Effect of Ethylene Oxide Sterilization on Humic Acid Modified Gelatin-Alginate Hydrogels

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Abstract: The work is devoted to the clarification of the preservation of humic acid biomolecules encapsulated in biopolymer gelatin-alginate hydrogels during conventional sterilization with ethylene oxide (EO). According to SEM and EDS data, immediately after fabrication dried biopolymer hydrogels have smooth surface, layered structure and relatively uniform distribution of elements inherent in biopolymers C, O, N, and Na across all samples. According to the microbiological experiments, all of them contain a small number of fungi, but the contamination of Hana 2.5 and Hana 5 hydrogels with *E. coli* is high due to the encapsulated Hana biomolecules. The morphology of the biopolymer hydrogels was partially damaged but not completely destroyed after *E. coli* contamination and ethylene oxide sterilization according to SIST EN 550:2000. Moreover, microbiological tests for the detection of *E. coli* after ethylene oxide sterilization revealed the persistence of these bacteria due to the encapsulation with Gal, Nana 2.5 and Hana 5 hydrogels. Microbiological tests have shown sufficient resistance of the microbiota encapsulated inside gelatin-alginate biopolymer hydrogels to EO sterilization. In addition, it has been experimentally confirmed that the gelatin-alginate hydrogels modified with humic acids that we developed mainly retain their morphology and chemical composition during this sterilization.

Keywords: Biopolymer hydrogel, sterilization, encapsulation, humic acids, gelatin, sodium alginate.

1. INTRODUCTION

Hydrogel materials are widely used for encapsulation of biological molecules in controlled drug delivery to protect them from degradation and/or immune recognition [1-3]. According to [4-6], hydrogel encapsulation is a promising method for drug delivery due to its ability to protect encapsulated objects from harsh physiological conditions and increase their therapeutic efficacy and biocompatibility. Among them, non-toxic and highly bio-friendly biopolymer hydrogels may be particularly useful for creating systems that will deliver desired therapeutic agents in a controlled manner by encapsulating bioactive drugs that will be released from these systems [7,8]. Biosafe biopolymer hydrogels based on natural polymers such as chitosan, alginate, gelatin are well compatible with tissues, create a moist healing microenvironment and minimize

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the local inflammatory response, which reduces pain and accelerates regeneration [9,10]. They work as platforms for the controlled release of bioactive agents (antiseptics, anti-inflammatory compounds, growth factors, cells), maintaining therapeutic concentrations locally and reducing the risk of systemic toxicity [11]. For example, dietary supplementation of humic acids (Hana) for medicinal and health-promoting purposes is deeply rooted in cultural traditions as it has profound effects on the healthy colonic microbiome [12]. In our recent works [13,14] Hana was used as a modifier of thermosensitive, hemostatic and wound healing gelatin-alginate biopolymer hydrogels. When using composite biomaterials, including for hemostasis and wound healing, terminal sterilization is a crucial step [15-18]. The problem is that, according to [15-17], sterilization of biopolymer hydrogels is challenging due to the sensitivity of this type of materials to common sterilizing agents such as gamma radiation and heat. In addition, each biopolymer system requires individual testing to select the suitable and effective sterilization method, which will keep the basic properties unchanged [15,16]. When sterilizing hydrogels to release the active compound/drug, the ability of the hydrogels and the drugs/molecules contained therein to withstand sterilization processes is of primary importance [15]. When it comes to preserving the biomolecules encapsulated in these hydrogels, it is important to know how sterilization affects their morphology and properties. At the same time, according to data [15,16,18], the influence of sterilization methods on the internal properties of systems based on biopolymer hydrogels has not been sufficiently studied and further research and careful selection of a suitable sterilization method are necessary.

Sterilization with ethylene oxide (EO) gas is an effective method for sterilizing a wide range of materials including plastics, rubber, textiles and electronics without causing damage from heat or moisture [19,20]. Validation of EO sterilization processes, which includes physical and microbiological performance qualification, is described in detail in the European Standard (EN) SIST EN 550:2000 Sterilization of health care products - Validation and routine control of ethylene oxide sterilization. Recently, great success has emerged in hydrogels modified with humic acids (HA), which demonstrate improved antibacterial potency, increased stability, and the ability to regulate thermoresponsive behavior and fluidity polished exudate. Any hydrogel used for clinical stagnation may undergo sterilization, and this very stage often causes degradation of the polymer structure, change in mechanical properties and destruction functionality of the material [13,14]. EO sterilization is one of the most extensive methods for temperature-sensitive germs and is used for sterilization of over 20 billion medical devices in the world [18]. For hydrogels, EO is a potentially delicate method, which may cause excessive toxicity, as well as chemical modification of polymers, including partial destruction or additional cross-linking. However, there is no information in the modern literature on the use of EO sterilization for biopolymer hydrogels, especially for humic acid-modified gelatin-alginate hydrogels [21]. Thus, this work is devoted to studying the effect of ethylene oxide sterilization according to the conditions of the SIST EN 550:2000 standard on micromorphology, elemental composition, and assessment of the presence and levels of microorganisms in the initial hydrogels, as well as after contamination using a microbiological McFarland test and after EO sterilization.

