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M. Foox, M. Ben-Tzur, N. Koifman, and M. Zilberman

Department of Biomedical Engineering, Tel-Aviv University, Tel-Aviv, Israel

ABSTRACT

Tissue bioadhesives are gaining popularity as an alternative for sutures and staples. The authors have previously developed novel bioadhesives based on gelatin and alginate, crosslinked with carbodiimide. However, the bioadhesives must be sterilized before proceeding to clinical trials. The effect of gamma irradiation, a common sterilization method, was investigated in the current study. The viscosity was significantly decreased, while the bonding strength to the tissue (a) and the gelatin release from the bioadhesives were not affected significantly due to the exposure to gamma radiation. The results also indicate that a gamma radiation dose of 25 kGy, as is customary for biomedical applications, has a minor effect on human fibroblast viability when using formulations based on low concentration of carbodiimide (b). These results enabled the authors to positively consider gamma irradiation as a sterilization method for their bioadhesives.

1. Introduction

1.1. Topical skin bioadhesive

Sixty percent of wound closures are performed using sutures, staples, or other mechanical procedures [1]. However, these procedures are very painful and time consuming. Great effort has therefore been made to develop bioadhesives that could replace them. The global market for sutures and staples is predicted to decrease by 2% by 2017, while the global market for tissue bioadhesives is predicted to increase by 3%, to an estimated $38 billion [2–4]. The use of bioadhesives offers many advantages that might enable a faster return to normal activities after an injury or surgery. These advantages include a decrease in pain with no need of sedation or anesthetic drugs, shorter application time than sutures, no requirement for follow-up visits for removal due to their spontaneous peeling and degradation, no risk of needle stick injury from skin suture needles [5] and good tensile strength. Some bioadhesives also have microbial barrier properties. There is a similar or better cosmetic outcome compared to other methods and these materials have a cost advantage over the common treatments [6].

However, developing tissue bioadhesives is challenging because they must fulfill many requirements. They must have strong and rapid adhesion to living tissue in the moist environment of fluids or blood. They should enable wound healing and maintain biocompatibility with no toxic or harmful side effects. Most bioadhesives that have been approved for use are either too toxic or have a weak bonding strength to the tissue, and none seem to be superior to the mechanical methods. Novel bioadhesives based on a combination of natural polymers such as gelatin and alginate, with N-ethyl-N-(3-dimethylaminopropyl)
carbodiimide (EDC) as a crosslinker, were therefore developed and studied by us [7]. N-hydroxysuccinimide (NHS) is known to improve the crosslinking reaction of EDC and reduce the number of side reactions [8]. Incorporating NHS enables using a lower EDC concentration. This results in a decrease in the cytotoxic effect, without decreasing the bonding strength [9].

However, bioadhesives must be sterilized before they can advance to the clinical trial phase. The sterilization process often has a dramatic effect on the physical, chemical and mechanical properties of biomaterials [10]. It is essential that a particular sterilization technique can be selected following a careful investigation of possible effects of the sterilization method on the biomaterial. Predicting the outcome of a sterilization process on a specific biomaterial can be complicated. The best way to determine how a specific sterilization process will affect a particular biomaterial is to carry out a comprehensive examination of the biomaterial’s properties before and after sterilization.

1.2. Biomaterial sterilization methods
Sterilization is an important process that can be used in order to kill or eliminate almost all types of microorganisms. There exist several sterilization methods, which are used according to the purpose of the sterilization and the biomaterial that needs to be sterilized. The sterilization process can be carried out by dry heat, pressured vapor, ethylene oxide, formaldehyde, gas plasma (H2O2), peracetic acid, ultraviolet (UV) light, and visible light. The efficiency of each method depends on the chemical and physical properties of the biomaterial and the type of microorganism that must be eliminated. Some microorganisms are very resistant to killing, while others are very sensitive. There is no single sterilization process for all pharmaceuticals and medical devices, and each method has advantages and disadvantages. Ethylene oxide and autoclave sterilization are commonly used in hospitals, whereas gamma radiation and e-beam sterilization are mainly used in industry for the sterilization of pharmaceuticals [11]. The superiority of sterilization by irradiation compared to ethylene oxide and other sterilization methods is known [11,12].

