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Gentamicin extended release from an injectable polymeric implant

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Abstract

Gentamicin sulfate, a potent antibiotic agent, is currently used for treatment of osteomyelitis mainly by intravenous injection with a long-term indwelling catheter, local implant of antibiotic containing polymethylmethacrylate beads or calcium phosphate (bone cements). Searching for more effective treatments, this study was designed to evaluate biodegradable injectable gelling polymeric devices for the controlled release of gentamicin sulfate in the treatment of invasive bacterial infections. Gentamicin sulfate was incorporated in poly(sebacic-co-ricinoleic-ester-anhydride P(SA-RA)) paste at 10–20% *w/w* and its release in buffer solution was monitored. The in vitro activity of the formulations was determined against *Staphylococcus aureus*. A constant release of active gentamicin for over 28 days was found. The stability of the formulation was determined under different storage conditions. The formulations were stable to sterilization by γ-irradiation and long term storage under freezing. The toxicity of the polymer and the formulations with gentamicin was examined by subcutaneous injection to rats. Four weeks after implantation, histopathological examination of the tissues surrounding the implant showed no inflammation. A preliminary study revealed positive effect of gentamicin containing P(SA-RA) on established osteomyelitis in a rat model. In conclusion this study suggests that poly(sebacic-co-ricinoleic-ester-anhydride) 3:7 loaded with 10%–20% gentamicin sulfate, might be used as an injectable biodegradable device for in situ treatment of osteomyelitis induced by *S. aureus*.

Keywords: Osteomyelitis; Injectable polyner; poly(ester-anhydride); *Staphylococcus aureus*; Gentamicin; Antibiotic

1. Introduction

Staphylococcal infections are an emerging problem with a major economic impact [1,2]. Osteomyelitis is a deep bone infection mostly staphylococcal, that can occur from trauma or nosocomial infection or from the treatment of trauma that allows organisms to enter bone e.g. after hip or knee replacement surgery [3,4]. Osteomyelitis is a particularly complicated infection to treat [5] because it is difficult to achieve a sufficient concentration of antibiotic at the site of infection by systemic administration. This can be attributed to a number of factors: the short half-life of the antibiotic, poor blood circulation to the infected area and systemic toxicity of the antibiotic, which prohibits the use of the required high systemic dose. In addition, it is difficult to alleviate osteomyelitis because the infecting bacteria are in a biofilm mode of growth. Biofilm formation on devascularized surfaces protects bacteria from antibiotics [6].

Because of the ischemia and slow healing seen in infected tissue and the relatively low blood flow in bone, antibiotics are given for extended periods. Soft tissue infections and septic joints are usually treated with parenteral antibiotics for two to four weeks, whereas chronic osteomyelitis or infected arthroplasties are treated for four to six weeks or longer.

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The two methods currently available for the administration of antibiotics are intravenous injection with a long term indwelling catheter [6] and local implant of antibiotic containing polymethylmethacrylate beads or calcium phosphate (bone cements) [7,8]. Both of these methods have significant disadvantages. Although the intravenous route produces adequate blood concentrations, it requires an invasive procedure for catheter placement and multiple daily antibiotic doses. Complications and difficulties associated with the intravenous catheter include infection, catheter failure and the high cost of the antibiotics and supplies [9]. Administration of antibiotics containing polymethylmethacrylate beads has an advantage over intravenous antibiotics, as high concentrations of antibiotics are delivered locally, while systemic levels are low, thereby producing minimal systemic complications or allergic reactions. However, polymethylmethacrylate beads alone usually provide locally bactericidal levels of antibiotics for only two weeks, so supplemental parenteral antibiotics are often needed. In addition, polymethylmethacrylate beads require a second intervention for their removal, after which the cavity may require reconstruction. Moreover, resistant bacteria protected within the beads may be a cause of treatment failure or late recurrence [10].

In recent years, various biodegradable systems have been evaluated for local delivery of antibiotics in the treatment of bone infections [11]. Polyanhydrides are one of several types of biodegradable polymeric carriers which show promise as a highly effective treatment modality [12,13]. Additional biodegradable polymers such as poly(lactic acid) [11], poly(glycolic acid) [14], their copolymer poly(lactide-co-glycolide) [15], collagen [16], chitosan [17] and polyhydroxyalkanoates [18] have also been tested, showing their potential effectiveness as antibiotic carriers in the local treatment of infections.

