Novel soy protein blend scaffolds loaded with antibiotics: Drug release profile—bacterial inhibition effects

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Soy protein is a natural biocompatible and biodegradable material and can therefore be used in biomedical applications. However, the practical use of pure soy protein is limited, due to its unsatisfactory physical properties. We hypothesized that cross-linking and blending soy protein with other natural polymers would enhance its biostability and potential use for skin regeneration applications. In the current study, gelatin and alginate were chemically cross-linked to soy protein using the cross-linking agent carbodiimide, and a porous blend structure was obtained through lyophilization. The antibiotic drug clindamycin was incorporated into the matrix for local controlled release, enabling a continuous bactericidal effect. The study focused on the microstructure and weight loss as well as the release profile of clindamycin from the porous blend structure and its effect on bacterial inhibition. Our results show that these blend structures can be assembled into porous three-dimensional structures. The soy protein-alginate blends remained intact and stable for longer than the soy protein-gelatin blends. Clindamycin release from the soy protein-based scaffolds exhibited a high burst effect (70%) accompanied by a decrease in the release rate for up to 4 days. The clindamycin release was correlated with *in vitro* bacterial inhibition profiles which demonstrated a significant decrease in the bacterial viability and effectively inhibited *S. aureus* and *S. albus* for 4 days. These soy protein porous blend scaffolds may be potentially useful as a unique drug/cells carrier platform for skin regeneration applications.

Keywords: soy protein, scaffold, tissue regeneration, antibiotics release, bacterial inhibition

1. Introduction

Tissue loss caused by various types of wounds is one of the most frequent and devastating problems in healthcare. Serious injuries to the skin, such as burns, trauma or chronic ulcers, have a limited capability for spontaneous regeneration. In the United States alone, more than 1.25 million people experience burns every year, and 6.5 million experience various chronic skin ulcers such as diabetic ulcers and pressure sores. The damaged full-thickness skin requires immediate coverage to facilitate repair and restore skin function [1]. The goal of skin tissue engineering and wound dressings is therefore to restore the milieu required for skin regeneration by protecting the wound from environmental threats such as the penetration of bacteria, and by maintaining a moist healing environment.

Wound dressings and skin substitutes should have some ideal characteristics. They should be easy to handle and apply to the wound site, comprise a barrier against bacteria and environmental threats while supplying an appropriate moist environment [2]. They should also have appropriate physical and mechanical properties; undergo controlled degradation; be sterile, non-toxic, non-antigenic and as far as is feasible be able to release growth factors, bioactive molecules, enzymes and pharmacological agents in a controlled manner. They should achieve aesthetic results with minimal scarring and pain, while still being cost effective. The ultimate goal in wound management is to satisfy most, if not all, of these criteria when producing novel smart wound dressing and skin replacement therapies in order to promote optimal tissue repair and regeneration of full-thickness skin wounds [3]. The repair options for serious injuries to the skin are limited. The availability of products with clinical relevance is still small, especially if we limit the application to the drug delivery field for wound healing applications [4].

The main current approaches in modern dressings are designed to model the properties of the extracellular matrix (ECM). A range of dressing formats based on films, hydro-

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philic gels and foams are available or have been investigated. The porous structures of biodegradable polymers are frequently applied in skin tissue engineering both to deliver cells or growth factors, and to serve as a template for tissue regeneration. Bacterial contamination of a wound seriously threatens its healing. In burns, infection is the major complication after the initial period of shock, and it is estimated that about 75% of the mortalities following burn injuries are related to infections rather than to osmotic shock and hypovolaemia [5]. This has encouraged the development of improved wound dressings that provide an antimicrobial effect by eluting germicidal compounds such as iodine (Iodosorb®, Smith & Nephew), chlorohexidine (Biopatch®, J & J) or, in most cases, silver ions (e.g. Acticoat® by Smith & Nephew, Actisorb® by J & J and Aquacell® by ConvaTec). Such dressings are designed to provide controlled release of the active agent through a slow but sustained release mechanism which avoids toxicity yet ensures delivery of a therapeutic dose to the wound. However, some safety concerns have been raised regarding the silver ions included in most products.

Various natural, synthetic, and semi-synthetic materials are currently being utilized in the fabrication of scaffolds for skin tissue engineering. The use of synthetic biomaterials has some limitations in biomedical engineering, including high infection, lack of degradation and improper tissue regeneration [6]. Biopolymers from plant and animal origins are mostly nontoxic, biocompatible, biodegradable, and hence suitable for the development of biomaterials. Biopolymers, such as polylactic acid and collagen, are the most widely studied materials for the regeneration of damaged tissues, acting as artificial supports (scaffolds) for cell growth and for the growth of several tissue types [7]. Other extensively used natural materials include protein-based materials (e.g., gelatin, fibrin, elastin and silk fibroin) and polysaccharide-based materials (e.g., chitosan, alginate, glycosaminoglycans, and hyaluronic acid).