1.3. Gamma irradiation of biomaterials
Radiation processing has become a well-accepted technology worldwide, with diverse uses ranging from irradiation of food products, polymer crosslinking and curing to sterilization and surface modification of medical device [13]. Gamma radiation, beta radiation (high-energy electrons), UV light, and visible light are the most commonly used energy sources for irradiation of biomaterials. Exposure of a polymer to radiation, especially ionizing radiation (gamma), can lead to ionization and excitation, chain scission, or crosslinking and to changes in bulk and surface properties [12].

Gamma radiation is an ionizing radiation that utilizes short wavelengths with high-intensity radiation. Gamma rays or electron beams are the most commonly used ionizing radiations for the sanitization/sterilization of medical devices. In these procedures, secondary electrons activate chemical reactions that induce oxidative degradations in the presence of oxygen [14]. Gamma or beta radiation can damage cellular components, including the DNA structure, resulting in cell death. This sterilization technique is based on the radiation’s penetration ability and on the ease of delivery of the necessary doses [11,12]. Gamma rays are emitted from a nuclear source 60 Co or 137 Ce. This sterilization is a simple, rapid and effective process with no chemical residues. It thus enables use of the biomaterial immediately after sterilization. Furthermore, it does not require very high temperatures and the sterilized biomaterial’s temperature is elevated only moderately. The main interactions between polymers and gamma rays or electron beams are based on the same reactions. However, there are minor differences. Gamma rays are electromagnetic radiation with a very low dose rate (kGy/h), while an electron beam is corpuscular radiation with a very high dose rate (kGy/s).

It was shown that when polymer systems are submitted to sterilization by gamma radiation, the irradiation conditions (such as the temperature, dose rate, process time) and the chemical structure (i.e., the thickness and volume of the polymer) determine the effect of the radiation on the polymer [11]. The chosen sterilization method must therefore be well matched, with a specific duration, contact, and temperature, in order to avoid damage to the biomaterial.

1.4. Irradiation effect on our bioadhesives’ components
Several studies have been conducted in order to elucidate the effect of gamma radiation. In Zaman et al.’s [15] study, pure gelatin films were irradiated with gamma radiation. Gamma radiation-treated gelatin films were found to have higher tensile strength and elongation at break compared to untreated ones. At lower doses, free radicals are stabilized by a reaction that results in the photo crosslinking of gelatin. Poor tensile properties are obtained, probably due to weak intramolecular bonds. As the radiation dose increases, strong crosslinking may occur, such that the tensile properties are improved. However, gamma radiation doses higher than 1 kGy can also cause substantial degradation of the polymer and therefore weaken its mechanical properties proportionally to the increase in the radiation dose [12,15]. It was also shown that exposure to gamma irradiation at doses of up to 50 kGy significantly improved the tensile strength of fish gelatin, with no change in the elongation at break value. The irradiation did not affect the melting temperature of the films. However, the glass transition temperature had a slight tendency to increase with increasing radiation doses. FTIR spectra confirmed that the improvement in the mechanical and thermal properties of the irradiated film was caused by a crosslinking reaction that generated peptide linkages between gelatin molecules. The researchers suggested that gamma irradiation causes water radiolysis that generates free radicals. The free radicals ionize carboxyl groups and the ionized carboxyl groups react with amine groups to form amides [16]. In another research, gamma radiation was found to have a strong effect on alginate films. At low doses, their mechanical strength improved. However, it began to decrease after exposure to a 5 kGy dose [17]. Seto et al. [18] investigated the effect of gamma irradiation on EDC-treated and untreated tendon tissue. Pretreatment with EDC crosslinking was found to provide better maintenance
of native tendon properties after exposure to gamma irradiation, whereas degenerative effects occurred when the native tendon did not undergo such pretreatment. According to another study, EDC/NHS-crosslinked collagen with relatively high crosslink densities demonstrated improved biomaterial properties after gamma sterilization [19].

In conclusion, high doses of radiation can be harmful and may affect the mechanical properties of biomaterials [11,12,15,17–19]. Therefore, in the current research, we studied the effect of gamma irradiation on the cytotoxicity and mechanical and physical properties of our bioadhesive.