Although there are many different types of biodegradable polymers that can potentially be used in the preparation of drug delivery systems [19], the pasty hydrophobic polymers represented by P(SA-RA) posses certain advantages over other polymeric delivery systems. The incorporation of drugs is done at room temperature by gentle mixing of the drug powder in the oily polymer with no solvents, additives or water involved and without any mechanical forces. The resulting paste can be injected via a common syringe into the desired site. Once the loaded polymer encounters aqueous medium it gels, forming hydrophobic protective environment for the entrapped compound. The hydrophobic gel degrades by hydrolysis, mainly from the surface, while releasing the entrapped drug. This method is superior to the common incorporation methods for polymeric delivery systems which require either mixing the drug in a hot polymeric melt, use of organic solvents and mixtures with water, and for the use of additives such as surfactants and organic solvents, which may deteriorate the drug [20–22]. The P(SA-RA) is hydrophobic and does not contain hydrophilic segments as poly(ethylene glycol) in thermogelling systems [23], and the hydrophobic polymeric matrix protects drugs from possible deterioration effects of aqueous medium and the interface of water-organic medium. According to our previous study [24], the hydrophobic P(SA-RA) can be compared with a hydrophobic organogel system, which is composed of water-insoluble amphiphilic lipids that swell in water and form various types of lyotropic liquid crystals [25]. However the organogels suffer from the following disadvantages: purity of waxes and stability of oils–oils need a stabilizer, antioxidant and preservative to increase their shelf life and stability. The hydrophobic pasty P(SA-RA), with or without drug, can be kept under refrigeration for months [26] without any stabilizers. Moreover, the difference between the melting point of waxes and oils makes the organogels system susceptible to phase separation. In addition, the typical equilibrium of the water content of the organogel formed is approximately 35%, which therefore produces relatively short release duration for hydrophilic drugs. The gentamicin that was presented in this study was released from the polymer in a time frame of one month. This can be compared to the release of some compounds such as heparin and immunoglobulin from polyanhydride microspheres prepared by a solvent evaporation technique method using a double emulsion [27].

This study focuses on an in situ formation of a biodegradable, injectable, polymeric depot for controlled release treatment of regional bacterial infections. Recent studies in our laboratory have explored the use of this injectable biodegradable poly(sebacic-co-ricinoleic-ester-anhydride)s (P(SA-RA)s) for the treatment of solid tumors [28]. P(SA-RA)s containing more than 65% ricinoleic acid are injectable at room temperature. The gentamicin sulfate loaded formulations could be injected directly into infected bone. According to our previous studies, once these polymers meet an aqueous environment they increase in viscosity and become semi-solid gels. This semi-solid polymeric matrix will release gentamicin until the polymer disintegrates. The goal of the present study was to determine the in vitro efficacy of P(SA-RA) 3:7 w/w loaded with 10% and 20% of gentamicin, the in vivo toxicity of these formulations, their stability under γ-irradiation and their storage stability.

2. Experimental

2.1. Polymers

Poly(sebacic-co-ricinoleic-ester-anhydride) 3:7 *w/w* ratio (P(SA-RA)) with different molecular weights were synthesized as described elsewhere [28,29]. The physical properties of the polymers are presented in Table 1.

Table 1
Molecular weights (Mn and Mw) and melting temperatures (m.p.) of P(SA:RA) 3:7 *w/w* ratio

Polymer assign [a]	Mn (Da) [b]	Mw (Da) [b]	m.p. (C) [c]
P(SA-RA) 3:7 4500	4500	8200	32
P(SA-RA) 3:7 5600	5600	10,000	34
P(SA-RA) 3:7 18,000	18,000	43,000	41
P(SA-RA) 3:7 33,000	33,000	50,000	45

[a] The polymers were prepared as described elsewhere (28,29).
[b] The weight-average molecular weight (Mw) and number-average molecular weight (Mn) were determined by GPC.
[c] Melting point (m.p.) was recorded by differential scanning calorimeter (DSC) (Mettler-Toledo, Schwerzzenbach, Schweiz) at 10 °C/min.