In recent years, there has been a growing tendency to replace synthetic and biological animal-origin polymers with natural, abundant, non-animal origin and low-cost biodegradable materials such as soy protein [8, 9]. These plant proteins can be used to meet the high demand for new materials that are needed for wound healing applications. Soy protein has advantages over the various types of natural animal proteins currently employed for biomedical applications due to its non-animal origin and therefore lower immunogenicity, relatively long storage time and stability, degradation into natural components as well as low price. It is also well known that soy protein possesses medicinal properties that accelerate wound healing and tissue regeneration (anti-inflammatory genistein isoflavones) [10, 11]. However, some studies reported that neat soy protein as dense films is rather brittle and exhibits relatively poor mechanical properties [12]. One approach to improve the physical properties of soy protein materials is to prepare blend films. It is assumed that the blending of polymers results in materials with better performance than their separate components, while promoting cell adhesion to the extracellular matrix as well as regulating the development of various tissues.

In many tissue engineering applications it is assumed that three-dimensional structures more closely mimic the morphology of human tissues and are therefore preferable over two-dimensional structures. In the fields of tissue engineering and wound healing, for instance, extensive research has been dedicated to the introduction of solutions for producing porous structures from blended natural polymers. These include chitosan/gelatin freeze-dried scaffolds [13], cellulose/soy protein composite freeze-dried sponges [14], collagen–chitosan scaffolds [15] and others. However, none of the existing solutions can combine all the essential characteristics for the creation of "smart" hybrid biomaterials with optimal properties. It can therefore be said that there is an increasing need for development of new biodegradable materials for use in wound healing applications and that a great deal of research must still be performed in order to develop successful natural material-based blend systems in this area [16, 17].

Despite the availability, low cost and stability of soy protein, only few groups in the biomedical field have investigated its potential as a natural material for various biomedical applications [18–20]. Also, only a few studies reported on the preparation of soy protein-based three-dimensional porous structures [14, 21]. Additionally, there have been no published studies to date on the preparation of chemically cross-linked soy protein–natural polymer blend porous structures for biomedical applications.

In light of the above, we recently developed and studied novel three-dimensional soy protein-based porous blend structures loaded with antibiotics as potential new materials for biomedical applications. These new materials can be used as either an acellular matrix (e.g., wound dressing) or as a carrier system for cells or active biomolecules in the field of tissue engineering, mainly for skin and cartilage tissues. Soy protein was blended with suitable biopolymers (gelatin, alginate and pectin) and cross-linked with nontoxic agents. These structures were characterized for their mechanical and physical properties [22] as well as for their microstructure and cytotoxicity [23]. In continuation of our research, in the current study the antibiotic drug clindamycin was incorporated into the selected scaffold formulations in order to inhibit bacterial infections upon its release to the surrounding tissue. Clindamycin was selected as the preferred drug since it is inert toward the cross-linking reaction. It is used for treatment of a wide spectrum of diseases, such as infections of the respiratory tract, skin and soft tissue infections, osteomyelitis, and gynecological infections [24, 25]. In the current study, we characterized the drug release profile and the resulting antimicrobial effects and investigated the effect of clindamycin incorporation into

the soy protein porous blend on the degradation behavior of the scaffolds.

2. Materials and methods

2.1. Materials

Primary component. Soy protein source: Non-GMO soy protein isolate (SPI, Solpro 910™, minimum 90% w/w protein, on dry basis) was produced by Solbar™ (Ashdod, Israel) and obtained as a donation.

Additional natural polymers. Gelatin "type A" from porcine skin (90–100 bloom) was purchased from Sigma-Aldrich, Rehovot, Israel (G6144).

Alginic acid sodium salt (viscosity ~250 cps, 2% (25°C)) was purchased from Sigma-Aldrich, Rehovot, Israel (A2158).

Cross-linkers. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride—a water soluble carbodiimide (EDC) E7750 and glyoxal (50649) were purchased from Sigma-Aldrich, Rehovot, Israel.

Drug. Clindamycin hydrochloride (PHR1159-1G) was purchased from Sigma-Aldrich, Rehovot, Israel.

2.2. Preparation of soy protein-based porous blend structures

In order to prepare the soy protein blends, alginate and gelatin were separately prepared by dissolving the second component at 4°C in double-distilled water at a concentration of 5 wt % overnight to obtain a homogeneous solution. For all blends, a final soy protein concentration of 5 wt % was reached by slowly suspending the soy protein powder under constant stirring in double-distilled water. The dispersion was heated in a water bath to 80°C for 30 min. The soy protein slurry was then cooled to 60°C. The soy protein slurry and the second component solution were then mixed together to obtain blends with a second biopolymer content of 25 wt % relative to the soy protein (4 : 1 weight ratio compositions). Then the clindamycin was added to the mixture for a final concentration.

