 slightly higher ($73.25 \pm 0.59\%$, $76.21 \pm 0.46\%$ and $81.01 \pm 1.61\%$, respectively, **Figure 3**) in
319 comparison to CPM obtained with the use of PUR/PLA composition. One-way ANOVA
320 statistical evaluation determined, that mean porosity values for PUR/10PVA and PUR/20PVA
321 were not statistically different ($\alpha = 0.05$). However, Tukey test evaluated that porosity for
322 PUR/30PVA was significantly higher than for PUR/10PVA and PUR/20PVA ($\alpha = 0.05$). It can
323 be considered that the type of added component in a composite, in this case PLA or PVA, affects
324 porosity of obtained composites.

325 PUR/PVA samples were characterized by a porosity higher by 5% on average in comparison
326 to PUR/PLA samples. Despite this identified difference both CPM (PUR/PLA and PUR/PVA)
327 were described as suitable for the purpose of skin engineering.

328 Computer analysis of CPMs images (**Figure 2**) revealed that PUR/10PLA matrix was
329 characterized by high average pore sizes equal to $123.27 \pm 31.41 \mu\text{m}$ (**Figure 4**). This value
330 was slightly higher than average pore size calculated for PUR/20PLA and PUR/30PLA
331 matrices, which reached $92.76 \pm 29.99 \mu\text{m}$ and $68.47 \pm 15.16 \mu\text{m}$, respectively ($\alpha = 0.05$).

332 For PUR/10PVA, PUR/20PVA and PUR/30PVA samples average pore sizes were similar with
333 the amount of PVA in a composition: $88.20 \pm 25.92 \mu\text{m}$, $97.66 \pm 26.28 \mu\text{m}$ and 103.07 ± 59.72
334 μm , respectively. One-way ANOVA test proved that pore size values for PUR/PVA composite
335 matrices were not statistically different ($\alpha = 0.05$). It was also found that the addition of PLA
336 causes a decrease in porosity with its increasing amount, while the growing addition of PVA
337 increases the average pore size diameter.

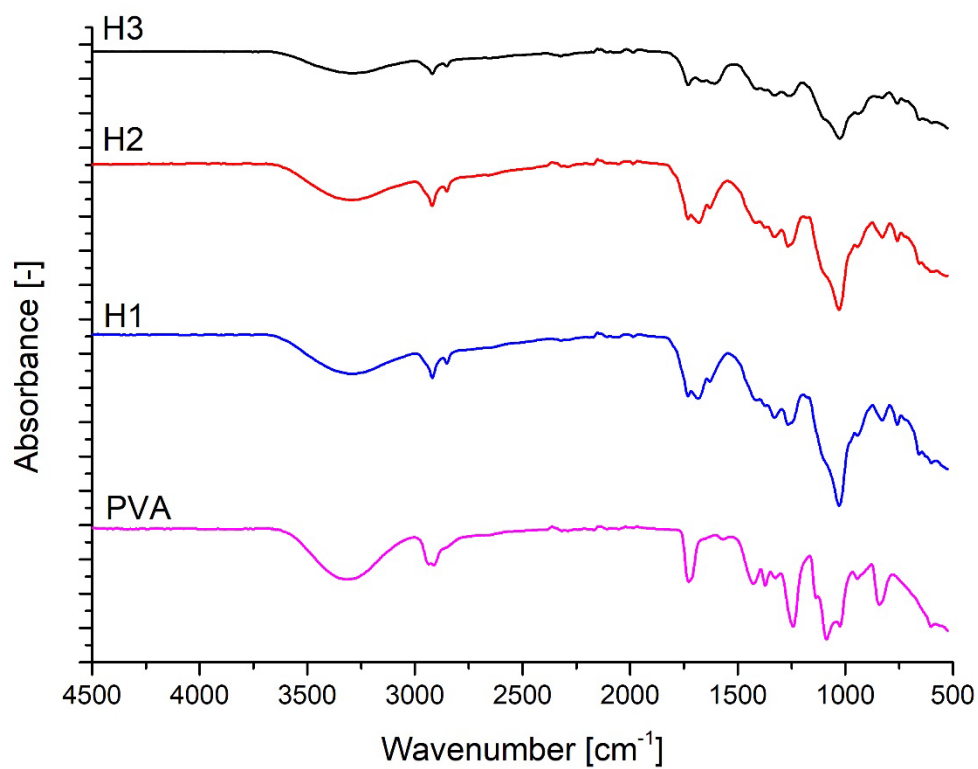
338 3.2. Unmodified hydrogels (UnMHs) and Cipro-Loaded Hydrogels (CLHs)

339 Characterization

340 3.2.1. Fourier transform infrared spectroscopy (FTIR)

341 FTIR spectra of native PVA, and UnMHs (**Table 2**, H1-H3) were presented in **Fig. 5**. Detailed
342 band assignments were given in **Table 4**.

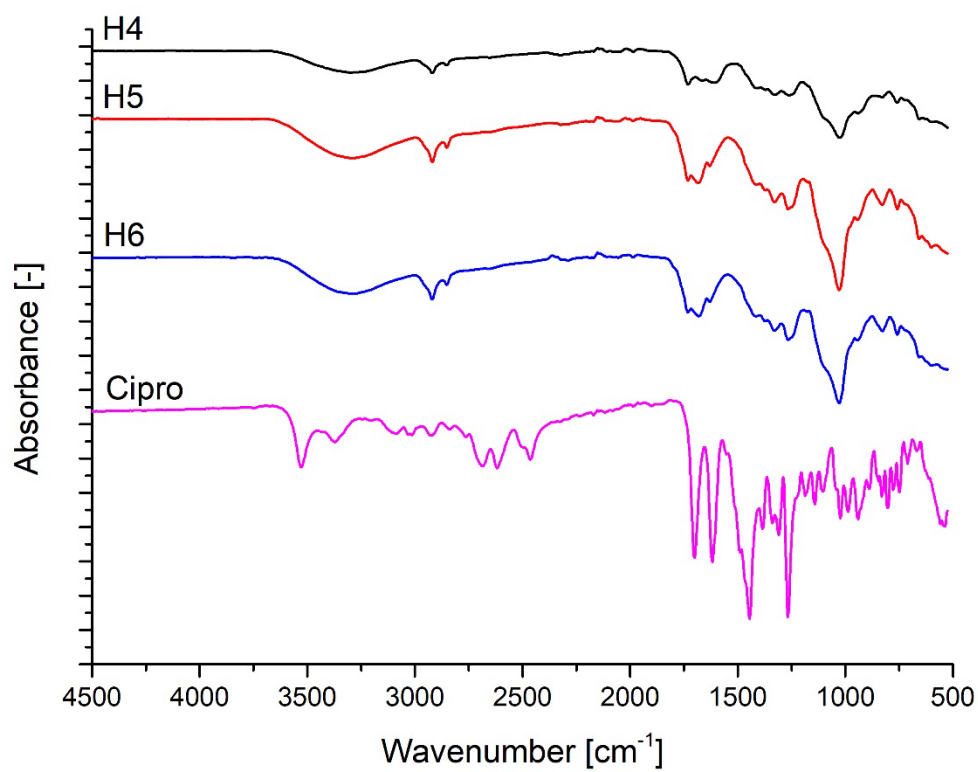




343

344

Figure 5. FTIR spectra for native PVA and UnMHs.



345

346

Figure 6. FTIR spectra for CLHs and Cipro.

347
348
349
350

Table 4. Detailed band assignment of Fourier transform-infrared spectroscopy (FTIR) spectra presented in Fig. 5 (PVA and UnMHs).

Wavelength [cm ⁻¹]	Assignment
3600-3100	ν (O-H) stretching vibrations from hydroxyl groups of PVA and H1, H2, H3 hydrogels
2930	ν (C-H) symmetrical stretching vibrations from CH ₂ groups
2850	ν (C-H) asymmetrical stretching vibrations from CH ₂ groups
1740	ν (C=O) stretching vibrations from acetyl groups
1500-1300	δ (C-H) deformation vibrations from CH ₂ groups
1290	ν (B-O-C) stretching vibrations from H1, H2 and H3 hydrogels
1250	ν (C-O) stretching vibrations from acetyl groups
1130	ν (B-O-C) stretching vibrations from H1, H2 and H3 hydrogels
1100	ν (C-O) stretching vibrations from C-O-H bonds
1100-1000	γ (C-C) skeletal vibrations from PVA chains
700	δ (O-H) deformation vibrations from C-O-H bonds

351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370

Broad band noted at 3600-3100 cm⁻¹ was described as stretching vibrations of O-H bonds of PVA molecules present in UnMHs, obtained by crosslinking of PVA with borax at different ratios (3:1, 2:1 and 1:1 w/w). Within hydroxyl groups observed at 3600-3100 cm⁻¹ range, borax anions were identified [34]. The absorption bands at 2930 and 2850 cm⁻¹ were related to the asymmetrical and symmetrical stretching vibrations of the C-H bonds of CH₂ groups, present in the linear structure of PVA [35] and obtained UnMHs. The peak at 1740 cm⁻¹ is characteristic for acetic groups derived from partial hydrolysis of polyvinyl acetate to polyvinyl alcohol [36]. At the wave number of approx. 1500-1300 cm⁻¹, deformation bands of C-H bonds of CH₂ groups located in the chains of PVA and obtained unmodified hydrogels can be observed [36]. Bands at 1250 and 1100 cm⁻¹ were related to C-O stretching bonds from acetic groups and hydroxyl groups in PVA [36] and they slightly disappear in case of UnMHs. Peaks occurring at 1290 and 1130 cm⁻¹ are characteristic for stretching vibrations of Borax-O-C groups and they confirm the formation of covalent bonds between PVA chains and Borax anions [36]. The intensity of mentioned bands increases with the growing amount of Borax in hydrogel compositions. To provide sufficient FTIR spectra analysis of CLHs (**Figure 6**) it was needed to perform detailed analysis of FTIR spectra of Cipro. Detailed band assignments were given in **Table 5**.