2.2. Bacteria

Staphylococcus aureus (*S. aureus*) (strain ATCE 29213) was selected for the bacterial inhibition study due to its presence in the majority of orthopedic infections [30] and grown in a tube containing Tryptic Soy Broth (TSB, Becton, Dickinson and Company) at 37C.

2.3. In vitro gentamicin release

Gentamicin was incorporated in P(SA-RA)s by mixing the drug powder (10% and 20%, *w/w*) with the polymer at room temperature until a homogeneous mixture was achieved. The mixing was performed by trituration, meaning that small amounts of gentamicin and polymeric paste were added each time to the mortar and mixed together till a homogenic mixture was achieved. Although the paste melting point was above room temperature, it was soft enough to allow mixing. The particle size of the gentamicin in the formulation was less than 100 microns, as determined by SEM. The release study was conducted by injecting the formulation onto the side of the vial prior to adding the buffer releasing medium. The solution was periodically replaced with fresh buffer solution and the gentamicin concentration was determined by the fluorescamine method described above.

The gentamicin did not affect the polymer molecular weight and structure, as confirmed by gel permeation chromatography (GPC) (Waters MA) and ^{1}H NMR (Varian, Palo Alto, CA). Drug release studies were conducted by placing 200 mg formulation in 50 ml phosphate buffer solution (0.1 M, pH 7.4) at 37 °C with constant shaking (100 RPM). The experiment was performed in duplicate. To simulate the flow of a biological liquid, the buffer solution was replaced every 48 h and the replaced solutions were kept for gentamicin analysis.

The in vitro release assay was also conducted in a plastic tray as follows: 25 μl of the formulation was placed at the bottom of each well in a 24-well microtitre flat bottom tray (Nonc, Copenhagen, Denmark). One ml of phosphate buffer saline (PBS) was added and the trays were incubated in a humid chamber at 37C. At each time point the medium was collected, the formulation was washed with 1 ml PBS, and 1 ml of fresh PBS was added. The collected medium was centrifuged and the supernatant was kept for the bacterial study and gentamicin concentration analysis.

The collected samples were diluted 100 times before the analysis. 0.2 ml of the diluted samples were reacted with fluorescamine (Sigma, Israel) solution (acetone, 0.1 mg/ml), diluted to 2 ml with buffer borate, pH 7.0, incubated for 15 min at room temperature and the complex was analyzed by a spectofluorometer (Jasco, Japan) at excitation wavelength 392 nm and emission wavelength 480 nm [31]. The experiment was performed in triplicates.

2.4. Anti bacterial activity

The antibacterial activity of the releasing solutions was examined in *S. aureus* cultures. The collected supernatants were diluted 100 times, 1000 times and 10,000 times with PBS and added to the wells in a 96 well microtitre flat bottom plate containing 10^{6} CFU of *S. aureus* in TSB. The plate was incubated at 37C inside a temperature controlled microplate spectrophotometer (VERSAmax, Molecular Devices Corporation, CA, USA) for 24 h. Optical density (OD) measurements were performed every 2 h to determine bacterial concentrations in the wells at 650 nm. All counts were determined in triplicate.

2.5. Stability to γ-irradiation

P(SA-RA) 3:7 *w/w* loaded with 20% *w/w* gentamicin was prepared by mixing the drug in the pasty polymer at room temperature until a homogeneous mixture was obtained. The formulation mixture was loaded into 1 ml lock plastic syringes (Sewa Medicals, Pune, India). Syringes containing 1 ml of blank polymer were also prepared and served as control. All polymers were irradiated with an absorbed dose of 2.5 Mrad by means of a ^{60}Co source (450 000 Ci; 8 h). The irradiation was conducted at Sor-Van Radiation Ltd. (Kiryat Soreq, Yavne, Israel)

All samples were analyzed for changes in molecular weight by GPC, changes in chemical structure by infrared spectrophotometer (IR) (Ettlingen, Germany), and for drug content, shortly after the preparation of the formulation and after irradiation. To determine the gentamicin content in the polymers before and after irradiation, formulation samples were dissolved in dichloromethane and the drug was extracted by double distilled water (DDW) 3 times and analyzed by spectrofluorometer.