In order to create porous structures, a cross-linker solution containing EDC in double-distilled water (1 or 4 wt % relative to the soy protein) was cast into a 3-cm diameter Petri dish at a volume of 300 μl. The cross-linker solution was then frozen at –40°C for 30 min. Three milliliters of the soy mixture (containing either gelatin or alginate) were then quickly poured on top of the frozen cross-linking solution, then left to react at room temperature for 1 h. The cross-linked samples were then washed three times with saline. Finally, the sponge-like three-dimensional structures were obtained by freezing at –40°C for 4 h, followed by freeze-drying at –20°C for 18–24 hours.

2.3. Morphological characterization

The morphology of the different types of soy protein-based porous blend structures (cross-section and surfaces of cryogenically fractured scaffolds) was observed using a Quanta 200 FEG environmental scanning electron microscope (ESEM) in a high vacuum mode with an acceleration voltage of 10 kV (n = 2 per group). The selected samples were Au-sputtered prior to observation.

2.4. In vitro drug release studies

The soy protein-based blend scaffolds loaded with 3 wt % clindamycin (triplicate samples) were immersed in saline (pH 7.4) at 37°C for 7 days in order to determine the drug release kinetics from these porous structures. The release studies were conducted in closed vials containing 3.0 ml saline. Sodium azide (0.02% w/v) was added to the solution in order to prevent bacterial growth. The saline solution was completely removed periodically, at each sampling time, and fresh solution was introduced at 1, 3, 5, and 24 h, and 3 and 7 days. The results are presented as cumulative release data.

Clindamycin assay. The medium clindamycin content was determined using a Jasco HPLC (Jasco Corp., Tokyo, Japan) with a UV 2075 plus detector and a reverse phase column (ACE®, Aberdeen, Scotland, 5 μm, inner diameter d = 4.6 mm, length = 250 mm), kept at 35°C.

The mobile phase consisted of a mixture of saline (pH 7.4) and acetonitrile (55/45, v/v) at a flow rate of 1 ml/min with a quaternary gradient pump (PU 2089 plus) without gradient. Twenty-microliter samples were injected with an autosampler (AS 2057 Plus). The column effluent was eluted for 15 min and detected at 210 nm. The area of each eluted peak was integrated using the EZstart software version 3.1.7 (Scientific Software Inc., Pleasanton, CA). A calibration curve was prepared for concentrations ranging from 25.0 to 1500.0 μg/ml (correlation coefficient >0.99, slope 0.0007).

2.5. Microbiological evaluation

Two bacterial strains, *Staphylococcus aureus* and *Staphylococcus albus*, were chosen for this study, due to their frequent presence on human skin and their high involvement in infections during wound management. Both strains were clinically isolated at the Microbiological Laboratory of Rambam Medical Center (Haifa, Israel), and their minimal inhibitory concentration (MIC) values were determined. The strains were grown overnight on Mueller–Hinton (Difco) agar plates at 37°C prior to use. The bacteria were then collected and re-suspended in saline, and adjusted to 1×10^6 CFU/mL (colony forming units). Two groups of soy protein-based scaffolds were tested for drug release-induced bacterial inhibition. The scaffolds loaded with 3 wt % clindamycin were cut into 6.5×6.5×3.5 mm cube-shaped samples. Drug release-induced bacterial inhibition from these samples was evaluated using the following two methods.

Corrected zone of inhibition method. The corrected zone of inhibition test (CZOI) was used to determine the antimi-

crobial activity of the soy protein blend scaffolds, which is time-dependent. In this modified version of the Kirby–Bauer method, cube-shaped samples of the selected scaffolds loaded with clindamycin were placed on a bacterial lawn (100 μl of inoculums, ~10^6 CFU/ml, seeded on Mueller–Hinton agar plates), and incubated overnight at 37°C and then photographed, instead of the disk diffusion test which is typically used to determine bacterial susceptibility to antibiotics. Bacterial inhibition zones around the dressings were assessed after 24 h of incubation. Similar cube-shaped samples prepared without the drug were used as controls. This procedure was repeated on soy protein blend samples which were incubated in PBS for 24, 48, and 96 h prior to testing. The test was performed in duplicates for each of the microorganisms.

Viable counts method. A release study from the cube-shaped soy protein blend scaffolds in the presence of bacteria was performed in order to study the effect of drug release on the kinetics of residual bacteria. One milliliter of PBS containing the bacterial strains at a concentration of 1×10^5 CFU/ml was added at the beginning of the release study, and the effect of the antibiotic released from the samples on residual bacteria was tested. Microorganisms in the presence of PBS only and cube-shaped samples prepared without the drug served as the control. Ten-microliter samples were collected at time 0 and after 96 h, and were spread on Mueller–Hinton agar plates. CFU/ml were counted after 24 h incubation at 37°C. The test was performed in duplicate for each of the microorganisms.