2. MATERIALS AND METHODS

Similarly to what was described in [22,23], in this work we used food gelatin (GN) and sodium alginate (SA) to prepare the gelatin-alginate base of all hydrogels. For their modification, Hana humic acids were obtained by extraction from lignite with a solution of tetrasodium pyrophosphate $Na_4P_2O_7$ and further extraction with 1 wt.% NaOH followed by precipitation with HCl. The Hana extraction method consisted of the stages described in detail in [24].

The preparation of hydrogels was carried out in accordance with the procedure described in [28,29]. The appropriate amount of gelatin was placed in distilled water, preheated to 90 ± 2°C, and stirred in a water bath during 30 minutes using a VEVOR 85-2 magnetic stirrer until a pure GN sol was obtained. Then dry sodium alginate SA was added to the GN sol and stirred with a VEVOR 85-2 magnetic stirrer with a heating plate until a homogeneous gelatin-alginate sol, called Gal, was obtained. Before adding to the Gal sol, the Hana humic acids were partially dissolved in an aqueous alkaline solution of 1 wt. % NaOH. Table **1** shows the percentage ratio of the components without indicating the water content in the studied sols/hydrogels.

Then, similarly to that described in [27,28], 1.8 g of each sol/hydrogel was naturally dried in a special mold

Table 1: Biopolymer Hydrogels Studied in this Work

Hydrogel symbol	Hydrogel Composition, Wt. (SD)		
	GN	SA	Hana
Gal	14	6.4	-
Hana2.5	14	6.4	2.5
Hana5	14	6.4	5

for 48 h, obtaining dry hydrogel washers with a diameter of ~1.5 cm and a height of ~0.1 cm. These washers were cut into pieces and used in experiments to determine the initial sterility of hydrogels and in experiments to sterilize hydrogels with ethylene oxide. The samples were first weighed before the experiment, then placed in 10 ml of aqueous phosphate-buffered saline (PBS) (pH 7.2) containing sodium chloride, potassium chloride, potassium dihydrogen phosphate, and disodium hydrogen phosphate, and gently shaken in a Falcon 50 ml tubes for 2 h, 135 RPM, using Benchmark scientific mixer under the room temperature. The weights of the hydrogels before and after washing in PBS are shown in Table **2** [29].

To assess the initial sterility of the hydrogel samples, the Plate Count Method was employed in accordance with the USP 43-NF 38 standards of with the microbiological examination of nonsterile products. Samples were subjected to serial decimal dilutions ranging from 10^{-1} to 10^{-}□ and analyzed using the pour plate technique. For each dilution, 1 mL was aseptically transferred and plated under the agar. The agar plates were incubated at 37 °C for 18–24 hours under aerobic conditions. Two types of culture media were utilized for microbial enumeration: Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) (1). The control group (Ctr1) consisted of sterile phosphate-buffered saline (PBS) and just agar as a negative control (Ctr).

The volume of colony-forming units (CFU) in 1 ml was calculated as follows:

$$\frac{CFU}{ml} = \left(\frac{\text{NUMB.COLON.} \cdot \text{DIL.FACT.})}{\text{VOL.PLAT.(IN ML.)}}\right), \quad (1)$$

The colony-forming unit (*CFU*) counts obtained from the microbial analysis are presented in Table **3**.

Table 2: Weight of Hydrogels Before and After Washing in PBS

Hydrogel	Hydrogel weight, g			
	Initial	After washing in PBS and drying overnight in an incubator at 30°C	Before sterilization process	In sterile Petri dish before EO sterilization
Gal	0.21±0.01	0.19±0.01	0.09±0.01	0.072±0.001
Hana2.5	0.23±0.01	0.21±0.01	0.10±0.01	0.074±0.001
Hana5	0.12±0.01	0.09±0.01	0.10±0.01	0.085±0.001

Table 3: Data on Initial Contamination of Biopolymer Hydrogels

Hydrogel and concentration	Medium	Colony-forming units results, CFU/mL (SD)		
		First plate	Second plate	Third plate
Gal (10)	TSA (*E. coli*)	1±0.1	1±0.1	1±0.1
Gal (10^{-1})		0	0	0
Hana 2.5 (10)		478±0.1	474±0.1	498±0.1
Hana 2.5 (10^{-1})		40±0.1	44±0.1	48±0.1
Hana 5 (10)		2±0.1	2±0.1	3±0.1
Hana 5 (10^{-1})		0	0	0
Gal (10)	SDA (fungi)	6±0.1	14±0.1	11±0.1
Hana 2.5 (10)		2±0.1	3±0.1	4±0.1
Hana 5 (10)		30±0.1	27±0.1	33±0.1

