polysaccharides

MDPI

Article

The Chitinous Skeleton of *Ianthella basta* Marine Demosponge as a Renewable Scaffold-Based Carrier of Antiseptics

Izabela Dziedzic [1,2,*], Kamil Dydek [2,3], Alona Voronkina [4,*], Valentin Kovalchuk [5], Teofil Jesionowski [6] and Hermann Ehrlich [2,6]

1 Faculty of Chemistry, Adam Mickiewicz University, Uniwersytetu Poznańskiego 8, 61-614 Poznan, Poland
2 Center of Advanced Technology, Adam Mickiewicz University, Uniwersytetu Poznańskiego 10, 61-614 Poznan, Poland; kamil.dydek@pw.edu.pl (K.D.); hererh@amu.edu.pl (H.E.)
3 Faculty of Materials Science and Engineering, Warsaw University of Technology, 141 Wołoska St., 02-507 Warsaw, Poland
4 Department of Pharmacy, National Pirogov Memorial Medical University, Vinnytsya, Pirogov Street 56, 21018 Vinnytsia, Ukraine
5 Department of Microbiology, National Pirogov Memorial Medical University, Vinnytsya, Pirogov Street 56, 21018 Vinnytsia, Ukraine; valentinkovalchuk2015@gmail.com
6 Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan University of Technology, Berdychowo 4, 60-965 Poznan, Poland; teofil.jesionowski@put.poznan.pl
* Correspondence: izadzi@amu.edu.pl (I.D.); voronkina@vnmu.edu.ua (A.V.)

Abstract: The chitinous skeleton of the marine demosponge *Ianthella basta* exhibits a unique network-like 3D architecture, excellent capillary properties, and chemical inertness, making it highly suitable for interdisciplinary research, especially in biomedical applications. This study investigates the potential of renewable *I. basta* chitinous scaffolds for drug delivery and wound dressing. The scaffolds, characterized by a microtubular structure, were impregnated with selected commercially available antiseptics, including solutions with hydrophilic and hydrophobic properties. Evaluations against selected clinical strains of bacteria, as well as fungi, demonstrated significant zones of growth inhibition with antiseptics such as brilliant green, gentian violet, decamethoxine, and polyhexanide. Notably, the antibacterial properties of these antiseptic-treated chitin matrices persisted for over 72 h, effectively inhibiting microbial growth in fresh cultures. These findings highlight the considerable potential of *I. basta* chitin scaffolds as sustainable, innovative biomaterials for controlled drug release and wound dressing applications.

Keywords: chitin; *Ianthella basta*; scaffolds; sponges; antiseptics

Citation: Dziedzic, I.; Dydek, K.; Voronkina, A.; Kovalchuk, V.; Jesionowski, T.; Ehrlich, H. The Chitinous Skeleton of *Ianthella basta* Marine Demosponge as a Renewable Scaffold-Based Carrier of Antiseptics. *Polysaccharides* **2024**, *5*, 540–551. https://doi.org/10.3390/polysaccharides5040034

Academic Editors: San-Lang Wang and Karin Stana Kleinschek

Received: 26 July 2024
Revised: 26 August 2024
Accepted: 29 September 2024
Published: 1 October 2024

1. Introduction

Chitin is the world's second most widespread polysaccharide after cellulose. It is composed of N-acetyl-D-glucosamine units connected by β-1,4-glycosidic bonds [1,2]. This structural aminopolysaccharide naturally occurs in the skeletal structures of numerous organisms including fungi, invertebrates such as squids and other mollusks, and arthropods such as spiders, insects and crabs [3]. Chitin possesses extraordinary properties such as biocompatibility and biodegradability; moreover, it is nontoxic, non-allergenic, and chemically inert [1]. Because of these properties, chitin has found application in the food industry [4,5], in the medical and pharmaceutical industry as a wound-dressing material [6–9] as well as for controlled drug release [9–12] and tissue engineering [3], and in the removal of water pollutants [13,14] (for an overview, see [15]). Chitin can occur in three different polymorphic configurations: α-, β-, and γ-chitin [3,16,17]. They are characterized by different arrangements of polysaccharide chains, with γ-chitin being structurally closer to the α form [18]. Alpha chitin is the most abundant form in crabs and shrimps, while the beta and gamma forms occur in squids and loligo, respectively [18].

Chitin in sponges (Porifera) was discovered for the first time in 2007 by Ehrlich et al. [3]. The authors showed that sponges also possess the α-chitin polymorphic form. Chitin of poriferan origin, in addition to typical properties of the biopolymer such as biocompatibility, biodegradability, chemical inertness, and resistance to dissolution [3,19], also exhibits unique structural features. It has a 3D architecture composed of microtubular fibers with a well-developed system of intercalated internal channels [3]. This morphology corresponds to a large inner surface area, which contributes to the excellent capillary action of these structures [20], and, consequently, their unusual ability to swell [20], including high absorption activity [13,14,20]. The purified chitin scaffold isolated from *Aplysina archeri* demosponge is hydrophilic, yet chitin immediately absorbs crude oil, blood, and water solutions of dyes (e.g., methylene blue). This may be attributed to capillary action within the 3D tubular network and the influence of van der Waals forces [20].

Recently, selected mechanical properties of chitin isolated from diverse sponge species were examined and described by Duminis et al. [21]. Due to their mechanical stiffness, poriferan chitinous scaffolds are an excellent base for the cultivation of cells, including chondrocytes and mesenchymal stromal cells [3]. Recently, chitinous scaffolds isolated from the *Ianthella labiryntus* demosponge have been demonstrated to be suitable for the cultivation of human induced pluripotent stem cells (iPSC-CMs) [3].

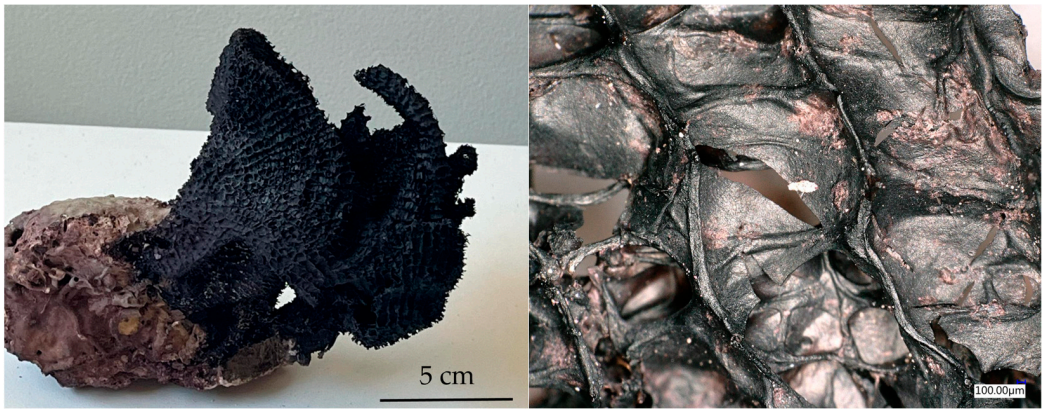
With respect to technological applications, it has been reported [3] that sponge chitin can enhance the properties of chitosan membranes for supercapacitors. A chitosan/sponge chitin membrane exhibited superior electrochemical properties compared with a chitosan membrane without the sponge-derived chitin scaffold. The improvement in electrochemical and mechanical properties is likely due to the unique structure of the chitinous scaffold [3,21]. Mechanically robust poriferan chitin scaffolds have also been used as templates for the electrochemical deposition of copper nanocrystallites [3] and for the nucleation and growth of diverse metal-based composites [3].

