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Research Article

Effect of Gamma-irradiation Sterilization on the Antibacterial Efficacy and the Properties of a Hybrid Burn Dressing

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Abstract

A new bio restorable hybrid burn dressing which combines a synthetic porous top layer with a spongy collagen sub layer was developed and studied. The top layer contained the antibiotic drug gentamicin for controlled release to the wound site. Our investigation focused on the effects of gamma-irradiation sterilization (10, 25, 35 and 50 kGy) on the antibacterial efficacy and on the mechanical and physical properties of this wound dressing. The gentamicin release profile from the hybrid wound dressing was very effective against three relevant bacterial strains, *Staphylococcusalbus*, *S. aureus* and *Pseudomonas aeruginosa*, for at least 14 days. This effectiveness did not decrease after gamma-irradiation sterilization, even at high irradiation doses. The changes in the mechanical properties, which were probably due to a combination of cross linking and chain scission of the hybrid layers by the gamma radiation, are beneficial when the radiation doses are in the range of 10-35 kGy.

INTRODUCTION

Wound dressings and burn infections

Over the last decades, wound dressings have evolved from the crude traditional gauze dressing to tissue-engineered scaffolds. Many types of wound dressing formats designed for specific wound-healing functions are commercially available or have been investigated [1]. A wound dressing should ideally provide an optimal healing environment which enables rapid healing. It should maintain a moist environment at the wound surface, allow gas exchange, act as a barrier to microorganisms and remove excess exudates [2]. Some modern dressings are therefore designed according to the well-accepted bilayer structure in order to provide a better healing environment compared to homogeneous films. The dense top layer is designed to control moisture transmission, prevent bacterial penetration and afford mechanical protection to the wound. The spongy sub layer is designed to absorb wound exudates, smoothly adhere to the wet wound bed and accommodate newly formed tissue [3,4].

Based on these principles, various bilayer structures in which both layers are based on natural [5-8] or synthetic polymers [4] have been designed over the years. Natural polymers have the advantage of being similar or identical to macromolecules in our body. However, they suffer from the disadvantage of undergoing rapid *in vivo* degradation by proteases [9]. The incorporated drug diffuses out rapidly, through a combination of diffusion and natural enzymatic breakdown of the protein [5,6]. On the other hand, dressings based on synthetic polymers do not fully promote cell adhesion and proliferation due to their inherently inert surface chemistry [10], and do not allow smooth adherence to the wound bed, which may lead to bacterial infection.

Hybrid bilayer wound dressings are very promising, since they enable combining the advantageous properties of a natural sub layer and a synthetic top layer. Such a design is very challenging, due to the different nature of synthetic and natural polymers, which leads to difficulties in binding between them.

Controlled release of bioactive agents from wound dressings has also been studied. Much attention has focused on wound dressings that provide an inherent antimicrobial effect by eluting

germicidal components in order to prevent bacterial infection [5,11,12]. To date, not enough research has focused on local release of antibiotics.

We have recently reported the development of hybrid bilayer wound dressings which combine a drug-loaded porous poly (DL-lactic-co-glycolic acid) (PDLGA) top layer with a spongy collagen sub layer [13]. The top layer can be tailored to produce the desired drug-release kinetics as well as to control moisture evaporation from the dressing. The spongy collagen sub layer is designed to maintain high absorption of wound exudates and to accommodate newly formed tissue [13]. We also reported a simple methodology for integrating the collagen sponge layer with a synthetic PDLGA layer into a unique hybrid structure.

Burn wound infections are among the most important and potentially serious complications that occur during the acute period following burn injury. Burn wound surfaces are sterile immediately after the thermal injury. However, colonization with autogenous microorganisms or through contact with the contaminated environment usually occurs within 48 hours [14,15]. Inadequate wound perfusion restricts migration of the host's immune cells and delivery of antimicrobial agents to the wound, thus limiting the effectiveness of systemic treatments. The local antibiotic concentration may be insufficient and may lead to bacterial resistance. Application of a topical antimicrobial agent on the open burn wound surface can substantially reduce the microbial load and the risk of infection [16]. However, such treatment requires frequent changes of the dressing material, causes inconvenience to the patient and places a financial burden on the healthcare system. Uncomplicated skin infections account for almost 200 million annual physician-office visits in the US, and treatment of these infections is estimated to cost over $350 million annually [17]. Our above-described novel hybrid wound dressing was also loaded with an antibiotic drug and studied *in vivo* on a guinea pig model [18]. Our study indicated promising results for the hybrid dressing material with controlled gentamicin release. It does not require bandage changes and offers a potentially valuable and economical approach for treating the life-threatening complication of burn-related infections. Our new antibiotic-eluting wound dressing platform is advantageous over current popular dressing materials that provide controlled release of silver ions as the antibacterial agent, since these may have toxic effects on cells and may delay wound healing. It should be indicated that biodegradable drug-eluting wound dressings which present an alternative to silver ion-eluting dressings are not available on the market to date. However, it is important to find an appropriate sterilization method for this new type of wound dressing. The effects of gamma radiation on the antimicrobial activity and on the mechanical properties of the biodegradable drug-eluting wound dressing were therefore investigated in the current study.