371 **Table 5.** Detailed band assignment of Fourier transform-infrared spectroscopy (FTIR) spectra
 372 presented in Fig. 6 (CLHs and Cipro).

373

Wavelength [cm ⁻¹]	Assignment
3500-3368	$\nu(\text{O-H})$ stretching vibrations from hydroxyl groups, intramolecular H-bonded
3035-3011	$\nu(\text{C-H})$ and $\nu(\text{Ar-H})$ from aromatic cyclic enes
2927, 2907	$\nu(\text{C-H})$ asymmetrical and symmetric stretching vibrations from CH ₂ groups
2697-2457	$\nu(>\text{C}=\text{O})$ and $\nu(>\text{N}-<)$ stretching vibrations from COOH and N-bonded and NH
1702	$\nu(\text{C}=\text{O})$ stretching vibrations from carbonyl
1625-1603	Quinolines, $\delta(\text{N-H})$ bending vibrations
1556	$\delta(\text{C-H})$ deformation vibrations from CH ₂ groups
1494-1386	$\nu(\text{C-O})$ stretching vibrations from carbonyl groups
1333-1211	$\delta(\text{O-H})$ bending vibrations of hydroxyl groups
1050-1033	$\nu(\text{C-F})$ stretching vibrations of C-F bond, fluorine group

374

375 **Table 6.** Detailed band assignment of Fourier transform-infrared spectroscopy (FTIR) spectra
 376 presented in Fig. 6(CLHs, H4-H5).

377

Wavelength [cm ⁻¹]	Assignment
3615-3001	$\nu(\text{O-H})$ stretching vibrations from hydroxyl groups of PVA and H1, H2, H3 hydrogels
2923	$\nu(\text{C-H})$ symmetrical stretching vibrations from CH ₂ groups
2848	$\nu(\text{C-H})$ asymmetrical stretching vibrations from CH ₂ groups
1727	$\nu(\text{C}=\text{O})$ stretching vibrations from acetyl groups
1674-1627	Quinolines, $\delta(\text{N-H})$ bending vibrations
1333	$\nu(\text{B-O-C})$ stretching vibrations from H1, H2 and H3 hydrogels
1274	$\nu(\text{C-O})$ stretching vibrations from acetyl groups
1180	$\nu(\text{B-O-C})$ stretching vibrations from H1, H2 and H3 hydrogels
1106	$\nu(\text{C-O})$ stretching vibrations from C-O-H bonds
1025-950	$\gamma(\text{C-C})$ skeletal vibrations from PVA chains
823, 770	$\delta(\text{O-H})$ deformation vibrations from C-O-H bonds

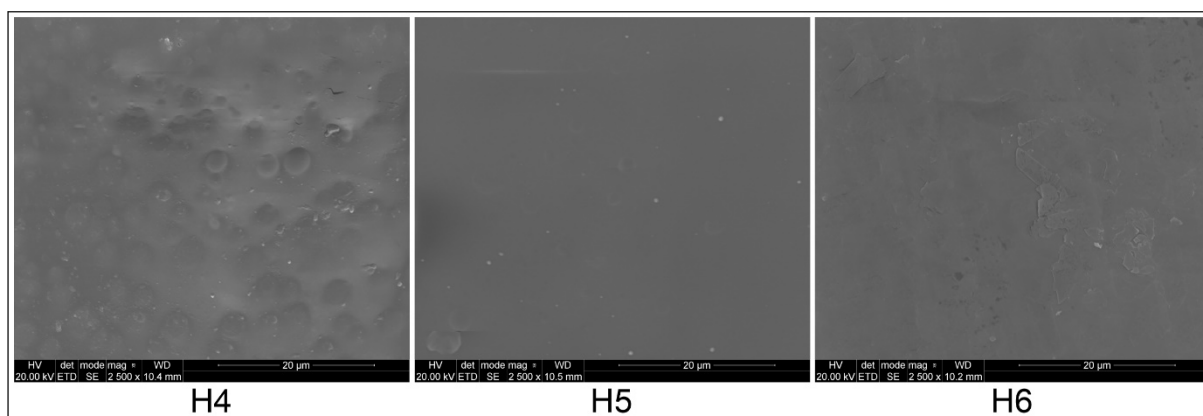
378

379 The prominent and characteristic peak for Cipro was identified between 3500-3368 cm⁻¹. It was
 380 assigned to an O-H stretching vibration (intermolecular hydrogen bonding). Band observed
 381 between 3035-3011 cm⁻¹ represented alkenes and aromatic C-H stretching, related to stretching
 382 vibrations of aromatic enes. Bands at 2927 cm⁻¹ and 2907 cm⁻¹ were described as asymmetric
 383 and symmetric stretching vibrations coming from CH₂ groups. In the range of 2697-2457 cm⁻¹
 384 stretching vibrations of C=O and >N- coming from COOH and N-bonded were identified. Band
 385 at 1702 cm⁻¹ showed carbonyl stretching vibrations in the Cipro molecule. Range of 1625-1603
 386 cm-1 shows N-H bending vibrations of Quinolines. At 1556 cm⁻¹ deformation vibrations of C-
 387 H from CH₂ groups were observed. Range of 1494-1386 cm⁻¹ represented C-O stretching
 388 vibrations from carbonyl and carboxylic acid groups. In between 1333 and 1211 cm⁻¹ bending
 389 vibrations of hydroxyl groups were observed. Finally, the range between 1050-1033 cm⁻¹

390 Analysis of CLHs FTIR spectra (**Fig. 6**) did not reveal large differences in band assignments
391 (**Table 6**), with one significant exception. Bands observed in the range between 1674-1627 cm⁻¹
392 were not noted in case of UnMHs, but were identified in case of Cipro FTIR spectra analysis
393 (**Table 5**). These bands were assigned to Quinolines, $\delta(\text{N-H})$ bending vibrations, what could
394 confirm presence of Cipro in CLHs.

395 3.2.2. Scanning electron microscopy (SEM) with Energy-dispersive X-ray 396 spectroscopy (EDX)

397 CLHs surface (**Table 2**, H4-H6) were studied by SEM imaging (**Fig. 7**). H4 hydrogel was
398 characterized by a surface at which craterous-like pattern was observed. Single crater-like
399 structure sizes were in the range of 2-5 μm . Observed craterous-like structures were distributed
400 evenly over the entire surface of H4 CLH. This structure seems to be interesting according to
401 the potential place of Cipro bonding in a CLH and its further release from CRH network.
402 Craterous-like structures were not identified further for H5 and H6 hydrogel.



403
404 **Figure 7.** Selected SEM images showing craterous-like structures at the surface of H4 CLH
405 and lack of these structures at the surface of H5 and H6 CLHs. Magnification 2500x.

406
407 EDX spectra (**Figure 8**) confirmed presence of such elements like carbon and oxygen, which
408 were core of polymers used in CLHs fabrication. Fluorine and chlorine presence may be
409 additional confirmation of Cipro presence in CLH. This would suggest successful hydrogels
410 modification. Detected sodium element can come from unreacted Borax used as a hydrogels
411 crosslinker. This would suggest that further purification process could be thought after CLHs
412 synthesis. Samples were sputtered with gold before testing; therefore peaks from Au were
413 present.

414

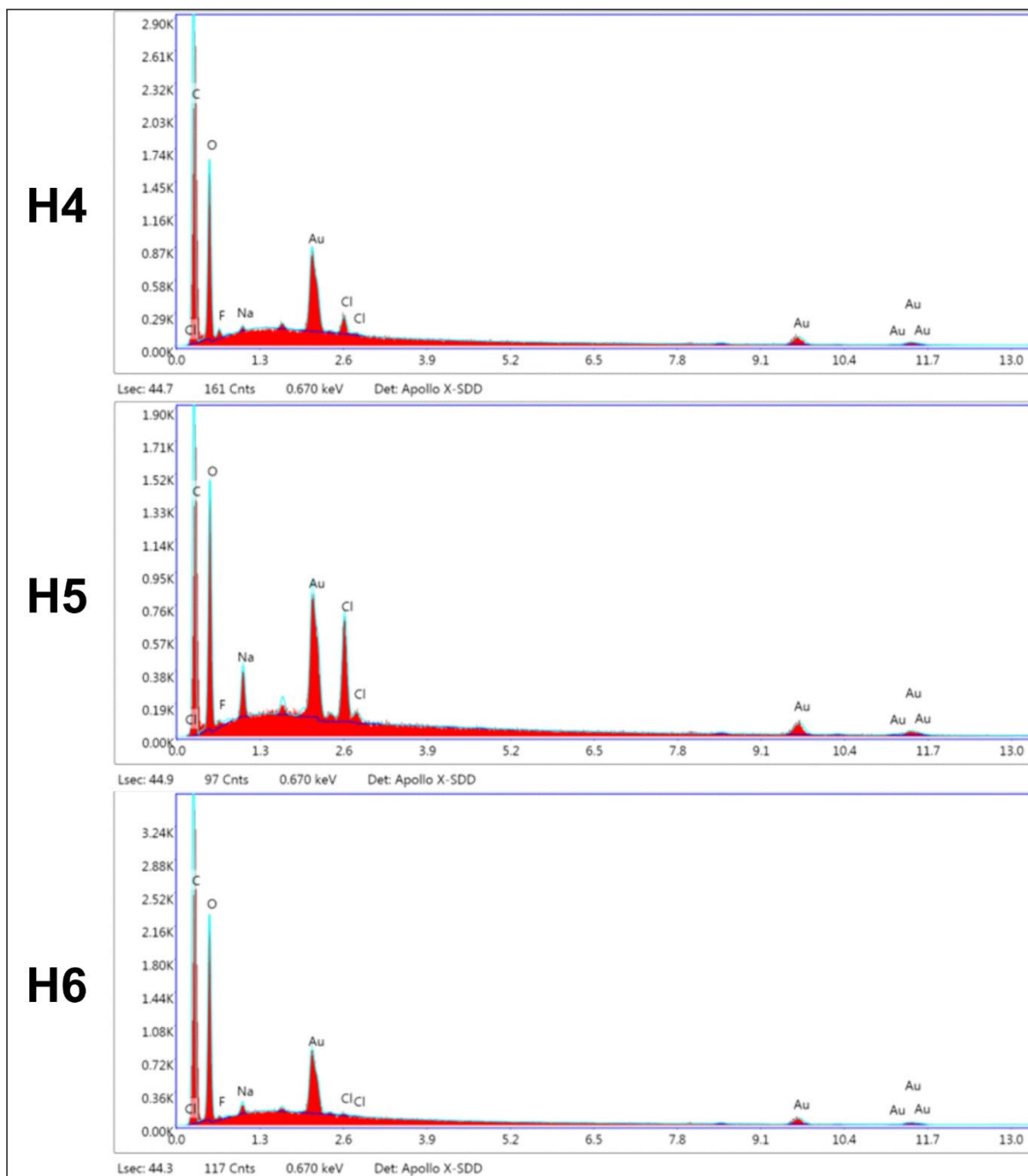


Figure 8. EDX spectra of H4-H6 of CLHs.

