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Perivascular graft heparin delivery using biodegradable polymer wraps

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Abstract

Heparin remains the gold-standard inhibitor of the processes involved in the vascular response to injury. Though this compound has profound and wide-reaching effects on vascular cells in culture and animal models, its clinical utility has been questionable at best. It is clear that the mode of heparin delivery is critical to its potential and it may well be that routine forms of administration are insufficient to observe benefit given the heparin's short half-life and complex pharmacokinetics. When ingested orally, heparin is degraded to inactive oligomer fragments while systemic administration is complicated by the need for continuous infusion and the potential for uncontrolled hemorrhage. Thus alternative heparin delivery systems have been proposed to maximize regional effects while limiting systemic toxicity. Yet, as heparin is such a potent antithrombotic compound and since existing local delivery systems lack the ability to precisely regulate release kinetics, even site-specific therapy is prone to bleeding. We now describe the design and development of a novel biodegradable system for the perivascular delivery of heparin to the blood vessel wall with well-defined release kinetics. This system consists of heparin-encapsulated poly(DL lactide-co-glycolide) (PLGA) microspheres sequestered in an alginate gel. Controlled release of heparin from this heterogeneous system could be obtained over a period of 25 days *in vitro*. The experimental variables affecting heparin release from these matrices were investigated. Gel permeation chromatography (GPC) and scanning electron microscopy (SEM) were used to monitor the degradation process and found to correlate well with the release kinetics. Heparin-releasing gels inhibited growth of bovine vascular smooth muscle cells in tissue culture in a dose-dependent manner. Moreover, gel release controlled vascular injury in denuding and interposition vascular graft animal models of disease even when uncontrolled bleeding was evident with standard matrix-type release. This system may therefore provide an effective means of examining the effects of various compounds in the control of smooth muscle cell proliferation in accelerated arteriopathies and also shed light on the biologic nature of these processes. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Local drug delivery; Polymeric drug delivery; Restenosis; Vascular biology

1. Introduction

Heparin is widely used for a variety of important diseases, including deep venous thromboses [1], pulmonary emboli, unstable angina [2,3], myocardial infarction [4], and cardiovascular interventions such as bypass surgery and angioplasty [5]. Recent technological advances have provided exciting alternatives to cardiothoracic surgery for the relief of symptoms arising from

obstructive proliferative vascular disease. Stainless-steel endovascular stents and the like can be inserted within coronary and peripheral arteries within minutes with few failures. Yet, the metals used are highly thrombogenic, and acute occlusion from *in situ* thrombosis can occur in 1–5% of patients. Heparin is used in virtually all of these cases, often for days and as a continuous infusion. Unlike other agents that reduce thrombosis and hemostasis, heparin has profound effects on many of the other events that accompany vascular repair. Heparin markedly and rapidly inhibits DNA and RNA synthesis in cultured vascular smooth muscle cells, limits leukocyte adhesion to injured endothelium, restores endothelial integrity, enhances complement biology, interacts with vascular

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growth factors and a number of steps in their signaling cascade among other effects. Thus, the continuous systemic infusion of heparin is the gold standard by which all agents touted to reduce intimal hyperplasia after vascular interventions are to be evaluated [6–10].

When administered in this fashion, however, complications invariably ensue. Shifts in potassium and bone mineral can be devastating, but pale in comparison to the bleeding potential. In some angioplasty trials evaluating heparin up to 40% of heparinized patients required blood transfusion post-procedure. Heparin is degraded to ineffective fragments after oral ingestion [11], and while subcutaneous administration is efficacious, it is uncomfortable and associated with poor patient compliance [12]. Transdermal and intrapulmonary delivery [13] have so far met with limited success. Methods for local drug infusions can circumvent systemic administration, but may even produce heightened local concentrations. Thus, not unexpectedly increased bleeding can result when local controlled release of heparin is attempted. There is, therefore, an immediate need for precise controlled heparin release from devices that can be implanted in and around arterial structures.