Sterilization and its effect on polymeric biomaterials

Use of bio absorbable polymeric biomaterials in the medical field is constantly increasing. Devices made of such materials must be produced aseptically or be sterilized before use. Irradiation with gamma rays or beta particles (electrons from an electron beam generator) in doses of 5-50 kGy guarantees sterility of the product. However, it has been shown that irradiation causes degradation via chain scission and cross linking, even at minimal doses [19]. Polymers vary greatly in their interaction with ionizing radiation, and it is often difficult to predict the specific properties of a polymer that will be affected by the radiation. Sterilization using gamma radiation or ethylene oxide (EtO) is currently the most commonly used sterilization methods in the industry [20], and were studied intensively with reference to bio restorable polymers [21]. It is reported that gamma radiation causes chain scission and cross linking of PDLGA, leading to changes in the polymer's properties [22-25]. When exposed to gamma radiation, PDLGA chain scission is more significant than crosslinking. This leads to a decrease in the molecular weight of the polymer.

The current study describes the effect of gamma radiation doses and conditions on the antibacterial activity of our new PDLGA-collagen hybrid wound dressing and on its mechanical properties. It may therefore enable finding an appropriate sterilization process for this wound dressing that will not have a deleterious effect on its function.

MATERIALS AND METHODS

Materials

Synthetic polymer: Poly(DL-lactic-co-glycolic acid) with a co-polymeric ratio of 50% lactic acid and 50% glycolic acid (50/50 PDLGA), inherent viscosity (i.v.)= 0.65dL/g (in $CHCl_3$ at 30°C) (molecular weight approximately 50 kDa), Absorbable Polymer Technologies, Inc., USA.

Natural polymer: Collagen-Klee® 10x10x0.5 cm (a natural restorable spongy membrane from porcine dermis consisting of a minimum of 96.75% native collagen type 1), Medical Biomaterial Products GmbH, Germany (1010S).

Drug: Gentamicin sulfate (Sigma, G-1264).

Reagents: Reagent kit (8-1P31-25) and calibration kit (8-1P31-01) for the analysis of gentamicin concentrations were purchased from Sigma-Aldrich, Rehovot, Israel.

Hybrid wound dressing preparation and sterilization

The preparation of the hybrid wound dressing is based on two stages. First, an inverted emulsion loaded with the drug molecules is prepared. The bilayer hybrid wound dressing is then prepared, based on the freeze-drying of an inverted emulsion, as follows:

Preparation of the inverted emulsion: The aqueous phase of the inverted emulsion was based on double-distilled water and the drug gentamicin (20% w/w) was included in it. The organic phase of the inverted emulsion contained 17.5% (w/v) of 50/50 PDLGA dissolved in chloroform. Homogenization of the two phases was performed for 90 s at 16,000 RPM using a Kinematica PT-2500 E Polytron homogenizer. Anorganic: aqueous (O: A) phase ratio of 6:1 was chosen.

Preparation of the bilayer structures: An aluminum tube with rounded and homogenously dispersed holes on its lower surface (D=5 cm), used as a dip-coating instrument, was connected to a vacuum source to hold the collagen sponge. The sponge was then dip-coated in a fresh inverted emulsion for a few

seconds and then immediately frozen in a liquid nitrogen bath. The samples were then placed in a pre-cooled(-105°C) freeze-dryer (Virtis 101 equipped with a nitrogen trap) and freeze-dried to preserve the microstructure of the emulsion-based structures. Drying was performed in two stages: The freeze-dryer chamber pressure was reduced to 100 mTorr while the temperature remained at -105°C. After 5 h, a hot plate was turned on to -30°C overnight. The condenser was then turned off and its plate temperature gradually increased to room temperature while the pressure was monitored between 100 and 700 mTorr. During this step, the liquid nitrogen trap condensed the excess water and solvent vapors. The dried samples were stored in desiccators until use.

Sterilization of the hybrid wound dressings using gamma radiation: Sterilization was performed using gamma radiation at doses of 10, 25, 35 and 50 k Gyin order to study the effect of the sterilization process on the mechanical and physical properties and on the antibacterial activity of the wound dressing. Two series of samples were studied. In the first, irradiation was carried out at the ambient room temperature (25°C) and in the second, irradiation was carried out in liquid nitrogen. Gamma-irradiation was carried out using a 60 Co source (MDS Nordion, Canada, Cobalt 60 irradiator type JS-9500) with a dose rate of about 8kGy/h.

Microbiological studies

Two types of microbiological studies were performed: sterilization efficacy and bacterial inhibition.

Sterilization efficacy: The efficacy of gamma radiation for sterilizing the wound dressing was tested for each dose in order to determine the minimum dose required to achieve sterilization. Another aim of this test was to investigate the effect of gamma radiation on the antibiotic drug's activity. Two sets of gamma-irradiated wound dressings were used (10,25,35,50 kGy) and an unsterilized wound dressing was used as control for each set. The first set was performed with a wound dressing that did not contain the antibiotic drug and the second set was performed with a drug-containing wound dressing. The wound dressings were cut into 1x1 cm rectangle specimens and were placed in sterile 50 mL test tubes with 3 mL of Müller-Hinton liquid medium under sterile conditions. Specimens were incubated for 48 h at 37°C. An observed change in the turbidity of the medium was regarded as a sign of infection.