The storage stability of the irradiated specimens was studied at four different temperatures. After irradiation, syringes loaded with the formulations were stored at 37 °C, 20 °C, 4 °C and −17 °C; non-irradiated samples were used as control. At each time point a sample was withdrawn and analyzed for molecular weight, IR spectra and drug content. At some time points a short drug release study was performed in order to detect possible changes in drug release because of the storage conditions. At the same time points gentamicin was recovered from the formulations by dissolving the formulation in dichloromethane and extracting it three times with buffer phosphate pH 7.4, 0.1 M. The study was performed in triplicates over six months.

2.6. In vivo toxicity of polymer-gentamicin loaded implant

Male Wistar rats, 10 week old (Harlan laboratories, Jerusalem, Israel) were kept under specific pathogen free conditions and given free access to irradiated food and acidified water throughout the experiment. The ethics committee at the Hebrew University of Jerusalem (National Institutes of Health approval number: OPRR-A01-5011) reviewed the application for animal study and found it compatible with the standards for care and use of laboratory animals (ethics committee research number: MD-80.04-3).

200 μl of gentamicin-loaded semi-solid formulation (0%, 10% and 20% w/w) were injected subcutaneously in the front abdomen via 19 G needle into groups of three male Wistar rats. One group was injected with 200 μl blank P(SA-RA) 3:7 (0% gentamicin) on the left side (control) and 200 μl of P(SA-RA) 3:7 loaded with 20% gentamicin on the right side. A second group was injected with 200 μl blank P(SA-RA) 3:7 (control)

on the left side and 200 μl of P(SA-RA) 3:7 loaded with 10% gentamicin on the right side. The animals were observed for local toxicity signs. Four weeks after injection the rats were sacrificed, the implant was taken for chemical analysis and the surrounding tissue was fixed in 4% neutrally buffered formaldehyde for histopathological examination.

2.7. In vivo effect of soft polymer on osteomyelitis in rats

Male Wistar male rats weighing 300 gram were anesthetized by intra-peritoneal injection of a mixture of xylazine and ketamine. The operated area was shaved, the skin was disinfected with ethanol between the knee and the ankle, and the tibia was exposed and drilled using 1 mm width kw. The entering site, drilled down for 1 cm, was medial to the patellar tendon, at the height between the tibial plateau and the tibial tuberosity. Following drilling it was injected with 50 μl saline, which contained 10^5 CFU of *S. aureus*. The skin was disinfected and closed with stitches. Two weeks later the rats were identically operated and treated by intra-tibial injection of a 50 μl soft polymer with gentamicin in the right tibia and without gentamicin in the left tibia. After an additional 3 weeks the rats were anesthetized and examined by radiology.

3. Results and discussion

3.1. In vitro gentamicin release

The minimum inhibitory concentration of gentamicin for the *S. aureus* used in this study is 2–4 μg/ml. The formulation prepared for this study contained 20–40 mg of gentamicin. Gentamicin loaded polymers were prepared by simple mixing of the drug powder in the polymer paste. No chemical interactions between the drug salt and the polymer occurred. The rate of gentamicin release from the pasty polymers into buffer phosphate is shown in Fig. 1. It can be seen that formulations with 20% gentamicin have slower release profiles than that of

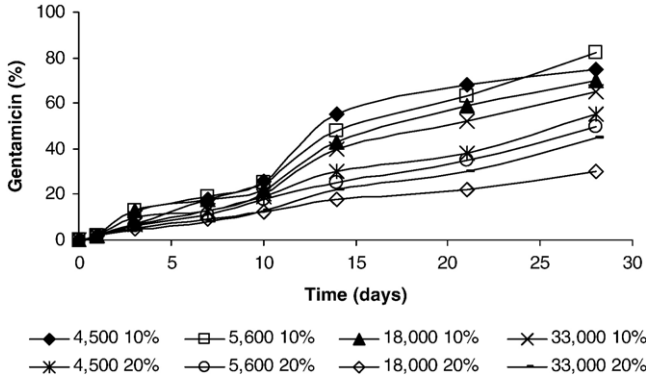

Fig. 1. Release of gentamicin sulfate from P(SA-RA) 3:7 *w/w* with different number molecular weight (Mn) and amount of drug. The gentamicin sulfate release was performed in 0.1 M buffer phosphate pH 7.4 at 37C with constant shaking at 100 RPM. The gentamicin sulfate content in solution was determined by spectrofluorometer at excitation wavelength 392 nm and emission wavelength 480 nm. The experiment was performed in duplicates and the standard deviation did not exceed ±7%.