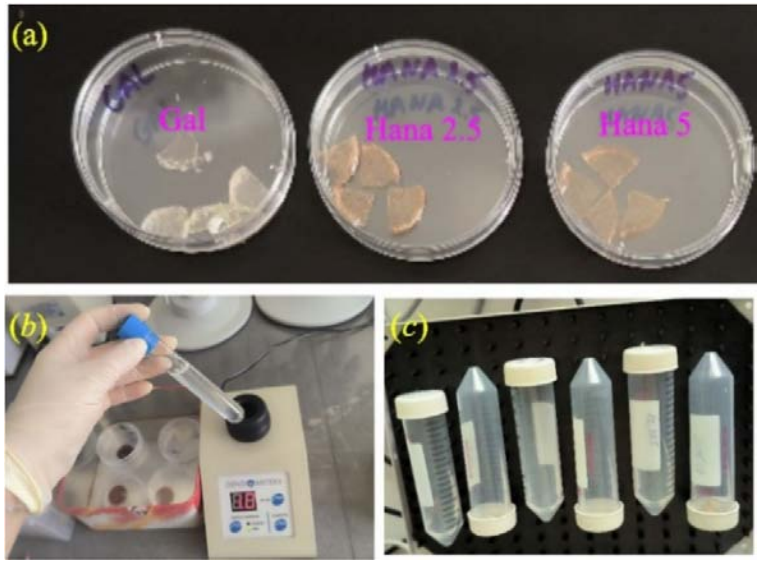

Figure 1: Photographs of cut-in-piece biopolymer hydrogel washers in sterile Petri dishes before the experiment (**a**), to which 10 ml of PBS buffer was added (**b**) and placed in Falcon tubes (**c**).

Figure **1a** shows photographs of the samples used in the pre-sterilization process, in the form of cut-in-piece biopolymer hydrogel washers weighed into a sterile Petri dish before the experiment, the weights of which are presented in Table **2**. Then they were placed in Falcon tubes as shown in Figure **1b** and **1c** and then 10 ml of PBS buffer was added to each and shaken gently at 135 rpm for 2 hours on a Benchmark scientific mixer. Before sterilization with ethylene oxide according to the conditions of the SIST EN 550:2000

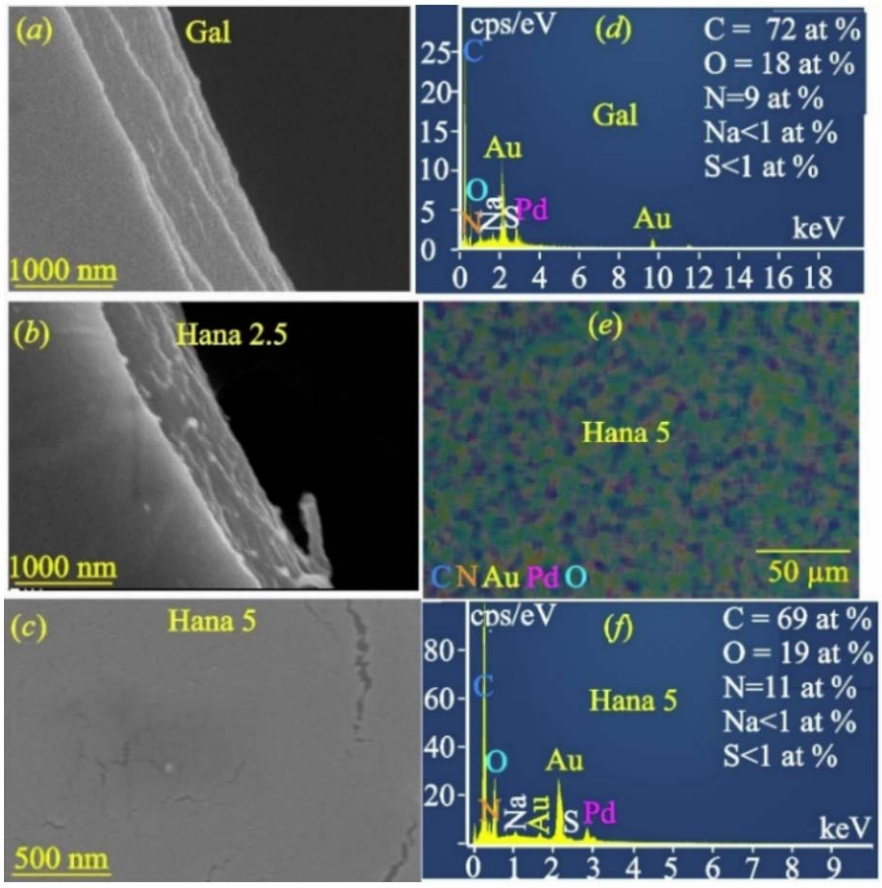

Figure 2: SEM images, EDS spectra and EDS map of dried biopolymer hydrogels immediately after fabrication (hydrogels coated with a 10 nm thick Au80Pd20 film).

standard, *E. coli* bacteria were added to the non-sterile samples as a contaminating agent (McFarland test = 1.0 (3*10□)). To test the effect of ethylene oxide sterilization according to the SIST EN 550:2000 standard on the presence and levels of *E. coli* in hydrogels after contamination using the McFarland microbiological test after ethylene oxide sterilization, the hydrogels were placed in sterile bags and incubated for 1 week in TSA medium with PBS in sterile Petri dishes. The control sample (Ctr) contained TSA or SDA in PBS medium without biopolymer hydrogel.

Prior to ethylene oxide sterilization, conducted in accordance with the SIST EN 550:2000 standard, Escherichia coli (*E. coli*) was intentionally introduced into non-sterile hydrogel samples to simulate microbial contamination. The bacterial suspension was prepared to a turbidity equivalent to McFarland standard 1.0, corresponding to approximately 3 × 10□ *CFU*/mL.

To evaluate the efficacy of ethylene oxide sterilization on the elimination of *E. coli* from the contaminated hydrogels, samples were subjected to the standardized sterilization process [12].

