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Preparation and Characterization of Novel Amidated Pectin-Gelatin-Oxidized Tannic Acid Hydrogel Films Supplemented with In-Situ Reduced Silver Nanoparticles for Wound-Dressing Applications

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Abstract

Wound dressings play a pivotal role in shielding injured tissues and facilitating the healing process. However, fabrication of antibacterial wound dressing often entails intricate procedures and incorporates toxic constituents. In this study, we developed a novel hydrogel film (AP:GE@OTA/Ag) consisting of amidated pectin (AP), gelatin (GE), oxidized tannic acid (OTA) in different contents (from 0 to 5% wt.%), and in-situ formed silver nanoparticles (AgNPs). Our approach, grounded in an economically feasible method, harnesses the potential of a biocompatible polysaccharide to promoting wound healing. The FTIR and XRD patterns showed that the crosslinking process is the result of interactions between OTA quinone groups and the available free amino groups in AP and GE. SEM images showed homogenous and interconnected structures characterized by sought dispersion of the AgNPs on the surface. Furthermore, TG measurements revealed a good improvement of the AP:GE@OTA/Ag thermal stability compared to the AP:GE film. The fabricated AP:GE@OTA/Ag films exhibited not only improved fluid uptake ability (90.96% at 2h), water retention capacity (91.69% at 2h), water vapor transmission rate (1903.29 g/m²/day), but also demonstrated enhanced tensile strength (38 MPa). Moreover, they displayed cytocompatibility and sustained potent antimicrobial activity against both *S. aureus* and *E. coli* at remarkably low AgNPs loadings of $1.02 \pm 0.13 \mu\text{g}/\text{cm}^2$. Furthermore, NIT-1 mouse insulinoma cells exhibited robust proliferation when cultured with the prepared dressings. These composite structures significantly accelerated wound repair in a skin excision model, suggesting their potential clinical applicability for hemostasis and wound healing.

Keywords: Amidated pectin; wound dressing; oxidized tannic acid; covalent crosslinking; silver nanoparticles.

41 **1. Introduction**

42 Nowadays, the scope of wound dressings has transcended their conventional role of simply
43 protecting wounds. Incorporating diverse functional elements into dressings enhances the
44 wound-healing process and enables monitoring of biological parameters at the wound site [1].
45 Developing biocompatible and biodegradable films with sufficient physicochemical
46 characteristics, mechanical properties and excellent retention capacity of physiological fluids
47 that are cytocompatible, hemostatic, and own both anti-inflammatory and antimicrobial
48 properties emerged as one of the most promising research areas in this field [2]. Indeed, these
49 multifunctional films allow for accelerating wound healing and simultaneously provide higher
50 protection against bacterial infection with minimal time and cost [3].

51 Extensive research in the realm of wound dressings has investigated the use of hydrogels,
52 exploring both synthetic polymers and natural polymers either individually or in combinations
53 [4]. In our study, we have specifically focused on natural polymers, namely pectin and
54 gelatin, selected for their remarkable biocompatibility and biodegradability attributes [5, 6].

55 Blending pectin and gelatin has been extensively explored by the scientific community,
56 particularly in the context of investigating protein-polysaccharide interactions aimed at the
57 development of innovative biomaterials [7]. Recently, photocrosslinked hydrogels were
58 developed by Bostancı et al. [8] by employing methacrylated forms of pectin and gelatin,
59 loaded with varying concentrations of curcumin to serve as stable antibacterial wound
60 dressings.

61 Osetrov et al. [9] have introduced the potential use of small oxidative agents like DMSO and
62 H₂O₂ to synthesize gelatin-tannin hydrogels. These hydrogels exhibit improved water and
63 oxygen permeability, as well as favorable mechanical properties, rendering them highly
64 suitable for wound dressing applications when compared to KIO₄ and KMnO₄. Similarly,
65 Yang et al. [10] have explored the incorporation of both OTA and ferric ions into a gelatin
66 matrix to create composite hydrogels characterized by high mechanical strength, mechanical
67 tunability, a high gelation temperature, rapid self-healing capabilities, and notably low
68 gelation times.

69 Nevertheless, it is worth noting that bacterial infection remains a significant impediment to
70 wound healing. To the best of our knowledge, the incorporation of OTA and the AgNPs to
71 enhance toughness, increase water resistance, and provide antibacterial properties to AP:GE

72 hydrogel films through Schiff base reactions and metal coordination remains an unexplored
73 area of study.

74 Pectin, an anionic heteropolysaccharide abundant in fruits and vegetables, is widely used in
75 pharmaceutical applications such as cancer targeting, wound healing, and drug administration
76 [11]. However, due to their hydrophilic nature, limited thermal stability, and poor mechanical
77 properties, films derived from pectin manifest a predisposition to readily absorb water and
78 undergo disintegration within aqueous environments; hence blending with alternative
79 biopolymers should be performed ahead.

80 On the other hand, gelatin is a biodegradable protein-based macromolecule that shares
81 chemical similarities with the extracellular matrix of the skin and develops an influential
82 nanofibrous structure for skin regeneration [12]. However, its weak mechanical strength and
83 poor water barrier prevent its biomedical application when it is used alone [13]. For instance,
84 gelatin is often combined with pectin via intermolecular hydrogen bonding and electrostatic
85 interactions, yielding blend films with enhanced hydrophilicity, improved physicochemical
86 properties, and heightened wound healing capabilities [14-16]. Incorporating cross-linking
87 agents is required to create a compact cross-linking network within the film matrix, thereby
88 ensuring the stability and mechanical resilience of the wound dressing [17].

89 However, most of the chemical crosslinking agents such as glutaraldehyde, carbodiimides,
90 and polyepoxy compounds are unsuitable for blending gelatin with a natural polymers due to
91 their residues, which may lead to toxic effects and reduce biocompatibility [18]. Thus, there is
92 growing interest in naturally derived cross-linking agents [19]. Tannic acid (TA) emerges as a
93 promising candidate, being a biocompatible, nontoxic, and abundant polyphenolic plant
94 extract. TA exhibits diverse pharmacological properties, including anti-inflammatory and
95 antimicrobial effects, as well as the ability to promote cell proliferation and enhance wound
96 healing through multiple binding sites and interactions [20]. Furthermore, the antioxidant and
97 antitumor attributes of TA have garnered considerable attention in recent decades [21].

98 Silver nanoparticles (AgNPs) are commonly incorporated in biopolymer-based composite
99 films to tune antibacterial activity [22]. To mitigate undesired aggregation and excessive
100 cytotoxicity associated with direct AgNPs addition recent studies have demonstrated the
101 efficacy of an in-situ reduction method using TA as both a natural reducing and stabilizing
102 agent [23]. This approach enables the preparation of AgNPs with controlled size and
103 improved dispersibility in polymer networks, which can be adjusted according to the degree
104 of crosslinking density [24].

105 It should be noted that in the current study, straightforward, environmentally safe, and cost-
106 effective green methodologies have been employed for the creation of multifunctional
107 hydrogels based on pectin and gelatin. The basic requirement for the eco-friendly synthesis of
108 AgNPs is silver nitrate in combination with a naturally derived reducing agent.

109 Therefore, this work aimed to develop and characterize, as a first attempt, antibacterial wound
110 dressings, composed of pectin functionalized with primary amine groups (AP) and GE,
111 supplemented with OTA and AgNPs through a Schiff base reaction and in-situ reduction,
112 respectively. The effect of the mass fraction of the crosslinking agent OTA on the
113 physicochemical, mechanical, thermal, and barrier properties of the prepared films was also
114 investigated using various analytic techniques, e.g. FTIR, XRD, SEM, μ XRF, TGA, and
115 tensile strength tests. The hemo-compatibility and in vivo performance of the developed
116 dressings has been compared to that of a commercially available dressing, Hydrocoll®. The
117 cytotoxicity, antibacterial activity, and silver release were also assessed.

118 **2. Experimental and methods**

119 **2.1. Materials**

120 Pectin (from citrus) USP with a degree of methoxylation of 65% and galacturonic acid
121 content of 74% was used for the preparation of films (Spectrum chemical, Spain). The gelatin
122 used, in powder form, was of pharmaceutical grade, and bovine origin (Type B, 80-100
123 Blooms), with a molecular weight of 41.000 g/mol, density = 1358 kg/m³ and a pH of 4.5 to
124 5.5 at 25°C (PanReac AppliChem, Spain). Chemicals of analytical grade, namely, tannic acid
125 (TA) (powder form, food grade, VWR International, France), glycerol and sodium azide
126 (NaN₃) (Biochem Chemopharma Co, Canada), silver nitrate (AgNO₃) and sodium hydroxide
127 (NaOH) (Merck, Germany), hydrogen peroxide (H₂O₂), 2,4,6-Trinitrobenzene sulfonic
128 acid/TNBS 5% (Sigma-Aldrich, Germany), sodium dodecyl sulfate SDS (Sigma-Aldrich,
129 Germany), the simulated body fluid (SBF) (Biochemazone, Canada), silver standard stock
130 solution (Sigma-aldrich, Germany), and Phosphate-Buffered Saline/PBS (Scharlau, Spain),
131 have been used. Hydrocoll®, a commercial dressing consisting of a wound contact layer of
132 caboxymethylcellulose hydrocolloid particles contained within an outer gas permeable
133 polyurethane film, was procured from Hartmann, France. Furthermore, ultrapure water
134 obtained using an ELGA PURELAB Option-Q water purification system was employed.

135 **2.2. AP synthesis**

136 The experimental protocol reported by Reitsma et al. was carefully followed to synthesize AP
137 [25]. Briefly, commercial pectin (10g) was dissolved with stirring in a pre-cooled mixture of

138 40 mL ammonia solution (33%) and 60 mL of isopropanol. The reaction occurred in a closed
139 flask immersed in a water bath maintained at 5°C for 24 hours. The pectin solute was
140 collected by filtration using Whatman® Grade 42 filter paper. Subsequently, the collected
141 solute was resuspended in acidic isopropanol (pH 1.5–2.0) and stirred for 10 minutes,
142 followed by another filtration step. To remove the remaining chlorine, the obtained solid was
143 subjected to multiple washes with 70% isopropanol. Finally, the resulting AP was further
144 washed with pure isopropanol, and the purified substance was air-dried to obtain AP powders.

145 **2.3. TA oxidation**

146 The synthesis of oxidized tannic acid (OTA) was carried out following the experimental
147 protocol reported by Osetrov et al. [26]. Briefly, 0.26g of TA was added to 0.6 mole of a
148 hydrogen peroxide solution (3% H₂O₂, pH=10). The pH was adjusted by adding NaOH (2M).
149 The mixture was continuously stirred at 70°C for 30 minutes. Subsequently, the suspension
150 was brought to a neutral pH of 7 using 6M HCl, and the resulting OTA was recovered by
151 filtration process and rinsed with ultrapure water. The obtained OTA was dried at 40°C and
152 kept in desiccators for subsequent use.

153 **2.4. Preparation of the AP:GE@OTA/Ag hydrogel films**

154 A mixture of 5g of GE and AP in a weight ratio of 3:2 was suspended in 100 mL of ultrapure
155 water at 60°C. This ratio of the constituents was chosen following previous investigations
156 [16]. Sodium azide was added at a concentration of 0.02% as an antimicrobial agent to
157 prevent fungal contamination. Continuous stirring was maintained until the complete
158 dissolution of the biopolymers was achieved. Subsequently, an OTA solution at different
159 concentrations (0%, 0.5%, 1%, 2%, and 5% based on the total weight of the AP and GE,
160 respectively) was added dropwise over the polymer blend under stirring at 60°C for 30
161 minutes to facilitate the cross-linking. After, glycerol was added as a plasticizer to each
162 crosslinked blend at 40% w/w of the total dry biopolymer weight and stirred continuously for
163 16 hours at 60°C to obtain a homogeneous solution. The plasticized dispersions were then
164 degassed and cast by pouring onto Petri dishes (90 mm diameter) in quantities of
165 approximately 20g. The cast films were allowed to dry in an open-air environment at room
166 temperature for 3 days.

167 After drying, the films were peeled off their supports and immersed in a solution of silver
168 nitrate (20 mg/L, pH of 7.6) for 30 minutes [27]. Subsequently, the films were subjected to

169 multiple washes using ultrapure water, air-dried, and conditioned in desiccators at a relative
170 humidity of 53% and a temperature of 25°C for 7 days prior to testing.

171 In the upcoming section, the resulting biopolymer films with different OTA concentrations,
172 are labeled as follows: AP:GE, AP:GE@OTA0.5%/Ag, AP:GE@OTA1%/Ag,
173 AP:GE@OTA2%/Ag and AP:GE@OTA5%/Ag.

174 2.5 Characterization

175 2.5.1 Determination of degree of amidation (DA)

176 The degree of amidation was obtained by deconvolution of FTIR spectra (1800–1500 cm⁻¹ of
177 AP). This analysis was carried out using Origin software. The DA was calculated as the ratio
178 of the area of the band at 1667 cm⁻¹ (A_{amide}) on the sum of areas of free, methyl-esterified and
179 amidated galacturonic acids (A_{tot}) at 1724, 1584 and 1667 cm⁻¹, respectively, according to the
180 following equation [28] :

$$181 \quad DA (\%) = \frac{A_{amide}}{A_{tot}} \times 100 \quad (1)$$

182 2.5.2 Determination of the amine content and the degree of crosslinking

183 The quantification of uncrosslinked primary amino groups (-NH₂) in AP and GE matrices
184 was performed using a UV assay with TNBS [29]. Crosslinking extent was assessed by the
185 difference in chemically determined free primary amine groups before and after crosslinking
186 [30]. Each sample with a mass of 11mg was mixed with a freshly prepared TNBS solution (1
187 mL of 0.5% w/v TNBS, 1 mL of 4% w/v NaHCO₃). After a 4 h incubation at 40°C, 3 mL of
188 6N HCl were added, followed by autoclaving (120°C, 1 h). The resulting solution was diluted
189 with ultrapure water (5 mL), extracted with 8 mL of ethyl ether to eliminate excess unreacted
190 TNBS. Finally, a 5 mL aliquot from each sample was heated in a hot water bath for 15 min to
191 evaporate residual ethyl ether, then diluted with 15 mL of ultrapure water. Absorbance at 346
192 nm was measured with a Shimadzu model UV-1900i spectrophotometer against blank
193 samples prepared using the same procedure, except HCl was added before TNBS to prevent
194 any reaction between TNBS and biopolymers. Free primary amino groups per gram of
195 material (Nε) in non-crosslinked and crosslinked samples were calculated using Equation (2).

$$196 \quad N\epsilon = \frac{\text{Mol of } \epsilon\text{-amino}}{\text{g of material}} = \frac{2(\text{absorbance})(V)}{\left(1.46 \times 10^4 \frac{\text{L}}{\text{mol.cm}}\right).b.x} \quad (2)$$

197 Where the molar absorptivity coefficient of the formed colorimetric complex (TNP-lys) is
198 represented as 1.46×10⁴ L/mol.cm, with V = 0.02 L indicating the volume of the water
199 solution containing hydrolyzed samples post- ether evaporation. The variables b = 1 cm

200 denote the cell path length, and “x” corresponds to the sample weight (g). The crosslinking
201 degree (f) was subsequently deduced according to Equation (3).

$$202 \quad f (\%) = \left(1 - \frac{\text{Absorbance of crosslinked sample}}{\text{Absorbance of uncrosslinked sample}} \right) \times 100 \quad (3)$$

203
204 Each determination was conducted in triplicate. The non-crosslinked samples were considered
205 to contain 100% of the initially available free amine groups, serving as a reference for
206 estimating the percentage of remaining free amine groups after the crosslinking reaction.

207 ***2.5.3 Structural, morphological, thermal, and mechanical properties***

208 The structural analysis of the AP:GE@OTA/Ag hydrogel films was conducted using various
209 analytical techniques. The FTIR spectra of the AP:GE@OTA and AP:GE@OTA/Ag films
210 were recorded in ATR mode using an Alpha II FTIR spectrometer (Bruker, Germany). The
211 measurements were performed between 400 to 4000 cm^{-1} by adding 32 scans with a
212 resolution of 1 cm^{-1} . The ^1H -NMR spectra of PE and AP were recorded in Deuterium Oxide
213 (D_2O) solution using an NMR spectrophotometer (Ascend 400 MHz, Bruker, Switzerland).

214 The morphologies and distribution patterns of metal ions within the films were observed by
215 scanning electron microscope-energy dispersive spectrometry (SEM-EDS, FEI Inspect-S50,
216 Czech Republic). Additionally, X-ray diffraction (XRD) patterns of PE, AP, and both the
217 AP:GE@OTA and AP:GE@OTA/Ag films were recorded on a SmartLab X-ray
218 diffractometer (Rigaku Corp., Japan), scanning from $2\theta = 5^\circ$ to 80° with steps of 0.01° . The
219 chemical composition of the hydrogel films was determined using a micro-X-ray fluorescence
220 spectrometer (μXRF , XGT-9000 Pro, Horiba-Jobin-Yvon) working for elements between F
221 and Am. Based on these spectra, 2D elemental maps of the specimens were generated, with
222 each distinct color and its relative intensity representing a specific element and its content. A
223 background color signifies the absence of an element or the presence of hollow regions. To
224 assess the thermal behavior, thermogravimetry (TG) experiments were conducted using a
225 Q600 SDT instrument (TA Instruments, USA) at a heating rate of $10^\circ\text{C}\cdot\text{min}^{-1}$ and under a
226 nitrogen atmosphere.

227 The film thickness parameter was determined using a Mitutoyo micrometer (Tokyo, Japan),
228 and the measurements were performed at five random locations on each hydrogel film, and
229 the average thickness values of the films were computed.

