Glow Discharge Plasma Deposition (GDPD) Technique for the Local Controlled Delivery of Hirudin from Biomaterials

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Received October 24, 1997; accepted February 20, 1998

Purpose. Biomaterials which release locally high concentrations of antithrombotic agents should lessen the thrombogenicity of the materials. To evaluate this approach, we prepared novel polyurethane matrices loaded with hirudin and coated them with 2-hydroxyethyl methacrylate (HEMA) by glow discharge plasma deposition (GDPD) to reduce the release rate.

Methods. Polyurethane (BioSpan®) matrices containing hirudin and pore former (d-mannitol or BSA) were prepared by the solvent casting method. HEMA plasma deposition was then applied using GDPD technique to create a diffusional barrier film on the surface of the matrices. The effect of pore former and HEMA plasma coating on the release of hirudin was systematically investigated. Surface properties of matrices was also studied using Scanning Electron Microscopy (SEM) and Electron Spectroscopy for Chemical Analysis (ESCA).

Results. The release of hirudin from BioSpan® matrix could be controlled by changing the weight fraction and particle size of pore former. HEMA plasma treatment of matrices produced a thin, highly cross-linked film on the surface. The initial burst and subsequent release of hirudin was significantly reduced after HEMA plasma coating, which suggested that the plasma disposition acted as a diffusional barrier and limited the release of hirudin incorporated in the polyurethane matrix.

Conclusions. The plasma coating served as a diffusional barrier, and could work to control the release kinetics of hirudin by changing the various plasma coating conditions. Local delivery of hirudin using these biomaterials at the site of cardiovascular diseases can have the advantage of regional high levels of hirudin, as well as lowering systemic hirudin exposure, thereby minimizing the possibility of side effects.

KEY WORDS: local drug delivery; hirudin; glow discharge plasma deposition (GDPD); biomaterial; electron spectroscopy for chemcal analysis (ESCA).

INTRODUCTION

Hirudin is a potent anti-thrombotic peptide extracted from leeches (*Hirudo medicinalis*) that can be used for prophylaxis and treatment of various cardiovascular disorders, such as restenosis (1). Local delivery of hirudin from a biomaterial can offer the advantages of regional high levels of hirudin into the near wall zone, thus preventing blood clotting on the biomaterial. In addition, implants of this type may be useful for anti-coagulant delivery at the site of cardiovascular diseases (2,3). This report presents a study directed towards the development of new biomaterials that can release hirudin at a desired therapeutic rate for an extended period of time. The desired release rate of hirudin from the polymer matrix is to be achieved by depositing a rate-controlling barrier using the radio-frequency (RF) glow discharge plasma deposition (GDPD) technique.

GDPD is a novel technology that has been used to obtain surfaces that are resistant to protein adsorption and cellular attachment (4), or to create a diffusional barrier film on a polymer matrix loaded with biologically active agent (5). In this report, hirudin was incorporated into a polyurethane matrix using either d-mannitol or bovine serum albumin (BSA) as a pore former. GDPD of an organic monomer, 2-hydroxyethyl methacrylate (HEMA), was then applied on the polymer matrix. The effects of HEMA coating on the release rate of hirudin and on the surface morphology of the matrix were studied.

EXPERIMENTAL METHODS

Materials

Native hirudin from leeches (2000 units, lyophilized), d-mannitol, BSA and gentamicin sulfate were obtained from Sigma Chemical Co. (St. Louis, MO). Segmented polyurethane (BioSpan®) was obtained as a solution in dimethylacetamide (DMAc) from the Polymer Technology Group (Emeryville, CA). Ophthalmic-grade HEMA was obtained from Polysciences, Inc. (Warrington, PA). All other chemicals were reagent grade and used as purchased.

Preparation of Polymer Matrix

The mixed aqueous solution of hirudin and pore former (d-mannitol or BSA) was lyophilized, and sieved to fractionate various particle sizes (< 63, 63–90, 90–125 μm). This powder with specific particle sizes was mixed with a 15% BioSpan® in DMAc to make various weight fraction of pore former. This mixture was then cast in a PTFE tray, and dried in a 60°C oven for 24 hours. The matrices were vacuum-dried at room temperature for another 48 hours. Final matrices with thickness of 1.0–1.3 mm were then trimmed to 1 cm^2, and stored at 4°C until used.