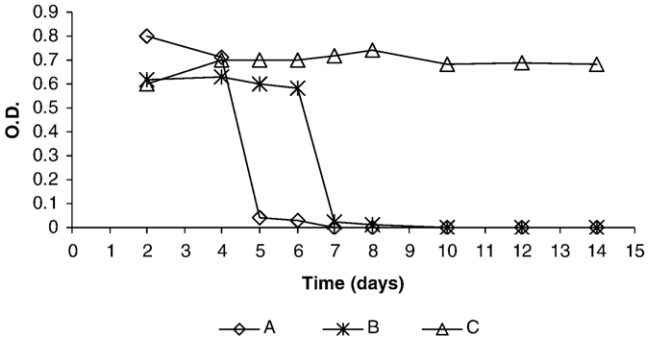

Fig. 2. Gentamicin sulfate effect on *S. aureus*. Supernatant collected at various time point of the gentamicin sulfate release system was diluted 100 (A), 1000 (B) and 10,000 (C) times with phosphate buffer saline and incubated with *S. aureus* for 24 h in an VERSAmax microplate spectrophotometer for 24 h. Optical density (OD) measurements were performed every 2 h to determine bacterial concentrations in the wells at 650 nm. One O.D. corresponds to 10^{13} colonies (based on dilution in PBS and plating in TSB agar plates). The experiment was performed in duplicates and the average O.D. is presented.

formulations with 10% gentamicin, probably due to salt formation between gentamicin and the fatty degradation products of the polymer [13]. Increasing the molecular weight of the polymer slightly decreased the gentamicin release rate. P(SA-RA) 3:7 (Mn=4500) loaded with 20% gentamicin was chosen for further investigation in order to achieve a release rate which would enable: relative high concentration of gentamicin during a prolonged period, and in order to achieve an injectable formulation. Gentamicin- loaded polymers with higher molecular weight are too viscous for injection.

In a second in vitro release study, gentamicin was released into a 24-well tray (into 1 ml solution/well). The formulation prepared for this study contained ~5 mg of active gentamicin. The release profile (data not shown) was similar to the release profile obtained when releasing in a large volume of water (Fig. 1). For example, after eight days 9% (about 0.45 mg) of the loaded gentamicin was released. The use of 2 different experimental set-ups providing similar results, suggests a larger scope for practical applications. The overall results (see also Fig. 5) indicate a constant release of gentamicin during a long period, which is an advantage in the treatment of a chronic infection or for prevention of a recurrent infection. The release of antibiotics from P(SA-RA)s is controlled by diffusion through the polymer mass and by degradation of the polymer, resulting in complete elimination of both the drug and the polymer from the site of delivery.

The solutions were incubated with *S. aureus* in TSB in order to check the antibacterial activity of the released antibiotic.

3.2. Bacterial study

The supernatant solutions from the release study of polymer containing gentamicin were added to cultures of *S. aureus*. The results of the VERSAmax spectrophotometer are summarized in Fig. 2. After 6 and 8 days, the gentamicin concentration was sufficient to eradicate *S. aureus* when the solutions were diluted 100 (4.5×10^{-3} mg/ml) and 1000 (4.5×10^{-4} mg/ml) times, respectively (A and B in Fig. 2). In the case of 10,000 time dilution