***In vitro* bacterial inhibition:** The zone of inhibition test was performed in order to study the effect of the sterilization process on the bacterial inhibition of the wound dressings. Three bacterial strains, *Staphylococcus aureus* (*S. aureus*), *Staphylococcus albus (S. Albus)* and *Pseudomonas aeruginosa (P. aeruginosa)*, were chosen for this study, due to their frequent presence on human skin and their high involvement in infections during wound management. All three strains were clinically isolated at the Microbiological Laboratory of Rambam Medical Center (Haifa, Israel), and their minimal inhibitory concentration (MIC) values were determined (Table 1). The strains were grown overnight on Müller-Hinton (Difco) agar plates at 37°C prior to use. The bacterial cells were collected and re-suspended in saline, and adjusted to $1x10^7$ CFU/mL (colony forming units) by visual comparison with a 0.5 McFarland standard. The zone of inhibition test was used to determine the time-dependence of the antimicrobial activity of the wound dressing. In this modified version of the Kirby-Bauer disc diffusion test, which is typically used to determine bacterial susceptibility to antibiotics, round pieces of dressing material (1 cm^2) were placed on a bacterial lawn (100 µL of inoculum, $\sim 10^7$ CFU/mL, seeded on Müller-Hinton agar plates), incubated overnight at 37°C, and then photographed. The diameter of inhibition around the dressings was measured from the images.

This procedure was repeated on dressing materials which were incubated in PBS (1.5 mL) for 1, 2, 5, 8, 14, and 28 days prior to testing. The test was performed in triplicate for each type of dressing and for each of the three microorganisms: *S. aureus*, *S. Albus* and *P. aeruginosa*.

Morphology, drug release and mechanical properties

Morphological characterization: The morphology of the wound dressing structure was observed using an environmental scanning electron microscope (ESEM) with a Quanta 200 FEG at a high vacuum mode and accelerating voltage of 10 kV. The cryogenically fractured surfaces were Au-sputtered prior to observation.

***In vitro* drug-release studies:** Small disc-shaped (D=1.5 cm) pieces (triplicates) were immersed in phosphate buffered saline (PBS, pH 7.0) and kept at 37°Cfor 56 days in order to determine the various drug release kinetics from these structures. The release studies were conducted in closed glass vessels containing 2.5 mL PBS. Sodium azide (0.02w/v) was added in order to prevent microbial contamination. The medium was removed (completely) periodically at each sampling point (6 h,1,2,3,7,14,21,28,35,42,56 days), and fresh medium was introduced. Residual drug recovery from the wound dressings was carried out using a Trypsin A solution to dissolve the remaining collagen sponge. Next, 1mL of methylene chloride and 2 mL of double-distilled water were used to dissolve the hydrophilic drug residues.

Determination of the medium's gentamicin content was carried out using the Architect i2000SR (Abbot Laboratories) according to the manufacturer's instructions. This machine enables determination of the gentamicin concentration based on a chemiluminescent micro particle immunoassay (CMIA). An Architect iGentamicin Reagent kit (1P31) was purchased for the analysis. The measurable concentration range without dilution is 0.0-10.0 mg/mL. Higher drug concentrations were measured after carrying out manual dilutions.

Tensile mechanical properties

The wound dressings' tensile mechanical properties before and after the sterilization process were evaluated at room temperature, under unidirectional tension at a rate of 10 mm/min, using a 5500 Instron machine equipped with a 5 kg load cell. Dry wound dressing samples (n=5) were cut into a dog bone shape (neck length 3 cm, width 1 cm). Engineering stress and strain were calculated from the load and displacement data. The tensile strength was defined as the maximum strength at which one layer experienced failure. Maximal strain was defined as the breaking strain. Elastic modulus was defined as the slope of the stress-strain curve in the linear region.

RESULTS AND DISCUSSION

Structure and gentamicin release

As mentioned in the introduction section, our new hybrid wound dressing combines a porous drug-eluting PDLGA top layer with a spongy collagen sub layer which is in contact with the damaged skin. The spongy collagen layer is designed to absorb wound exudates, smoothly adhere to the wet wound bed as well as to accommodate newly formed tissue. The advantages of collagen-based dressings over other systems are due to their unique features such as weak antigenicity, biodegradability and superior biocompatibility [6,26]. Such systems have been reported to perform better than conventional and synthetic dressings in accelerating granulation tissue formation and epithelialization [6,27].The porous synthetic PDLGA top layer is designed to control moisture transmission, prevent bacterial penetration, as well as to act as a drug reservoir. PDLGA is a mechanically reliable polymer that has been proven to perform well in various implants and long-term drug delivery systems [28,29]. Taken together, both materials synergistically produce properties which are not available in the individual constituent materials. A similar concept was used in several commercially available dressings, such as Integra® which uses a silicone upper layer and a collagen-glycosaminoglycan sub layer. It is important to note that contrary to other systems, all of the structural constituents in our new systems are biodegradable. Dressings made from these constituents therefore do not need to be removed from the wound surface once they have fulfilled their role. Furthermore, none of the commercially available bilayer wound dressings release drugs to the wound site in a controlled manner.

A photograph of our hybrid wound dressing is presented in Figure (1a) and an ESEM micrograph of the top drug-eluting layer is presented in Figure (1b). The latter shows a highly porous structure with an average porosity of 63% and a pore diameter of 1.4μm. In our unique hybrid structure, both layers are bonded by a third interfacial layer (Figure 1c), where they are physically mixed.