415
416
417
418
419
420
421
422
423
424

The results of EDX analysis (**Figure 8, Table 7**) of CLHs revealed that H6 sample was characterized by the slightly higher atomic content of fluorine ($1.33 \pm 0.05\%$). For H4 and H5 hydrogels atomic content of fluorine reached $0.44 \pm 0.03\%$ and $0.58 \pm 0.03\%$, respectively. Based on this analysis, we can assume, that slightly higher Cipro content was enclosed in H6 hydrogel. One-way ANOVA and Tukey test evaluated that all those values were statistically different ($\alpha = 0.05$).

425 **Table 7.** Weight and atomic content of elements in H4, H5 and H6 hydrogels.

426

Element	H4		H5		H6	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
C	53.95 ± 0.34	61.21 ± 0.34	53.92 ± 0,41	62.64 ± 0.50	56.87 ± 0.16	64.25 ± 0.19
O	44.24 ± 0.36	37.67 ± 0.33	38.22 ± 0.81	33.34 ± 0.69	39.83 ± 0.41	33.78 ± 0.33
F	0.61 ± 0.05	0.44 ± 0.03	0.79 ± 0.04	0.58 ± 0.03	1.86 ± 0.07	1.33 ± 0.05
Na	1.08 ± 0.01	0.64 ± 0.01	3.15 ± 0.07	1.91 ± 0.04	0.46 ± 0.13	0.27 ± 0.07
Cl	0.11 ± 0.07	0.04 ± 0.03	3.92 ± 0.31	1.54 ± 0.12	0.98 ± 0.06	0.38 ± 0.02

427

428

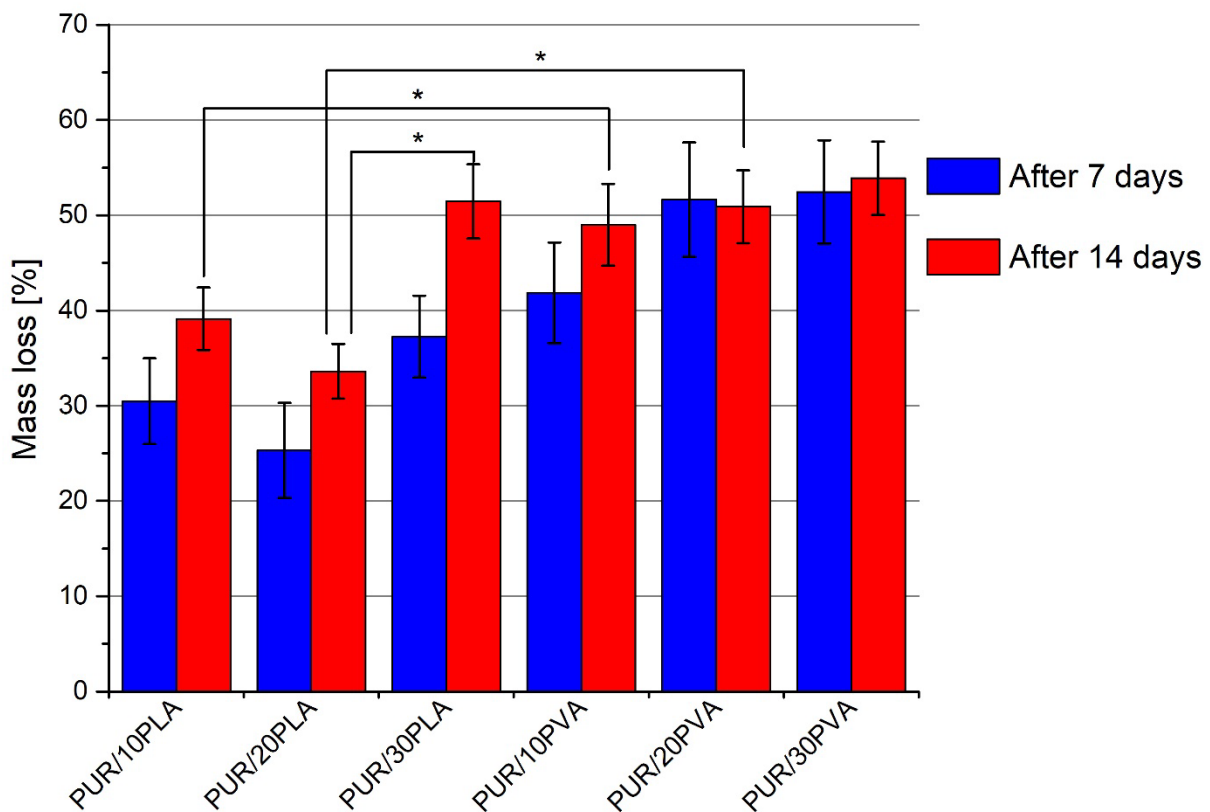
429 Growing trace of carbon in hydrogels was observed along with the growing PVA/borax ratio.
 430 For example, one-way ANOVA evaluation and Tukey test confirmed that for H4 sample
 431 (PVA/borax = 1:1 w/w) C atomic content was slightly lower ($61.21 \pm 0.34\%$) than for H6
 432 sample ($64.25 \pm 0.19\%$) with PVA/borax ratio equal 3:1 w/w ($\alpha = 0.05$). In the case of oxygen
 433 content, for H4, H5 and H6 values were close to be equal $37.67 \pm 0.33\%$, $33.34 \pm 0.69\%$ and
 434 $33.78 \pm 0.33\%$, respectively. One-way ANOVA and Tukey test evaluated that oxygen content
 435 for H4 was significantly higher than for H5 and H6, which were statistically the same ($\alpha =$
 436 0.05). Statistical analysis of H4, H5 and H6 samples confirmed the impact of the PVA/borax
 437 ratio on the carbon, oxygen and fluorine content in the tested hydrogels.

438 3.3. Hybrid Cipro-Loaded Wound Dressings (H-CLWDs) Characterization

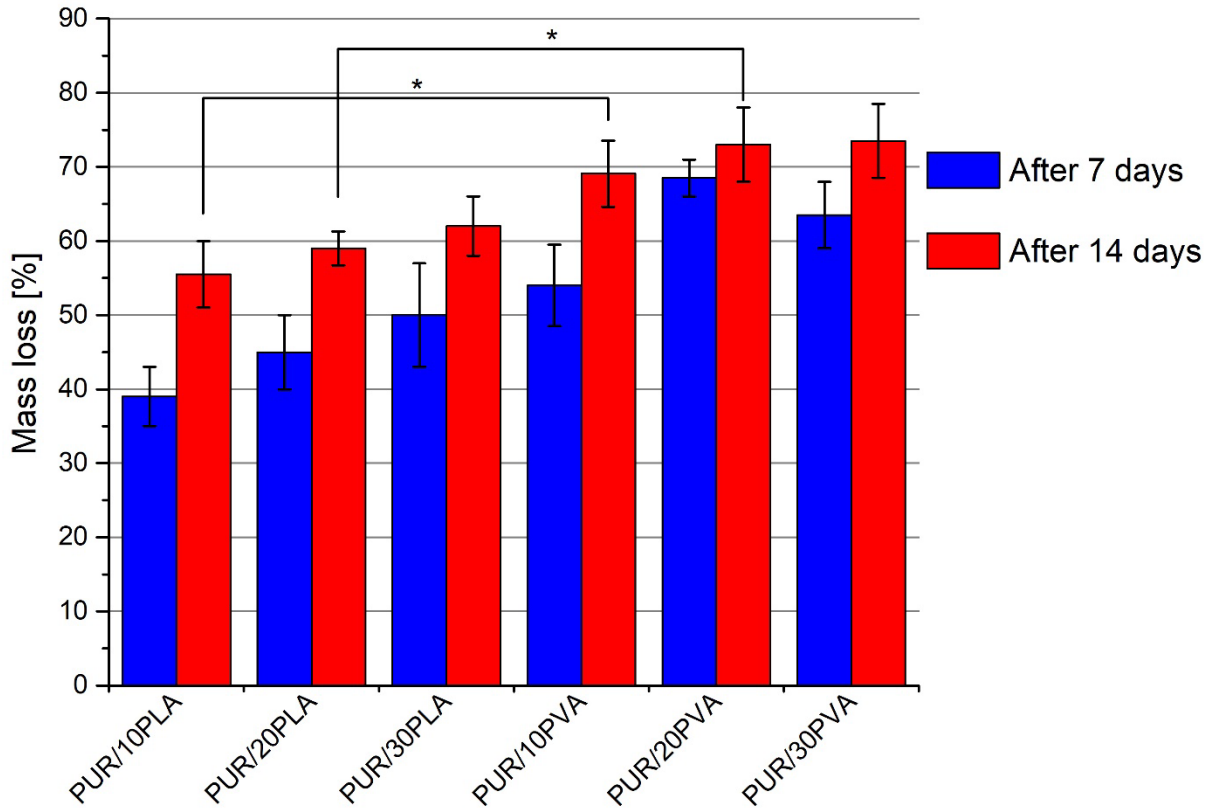
439 3.3.1. Short-term degradation (STD) study