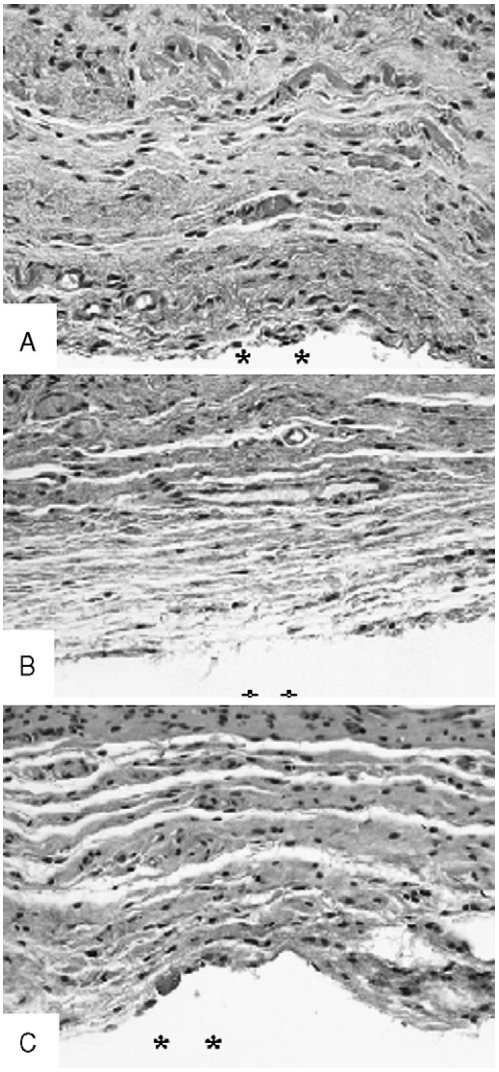

Fig. 3. Photographic presentation (×200, hematoxylin and eosin) of a typical capsular reaction in the four types of implanted material, A—sample of P(SA-RA) 3:7 blank, B—sample of (P(SA-RA) 3:7+10% gentamicin sulfate, C—sample of (P(SA-RA) 3:7+20% gentamicin sulfate. Note: in all cases the capsule has a comparable thickness, composed of mature connective tissue and minimal presence of dispersed mononuclear cells.

(C in Fig. 2) the concentration was not inhibitory. The control wells with the blank P(SA-RA) 3:7 w/w (Mn=4500) did not affect the growth of bacteria. No effect was found in these wells: the O.D. was approximately 0.7. In wells that contained *S. aureus* in TSB only, an O.D. of approximately 0.6 was found. In view of the results of gentamicin release (Fig. 1) and the profound bacterial inhibition by the diluted solutions (Fig. 2), there is probably a significant antibacterial activity in the release medium at an earlier stage.

3.3. Toxicity of polymer-gentamicin implant

The toxicity of polymer implants was assessed in rats. A subcutaneous injection enables an accurate examination of tissue response [32]. All rats survived the entire period of the experiment. The histopathological evaluation indicated that in the samples from the rats (2 groups) in which 10% or 20% gentamicin formulations were administrated, the degree of capsular tissue reaction was comparable to that formed when the blank polymer alone was present, suggesting no adverse reaction upon application of the combined therapeutic modality. The formed capsule was predominantly composed of mature collagen deposition, associated with presence of fibroblasts, blood vessels and sparse hystiocytes or other mononuclear cells. No evidence of active inflammatory reaction or tissue irritation was present within the capsule or extending beyond the local capsule. In all cases, the thickness of the capsule was relatively comparable. A photographic representation of the tissue reaction (e.g. capsule) observed in various groups is shown in Fig. 3(A–C). The local tissue reaction typically consisting of thin envelope capsule is interpreted as scarring chronic inflammatory reaction. No evidence of granulomatous foreign-body or lymphoid cell aggregation was noted, indicating good tolerability and lack of immunological stimulation.

3.4. Treatment of osteomyelitis in a rat model by gentamicin loaded P(SA-RA)

Osteomyelitis was induced by injection of *S. aureus*. The rats were treated 2 weeks later by soft polymer P(SA-RA) with or without gentamicin. At this time, the tibia was much softer for drilling, compared to its situation in the onset of the experiment. In addition, the surrounding soft tissue was hyperemic, suggesting inflammation. After an additional 3 weeks, the rats were analyzed by radiology. A representative radiograph shows clear lysis of the medullary area with blearing of the cortex in the left control tibia and no clinical signs in the right one (treated with P(SA-RA) (Fig. 4).