One of the challenges in fabricating a bilayer structure that can fulfill its function is to ensure adhesion between the two distinct layers. Integration between a synthetic and a natural polymer is challenging due to their different structural and chemical properties. Contrary to previously described methodologies for chemically combining natural and synthetic polymers [5,30], our wound dressing preparation method is based on a simple dip-coating technique for physically binding between the natural polymer collagen and the synthetic polymer PDLGA, which enables the penetration of the inverted emulsion into the collagen pores when vacuum is used. This results in an interface between the collagen and the PDLGA porous layer in the solid state, which actually behaves like an interphase in which both materials are mechanically mixed and therefore the two layers are well held together. Superior mechanical properties such as tension, as well as physical properties (water uptake, water vapor transmission rate, etc.), were obtained and are described elsewhere [13].

The main challenge in designing a device for the release of low molecular weight hydrophilic bioactive agents (such as the antibiotic used in the current study) is to overcome their rapid release from the device. A drug-eluting bilayer structure is even more challenging, especially when the drug is incorporated in the top layer and its discharge from the device also depends on the swelling rate of the sub layer. We used a non-cross linked collagen sponge with high porosity and swelling rate in our system so that it would not decrease the drug release rate from the top layer to the wound bed. The cumulative release of the antibiotic drug gentamicin from our hybrid dressing is presented in Figure (2). Amedium burst release of gentamicin (38%), followed by a gradual release in a decreasing rate over time with 80% release of the encapsulated drug within 4 days, and was observed. Most of the remaining 20% drug was released slowly within the following 30 days. It should be noted that most of the gentamicin was released within the first 4 days of the study, due to the hydrophilic nature of the antibiotic drug. An unfavorable faster drug release rate has been reported in the literature for other antibiotic-eluting systems [6]. Thus, our new antibiotic-eluting structure is advantageous over other systems.

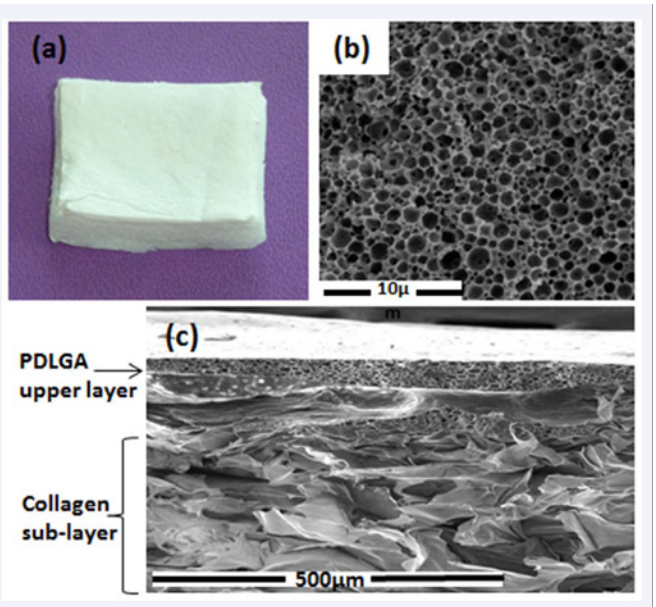

Figure 1 The structure of the biodegradable hybrid wound dressing composed of a PDLGA top layer loaded with gentamicin and a collagen sublayer. (a) Photograph of the wound dressing, (b) High magnification SEM image of the porous top layer, (c) Cross-sectional cryo-fractured SEM image demonstrating the two layers and their interface.

The strategy of drug release to a wound depends on the condition of the wound. After the onset of an infection, it is crucial to immediately respond to the presence of large numbers of bacteria (>10^5 CFU/mL) which may already be present in the biofilm [31], and which may require antibiotic doses of up to 1000 times those needed for bacteria in suspension [32]. Following the initial release, a sustained release at an effective level over a period of time can prevent the occurrence of a latent infection. We have shown that the proposed new hybrid system can comply with these requirements, since the gentamicin release profile from our hybrid dressings exhibited a combination of a medium burst release followed by release in a decreasing rate over 30 days (Figure 2).

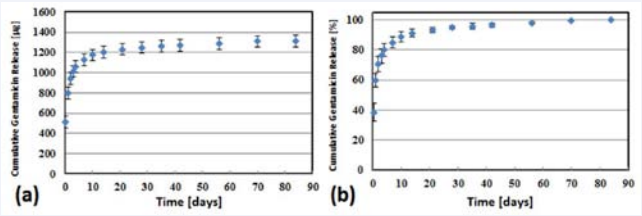

Figure 2 Cumulative release of gentamicin from the hybrid wound dressing. (a) Results in micrograms, (b) Results in % of the encapsulated quantity. Mean ± SEM are presented.

Microbiological studies

Specimens that did not contain the drug and were sterilized with different doses of gamma radiation were used to evaluate the efficacy of the sterilization in inhibiting the growth of random infections during the fabrication process, after incubation for 48 h in Müller-Hinton liquid medium under sterilized conditions. The results in Table (2) show that a minimal sterilization dose of 25 kGy is needed in order to achieve sterility for a random environmental infection, as is usually required for medical devices. In addition, drug-containing specimens that were sterilized with different doses inhibited the growth of random infections at all radiation doses (Table 2), thus indicating that the sterilization process did not affect the antibiotic drug's activity.