Sponges of the order Verongiida, including *Ianthella basta* (Pallas, 1766) [22–24] (Figure 1), are characterized by an extraordinary ability to regenerate their skeletons, which enables their cultivation under marine farming conditions [25], providing a renewable source for the sustainable production of unique, naturally pre-structured 3D chitin. This exclusively marine demosponge, colloquially called "elephant ear" sponge due to its size and morphology, grows in extensive formations resembling large fans and funnel shapes, reaching sizes of up to 1.7 m in height and 9.5 m in circumference [25,26]. *I. basta* can grow at a fast rate of more than 10 cm per year [25]. The *I. basta* chitin scaffold closely mimics the shape of the sponge skeleton, made of interconnected fibers forming a 3D flat network with almost square chambers (Figure 1b). *I. basta* sponges contain partially disordered chitin, structurally closer to α-chitin than to the β type. The chitin content in the native *I. basta* skeleton mineralized with nanostructured calcite [27] is approximately 7 wt.%, as determined by the measurement of chitin in a skeleton sample of known mass [27]. Chitin isolated from the *I. basta* sponge exhibits a rough, fissured surface and is composed of cross-linked, loosely packed fibers with diameters ranging from approximately 40 to 100 nm [27].

Poriferans of the order Verongiida not only possess chitin as their skeleton for structural rigidity but also produce bromotyrosines—brominated derivatives of tyrosine—as a chemical means of defense against pathogens and other microorganisms [28]. Bromotyrosines have been identified as multi-target marine drugs due to their antibacterial and antiviral activity and other therapeutic properties [28–31]. They also offer potential activity against COVID-19 [32]. According to a recent report [21], bromotyrosines may be responsible for the stability of chitinous skeletons in sponges, acting as cross-linking agents.

In the native sponge skeleton, bromotyrosine components are intercalated into the chitin scaffold, preventing the formation of unperturbed crystalline chitin [27]. It is notable that so-called spherulocytes, the cells that produce bromotyrosines, occur within the chitinous skeletal fibers of verongiid demosponges. These spherulocytes can degenerate, re-

leasing bromotyrosines into the intercellular matrix and subsequently into the surrounding seawater [33], where they can act as antibiotics.



(**a**) (**b**)

Figure 1. Dried *Ianthella basta* demosponge specimen on a rock fragment as collected (**a**) and a digital microscope image of its surface (**b**).

Thus, chitinous skeletons of verongiid sponges originally loaded with bromotyrosines can be used as model systems in drug release and wound healing. Previous research has shown that poriferan chitin scaffolds from the demosponge *Ianthella flabelliformis* impregnated with decamethoxine solution inhibit the growth of the pathogen *Staphylococcus aureus*, as demonstrated by an agar diffusion test [19].

The aim of the present research was to evaluate the in vitro release of selected antiseptics from the up to 3 mm thick, flat chitinous matrix of *I. basta*, demonstrating its potential for future application as a natural carrier for selected antiseptics that mimics artificial materials already in use for wound dressing. Antiseptics were chosen from diverse Anatomical Therapeutic Chemical (ATC) groups, including dyes, iodine compounds, oxidizers, quaternary ammonium compounds, and biguanides. The substances used include gentian violet, potassium permanganate, Rivanol, iodine, bromotyrosine glycerin extract, brilliant green, sea buckthorn oil, decamethoxine, and polyhexanide (Tables 1 and 2).

Table 1. Characteristics of the antiseptics used in the study.

Antiseptic	Active Ingredient	Chemical Name	Molecular Formula	Molecular Weight [g/mol]	References
Gentian violet	1% (*w*/*v*) methylrosaniline chloride	Hexamethylpararosaniline chloride	$C_{25}H_{30}N_3Cl$	407.98	[34]
Potassium permanganate	1% (*w*/*v*) potassium permanganate	Potassium permanganate	$KMnO_4$	158.03	[35]
Rivanol	0.1% (*w*/*v*) ethacridine lactate monohydrate	2-ethoxy-6,9-diamino acridine monolactate monohydrate	$C_{18}H_{21}N_3O_4$	343.38	[36]
Iodine	3% (*w*/*v*) iodine	Iodine	I_2	253.81	[37]
Bromotyrosine glycerin extract	Glycerin extract from *Aplysina aerophoba* sponge	-	-	-	[33]
Brilliant green	1% (*w*/*v*) brilliant green	[4-[[4-(diethylamino)phenyl]-phenylmethylidene]cyclohexa-2,5-dien-1-ylidene]-diethylazanium;hydrogen sulfate	$C_{27}H_{33}N_2.HO_4S$	482.63	[38]
Sea buckthorn oil	Sea buckthorn oil 100%	-	-	-	[39]

Table 1. *Cont.*

Antiseptic	Active Ingredient	Chemical Name	Molecular Formula	Molecular Weight [g/mol]	References
Decamethoxine	0.1% (*w*/*v*)	10-[dimethyl-[2-[(1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexyl]oxy-2-oxoethyl]azaniumyl]decyl-dimethyl-[2-[(1R,2S,5R)-5-methyl-2-propan-2-ylcyclohexyl]oxy-2-oxoethyl]azanium;dichloride	$C_{38}H_{74}Cl_2N_2O_4$	693.92	[40]
Polyhexanide	0.1% (*w*/*v*) polyaminopropyl biguanide	poly(hexamethylene biguanide)	$(C_8H_{17}N_5)$n.xHCl, n ≤ 40	from 500 to 6000	[41]

Table 2. List of antiseptics used in the study.