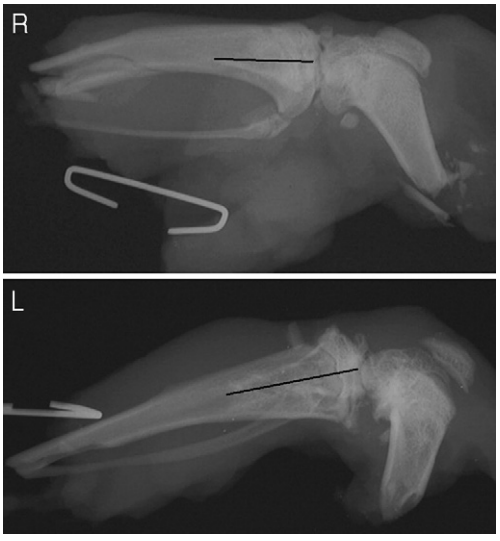

Fig. 4. Treatment of osteomyelitis in a rat model by P(SA-RA). Osteomyelitis was induced by injection of *S. aureus*. The rats were treated 2 weeks later by soft polymer P(SA-RA) with gentamycin. The rats were analyzed by radiology after additional 3 weeks. A representative radiograph shows lysis of the medullary area with blearing of the cortex in the left tibia, suggesting osteomyelitis (L) and no radiogloic signs in the right one (R). The line indicates the affectedarea.

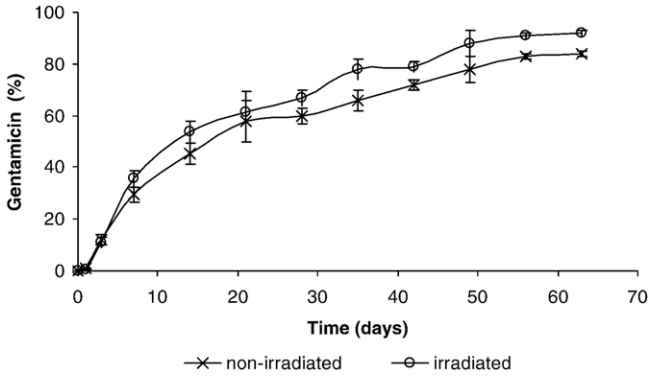

Fig. 5. Gentamicin sulfate release from irradiated and non irradiated P(SA-RA) 3:7 loaded with 20% drug. The gentamicin sulfate release and detection were performed under conditions described in Fig. 1. The experiment was performed in duplicate.

3.5. Stability to γ-irradiation

The expected applications of these poly(ester anhydride)s include injectable drug carriers. It is, therefore, essential to properly sterilize the formulations before their administration. The most effective method for the terminal sterilization of moisture and heat-sensitive polymers is exposure to ionization radiation. The irradiation resistance of various microorganisms varies with the type of organism; it is currently thought that a radiation dose of 2.5 Mrad is sufficient for the sterilization of medical implants [33].

To determine changes in the molecular weight of the polymers before and after irradiation, samples were dissolved in chloroform and analyzed by GPC. All samples were checked by IR spectroscopy to confirm anhydride bonds. All irradiated polymers showed an anhydride peak (1813 cm^{-1}), an ester peak (1731 cm^{-1}) and no evidence of carboxylic acid peak (1700 cm^{-1}), which indicates no hydrolysis of the polymers.

There was no difference between gentamicin content in the irradiated (21.5%±2%) and non-irradiated formulations (19.8%±2%). A drug release study showed that irradiation had a minimal effect on gentamicin release; gentamicin release from the irradiated formulation was slightly higher (Fig. 5).

The stability of P(SA-RA) 3:7 with or without (20% *w/w*) gentamicin stored at −17 °C, 4 °C, 25 °C and 37 °C was studied. The changes in the molecular weight and melting temperature of these samples were determined for a period of six months (Table 2). Both loaded and blank polymers kept their initial molecular weights for 30 days at 4 °C, but they dropped to about one-third of that value after storage at room temperature. The formulations stored at −17 °C still retained their initial molecular weight even after six months. The formulations stored at 37 °C kept their initial molecular weight for 48 h followed by degradation to short oligomers of ~1000 Da over the following two weeks.