2.6. Weight loss

The degradation behavior of the soy protein-based porous blend structures was examined by weight loss from the clindamycin drug-loaded samples. The *in vitro* weight loss of the soy protein porous blend samples was studied in an aqueous cell culture medium. Round dry specimens with a 30 mm diameter (n = 3 specimens per group) were preweighed after lyophilization and immersed in 4 ml of a modified Eagle's cell culture medium (MEM) with 10% fetal bovine serum (FBS) and 3% penicillin–streptomycin–nystatin (pH = 7.4) at 37°C in an incubator which mimics the *in vivo* environment. The scaffolds were taken out after 14 days, redried by freeze-drying and weighed.

2.7. Statistics

Statistics were calculated using the SPSS 10 software. All data are expressed as means±standard deviation (SD). Statistical comparison between more than two groups was performed using the ANOVA (Tukey Kramer) and P values less than 0.05 were considered significant.

3. Results and discussion

A series of preliminary experiments under various conditions and with different natural polymers (gelatin, and alginate) with a final soy protein content of 5 wt % and a second biopolymer content of 12, 25, and 50 wt % relative to the soy protein (8 : 1, 4 : 1 and 2 : 1 weight compositions, respectively) were carried out in order to try to create homogeneous soy protein porous blend structures. Only the 4 : 1 composition produced homogeneous blend mixtures and yielded the most suitable soy protein-based porous blend structures.

3.1. Physical properties

Based on our preliminary study, we focused on two blend structures: 4 : 1 soy protein:alginate and 4 : 1 soy protein : gelatin, both cross-linked using 1% w/w EDC (relative to soy protein). These are termed soy protein-alginate and soy protein-gelatin throughout the entire article.

3.1.1. Morphological characterization

Figure 1 shows ESEM fractographs of the two (drug free) blends that were used in the current study (soy protein-gelatin and soy protein-alginate). Based on environmental scanning electron microscopy (ESEM) morphological characteristics at high magnifications, these blends exhibited homogenous dispersion of the gelatin or alginate in the soy protein. Porous soy protein-alginate samples (Figs. 1c and 1d) yielded much higher pore interconnectivity morphology compared with the less interconnected porous structure of the soy protein-gelatin blend (Figs. 1a and 1b). The pore size and distribution were relatively similar and relatively high for both types of blends, i.e. pore size in the range of 100–300 μm and bulk porosity of approximately 60%.

Structural properties of biomaterial scaffolds play a critical role in tissue engineering. High porosity and interconnected porous networks are desirable in order to permit the attachment and ingrowth of cells and the regeneration of new tissue. The soy protein-alginate samples have the advantage of greater pore connectivity than the soy protein-gelatin samples and therefore show a higher potential for tissue engineering applications.

3.1.2. Weight loss

The effect of the drug incorporation on the total weight loss of the two soy protein-alginate and soy protein-gelatin samples over 14 days is presented in Fig. 2. All soy protein-alginate porous blend samples lost 10–20% of their initial weight after 14 days of immersion in the cell culture medium, with no significant difference between samples. During the same period, all porous soy protein-gelatin samples degraded at a significantly faster rate, losing a total of 25–40% of their original mass. The mass loss of the reference soy protein-gelatin blend (without drug) was approximately 3 times higher than that of the reference soy protein-alginate formulation ($p < 0.05$). The samples loaded with 3 and 6 wt % clindamycin showed slightly higher mass loss compared to the corresponding reference formulations, with no statistical difference. It is important to mention that