Antiseptic	Active Ingredient	Solvents/Excipients	Manufacturer	ATC WHO
Gentian violet	1% (*w*/*v*) methylrosaniline chloride	Ethanol 96%	PPF HASCO-LEK Wroclaw, Poland	D01AE02 Other antifungals for topical use G01AX09 Other antiinfectives and antiseptics
Potassium permanganate	1% (*w*/*v*) potassium permanganate	Purified water		D08AX06 Other antiseptics and disinfectants, oxidizer
Rivanol	0.1% (*w*/*v*) ethacridine lactate monohydrate	Purified water	PPF HASCO-LEK Wroclaw, Poland	D08AA01 Acridine derivatives
Iodine	3% (*w*/*v*) iodine	Ethanol 96% Potassium iodide 1%	AVENA Osielsko, Poland	D08AG03 Iodine products
Bromotyrosine glycerin extract	Glycerin extract from *Aplysina aerophoba* sponge	Glycerin	-	-
Brilliant green	1% (*w*/*v*) brilliant green	Ethanol 60%	BORSHCHAHIVSKY CFP, Kyiv, Ukraine	D08AX06 Other antiseptics and disinfectants, dyes
Sea buckthorn oil	Sea buckthorn oil 100%	-	FITOLIK, Ivano-Frankivsk, Ukraine	D11A Other dermatological preparations
Decamethoxine	0.1% (*w*/*v*)	Purified water / Ethanol 96%	YURIA-PHARM, Kyiv, Ukraine	D08AJ10 Quaternary ammonium compounds
Polyhexanide	0.1% (*w*/*v*) polyaminopropyl biguanide	Purified water	BLANIDAS, Kyiv, Ukraine	D08AC05 Biguanides

2. Materials and Methods

2.1. Materials

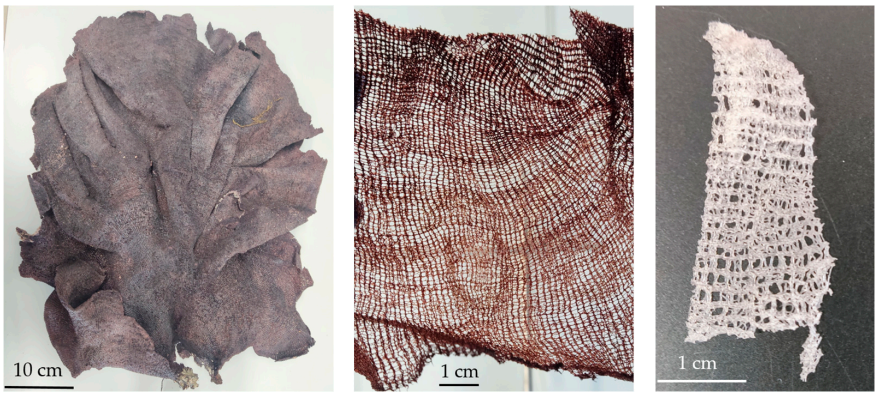
A dried *Ianthella basta* demosponge was procured from INTIB GmbH (Freiberg, Germany). The samples were collected underwater at station SOL47/W/A042 (1536′46.10″ S, 12,404′22.92″ E to 1536′44.77″ S, 12,404′22.38″ E, Kimberley, Western Australia) in March 2015 at a depth of 35.3–35.5 m. The characteristics and sources of antiseptics used for this study are described in Tables 1 and 2.

2.2. Methods

2.2.1. Isolation of Chitin Scaffolds

Isolation was performed according to the well-established standard method [3,20]. The sponge, measuring 50 × 35 cm (Figure 2a), was first soaked in distilled water for 2 h to

remove salts and then cut into 4 × 4 cm pieces. These pieces were immersed in 20% acetic acid solution at room temperature for 24 h. Following this, the skeletal pieces were rinsed with water until neutral pH was attained and then immersed in 10% NaOH solution at 37 °C for 48 h (Figure 2b). This alternating treatment between acid and base was repeated over 5 days to obtain colorless chitinous scaffolds for the study (Figure 2c).



(**a**) (**b**) (**c**)

Figure 2. (**a**) Dried *I. basta* sponge before chitin isolation; (**b**) *I. basta* sponge skeleton after treatment with acetic acid and sodium hydroxide solution; (**c**) fragment of the resulting purified chitin scaffold.

2.2.2. Microbiological Investigation

The prepared 3D chitin scaffolds of *I. basta* were cut into 1 cm^2 squares, impregnated with solutions of antiseptics (Table 2) for 2 h, and dried with sterile filter paper and then at a thermostatically controlled temperature of 37 °C for 6 h. Samples used as controls were placed in sterile distilled water or 96% ethanol.

Dry samples were placed on a Petri dish with fresh cultures of clinical strains of Gram-positive (*Staphylococcus aureus*) or Gram-negative (*Escherichia coli*) bacteria on tryptic soy agar (TSA) (GRASO BIOTEC, Poland) or fungi (*Candida albicans*) on Sabouraud Dextrose Agar (SDA) (GRASO BIOTEC, Poland) and cultivated for 24 h at 37 °C. After 24 h, the zones of growth inhibition at first use were observed. Samples showing antimicrobial activity against *S. aureus* were moved with sterile forceps to a Petri dish with a fresh daily culture of the same microorganism. Cultivation was repeated five times with the same samples of the chitin scaffold and fresh cultures. All tests were conducted with proper sterility control of the nutritive environment (TSA and SDA) and included control samples to measure microorganism growth in the absence of the compound. Figure 3 contains a schematic representation of the procedure.

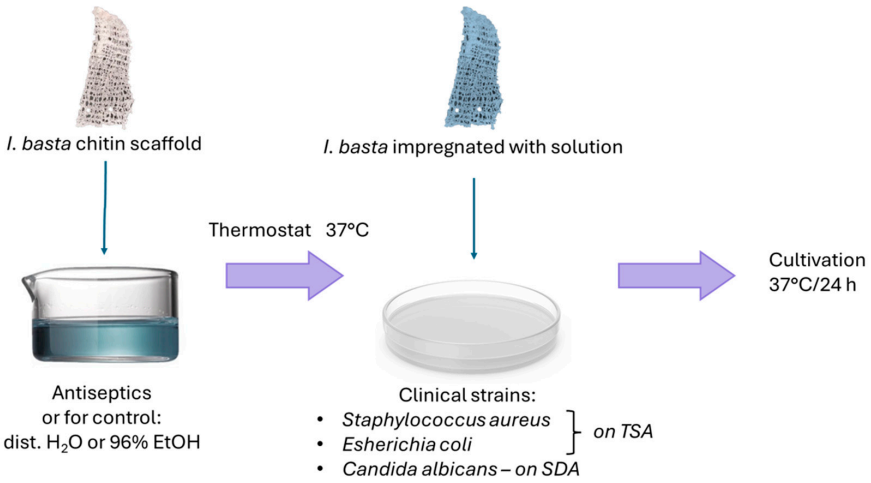


Figure 3. Schematic diagram of the procedure for investigation of antimicrobial properties.