440 **Figure 9** and **Figure 10** showed mass loss of obtained H-CLWDs, calculated after short-term
 441 degradation assessment in acidic (2M HCl) and alkali (5M NaOH) media respectively. Each
 442 type of biomaterial may be considered as possibly degradable in physiological conditions. It
 443 was observed that in the alkali environment all of the tested samples were characterized by the
 444 higher average mass loss than in the acidic medium. In the alkali environment degradation rates
 445 were approximately 13% and 19% higher than in the acidic conditions after 7th and 14th day
 446 respectively ($\alpha = 0.05$). For instance, after 14th day of degradation PUR/10PVA showed 49.58
 447 $\pm 4.29\%$ and $69.08 \pm 4.47\%$ mass loss in HCl and NaOH respectively. Moreover, two-way
 448 ANOVA examination revealed that, in both acidic and basic environment, the type of composite
 449 based on PLA or PVA had a strong impact on the mass loss results ($\alpha = 0.05$). Obtained results
 450 and two-way ANOVA test revealed, that for H-CLWD obtained by using PVA (PUR/10PVA,
 451 PUR/20PVA and PUR/30PVA), the mass loss was significantly higher than for H-CLWDs
 452 made by using PUR combined with PLA: PUR/10PLA, PUR/20PLA and PUR/30PLA ($\alpha =$

453 0.05) for both acidic and alkali media. For example, after 14th day of assessment in HCl, for
 454 PUR/20PLA mass loss reached $26.01 \pm 4.78\%$ and for PUR/20PVA it was equal to $50.89 \pm$
 455 6.03% . **Figure 11** shows mass loss of obtained H-CLWDs degradation assessment in solution
 456 mimicking physiological fluids (0.01M PBS). The study confirms findings from degradation in
 457 acid and alkali media. The highest degradation after 56 days was observed for PUR/30PVA
 458 ($24.55 \pm 1.88\%$) and the lowest for PUR/10PLA ($12.00 \pm 0.57\%$). Two-way ANOVA
 459 examination revealed that both the type of composite (PLA or PVA) and the concentration of
 460 additive (PLA or PVA) concentration had a strong impact on the mass loss results on 14th day
 461 and 56th day ($\alpha = 0.05$). On 28th day the impact of composite type was not statistically important.
 462



463 **Figure 9.** Average mass loss of H-CLWDs based on PUR and PLA composition in 2M HCl
 464 (* samples significantly different: $\alpha = 0.05$, $n=3$).
 465

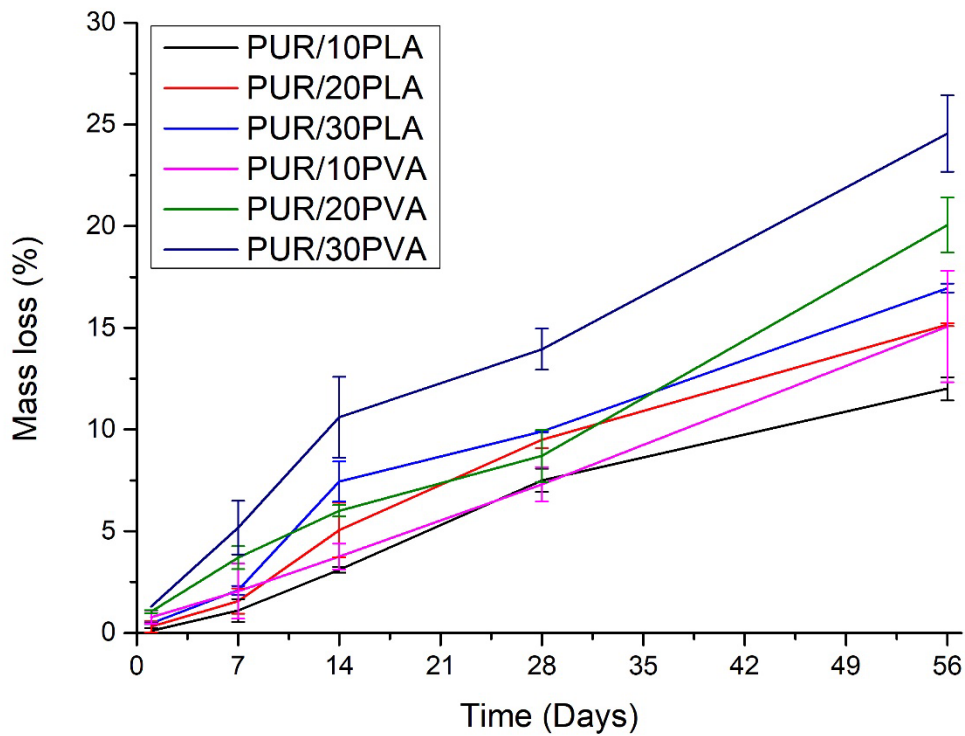


466

467 **Figure 10.** Average mass loss of H-CLWDs based on PUR/PLA or PUR/PVA composition in

468

5M NaOH (* samples significantly different: $\alpha = 0.05$, $n=3$).



469

470 **Figure 11.** Average mass loss of H-CLWDs based on PUR/PLA or PUR/PVA composition in
471 0.01M PBS.

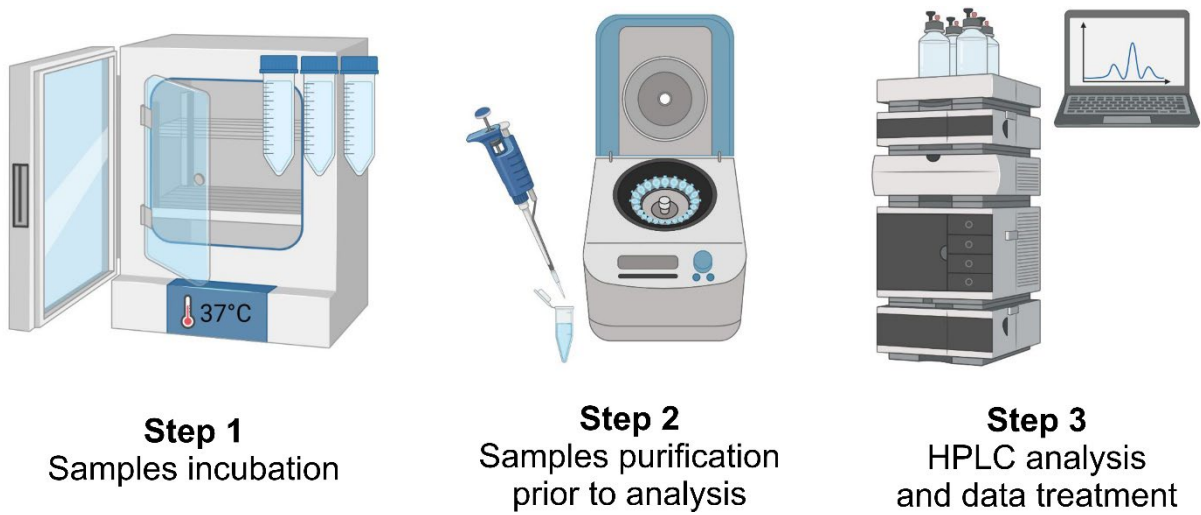
472
473

474 3.3.2. High Performance Liquid Chromatography

475 HPLC (**Fig. 12**) was used to determine the release of ciprofloxacin hydrochloride from obtained
476 composite dressings after short-term (5 and 15 minutes - **Figures 13-14**) and long-term (1 and
477 7 days – **Figures 15-16**) exposure to hydrolytic conditions. **Figure 13** showed the amount of
478 released Cipro, depending on the type of hydrogel and matrix used to obtain composite dressing.
479 These values were also converted into Cipro content per 1 μg of the dressing (**Figure 14**). It
480 was observed that all the produced dressings show the ability to release Cipro. According to
481 **Figure 13**, D6 was characterized by the highest Cipro release for both 5 and 15 minutes (18.7
482 $\pm 1.8 \mu\text{g/ml}$ and $21.76 \pm 0.64 \mu\text{g/ml}$, respectively) in comparison to the rest of tested materials.
483 It was also evaluated that for dressings PUR/30PVA: (D4, D5, D6) the concentrations of eluted
484 drugs were approximately 9% and 3% higher on average (for 5 and 15 min, respectively) than
485 for D1, D2, D3 matrices ($\alpha = 0.05$). Obtained results may be related to higher porosity of D4,
486 D5, D6 PUR/30PVA matrices. Additionally, it was observed that the type of used hydrogel also
487 had strong impact on Cipro releasing process. For example, after 15 min of releasing for D1,
488 D2 and D3 patches (**Figure 13**) containing PUR/30PLA porous matrices, the quantity of Cipro
489 increased with the growing proportion of PVA and borax solutions (from $13.0 \pm 2.2 \mu\text{g/ml}$ for
490 D1 to $18.1 \pm 1.3 \mu\text{g/ml}$ for D3). The same situation can be observed for D4, D5 and D6 patches
491 including PUR/30PVA matrices. For D4 with PVA/borax solutions ratio equal to 1:1 w/w,
492 amount of released Cipro after 15 min ranged $17.2 \pm 1.5 \mu\text{g/ml}$ and for D6 with PVA/borax
493 solutions ratio it was equal $21.76 \pm 0.64 \mu\text{g/ml}$. **Figures 15-16** showed results of prolonged
494 release studies for D6 composite dressing. Performed one-way ANOVA test proved that
495 released drug values were not statistically different after 15 min of test ($\alpha = 0.05$).