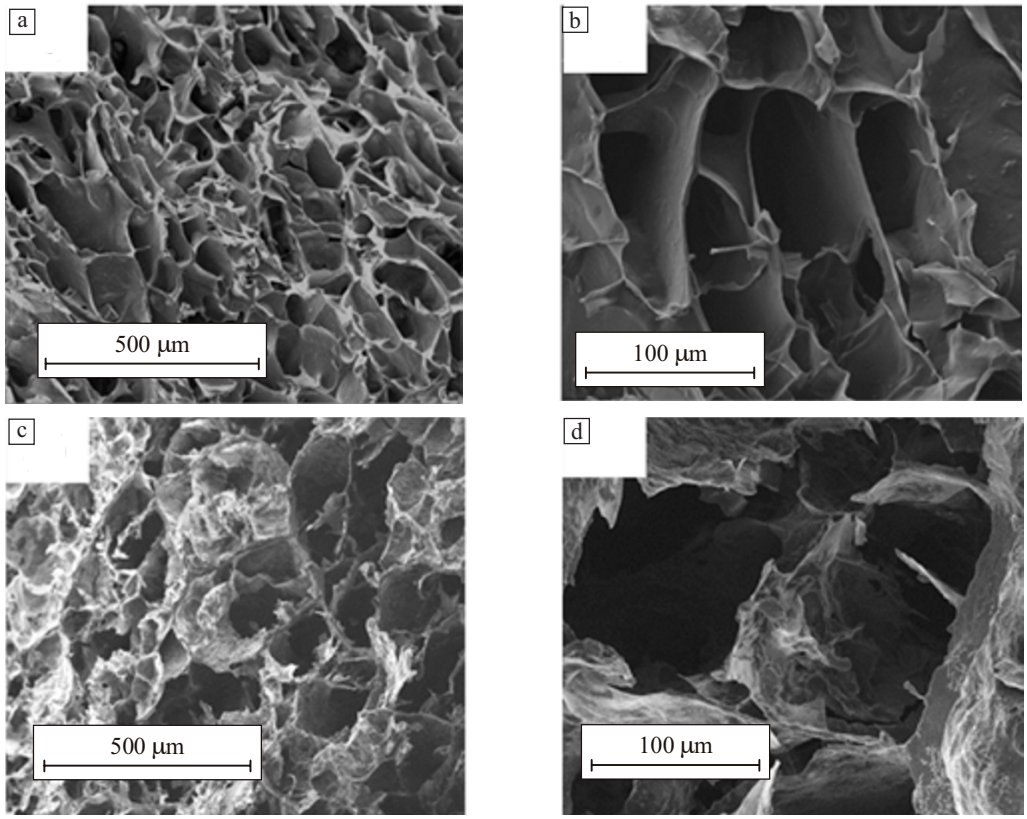

Fig. 1. ESEM fractograhs of 4 : 1 soy protein:natural polymer blends cross-linked with 1 wt % EDC: soy protein-gelatin (a, b) and soy protein-alginate (c, d).

the pure cross-linked soy protein and the non-cross-linked porous blend samples were completely dissolved in the cell culture medium after approximately 3 days. It is known that the resistance of different protein-based materials to weight loss as a result of degradation is directly proportional to the degree of cross-linking [26]. Thus, we assume that such behavior is attributed to a less effective cross-linking reaction in the soy protein-gelatin system. Clindamycin-loaded samples of both blend types showed relatively (insignificant) increased weight loss values, which can be caused by some steric interference of the small drug molecules with the soy protein-natural polymer cross-linking process.

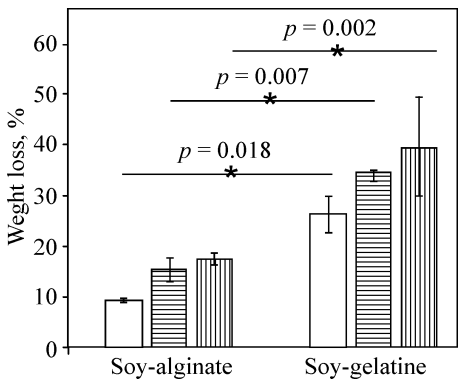

Fig. 2. Total weight loss of clindamycin-loaded porous blends after 14 days of immersion in a cell culture medium: □—unloaded samples, ▤—samples containing 3 wt % clindamycin, and ▥—samples containing 6 wt % clindamycin. The soy protein blend compositions are indicated. Data represent the mean ± standard deviation of three independent samples.

3.1.3. *In vitro* clindamycin release

Skin infections limit the healing process, may attract harmful organisms, and may lead to bacteremia, sepsis, or multi-system failure. It is therefore crucial to respond immediately to the presence of large numbers of bacteria. In order to avoid such risks, a local antibiotic release profile should exhibit a high initial release rate followed by a sustained release phase at an effective inhibitory level [27]. In the current study, the soy protein-gelatin and soy protein-alginate structures were loaded with 4.5 mg of clindamycin per scaffold (3 wt % relative to the soy protein) and the clindamycin release kinetics were studied for 7 days. The results are presented in Fig. 3. The release profile of clindamycin from both studied scaffolds was very similar and can be divided into two main stages:

1. Burst release of 68% and 76% for soy protein-alginate and soy protein-gelatin, respectively, during the first 5 h of release.

2. Approximately constant release at a relatively low rate until 72 h. Approximately 95% of the encapsulated clindamycin was released within 4 days.