We have previously demonstrated that controlled release of heparin from ethylene-vinyl acetate (EVAc) matrices placed adjacent to balloon-denuded arteries, can deliver the drug at a dose low enough to avoid systemic effects but high enough to provide local protection against vascular disease [14,15]. Although EVAc is biocompatible and readily applicable for the prolonged release of macromolecules [16], the polymer is nondegradable under physiological conditions. Moreover, it is not clear whether the optimum configuration of such a delivery device would require maximum contact or exposure to the blood vessel. It is possible that drug distribution may be more efficient if drug release is circumferentially symmetric. Thus a portion of a blood vessel can be sheathed with a drug-loaded film to maximize delivery of heparin. Hydrophobic materials such as EVAc are noncompliant and nondeformable. Films of these materials have limited direct contact with the blood vessel wall, and the interaction they do have may injure crucial structures such as the vasa vasorum. Silastic and polyethylene sleeves placed around arteries from a number of vascular beds have been reported to cause medial necrosis and loss of planar integrity of the vessel compartments [17,18]. Hydrophilic materials are more tissue compatible as they are more pliable and have a water content closer to physiologic tissues. Yet, their release kinetics is uncontrollable and the bulk of the embedded drug is released within a few hours.

We now report the design and development of a novel biodegradable delivery system for heparin comprising of hydrophobic poly(DL lactide-co-glycolide) microspheres sequestered in a hydrophilic calcium alginate matrix. This system combines the tissue-like property of hydro-

gels with the slow release properties of hydrophobic polymers. The experimental variables affecting heparin release from these matrices were investigated *in vitro*. The antiproliferative effect of heparin released from gels on bovine aortic smooth muscle cells was examined in tissue culture, and the effect on intimal hyperplasia examined in two different animal models of vascular disease.

2. Materials

Poly(D,L-lactic : glycolic) acid 50 : 50 (pLGA, MW 100 000) was obtained from Boehringer Ingelheim, Germany, Poly(D,L-lactic : glycolic) acid 70 : 30 (pLGA, MW 45 000) and poly(vinyl alcohol) (PVA, MW 25 000, 88% hydrolyzed) were obtained from Polysciences Inc., Warrington, PA. Heparin was a kind gift from Glycogen Inc, Alameda, CA. ³H-labeled heparin was obtained from New England Nuclear and mixed with unlabeled heparin (specific activity = 0.76 μ Ci / mg). Sodium alginate was obtained from Sigma Chemical Co., St. Louis, MO. Other materials were reagent grade.

3. Methods

3.1. Heparin-loaded pLGA microspheres

A double emulsion solvent extraction procedure was used to prepare the heparin-loaded pLGA microspheres [19]. 100 μ l of an aqueous solution of heparin (400 mcg/ml) was emulsified in 2 ml of a methylene chloride solution of pLGA (100 mg/ml). The pLGA-heparin mixture was then sonicated at 50 W for 10 s. (ultrasonic probe, Sonics & Materials Inc.): 4 ml of 1% poly(vinyl alcohol) (PVA) was added to this solution and vortexed for 10 s. This was then diluted in 200 ml of 0.3% PVA solution and stirred for 5 min. 400 ml of an aqueous solution of isopropanol (2% by volume) was added to accelerate the extraction of the solvent. After 45 min of stirring, the microspheres were collected by centrifugation (Sorvall Dupont Model RC-5B, 1000 *g* for 10 min), sized using sieves with apertures of 150 μ m, washed three times in distilled water and freeze-dried overnight. Microspheres containing ³H-labeled heparin (specific activity = 0.76 μ Ci/mg) were prepared in a similar fashion. The efficiency of drug encapsulation was evaluated. 5–10 mg of the microspheres containing ³H-labeled heparin were placed in an ampule with 1 ml of 5 N HCl. The ampule was sealed under vacuum and heated overnight at 110°C to degrade the microspheres completely. The solution was then diluted 10-fold and 1 ml of this solution was counted for radioactivity by scintillation counting. Microsphere shape and size were determined by a semi-automated computer-directed video microscopy morphometry system using a light microscope (Nikon). Samples of pLGA microspheres were

suspended in aqueous solutions and placed on glass microscope slides. Images of the microspheres were acquired and analyzed by computer-guided morphometric analysis. Microsphere size distribution in a given field was analyzed according to a reference scale. The molecular weight of pLGA polymers before and after microsphere preparation and during degradation studies was measured on a Perkin–Elmer GPC system with a refractive index detector. Samples of fresh microspheres and microspheres from degradation experiments were freeze-dried, dissolved in chloroform, and filtered through a 0.22 μm filter. The samples were eluted with chloroform through a phenogel column. The molecular weight was determined relative to polystyrene standards (Polysciences, MW range: 1250–233 000).