230 The tensile properties of the AP:GE@OTA/Ag films were evaluated through the
231 determination of the tensile strength (TS), elongation at break (EB), and Young's modulus

232 (YM) using a universal tensile machine (MTS Criterion universal testing machine, model 45,
233 USA) in accordance with the ASTM D-882-88 standard test method [31]. Samples were
234 prepared as pieces measuring 25.4×150 mm (width \times length). The experiments were carried
235 out with a gauge length of 50 mm and a crosshead speed of 50 mm/min. Five tests were
236 performed to evaluate the average tensile strength, the percentage of elongation at break, as
237 well as Young's modulus based on the stress-strain curves.

238 ***2.5.4 Fluid absorption ability, water storage capacity, and water vapor transmission rate***

239 The determination of the fluid absorption capacity of dried AP:GE@OTA/Ag films was
240 evaluated gravimetrically in triplicate with an accuracy of $\pm 5\%$ [32]. Beforehand, the samples
241 were subjected to a drying process until they reached an invariant weight. The samples were
242 then precisely cut into strips of uniform dimensions (30×30) mm², weighed, and submerged
243 in vials filled with 15 mL of PBS solution (pH 7.4) at a temperature of 37°C. At each
244 predefined time interval, the strips were carefully removed from the vials and the extra
245 amount of PBS solution present on the surface was softly blotted with a filter paper. The
246 swollen samples were then reweighed to determine the weight change. This process was
247 repeated multiple times until no further alteration in weight was observed. The swelling
248 equilibrium (%) was then calculated using Equation (4):

$$249 \quad \text{Equilibrium of fluid content (\%)} = \left(\frac{W_t - W_0}{W_0} \right) \times 100 \quad (4)$$

250 where W_t is the weight of the swollen and W_0 stands for the weight of the dry film.

251 For the assessment of the water retention capacity, each film was immersed in ultrapure water
252 for 24 hours. The samples were removed from water and the remaining extra amount of water
253 on the surface was softly removed by blotting with a filter paper. The wet films were then
254 weighed (W_0) and kept in an open-air environment ($T= 25^\circ\text{C}$, $\text{RH}= 75\%$) for specific time
255 intervals (W_t). The water storage ratio was determined in triplicate according to Equation (5):

$$256 \quad \text{Water storage capacity (\%)} = \left(\frac{W_t}{W_0} \right) \times 100 \quad (5)$$

257 The measurement of the water vapor transmission rate (WVTR) was performed
258 gravimetrically in triplicate, following ASTM standard method [33]. Briefly, 10 mL of
259 distilled water was poured into a cylindrical glass vial with a diameter of 34 mm. The vial's
260 opening was then completely sealed with a test specimen, firmly affixed using Teflon tape
261 along the edges to prevent any water vapor leakage through the borders. Subsequently, the
262 vials were placed inside an incubator ($T=37^\circ\text{C}$, $\text{RH}= 35\%$) for 48 hours. The evaporation-

263 induced loss of water through the sample was periodically measured by weighing. By
264 analyzing the weight loss versus time plot, the WVTR of each tested specimen (in $\text{g/m}^2/\text{day}$)
265 was calculated according to Equation (6):

$$266 \quad \text{WVTR (g/m}^2 \text{/day)} = (\text{slope} \times 24)/A \quad (6)$$

267 where A (mm^2) denotes the permeation area of the film.

268 **2.6 Biological characterization**

269

270 **2.6.1. Antibacterial activity test**

271 To reveal the infection-resistant efficacy of both AP:GE@OTA and AP:GE@OTA/Ag
272 formulations in comparison to the AP:GE film, an inhibition zone method was employed
273 against *Staphylococcus aureus* (*S. aureus*, ATCC 6538, gram-positive bacteria) and
274 *Escherichia coli* (*E. coli*, ATCC 8739, gram-negative bacteria). Briefly, each film was cut into
275 6 mm diameter discs and underwent sterilization under a UV lamp for 30 min before being
276 placed on the freshly prepared LB agar plates, where 100 μL of a bacteria suspension (10^8
277 CFU/ml) was uniformly spread over the surface. Following incubation at 37°C for 24 h, the
278 diameter of the resulting inhibition zone around each sample was measured from ImageJ
279 software with digital images of plates [34]. The experiment was conducted in triplicate within
280 a biosafety cabinet.

281 **2.6.2. Hemocompatibility test.**

282 Hemolysis and anticoagulant assays were performed to evaluate the hemocompatibility of the
283 prepared dressings. In the hemolysis assay, human red blood cells (HRBCs) were acquired by
284 eliminating serum through centrifugation, followed by five washes with PBS, as previously
285 described [35]. Afterward, the HRBCs were appropriately diluted tenfold with PBS.
286 Subsequently, 2 mL of the diluted HRBCs suspension was dispensed into separate 5 mL
287 sample vials, each containing 1mg of the corresponding dressings, namely AP:GE,
288 AP:GE@OTA0.5%, AP:GE@OTA5%, and AP:GE@OTA0.5%/Ag, AP:GE@OTA5%/Ag.
289 As controls, two additional sample vials were used: one with 0.4 mL of diluted HRBCs and
290 1.6 mL of water (positive control), and other with 1.6 mL of PBS solution (negative control).
291 After incubation at 37°C for 2 h, the above mixtures underwent centrifugation at 10,000 rpm
292 for 1 minute and the absorbance (A) of the resulting supernatants was measured at 541 nm
293 using a Shimadzu model UV-1900i spectrophotometer. The hemolytic percentage (HP) was
294 then calculated as follows [35],

$$295 \quad \text{HP (\%)} = \frac{(A_{\text{sample}} - A_{\text{negative control}})}{(A_{\text{positive control}} - A_{\text{negative control}})} \times 100 \quad (7)$$

296
297 The blood coagulation index (BCI) was assessed by the kinetic clotting time method,
298 following the procedures outlined by Wang et al. [36]. Initially, the prepared films and
299 Hydrocoll® commercial wound dressing were cut into disk-shaped specimens (6 mm
300 diameter) and sterilized under UV light for 1 h, followed by triple rinsing with PBS. These
301 samples were then placed in flat beakers, onto which 0.25 mL droplets of freshly collected
302 ACD human blood were dispensed, followed by the addition of 0.02 mL of CaCl₂. After
303 incubation at 37°C for 10, 20, 30, and 50 min, 50 mL of distilled water was sequentially
304 added at each time point. Following an additional 5-min incubation, hemolyzed blood was
305 collected and measured spectrophotometrically at 542 nm. Each experimental group was
306 conducted in triplicate. The BCI was calculated using the following formula:

$$\text{BCI (\%)} = \left(\frac{\text{Absorbance of blood which had been in contact with sample}}{\text{Absorbance of solution of distilled water and ACD blood}} \right) \times 100 \quad (8)$$

2.6.3 In vitro silver release behavior

310 The evaluation of in vitro silver release through AP:GE@OTA/Ag films was conducted using
311 a Franz vertical diffusion cell with eggshell membranes positioned between the donor and
312 receptor compartments, following the methodology as described by Gupta et al. [37]. The
313 membranes were pre-equilibrated by immersion in SBF for 24 h. AP:GE@OTA/Ag dressings
314 (1 cm² each) were placed over the membranes in the donor compartment, ensuring continuous
315 contact with the receptor compartment filled with 30 mL of pH 7.4 SBF. The assembly was
316 maintained at 37°C with stirring at 50 rpm. At specific intervals over a 48-hour period, 3 ml
317 aliquots were collected and replaced with an equal volume of SBF to maintain the receptor
318 phase at 30 ml. Silver content in each aliquot was analyzed using a graphite furnace atomic
319 absorption spectrometer (GFAAS) (AA7000, Shimadzu, Japan) at 328.1 nm. Calibration
320 standards (0.5 ppm to 5 ppm) were prepared from a 1000 ppm silver standard stock solution
321 for generating a calibration curve. Cumulative silver permeation per square centimeter of
322 dressings was plotted against incubation time. To determine overall silver loading, 20mg of
323 each AP:GE@OTA/Ag formulation was digested in 30 mL of 70% nitric acid, and a 3 mL
324 sample was analyzed using GFAAS. Each experiment was replicated three times.

2.6.4 Cytotoxicity test using XTT bioassay

2.6.4.1 Cell culture conditions

327 The NIT-1 mouse insulinoma cell line (CRL-2055) were obtained from the American Type
328 Culture Collection (ATCC) in Washington, DC, USA. These cell lines were cultured using
329 RPMI-1640 medium, enriched with 10% fetal bovine serum (FBS) and 1%
330 penicillin/streptomycin. The culturing process was carried out in an incubator set to a 5% CO₂

331 atmosphere at a constant temperature of 37°C. A traditional trypan blue staining method was
332 employed alongside the Neubauer counting chamber for cell quantification, allowing for
333 accurate cell viability assessment and counting.

334 **2.6.4.2 Cytotoxicity assay**

335 NIT-1 mouse insulinoma cells (CRL-2055) were used to assess cytotoxicity of the prepared
336 dressings using XTT assay [38], employing XTT cell proliferation kit sourced from Roche
337 Company (Bale, Switzerland). The NIT-1 cells were seeded into a 96-well microplate and
338 incubated at 37°C in a 5% CO₂ atmosphere for 24 h for cell attachment and growth. Each
339 sterilized dressing (6 mm diameter) was dissolved in PBS, and dilutions ranging from 100
340 µg/ml to 12.5 µg/ml were prepared. After the initial 24-hour incubation period, the samples
341 were added to experimental wells in triplicate at varying concentrations. Subsequently, cells
342 were further incubated for 72 h. Post-incubation, the XTT reagent was applied to the cells,
343 and cell viability was assessed by comparing their absorbance with those of the PBS at 490
344 and 660 nm using a microplate reader. Cell viability was calculated by using the following
345 equation [39] :

$$346 \quad \text{Cell Viability (\%)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of control group}} \times 100 \quad (9)$$

347 **2.6.5 In vivo wound healing studies**

348 Full-thickness surgical excisions were performed to assess the wound healing-promoting
349 capabilities of the hydrogel films. The animal procedures were conducted in accordance with
350 the guidelines of the Algerian Association for Animals Experimentation Science, as approved
351 under permission number XXXX, dated X X, 20XX. Twenty-one Wistar albino rats (male, age
352 6 months, 200±20g) from the animal household of the pharmacotoxicology department of the
353 National Pharmaceutical Products Control Laboratory (NPPCL) were randomly divided into
354 four groups, each comprising seven rats. The group allocations were as follows:

355 Group I: Control (Wound + Hydrocoll®)

356 Group II: Wound + AP:GE dressings

357 Group III: Wound + AP:GE@OTA5% dressings

358 Group IV: Wound + AP:GE@OTA0.5%/Ag dressings

359 The rats were individually housed in hygienic conditions for several days before the study to
360 acclimatize to the laboratory environment (22 ± 2°C, RH of 50%). They were provided with
361 standard rodent feed and water ad libitum throughout the study, while maintaining a 12 h

362 light/dark cycle. Before surgery, anesthesia was induced via intraperitoneal injection of
363 Thiopental at a dosage of 45mg/kg body weight. Dorsal hairs were shaved, and the skin was
364 sterilized with 70% ethanol. Using a surgical blade and forceps, full-thickness round wounds
365 measuring 3 mm in diameter were created on the cervicodorsal area under strict aseptic
366 conditions. Excess blood was absorbed using sterile surgical compresses, and wound margins
367 were cleaned with surgical alcohol. Postoperatively, rats received a single intravenous
368 injection of 10mg of paracetamol to alleviate pain, adhering strictly to guidelines for the Care
369 and Use of Laboratory Animals, given the susceptibility of rodents to self-mutilation in
370 response to severe pain. Subsequently, the wounds were covered with freshly prepared
371 AP:GE, AP:GE@OTA5% and AP:GE@OTA0.5%/Ag dressings, while the positive control
372 group received Hydrocoll®. Dressings were changed daily. Wound healing progression was
373 monitored and documented via digital photography on the 2nd, 4th, 6th, 8th, 10th and 12th
374 postoperative days. Throughout this period, rats were handled gently to minimize stress and
375 acclimate them to the laboratory environment. The repair area was calculated using Image J
376 software, and the wound contraction was determined by the following equation :

377

$$378 \quad \text{Wound contraction}(\%) = \frac{\text{initial wound area} - \text{wound area at } D_x}{\text{initial wound area}} \times 100 \quad (10)$$

379

379 **2.7 Statistical Analysis**

380 The experimental results were statistically analyzed using SPSS statistics V26 software
381 (IBM Corp., Armonk, NY, USA) employing a one-way analysis of variance (ANOVA) and a
382 confidence level of 95%. To identify significant differences among the groups, Tukey's test
383 was performed, and a p-value <0.05 was considered indicative of statistical significance.

384 **3. Results and discussion**

385 **3.1. AP characterization**

386 Commercial pectin (PE) was chemically modified before the film preparation by an amide
387 reaction leading to AP. Figure 1(a) displays the FTIR spectra of both types of pectin, i.e., the
388 non-amidated and amidated ones. In the pectin spectrum, the peak at 3370 cm⁻¹ is attributed to
389 the stretching vibration of -OH groups. The peak at 2932 cm⁻¹ corresponds to the C-H
390 stretching vibration, while the peak at 1724 cm⁻¹ indicates the stretching vibration of the
391 methyl ester. The peaks at 1436 cm⁻¹ and 1340 cm⁻¹ can be assigned to -CH₂ scissor and -OH
392 bending vibrations, respectively [40].

393 Furthermore, the sharp peak spotted at 1006 cm⁻¹ is assigned to the glycosidic bonds that
394 connect two galacturonic sugar units [41]. The FTIR spectrum of AP showed a broadening

395 accompanied by a significant increase of the peak at 3370 cm^{-1} , which is attributed to the
396 amide N-H peak and the strong broad O-H [42]. Additionally, the peaks observed at 1667 cm^{-1}
397 1 (amide I) and 1584 cm^{-1} (amide II) were the result of the electron doping from $-\text{NH}_2$,
398 which is in agreement with the reported amide band FTIR peak at 1590 cm^{-1} [28]. The AP
399 spectrum displayed also a weak peak at around 1423 cm^{-1} , suggesting that the amide group
400 was successfully incorporated into the pectin molecules. The DA of AP obtained by
401 deconvolution FTIR spectra was $38.62\pm 0.04\%$.

402 Figure 1(b) shows the X-ray diffractograms of PE and AP. The obtained pattern obtained for
403 the unmodified pectin exhibits well-defined peaks at 2θ angles of 9° , 18.54° , 28.44° , and
404 40.21° , indicating its semi-crystalline nature. In contrast, the pattern of AP shows two weak
405 and broad diffraction peaks at around 13.34° and 20° (2θ). The incorporation of the $-\text{NH}_2$
406 groups could have obstructed the rearrangement of pectin molecular chains, causing the
407 disruption of its crystalline structure.

408 Figure 2 displays the $^1\text{H-NMR}$ spectra of PE and AP. The NMR spectrum of AP showed
409 prominent peaks within the 3-5.5 ppm range, these chemical shifts correspond to hydrogen
410 atoms on the polygalacturonic acid chain of pectin [43]. Furthermore, the strong peak in the
411 range of 3-4 ppm were assigned to the methyl protons on the nitrogen of primary amine
412 inserted in the PE chains. This suggests the partial methylation of the methoxy groups in the
413 pectin molecules [42], indicating the successful preparation of AP.

414 The results provided by the spectroscopy techniques (FTIR, XRD, and $^1\text{H-NMR}$) allow
415 concluding that the PE was successfully functionalized with primary amine groups according
416 to the reaction presented in Figure 3, leading finally to AP.

417 **3.2. AP:GE@OTA/Ag film characterization**

418 AP:GE@OTA films were initially created through covalent cross-linking of amino groups in
419 both AP and GE with the carbonyl reactive sites of OTA. Subsequently, under alkaline
420 conditions, OTA phenolic hydroxyls acted as a natural reducing agent for silver ions, leading
421 to the formation of AgNPs. Meanwhile, the existing carbonyl groups on OTA could form
422 coordinate bonds with AgNPs, creating a protective layer that delayed the release of Ag^+ and
423 prevented direct contact between AgNPs and cells [27]. The obtained films are shown in
424 Figure 4 and their formation mechanisms are detailed in the scheme reported in Figure 5. The
425 TNBS reaction has been used for the specific measurement of amino groups in proteins,
426 foodstuffs and peptides [44]. The degree of crosslinking was subsequently calculated as the
427 difference between the chemically determined amount of free amino groups before and after
428 crosslinking. It is presumed that each lost amino group contributes to the formation of a single

429 crosslink. The data in Table 1 represent the values of free amine groups per gram of material,
430 before and after the crosslinking process, along with the extent of crosslinking in the prepared
431 films. The number of free amine groups in GE was comparable to previous studies [30, 45],
432 and higher than that in AP samples ($31.8 \pm 1.35 \times 10^{-5} \text{ mol.g}^{-1}$ compared to $8.5 \pm 0.75 \times 10^{-5}$
433 mol.g^{-1}). After the crosslinking process, a significant reduction in the number of free amino
434 groups occurred in the AP and GE matrices, confirming the active involvement of amino
435 groups as highly nucleophilic agents in the film crosslinking. Additionally, Figure 6
436 demonstrates the impact of OTA content on the crosslinking process concerning amino
437 consumption and the degree of crosslinking. An increase in OTA content correlated with
438 elevated amino consumption. The number of free amine groups in all AP:GE@OTA films
439 decreased after crosslinking within the range of $7.1\text{--}29.2 \times 10^{-5} \text{ mol.g}^{-1}$. As a result, the extent
440 of crosslinking exhibited an increase, reaching $58.52 \pm 2.68\%$ for AP:GE@OTA2% and
441 $78.27 \pm 4.47\%$ for AP:GE@OTA5%, respectively.