Our results indicate that the drug release mechanism in our system is probably diffusion, which is strongly enhanced by the high porosity of the matrix. In the aqueous medium, water penetrates through the scaffold pores due to its hydrophilic nature, thus facilitating clindamycin driving force to diffusion to the surrounding. Drug diffusion is affected by the physical and chemical characteristics of the drug and matrix and generated by a gradient in the concentration of the drug as a driving force. Diffusion-based systems exist as monolithic systems where the drug is dispersed throughout the polymer matrix and its release is controlled by diffusion from the matrix. The drug release profile from soy-gelatin and soy-alginate scaffolds demonstrated a linear ratio between cumulative release and square root of time during the first 5 h (not shown) which confirms that drug is release by a diffusion-controlled mechanism). After 1 h, the soy protein-gelatin and soy protein-alginate structures released approximately 53% and 45% of their total drug content, respectively. After 24 h, approximately 83% and 73% of the clindamycin was released from these structures, respectively (Table 1). Such release kinetics are critical for eradicating infections. The slightly higher burst release of clindamycine from soy protein-alginate blends compared

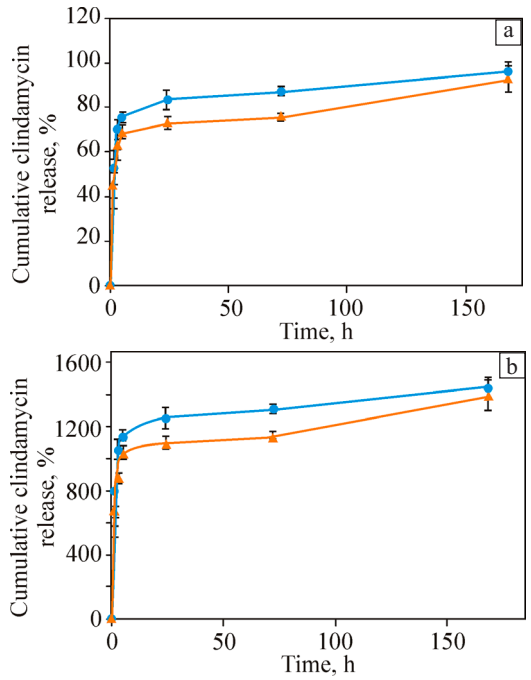

Fig. 3. Cumulative clindamycin release profiles from the selected scaffolds: Soy protein-gelatin (●) and soy protein-alginate (▲) containing the 4:1 soy protein:natural polymer ratio, cross-linked with 1 wt % EDC and loaded with 3 wt % clindamycin relative to the soy protein.

Table 1. Clindamycin percentage of release from soy protein scaffolds at various time points

Sample	72 h	24 h	1 h
Soy-gelatin	87.04%	83.33%	53.12%
Soy-alginate	75.86%	73.28%	45.58%

to the soy protein-gelatin blends (Fig. 3) probably results from the interconnected pore structure of the former, compared to the closed morphology of the latter, and can be affected by the faster degradation of the soy protein-gelatin blend and the release of its soluble fraction.

The phenomenon of fast drug release is also attributed to the antibiotic's low MW and its hydrophilic nature. This relatively short-term antibacterial effect has been reported for various porous antibiotic-eluting devices, including periodontal devices and wound dressings [28, 29]. Such devices provide only a short release, varying between hours to only several days [30], along with a high fraction of burst release. However, the obtained release profile can be beneficial for our application of antibiotic-eluting scaffolds. Some studies report that during the first hours of the wound, it is essential to release a relatively high quantity of an antimicrobial drug in order to eliminate various infections that might create a resistant biofilm [31]. Later on, the continued low release rate should keep the wound "infection-free" for about 5 days. We have shown that our proposed systems can comply with these requirements.

3.2. Microbiological evaluation

Drug-eluting wound dressings offer many advantages compared to conventional dosage forms or wound management without antibiotic drugs. The incorporation of antibiotic drugs can increase the therapeutic effect and help the healing process. It is therefore important to examine the effect of the drug release profile on the inhibition of bacterial growth. The time-dependent antimicrobial efficacy of our selected scaffolds loaded with clindamycin was tested *in vitro* using two complementary methods, the corrected zone of inhibition (CZOI) and the viable counts tests.

The minimal inhibitory concentration (MIC) values of clindamycin were first determined. The obtained MIC values against Gram-positive bacteria (*S. aureus* and *S. albus*) were 0.064 and 0.047 μg/ml, respectively.