3.2. Heparin-loaded alginate films

70 mg of microspheres were suspended in 10 ml of 1% alginate solution (viscosity 7000 cP). The solution was poured into a leveled Petri dish sandwiched between two Petri dishes containing liquid nitrogen. Cooling time was approximately 10 min. There was a need for rapid freezing to ensure uniformity within the film, with the microspheres suspended evenly. The film was then lyophilized overnight and weighed. The alginate films were cut into 30 mg pieces and immersed in 3% CaCl_2 for 30 min, and then washed three times for 1 min each, with 1 mM CaCl_2 in saline. The crosslinked alginate films were used immediately for in vitro drug-release experiments and implantation in vivo.

Release kinetics were defined on cross-linked films placed in Teflon vials containing 2 ml of 1 mM CaCl_2 in saline and kept at 37°C in an incubator-shaker. For release from free microspheres, 30 mg of microspheres containing ^3H -labeled heparin were suspended in the same buffer with 0.02% Tween 80, added to prevent the microspheres from clumping. Aliquots were removed periodically, and analyzed for released heparin by β -counting. Free microspheres were centrifuged for 10 min at 2000 rpm prior to aliquot removal. Release experiments were performed independently in triplicate. The morphology and surface appearance of pLGA microspheres and alginate films were examined by scanning electron microscopy (Cambridge Instruments, 250 MK). Samples for SEM were freeze-dried, mounted on metal stubs with double-sided tape, and coated with gold to a thickness of 200–500 Å. The stability of the ^3H heparin was determined by size exclusion chromatography over the entire release period. One ml of buffer was removed at time points corresponding to those in the above drug-release experiments, loaded on Econo-Pac 10 DG columns and eluted with 1 mM CaCl_2 in saline. Thirteen fractions of 1 ml each were collected and the distribution of radioactivity in the fractions was determined by β -counting.

3.3. SMC proliferation in tissue culture

Bovine aortic smooth muscle cells (SMCs) were isolated as described [20] and used at passage 4. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Grand Island, NY), supplemented with penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$) and 10% calf serum (CS, Hyclone, Logan, Utah). Cells were plated at 4×10^3 cells per well in a Costar cluster 12-well plate and allowed to attach for 5–6 h. After attachment, cells were washed with phosphate-buffered saline (PBS) and growth arrested by replacing the medium with 0.05% CS-DMEM. Cells were kept growth-arrested for 72 h and then released from G_0 by the addition of 10% CS. Cell culture inserts (0.4 μm pore size, 12 mm diameter, Costar, Cambridge, MA) containing heparin-releasing alginate films were placed above the SMCs in such a way that the films were completely submerged in media. On day 7, the cells were washed with PBS, released from the dish by trypsinization, and cell number was determined using a Coulter Counter. Each data point is presented as mean \pm SE for two separate experiments performed with duplicate wells at each dose.

3.4. Heparin and intimal hyperplasia in a rat arterial denudation model

Male Sprague-Dawley rats weighing from 300 to 350 g (Charles River breeding Laboratory, Wilmington, MA) were anesthetized with an intraperitoneal injection of ketamine and xylazine. Endothelial denudation of the left common carotid artery in the rats was performed with a 2F balloon catheter [21,22]. A midline incision exposed the distal left common and external carotid arteries. The balloon catheter was introduced into the external carotid artery and passed three times with the balloon distended sufficiently with air to generate slight resistance. Upon removal of the catheter the external carotid artery was ligated. Heparin-releasing alginate films ($n = 12$) and control alginate films ($n = 3$) were then wrapped around endothelial-denuded artery. The strip ends overlapped ensuring complete encircling of the artery. Fascial planes were sutured closed to further immobilize the device.