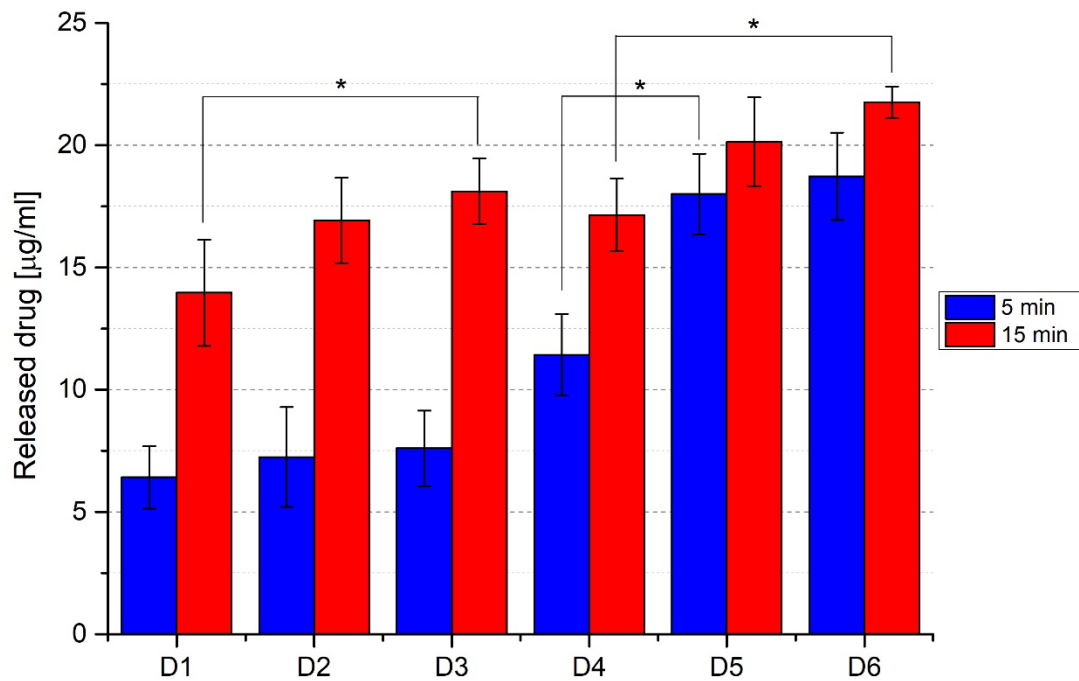
496





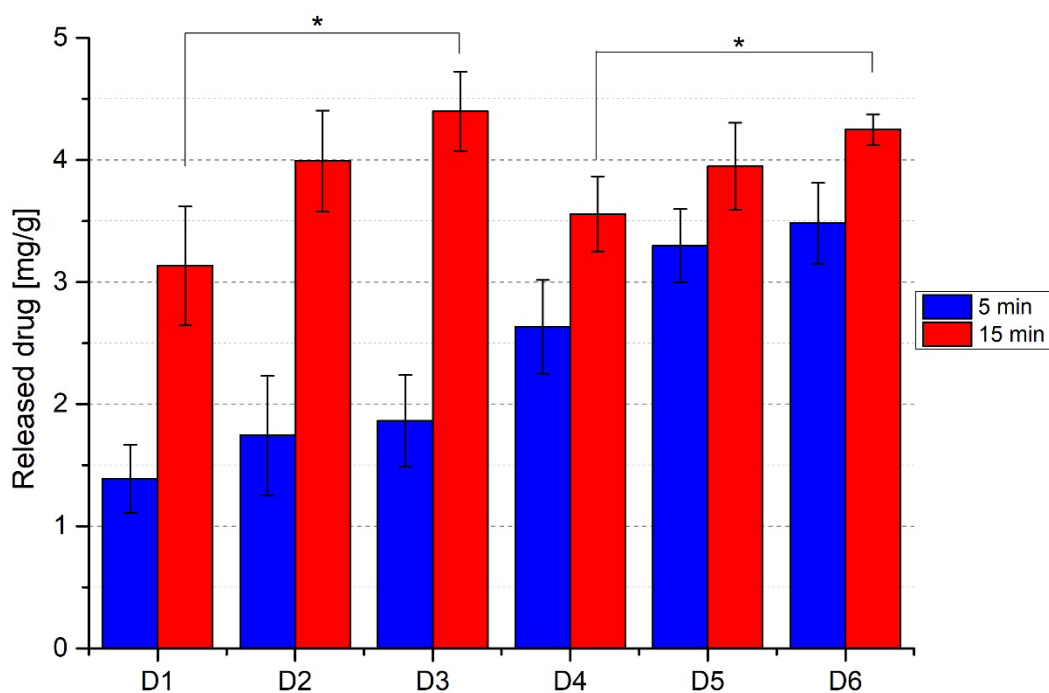
497
498
499

Figure 12. Scheme of sample preparation for determination of Cipro released in time.

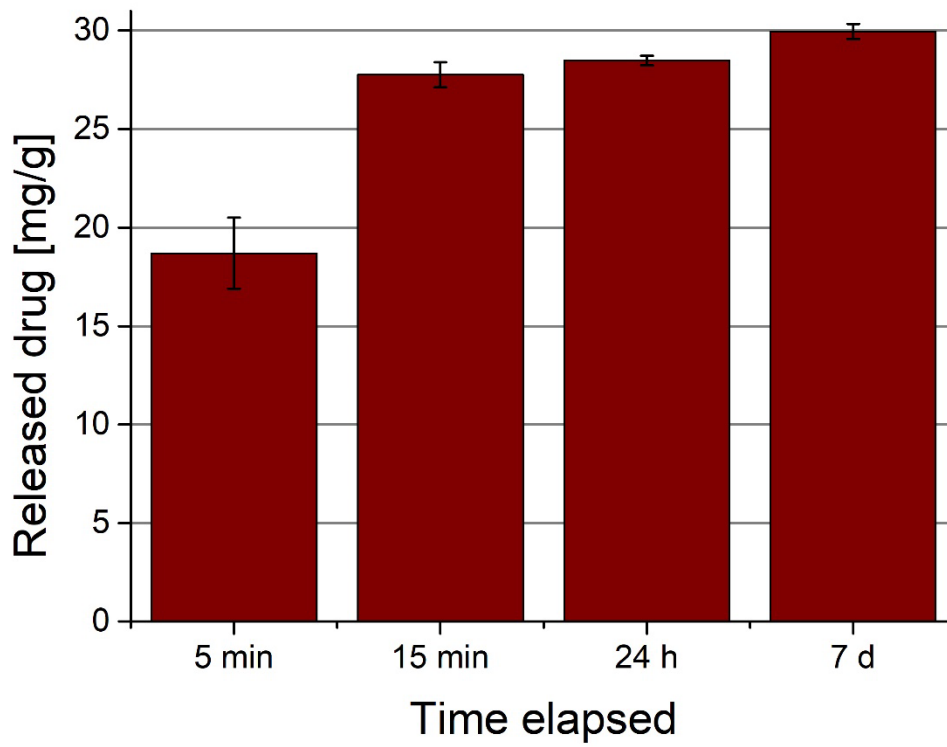


500
501
502
503

Figure 13. Concentration of released Cipro after 5 and 15 min for D1, D2, D3, D4, D5 and D6 composite dressings (* samples significantly different: $\alpha = 0.05$, $n=3$).



504
 505 **Figure 14.** Amount of released Cipro after 5 and 15 min for D1, D2, D3, D4, D5 and D6
 506 composite dressings per 1 mg of dressing (* samples significantly different: $\alpha = 0.05$, $n=3$).
 507



508

509

Figure 15. Amount of released Cipro after 5 and 15 min, 24 h and 7 d for D6 composite dressing.

510

511

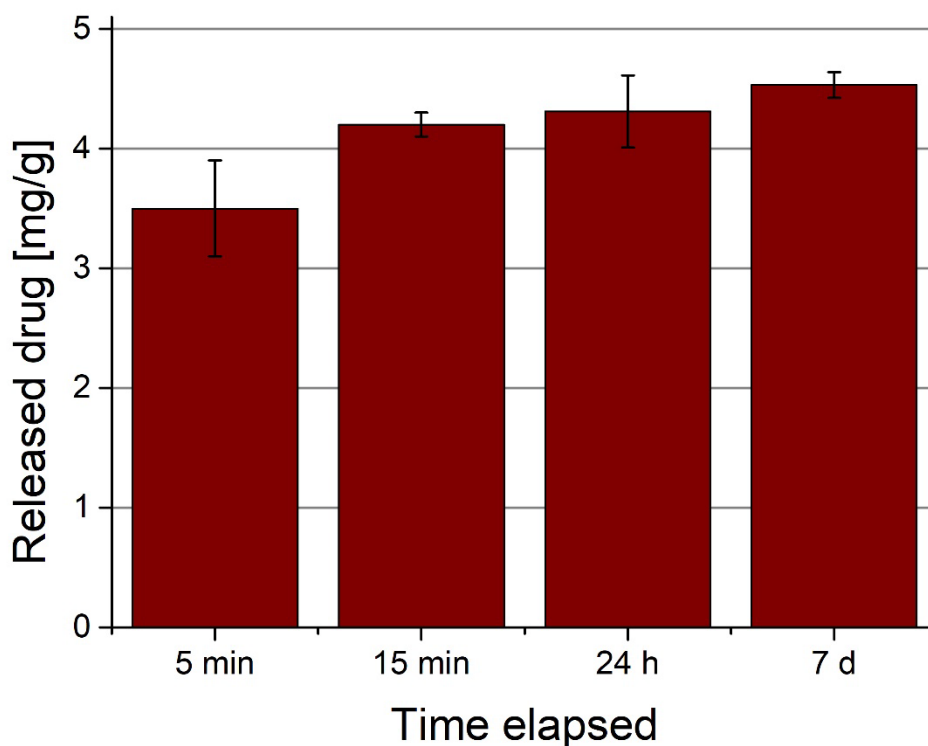


Figure 16. Amount of released Cipro after 5 and 15 min, 24 h and 7 d for D6 composite dressing per 1 mg of dressing.