For determination of the antimicrobial activity of the experimental matrices, clinical strains of *S. aureus* and *E. coli* (isolated from purulent burnt wounds) and *C. albicans* (isolated from a patient with acute stomatitis) were used. The clinical strains were isolated and cultured at the Department of Microbiology of the National Pirogov Memorial Medical University, Vinnytsya, Ukraine. Ethics approval was not required for this study as the microbial strains were isolated as part of routine clinical diagnostics.

3. Results

Growth inhibition of *S. aureus* was observed for the samples impregnated with gentian violet, brilliant green, both water and alcohol solutions of decamethoxine, and polyhexanide solution. For some samples, i.e., potassium permanganate, Rivanol, and iodine, bacterial culture growth was not observed directly on the matrix but neither were growth inhibition zones on TSA. Samples impregnated with sea buckthorn oil, iodine, and Br-tyrosine glycerin extract did not differ from the control, where the growth of bacteria was observed directly on the matrix.

For *S. aureus*, the largest inhibition zones were for the chitin matrix impregnated with gentian violet, brilliant green, and both decamethoxine solutions. For polyhexanide, the inhibition zone was slightly smaller.

Matrices impregnated with dyes were less active against *E. coli*: slight growth inhibition was observed for brilliant green, and a growth inhibition zone less than 1 mm from the edge of the matrix was observed for gentian violet. Polyhexanide solution was slightly more effective, but good results were achieved with both water and alcohol solutions of decamethoxine.

In contrast to the case of Gram-negative bacteria, the most promising results against *C. albicans* were achieved by dyes: gentian violet and brilliant green. Matrices impregnated with water and alcohol solutions of the quaternary ammonia compound decamethoxine appeared less effective but still exhibited growth inhibition, while samples with biguanide polyhexanide produced no effect. The results are presented in Table 3 and Figure 4.

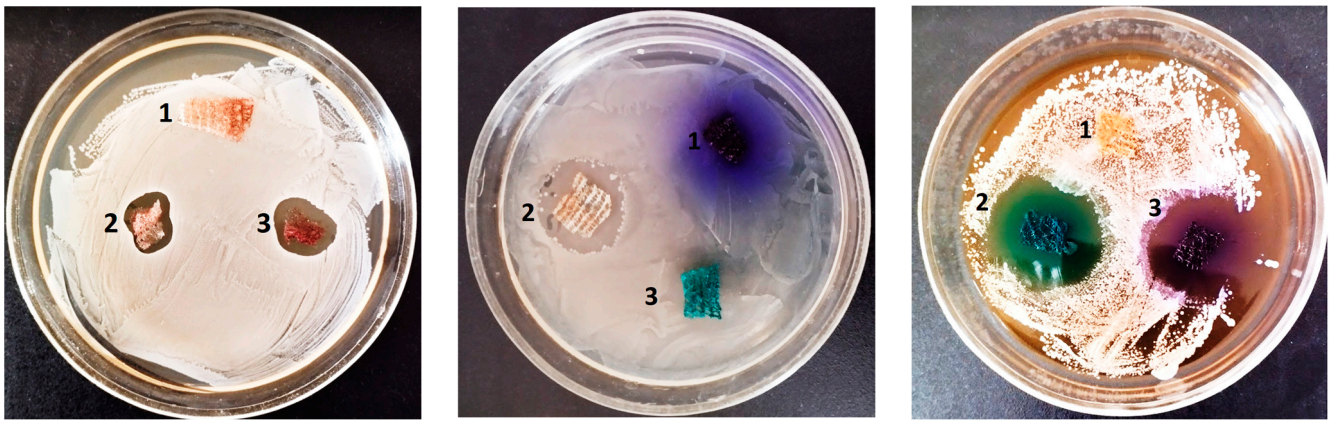
Table 3. Results of microbial activity test using different antiseptics for *S. aureus*, *E. coli*, and *C. albicans* strains.

	S. aureus	***E. coli***	***C. albicans***
Control	-	-	-
Gentian violet 1%	+ + +	+	+ + +
Potassium permanganate 1%	-	-	-
Rivanol (ethacridine lactate)	-	-	-
Iodine 3%	-	-	-
Br-tyrosine glycerin extract	-	-	-
Brilliant green 1%	+ + +	+	+ + +
Sea buckthorn oil	-	-	-
Decamethoxine water solution	+ + +	+ + +	+
Decamethoxine ethanol solution	+ + +	+ + +	+
Polyhexanide	+ +	+	-

"-"—no growth inhibition observed; "+"—weak growth inhibition (less than 1 mm from the matrix edge; no bacterial growth under or on the matrix); "+ +"—moderate growth inhibition (1–2 mm from the matrix edge); "+ + +"—strong growth inhibition (more than 2 mm from the matrix edge).

For most combinations of the *I. basta* chitin matrix with gentian violet, brilliant green, and decamethoxine solutions, the capacity for antiseptic release from the matrix was evaluated. When after every 24 h of exposition the matrix impregnated with the antiseptic was transferred to a fresh culture, the growth inhibition zones for *S. aureus* were almost constant (from 25 to 30 mm in diameter) in the first 72 h. After the fourth transfer, the size

of the growth inhibition zone started to decrease visibly, and after 120 h, growth inhibition was observed only under the matrix (Figure 5).



(**a**) (**b**) (**c**)

Figure 4. Examples of Petri dishes with growth inhibition zones for (**a**) clinical strain of *S. aureus*: **1**—control, **2**—chitinous matrix impregnated with decamethoxine water solution, and **3**—chitinous matrix impregnated with decamethoxine alcohol solution; (**b**) clinical strain of *E. coli*, **1**—chitinous matrix impregnated with gentian violet, **2**—chitinous matrix impregnated with decamethoxine alcohol solution, and **3**—chitinous matrix impregnated with brilliant green; (**c**) clinical strain of *C. albicans*, **1**—chitinous matrix impregnated with solution of polyhexanide, **2**—chitinous matrix impregnated with brilliant green, and **3**—chitinous matrix impregnated with gentian violet.