On the 14th post-operative day, animals were euthanized and perfused clear via the left ventricle with Ringer's lactate solution followed by immersion fixation with Carnoy's fixative (60% methanol, 30% chloroform, 10% glacial acetic acid). The location of the implanted films was noted and the films recovered with the entire length of the intact arteries. The carotid arteries were harvested and cut into five equal segments, three including the hydrogel wrap and one segment each above and below the wraps. Segments were paraffin embedded and 6 μm sections obtained along the length of each segment. After staining with hematoxylin/eosin or verHoeff's elastin stain, the intimal, medial and adventitial areas, the

intima : media area ratio and the percent of luminal occlusion were calculated using computerized digital planimetry with a dedicated video microscope and customized software.

3.5. Heparin and intimal hyperplasia in a rat vein-graft model

Male Sprague-Dawley rats whose weight matched the balloon-denuded group were anesthetized with identical intraperitoneal injections of ketamine and xylazine. The right groin was shaved, prepared aseptically, and opened through a vertical incision. Under microscope magnification (6–18 \times), the common femoral artery (CFA) and epigastric vein (EV) were isolated and the adventitia dissected free from the central portion of the CFA as well as from either end of a 1 cm segment of EV. A constant profunda branch of the CFA was coagulated with diathermy and divided. A double-anastomosis clamp was placed on the CFA and a 3–4 mm segment of artery resected. The ends of the arteries were gently distended with a microforceps and the ends flushed with a sterile solution of Hanks's Hepes buffer. One centimeter of epigastric vein was then harvested, the graft ends dilated with forceps, and the graft gently flushed. The proximal and distal anastomoses were then sutured with 10-0 nylon using an open-interrupted technique. Between 8 and 10 sutures were needed for each anastomosis. Once the anastomoses were completed, the distal clamp was released and the graft back-perfused with blood for approximately 1 min. The proximal clamp was then released and any anastomotic leak was treated with gentle pressure. Heparin-releasing alginate films ($n = 10$) and control-alginate films ($n = 4$) were then wrapped around the vein graft. The wound was then closed with a single layer of running 4-0 nylon sutures. Graft ischemia time, from harvest to restitution of flow, was standardized to 50 min.

4. Results

4.1. Characteristics of microspheres and films

pLGA microspheres containing heparin were prepared in high yields (>90%) by a double emulsion solvent extraction technique [19]. The number average molecular weight of the polymers used in this study were 100 000 for pLGA 50 : 50 and 45 000 for pLGA 70 : 30 as determined by gel permeation chromatography relative to polystyrene standards. The microspheres were spherical in shape and uniform in size with an average microspheres diameter of $104 \pm 6 \mu\text{m}$. The encapsulation efficiency was determined using ^3H -labeled heparin following complete degradation of the microspheres using 5 N HCl and was found to be consistently high

(70–80%). The microspheres were subsequently immobilized in alginate films followed by cross-linking with 3% CaCl_2 . At this concentration, the films obtained were strong and the microspheres were held in place by the Ca^{2+} cross-links.

4.2. In vitro release of heparin

pLGA microspheres demonstrated prolonged controlled heparin release with release kinetics determined by comonomer ratios and molecular weights (Fig. 1). Drug release from the 50 : 50 copolymer spheres was triphasic. An initial burst (18%) was followed by a lag phase of 11 days, and then by near constant release for an additional 69 days. In contrast, the lower molecular weight pLGA 70 : 30 copolymers showed a smaller initial burst (10%) and faster, constant release over the ensuing 2 weeks (Fig. 1). As elements of vascular repair commence early after injury we chose the latter configuration (pLGA 70 : 30) for further investigation. The burst of drug release within the first hours of hydration increased with increasing concentration of heparin relative to pLGA material. A two-fold increase in heparin concentration led to a 2.16-fold increase in the amount of heparin released over the first 24 h.

When the pLGA 70 : 30 microspheres were sequestered in alginate gels, a near constant release was obtained over 25 days, after an initial burst of 13.5% over 24 h (Fig. 2). Investigation of the effect of alginate and calcium chloride concentrations demonstrated that the gels primarily reduced the burst effect without substantially changing the later phases of release. The rate of release decreased with increasing alginate concentration (Fig. 3), but not with increased CaCl_2 concentrations.