2. Experimental

2.2. Materials

Gelatin “type A” from porcine skin (90–110 g bloom), alginic acid sodium salt (viscosity ∼250 cps, 2% [25°C]), N-(3-dimethylaminopropyl)-N’ ethylcarbodiimide, hydrochloride (EDC), and NHS were purchased from Sigma Aldrich, Rehovot, Israel.

2.3. Bioadhesive preparation

The preparation of the bioadhesive was based on dissolving 200 mg/mL gelatin and 40 mg/mL alginate in double-distilled water, under heating up to 60°C. EDC and NHS were dissolved in double-distilled water, and various amounts of these solutions were added to the gelatin-alginate solution immediately prior to the bioadhesive’s use. The formulations are presented in the form of EDC-NHS, where EDC is the concentration of the carbodiimide crosslinking agent (mg/mL) and NHS is the concentration of the NHS (mg/mL).

2.4. Sterilization by gamma radiation

Both crosslinked and noncrosslinked bioadhesives were packed in a closed carton box in an atmospheric environment. Gamma irradiation was carried out at Sorvan Radiation, Ltd., using a 60 Co source (MDS Nordion, Canada, Cobalt 60 irradiator type JS-9500) with a dose rate of about 8 kGy/h using a MDS Nordin produced Cobalt 60 irradiator (type JS-9500), at a temperature of 55°C. The validated dose was 25 kGy.

2.5. Ex vivo bonding strength measurements

Porcine skin (Kibbutz Lahav, Israel) was used as a soft tissue model. The porcine skin was cut into 2 × 2 cm² square-shaped pieces, and their epidermis side was firmly attached to metal testing holders with a matching surface area (all dimensions of the holders are specified elsewhere [7]). Then, 140 µL of the bioadhesive were spread uniformly on the dermis side of two porcine skin pieces (that were attached to the testing holders). These two porcine skin pieces were immediately attached by applying a load of 1.25 N on them and were incubated at 37°C and 100% humidity. After 30 min, the bonding strength was measured in tension mode at room temperature using a 3500 Instron Universal Testing Machine (Instron Engineering Corp., Norwood, MA) and a 10 N load cell. The two parts of the joint were strained at a constant velocity of 2 mm/min until separation was achieved. The mechanical testing procedure was inspired by the standard test method ASTM F-2258-03. The bonding strength was defined as the maximum strength in the stress–strain curve, measured by the Instron Merlin software. Ten repetitions were performed for each formulation.

2.6. Swelling ratio

The bioadhesives, with varying concentrations of crosslinking agents, were poured into a 6.2 × 6.2 × 3.5 mm³ silicon mold. After gelation, the bioadhesives were weighed (W₀), immersed in PBS and placed in a static incubator at 37°C and 100% relative humidity. The bioadhesives’ weight (W) was measured after 2, 4, 6, 24, 48, and 72 h by removing the bioadhesives from the PBS and blotting the excess liquid using Kimwipes. The bioadhesives were then left to dry for another 24 h and were weighed again at the end of this period (W_f). The swelling ratio was calculated according to Eq. 1:

Swelling ratio(%) = \( \frac{W_f - W}{W_f} \times 100\% \) (1)

2.7. Gelatin release ratio

Samples were prepared as described in section 2.6. After gelation, the cuboids were carefully removed and dried in air. The samples (n > 3) were immersed in 1 mL phosphate-buffered saline (PBS) and placed in a static incubator at 37°C and 100% relative humidity. The PBS was removed completely, and fresh medium was added at 2, 4, 6, 24, 48, and 72 h. The detection of the gelatin release ratio was performed using the Bio-Rad DC Protein Assay. The Bio-Rad DC Protein Assay is a colorimetric assay for protein concentration following detergent solubilization. It is based on the reaction of protein with an alkaline copper tartrate solution and Folin reagent. Similarly to the Lowry assay, it includes two steps that lead to color development: The reaction between protein and copper in an alkaline medium, and the subsequent reduction of Folin reagent by the copper-treated protein. The gelatin content was determined using a spectrophotometer, at 750 nm. A calibration curve was prepared for concentrations ranging from 0 to 6000 µg/mL (correlation coefficient >0.98).