HEMA Plasma Deposition

The capacitatively-coupled RF plasma deposition reactor has been previously described (6). Matrices were etched briefly in an argon plasma (40 Watts, 150 mTorr, 5 min) immediately before HEMA deposition. HEMA monomer was heated to 60°C to increase its vapor pressure, and flow into the reactor was controlled by a Teflon stopcock. The coating was applied at various pressures, RF powers, and duration of the plasma deposition to investigate the effect of these parameters on the surface morphology of the coating and release rate of hirudin. The reaction was quenched with HEMA vapor for 10 minutes following plasma treatment.

Hirudin Release Study

The matrices were placed in vials containing 3–10 ml of 0.02M Tris-HCl buffer (pH 7.4) with 0.01% (w/v) gentamicin,

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0724-8741/98/0500-0783$15.00/0

and placed in a shaking water bath at 37°C. Aliquots of medium were taken at predetermined time intervals for 24 hours, and replaced with the same amount of fresh buffer. The amount of hirudin released was expressed as the percent of the starting amount in the matrix. Triplicate experiments were conducted for each study.

Analysis of Hirudin

Since hirudin is an inhibitor of thrombin, hirudin concentration was analyzed by a modified chromogenic assay for thrombin (7). Briefly, 200 μl of each sample was placed in a 96-well microplate, and 50 μl thrombin (5 unit/ml) solution was added. After an incubation time of 1 minute at room temperature, 50 μl Chromozym TH (1.9 mM, Sigma Chemical Co., St. Louis, MO) was added to react with residual thrombin. The change of absorbance as a function of time (ΔA/min) produced by 4-nitroaniline during the reaction of Chromzym TH and the residual thrombin was measured at 405nm for 3 minutes using a microplate reader (Vmax Kinetic Microplate Reader, Molecular Devices, Menlo Park, CA). The difference between thrombin base value and the sample $[(\Delta A/min)_{thrombin} - (\Delta A/min)_{sample}]$ was plotted as the function of hirudin concentration, and was linear from 0.1 to 3 μg hirudin/ml in sample.

Surface Morphology of Matrix

Scanning Electron Microscopy (SEM) was performed on Au/Pd sputter coated samples using a JEOL 35C SEM (JEOL, Japan). X-ray photoelectron spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA), was performed on SSX-100 and S-Probe spectrometers (Surface Science Instruments, Mountain View, CA).

RESULTS

Effect of Pore Former on the Release of Hirudin

Figure 1 shows the effect of d-mannitol amount, used as a pore former, on the release of hirudin from the BioSpan® matrix. Increase in the weight fraction of mannitol enhanced the release rate and the total amount of hirudin released from the matrix, but did not affect the time to reach the plateau level (about 10 hours). Increase in the particle size of pore former also enhanced the release rate and the total amount of hirudin released from the matrix containing 40% (w/w) pore former (Figure 2). It is interesting to note that more hirudin was released when d-mannitol was used as a pore former (Figure 2A) than BSA (Figure 2B), which might be related to the water solubility of pore former and requires further investigation.

Surface Morphology of HEMA Plasma Deposition

SEM studies revealed that HEMA plasma treatment of polymer produced a smooth overlaying film on the top of the matrices (data not shown). The structure of this film could be manipulated by changing deposition parameters to form films with compositions ranging from a lightly cross-linked network to a dense, highly cross-linked glassy polymer (5).

ESCA study shows that more fragmentation of HEMA monomer was observed when the coating process was conducted with higher power (Figure 3). The carbon/oxygen ratio of plasma-polymerized HEMA was also higher than that of the intact monomer (data not shown), which suggests the loss of oxygen and fragmentation of the starting HEMA monomer, and is consistent with increased cross-linking in the film.