In order to estimate gentamicin efficacy against typical skin microorganisms, particularly during release from the gamma-irradiated hybrid wound dressing, the Kirby-Bauer disc diffusion test was carried out with the three relevant bacterial strains (*S. aureus, S. albus* and *P. aeruginosa*). The samples were pre-immersed in PBS for 0,1,2,5,8,14 and 28 days prior to testing in order to evaluate the efficiency of the dressing over prolonged periods of application. Examples for bacterial inhibition of *P. aeruginosa* at t=0 are presented in Figure (3), and show a clear zone of inhibition around the unsterilized sample and around samples sterilized using radiation doses of 25 and 50 kGy. As expected, both irradiated samples began to lose their original rectangular shape, due to the irradiation effect. The change in the 50 kGy-irradiated samples was higher than in the 25 kGy-irradiated samples. The zone of inhibition at t=0 increased with the increase in the radiation dose, probably due to faster diffusion of the drug molecules from the more damaged wound dressing. This phenomenon was observed for *P. aeruginosa* but not for *S. aureus* or *S. albus*.

- Histograms showing the effect of gentamicin release on the zone of inhibition around the three types of gentamicin-eluting wound dressings (unsterilized, sterilized using 25 kGy and sterilized using 50 kGy) as a function of pre-incubation time for all 3 bacterial strains are presented in Figure (4). The MIC values of the three bacterial strains are presented in Table (1). These results demonstrate the following main effects: The three bacterial strains clearly show susceptibility towards gentamicin, with *S. albus* being the most sensitive and *P. aeruginosa* the most resistant. They were effectively inhibited by all the samples for a period of at least 14 days. At t=28 days, no inhibition was observed for all three wound dressings.
- The largest zones of inhibition are evident for all samples at t=0 (without pre-immersion).
- The zones of inhibition created around all 3 wound dressings slowly decreased with time, due to the decreasing rate of gentamicin release (Figure 2). After 14 days of release, when approximately 90% of the gentamicin was released, the wound dressings were no longer effective against the bacteria.
- All three studied hybrid wound dressings (unsterilized, sterilized using 25 kGy and sterilized using 50 kGy) showed very similar zones of inhibition, indicating that the gamma-irradiation sterilization process did not reduce the efficacy of the antibiotic drug.

The time-dependent antimicrobial efficacy of our new SPI-based wound dressing was tested *in vitro* by the disc diffusion test, which is a good representation of the clinical situation, where the dressing material is applied to the wound surface, allowing the drug to diffuse to the wound bed. The results from this method are dependent on the rate of diffusion of the active agent from the dressing, set against the growth rate of the bacterial species growing on the lawn, and are highly dependent on the physicochemical environment. Gentamicins showed great efficacy against microorganisms which are abundant on human

Table 1: Minimal inhibitory concentrations of gentamicin.

Bacterial Strain	MIC [μg/mL]
Staphylococcus albus	3
Staphylococcus aureus	4.5
Pseudomonas aeruginosa	6.3

Table 2: Sterilization efficacy microbial test.

Irradiation Dose [kGy]	Bacterial Growth (No Antibiotic)	Bacterial Growth (With Antibiotic)
Unsterilized hybrid	+	+
10	+	-
25	-	-
35	-	-
50	-	-

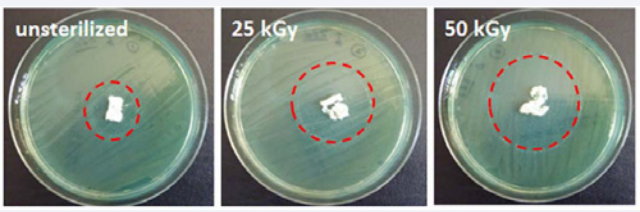

Figure 3 An example of the Kirby-Bauer diffusion test (zone of inhibition) results showing the inhibition of *P. aeruginosa* growth around the gentamicin-eluting hybrid wound dressing at time 0 (prior to incubation in PBS), of an unsterilized sample, a sample sterilized with a 25 kGy dose of gamma radiation and a sample sterilized at room temperature with a 50 kGy dose of gamma radiation. The zones of inhibition around the dressings are circled.