3.4. Biological performance of H-CRWDs

3.4.1. Microbiology tests

Performed microbiology tests showed that both types of obtained H-CRWDs based on PLA and PVA showed significant bacterial growth inhibition zones (**Figure 17**). This clearly confirmed that Cipro introduced to such kind of hybrid dressing revealed antibacterial activity against *S. aureus*.



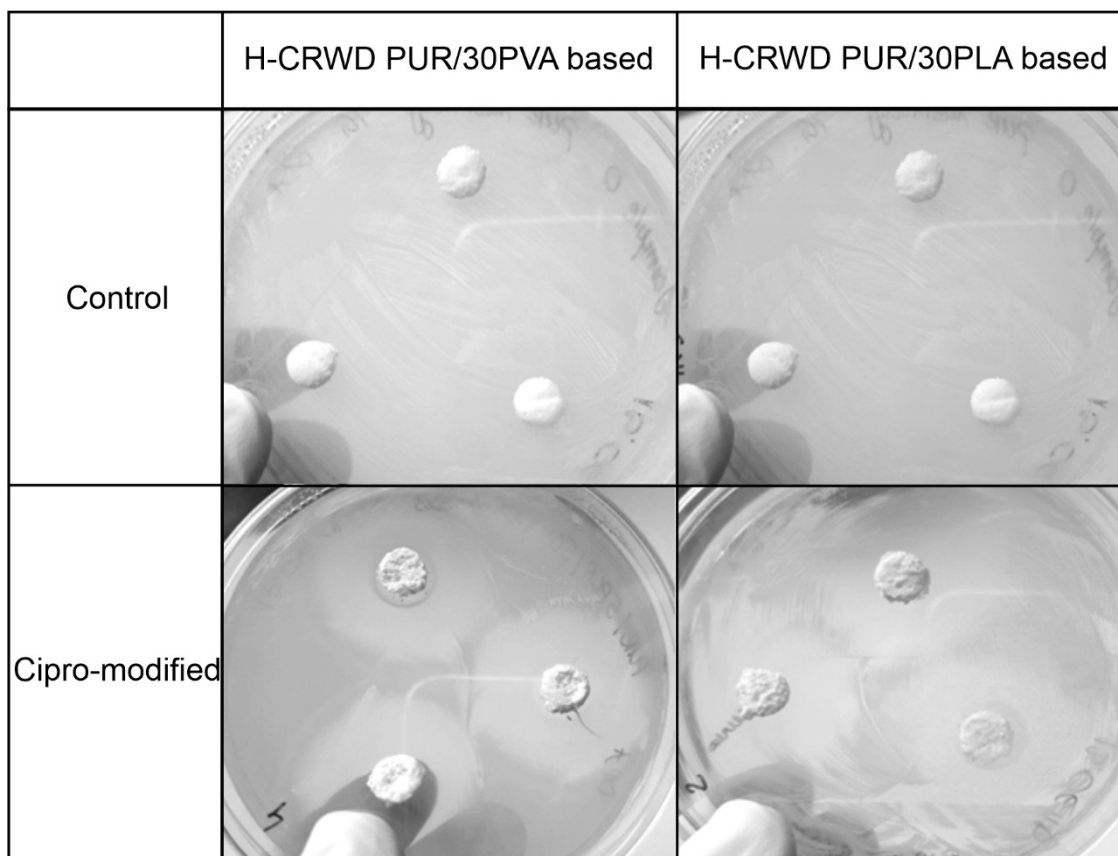
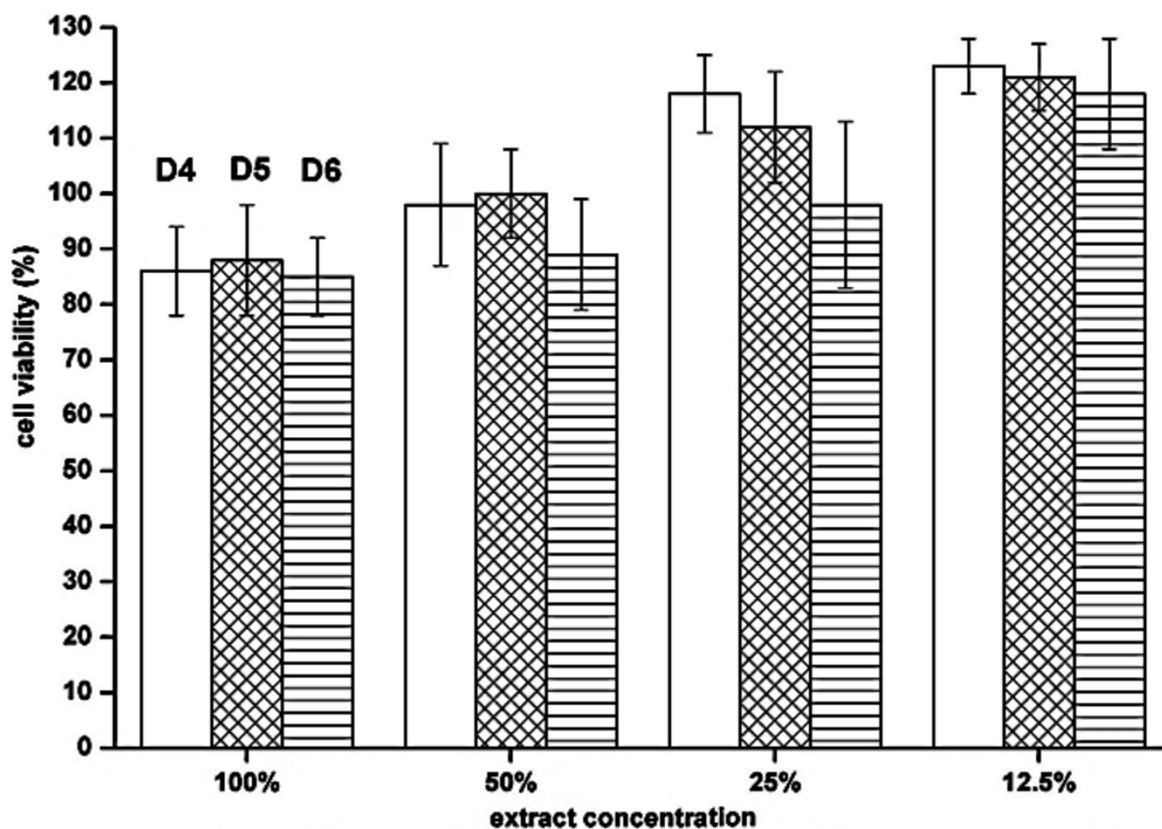


Figure 17. Inhibition zones of *S. aureus* growth observed for H-CRWD in comparison to Control (hybrid wound dressing not loaded with Cipro).

3.4.2. Cytotoxicity Assay

Analysis of **Figure 18** showed that obtained H-CRWDs with the use of PUR/30PVA were representing satisfactory biocompatibility levels at variety of extract concentrations. It could be seen that at 100% extract concentration the cell viability was lower in comparison to other concentrations (50%-12.5%). We assume few causes for this effect. First of them could be the fact, that obtained materials, despite fabrication methods using a lot of flushing, may need development of additional purification methods. Additionally, LAA used for CLHs modification may still be present in the H-CRWDs structure and impact cells growth. Despite this nuisance, obtained materials were considered as biocompatible according to ISO 109993-5 standard. Optical microscope images (**Figure 19**) confirmed this fact. Obtained H-CRWDs by using PUR/30PVA allowed morphology maintenance of CC1163 cell line. Presence of 1.5 wt% of Cipro in this systems was not disturbing neither cell growth nor their morphology.



540

541

Figure 18. Cell viability of CCI163 cells depending on extract concentration.

Sample/ Extract Concentration	0%	100%	75%	50%	12.5%
D4					
D5					
D6					

542

543

Figure 19. Morphology of CCI163 cells after 24h contact with extracts at different concentrations.

544

545

546

547

4. Discussion

548 Skin is one of the most important organs of human body and development of its functional
549 substitutes are one of the highly demanded products, because chronic wounds treatment like
550 burns, ulcers or infected wounds belongs to one of the biggest challenges of XXIst century. In
551 the case of small, uninfected wounds no medical intervention is necessary due to the high ability
552 of the skin to regenerate. For chronic wounds and large burns the process of natural regeneration
553 is much more complicated, often impossible or lasts long. One of the modern solutions for
554 problems associated with healing of skin injuries is the application of different types of wound
555 dressings selected for specific types of wounds. In this paper we proposed as a promising
556 solution a H-CRWD composed of CPMs based on PUR/30PVA and CLHs releasing Cipro in
557 vitro.

558 The solid porous matrices of CPMs were based either on PUR/PLA composition either
559 PUR/PVA composition (10-30 wt%). Average porosity of CPMs was in the range of 69-81%
560 and the highest porosity values were observed for composites containing of 30 wt% either PUR
561 either PLA. On this basis CPMs consisting of PUR/30PLA or PUR/30PVA were selected for
562 further fabrication of H-CRWDs. What is worth to mention is the fact that CPMs composed of
563 PUR/PVA had of 5% higher porosity than those composed of PUR/PLA. Type of composition
564 had an effect on CPMs porosity. The higher porosity the better scaffold availability for cells to
565 diffuse through the scaffold [38] and controlled degradation [39,40] due to the equal medium
566 flow through the scaffold. Based on porosity CPMs obtained with the use of PUR/PVA
567 composition seemed to be more favorable for skin engineering at this stage of studies.