The morphology and chemical composition of the dry hydrogel samples immediately after preparation and after sterilization with ethylene oxide were investigated by scanning electron microscopy (SEM) and energy-dispersive X-ray spectrometry (EDS) using a Zeiss ULTRA Plus SEM (ZEISS, Jena, Germany) scanning electron microscope equipped with an OXFORD X-Max 20 EDS detector (Oxford Instruments, Abingdon, UK). Considering the dielectric properties of biopolymer hydrogels, before their analysis using SEM and EDS, 10 nm thick Au80Pd20 alloy films were deposited on the surface of the hydrogels using the radio frequency magnetron sputtering method. EDS maps of hydrogels with a resolution of 500 × 500 pixels were superpositions of the signal obtained from the electron backscatter detector, colored white, and the characteristic colors of the elements C, O, N, Na, etc.

The obtained data were presented as average values and standard deviations. Data normality was assessed for statistical analysis using Tukey's multiple comparison post hoc test using ANOVA with sample size (n) and standard deviation (SD).

3. RESULTS AND DISCUSSION

Figure **2a**, **2b**, **2c** shows the morphological characterization of dried biopolymer hydrogels immediately after fabrication using scanning electron microscopy, which their smooth surface and layered structure. The cracks seen in Figure **2c** are the result of the hydrogel compression during its drying. The elemental composition of the dried hydrogels Gal and Hana5 shown in the EDS spectra in Figure **2d** and (f), respectively, contain elements inherent in biopolymers: carbon and oxygen from GN, SA and Hana, nitrogen from GN and Hana, and sodium from SA and Hana.

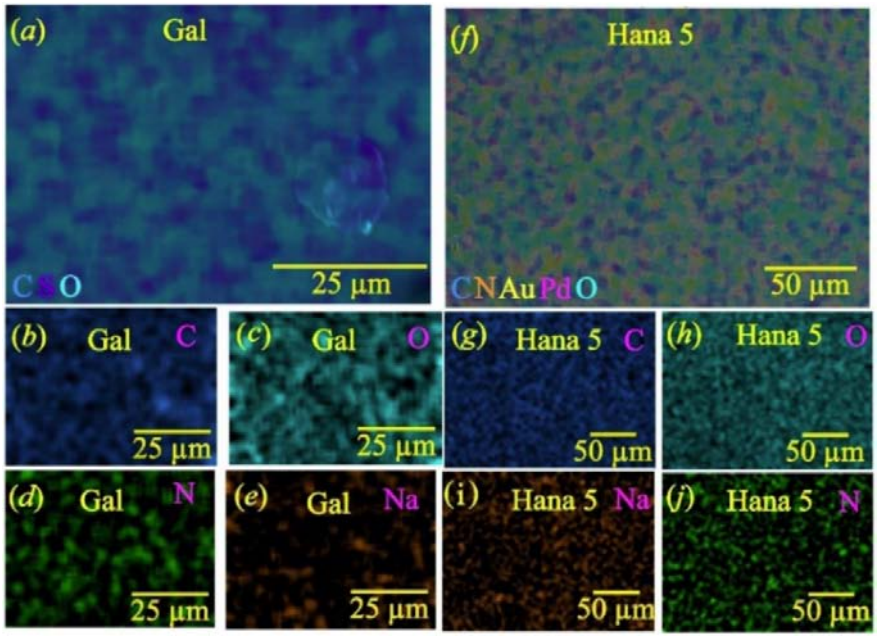

Figure 3: Total and elemental EDS maps of dried biopolymer hydrogels immediately after fabrication (hydrogels coated with a 10 nm thick Au80Pd20 film).

The overall EDS map in Figure **2e** and the overall and elemental maps in Figure **3** show a relatively uniform distribution of C, O, N, and Na across all samples of dried biopolymer hydrogels immediately after fabrication.

The photographs in Figure **4** and the data in Table **3** show that immediately after fabrication, the biopolymer hydrogels contain a small amount of fungi. At the same time, the contamination of Hana2.5 and Hana5 hydrogels with *E. coli* is high due to the encapsulated Hana biomolecules.

SEM images of dried biopolymer hydrogels after ethylene oxide sterilization according in Figure **5** show that the morphology of the biopolymer hydrogels was partially damaged but not completely destroyed. Etching spots can be seen in Figure **6b**, **6d**, **6e**, **6f**, as well as additional cracks on the surface of all hydrogels after their sterilization with ethylene oxide. The above is confirmed by the total and elemental EDS maps in Figure **6**. The EDS spectra of the dried biopolymer hydrogels Gal and Hana5 after sterilization with ethylene oxide in Figure **6b** and **6h**, respectively, are similar to the spectra for these samples immediately after fabrication.