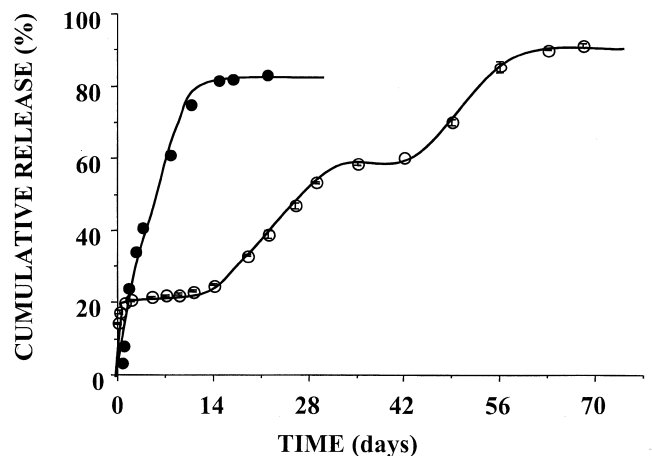


Fig. 1. In vitro cumulative heparin release from pLGA 50 : 50 microspheres ($M_n = 100\,000$, closed circles), and from pLGA 70 : 30 microspheres ($M_n = 45\,000$, closed circles).

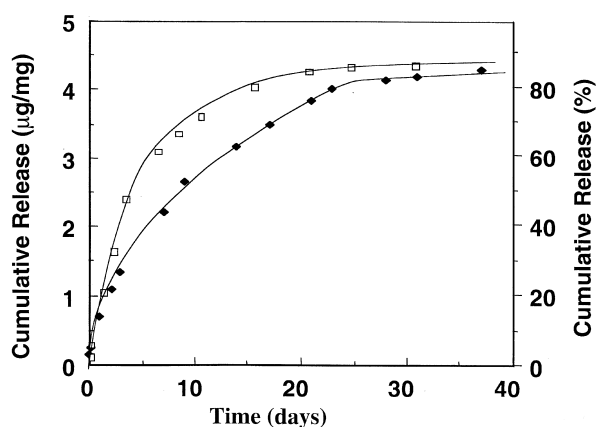


Fig. 2. In vitro cumulative heparin release from pLGA 70:30 microspheres ($M_n = 45\,000$) in isolation (open circles) and encapsulated within alginate films (closed circles).

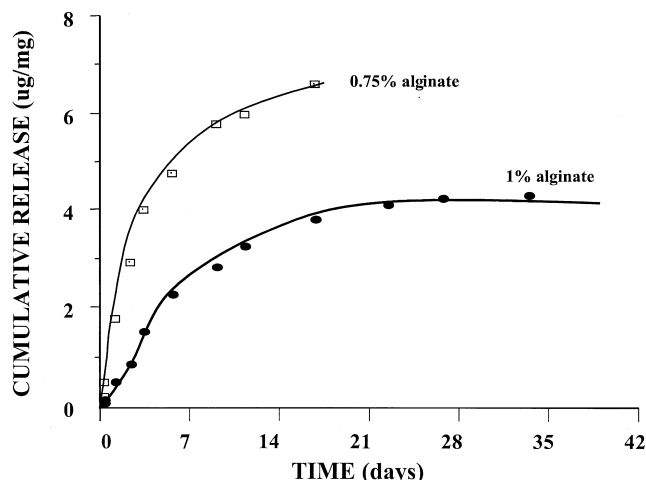


Fig. 3. In vitro cumulative heparin release from pLGA 70:30 microspheres sequestered in an alginate gel: (closed circles) 0.75% alginate, (closed circles) 1% alginate.

4.3. In vitro degradation of pLGA microspheres

The degradation of heparin-loaded pLGA microspheres was characterized in terms of molecular weight distribution using GPC. The molecular weights of pLGA before and after microsphere preparation were identical. Upon degradation, there was a distinct shift in the pLGA peak to higher elution times, corresponding to a decrease in the modal molecular weight of pLGA polymers. The molecular weight distribution throughout the degradation period was unimodal and narrow (1.6 ± 0.09) and degradation followed a linear curve with the number average molecular weight decreasing from 43 000 to 1000 over a period of 8 weeks (Fig. 4). Immediately after