568 Pore sizes of CPMs obtained by using PUR/PLA were in the range from 123-68 μm and pore
569 sizes of PUR/10PLA were statistically higher than other two described compositions. In case
570 of PUR/PVA compositions pore sizes were in the range from 88-103 μm and were not
571 statistically different for described compositions. In literature Chitrattha et al. [41] obtained
572 composite porous dressings consisted of PLA and PEG loaded with gentamicin sulphate (GS)
573 or metronidazole (MZ). Their porosities differed from $53 \pm 2\%$ to $55 \pm 2\%$ and they were
574 characterized by high antimicrobial activity against e.g. *S. aureus*. O'Brien et al. evaluated that
575 the suitable pore size for skin tissue regeneration was in the range of 20 – 120 μm . The pore
576 size of obtained CPMs was similar, thus optimal for cell migration, adhesion and proliferation
577 favouring with skin regeneration [42,43].

578 Obtained hydrogels were studied by FTIR spectroscopy. FTIR spectra analysis confirmed
579 presence of Cipro in obtained CLHs. Bands observed between 1674 and 1627 cm^{-1} were
580 assigned as bending vibration of N-H related to the quinolines, what found a confirmation in
581 other literature data [37]. Slight spectra shift was observed when ratio of PVA:Borax was

582 changed from 1:1 to 3:1 in obtained hydrogels, but it did not influence chemical structure
583 enough to impact hydrogels behavior within further characterization studies.

584 SEM analysis of UnMHs showed craterous-like surface for H4 hydrogel, which was not noted
585 for other types of UnMHs or CLHs. EDX results were another asset confirming presence of
586 fluorine and chlorine in obtained CLHs. Due to lack of proof in literature, this surface defects
587 can be treated as anomaly.

588 Short-term degradation studies determined that H-CRWDs were degrading faster in basic and
589 acidic media if CPMs were obtained with the use of PUR/PVA composition in comparison to
590 these CPMs obtained by using PUR/PLA composition. It could be caused by ease of hydrolysis
591 process observed for PVA compared to PLA, due to the fact that PVA is a polymer soluble in
592 water [44,45]. In this case, PVA occurred to act as a more favorable “fast-degradable polymer”,
593 what would be an asset in skin engineering expecting tissue scaffold to degrade within a suitable
594 time frame. Those results were confirmed with degradation studies in physiological fluid-like
595 medium (0.01 M PBS).

596 Studied drug release of H-CRWD based on PUR/30PVA composition revealed that the
597 concentration of eluted Cipro was approximately 9% and 3% higher in average (after 5 and 15
598 min, respectively) than for H-CRWD based on PUR/30PLA composition. Prolongated studies
599 on D6 sample showed that amount of released drug was not changed after 15 min of the test. It
600 could be caused by difficulties of active substance transport from inner parts of the sample. It
601 is worth noting that swelling is related to drug release, and swelling is mainly dependent from
602 area of contact between hydrogel and medium [46]. The active substance release can be then
603 lower in H-CRWD than it would be expected from hydrogel, mostly because presence of
604 polymer pores, which lowers the contact area and swelling ability.

605 Microbiology testing was another experiment, which confirmed successful Cipro-release of H-
606 CRWDs. Bacterial growth inhibition zones were observed for both types of CPMs, but in case
607 of CPMs obtained with the use of PUR/PVA compositions the growth inhibition zones (Fig.
608 14) were better distinguished in comparison to CPMs obtained with the use of PUR/PLA
609 composition. It was related to drug released studies, because it was found out that PUR/PVA
610 were releasing more of Cipro. Literature also proves presence of inhibition zones for Cipro-
611 modified hydrogels [33].

612 Performed MTT assay, according to ISO 10993-5 standard, proved that obtained H-CRWDs
613 based on PUR/30PVA were biocompatible (over 85% of cells viability at 100% of extract
614 concentration) and did not disturb CCl163 cell morphology. It was also shown that presence of
615 Cipro in the systems was not disturbing neither cell growth nor their morphology. Literature

616 presents both the significant reduction in cells viability [47] and biocompatibility of Cipro-
617 loaded hydrogels [48]. Probably the Cipro acts differently with different cell assays, but in
618 presented study, the MTT assay was performed accordingly to ISO 10993-5 standard and
619 suitable assay.

620 Summarizing all the results, obtained H-CRWDs presented suitable characteristics to be
621 considered as potential skin wound dressings. The best results were obtained for H6 prototypes
622 produced using 30 wt% of PVA (PUR/30PVA) and a hydrogel made of 4% PVA, 1.5% Cipro
623 and 10% AA solution and mixed with a borax solution in ratio 3:1 (w/w) (H6).

624

625 5. Conclusions

626 In this scientific paper we proposed a promising solution of H-CRWDs composed of CPM
627 based on PUR/PVA and PUR/PLA mixes and CLHs releasing Cipro *in vitro* (21.76 ± 0.64
628 $\mu\text{g/ml}$ after 15 min). Simple and cost-effective methods were used to obtain H-CRWDs
629 components; CMPs were obtained by SC/PL and CLH simply by mixing PVA with Borax at
630 different ratios. Performed physicochemical studies, morphology analysis, drug-release studies,
631 short term degradation studies and biological performance verification lead to the conclusion
632 that only one system was favorable for application as skin wound dressing and it was H-CRWD
633 based on PUR/30PVA composition and hydrogel made of 4% PVA, 1.5% Cipro and 10% AA
634 and mixed with a borax solution in ratio 3:1 (w/w). This system showed the highest degradation
635 (up to 53% mass loss in 2M HCl, 72% in 5M NaOH after 14 days and up to 25% mass loss in
636 0.01M PBS after 56 days), and highest Cipro *in vitro* release (up to 4 mg of Cipro per 1 g of
637 wound dressing after 15 minutes). Cipro-loaded system showed significant inhibition zones
638 against *S. aureus*. Presented wound dressing prototypes showed also biocompatibility
639 accordingly to ISO 10993-5 standard (over 85% cell viability at 100% extract). With the use of
640 this material we will continue to design and develop new solutions to provide easy to do and
641 affordable solutions to treat chronic wounds of high potential to become infected wounds, thus
642 antibacterial effect is in great demand.

643

644 Data Availability

645 The raw/processed data required to reproduce these findings cannot be shared at this time as
646 the data also forms part of an ongoing study.

647

648 References

- 650 [1] Granick MS, Sood A, Tomaselli NL. Wound Dressings and Comparative Effectiveness
651 Data 2014;3:511–29. <https://doi.org/10.1089/wound.2012.0401>.
- 652 [2] Nosrati H, Aramideh Khouy R, Nosrati A, Khodaei M, Banitalebi-Dehkordi M, Ashrafi-
653 Dehkordi K, et al. Nanocomposite scaffolds for accelerating chronic wound healing by
654 enhancing angiogenesis. *J Nanobiotechnology* 2021;19:1–22.
655 <https://doi.org/10.1186/s12951-020-00755-7>.
- 656 [3] Sadeghzade S, Emadi R, Labbaf S. Hardystonite-diopside nanocomposite scaffolds for
657 bone tissue engineering applications. *Mater Chem Phys* 2017;202:95–103.
658 <https://doi.org/10.1016/j.matchemphys.2017.09.018>.
- 659 [4] Liu H, Wang C, Li C. RSC Advances dressing and drug delivery system in the treatment
660 of wound healing. *RSC Adv* 2018;8:7533–49. <https://doi.org/10.1039/C7RA13510F>.
- 661 [5] Kopecki Z. Development of next-generation antimicrobial hydrogel dressing to combat
662 burn wound infection. *Biosci Rep* 2021. <https://doi.org/10.1042/BSR20203404>.
- 663 [6] Saghazadeh S, Rinoldi C, Schot M, Saheb S, Shari F, Jalilian E, et al. Drug delivery
664 systems and materials for wound healing applications ☆ 2018;127:138–66.
665 <https://doi.org/10.1016/j.addr.2018.04.008>.
- 666 [7] Thaarup IC, Bjarnsholt T. Current in vitro biofilm-infected chronic wound models for
667 developing new treatment possibilities. *Adv Wound Care* 2021;10:91–102.
668 <https://doi.org/10.1089/wound.2020.1176>.
- 669 [8] Xu Z, Han S, Gu Z, Wu J. Advances and Impact of Antioxidant Hydrogel in Chronic
670 Wound Healing. *Adv Healthc Mater* 2020;9:1901502.
671 <https://doi.org/10.1002/adhm.201901502>.
- 672 [9] Borda LJ, Macquhae FE, Kirsner RS. Wound Dressings : A Comprehensive Review.
673 *Curr Dermatol Rep* 2016. <https://doi.org/10.1007/s13671-016-0162-5>.
- 674 [10] Vilar G, Tulla-puche J, Albericio F. *Polymers and Drug Delivery Systems* 2018:1–28.
- 675 [11] Zhang X, Shu W, Yu Q, Qu W, Wang Y, Li R. Functional Biomaterials for Treatment
676 of Chronic Wound. *Front Bioeng Biotechnol* 2020;8:516.
677 <https://doi.org/10.3389/fbioe.2020.00516>.
- 678 [12] Koehler J, Brandl FP, Goepferich AM. Hydrogel Wound Dressings for Bioactive
679 Treatment of Acute and Chronic. *Eur Polym J* 2017.
680 <https://doi.org/10.1016/j.eurpolymj.2017.12.046>.
- 681 [13] Unnithan AR, Barakat NAM, Pichiah PBT, Gnanasekaran G, Nirmala R, Cha Y, et al.
682 Wound-dressing materials with antibacterial activity from electrospun polyurethane –