Effect of Plasma Deposition on the Release of Hirudin

As shown in Figure 4, the release of hirudin was significantly reduced after HEMA plasma treatment on the matrix, which suggests that HEMA coating worked as a diffusional

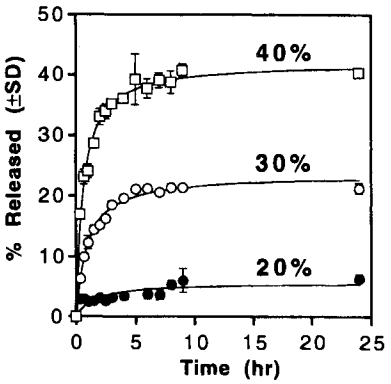

Fig. 1. The effect of the weight fraction of d-mannitol (particle size; <90 μm) on the release of hirudin from BioSpan® matrix; □ 40%, ○ 30% ● 20% (w/w) mannitol in BioSpan®.

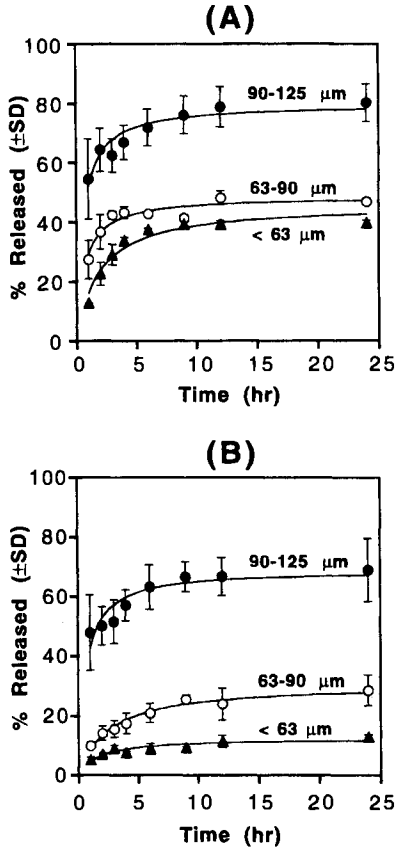

Fig. 2. The effect of the particle size of pore former on the release of hirudin from BioSpan® matrix when 40% (w/w) of (A) d-mannitol or (B) BSA was used as a pore former.

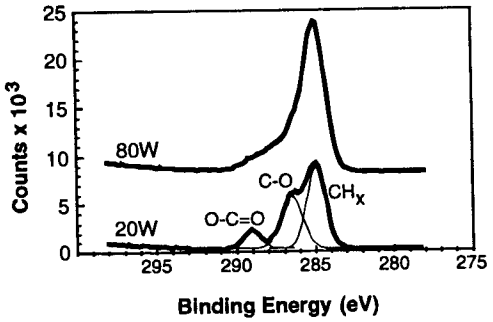

Fig. 3. Comparison of ESCA study of HEMA plasma deposited on the surface of BioSpan® matrix for 20 min with low (20Watts) or high (80Watts) radio-frequency power at 200 m Torr.

barrier. This result is consistent with our previous report that the release of echistatin also significantly reduced after HEMA plasma treatment on the matrix to a degree which depended upon deposition parameters (5). SEM of cross-section of matrix showed the typical channel formation after 24 hours release study (Figure 5).

DISCUSSION

Plasma polymerization by GDPD is an useful technique for modifying surfaces and depositing thin films (6). Plasma-generated films have been suggested as corrosion protective coatings, photoconductive films, semipermeable membranes, and electrically insulating films, to name a few of the potential applications. In the biomedical area, GDPD technique has been used to modify the surface to enhance the blood compatibility of biomaterials by changing the wettability and biologic reactivity of the surface (6). Thus, this technique is becoming increasingly popular in biomedical device fabrication (8). In this study, HEMA plasma deposition was applied on the surface of polyurethane matrix containing hirudin, and the feasibility of controlling the release kinetics of hirudin by changing the GDPD deposition conditions was systematically investigated.