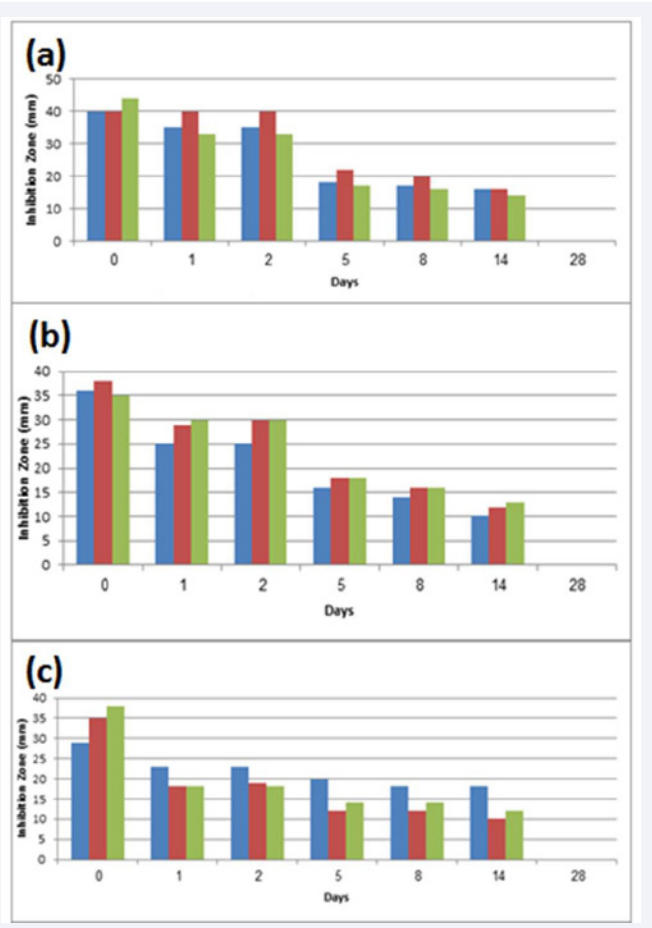

Figure 4 Histograms showing the effect of gentamicin release on the zone of inhibition around: ■ - unsterilized wound dressing, ■ - wound dressing sterilized with a 25 kGy dose of gamma radiation, ■ - wound dressing sterilized with a 50 kGy dose of gamma radiation, as a function of pre-incubation time in PBS. (a) *S. albus,* (b) *S. aureus*, (c) *P. aeruginosa.*

skin and are usually responsible for wound infections. *S. albus* exhibited the largest zone of inhibition, *S. aureus* exhibited a slightly smaller zone of inhibition and *P. aeruginosa* exhibited the smallest zone of inhibition at all time points (Figure 4). However, the differences are small, mainly due to the relatively large quantities of released drug (Figure 2), and are related to the bacterial sensitivity to gentamicin, as expected from the MIC values (Table 1).

Drug quantities higher than the MIC values should be released in order to eradicate all bacteria within a few days and prevent infection. In fact, a release profile such as the one presented by our hybrid wound dressings, with a medium burst effect followed by a decreasing release rate, is preferable. This study demonstrates that our gentamicin-loaded hybrid wound dressing is effective against the relevant bacterial strains and remains effective after the tested sterilization processes, even at high radiation doses.

The effect of gamma radiation on the mechanical properties

The effects of four doses of gamma radiation (10,25,35 and 50 kGy) on the tensile mechanical properties of the hybrid wound dressing and on their physical properties were studied. The doses were applied at room temperature and in liquid nitrogen. A standard dose of 25 kGy is usually considered as sufficient in the medical devices industry. However, if the material is too sensitive for the 25 kGy dose, a relatively clean process of preparation should be applied, in order to use a relatively low radiation dose of only 10 kGy, as is customary in the food industry. Doses that are higher than 35 kGy are seldom needed, except when the process of preparation requires it. The tensile testing provides an indication of their mechanical properties, which can be reflected by maximal tensile strength, modulus and tensile strain. It is important to maintain the desired mechanical properties also after the sterilization process.

The stress-strain curves of the hybrid system, both unsterilized and after sterilization using the four radiation doses, compared to that of the unsterilized collagen sponge, are presented in Figure (5), and their mechanical properties are summarized in Table (3). The collagen sponge exhibited a "linear" region (0 to ~90% of the ultimate stress) followed by a non-linear "failure" region Figure (5). Its tensile modulus is relatively low (2.88 ± 1.07 MPa), its maximal tensile strength is moderate (4.59 ± 0.26 MPa), and its strain is relatively high (173.5 ± 3.2 %). Higher modulus (6.58 ± 0.15MPa), slightly higher tensile strength (5.47±0.25 MPa) and lower maximal strain (95.7 ± 4.0%) were obtained for the PDLGA/collagen hybrid. As expected, this improvement

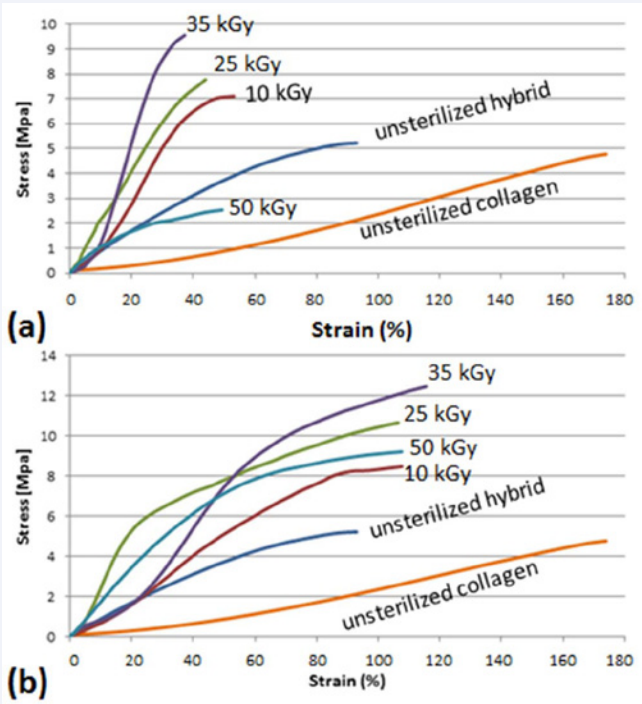

Figure 5 Tensile stress-strain curves of the hybrid unsterilized wound dressing and the wound dressing after sterilization at room temperature with gamma radiation doses of 10, 25, 35, and 50 kGy, compared to the unsterilized collagen layer. (a) Sterilization performed at room temperature, (b) Sterilization performed in liquid nitrogen. The radiation dose is indicated for each sample near the stress-stress curve.

Table 3: The tensile mechanical properties of the wound dressings as affected by the radiation dose at room temperature (RT) and in liquid nitrogen (LN).