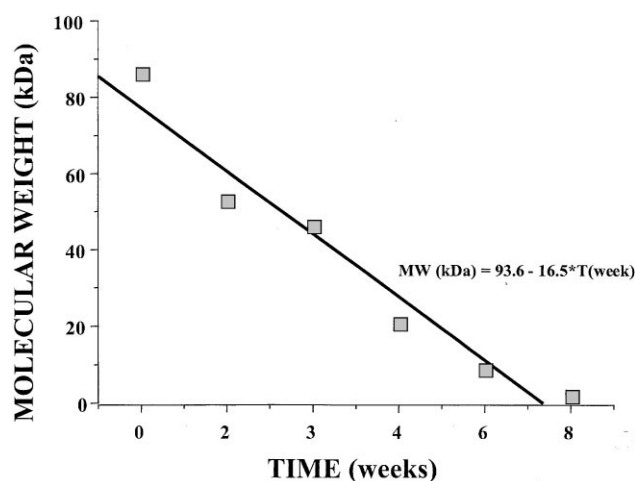


Fig. 4. GPC determined number-average molecular weight as function of time at 37°C represent the linear degradation of polymer microspheres over 8 weeks.

preparation, the microspheres showed an overall intact outer surface on scanning electron microscopy. Small micropores scattered over the entire microsphere surface were noticeable upon close examination. The spherical nature of the microspheres was retained from 0 to 3 weeks. At 4 weeks, the microspheres appeared highly eroded and porous while at 6 weeks, a significant change in morphology was noted with propagation of huge cracks and subsequent microsphere fracture. The microspheres no longer retain their characteristic spherical shape such that by 10 weeks, no semblance of the microspheres remained, and only polymer degradation products were observed.

4.4. Inhibition of SMC proliferation in tissue culture and animal models

The antiproliferative activity of heparin released from alginate-pLGA hybrid matrices was tested on cultured bovine aortic smooth muscle cells. Alginate films containing no heparin were used as controls. At 6 days after exposure, a linear dose-dependent inhibitory effect on cell proliferation was observed. As the dose of heparin administered was doubled from 20 to 40 μg , the percent inhibition relative to controls increased from 17 to 31%, suggesting that controlled release of heparin from alginate-pLGA matrices could be achieved without loss of activity.

When the EVAc matrix heparin releasing devices (33% heparin:polymer) that inhibited intimal hyperplasia after rat carotid arterial denudation by over 70% were placed adjacent to femoral arterial-venous interposition grafts all animals bled to death. In contrast heparin-releasing alginate films containing pLGA microspheres substantially reduced intimal hyperplasia without any

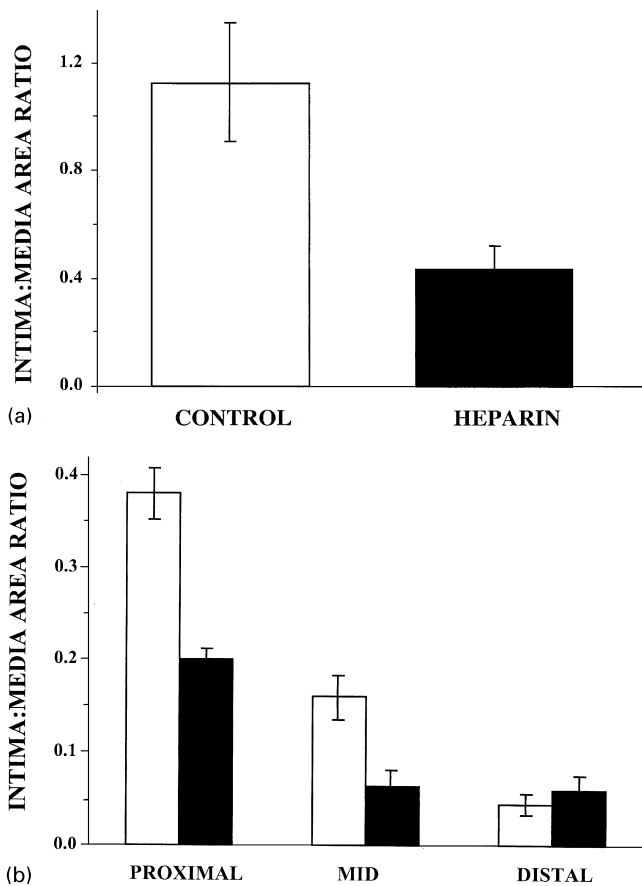


Fig. 5. Heparin released from the composite alginate-microsphere films reduced intimal hyperplasia after balloon denudation of the left common carotid artery 62% compared to controls ($p < 0.005$) (a), and in grafted arteries as well (b).