A drug release study performed at several time points (Fig. 6) demonstrated that the storage conditions had an influence on the controlled release of gentamicin. Samples that were kept at −17 °C released the drug at the same rate for the duration of the study. It can be seen that samples stored at room temperature kept their ability to release drug for four weeks (25 °C, 4 weeks) but doubled the release rate after seven weeks of study (25 °C, 7 weeks). Samples that were stored at 4 °C for 12 weeks increased

Table 2
Changes in molecular weights (Mn and Mw) and melting temperatures (m.p.) of stored polymers

Storage conditions	Sampling	Mn (Da) [a]			m.p. (°C) [b]
		Irradiated formulation	Non-irradiated formulation	Irradiated blank polymer	
37 °C	0 day	5000	5000	5000	32
	1 day	4000	4200	5000	32
	3 days	2000	2000	2500	30
	7 days	2000	2100	2000	30
	10 days	1700	1700	1600	27
	14 days	1300	1200	1300	27
20 °C	0 day	5000	5000	5000	32
	1 day	5000	5000	5000	32
	7 days	5000	5000	5000	32
	14 days	4000	3900	4100	32
	21 days	4000	4000	4200	32
	28 days	3500	3200	3000	31
	35 days	2100	2000	2200	31
	42 days	1700	1800	1600	29
	49 days	1700	1500	1400	28
	56 days	1500	1500	1400	28
4 °C	0 days	5000	5000	5000	32
	28 days	5000	5000	5000	32
	56 days	3700	3500	4100	32
	100 days	2000	2000	2000	29
−17 °C	0 days	5000	5000	5000	32
	56 days	5000	5000	5000	32
	100 days	5000	5000	5000	32
	150 days	5000	5000	5000	32
	200 days	5000	5000	5000	32

[a] The weight-average molecular weight (Mw) and number-average molecular weight (Mn) were determined by GPC.

[b] Melting point (m.p.) was recorded by DSC at 10 °C/min for irradiated samples only.

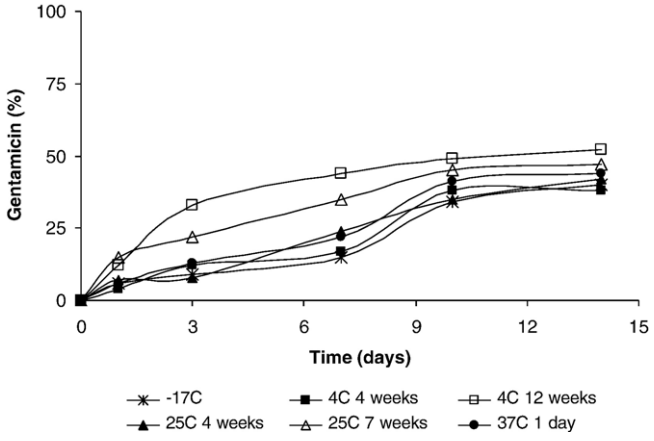

Fig. 6. Gentamicin sulfate release from irradiated P(SA-RA) 3:7 loaded with 20% drug stored at different temperatures. The gentamicin sulfate release and detection were performed under conditions described in Fig. 1. Samples stored under freezing (−17 °C) served as control. The experiment was performed in duplicate and the standard deviation did not exceed ±8%.

the release rate significantly after 12 weeks of study. Samples from 37 °C could be applied for drug release experiments only after one day of incubation, because incubation for longer period resulted in decomposition of the formulation after three days of drug release. The recovery of gentamicin showed presence of 97% of drug in samples that were kept under freezing, 95% of drug in samples that were kept at 4 °C and room temperature and 92% of drug in samples that were kept at 37 °C.

4. Conclusions

Injectable P(SA-RA) immediately forms a semi-solid depot when injected. It is a synthetic polymer made from common fatty acids, which is presumed to be free of immunogenicity and its physicochemical properties are relatively predictable, reproducible and easy to modify. Poly(sebacic-co-ricinoleic-ester-anhydride) 3:7 constantly releases the incorporated gentamicin at an effective concentration, sufficient to eliminate *S. aureus*. The formulations are resistant to sterilization by γ-irradiation and long term storage at −17 °C. Four weeks following implantation of gentamicin containing polymers into rats, there is no inflammation of the tissue surrounding the implant. There was a correlation between the in vitro and the in vivo results: gentamicin was effective in vitro on *S. aureus*, the main bacteria that cause osteomyelitis. The activity over time demonstrates the release of active drug from the device, as expected to be in vivo, a preliminary study reveals a positive effect of gentamicin containing P(SA-RA) on established osteomyelitis in a rat model.

The overall results suggest that the gentamicin containing soft polymers are suitable candidates for the preparation of biodegradable drug-eluting devices for treatment of internal bacterial lesions. They may interfere with bacterial attachment and biofilm production, kill the bacteria, and prevent development of resistance to the antibiotics.

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