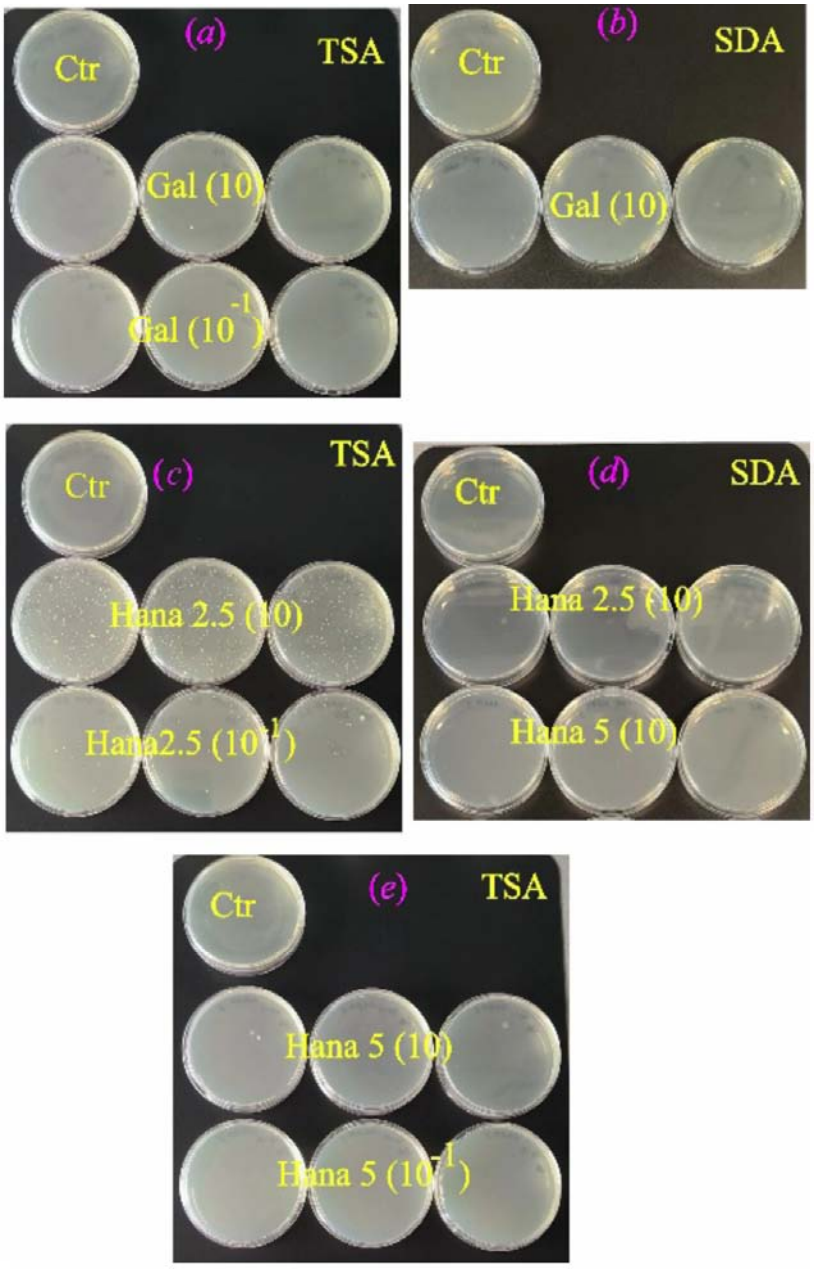

Figure 4: Photographs demonstrating the detection of microorganisms in Tryptone Soy Agar medium (**a**), (**c**), (**e**) and fungi in Sabouraud Dextrose Agar medium (**b**), (**d**) in solutions obtained from biopolymer hydrogels.

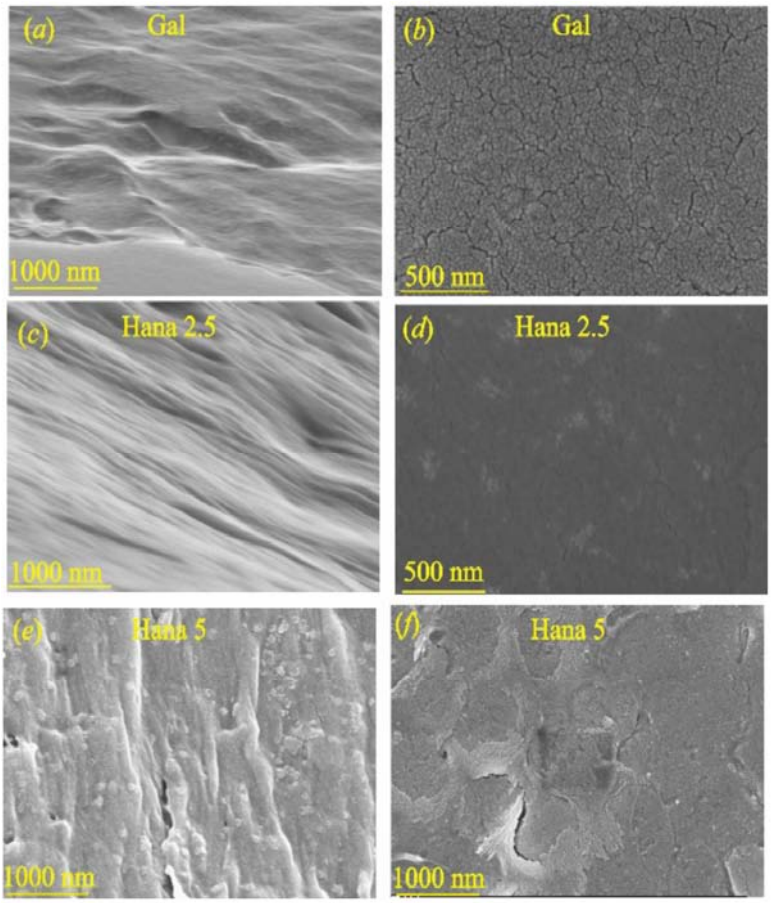

Figure 5: SEM images of dried biopolymer hydrogels after contamination *E. coli* and ethylene oxide sterilization according to the SIST EN 550:2000 (hydrogels coated with a 10 nm thick Au80Pd20 film).