3.2.1. Corrected zone of inhibition results

A modified Kirby–Bauer disc diffusion test was carried out with cube-shaped samples of our scaffolds in order to estimate clindamycin efficacy against typical skin microorganisms, particularly during release from the soy protein-based matrix. This method represents the situation when the sample is applied on the wound surface and the drug can diffuse to the wound area. The presence of bacterial inhibition in an area that exceeds the dressing material

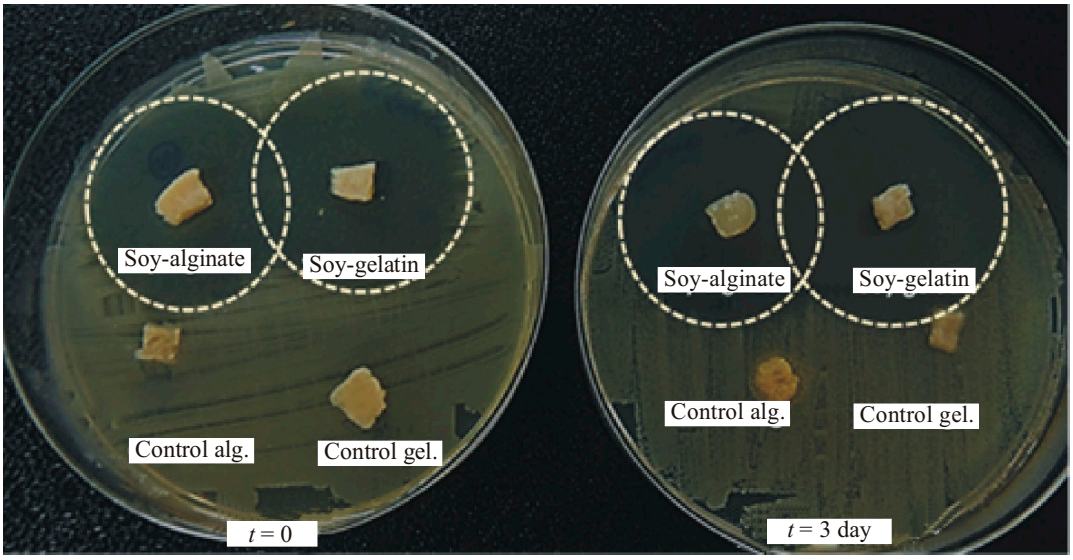

Fig. 4. Representative photographs of inhibition of *S. albus* growth around the selected soy protein-based scaffold samples containing 3 wt % clindamycin at the initial time (left) and after 96 h of immersion (right), with zones of inhibition around the cubes, compared with an unloaded sample (control) with no inhibition effect.

(CZOI > 0) can be considered beneficial in keeping the surrounding sterile.

The test was performed on two relevant bacterial strains (*S. aureus* and *S. albus*). The samples were pre-immersed in PBS for 0, 24, 48, 96 h prior to testing, in order to evaluate the efficiency of the sample over different periods of application. Examples for bacterial inhibition as well as for the control group are presented in Fig. 4.

Histograms showing the effect of clindamycin release on the corrected zone of inhibition around the two types of clindamycin-eluting soy protein porous blend samples as a function of pre-incubation time for the two bacterial strains are presented in Fig. 5. These results demonstrate that both types of selected soy protein-based blends effectively inhibited both types of microorganisms, *S. aureus* and *S. albus*, for at least 4 days.

Large and similar zones of inhibition were evident around both types of samples loaded with 3 wt % clindamycin at the initial time point (without pre-immersion) as well as after 24, 48 and 96 h of immersion, with no significant differences between samples. In contradistinction, extensive bacterial growth was observed around the control samples (without the antibiotic) (Fig. 4).

As mentioned above, the *in vitro* clindamycin release results showed that approximately 95% of the clindamycin was released within 4 days. Although most of the drug was released from the samples during the first 24 h (approximately 80%), the levels of bacterial inhibition for both types of specimens loaded with 3 wt % clindamycin were constant during the 4 days of the study. This can be explained by the ability of the soy protein-based scaffold cubes to absorb the surrounding liquid, which contains drug that was already released while they were incubated in PBS prior to exposure to the bacteria. This probably occurred due to their high surface area within the porous structures, together with high adsorption capacities.

3.2.2. Viable counts results

An additional bacterial test was performed in order to monitor the effectiveness of cumulative antibiotic release from the selected scaffolds in terms of the residual bacteria

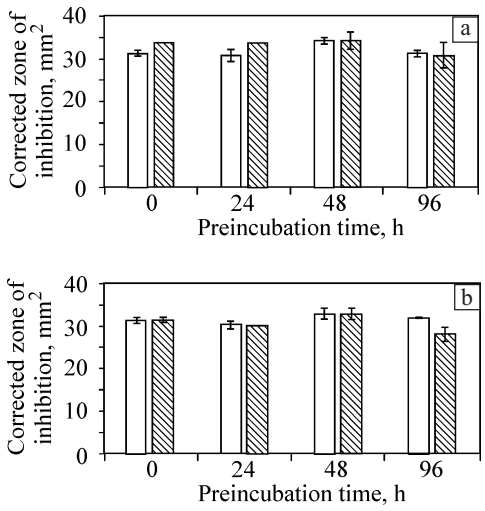

Fig. 5. Histograms showing the effect of clindamycin release on the corrected zone of inhibition of *S. aureus* (a) and *S. albus* (b), around soy protein-based scaffold cubes containing 3 wt % clindamycin: soy protein-gelatin (□) and soy protein-alginate (▨) at the initial point and as a function of pre-incubation time in PBS.