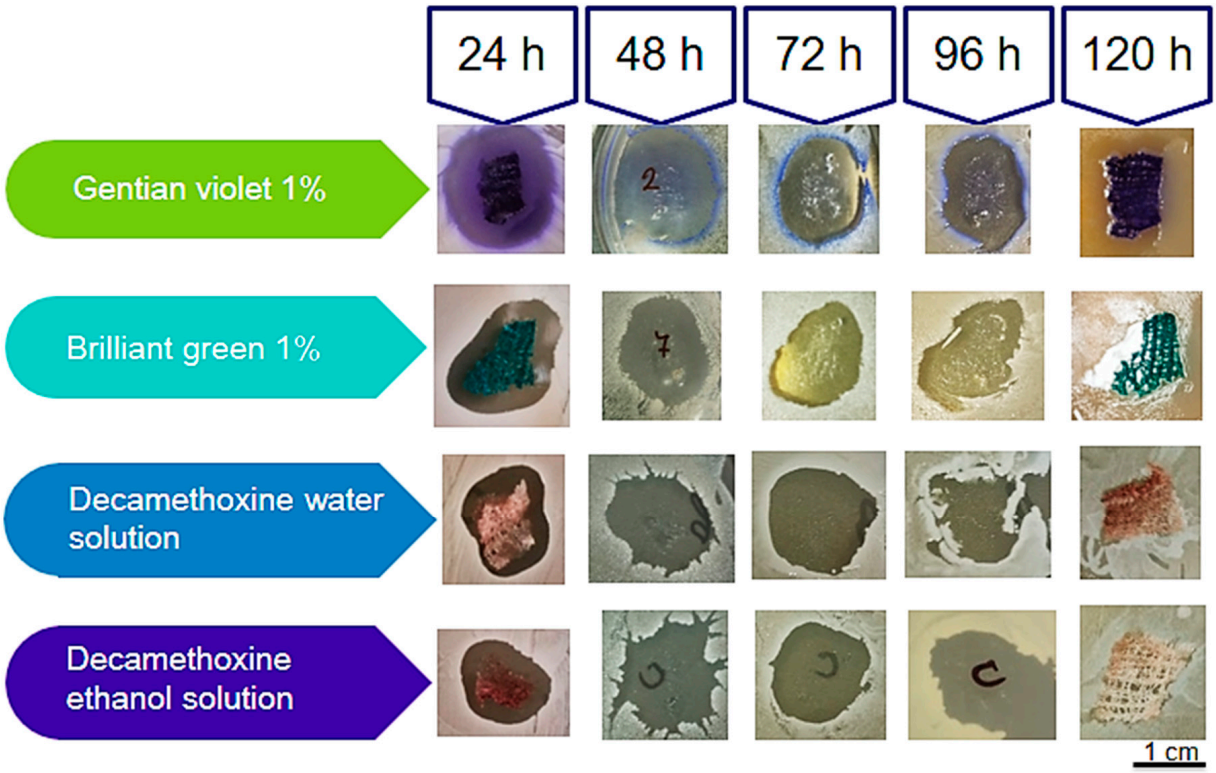


Figure 5. Results of the capacity test for the release of the antiseptics that exhibited the highest activity (gentian violet, brilliant green, decamethoxine water, and alcohol solutions) over 5 days. Images at 48, 72, and 96 h were obtained after moving the impregnated matrix to the fresh culture.

4. Discussion

The antimicrobial efficacy of chitinous scaffolds impregnated with various antiseptics is largely influenced by the chemical structure of the antiseptics, the minimum inhibitory concentration (MIC), the concentration used, and the adsorption and swelling properties of the chitin matrix. The study used antiseptics with different chemical structures, solubility, mechanisms of action, and qualitative and quantitative antibacterial activities. The chitinous scaffold of *I. basta* sponge origin, with its unique 3D microtubular structure and capillary properties [19–21], offers a high surface area for adsorption, significantly impacting the interaction with antiseptic compounds.

Antiseptics such as gentian violet and brilliant green, both of which are triarylmethane dyes with three aromatic rings connected to a central carbon, displayed strong antimicrobial activity, particularly against *S. aureus* and *C. albicans*. Their efficacy can be attributed to their molecular structure, which enables efficient adsorption onto the chitin surface. The strong cationic nature (due to the presence of multiple amine groups) of gentian violet and brilliant green allows them to interact robustly with the negatively charged sites on chitin. Brilliant green also includes additional alkyl groups, which increase its hydrophobicity and may further enhance its adsorption through hydrophobic interactions with the less polar regions of the chitin surface. The planar aromatic structure of these dyes likely facilitates strong van der Waals π-π stacking interactions with the chitin matrix, resulting in effective binding and sustained antimicrobial activity. The results obtained are similar to those reported for other dyes [13,20] such as methylene blue, for which chitin has been found to be an effective sorbent [20,42].

Despite Rivanol also being a dye with a planar three-ring system and a nitrogen atom, making it a weak base, it is an acridine derivative [36,43] and is less cationic than triarylmethane dyes. This difference, coupled with its lactate form, increases its hydrophilicity, leading to less effective adsorption. Consequently, the dye may not adhere as strongly to the hydrophobic regions of chitin. Additionally, the fact that for Rivanol, bacterial growth was not observed directly on the matrix—nor were inhibition zones observed on TSA plates—can be explained by the insufficient concentration of the antiseptic: commercially available ethacridine lactate solution is 0.1% *w*/*v*, in contrast to the 1% *w*/*v* solutions of brilliant green and gentian violet. The MIC of Rivanol against the microorganisms investigated is much higher [43,44] than that of triarylmethane dyes [38].

The quaternary ammonium compound decamethoxine [45,46] demonstrated good activity, especially in water and alcohol solutions. The long hydrophobic tail and the cationic nature of decamethoxine [40,47] likely promote strong adsorption to the negatively charged chitin matrix, thereby enhancing its antimicrobial properties. Polyhexanide, another biguanide compound, was slightly less effective, which may be due to its larger molecular size, leading to steric hindrance and reduced adsorption [41,48].

In contrast, antiseptics such as potassium permanganate [35] and iodine [37], which are smaller and less complex molecules, showed limited efficacy, particularly against *S. aureus* and *E. coli*. This may be due to their lower adsorption onto the chitin scaffold, which is possibly a consequence of their smaller molecular size and the absence of multiple aromatic rings that would otherwise enhance interactions with the chitinous material, or of possible degradation under the study conditions. This is particularly notable for iodine, traditionally known for its antiseptic properties [37]. Iodine's volatility and the potential for rapid sublimation might reduce its sustained release from the matrix, leading to lower observed activity. As a result, these antiseptics were less effective in forming a sustained inhibition zone.

Similarly, samples impregnated with sea buckthorn oil [39] and bromotyrosine glycerin extract [33] did not display significant antimicrobial activity compared with the control. Bacterial growth was observed directly on the matrix, indicating that these substances were ineffective in inhibiting bacterial proliferation under the tested conditions. For sea buckthorn oil, this may be caused by the much lower absorbance capacity of the oil compared with water [14,49,50], as well as inadequate release from the chitin matrix or

poor penetration into microbial cells of the bioactive compounds (such as flavonoids, carotenoids, and fatty acids) due to their hydrophobic nature.