Radiation Dose [KGy]	Elastic Modulus (MPa)		Ultimate Tensile Strength (MPa)		Ultimate Tensile Strain (%)	
	R.T.	L.N.	R.T.	L.N.	R.T.	L.N.
Unsterilized collagen	2.88 ± 1.07		4.59 ± 0.26		173.5 ± 3.2	
Unsterilized hybrid	6.58 ± 0.15		5.47 ± 0.25		95.7 ± 4.0	
10	23.58 ± 1.02	8.10 ± 0.58	6.28 ± 0.17	5.81 ± 0.16	52.8 ± 2.0	129.8 ± 6.2
25	25.36 ± 1.25	15.66 ± 0.95	7.56 ± 0.62	6.23 ± 0.17	43.3 ± 0.8	129.1 ± 4.7
35	27.82 ± 1.19	18.37 ± 0.59	8.67 ± 0.64	10.42 ± 0.24	41.4 ± 2.3	131.9 ± 5.6
50	8.94 ± 0.87	12.59 ± 0.45	3.14 ± 0.54	8.418 ± 0.29	54.9 ± 3.7	123.2 ± 7.3

in mechanical properties is due to some reinforcing effect of the porous PDLGA top layer. The mechanical properties of a wound dressing are highly important for its performance. In the clinical setting, wound dressings should possess appropriate strength, stiffness and flexibility, because they must be durable and stress-resistant in order to withstand the normal stress encountered during their application and handling.

Our results show that even a relatively low radiation dose of 10 kGy, applied at room temperature, increases the strength and modulus of the hybrid wound dressing and decreases its tensile strain (Figure 5a). An increase in the radiation dose from 10 to 35 kGy changes the mechanical properties only slightly (Table 3, Figure 5a). These changes in mechanical properties probably result from a crosslinking reaction, mainly in the collagen layer, which increases the stiffness and decreases the flexibility of this layer. The slight increase in the tensile strength is beneficial. When the highest radiation dose (50 kGy) was used, the tensile strength, modulus, and strain were dramatically decreased, probably due to massive chain scission, which is not desired.

When the irradiation process was performed in liquid nitrogen, the changes in the mechanical properties were much smaller (Table 3, Figure 5b), indicating that there is practically no crosslinking reaction at such a low temperature. It can therefore be concluded that using radiation doses of 10-35 kGy at room temperature for sterilization of the wound dressing can be beneficial. However, if these are not desired, the sterilization process should be performed in liquid nitrogen, where the cold temperature practically prevents crosslinking and/or chain scission which affect the material.

SUMMARY AND CONCLUSIONS

A novel bio restorable hybrid wound dressing which combines a poly (DL-lactic-co-glycolic acid) porous drug-loaded top layer with a spongy collagen sub layer was developed and studied. The top layer was prepared using the freeze-drying of inverted emulsions technique and contained the antibiotic drug gentamicin for controlled release to the wound site. Our investigation focused on the effects of gamma-irradiation sterilization on the antibacterial function and on the mechanical properties of this new hybrid wound dressing. Samples irradiated with 10, 25, 35 and 50 kGy were compared to the unsterilized ones and the antibacterial efficacy was studied using the zone of inhibition test. The tensile mechanical properties, weight loss, water uptake and water vapor transmission rate of the hybrid wound dressing were studied as well.

The gentamicin release profile from the hybrid wound dressing demonstrated a medium burst release, followed by a gradual release in a decreasing rate over time. Eighty percent of the encapsulated drug was released within 4 days and most of the remaining 20% drug was slowly released within the following 30 days. This release profile enabled high effectiveness against the three relevant bacterial strains, *S. albus*, *S. aureus* and *P. aeruginosa*, for at least 14 days. The zone of inhibition results showed that the gamma-irradiation sterilization process had practically no effect on the antibacterial efficacy of the wound dressing, even when high radiation doses were used.

The hybrid wound dressing exhibited good mechanical properties. The strength in tension and modulus increased with the increase in the radiation dose at room temperature and the strain decreased, probably due to a crosslinking reaction in the collage layer. These trends were smaller when the sterilization process was performed in liquid nitrogen.

In conclusion, gamma-irradiation sterilization of our hybrid wound dressing using doses between 10-35 kGy is beneficial. It does not change the antibacterial efficacy of the wound dressing. The changes in the mechanical properties are positive. If these changes are not desired, gamma-irradiation sterilization can be performed in liquid nitrogen.

REFERENCES

1. Boateng, JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: a review. J Pharm Sci. 2008; 97: 2892-2923.
2. Abdelrahman T, Newton H. Wound dressings: principles and practice. Surgery. 2011; 29: 491-495.
3. Jones I, Currie L, Martin RA. A guide to biological skin substitutes. Br J Plast Surg. 2002; 55:185-193.
4. Martineau L, Shek PN. Evaluation of a bi-layer wound dressing for burn care I. Cooling and wound healing properties. Burns. 2006; 32: 70-76.
5. Mi FL, Wu YB, Shyu SS, Schoung JY, Huang YB, Tsai YH, et al. Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery. J Biomed Mat Res. 2002; 59: 438-449.
6. Sripriya R, Kumar MS, Sehgal PK. Improved collagen bilayer dressing for the controlled release of drugs. J Biomed Materials Res. 2004; 70: 389-396.