442 **3.2.1 Fourier Transform Infrared Spectroscopy (FTIR) measurements**

443 Figure 7(a) displays the FTIR spectra of both TA and OTA. In the case of TA, peaks were
444 observed in the $3200\text{--}3700 \text{ cm}^{-1}$ range, and around 1316 cm^{-1} , which were assigned to the
445 stretching vibration of the phenolic hydroxyl groups. Peaks at 1192 cm^{-1} and 1536 cm^{-1} were
446 associated, respectively, with the stretching vibrations of the C=O and C=C bonds in the
447 aromatic ring. Additionally, the peak at 1023 cm^{-1} was associated with the ether bonds
448 (C–O–C) stretching vibration. Hydrogen peroxide has the capacity to oxidize phenolic
449 compounds, leading to their conversion into reactive quinones that may react with GE and AP
450 [9]. Moreover, the OTA spectrum highlighted a distinctive peak that emerged at 1626 cm^{-1} ,
451 indicating carbonyl stretching vibrations, which may be explained by the formation of an
452 intermediate quinone [10].

453 The FTIR analysis was conducted to confirm the crosslinking of the blended hydrogel films
454 and to investigate the molecular structures of the AP:GE@OTA and AP:GE@OTA/Ag
455 blends. For a comparative study, the spectra of AP, GE, and OTA were also recorded. From
456 Figure 7(b), it is evident that the GE samples exhibited distinct transmittance peaks in the
457 frequency range of $3320 \text{ cm}^{-1}\text{--}3520 \text{ cm}^{-1}$ due to the establishment of hydrogen bonds between
458 water and amide-A. Furthermore, peaks at 1630 cm^{-1} (amide-I), 1330 cm^{-1} (amide II), 1235
459 cm^{-1} (amide-III), and a series of peaks spanning from 1460 cm^{-1} to 1380 cm^{-1} were attributed
460 to the symmetric and asymmetric bending vibrations of methyl groups [46].

461 The FTIR spectrum of AP:GE films displayed transmittance peaks in the range 3200 cm⁻¹-
462 3400 cm⁻¹, representing the stretching vibrations of NH and OH groups, indicative of
463 hydrogen bonding between these functional groups and water molecules [47]. The C=O
464 stretching (amide I) absorption at 1637 cm⁻¹ and N-H bending (amide II) bands at 1553 cm⁻¹
465 are representatives of the AP and GE structures. The IR spectrum of AP:GE films suggests a
466 possible physical interaction between the positively charged GE-NH₃⁺ derived from gelatin
467 and the negatively charged pectin-COO⁻. The C-N stretching vibration peak at 1553 cm⁻¹ is
468 significantly stronger in the AP:GE@OTA5% sample compared to that in the AP:GE film.
469 The characteristic peaks in the AP:GE@OTA5% are significantly stronger than those in the
470 AP:GE film, indicating the establishment of new C-N bonds formed through the Michael
471 addition reaction [48]. At the same time, stretching vibration of the carbonyl group spotted at
472 1630 cm⁻¹ was shifted to 1648 cm⁻¹ after crosslinking, thus confirm the formation of the
473 imine bond (-C=N) imine bond during Schiff reactions [10]. Furthermore, the presence of -
474 CH stretching vibration peaks in the crosslinked films is indicated by the peaks spotted at
475 2940 cm⁻¹ to 2960 cm⁻¹ [49]. The transmission peak observed at 1238 cm⁻¹ is attributed to the
476 stretching vibrations of C-O bonds. As shown in Figure 7(c), a decrease in the intensity of the
477 -OH stretching vibration peak in the FTIR spectra of AP:GE@OTA/Ag hydrogel films is
478 recorded compared to the AP:GE@OTA films. This can be attributed to the increase in
479 hydrogen bonding or stronger intermolecular interactions among OTA, AP, and GE within the
480 hydrogel structure.

481 **3.2.2 X-ray diffraction (XRD) analysis**

482 Figure 8(a) shows the X-ray diffractograms for AP:GE, AP:GE@OTA films, and for neat GE.
483 GE exhibited a single diffraction peak within the 2θ range of 15–25°, which aligns with the
484 typical XRD profile of pure gelatin, stemming from the α-helix and triple-helical structure
485 from the collagen renaturation process [50]. The X-ray diffractograms of AP:GE samples
486 revealed broad and diffused peaks at 2θ equal to 15.91° and 38.19°, indicative of the
487 amorphous nature of the blend film. The disappearance of X-ray diffraction peaks
488 corresponding to AP confirms the intermolecular interactions between these two biopolymers,
489 which is in agreement with the results of the work of Mishra et al. [51].

490 The X-ray diffractograms of AP:GE@OTA formulations exhibited differences compared to
491 those observed in the AP:GE films. When the OTA mass fraction increased, the intensity of
492 the crystal peak of gelatin at 8.10° (2θ) decreased in intensity, while new peaks at 2θ values of
493 22.95, 21.48, 21.35 and 21.07° appeared for AP:GE@OTA0.5%, AP:GE@OTA1%,

494 AP:GE@OTA2%, and AP:GE@OTA5%, respectively. These findings suggest that the
495 characteristic helical structure of GE was modified by AP chains during the crosslinking
496 process with OTA. This modification was achieved by disrupting hydrogen bonds and
497 hydrophobic interactions while also creating new Schiff base ($-C=N$) connections because of
498 the condensation of amine groups in both GE and AP with the carbonyl group present in
499 OTA. These findings highly corroborate the FTIR results discussed previously. Additionally,
500 the first diffraction peak of gelatin at 8° , which is related to the diameter and rate of the triple
501 helix [52], is often reduced in intensity by the addition of polyols such as tannin [53], and
502 glycerol [53].

503 On the other hand, the XRD patterns of the AP:GE@OTA/Ag samples, shown in Figure 8(b),
504 revealed four prominent peaks ranging from 30° to 80° . To ascertain the precise nature of the
505 silver nanoparticles formed in our experiments, a comparative analysis of the obtained
506 diffractograms with those of pristine crystalline silver structures, as cataloged by the Joint
507 Committee on Powder Diffraction Standards (reference file nos. 04-0783) was conducted. The
508 result clearly confirmed the formation of silver nanoparticles with the face-centered cubic
509 (fcc) structure [54]. This confirmation was derived from the observed peaks at angles of
510 38.72° , 43.96° , 64.29° , and 77.40° , corresponding to the (111), (200), (220), and (311)
511 crystallographic planes of silver, respectively.

512 The determination of the nanoparticle size was achieved through the utilization of the full
513 width at half maximum (FWHM) values observed for these reflection planes in conjunction
514 with the Debye–Scherrer equation. The calculated particle sizes derived from the XRD line
515 broadening analysis were in concordance with those ascertained through scanning electron
516 microscopy (SEM) analysis (Table 2). Consequently, these results establish an average
517 particle size of approximately 56 nm.

518 **3.2.3 Scanning electron microscopy (SEM) Analysis**

519 The SEM image obtained for the AP:GE film conjointly with those obtained for all the
520 AP:GE@ OTA films are shown in panel (a) of Figure 9. It is obvious that all the investigated
521 films exhibited translucent, smooth, and homogenous surfaces, indicating that the addition of
522 OTA and AgNPs causes the formation of a compact network structure within
523 AP:GE@OTA/Ag films (see Figure 9(a)). There were no visible pores or cracks on the
524 surface. It is not possible to detect a significant difference in the films produced with different
525 concentrations of OTA. Moreover, a well-dispersed AgNPs had been achieved for the film
526 treated with 2% and 5%. The size of the AgNPs was confirmed by SEM analysis and was
527 found around 57 nm.

528 EDS analysis was also carried out on the AP:GE@OTA5%/Ag film and the obtained results
529 were illustrated in plot (b) of Figure 9. A distinct peak in the silver region could be easily
530 observed, thus confirming AgNPs formation. The uniform dispersion of AgNPs within the
531 films further highlights the successfulness of the cross-linking process.

532 **3.2.4 Micro X-ray fluorescence (μ XRF) analysis**

533 Table 3 presents the relative abundance of elements present in the AP:GE@OTA/Ag hydrogel
534 films, as determined by the μ XRF analysis, at the level of impurities. The obtained results
535 confirmed the effective incorporation as well as the homogeneous distribution of the AgNPs
536 over the films for all the investigated samples (Figure 10). These findings strongly
537 corroborate those from the SEM-EDS and XRD analyses. Results revealed that sulfur (S),
538 calcium (Ca), and copper (Cu) are the main elements forming the AP:GE@OTA/Ag hydrogel
539 films. Exception is made for the AP:GE@OTA5%/Ag film, in which, the relative abundance
540 of S, Ca and Ag has been respectively found to be 75.31%, 24.54% and 0.14%.

541 **3.2.5 Mechanical properties of hydrogel films**

542 The tensile testing offers insights into the film's strength and flexibility, as evidenced by its
543 tensile strength and elongation at break. For wound dressing application, it is suggested that
544 films should preferably exhibit both strength and flexibility. This is necessary to ensure
545 durability and enough stress-resistant to meet the requirements for application, handling, and
546 storage. Hence, glycerol, a commonly employed component in hydrogel formulations, was
547 added as a plasticizer (40% w/w of the total dry biopolymer weight) to each crosslinked blend
548 to enhance the flexibility of the film. Table 4 summarizes the mechanical properties obtained
549 for the studied hydrogel, namely, the thickness, the tensile strength, the elongation at break,
550 and Young's modulus.

551 The thickness parameter was measured using a thickness gauge. The results showed that the
552 thickness ranged from 0.27 mm to 0.41 mm. The uncertainty associated with the thickness is
553 found to be less than 0.08 mm. Furthermore, it was found that the thickness, the tensile
554 strength, and Young's modulus increased with the incorporation of OTA as well as the
555 increase in its mass fraction. Nevertheless, the elongation at break (%) evolution showed an
556 opposite trend.

557 Results showed (Table 4) that AP:GE@OTA0.5%/Ag film exhibited a significantly higher
558 percentage of elongation (37.73%) and lower tensile strength (23.11 MPa) and Young's
559 modulus (1218 MPa) than those of AP:GE@OTA1%/Ag, AP:GE@OTA2%/Ag and
560 AP:GE@OTA5%/Ag ($p < 0.05$). This result suggested that the addition of 0.5 wt.% OTA was
561 enough to generate a flexible AP:GE@OTA/Ag hydrogel film, by improving its mechanical

562 properties. The highest mass fraction used of OTA (5%) confers to the film the highest
563 toughness and rigidity. This may be well appreciated through the high values of tensile
564 strength (38.49 MPa) and Young's modulus (1512.98 MPa), along with a low value of
565 elongation at break (32.42%) ($p < 0.05$). The AP:GE@OTA2%/Ag films demonstrated
566 slightly diminished tensile strength (30.07 MPa) and Young's modulus (1438.30 MPa), along
567 with an increased elongation percentage (33.98%), when compared to the
568 AP:GE@OTA5%/Ag films.

569 Figure 11 shows the evolution of the tensile strength (TS) and the elongation at break (EB)
570 parameters of crosslinked films at different mass fractions of OTA. It is evident that as the
571 mass fraction of the crosslinking agent increased, there was a noticeable increase in the tensile
572 strength (TS) accompanied by a decrease of the elongation at break (EB) when compared to
573 the AP:GE film. This is mainly caused by the ability of OTA to increase intermolecular forces
574 along the polymer chains [55]. On the other hand, as the OTA concentration increased, the
575 treated films maintained their transparency but took on a honey-yellow hue when observed
576 with the naked eye, owing to the inherently honey-yellow coloration of the OTA solution. In
577 summary, it could be concluded that both OTA and AgNPs exhibited crosslinking effects on
578 the mechanical properties of the AP:GE films. **3.2.6 Thermogravimetry (TG)**
579 **measurements**

580 The thermal behavior of the AP:GE@OTA/Ag films was investigated by thermogravimetric
581 analysis under a nitrogen atmosphere. The obtained TG curves are shown in Figure 12. Three
582 decomposition steps occurred during the thermal degradation of the investigated hydrogel
583 films. Furthermore, the rate of weight loss in these three steps for the AP:GE@OTA/Ag
584 samples was found to be lower than those obtained for the AP:GE film. The first step, which
585 occurred at 50–100°C, is attributed to the adsorbed water evaporation. In the temperature
586 range of 190 to 230°C, corresponding to the second decomposition step, the
587 AP:GE@OTA/Ag samples showed a loss of approximately 30% of their mass, potentially
588 assigned to the thermal oxidation of the films [51]. As the mass fractions of OTA increased in
589 the film, the residual weights exceeded those observed for AP:GE film, which were
590 approximately 30 wt.%. This finding suggests that the incorporation of both OTA and AgNPs
591 within the AP:GE hydrogel network enhances the thermal stability of the blend, due to the
592 formation of strong interactions between the carbonyl groups of OTA and the amino groups
593 of AP and GE through a Schiff base reaction to obtain a new functional group (imine). The
594 final weight loss evidenced at 300–320°C was caused by the final thermal decomposition of
595 the films. The nonvolatile material remaining as a residue at 320°C was found to be around
596 20% of the original mass of the film sample.

597 **3.2.7 Fluid uptake ability, water retention capacity, and water vapor transmission rate**
598 **of hydrogel films**

599 To identify suitable wound dressing materials, crucial factors such as fluid uptake ability,
600 water retention capacity, and water vapor transmission rate had to be accurately evaluated.

601 The fluid uptake capacities of AP:GE@OTA/Ag hydrogel films were assessed by incubating
602 them in PBS at 37 °C. The obtained results are presented in plot (a) of Figure 13. Except for
603 the AP:GE sample, the hydrogel films exhibited similar patterns, in which an equilibrium
604 state of fluid uptake is reached after 2 hours (approximately 90%). Furthermore, both AP-GE
605 and AP:GE@OTA0.5%/Ag films presented lower fluid uptakes compared to the other
606 formulations, which might be attributed to the poor mechanical properties affecting their fluid
607 uptake ability [56]. From plot(a) in Figure 13, there was no notable disparity observed from 2
608 to 8 hours for the hydrogel films containing OTA and glycerol (acting as a humectant),
609 enabling these films to attain their early saturation in fluid uptake at 2 hours.

610 The water retention capacities of the hydrogel films were evaluated by measuring the loss of
611 water content when exposed to dry air conditions. The results shown in plot (b) in Figure 13
612 clearly revealed that the incorporation of both OTA and AgNPs led to an increase in the water
613 retention capacity of the film. Moreover, it was found that these capacities depend on the
614 mass fraction of the crosslinker used during their preparation. Among all the prepared films,
615 the AP:GE@OTA5%/Ag film displayed the highest capacity for retaining water. This can be
616 attributed to the greater rigidity and decreased flexibility of the AP:GE@OTA/Ag
617 macromolecular chains. Besides, the assessment of water retention capacity over 24 hours
618 revealed a sensitive increase when the amount of OTA in the film exceeds 1%. Above this
619 typical value, the change in the capacities is found relatively low (2%-5%), indeed hydrogel
620 films with a rigid structure tend to absorb less fluid, but exhibit a higher percentage of water
621 retention capacity.

622 Water vapor transmission rate (WVTR) is a critical factor in moisture management in wound
623 areas. In general, it is agreed that a rate of 2000-2500 g/m²/day water loss can maintain an
624 appropriate level of moisture in wounds without causing dehydration [57]. A lower value
625 results commonly in an accumulation of wound exudates, while a higher WVTR can lead to
626 dehydration of the wound surface. Histograms presenting the obtained WVTR for all the
627 investigated hydrogel films are provided in the plot (c) in Figure 12. Values were found to
628 range from 1653 to 1896 g/m²/day, which closely align with the suggested range for an
629 optimal wound dressing. Overall, the incorporation of OTA and AgNPs increases the WVTR
630 parameter. As well the increase of the OTA amount in the film until 2% results in an increase
631 of WVTR value. Exceeding this amount, the trend was inversed. This could be attributed to
632 the intensity level of the bonding interactions in the polymer network, along with the presence

633 of a humectant. Consequently, the prepared biofilms are expected to promote cell migration
634 and facilitate re-epithelialization.

635 **3.10 Biological characterization**

636 The antibacterial efficacy is a crucial requirement for wound dressings intended for
637 use in wound healing. The assessment of antibacterial activity involved the measurement of
638 inhibition zones induced by both AP:GE@OTA and AP:GE@OTA/Ag dressings against *S.*
639 *aureus* and *E. coli*, prevalent pathogens in infected skin wounds. As illustrated in Figure 14,
640 both formulations effectively eliminated surrounding *E. coli* and *S. aureus*, while the AP:GE
641 group showed no discernible bacteriostatic effect. With increasing OTA content, the
642 antibacterial zone of AP:GE@OTA dressings consistently expanded. Previous research has
643 demonstrated that TA exhibits a noteworthy inhibitory effect on the growth of both Gram-
644 positive and Gram-negative bacteria, displaying bacteriostatic rather than bactericidal activity
645 [58]. This is attributed to its structural abundance of phenolic hydroxyl groups, recognized for
646 effectively neutralizing free radicals and safeguarding against microorganisms [59]. The
647 AP:GE@OTA/Ag group exhibited significantly larger inhibition zones against both bacterial
648 types, ranging from 12.94 ± 0.23 to 31.77 ± 0.57 mm (Figure 14). This heightened antimicrobial
649 activity is attributed to the synergistic effect of OTA and AgNPs. Interestingly, an inverse
650 relationship was observed between OTA content and the antibacterial zone of
651 AP:GE@OTA/Ag dressings against *E. coli* and *S. aureus*. Particularly, the
652 AP:GE@OTA0.5%/Ag dressing showcased the most pronounced bacteriostatic zones,
653 measuring approximately 24.25 ± 0.24 mm and 31.77 ± 0.57 mm for *E. coli* and *S. aureus*,
654 respectively. The observed phenomenon is attributed to a change in crosslink density
655 occurring through metal coordination during Ag loading. These findings closely align with
656 outcomes from in vitro silver release studies. The inhibition zone was induced by the
657 diffusion of silver ions, which are trapped within the AP:GE@OTA/Ag matrix, into the
658 surrounding nutrient medium. This diffusion process, facilitated through swollen matrix
659 pores, involves the conversion of Ag^0 to Ag^+ upon exposure to moisture. The resulting Ag^+
660 ions interact effectively with thiol groups in crucial enzymes linked to bacterial respiration,
661 ultimately leading to cellular demise [60]. In an alternative mechanism, Ag^+ ions may interact
662 with the negatively charged bacterial cell wall, inducing membrane disintegration or
663 interfering with DNA replication, thereby inhibiting bacterial growth. If small enough, AgNPs
664 can penetrate the bacterial cell wall, causing the leakage of cellular constituents and eventual
665 cell death [61]. Overall, these results underscore the potent antibacterial activity of

666 AP:GE@OTA0.5%/Ag dressings against both bacterial classes, rendering them suitable
667 candidates for selection in in vivo wound healing assays.