2.8. Cytotoxicity evaluation

Human neonatal foreskin fibroblast cell cultures were exposed to bioadhesive extracts for 24 and 48 h, as described in the ISO 10993 Standard (parts 5 and 12) for biological evaluation of medical devices [20,21], in order to evaluate the cytotoxic effect of exposure of bioadhesive formulations to gamma radiation.

2.8.1. Cell seeding

Primary human fibroblast cultures were obtained from neonatal foreskins. The cells were thawed and cultured in 75 mm³ flasks with modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine,
and 1% penicillin-streptomycin-nystatin. The cultures were kept in a humidified 37°C and 5% CO2 environment. After reaching a confluence of 70%, the cells were separated from the bottom of the flasks using a trypsin A solution and were seeded into 96-well plates at concentrations of 5000 cells per well with 0.2 mL of fresh culture medium and incubated for 24 h. This cell concentration was chosen based on preliminary experiments, which demonstrated that optimal growth is obtained using this initial cell concentration. After 24 h, the medium was removed and replaced with bioadhesive extract. Cells cultured without bioadhesive extract served as a negative control. The cells were cultured for an additional 24 and 48 h. Three repetitions were carried out for each formulation at each time point.

2.8.2. Preparation of bioadhesive extract

The bioadhesives were poured into $6.2 \times 6.2 \times 3.5$ mm silicon molds. After gelation, the bioadhesive cubes were carefully removed and weighed. These samples were sterilized using gamma radiation. Bioadhesive extracts were obtained by immersing the sterilized samples in culture medium at a concentration of 0.2 g/mL and incubation for 24 h at 37°C. Cell viability was evaluated quantitatively using the Alamar Blue (AB) assay and qualitatively with a microscope.

2.8.3. Alamar blue assay

AB was used to evaluate human fibroblast growth and viability in the presence of bioadhesive extracts after exposure to gamma radiation. The AB assay was performed at 24 and 48 h after the addition of the bioadhesive extracts to the wells. The procedure included replacing the original medium with 0.25 mL of fresh medium containing 10% (v/v) AB and incubating the cells for 4 h. Subsequently, 100 μL duplicates from each well were transferred into a 96-well plate for spectrophotometer analysis (Spectra max 340 PC384, Molecular Devices). The percent reduction of the AB was calculated according to the manufacturer’s protocol. The %AB reduction after exposure to the adhesive extracts for different periods was compared to the %AB reduction in the control cells’ environment (cells that were not exposed to the extracts), in order to evaluate the influence of the irradiation on the bioadhesives’ cytotoxicity.

2.8.4. Qualitative evaluation of cell viability by microscopy

Human fibroblast cells at a concentration of 100,000 cells per mL were seeded into 6-well plates and incubated with 2 mL growth medium for 24 h. This cell concentration was chosen based on preliminary experiments, which demonstrated that optimal growth is obtained using this initial cell concentration. After 24 h, the medium was removed and replaced with bioadhesive extract. Cells cultured without bioadhesive extract served as a negative control. The cells were cultured for an additional 24 and 48 h. Three repetitions were carried out for each formulation at each time point.

2.9. Viscosity characterization

The initial viscosity of the gelatin alginate–based bioadhesive at the moment when it is applied on the tissue is affected mainly by the viscosity of the gelatin-alginate solution. Viscosity measurements of gelatin-alginate solutions before and after exposure to gamma radiation were performed using a hybrid rheometer (model: Discovery HR-3, TA Instruments Ltd.), fitted with a cone-and-plate geometry (1° cone angle, 40 mm diameter, 270 μm gap), at a constant temperature of 37°C, and a constant shear rate of 10 Hz in order to investigate the effect of gamma radiation on the bioadhesive’s initial viscosity.

2.10. Statistical analysis

All data were processed using the Excel software. Statistical comparison was evaluated by Student’s t test. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

In the current study, we investigated the effect of sterilization using gamma radiation on the cytotoxicity and mechanical and physical properties of our gelatin alginate–based bioadhesives. Based on previous studies [9,13], selected formulations with different concentrations of the crosslinking agents were chosen and were exposed to gamma radiation. The main results and their discussion are presented below. In all figures, significant differences are indicated by an asterisk.