- 683 dextran nanofiber mats containing ciprofloxacin HCl. *Carbohydr Polym* 2012;90:1786–
684 93. <https://doi.org/10.1016/j.carbpol.2012.07.071>.
- 685 [14] Vermeulen H, Ubbink DT, Goossens A, Vos R De, Legemate DA. Systematic review of
686 dressings and topical agents for surgical wounds healing by secondary intention
687 2005:665–72. <https://doi.org/10.1002/bjs.5055>.
- 688 [15] Mirafteb M, Collyer G. A critical review of modern and emerging absorbent dressings
689 used to treat exuding wounds 2012:1–12. [https://doi.org/10.1111/j.1742-
690 481X.2011.00923.x](https://doi.org/10.1111/j.1742-481X.2011.00923.x).
- 691 [16] Chattopadhyay S, Raines RT. Review Collagen-Based Biomaterials for Wound Healing
692 2014;101. <https://doi.org/10.1002/bip.22486>.
- 693 [17] Yao C, Lee C, Huang C, Chen K. Novel bilayer wound dressing based on electrospun
694 gelatin/keratin nanofibrous mats for skin wound repair. *Mater Sci Eng C* 2017.
695 <https://doi.org/10.1016/j.msec.2017.05.076>.
- 696 [18] Alarcon EI, Baker AB. Biomaterials and Nanotherapeutics for enhancing Skin wound
697 Healing 2016;4:1–20. <https://doi.org/10.3389/fbioe.2016.00082>.
- 698 [19] Pott FS, Meier MJ, Ribas JD. La efectividad del hidrocoloide versus otros apósitos en la
699 cicatrización de úlceras por presión en adultos y ancianos: revisión sistemática y
700 metanálisis 1 Introducción Método 2014;22:511–20. [https://doi.org/10.1590/0104-
701 1169.3480.2445](https://doi.org/10.1590/0104-1169.3480.2445).
- 702 [20] K PS, Babu M. Collagen based dressings Ð a review 2000;26:54–62.
- 703 [21] Queen D, Orsted H, Sanada H, Sussman G. A dressing history 2004;1.
- 704 [22] Ono S, Imai R, Ida Y, Shibata D, Komiya T, Matsumura H. ScienceDirect Increased
705 wound pH as an indicator of local wound infection in second degree burns. *Burns*
706 2014;41:820–4. <https://doi.org/10.1016/j.burns.2014.10.023>.
- 707 [23] Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound Healing Dressings and
708 Drug Delivery Systems: A Review. *J Pharm Sci* 2008;97:2892–923.
709 <https://doi.org/10.1002/jps.21210>.
- 710 [24] Leaper DJ, Schultz G, Carville K, Fletcher J, Swanson T, Drake R. Extending the TIME
711 concept : what have we learned in the past 10 years ?* 2012.
- 712 [25] Gogolewski S, Gorna K, Zaczynska E, Czarny A. Structure-property relations and
713 cytotoxicity of isosorbide-based biodegradable polyurethane scaffolds for tissue repair
714 and regeneration. *J Biomed Mater Res - Part A* 2008;85:456–65.
715 <https://doi.org/10.1002/jbm.a.31481>.
- 716 [26] Grzesiak J, Marycz K, Szarek D, Bednarz P, Laska J. Polyurethane/poly lactide-based



- 717 biomaterials combined with rat olfactory bulb-derived glial cells and adipose-derived
718 mesenchymal stromal cells for neural regenerative medicine applications. *Mater Sci Eng*
719 *C* 2015;52:163–70. <https://doi.org/10.1016/j.msec.2015.03.050>.
- 720 [27] Gubanska I, Kucinska-Lipka J, Janik H. The influence of amorphous macrodiol,
721 diisocyanate type and L-ascorbic acid modifier on chemical structure, morphology and
722 degradation behavior of polyurethanes for tissue scaffolds fabrication. *Polym Degrad*
723 *Stab* 2019;163:52–67. <https://doi.org/10.1016/j.polymdegradstab.2019.02.025>.
- 724 [28] Chen H, Xing X, Tan H, Jia Y, Zhou T, Chen Y, et al. Covalently antibacterial alginate-
725 chitosan hydrogel dressing integrated gelatin microspheres containing tetracycline
726 hydrochloride for wound healing. *Mater Sci Eng C* 2017;70:287–95.
727 <https://doi.org/10.1016/j.msec.2016.08.086>.
- 728 [29] Bergman B, Bishop MC, Bjerklund-johansen TE, Botto H, Lobel B, Cruz FJ, et al. EAU
729 Guidelines for the Management of Urinary and Male Genital Tract 2001:576–88.
- 730 [30] Sripriya R, Kumar MS. *Journal of Biomaterials Science*, Collagen bilayer dressing with
731 ciprofloxacin, an effective system for infected wound healing 2012:37–41.
732 <https://doi.org/10.1163/156856207779996913>.
- 733 [31] Choipang C, Chuysinuan P, Suwantong O, Ekabutr P, Supaphol P. *AC. J Drug Deliv Sci*
734 *Technol* 2018. <https://doi.org/10.1016/j.jddst.2018.06.025>.
- 735 [32] Kucińska-Lipka J. Polyurethanes crosslinked with poly(vinyl alcohol) as a slowly-
736 degradable and hydrophilic materials of potential use in regenerative medicine. *Materials*
737 (Basel) 2018;11. <https://doi.org/10.3390/ma11030352>.
- 738 [33] Iga C, Agata T, Marcin Ł, Natalia F, Justyna KL. Ciprofloxacin-modified degradable
739 hybrid polyurethane-poly lactide porous scaffolds developed for potential use as an
740 antibacterial scaffold for regeneration of skin. *Polymers (Basel)* 2020;12:171.
741 <https://doi.org/10.3390/polym12010171>.
- 742 [34] Devi S.A., Philip D. AG. Infrared, Polarized Raman, and SERS Spectra of Borax. *J Solid*
743 *State Chem* 1994;113:157–62.
- 744 [35] Brady J., Dürig T., Lee P.I. LJX. Chapter 7 - Polymer Properties and Characterization,
745 Developing Solid Oral Dosage Forms (Second Edition), *Pharmaceutical Theory and*
746 *Practice*. 2017.
- 747 [36] Marin E, Rojas J, Ciro Y. A review of polyvinyl alcohol derivatives: Promising materials
748 for pharmaceutical and biomedical applications. *African J Pharm Pharmacol*
749 2014;8:674–84. <https://doi.org/10.5897/AJPP2013.3906>.
- 750 [37] Sahoo S, Chakraborti C, Mishra S. Qualitative analysis of controlled release

- 751 ciprofloxacin/carbopol 934 mucoadhesive suspension. *J Adv Pharm Technol Res*
752 2011;2:195. <https://doi.org/10.4103/2231-4040.85541>.
- 753 [38] Kucińska-Lipka J, Gubanska I, Skwarska A. Microporous polyurethane thin layer as a
754 promising scaffold for tissue engineering. *Polymers (Basel)* 2017;9:1–16.
755 <https://doi.org/10.1023/B:WATE.0000026525.82039.ef>.
- 756 [39] Ballarin FM, Caracciolo PC, Blotta E, Ballarin VL, Abraham GA. Optimization of poly
757 (L -lactic acid)/ segmented polyurethane electrospinning process for the production of
758 bilayered small-diameter nano fibrous tubular structures. *Mater Sci Eng C* 2014;42:489–
759 99. <https://doi.org/10.1016/j.msec.2014.05.074>.
- 760 [40] Montini-Ballarin F, Caracciolo PC, Rivero G, Abraham GA. In vitro degradation of
761 electrospun poly(l-lactic acid)/segmented poly(ester urethane) blends. *Polym Degrad*
762 *Stab* 2016;126:159–69. <https://doi.org/10.1016/j.polymdegradstab.2016.02.007>.
- 763 [41] Chitrattha S, Phaechamud T. Porous poly (DL -lactic acid) matrix film with
764 antimicrobial activities for wound dressing application. *Mater Sci Eng C* 2016;58:1122–
765 30. <https://doi.org/10.1016/j.msec.2015.09.083>.
- 766 [42] Brien FJO, Harley BA, Yannas I V, Gibson LJ. The effect of pore size on cell adhesion
767 in collagen-GAG scaffolds 2005;26:433–41.
768 <https://doi.org/10.1016/j.biomaterials.2004.02.052>.
- 769 [43] Pietramaggiore G, Erba P. Foam Pore Size Is a Critical Interface Parameter n.d.:589–97.
770 <https://doi.org/10.1097/PRS.0b013e3182402c89>.
- 771 [44] Pal K, Banthia AK, Majumdar DK. Polyvinyl alcohol-gelatin patches of salicylic acid:
772 Preparation, characterization and drug release studies. *J Biomater Appl* 2006;21:75–91.
773 <https://doi.org/10.1177/0885328206056312>.
- 774 [45] Goyanes A, Kobayashi M, Martínez-Pacheco R, Gaisford S, Basit AW. Fused-filament
775 3D printing of drug products: Microstructure analysis and drug release characteristics of
776 PVA-based caplets. *Int J Pharm* 2016;514:290–5.
777 <https://doi.org/10.1016/j.ijpharm.2016.06.021>.
- 778 [46] Bouklas N, Huang R. Swelling kinetics of polymer gels: comparison of linear and
779 nonlinear theories. *Soft Matter* 2012;8:8194. <https://doi.org/10.1039/c2sm25467k>.
- 780 [47] Du J, El-Sherbiny IM, Smyth HD. Swellable Ciprofloxacin-Loaded Nano-in-Micro
781 Hydrogel Particles for Local Lung Drug Delivery. *AAPS PharmSciTech* 2014;15:1535–
782 44. <https://doi.org/10.1208/s12249-014-0176-x>.
- 783 [48] Bruk LA, Dunkelberger KE, Khampang P, Hong W, Sadagopan S, Alper CM, et al.
784 Controlled release of ciprofloxacin and ceftriaxone from a single otological

785 administration of antibiotic-loaded polymer microspheres and thermoresponsive gel.
786 PLoS One 2020;15:e0240535. <https://doi.org/10.1371/journal.pone.0240535>.
787