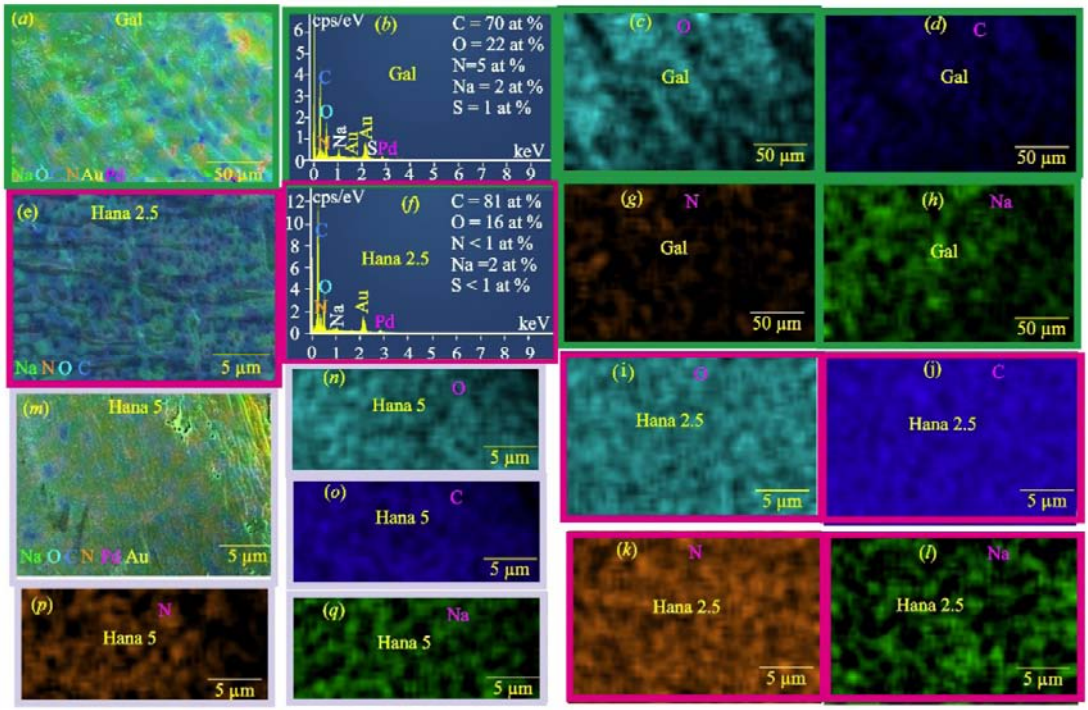

Figure 6: Total and elemental EDS maps and EDS spectra of dried biopolymer hydrogels after contamination with *E. coli* and sterilization with ethylene oxide (hydrogels coated with a 10 nm thick Au80Pd20 film).

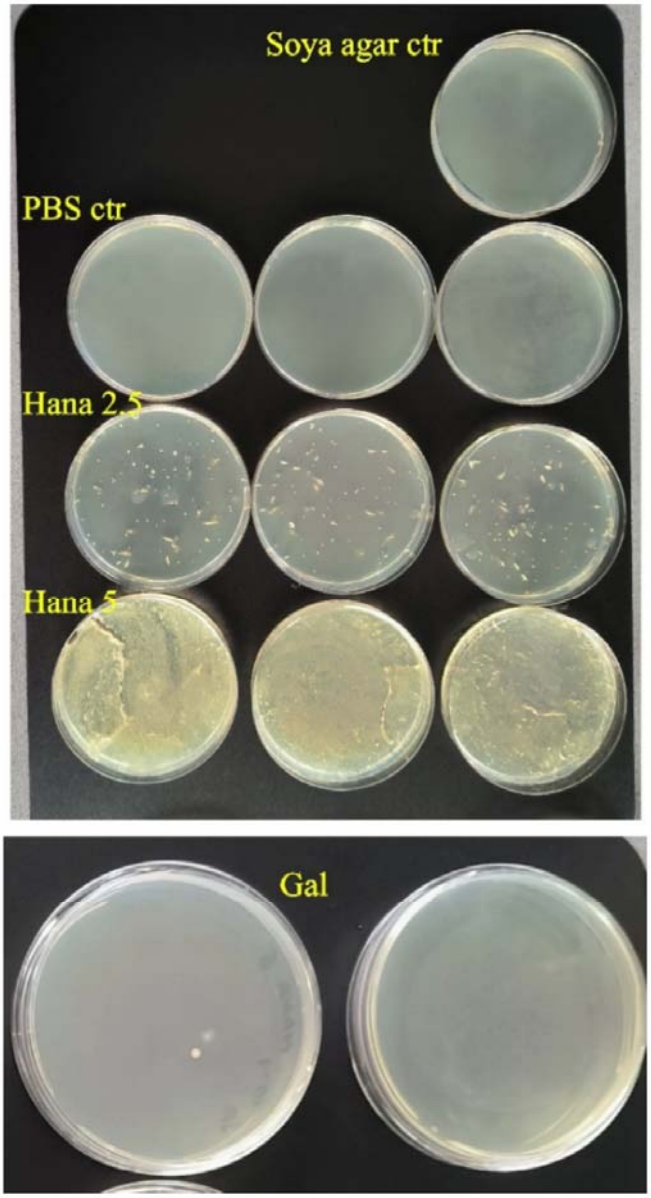

Figure 7: Photographs demonstrating the detection of *E. coli* in Tryptone Soy Agar medium, in PBS solution without biopolymer hydrogel, and in solutions obtained from biopolymer hydrogels Hana 2.5, Hana 5 and Gal.