compared with an initial bacterial concentration of at least 10^5 CFU/ml, which corresponds to infection. Bacteria in PBS only served as the control. The results are presented in Fig. 6. Relatively high numbers of bacteria (*S. aureus* and *S. albus*), above 10^5 CFU/ml, survived after 96 h in the presence of control scaffolds (without the antibiotic), whereas the release of clindamycin from the soy protein-based porous samples reduced the bacterial viability by at least 3 orders of magnitude, with no significant differences between treatment groups (Fig. 6).

The drug clindamycin is probably inert to our cross-linking reaction and therefore does not react with the cross-linking agent EDC. Our results show that the cross-linking of the polymers occurs as expected, and that the degradation time is not affected by the drug incorporation (Fig. 2). It will therefore be possible to increase the drug doses within the scaffolds in order to obtain total eradication of micro-organisms. Delivering this drug locally using our soy protein-based scaffolds could thus decrease the risk of systemic complications and increase the therapeutic effect of the scaffold wound dressing itself.

In conclusion, both types of microbiological studies showed that the investigated clindamycin-eluting scaffolds are effective against the two studied bacterial strains, *S. aureus* and *S. albus*. The zone of inhibition results showed that both types of clindamycin-eluting scaffolds preserved their antibacterial potency for at least 4 days. At this time point, the viable count results also demonstrated considerably reduced amounts of *S. aureus* and *S. albus*, to below 10^2 CFU/ml, which indicates that the infection was eradicated.

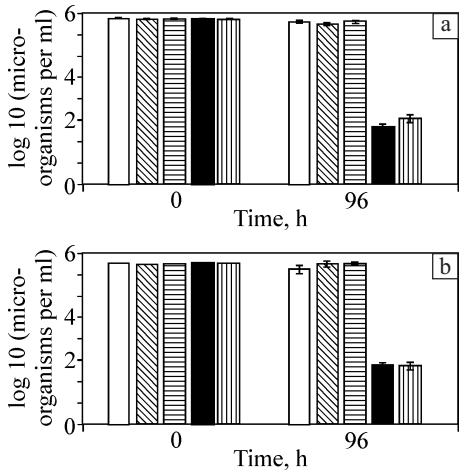

Fig. 6. Histograms showing the effect of 3 wt % clindamycin release on the number of CFU (at $t = 0$ and 3 days), when initial concentrations of at least 10^5 CFU/ml were used: *S. aureus* (a) and *S. albus* (b). The releasing samples: soy protein-gelatin (■) and soy protein-alginate (▥). Bacteria in the presence of phosphate buffered saline only served as control (□). The effect of the unloaded samples of soy protein-gelatin (▧) and soy protein-alginate (▤) is also shown.

4. Summary and conclusions

In the current study, novel soy protein-based porous blend scaffolds with antibiotic release were developed and studied. Such scaffolds can be used for wound healing and other applications. We investigated the physical properties of select soy protein-gelatin and soy protein-alginate samples, i.e. the clindamycin release profile, weight loss and microstructure. The effect of drug release on bacterial inhibition was studied as well using two methods of evaluation.

Structure characterization indicated high porosity for both blends, with the formation of large pores resulting mainly from the freeze-drying process. The soy protein-gelatin scaffold displays a more defined porous structure, while the soy protein-alginate scaffold displays a disorganized pore structure. According to the weight loss results, the soy protein-alginate blends are advantageous compared to the soy protein-gelatin blends due to their slower degradation rate.

The release of clindamycin from the soy protein-based scaffolds was relatively short. It exhibited an initial burst effect followed by a second phase of slow release rates, up to 4 days. The drug release mechanism is probably controlled by diffusion and by matrix degradation, mainly of the soluble fraction, and is strongly enhanced by the high porosity of the matrix and by the hydrophilic nature of the drug.

Clindamycin demonstrated activity as well as efficiency towards the relevant bacterial strains, which are abundant on human skin. The zone of inhibition results showed that soy protein-based scaffolds loaded with clindamycin could effectively inhibit *S. aureus* and *S. albus* infections for at least 4 days. The viable count results demonstrated a significant decrease (by 3 order of magnitude) in the bacterial viability after 4 days. We therefore conclude that our novel soy protein porous blend scaffolds may provide a potentially safer, lower-cost biomaterial system for various tissue regeneration applications.

Acknowledgments

The authors are grateful to the Israel Science Foundation, ISF, grant No. 1055/11/ for supporting this research. We would like to thank Solbar™ (Ashdod, Israel) for kindly providing the soy protein isolate used in this study.

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