668 Hemocompatibility has emerged as a fundamental criterion for assessing the suitability of
669 biomaterials, particularly when these materials are intended for direct contact with blood in
670 vivo [35]. The standard categorizes biomaterials based on their hemolytic potential: non-
671 hemolytic (0–2% hemolysis), slightly hemolytic (2–5% hemolysis), and hemolytic (>5%
672 hemolysis) [62]. As depicted in Table 5, the HP of the experimental groups tested at 37°C for
673 2 h is consistently below 2%. This suggests minimal disruption to membrane blood cells,
674 affirming the suitability of all samples for in vitro applications [63]. No significant difference
675 in hemolysis was evident between AP:GE@OTA and AP:GE@OTA/Ag dressings. The lower
676 hemolytic ratio exhibited by these films suggests its enhanced biocompatibility. Therefore, it
677 is noteworthy that the incorporation of OTA and AgNPs does not induce hemolysis,
678 indicating excellent hemocompatibility.

679 The hemostatic performance of the prepared dressings was also evaluated by a relative
680 parameter, BCI (Figure 15). As well known, the crucial factor of hemostasis is that hemostatic
681 biomaterials demonstrate a potent capability to enhance the enrichment of hemocytes. As
682 depicted in Figure 15, an increase in the duration of blood contact with the samples led to a
683 corresponding decrease in the BCI. As highlighted by Meena et al. [64], blood clotting is
684 related to swelling and rapid water absorption from the blood. This phenomenon contributes
685 to the formation of a concentrated pool of blood components, thereby facilitating the
686 formation of a dense clot.

687 The AP:GE@OTA0.5%/Ag and AP:GE@OTA5%/Ag films demonstrated a swift decline in
688 BCI within 10 min, whereas the control (Hydrocoll® commercial hydrocolloid dressing),
689 AP:GE@OTA0.5%, and AP/GE@OTA5% films exhibited a similar trend after 20 min. This
690 observation clearly indicates the capacity of AP:GE@OTA/Ag films to initiate blood
691 coagulation, thus displaying advantageous hemostatic properties. Therefore, the exceptional
692 hemostatic performance of AP:GE@OTA/Ag films may stem from a synergistic effect of
693 AgNPs, attributed to their positive charge [65].

694 The cytotoxicity of AgNPs has long been widely recognized as a significant challenge in their
695 biomedical applications, prompting extensive research into toxicological mechanisms
696 involved. The cytotoxicity of AgNPs continues to pose a significant challenge in biomedical
697 applications, prompting extensive research to investigate the underlying toxicological
698 mechanisms. Despite considerable efforts, the precise mechanisms and interactions with
699 biological entities remain incompletely understood [66, 67]. The cytotoxicity of AgNPs is

700 characterized by the release of Ag^+ ions, which interact with cells, affecting DNA and
701 ultimately leading to cell lysis [68]. Studies indicate that smaller AgNPs release elevated
702 concentrations of Ag^+ ions compared to larger counterparts, suggesting size-dependent
703 cytotoxicity [69]. In this context, it is deemed necessary to investigate the release of silver
704 from the AP:GE@OTA/Ag dressings under simulated physiological conditions. Initial silver
705 content was determined through acid digestion and GFAAS analyses, and the results are
706 presented in Table 6. AP:GE@OTA/Ag dressings showed an average loading ranging from
707 1.02 ± 0.13 to $3.11 \pm 0.76 \mu\text{g}/\text{cm}^2$. Cumulative silver release kinetics, illustrated in Figure 16,
708 showed a biphasic pattern across all AP:GE@OTA/Ag dressings, characterized by an initial
709 burst followed by sustained release. The linear fast release occurred over 2h, succeeded by
710 prolonged release attributed to gradual Ag^+ ion release of from the dressings. After 48h,
711 cumulative silver release percentages from initial loads of AP:GE@OTA0.5%/Ag,
712 AP:GE@OTA1%/Ag, AP:GE@OTA2%/Ag and AP:GE@OTA5%/Ag dressings were
713 $7.18\% \pm 0.13\%$, $3.5\% \pm 0.02\%$, $2.37\% \pm 0.01\%$ and $1.66\% \pm 0.01\%$, respectively. This suggests
714 that AP:GE@OTA/Ag dressings effectively control bacterial infection early in wound
715 infliction, providing sustained antibacterial activity over an extended duration. Previous
716 research investigated silver release from various commercial dressings, including
717 ACTICOAT™ Flex 3, ACTISORB1 Silver 220, AQUACEL1 Ag, and Mepilex1 Ag. In a
718 serum substitute, silver release ranged from 7% to 67%, with rates varying between 0.0001
719 $\mu\text{g}/(\text{h cm}^2)$ and $4099 \mu\text{g}/(\text{h cm}^2)$ [70]. Another study observed an approximately 86% release
720 of Ag^+ after 48 h from a bacterial cellulose silver nanocomposite immersed in distilled water
721 [71]. In contrast, AP:GE@OTA/Ag dressings, containing only 1.02 - $3.11 \mu\text{g}/\text{cm}^2$ of silver,
722 release only 1.63-7.15% of silver after 48h. The low Ag^+ concentrations suggest reduced
723 cytotoxicity, possibly due to AgNPs entrapment and encapsulation within three-dimensional
724 polymeric network [72].

725 In vitro silver release data were fitted using first-order, Korsmeyer–Peppas, and Sahlin-
726 Peppas models (Figure 17). The Peppas-Sahlin model showed the best fit (Table 7),
727 considering both diffusion and swelling mechanisms. Results indicated dominant diffusion (n
728 ≈ 0.5) and minimal swelling ($k_2 \rightarrow 0$), suggesting a diffusion-based silver release mechanism.
729 Overall, the results indicate that the crosslinking density of AP:GE@OTA/Ag films
730 effectively controls silver release, making them suitable for sustained-release wound dressings
731 with antibacterial properties.

732 Assessment of cytocompatibility is deemed a fundamental necessity in biomaterial
733 development for biomedical applications. The cytotoxic effects of the formulated dressings on

734 the NIT-1 cell line were evaluated at various concentrations over 72-hour period using the
735 XTT assay. Figure 18(a) demonstrates that both AP:GE@OTA0.5% and AP:GE@OTA5%
736 dressings exhibit favorable cell viability and the different OTA concentrations did not affect
737 cell growth. It is recognized that PE, GE and TA are bioactive and biocompatible substances
738 that can effectively enhance cell proliferation [73]. Even with AP:GE@OTA0.5%/Ag and
739 AP:GE@OTA5%/Ag dressings, no obvious cytotoxicity was observed on NIT-1 cells.
740 Notably, AP:GE@OTA0.5%/Ag dressing showed the highest cell viability compared with the
741 other dressings. Statistical analysis revealed significant differences compared to pristine cells.
742 In this study, AgNPs were intentionally reduced by OTA in situ on films, resulting in
743 significantly improved dispersity of the formed AgNPs, thereby reducing cytotoxicity.
744 Further, the changes in cell morphology were examined under a phase contrast microscope
745 (Figure 18(b)). All the tested biopolymeric matrices offered a favorable surface for cell
746 adhesion and growth. Therefore, AP:GE@OTA and AP:GE@OTA/Ag dressings are non-
747 toxic and capable of inducing cell proliferation for wound healing applications. Our findings
748 align with those reported in the literature, where TA and AgNPs were incorporated into a
749 chitosan polymeric matrix [48]. This study demonstrated the enhanced cytocompatibility of
750 CS-Ag-TA cryogel compared to the control, attributed to the presence of AgNPs and TA,
751 which promote cell proliferation and viability.

752 According to the above experiments, in vivo wound healing studies were conducted on wistar
753 albino rats using AP:GE@OTA5% and AP:GE@OTA0.5%/Ag dressings. Figure 19(a)
754 depicted the comparative decrease in wound area throughout the 12 days. The intricate
755 process of wound healing, wherein the skin barrier regenerates and seals post-injury, operates
756 through a multifaceted mechanism that starts promptly after skin injury. This mechanism
757 involves three interconnected phases, including inflammation, proliferation, and tissue
758 remodeling [74]. The inclusion of bioactive elements capable of impacting these pathways
759 may induce modifications in the wound healing process. Following treatment, all groups
760 exhibited a gradual decrease in wound area. In comparison to the Hydrocoll® group, both
761 AP:GE@OTA5% and AP:GE@OTA0.5%/Ag dressings demonstrated accelerated wound
762 healing, achieving reductions in wound size of $82.13 \pm 9.43\%$ and $86.59 \pm 2.35\%$, respectively,
763 within 8 days. Conversely, unhealed wounds were still observable in the AP:GE group,
764 exhibiting a reduction of $57.93 \pm 7.10\%$ within the same timeframe. Notably, wounds treated
765 with AP:GE@OTA0.5%/Ag dressings was almost completely closed after 12 days, whereas
766 the skin closure ratio of Hydrocoll® group (control) was $80.87 \pm 8.63\%$ (Figure 19(b)).
767 Statistical analysis indicated no significant differences among Hydrocoll®, AP:GE,

768 AP:GE@OTA5%, and AP:GE@OTA0.5%/Ag treated wounds, potentially attributed to the
769 substantial disparity in standard deviation. The esterified galacturonic acid residues on the AP
770 backbone provide a potent anti-inflammatory effect [75], while gelatin induces hemostasis
771 and supports cell attachment and proliferation, enhancing the wound healing process.
772 Additionally, AgNPs demonstrate strong antimicrobial properties, effectively eradicating
773 bioburden at the wound site, thereby promoting rapid healing. It was noted that the fabricated
774 dressings did not adhere to the wound throughout the application period, allowing for easy
775 removal without harming the skin. Additionally, wounds treated with AP:GE@OTA0.5%/Ag
776 films displayed enhanced tissue quality and reduced scarring (Figure 19(a)). Tummalapallia et
777 al. previously described the development of an in-situ hydrogel wound dressing based on
778 oxidized pectin-gelatin-nanosilver (OP-Gel-NS) [76]. The fundamental crosslinking
779 mechanism employed in the OP-Gel-NS systems mirrors that of the current AP:GE@OTA/Ag
780 systems. Thus, we aimed to compare the efficacy of these two hydrogel films in promoting
781 wound healing. Tummalapallia et al. employed AgNPs as the antimicrobial agent, resulting in
782 approximately 80% healing in 8 days when applied to full thickness excisional wounds on
783 C57BL/6J mice, compared to about 70% in control wounds treated with Bactigras®. On the
784 other hand, wounds treated with AP:GE@OTA0.5%/Ag exhibited a healing rate of
785 $86.59 \pm 2.35\%$ within the same timeframe. This comparison suggests that the efficacy of
786 polysaccharide-protein complexes as wound healing matrices, and for tissue regeneration, is
787 greatly affected by the incorporation of OTA and AgNPs as antimicrobial agents.

788 **Conclusions**

789 In summary, the biocompatible AP:GE@OTA/Ag hydrogel films, fabricated through an in-
790 situ crosslinking methodology exhibit significant promise in the realm of wound healing.
791 These biopolymeric dressings demonstrate superior mechanical strength, exceptional water
792 handling features, effective moisture management characteristics, and satisfactory moisture
793 management. The FTIR and XRD analysis testify to the formation of a new biopolymer
794 chemical structure without compatibility issues through the establishment of molecular bonds
795 with the used crosslinker (OTA). SEM/EDX analysis highlighted the formation of a compact
796 network structure and confirmed the successful incorporation of the AgNPs into the film as
797 well. Moreover, TG measurements demonstrated an increase in the thermal stability of the
798 prepared biofilms once the incorporation of the OTA crosslinker and AgNPs is performed.
799 Moreover, the in-situ incorporation of AgNPs as antibacterial agents achieves low
800 cytotoxicity and an excellent antibacterial effect, particularly at an AgNPs loading of $1.02 \pm$
801 $0.13 \mu\text{g}/\text{cm}^2$. NIT-1 mouse insulinoma cells displayed favorable cell viability when treated

802 with both AP:GE@OTA and AP:GE@OTA/Ag dressings. Finally, in-vivo experimentation
 803 revealed that the AP:GE@OTA0.5%/Ag facilitated rapid and thorough skin regeneration
 804 within 12 days, with no observable tissue toxicity in a Wistar albino rat model. Their efficacy
 805 was found better than that of a commercially available dressing (Hydrocoll®). Based on the
 806 above advantages, these nanobiocomposite dressings present promising prospects for
 807 effective wound management.

808

809 **List of tables**

810 **Table 1**

811 Number of moles of uncrosslinked amino groups ($\times 10^5$) in GE and AP matrices before and
 812 after covalent crosslinking, along with the degree of crosslinking determined by TNBS assay.
 813 The results present the mean of three determinations \pm standard deviation.

	Samples	Number of moles of free amine groups per gram of sample ($\times 10^5$)	Degree of crosslinking (%)
Before crosslinking	GE	31.8 ± 1.35	-
	AP	8.5 ± 0.75	-
	AP:GE	37.3 ± 1.51	-
After crosslinking	AP:GE@OTA0.5%	30.2 ± 1.50	19.03 ± 4.04
	AP:GE@OTA1%	21.3 ± 1.77	42.89 ± 4.76
	AP:GE@OTA2%	15.6 ± 1.00	58.52 ± 2.68
	AP:GE@OTA5%	8.1 ± 1.67	78.27 ± 4.47

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Table 2

Angular position of AgNPs diffraction peak, FWHM of the peak and derived particle size from XRD and SEM measurements.

2θ [°]	FWHM (dθ [°])	Particle size from XRD (nm)	Particle size from SEM (nm)
37.72	0.18	47.6	
43.96	0.130	68.8	
64.29	0.163	60.3	57
77.40	0.232	45.8	

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Table 3

Metal elements contained in AP:GE@OTA/Ag hydrogel films as determined by micro-X-ray fluorescence (μ XRF) elemental analysis. They were calculated considering detectable elements.

Hydrogel film	Element (wt.%)				Element (atomic %)			
	S	Ca	Cu	Ag	S	Ca	Cu	Ag
AP:GE	74.97	12.21	12.83	00	82.20	10.71	7.10	00
AP:GE@OTA0.5%/Ag	72.99	24.41	2.55	0.05	77.80	20.82	1.37	0.02
AP:GE@OTA1%/Ag	68.85	18.13	12.95	0.07	76.58	16.13	7.27	0.02
AP:GE@OTA2%/Ag	56.88	9.96	33.04	0.12	69.74	9.77	20.44	0.04
AP:GE@OTA5%/Ag	75.31	24.54	0.01	0.14	79.28	20.67	00	0.04

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Table 4
 Mechanical properties of the AP:GE@OTA/Ag hydrogel.

Hydrogel film	Thickness (mm)	Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)
AP:GE	0.25±0.07 ^a	17.22±3.50 ^a	45.86±3.43 ^a	838.69±50.74 ^a
AP:GE@OTA0.5%/Ag	0.27±0.07 ^a	23.11±3.35 ^{a,b}	37.73±1.19 ^b	1218.19±94.56 ^b
AP:GE@OTA1%/Ag	0.34±0.08 ^a	25.33±2.76 ^b	35.43±2.22 ^b	1347.15±46.63 ^c
AP:GE@OTA2%/Ag	0.37±0.08 ^a	30.07±3.49 ^b	33.98±0.96 ^b	1438.30±66.64 ^{c,d}
AP:GE@OTA5%/Ag	0.41±0.07 ^a	38.49±4.00 ^c	32.42±2.94 ^{b,c}	1512.98±67.82 ^d

In each column, means sharing the same superscript indicates non-significant differences. Conversely, means labeled with distinct letters indicate statistical differences (p-value < 0.05).
¹ Mean ± SD (n = 5).

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Table 5

Hemolytic assay of HRBCs after treatment with distilled water, PBS solution, AP/GE@OTA and AP:GE@OTA/Ag films for 2 h.

Sample	HP (%)
AP:GE	1.39±0.03
AP:GE@OTA0.5%	1.21±0.05
AP:GE@OTA5%	1.52±0.06
AP:GE@OTA0.5%/Ag	1.60±0.06
AP:GE@OTA5%/Ag	1.70±0.12

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Table 6

Initial silver loading in AP:GE@OTA/Ag dressings

Dressings	Silver content ($\mu\text{g}/\text{cm}^2$)	Percentage of silver content (% w/w)
AP:GE@OTA0.5%/Ag	1.02 ± 0.13	0.005
AP:GE@OTA1%/Ag	1.94 ± 0.15	0.009
AP:GE@OTA2%/Ag	2.63 ± 0.37	0.013
AP:GE@OTA5%/Ag	3.11 ± 0.76	0.015

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Table 7

Kinetic parameters obtained through fitting to several mathematical kinetic models of silver release profile in in SBF at 37°C, pH 7.4.