bleeding complication (Fig. 5). The wraps reduced intimal hyperplasia in the denuded vessels by 62% ($p < 0.005$ compared to controls, Fig. 5a), and in those parts of the grafts with substantial neointima. As is evident from Fig. 5b there was substantial neointima in the proximal and midgrafts and heparin reduced this by 47 and 60%, respectively ($p < 0.01$ and 0.005). There was minimal hyperplasia in the distal segment to start and no difference between the treated and untreated groups.

5. Discussion

Over 1.5 million interposition vascular bypass grafts operations are performed each year. Up to five vessels may be bypassed simultaneously, and each graft has two anastomoses. If a given vascular anastomosis has a 30% chance of *restenosing* within 2–5 yr, then there is virtual certainty that every patient will lose at least one graft in that time. The innovations that might provide for reduction in intimal hyperplasia following angioplasty are

difficult to apply directly to bypass grafting. The biology is far more complex and the surgical field far more demanding. In our study the same hydrophobic ethylene-vinyl acetate copolymer matrices that inhibited intimal hyperplasia so effectively after balloon denudation failed to have demonstrable effect in the bypassed vessel within the same animal species. When the dose of drug delivered was increased all animals bled to death. This likely arose because the suture line of the vascular graft requires some degree of hemostasis for acute healing. High doses of heparin prevent this from occurring. Thus, there must be enough heparin delivered for the duration of vascular vulnerability to intimal hyperplasia, but not too much to prevent all forms of clotting, especially at the suture line immediately post-procedure.

A heterogeneous device was engineered to provide controlled release of heparin. The drug was first encapsulated in degradable pLGA microspheres that were subsequently embedded in a crosslinked alginate gel. pLGA copolymers are biocompatible, biodegradable, and easily processible and can produce matrices whose performance characteristics cover a wide range dictated by the comonomer ratio and polymer molecular weight [23–26]. pLGA microspheres have been extensively investigated for the controlled release of pharmaceuticals [27–29]. In our study pLGA microspheres were prepared by a solvent extraction using a water-in-oil-in-water emulsion [19] procedure developed to encapsulate hydrophilic drugs such as large molecular weight proteins and peptides [27]. The resulting microspheres were spherical, with an average diameter of $104 \pm 6 \mu\text{m}$. Decreasing the polymer concentration created smaller microspheres.

Heparin release depended upon the comonomer ratios and ratio of drug to polymer. Release kinetics from pLGA 50 : 50 ($M_n = 100\,000$, Fig. 1) was triphasic with an initial burst followed by two distinct first-order release phases. The initial burst likely stemmed from release of drug molecules close to the microsphere surface. The subsequent induction phase of minimal release ($\sim 3\%$ over 11 days) is characteristic of matrices of high molecular weight polymers which require a defined degradation time before erosion of the polymer matrix becomes sufficient to release macromolecules [30]. This pattern of release is not acceptable for application of heparin to vascular injury. Because heparin is so soluble, rapidly degraded and has such a short half-life, 7 min when infused intravenously at clinical doses, it must be administered continuously to exert an effect in proliferative vascular disease. The intimal smooth muscle cell proliferation that follows controlled denudation of the arterial endothelium in animal models of vascular injury is maximal in extent at 10–14 days after injury [6,21,22,31,32]. Thus, the effect of heparin is only observed when the drug is infused for at least this period of time. Although the pLGA 50 : 50 microspheres can provide desirable release

kinetics after 11 days, the prolonged lag phase to their release precludes use for these vascular applications. Other pLGA compositions can provide decreased lag phase and faster drug release kinetics [27]. Heparin release from pLGA 70 : 30 ($M_n = 45\,000$, Fig. 1) was characterized by an initial burst, followed by fairly constant release over 2 weeks. A lag phase was not observed with this composition suggesting that the lower molecular weight results in drug release mainly through matrix erosion. Because this kinetic pattern was deemed more consistent with the use of heparin in vascular repair we used pLGA 70 : 30 ($M_n = 45\,000$) for all subsequent experiments.