7. Rivero S, Garcia MA, Pinotti A. Composite and bi-layer films based on gelatin and chitosan. J Food Engg. 2009; 90: 531-539.

8. Lee SJ, Khang G, Lee YM, Lee HB. Interaction of human chondrocytes and NIH/3T3 fibroblasts on chloric acid-treated biodegradable polymer surfaces. J Biomater Sci Polym Ed. 2002; 13:197-212.

9. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliv Rev. 2007; 59: 207-233.

10. Yang Y, Marie-Christine Porté, Pascal Marmey, Charles Baquey. Covalent bonding of collagen on poly (L-lactic acid) by gamma irradiation. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 2003; 207: 165-174.

11. Chen G, Sato T, Ushida T, Ochiai N, Tateishi T. Tissue engineering of cartilage using a hybrid scaffold of synthetic polymer and collagen. Tissue Eng. 2004; 10: 323-330.

12. Elsner JJ, Berdicevsky I, Zilberman M. In vitro microbial inhibition and cellular response to novel biodegradable composite wound dressings with controlled release of antibiotics. Acta Biomaterialia. 2011; 7: 325-336.

13. Shemesh M, Zilberman M. Structure-property effects of novel bioresorbable hybrid structures with controlled release of bioactive agents for wound healing applications. Acta Biomaterialia. 2014; 10: 1380-1391.

14. Altoparlak U, Erol S, Akcay MN, Celebi F, Kadanali A. The time-related changes of antimicrobial resistance patterns and predominant bacterial profiles of burn wounds and body flora of burned patients. Burns. 2004; 30: 660-664.

15. Erol S, Altoparlak U, Akcay MN. Changes of microbial flora and wound colonization in burned patients. Burns. 2004; 30: 357-361.

16. Murphy KD, Lee JO, Herndon DN. Current pharmacotherapy for the treatment of severe burns. Exp Opin Pharmacother. 2003; 4: 369-384.

17. Pangilinan R, Tice A, Tillotson G. Topical antibiotic treatment for uncomplicated skin and skin structure infections: review of the literature. Exp Rev Anti Infect Ther. 2009; 7: 957-965.

18. Zilberman M, Egozi D, Shemesh M, Mazor E, Baranes M, Keren A, et al. Hybrid Dressings with Controlled Release of Antibiotics: Results in a Guinea Pig Burn Model. Acta Biomater. 2015; 22: 155-163.

19. Bushell JA, Mike Claybournb, Helen E. Williamsb, Damien M. Murphy. An EPR and ENDOR study of γ-and β-radiation sterilization in poly (lactide-co-glycolide) polymers and microspheres. J Controlled Release. 2005; 110: 49-57.

20. Lambert BJ, Mendelson TA, Craven MD. Radiation and ethylene oxide terminal sterilization experiences with drug eluting stent products. AAPS Pharm Sci Tech. 2011; 12: 1116-1126.

21. Ražem D, Katušin-Ražem B. The effects of irradiation on controlled drug delivery/controlled drug release systems. Radiation Phy Chem. 2008; 77: 288-344.

22. Birkinshaw C, Martin Buggy, Henn GG, E Jones. Irradiation of poly-D, L-lactide. Polymer degradation and stability. 1992; 38: 249-253.

23. Suljovrujić E, Ignjatović N, Uskoković D. Gamma irradiation processing of hydroxyapatite/poly-L-lactide composite biomaterial. Radiation Phy Chem. 2003; 67: 375-379.

24. Nugroho P, Hiroshi Mitomo, Fumio Yoshii, Tamikazu Kume. Degradation of poly (L-lactic acid) by γ-irradiation. Polymer Degradation Stability. 2001; 72: 337-343.

25. Sun-Young Joa, Jong-Seok Parka, Hui-Jeong Gwona, Young-Min Shina, Myung-Seob Khilb, Young-Chang Nhoa, et al. Degradation behavior of poly (L-lactide-co-glycolide) films through gamma-ray irradiation. Radiation Phy Chem. 2012; 81: 846-850.

26. Pachence JM, Collagen-based devices for soft tissue repair. J Biomed Mater Res. 1996; 33: 35-40.

27. Kilian O, Hossain H, Flesch I, Sommer U, Nolting H, Chakraborty T, et al. Elution kinetics, antimicrobial efficacy, and degradation and microvasculature of a new gentamicin-loaded collagen fleece. J Biomed Mater Res B Appl Biomater. 2009; 90: 210-222.

28. Lambert WJ, Peck KD. Development of an in situ forming biodegradable poly-lactide-coglycolide system for the controlled release of proteins. J Controlled Release. 1995; 33: 189-195.

29. Middleton JC, Tipton AJ. Synthetic biodegradable polymers as orthopedic devices. Biomaterials. 2000; 21: 2335-2346.

30. Zhu Y, Chan-Park MB, Sin Chian K. The growth improvement of porcine esophageal smooth muscle cells on collagen-grafted poly (DL-lactide-co-glycolide) membrane. J Biomedical Materials Res. 2005; 75: 193-199.

31. Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz PM. A wound-isolated Pseudomonas aeruginosa grows a biofilm in vitro within 10 hours and is visualized by light microscopy. Dermatol Surg. 2003; 29: 631-635.

32. Gilbert P, Collier PJ, Brown MR. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle, dormancy, and stringent response. Antimicrob Agents Chemother. 1990; 34: 1865-1868.

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