Formulation	First order model			Sahlin-Peppas model			Korsmeyer-Peppas model			
	$Mt / M_{\infty} = a[1 - \exp(-bt)]$			$Mt / M_{\infty} = K_1 t^m + K_2 t^{2m}$			$Mt / M_{\infty} = K_m t^n$			
	a	b	Adj.R ²	K ₁	K ₂	m	Adj.R ²	K _m	n	Adj.R ²
AP:GE@OTA0.5%	6.16723	0.02933	0.80695	1.7194	-0.10547	0.26841	0.95857	2.2285	0.15382	0.91708
	±	±		±	±	±		±	±	
	0.29314	0.00607		0.22957	0.02786	0.0282		0.23359	0.01654	
AP:GE@OTA1%	3.00107	0.03099	0.75389	0.92733	-0.06269	0.2503	0.97724	1.1443	0.14631	0.94322
	±	±		±	±	±		±	±	
	0.15122	0.00688		0.08727	0.01133	0.02089		0.09177	0.01273	
AP:GE@OTA2%	2.018	0.02313	0.84487	0.4683	-0.02381	0.29083	0.96	0.62932	0.17329	0.92168
	±	±		±	±	±		±	±	
	0.09607	0.00458		0.06948	0.00696	0.03059		0.07419	0.01834	
AP:GE@OTA5%	1.48905	0.01853	0.89895	0.27419	-0.01139	0.33161	0.96054	0.42413	0.18298	0.88856
	±	±		±	±	±		±	±	
	0.06163	0.00309		0.04184	0.0036	0.02859		0.06554	0.02387	

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Figures Captions

Figure 1. FTIR (a) and XRDs patterns (b) of PE and AP samples.

Figure 2. ¹H-NMR of the soluble PE and AP samples in D2O solvent.

Figure 3. Conversion of AP from pectin.

Figure 4. Digital images of fabricated hydrogel films.

Figure 5. Schematic Illustration of the formation mechanism of AP:GE@OTA/Ag hydrogel film.

Figure 6. Moles of free amino groups (●) and degree of crosslinking (■) in AP and GE matrices before and after crosslinking, as determined via TNBS analysis. Reaction time 30 min, reaction temperature 60°C, pH 5.5.

Figure 7. FTIR Spectra of (a) TA and OTA, (b) AP:GE, AP:GE@OTA5% and AP:GE@OTA5%/Ag films, (c) AP:GE and AP:GE@OTA/Ag hydrogel films.

Figure 8. The X-ray diffractograms of (a) AP:GE@OTA films (b) AP:GE@OTA/Ag films.

Figure 9. (a) SEM images of the surface of AP:GE and AP:GE@OTA/Ag hydrogel films and (b) EDX spectrum of AP:GE@OTA5%/Ag film, confirming the formation of AgNPs.

Figure 10. μXRF spectra validating the incorporation as well as the distribution of AgNPs in AP:GE@OTA5%/Ag hydrogel film.

Figure 11. Effect of OTA content on the tensile properties and elongation at break of AP:GE@OTA/Ag hydrogel films. Gauge length 50 mm; strain rate 50 mm/min.

Figure 12. TG (a) and DTG (b) thermograms of AP:GE and AP:GE@OTA/Ag hydrogel films at heating rate 10 °C/min under nitrogen (100 ml/min).

Figure 13. Fluid uptake ability (a), water retention capacity (b), and water vapor transmission rate (c) of AP:GE@TA/Ag hydrogel films. *p-value < 0.05.

Figure 14. Antimicrobial evaluation by zone of inhibition of AP:GE@OTA and AP:GE@OTA/Ag dressings against E.coli and S.aureus.

Figure 15. Blood coagulation index (BCI) of AP:GE@OTA0.5%. AP:GE@OTA5%. AP:GE@OTA0.5/Ag. AP:GE@OTA5%/Ag and HYDROCOLL® (as a control).

1081 **Figure 16.** Silver release profiles from AP:GE@OTA/Ag Dressings in SBF at 37°C, pH 7.4.

1082 **Figure 17.** The silver release kinetics model fitting curves: (a) first-order kinetics model; (b)
1083 Sahlin-Peppas model; and (c) Korsmeyer-Peppas model.

1084 **Figure 18.** (a) In-vitro cell viability analysis assessed by XTT assay against NIT-1 mouse
1085 insulinoma cells after 72h. and (b) Cell morphology of NIT-1 after 72h treated
1086 with AP:GE@OTA and AP:GE@OTA/Ag dressings at 100 µg/ml. Images were
1087 taken with a Nikon eclipse Ts2 phase contrast microscope (20X magnification).

1088 **Figure 19.** (a) Images of the wound healing process at different time intervals. Wounds
1089 treated with Hydrocoll® (commercial product) served as the positive control
1090 groups. Full-thickness round wounds (diameter = 3 mm) were created on the
1091 cervicodorsal area of Wistar albino rats, (b) wound size reduction with time.

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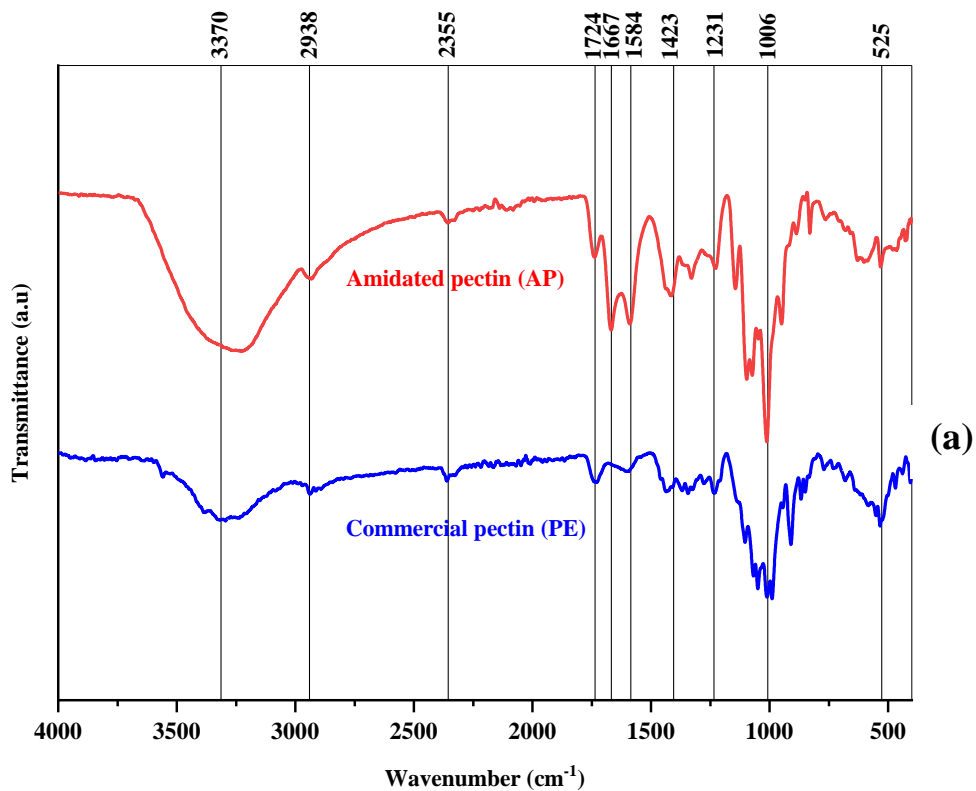
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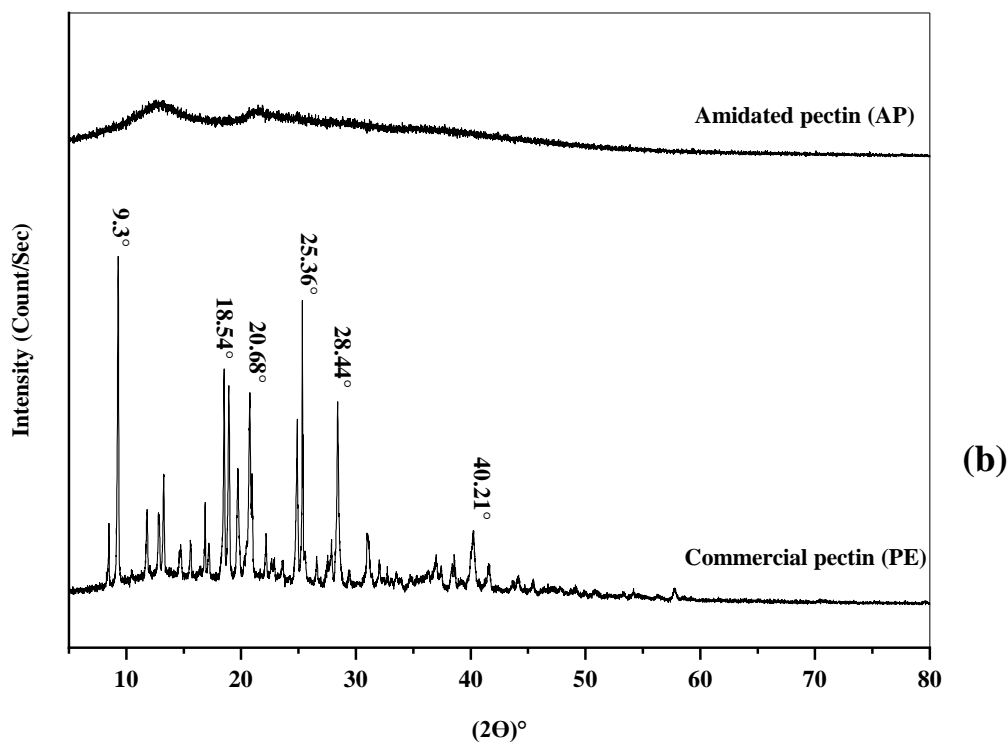
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Figure 1. FTIR (a) and XRDs patterns (b) of PE and AP samples.

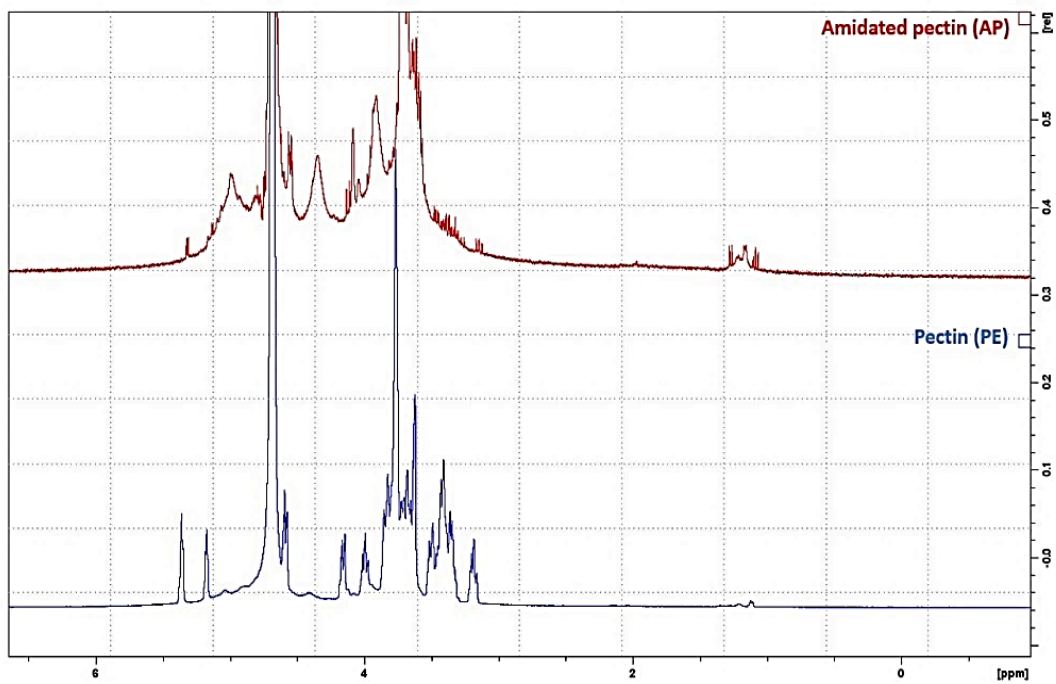


Figure 2. ¹H-NMR of the soluble PE and AP samples in D₂O solvent.

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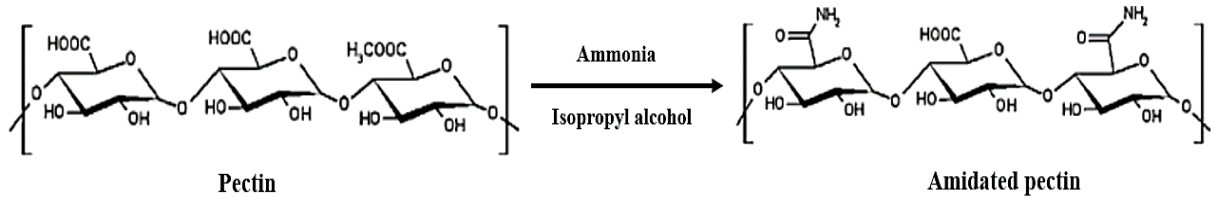
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Figure 3. Conversion of **AP** from pectin.

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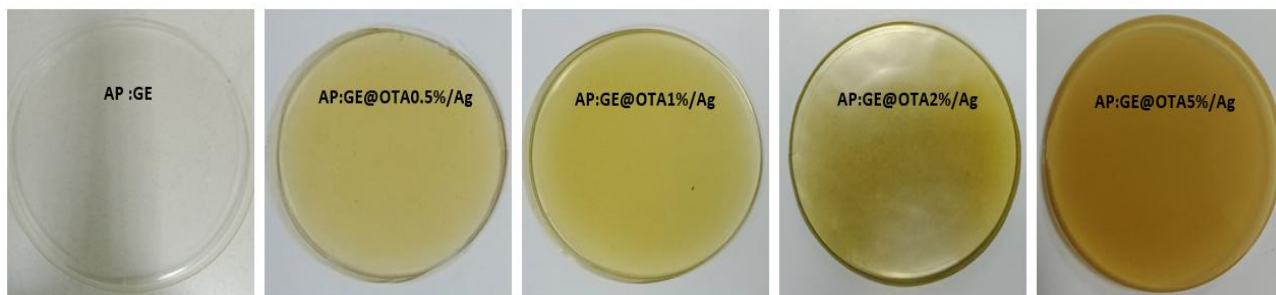
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Figure 4. Digital images of fabricated hydrogel films.

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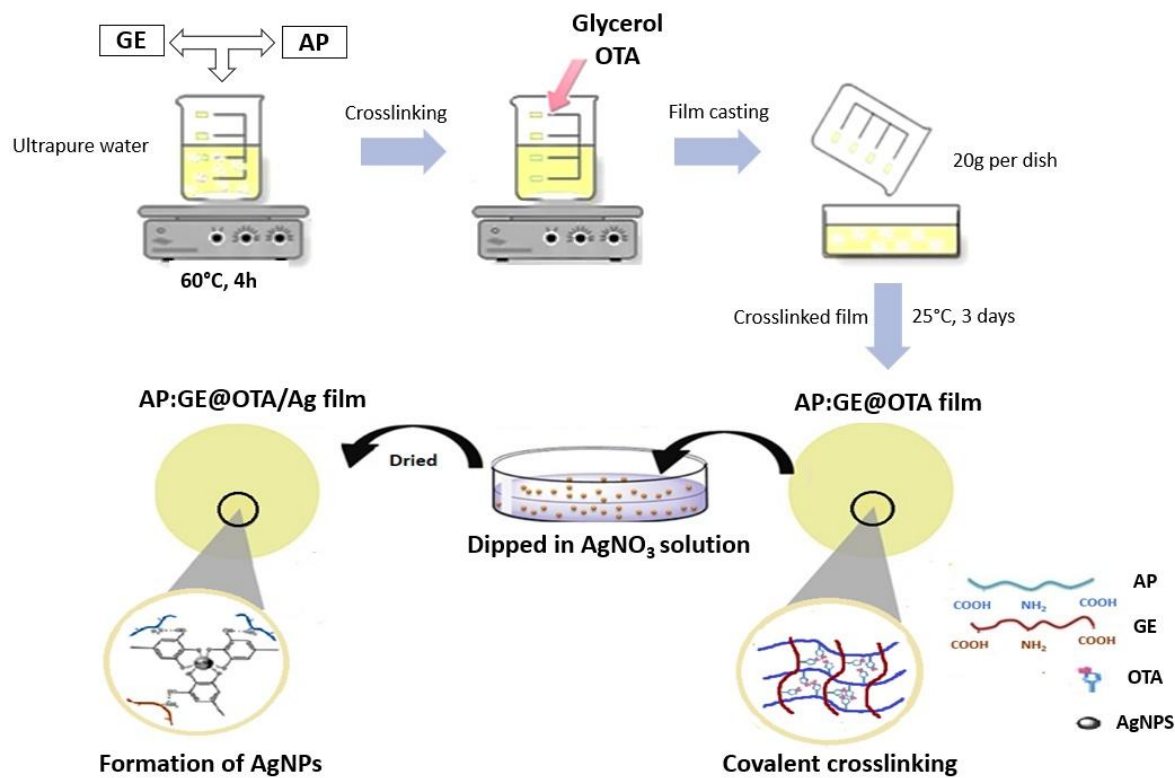
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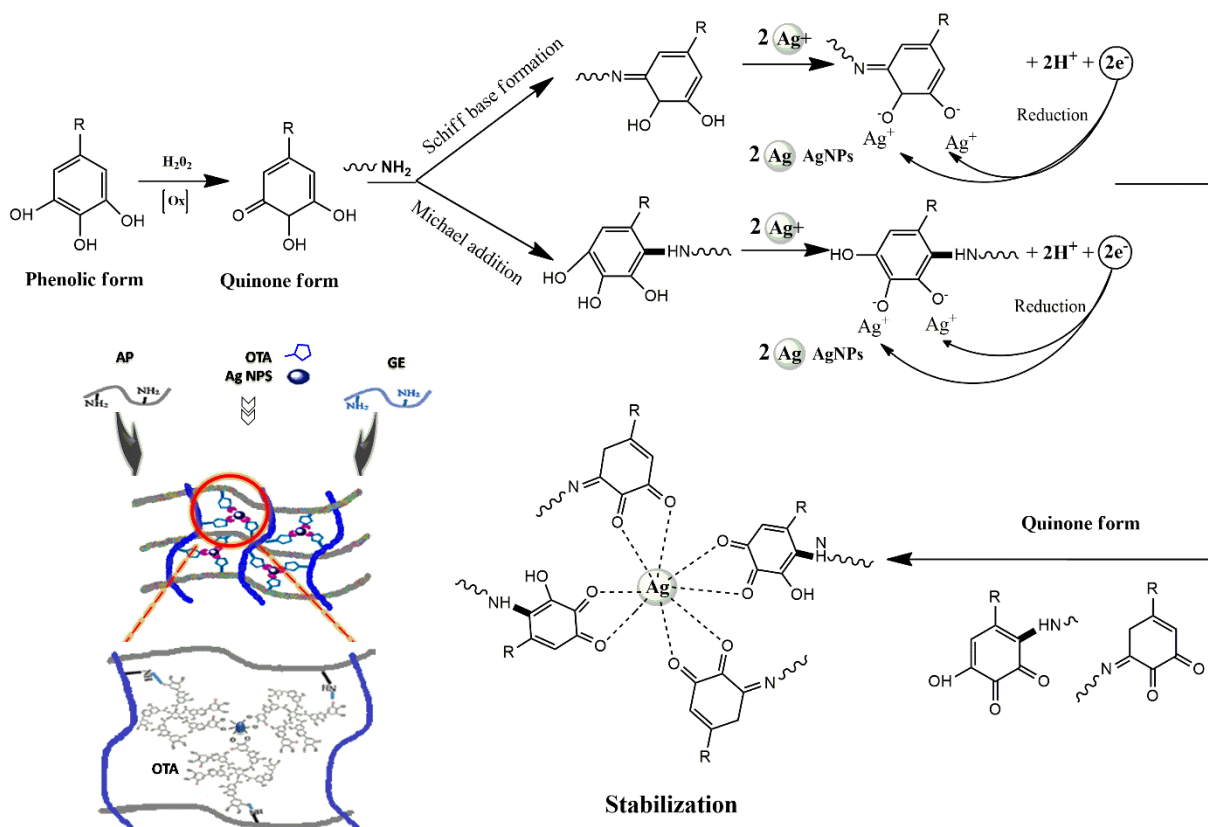
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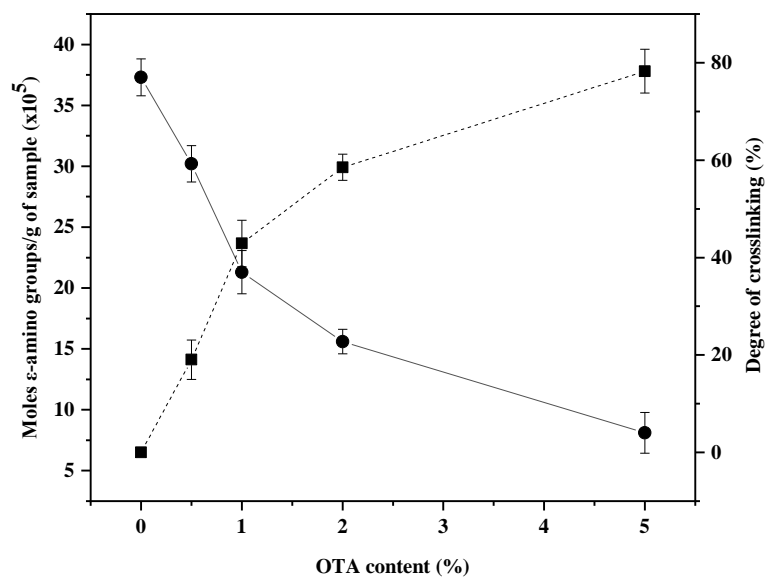