The bromotyrosine-glycerin extract [33] likely contained brominated aromatic structures, typical for natural marine products, which may enhance adsorption onto microbial surfaces. However, its effectiveness might be hampered by poor adsorption or instability within the chitinous matrix, leading to negligible antimicrobial activity.

The swelling properties of the chitin scaffold also play a crucial role in the diffusion and sustained release of the impregnated antiseptics. The hydrophilic nature of the chitinous matrices from the Verongiid sponges allows them to swell in aqueous solutions, with a swelling capacity of 255 $\pm$ 8% [20], facilitating the gradual release of antiseptics and maintaining prolonged antimicrobial activity. This property is particularly advantageous for antiseptics such as decamethoxine, where sustained release can ensure prolonged inhibition of microbial growth. Comparable swelling properties have been reported for chitin in water and alcohol solutions [51], explaining the consistent antimicrobial properties for both ethanol and water solutions of decamethoxine-impregnated matrices.

The *I. basta* chitin matrix continuously released these effective antiseptics for at least 72 h, demonstrating significant antimicrobial effects against *S. aureus*. This 3D chitinous *I. basta* sponge scaffold is an excellent and sustainable candidate for delivering selected antiseptics, offering a promising alternative to synthetic wound dressing materials.

Moreover, the antiseptics may diffuse both from the surface of the chitinous matrix and potentially from the inner regions of its microtubular and nanoporous structures [19]. The development of death zones around microbial colonies within 72 h of incubation confirms the antibiotic efficacy of the antiseptic via diffusion from the treated chitinous scaffold. Future research needs to include an in-depth analysis of both the Fickian and potential non-Fickian diffusion behaviors [52,53] of this previously unexplored microtubular chitin matrix. In the initial phase, we did not distinguish between the release of substances adsorbed on the matrix surface, those absorbed through nanopores, or those taken in and released via capillary action. Therefore, it is unclear whether a matrix-type or reservoir-type diffusion-controlled system is at play. Understanding the relationship between the structure and function of the sponge biomaterial system described in this study as a new antimicrobial drug delivery scaffold is essential for its successful implementation, including in fields such as dermatocosmetics against inflammatory skin diseases. Detailed studies on the drug release kinetics of antimicrobial agents in naturally pre-structured sponge chitin should be planned for the future.

5. Conclusions

Overall, the study highlights that the antimicrobial efficacy of chitinous scaffolds depends on a complex interplay of the chemical structure of the antiseptics, their concentration, and the inherent properties of the sponge chitin matrix, including its adsorption capacity and swelling behavior. This understanding is crucial for optimizing the use of chitin-based scaffolds in biomedical applications, particularly in wound dressing and controlled drug delivery systems.

Antiseptics with low MIC values, that are hydrophobic, have planar structures, and show strong adsorption to the matrix (including gentian violet and brilliant green) exhibit more potent and sustained antimicrobial effects. In contrast, those with higher MICs, poor adsorption, or rapid release (such as potassium permanganate and iodine) exhibit reduced efficacy in this context. The addition of antiseptics such as decamethoxine, with its strong adsorption and sustained release properties, further demonstrates the potential of chitinous scaffolds as effective delivery systems. Understanding these relationships can guide the selection and optimization of antiseptics for use in chitin-based scaffolds.

Author Contributions: Conceptualization, H.E., T.J. and I.D.; methodology, H.E. and V.K.; investigation, I.D., K.D., A.V. and V.K.; resources, H.E. and V.K.; writing—original draft preparation, I.D. and A.V.; writing—review and editing, H.E. and T.J.; visualization, I.D. and A.V.; supervision, H.E. and T.J.; project administration, H.E.; funding acquisition, H.E. and T.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre within the framework of the project OPUS 19 grant from the National Science Centre, Poland (2020/37/B/ST5/01909). A.V. was funded by the VW Foundation (Funding for Refugee Scholars and Scientists from Ukraine, Personal Ref. No. 05020407B, TU Bergakademie Freiberg, Germany). This research was performed with the financial support of the Ministry of Science and Higher Education, Poland (grant no. 0912/SBAD/2406).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: We would like to thank INTIB GmbH and BromMarin GmbH (Freiberg, Germany) for the sponge specimens, consulting, and technical support.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Rinaudo, M. Chitin and Chitosan: Properties and Applications. *Prog. Polym. Sci.* **2006**, *31*, 603–632. [CrossRef]
2. Roberts, G.A.F. *Chitin Chemistry*, 1st ed.; MacMillian: London, UK, 1992.
3. Tsurkan, D.; Wysokowski, M.; Petrenko, I.; Voronkina, A.; Khrunyk, Y.; Fursov, A.; Ehrlich, H. Modern Scaffolding Strategies Based on Naturally Pre-Fabricated 3D Biomaterials of Poriferan Origin. *Appl. Phys. A* **2020**, *126*, 382. [CrossRef]
4. Brandelli, A. Nanocomposites and Their Application in Antimicrobial Packaging. *Front. Chem.* **2024**, *12*, 1356304. [CrossRef] [PubMed]
5. Khatami, N.; Guerrero, P.; Martín, P.; Quintela, E.; Ramos, V.; Saa, L.; Cortajarena, A.L.; De La Caba, K.; Camarero-Espinosa, S.; Abarrategi, A. Valorization of Biological Waste from Insect-Based Food Industry: Assessment of Chitin and Chitosan Potential. *Carbohydr. Polym.* **2024**, *324*, 121529. [CrossRef] [PubMed]
6. Kumar, P.T.S.; Lakshmanan, V.-K.; Biswas, R.; Nair, S.V.; Jayakumar, R. Synthesis and Biological Evaluation of Chitin Hydrogel/Nano ZnO Composite Bandage as Antibacterial Wound Dressing. *J. Biomed. Nanotechnol.* **2012**, *8*, 891–900. [CrossRef]
7. Jayakumar, R.; Prabaharan, M.; Sudheesh Kumar, P.T.; Nair, S.V.; Tamura, H. Biomaterials based on chitin and chitosan in wound dressing applications. *Biotechnol. Adv.* **2011**, *29*, 322–337. [CrossRef]
8. Zhou, D.; Yang, R.; Yang, T.; Xing, M.; Gaoxing, L.G. Preparation of chitin-amphipathic anion/quaternary ammonium salt ecofriendly dressing and its effect on wound healing in mice. *Int. J. Nanomed.* **2018**, *13*, 4157–4169. [CrossRef]
9. Baharlouei, P.; Rahman, A. Chitin and Chitosan: Prospective Biomedical Applications in Drug Delivery, Cancer Treatment, and Wound Healing. *Mar. Drugs* **2022**, *20*, 460. [CrossRef]
10. Parhi, R. Drug Delivery Applications of Chitin and Chitosan: A Review. *Environ. Chem. Lett.* **2020**, *18*, 577–594. [CrossRef]
11. Prabu, P.; Kim, K.W.; Dharmaraj, N.; Park, J.H.; Khil, M.S.; Kim, H.Y. Antimicrobial drug release scaffolds of natural and synthetic biodegradable polymers. *Macromol. Res.* **2008**, *16*, 303–307. [CrossRef]
12. Shang, Y.; Ding, F.; Xiao, L.; Deng, H.; Du, Y.; Shi, X. Chitin-based fast responsive pH sensitive microspheres for controlled drug release. *Carbohydr. Polym.* **2014**, *102*, 413–418. [CrossRef] [PubMed]
13. Jóźwiak, T.; Filipkowska, U.; Bakuła, T.; Bralewska-Piotrowicz, B.; Karczmarczyk, K.; Gierszewska, M.; Olewnik-Kruszkowska, E.; Szyryńska, N.; Lewczuk, B. The Use of Chitin from the Molts of Mealworm (*Tenebrio molitor*) for the Removal of Anionic and Cationic Dyes from Aqueous Solutions. *Materials* **2023**, *16*, 545. [CrossRef] [PubMed]
14. De Freitas Barros, F.C.; Grombone Vasconcellos, L.C.; Vieira Carvalho, T.; Ferreira do Nascimento, R. Removal of petroleum spill in water by chitin and chitosan. *Electron. J. Chem.* **2014**, *6*, 70–74.
15. Younes, I.; Rinaudo, M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar. Drugs* **2015**, *13*, 1133–1174. [CrossRef]
16. Deringer, V.L.; Englert, U.; Dronskowski, R. Nature, Strength, and Cooperativity of the Hydrogen-Bonding Network in α-Chitin. *Biomacromolecules* **2016**, *17*, 996–1003. [CrossRef]
17. Montroni, D.; Fermani, S.; Morellato, K.; Torri, G.; Naggi, A.; Cristofolini, L.; Falini, G. β-Chitin samples with similar microfibril arrangement change mechanical properties varying the degree of acetylation. *Carbohydr. Polym.* **2019**, *207*, 26–33. [CrossRef]
18. Anitha, A.; Sowmya, S.; Kumar, P.T.S.; Deepthi, S.; Chennazhi, K.P.; Ehrlich, H.; Tsurkan, M.; Jayakumar, R. Chitin and Chitosan in Selected Biomedical Applications. *Prog. Polym. Sci.* **2014**, *39*, 1644–1667. [CrossRef]