Table 4: Data on Biopolymer Hydrogels Contaminated with *E. coli* and Sterilizated with EO

Hydrogel and concentration	Medium	Colony-forming units results, CFU/mL (SD)			
		First plate	Second plate	Third plate	Average
Gal (10)	TSA +*E. coli*	5±0.5	16±0.5	12±0.5	10±0.5
Hana 2.5 (10)		97±0.5	82±0.5	105±0.5	100±0.5
Hana 5 (10)		>2000±0.5	>2000±0.5	>2000±0.5	>2000±0.5

The photographs in Figure **7** and the corresponding data in Table **4** on the detection of *E. coli* after ethylene oxide sterilization of specially contaminated biopolymer hydrogels using the McFarland microbiological test revealed the persistence of these bacteria due to encapsulation with Gal, Nana2.5, and Hana5 hydrogels. Thus, microbiological tests showed sufficient resistance of microbiota encapsulated inside gelatin-alginate biopolymer hydrogels to EO sterilization.

CONCLUSIONS

Herein, the preservation of humic acid biomolecules encapsulated in biopolymer gelatin-alginate hydrogels during conventional sterilization with ethylene oxide was determined by studying their microbiological contamination, micromorphology and chemical composition. According to SEM and EDS data, immediately after fabrication dried biopolymer hydrogels have smooth surface, layered structure and relatively uniform distribution of elements inherent in biopolymers C, O, N, and Na across all samples. According to the microbiological experiments, all of them contain a small number of fungi, but the contamination of Hana 2.5 and Hana 5 hydrogels with *E. coli* is high due to the encapsulated Hana biomolecules. The morphology of the biopolymer hydrogels was partially damaged but not completely destroyed after *E. coli* contamination and ethylene oxide sterilization according to SIST EN 550:2000. Moreover, microbiological tests for the detection of *E. coli* after ethylene oxide sterilization revealed the persistence of these bacteria due to the encapsulation with Gal, Nana 2.5 and Hana 5 hydrogels. Thus, the study demonstrated sufficient resistance of the microbiota encapsulated inside the gelatin-alginate biopolymer hydrogels to EO sterilization. In addition, it has been experimentally confirmed that the gelatin-alginate hydrogels we developed, modified with humic acids, predominantly retain their morphology and chemical composition during this sterilization.

REFERENCES

[1] Pérez-Luna VH, González-Reynoso O. Encapsulation of biological agents in hydrogels for therapeutic applications. Gels 2018; 4(3): 61. https://doi.org/10.3390/gels4030061

[2] Far BF, Safaei M, Nahavandi R, *et al.* Hydrogel encapsulation techniques and its clinical applications in drug delivery and regenerative medicine: a systematic review. ACS Omega 2024; 9(27): 29139-58. https://doi.org/10.1021/acsomega.3c10102

[3] Yeh YW, Huang CC, Kuo WS, *et al.* Multifunctional hydrogel dressing that carries three antibiotics simultaneously and enables Real-Time ultrasound bacterial colony detection. ACS Omega 2023; 8(11): 10278-87. https://doi.org/10.1021/acsomega.2c07806

[4] Semenyuk N, Dudok G, Skorokhoda V. Hydrogels in biomedicine: Granular controlled release systems based on 2-Hydroxyethyl methacrylate copolymers. A review. Chemistry & Chemical Technology 2024; 18(2): 143-56. https://doi.org/10.23939/chcht18.02.143

[5] Cemka Z, Szarlej P, Piłat E, *et al.* Hydrogels based on natural polymers for cardiac applications. Chemistry & Chemical Technology 2022; 16(4): 564-72. https://doi.org/10.23939/chcht16.04.564

[6] Skorokhoda V, Semenyuk N, Melnyk J, *et al.* Hydrogels penetration and sorption properties in the substances release controlled processes. Chemistry & Chemical Technology 2009; 3(2): 117-21. https://doi.org/10.23939/chcht03.02.117

[7] Vazquez OR, Avila IO, Díaz JCS, *et al.* An Overview of mechanical tests for polymeric biomaterial scaffolds used in tissue engineering. Journal of Research Updates in Polymer Science 2016; 4(4): 168-7. https://doi.org/10.6000/1929-5995.2015.04.04.1

[8] Ghandali EV, Rastegari A, Mohammadi Z. Formulation and evaluation of vancomycin loaded chitosan/aloe vera hydrogel: a novel antibacterial biopolymeric system. Journal of Research Updates in Polymer Science 2024; 13: 147-154. https://doi.org/10.6000/1929-5995.2024.13.15

[9] Santhamoorthy M, Kim S-C. A Review of the Development of Biopolymer Hydrogel-Based Scaffold Materials for Drug Delivery and Tissue Engineering Applications. Gels 2025; 11(3):178. https://doi.org/10.3390/gels11030178

[10] Quazi MZ, Quazi AS, Song Y, Park N. Functional Hydrogels: A Promising Platform for Biomedical and Environmental Applications. International Journal of Molecular Sciences 2025; 26(18):9066. https://doi.org/10.3390/ijms26189066