3.1. In vitro cytotoxicity evaluation

One of the requirements from an ideal bioadhesive is to be safe, with minimal cytotoxic byproducts. Gelatin and alginate are natural biomaterials and are known to be biocompatible. However, the crosslinking agents used in our system may induce a cytotoxic effect on the tissue. Gamma radiation causes chain scission, which might release toxic materials. It is therefore important to evaluate the effect of radiation on the bioadhesives’ cytotoxicity. The elution method was performed on human fibroblasts, since they participate in the wound healing process, in order to determine their viability.

The cytotoxicity of our gelatin alginate–based bioadhesives was tested in previous studies [9]. It was found that at relatively low EDC concentrations (<15 mg/mL), cell viability was high (89–100%), while relatively high EDC concentrations (15 and 20 mg/mL) cause a decrease in cell viability. The addition of NHS at a very low concentration (less than 3 mg/mL) had almost no effect on cell viability, which remained at least 85%. However, at higher concentrations, there was a small decrease in cell viability, probably due to the slightly acidic nature of NHS [9]. Accordingly, in the current research, the effect of gamma radiation was studied on select formulations based on 200 mg/mL gelatin and 40 mg/mL alginate crosslinked with (a) 20 mg/mL EDC (20-0), (b) 10 mg/mL EDC (10-0), (c) 10 mg/mL EDC and 1 mg/mL NHS (10-1), and (iv) ddH2O as a control group. Figure 1 presents the viability of human fibroblasts as a function of indirect exposure to bioadhesives that were exposed to gamma radiation at a dose of 25 kGy. Significant differences in cell viability were found between all formulations compared to the control group after 24 and 48 h. After 24 h, all groups showed a cell viability higher than 76% (Figure 1a). After 48 h, exposure to a bioadhesive
based on an EDC concentration of 10 mg/mL resulted in 70% viability, which is considered to be nontoxic, while a relatively high EDC concentration (20 mg/mL) caused a decrease in cell viability to 66% (Figure 1b). The viability of human fibroblasts after indirect exposure to UV irradiated bioadhesives show similar tendency. However, higher viability rates of fibroblasts that were exposed to bioadhesive formulations crosslinked with low concentrations of EDC were found (Figure 1c). These results indicate that the gamma radiation had stronger effect on the bioadhesive formulations. Representative photographs of fibroblasts after indirect exposure to gamma or UV irradiated bioadhesives show the same effect (Figure 2). Fibroblasts that were indirectly exposed to gamma irradiated bioadhesive formulations that were crosslinked with a low concentration of EDC with or without NHS (10-1 and 10-0) demonstrated better viability than those exposed to formulations crosslinked with a high EDC concentration (20-0).

As mentioned in the Introduction, exposure of polymers to gamma radiation can cause chain scission and crosslinking. It is very probable that our bioadhesives underwent both processes, which resulted in a release of toxic materials to the culture medium, thus affecting the fibroblasts’ viability. Therefore, more toxic molecules were released when high concentrations of EDC were used. Our results indicate that a gamma radiation dose of 25 kGy, as is customary for biomedical applications, is safe when using formulations based on 10 mg/mL EDC (with or without NHS). Formulations with a relatively high EDC content of 20 mg/mL EDC necessitate a sterilization process using a lower radiation dose or preparation in relatively clean surroundings.

3.2. Viscosity

The viscosity of bioadhesives is a highly important characteristic which determines their ease of use. Rheological tests were performed in order to elucidate the effect of exposure to gamma radiation on the bioadhesive’s viscosity. The measurements were carried out on the bioadhesive’s polymeric solution at 37°C, without addition of crosslinkers. The viscosity of the gelatin-alginate solution before exposure to gamma radiation was 8.9 Pa.s, whereas it decreased to 3.6 Pa.s after exposure to 25 kGy. This might be due by the ability of gamma radiation to cause chain scission, which can affect the resistance of our gelatin alginate–based bioadhesives to shear or tensile stress. Lower viscosity may be more suitable for in vivo use, because it may enable better penetration into the surrounding tissue surface, thus improving the bonding to the tissue. However, the bonding strength should remain high.