19. Kovalchuk, V.; Voronkina, A.; Binnewerg, B.; Schubert, M.; Muzychka, L.; Wysokowski, M.; Tsurkan, M.V.; Bechmann, N.; Petrenko, I.; Fursov, A.; et al. Naturally Drug-Loaded Chitin: Isolation and Applications. *Mar Drugs* **2019**, *17*, 574. [CrossRef] [PubMed]
20. Klinger, C.; Żółtowska-Aksamitowska, S.; Wysokowski, M.; Tsurkan, M.V.; Galli, R.; Petrenko, I.; Machałowski, T.; Ereskovsky, A.; Martinović, R.; Muzychka, L.; et al. Express Method for Isolation of Ready-to-Use 3D Chitin Scaffolds from *Aplysina archeri* (Aplysineidae: Verongiida) Demosponge. *Mar. Drugs* **2019**, *17*, 131. [CrossRef]
21. Duminis, T.; Heljak, M.; Święszkowski, W.; Ereskovsky, A.; Dziedzic, I.; Nowicki, M.; Pajewska-Szmyt, M.; Voronkina, A.; Bornstein, S.R.; Ehrlich, H. On the Mechanical Properties of Microfibre-Based 3D Chitinous Scaffolds from Selected Verongiida Sponges. *Mar. Drugs* **2023**, *21*, 463. [CrossRef]
22. Pallas, P.S. Miscellanea Zoologica: Quibus Novae Imprimis Atque Obscurae Animalium Species Describuntur et Observationibus Iconibusque Illustrantur. Hagae Comitum: Apud Petrum van Cleef. 1766. [CrossRef]
23. Bergquist, P.R.; Kelly, M. Borges Systematics and biogeography of the genus *Ianthella* (Demospongiae: Verongida: Ianthellidae) in the south-west Pacific. *Rec. Mus. Art Gall. North. Territ.* **1995**, *12*, 151–176.
24. Andreakis, N.; Luter, H.M.; Webster, N.S. Cryptic speciation and phylogeographic relationships in the elephant ear sponge *Ianthella basta* (Porifera, Ianthellidae) from northern Australia. *Zool. J. Linn. Soc.* **2012**, *166*, 225–235. [CrossRef]
25. Rohde, S.; Schupp, P.J. Growth and Regeneration of the Elephant Ear Sponge *Ianthella basta* (Porifera). *Hydrobiologia* **2012**, *687*, 219–226. [CrossRef]
26. Freckelton, M.L.; Luter, H.M.; Andreakis, N.; Webster, N.S.; Motti, C.A. Qualitative variation in colour morphotypes of *Ianthella basta* (Porifera, Verongida). *Hydrobiologia* **2012**, *687*, 191–203. [CrossRef]
27. Kertmen, A.; Petrenko, I.; Schimpf, C.; Rafaja, D.; Petrova, O.; Sivkov, V.; Nekipelov, S.; Fursov, A.; Stelling, A.L.; Heimler, K.; et al. Calcite Nanotuned Chitinous Skeletons of Giant *Ianthella basta* Marine Demosponge. *Int. J. Mol. Sci.* **2021**, *22*, 12588. [CrossRef]
28. García-Vilas, J.A.; Martínez-Poveda, B.; Quesada, A.R.; Medina, M.Á. Aeroplysinin-1, a Sponge-Derived Multi-Targeted Bioactive Marine Drug. *Mar. Drugs* **2016**, *14*, 1. [CrossRef] [PubMed]
29. Franklin, M.A.; Penn, S.G.; Lebrilla, C.B.; Lam, T.H.; Pessah, I.N.; Molinski, T.F. Bastadin 20 and Bastadin O-Sulfate Esters from *Ianthella basta*: Novel Modulators of the Ry1R FKBP12 Receptor Complex. *J. Nat. Prod.* **1996**, *59*, 1121–1127. [CrossRef]
30. Calcul, L.; Inman, W.D.; Morris, A.A.; Tenney, K.; Ratnam, J.; McKerrow, J.H.; Valeriote, F.A.; Crews, P. Additional Insights on the Bastadins: Isolation of Analogues from the Sponge *Ianthella* cf. *reticulata* and Exploration of the Oxime Configurations. *J. Nat. Prod.* **2010**, *73*, 365–372.
31. Gartshore, C.J.; Salib, M.N.; Renshaw, A.A.; Molinski, T.F. Isolation of Bastadin-6-O-Sulfate and Expedient Purifications of Bastadins-4, -5 and -6 from Extracts of *Ianthella basta*. *Fitoterapia* **2018**, *126*, 16–21. [CrossRef]
32. Geahchan, S.; Ehrlich, H.; Rahman, M.A. The Anti-Viral Applications of Marine Resources for COVID-19 Treatment: An Overview. *Mar. Drugs* **2021**, *19*, 409. [CrossRef]
33. Muzychka, L.; Voronkina, A.; Kovalchuk, V.; Smolii, O.B.; Wysokowski, M.; Petrenko, I.; Youssef, D.T.A.; Ehrlich, I.; Ehrlich, H. Marine Biomimetics: Bromotyrosines Loaded Chitinous Skeleton as Source of Antibacterial Agents. *Appl. Phys. A* **2021**, *127*, 15. [CrossRef] [PubMed]
34. Abbas, M.; Harrache, Z.; Trari, M. Removal of Gentian Violet in Aqueous Solution by Activated Carbon Equilibrium, Kinetics, and Thermodynamic Study. *Adsorpt. Sci. Technol.* **2019**, *37*, 566–589. [CrossRef]
35. Abed, A.R.; Hussein, I.M. In Vitro Study of Antibacterial and Antifungal Activity of Some Common Antiseptics and Disinfectants Agents. *Kufa J. Vet. Med. Sci.* **2016**, *7*, 148–159. [CrossRef]
36. Unnisa, A.; Manasa, N.; Ravali, P.L. Development of Validated Spectrophotometric Methods for Estimation of Ethacridine Lactate in Pharmaceutical Formulations. *J. Chem.* **2011**, *8*, 274361. [CrossRef]
37. Mao, Y.-C.; Tsai, W.-J.; Wu, M.-L.; Ger, J.; Deng, J.-F.; Yang, C.-C. Acute Hemolysis Following Iodine Tincture Ingestion. *Hum. Exp. Toxicol.* **2011**, *30*, 1716–1719. [CrossRef]
38. Narat, J.K. Brilliant green: A clinical study of its value as a local antiseptic. *Ann. Surg.* **1931**, *94*, 1007–1012. [CrossRef]
39. Koskovac, M.; Cupara, S.; Kipic, M.; Barjaktarevic, A.; Milovanovic, O.; Kojicic, K.; Markovic, M. Sea Buckthorn Oil—A Valuable Source for Cosmeceuticals. *Cosmetics* **2017**, *4*, 40. [CrossRef]
40. Bagnyuk, N.A.; Nazarchuk, O.A.; Babina, Y.M.; Chornopyshchuk, R.M.; Kulyk, A.V. Antimicrobial Activity of Antiseptics in the Prevention of Postoperative Infectious Complications. *Biomed. Biosoc. Anthropol.* **2020**, *40*, 33–36. [CrossRef]
41. Carrijo-Carvalho, L.C.; Sant'ana, V.P.; Foronda, A.S.; de Freitas, D.; de Souza Carvalho, F.R. Therapeutic Agents and Biocides for Ocular Infections by Free-Living Amoebae of *Acanthamoeba* Genus. *Surv. Ophthalmol.* **2017**, *62*, 203–218. [CrossRef]
42. Dotto, G.L. Adsorption of Methylene Blue by ultrasonic surface modified chitin. *J. Colloid Interface Sci.* **2015**, *446*, 133–140. [CrossRef]
43. Jabri, T.; Khan, N.A.; Makhlouf, Z.; Akbar, N.; Gul, J.; Shah, M.R.; Siddiqui, R. Antibacterial Properties of Ethacridine Lactate and Sulfmethoxazole Loaded Functionalized Graphene Oxide Nanocomposites. *Antibiotics* **2023**, *12*, 755. [CrossRef]
44. Dydak, K.; Junka, A.; Dydak, A.; Brożyna, M.; Paleczny, J.; Fijalkowski, K.; Kubielas, G.; Aniołek, O.; Bartoszewicz, M. In Vitro Efficacy of Bacterial Cellulose Dressings Chemisorbed with Antiseptics against Biofilm Formed by Pathogens Isolated from Chronic Wounds. *Int. J. Mol. Sci.* **2021**, *22*, 3996. [CrossRef]
45. Paliy, G.K.; Nazarchuk, O.A.; Kulakov, O.I.; Paliy, V.G.; Nazarchuk, S.A.; Paliy, D.V.; Kordon, Y.V.; Gonchar, O.O. Substaniation of antimicrobial dressings use in surgery. *Med. Perspekt.* **2014**, *19*, 152–158. [CrossRef]