Device morphology was determined by initial drug and polymer concentrations and this in turn dictated release kinetics. Doubling the initial drug concentration resulted in a 2.16-fold increase in the initial burst, most likely attributed to the higher concentration of drug near the microsphere surface and increased amount of drug available for immediate release. Similarly drug release within the first hours of hydration was increased at lower polymer concentrations presumably because the microsphere size diminished and the device surface area to volume ratio rose. Immobilization of the microspheres within alginate films further reduced the burst effect without substantially altering the prolonged phase of release. After an initial burst of 13.5% near constant release was observed over 25 days (Fig. 2). The effects of alginate and calcium chloride concentrations on drug release were evaluated. Decreasing the alginate concentration from 1 to 0.75% resulted in faster release (Fig. 3). This may be secondary to a decrease in the degree of cross-linking that results from fewer carboxylic acid groups available for reaction with Ca^{2+} ions. In addition, the concentration of alginic acid affected the final film size and thickness, with the less-viscous solutions flowing faster onto the Petri dish during the freezing phase and resulting in thinner films. A 1.5% calcium chloride concentration was found to be optimal and increasing the Ca^{2+} concentration had no effect on the release.

Degradation of heparin-loaded pLGA microspheres *in vitro*, in buffer at pH 7.4 was monitored by GPC and the molecular weight was found to decrease linearly with time (Fig. 4). Degradation under these conditions takes place through hydrolytic cleavage of the ester groups. The molecular weight distribution showed a unimodal pattern suggesting that water penetrated throughout the matrix resulting in homogenous degradation. This has been observed previously using pLGA 75 : 25 of low molecular weights [27]. The absence of oligomeric peaks at the low molecular weight tail of the chromatogram suggests that pLGA degrades directly into water-soluble fragments [27]. Morphological scanning electron microscopy studies showed that pLGA microspheres retained their overall intact shape up to 3 weeks with progressive formation of pores all over the matrix. At

4 weeks, the microspheres appeared highly eroded and porous while at 6 weeks, a significant change in morphology was noted with propagation of huge cracks and subsequent microsphere fracture. The microspheres no longer retain their characteristic spherical shape such that by 10 weeks, no semblance of the microspheres remained, and only polymer degradation products were observed.

Alginate was used as the carrier film as this hydrophilic material has served well for a variety of biomedical applications, including dental impression material and wound dressings [33–38], drug delivery and even embedding of liposomes Weiner, 1985 [17]. More recently, alginates have been used to encapsulate living cells including islet cells of the pancreas and hepatocytes of the liver [39,40]. The alginate films remained intact in saline containing 1 mM CaCl_2 for prolonged periods of time. These films are however expected to degrade *in vivo*. Since calcium cross-linking of alginic acid is stabilized at acidic pH [41], heparin and pLGA degradation products (both of which are acidic) may stabilize alginate films *in vivo*. Further experiments need to be performed to confirm this and to determine the rate of degradation of the alginate.

Heparin released from alginate–pLGA composite matrices retained its ability to inhibit bovine aortic smooth muscle cells in culture and in animal models of vascular disease (Fig. 5). As the dose of heparin administered was increased, a linear decrease in cultured smooth muscle cell number was obtained. Control alginate films containing no heparin showed no effect on cell number. Heparin can therefore be encapsulated and controlled released from degradable alginate–pLGA films without loss of anti-proliferative activity. The effects on animal models of vascular disease were interesting. The dosing of heparin in grafted vessels, in contrast to denuded arteries, is far more delicate. When too much heparin is provided, as is the case with the EVAc matrices uncontrolled bleeding was observed and when heparin was delivered in a sustained fashion intimal hyperplasia was significantly reduced but the acute thrombotic events that limit the patency of these grafts was not entirely eliminated. Systems that offer the potential for precise control of drug release and site of administration will continue to be developed and provide greatest control of intimal hyperplasia. Their use in combination with novel compounds, including for example, chemically modified non-anti-coagulant heparin makes a most powerful potential therapeutic modality.

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