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1194 **Figure 5.** Schematic illustration of the formation mechanism of AP:GE@OTA/Ag hydrogel film.

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 1200 and after crosslinking, as determined via TNBS analysis. Reaction time 30 min, reaction temperature
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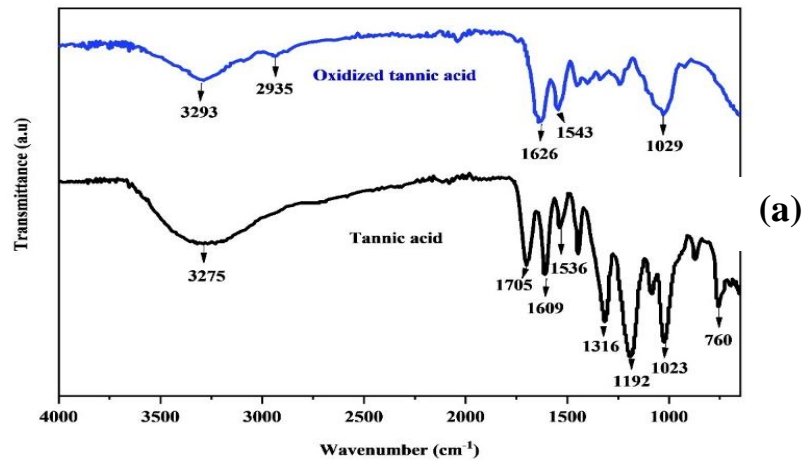
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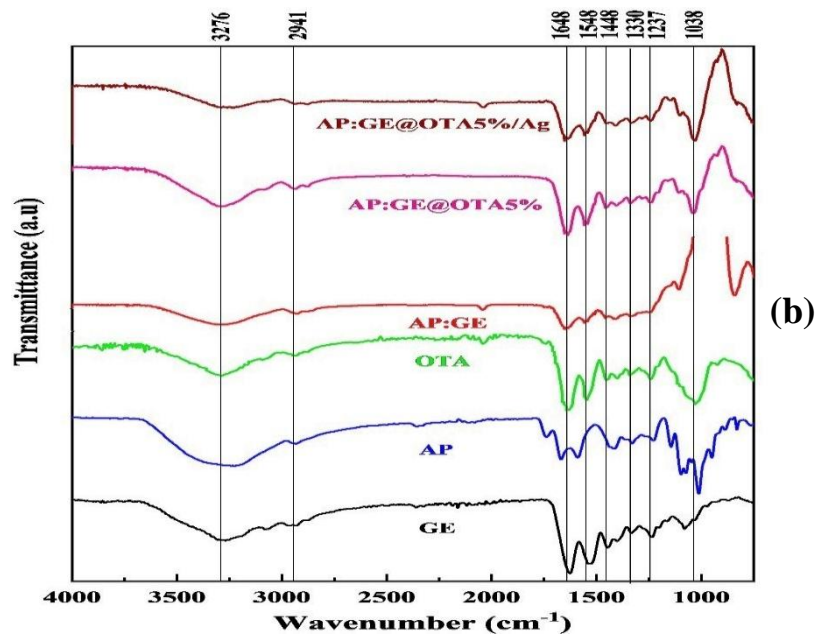
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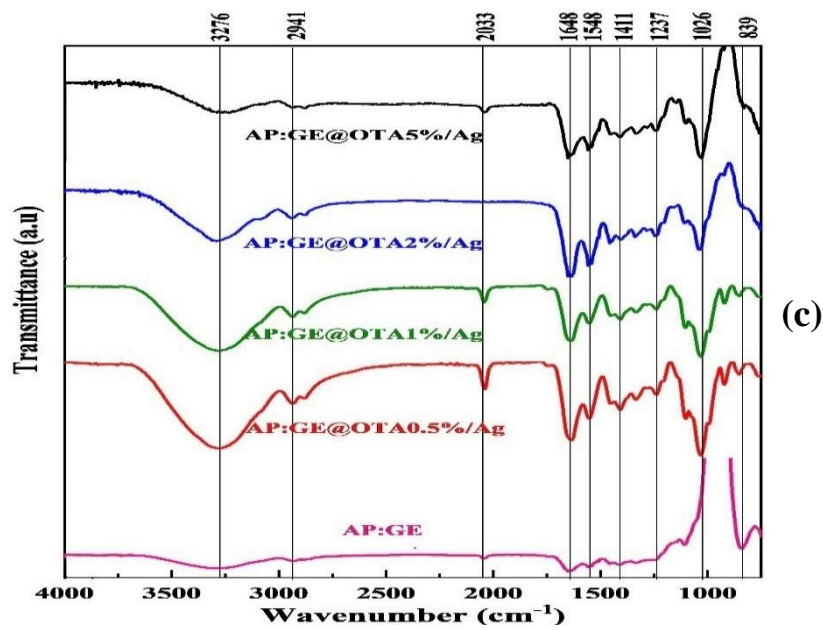
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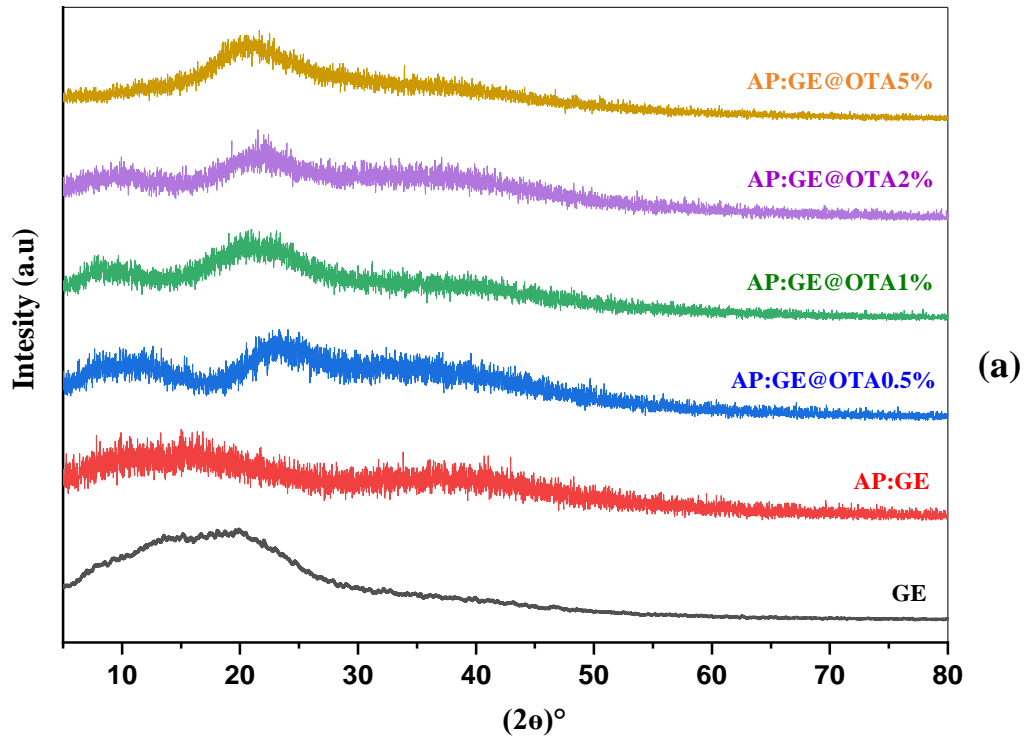


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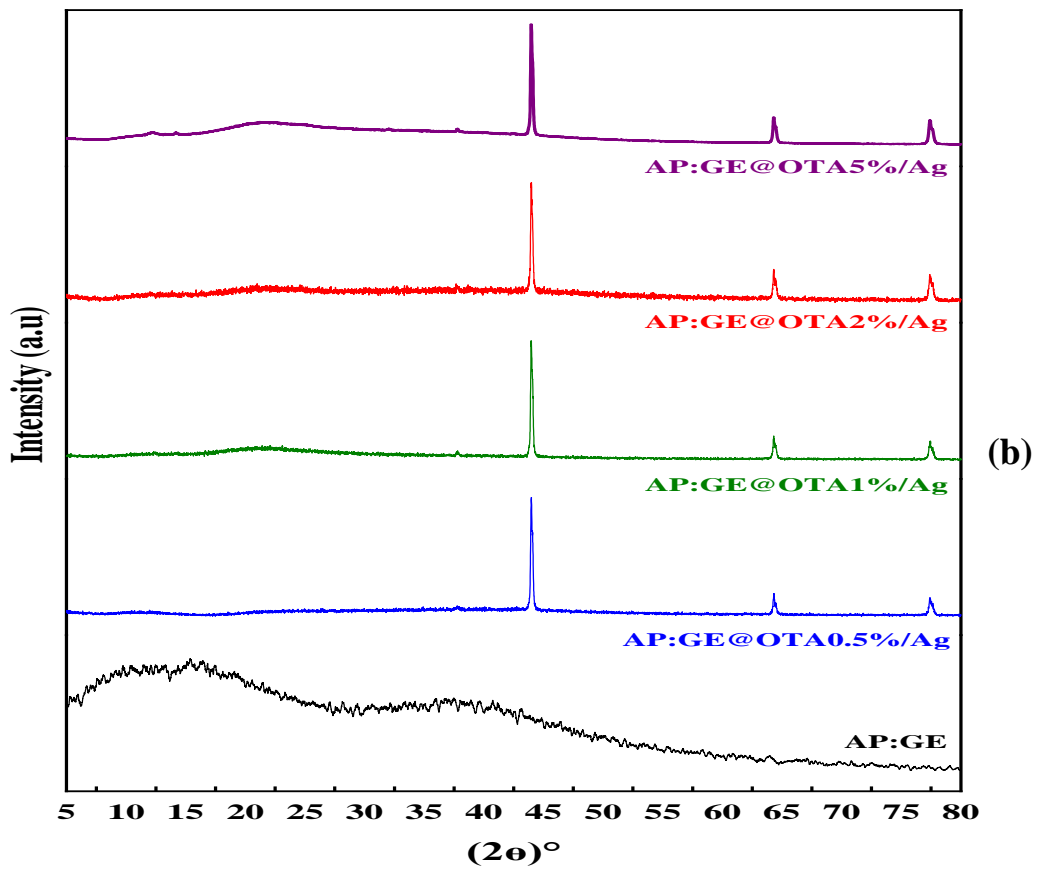


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Figure 7. FTIR Spectra of (a) TA and OTA, (b) AP:GE, AP:GE@OTA5% and AP:GE@OTA5%/Ag films, (c) AP:GE and AP:GE@OTA/Ag hydrogel films.



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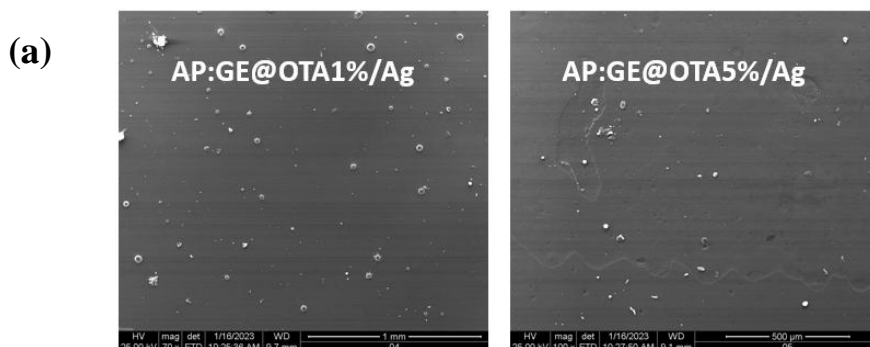
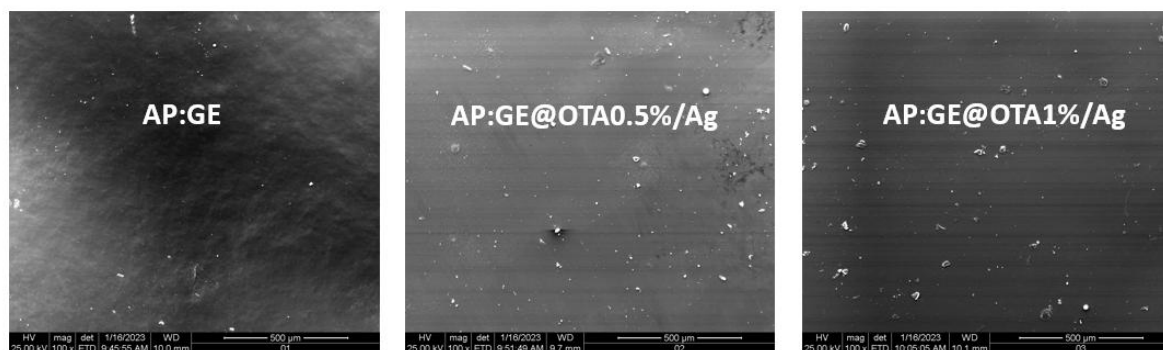


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1215 **Figure 8.** The X-ray diffractograms of (a) AP:GE@OTA films (b) AP:GE@OTA/Ag films.