46. Nazarchuk, O.A.; Chereshniuk, I.L.; Nazarchuk, H.H. The research of antimicrobial efficacy of antiseptics decamethoxin, miramistin and their effect on nuclear DNA fragmentation and epithelial cell cycle. *Wiad. Lek.* **2019**, *72*, 374–380. [CrossRef]
47. Fuss, J.; Palii, V.; Voloboyeva, A. Evaluating the effectiveness of antiseptic solution Decasan in treatment of necrotic soft tissue diseases. *Pol. J. Surg.* **2016**, *88*, 233–237.
48. Babalska, Z.Ł.; Korbecka-Paczkowska, M.; Karpiński, T.M. Wound Antiseptics and European Guidelines for Antiseptic Application in Wound Treatment. *Pharmaceuticals* **2021**, *14*, 1253. [CrossRef]
49. Duan, B.; Gao, H.; He, M.; Zhang, L. Hydrophobic Modification on surface of chitin sponges for highly effective separation of oil. *Appl. Mater. Interfaces* **2014**, *6*, 19933–19942. [CrossRef]
50. Setti, L.; Mazzieri, S.; Pifferi, P.G. Enhanced degradation of heavy oil in an aqueous system by a *Pseudomonas sp.* in the presence of natural and synthetic sorbents. *Bioresour. Technol.* **1999**, *67*, 191–199. [CrossRef]
51. Seito, Y.; Okano, T.; Gaill, F.; Chanzy, H.; Putaux, J. Structural data on the intra-crystalline swelling of β-chitin. *Int. J. Biol. Macromol.* **2000**, *28*, 81–88. [CrossRef]
52. Ritger, P.L.; Peppas, N.A.A. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *J. Control. Release* **1987**, *5*, 23–36. [CrossRef]
53. Peppas, A.N. Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta Helv.* **1985**, *60*, 110–111.