3.3. Ex vivo bonding strength

High bonding strength to the tissue is very crucial in the application of tissue bioadhesives. For most systems, the adhesion mechanism is considered to be a combination of mechanical interlocking and chemical adsorption [22,23]. Mechanical interlocking is derived from the ability of bioadhesive molecules to penetrate into porosities or irregularities on the surface of the adherend. Chemical adsorption is derived from the creation of intramolecular primary bonds (ionic, covalent, and metallic) or intermolecular secondary bonds (van der Waals and hydrogen bonds) between the bioadhesive molecules and molecules on the surface of the adherend.

The bonding strength of formulation 20-0, before exposure to gamma radiation, was lower than the bonding strength of formulation 10-1 (Figure 3). These results can be explained by the crosslinking reaction of the polymers. The crosslinking reaction of gelatin and alginate with EDC results in activated carboxylic acid residues that have a relatively short half-life. The addition of NHS creates an NHS-activated carboxylic acid group, which is less susceptible to hydrolysis, prevents rearrangements and extends the half-life of the activated carboxylic acid residues, thereby improving the crosslinking reaction and increasing the bonding strength.

The effect of gamma radiation on the bonding strength in tension of two selected bioadhesive formulations is presented in Figure 3. Formulations 20-0 and 10-1 that were not exposed
to gamma radiation exhibited a bonding strength of 20.0 and 13.9 Kpa, respectively. After exposure to gamma radiation, these values decreased to 16.5 and 10.6 Kpa, respectively. However, these differences were not significant.

As shown previously, gamma radiation decreased the bioadhesives’ viscosity. This improved their ability to penetrate porosities or irregularities on the surface of the skin samples. This is expected to increase the bonding strength due to more effective mechanical interlocking. However, some decrease in the bonding strength due to exposure to gamma radiation is probably achieved through weakening the other bonding mechanism: gamma radiation may decrease the chemical adsorption by decreasing the probability of creating bonds between the bioadhesive molecules and the skin. As mechanical interlocking was probably enhanced by the exposure to gamma radiation, while chemical adsorption was probably reduced, these two opposite effects resulted in very small changes in the bonding strength following gamma irradiation.

3.4. Gelatin release and swelling ratio

The gelatin release and swelling ratio of our bioadhesives are very important physical parameters, indicating the density of the network between the polymer chains formed by the physical and chemical crosslinking reactions. A high gelatin release indicates that the polymer chains detach from the network more easily or that the portion of polymer involved in the crosslinked network is smaller. A low swelling ratio can indicate a dense network structure. A denser polymer structure reduces the accessibility of water molecules to the hydrophilic parts of the polymer molecules and as a result less water can penetrate into the bioadhesive. In our study, two selected bioadhesive formulations were exposed to gamma radiation before and after crosslinking with EDC and NHS and their gelatin release and swelling ratios were studied.

3.4.1. Gelatin release

Gelatin release from formulation 20-0, before exposure to gamma radiation, was lower than the release obtained from formulation 10-1 (Figure 4). As can be seen in Figure 4, after 2 h only 3.5 mg/mL gelatin are released from formulation 20-0, while 8.1 mg/mL are released from formulation 10-1 after the same time. As was explained above, these results can be derived from the crosslinking reaction. The addition of NHS might lead to a shift in localization of the crosslinks from the amorphous regions towards the triple helix structures [8,24]. As fewer crosslinks are present in the amorphous regions, more water can penetrate, resulting in higher gelatin release.

The effect of gamma radiation on gelatin release from the bioadhesives was minor. In formulation 20-0 (Figure 4a), there was a small decrease in the swelling ratio after 24, 48, and 72 h only in samples that were exposed to gamma radiation after crosslinking. This effect was not significant in formulation
Crosslinking of the polymer solution before exposing it to gamma radiation may decrease the chain scission process [18], which decreases gelatin release. This protection may be more significant when the crosslinking is performed with a high concentration of EDC.

### 3.4.2. Swelling ratio

The swelling ratio of formulation 20-0, before exposure to gamma radiation, was also lower than the ratio obtained for formulation 10-1 (Figure 5). As can be seen in Figure 5, after 2 h, a swelling ratio of 258.1% and 623.8% was found for formulations 20-0 and 10-1, respectively. As was explained above, these results may be due to the crosslinking reaction. The addition of NHS might lead to fewer crosslinks in the amorphous regions, leading to a higher degree of water penetration, which leads to a high swelling ratio.