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(b)

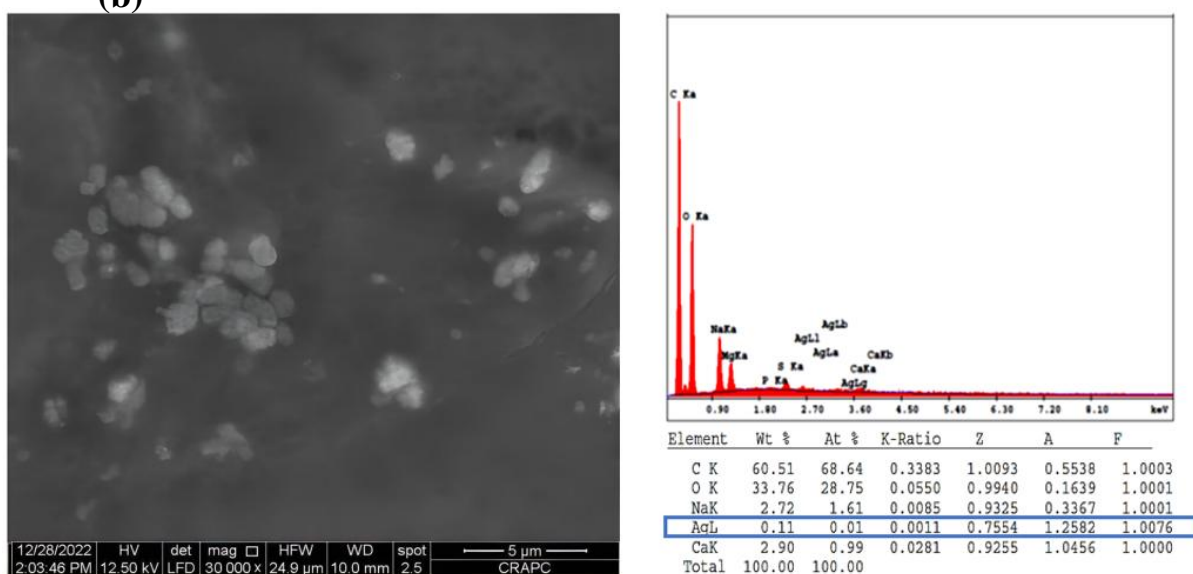
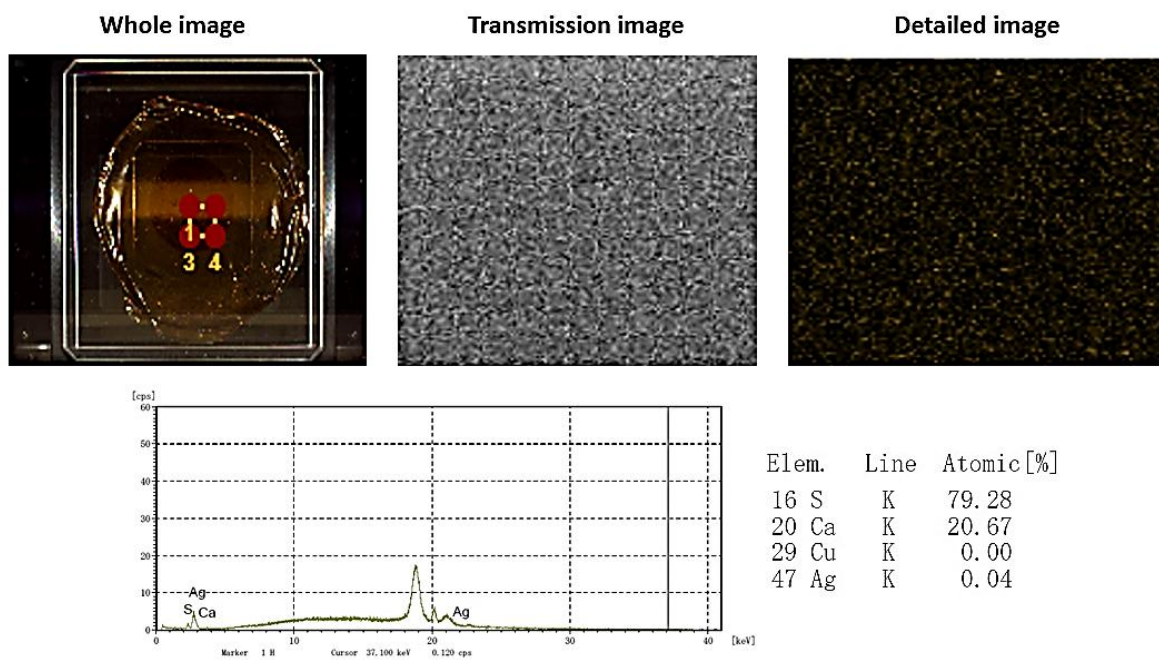


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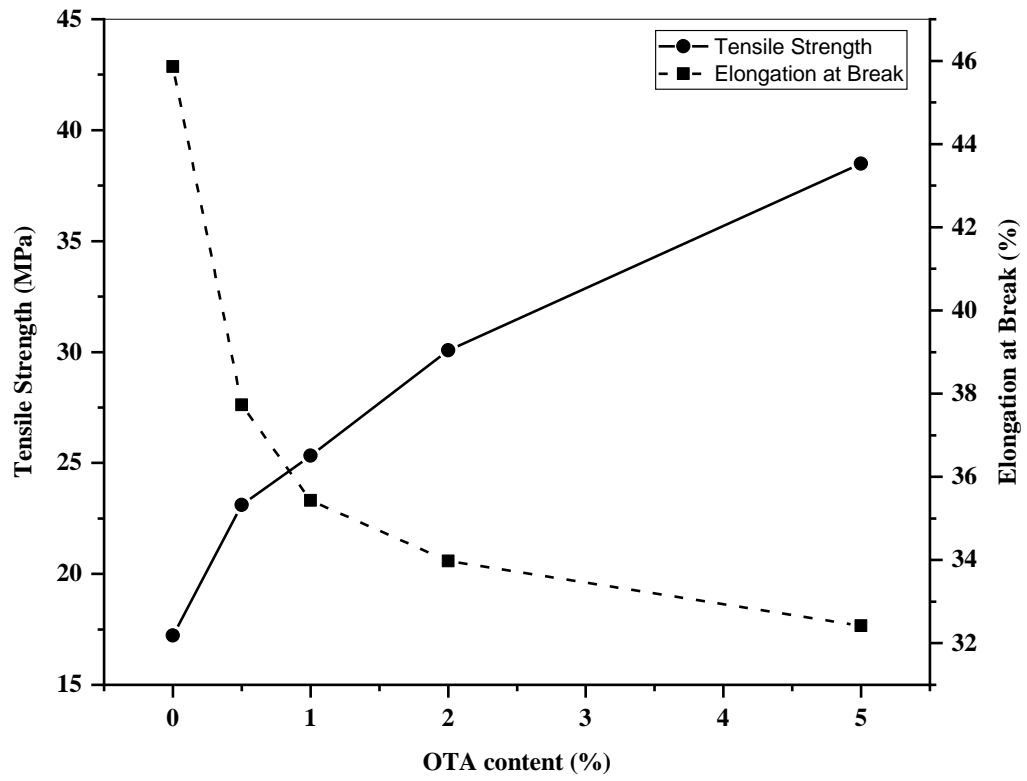
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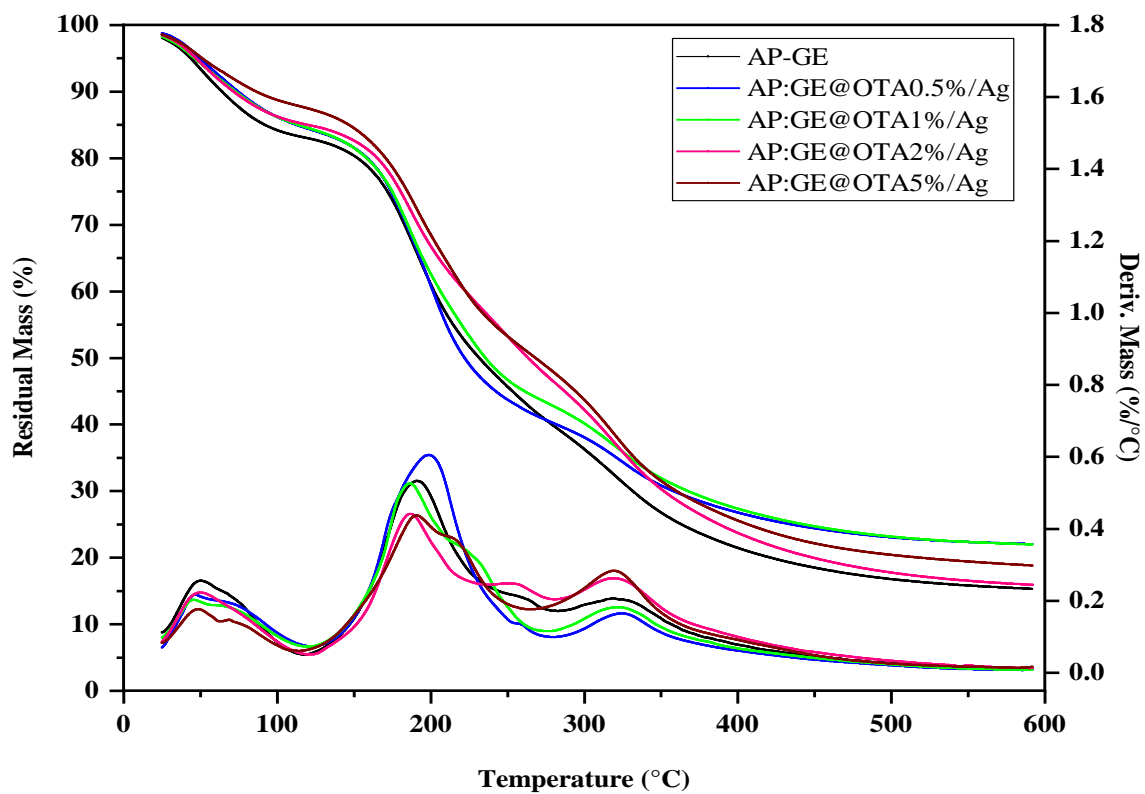
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1239 **Figure 11.** Effect of OTA content on the tensile properties and elongation at break of AP:GE@OTA/Ag hydrogel films. Gauge length 50 mm; strain rate 50 mm/min.

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1242 **Figure 12.** TG (a) and DTG (b) thermograms of AP:GE and AP:GE@OTA/Ag hydrogel films at
1243 heating rate 10 °C/min under nitrogen (100 ml/min).

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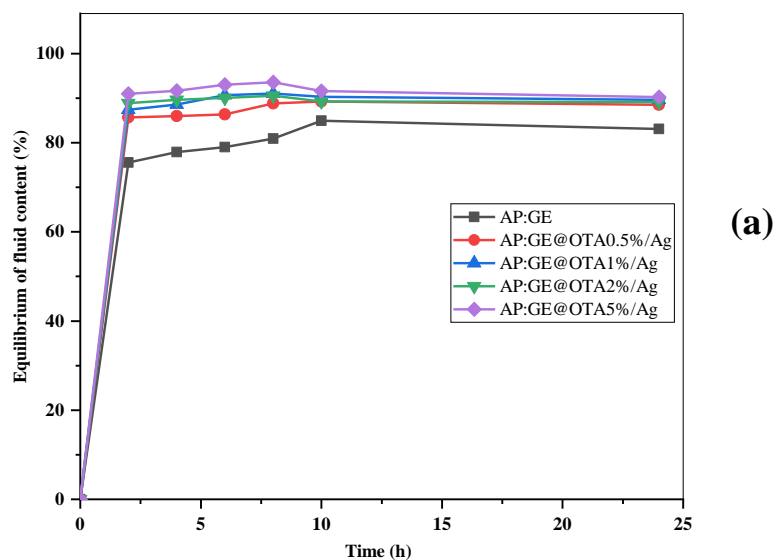
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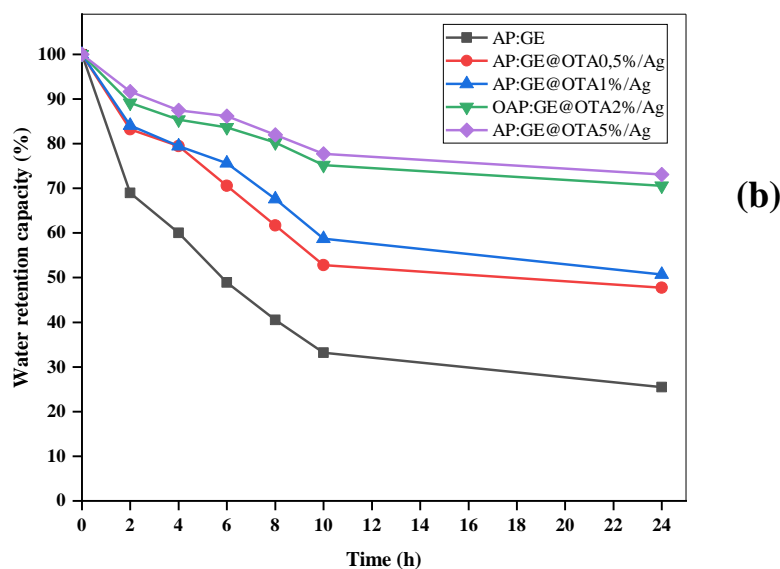
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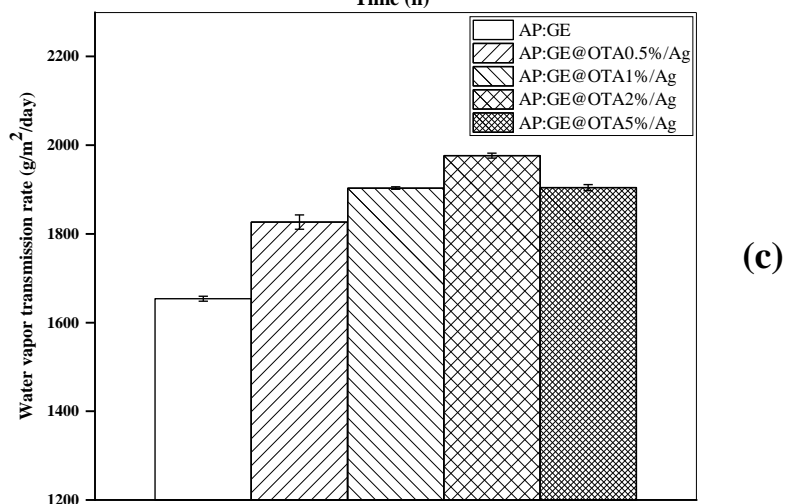
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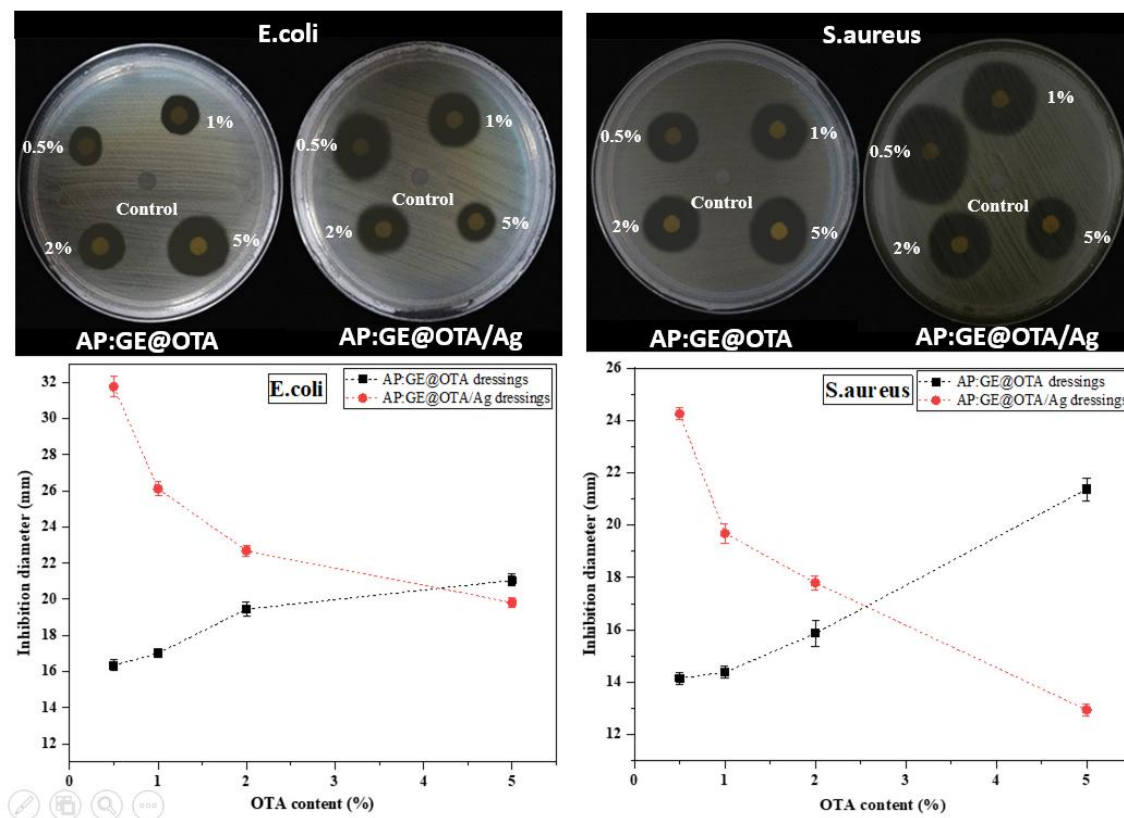
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1261 **Figure 13.** Fluid uptake ability (a), water retention capacity (b), and water vapor transmission rate
1262 (c) of AP:GE@TA/Ag hydrogel films. *p-value < 0.05.

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Figure 14. Antimicrobial evaluation by zone of inhibition of AP:GE@OTA and AP:GE@OTA/Ag dressings against *E. coli* and *S. aureus*.