[11] Segneanu A-E, Bejenaru LE, Bejenaru C, Blendea A, Mogoşanu GD, Biţă A, Boia ER. Advancements in Hydrogels: A Comprehensive Review of Natural and Synthetic Innovations for Biomedical Applications. Polymers 2025; 17(15):2026. https://doi.org/10.3390/polym17152026

[12] Swidsinski A, Dörffel Y, Loening-Baucke V, *et al.* Impact of humic acids on the colonic microbiome in healthy volunteers. World J Gastroenterol 2017; 23(5): 885-890. https://doi.org/10.3748/wjg.v23.i5.885

[13] Miroshnichenko D, Lebedev V, Lebedeva K, *et al.* Thermosensitive and wound-healing gelatin-alginate biopolymer hydrogels modified with humic acids. Journal of renewable materials 2024; 12(10): 1691-1713. https://doi.org/10.32604/jrm.2024.054769

[14] Petrushenko SI, Fijalkowski M, Adach K, *et al.* activity of thermosensitive, hemostatic, and wound healing biopolymer hydrogels. Gels 2025; 11(4): 301 https://doi.org/10.3390/gels11040301

[15] Galante R, Pinto TJA, Colaço R, *et al.* Sterilization of hydrogels for biomedical applications: A review. Journal of Biomedical Materials Research Part B Applied Biomaterials. 2017; 106(6): 2472-2492. https://doi.org/10.1002/jbm.b.34048

[16] Stoppel WL, White JC, Horava SD, *et al.* Terminal sterilization of alginate hydrogels: Efficacy and impact on mechanical properties. Journal of Biomedical Materials Research Part B Applied Biomaterials 2013; 102(4): 877-884. https://doi.org/10.1002/jbm.b.33070

[17] Zhang F, Scull G, Gluck JM, *et al.* Effects of sterilization methods on gelatin methacryloyl hydrogel properties and macrophage gene expression *in vitro*. Biomedical Materials 2022; 18(1): 015015. https://doi.org/10.1088/1748-605X/aca4b2

[18] Carranza T, Zalba-Balda M, Baraibar MJB, *et al.* Effect of sterilization processes on alginate/gelatin inks for three-dimensional printing. International Journal of Bioprinting 2022; 9(1): 645. https://doi.org/10.18063/ijb.v9i1.645

[19] Mendes GCC, Brandão TRS, Silva CLM. Ethylene oxide sterilization of medical devices: A review. American Journal of Infection Control 2007; 35(9): 574-581. https://doi.org/10.1016/j.ajic.2006.10.014

[20] Chen H, Liu J, Zeng A, Qin N. Analysis of sterilization efficiency and application cost of three low temperature

sterilization methods. Review of Scientific Instruments 2024; 95(4). https://doi.org/10.1063/5.0175121

[21] Da Costa RC, Pereira ED, Silva FM, De Jesus EO, Souza F. G Jr. Drug Micro-Carriers based on polymers and their sterilization. Chemistry & Chemical Technology 2018; 12(4): 473-487.

[22] Lebedev V, Miroshnichenko D, Vytrykush N, *et al*. Novel biodegradable polymers modified by humic acids. Materials Chemistry and Physics 2024; 313: 128778. https://doi.org/10.1016/j.matchemphys.2023.128778

[23] Lebedev V, Miroshnichenko D, Vytrykush N, *et al*. Novel biodegradable polymers modified by humic acids. Materials Chemistry and Physics 2024; 313: 128778. https://doi.org/10.1016/j.matchemphys.2023.128778

[24] Lebedeva K, Cherkashina A, Voronkin A, *et al*. Design and researching smart biologically active polymeric hydrogel transdermal nanomaterial's. 2023 IEEE 4th KhPI Week on Advanced Technology (KhPIWeek) 2023: 1-5. https://doi.org/10.1109/KhPIWeek61412.2023.10312985

[25] Lebedeva K, Cherkashina A, Masikevych YG, *et al*. Modeling of Smart Bio-Medical Active Polymeric Hydrogel Transdermal Materials. Journal of Engineering Sciences 2024; 11(1): C1-C7. https://doi.org/10.21272/jes.2024.11(1).c1

[26] Miroshnichenko D, Lebedeva K, Cherkashina A, *et al*. Study of hybrid modification with humic acids of environmentally safe biodegradable hydrogel films based on hydroxypropyl methylcellulose. C- Journal of carbon research 2022; 8: 71-10. https://doi.org/10.3390/c8040071

[27] Lebedev V, Miroshnichenko D, Bilets D, *et al*. Investigation of hybrid modification of Eco-Friendly polymers by humic substances. Diffusion and Defect Data, Solid State Data Part B, Solid State Phenomena/Solid State Phenomena 2022; 334: 154-161. https://doi.org/10.4028/p-gv30w7

[28] Lebedev V, Miroshnichenko D, Tykhomyrova T, *et al*. Design and research of environmentally friendly polymeric materials modificated by derivatives of coal. Petroleum and Coal 2023; 65(2): 334-340.

[29] Miroshnichenko DV, Pyshyev SV, Lebedev VV, *et al*. Deposits and quality indicators of brown coal in Ukraine. Naukovyi Visnyk Natsionalnoho Hirnychoho Universytetu 2022; (3): 5-10. https://doi.org/10.33271/nvngu/2022-3/005

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