Gamma radiation was found to have a major effect on the swelling ratio of the bioadhesives for formulation 10-1. In formulation 20-0 (Figure 5a), there was a significant decrease in the swelling ratio after 4, 48, and 72 h only in samples that were exposed to gamma radiation after crosslinking. However, in formulation 10-1, this decrease was significant in samples that were crosslinked before exposure to gamma radiation at all time points and also after 4 and 6 h in samples that were crosslinked after exposure to gamma radiation (Figure 5b).

As mentioned above, gamma radiation can cause chain scission and crosslinking. The tendency to a decrease in the swelling ratio in formulation 10-1 might be explained by both processes. Addition of NHS could shift the crosslinking to the triple helix structure, as suggested by Timkovich [8]. However, chain scission as a result of the gamma radiation probably occurs in amorphous regions, leaving fewer areas for the water to penetrate, which results in a decrease in the swelling rate.

The initial swelling and gelatin release occurred in the amorphous and noncrosslinked regions of the matrix. Although the swelling ratio of our bioadhesives is relatively high in an aqueous environment, it basically showed a minimal effect on the bonding of the injured tissue because it is applied in a very thin layer between the lacerated tissues [25]. Furthermore, due to technical reasons, the swelling ratio and gelatin release studies were performed on dry bioadhesive samples that were cast into cubic molds (3D instead of thin layers) and not on thin films between two skin layers, as will be applied in practice.

In this study, we investigated the effect of gamma radiation on the cytotoxicity, mechanical and physical properties of select gelatin-alginate bioadhesive formulations. The study was performed because bioadhesives must be sterilized before they can be examined in clinical trials. There is no ideal sterilization process that is suitable for all the pharmaceuticals and medical devices. Each sterilization method has its advantages and disadvantages. Gamma radiation is a commonly used sterilization method for medical devices and pharmaceuticals. It results in minimal increase in temperature, leaves no residue,
and does not require quarantine time postprocessing [26]. Therefore was chosen for use in this research. McDonnell and Lambert [27] claimed that cyanoacrylate bioadhesives can be sterilized in liquid form by gamma irradiation. The irradiated product should be comprised of a cyanoacrylate monomer and a combination of an anionic stabilizer and a free-radical stabilizer in effective amounts in order to stabilize the composition during irradiation and during storage prior to cure. Their results showed excellent retention of bonding performance on extended aging with no significant changes in overall purity. When tested in vivo, their adhesive was found to be safe and reliable as wound closure. Similarly, our current study shows that gamma-sterilization has minor effects on most studied properties of our new gelatin-alginate bioadhesives, when radiation dose of 25 Kg-1 was used.

4. Conclusions

In the current study, we investigated the effect of gamma radiation on the cytotoxicity and mechanical and physical properties of select gelatin-alginate bioadhesive formulations. The study was performed since the bioadhesives must be sterilized before they can be examined in clinical trials. Gamma radiation, a commonly used sterilization method for polymers, was chosen.

Exposure of our bioadhesives to gamma radiation of 25 Kg-1 resulted in some changes that stemmed from a combination of crosslinking and chain scission. The exposure to gamma radiation significantly decreased the bioadhesives’ viscosity, while the bonding strength to the tissue was not significantly changed due to the ability of gamma radiation to oppositely interfere with both adhesion mechanisms, mechanical interlocking and adsorption. Other physical properties, such as gelatin release and swelling ratio of the bioadhesives, were also affected by the gamma radiation. The effect on gelatin release from the bioadhesives was minor, whereas the swelling ratio was significantly decreased in formulations crosslinked with a low EDC concentration and NHS. The gamma radiation affected mainly the cytotoxicity of formulations with a relatively high EDC concentration.

In conclusion, elucidating the effect of gamma radiation on the bioadhesives’ properties convinced us to positively consider this as a suitable sterilization method. This enables us to continue our research and perform in vivo studies using sterilized samples. Further experiments can be performed using gamma-irradiated samples exposed to dry-ice flakes, in order to decrease the radiation effects.

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