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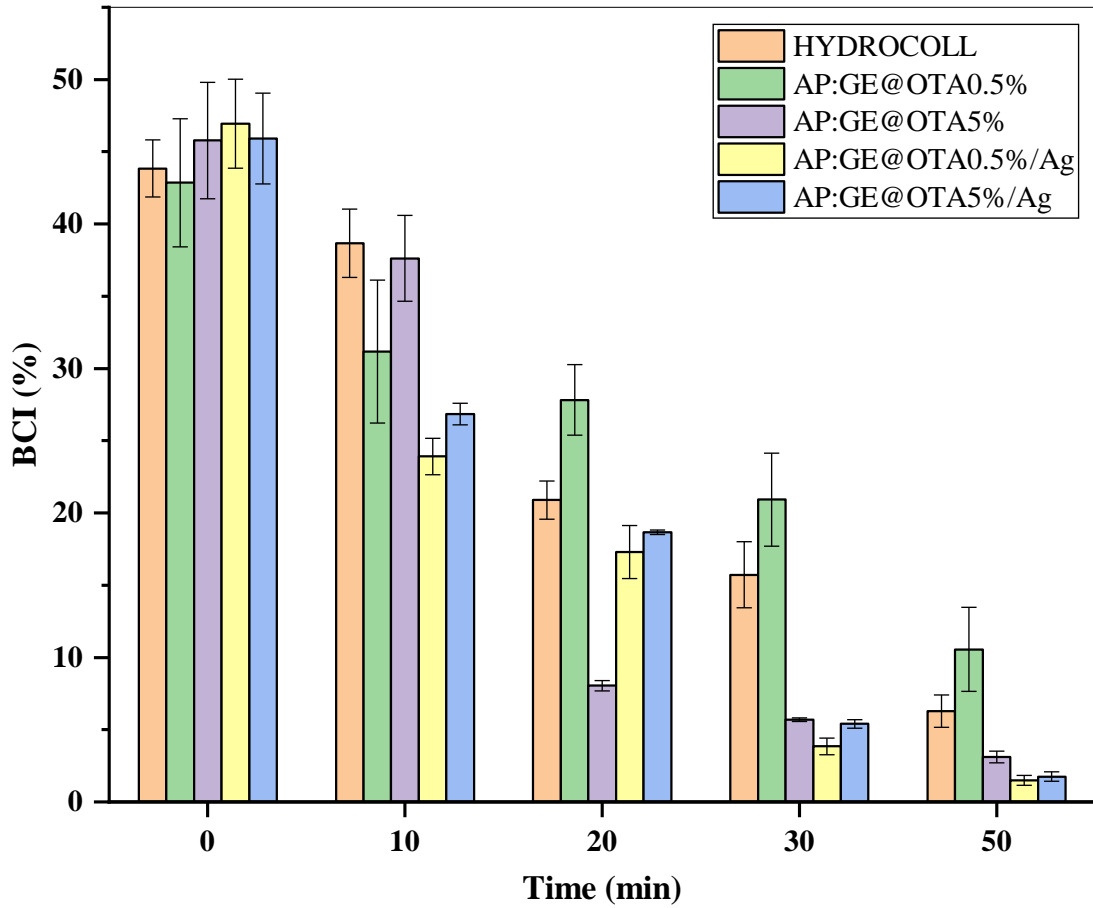
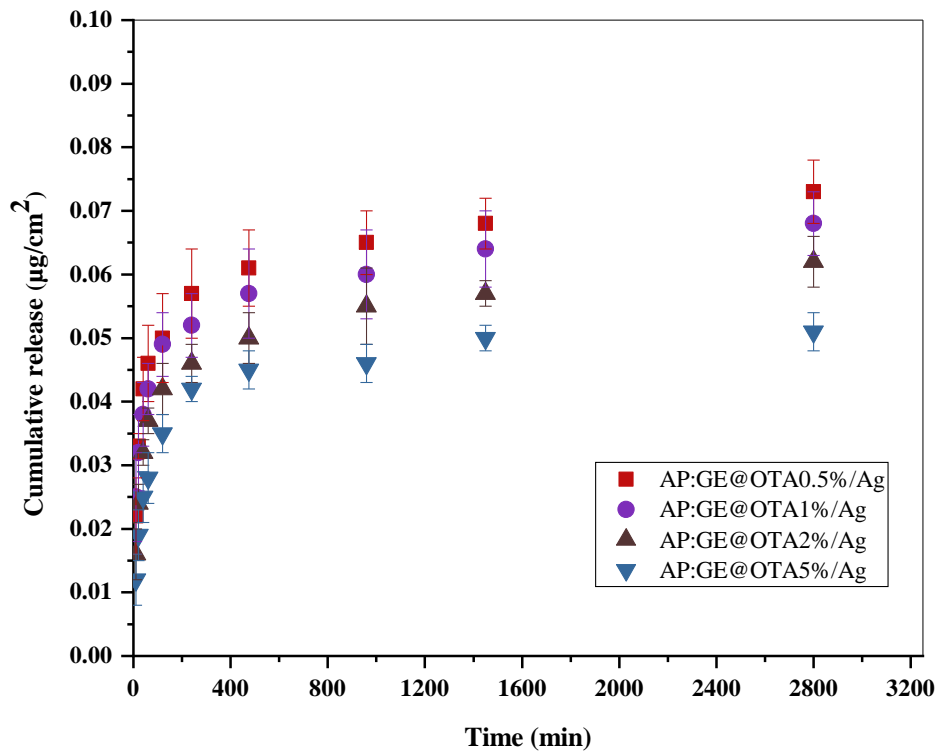


Figure 15. Blood coagulation index (BCI) of AP:GE@OTA0.5%. AP:GE@OTA5%. AP:GE@OTA0.5%/Ag. AP:GE@OTA5%/Ag and Hydrocoll® (as a control).

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1295 **Figure 16.** Silver release profiles from AP:GE@OTA/Ag Dressings in SBF at 37°C, pH 7.4.

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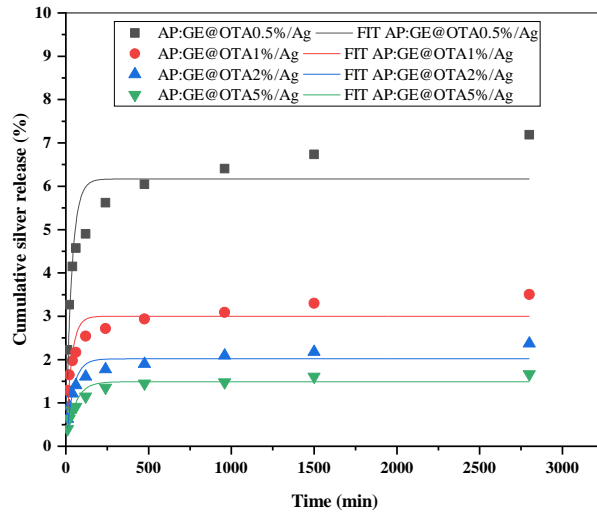
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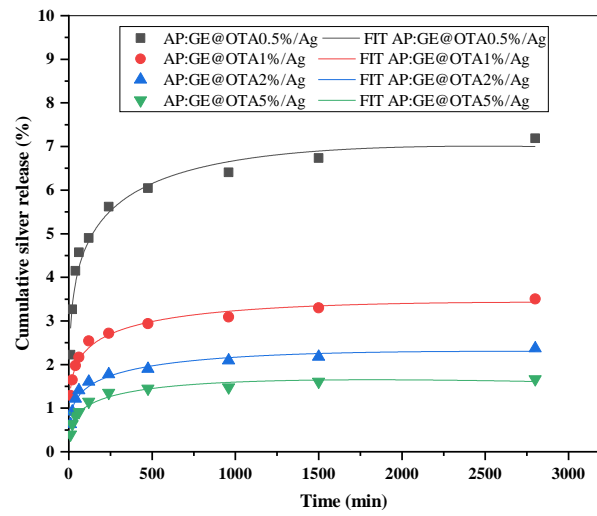
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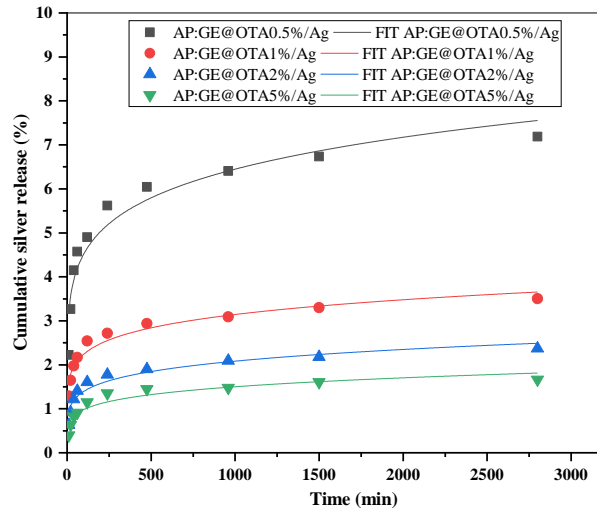
(a)

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(b)

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(c)

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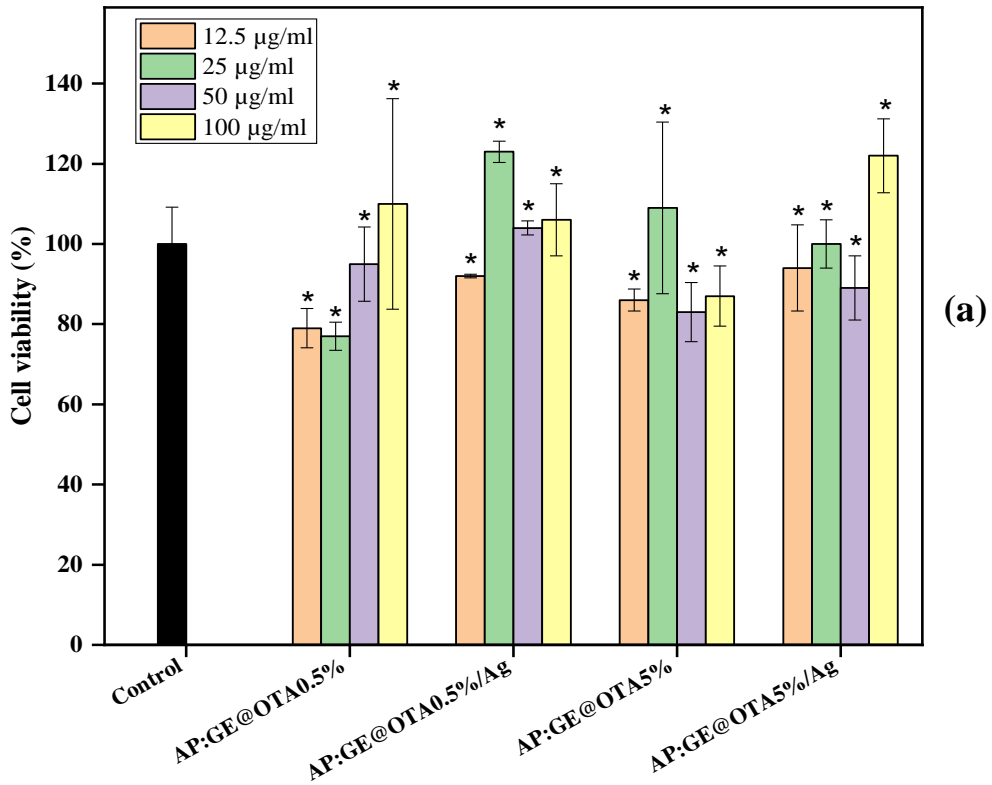
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Figure 17. The silver release kinetics model fitting curves: (a) first-order kinetics model; (b) Sahlin-Peppas model; and (c) Korsmeyer-Peppas model.

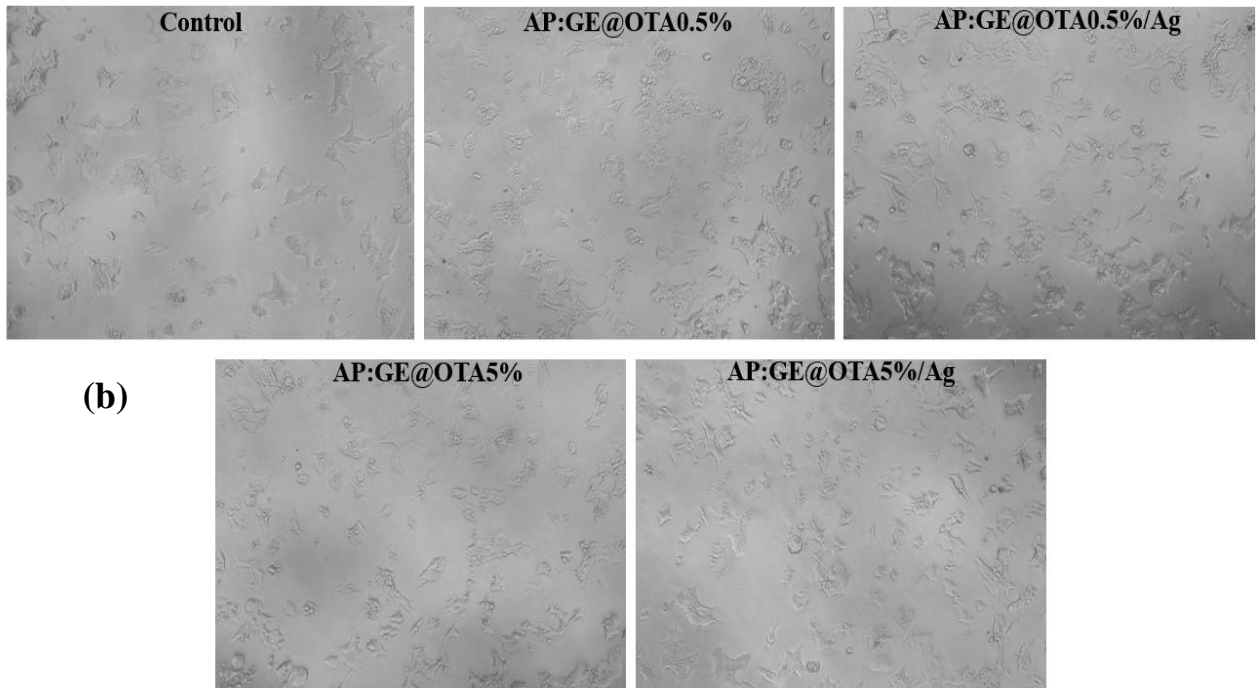
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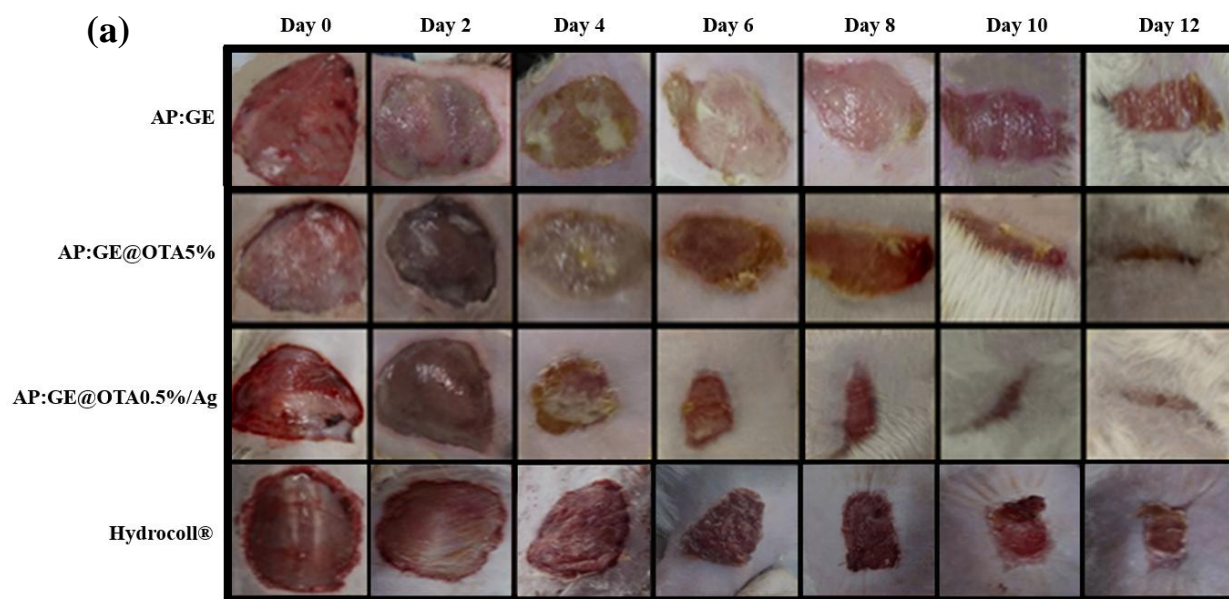
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Figure 18. (a) In-vitro cell viability analysis assessed by XTT assay against NIT-1 mouse insulinoma cells after 72h. and (b) Cell morphology of NIT-1 after 72h treated with AP:GE@OTA and AP:GE@OTA/Ag dressings at 100 µg/ml. Images were taken with a Nikon eclipse Ts2 phase contrast microscope (20X magnification).

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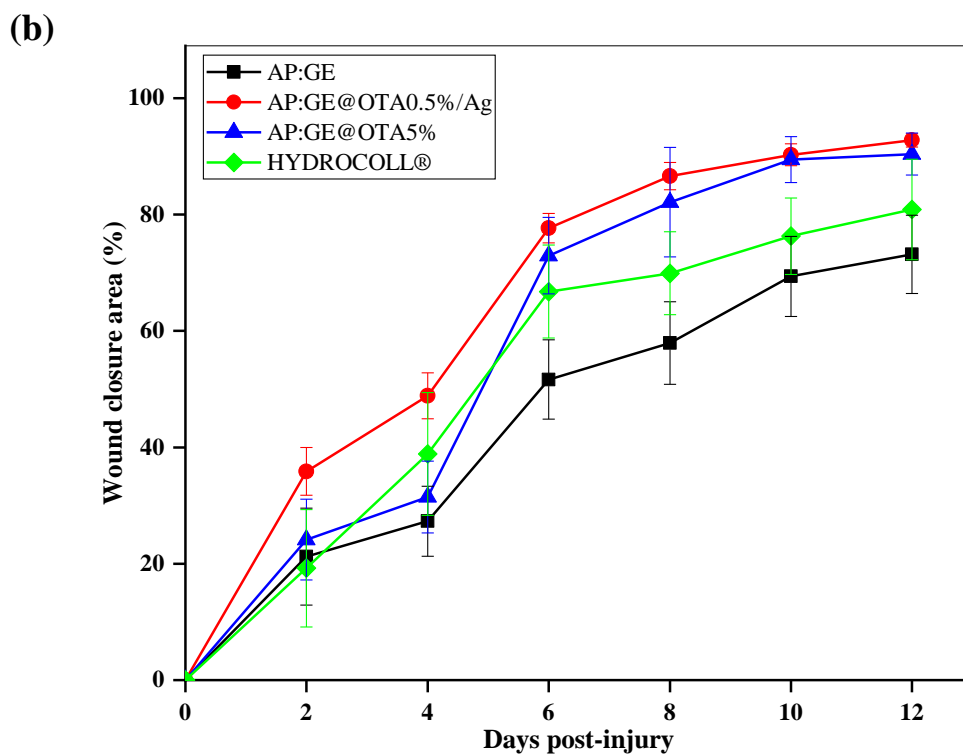


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Figure 19. (a) Images of the wound healing process at different time intervals. Wounds treated with Hydrocoll® (commercial product) served as the positive control groups. Full-thickness round wounds (diameter = 3 mm) were created on the cervicodorsal area of Wistar albino rats, (b) wound size reduction with time.

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