Before HEMA plasma deposition on the BioSpan® matrix, the effect of particle size and loading of pore former on the release kinetics was studied to maximize the release rate of hirudin from the matrix. An early study demonstrated that particle size and loading of pore former markedly affected the release kinetics of the macromolecular polymeric delivery system (9). As shown in Figure 1 and 2, increase in the weight fraction

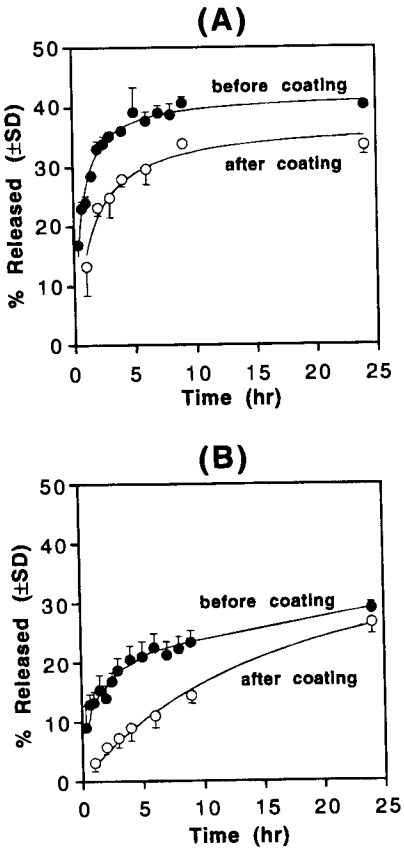

Fig. 4. The effect of HEMA plasma coating (40Watts/150mTorr/10min) on the release of hirudin from BioSpan® matrix when 40% (w/w) of (A) d-mannitol or (B) BSA was used as pore former (particle size; <90 μm); ○ after coating, ● before coating.

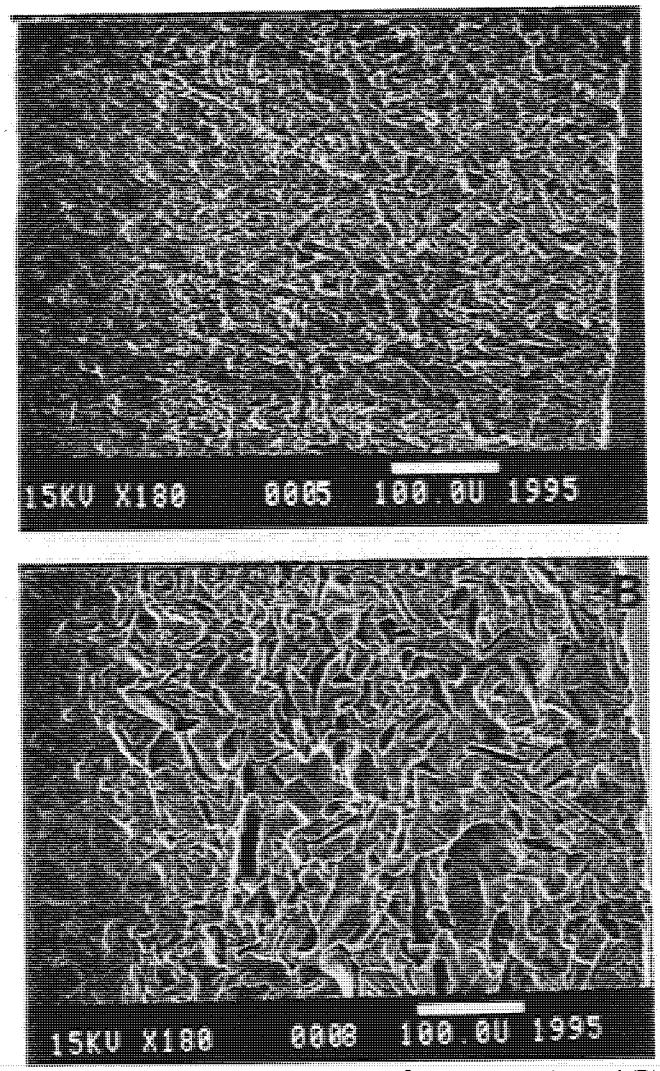

Fig. 5. SEM of cross-section of BioSpan® matrix (A) before and (B) after hirudin release study for 24 hours. Matrix contains 40% (w/w) BSA (particle size; <90 μm) as a pore former, and HEMA plasma coated for 10 min at 150 mTorr with 40 Watts RF power.

and the particle size of pore former enhanced the release rate of hirudin from the matrix. Enhanced release rate by increasing particle size may result from the formation of larger channels or pores in the polymer matrix. Similarly, increased loadings may provide simpler pathways (lower tortuosity) and greater porosity for diffusion, both of which facilitate the movement of water into, and protein out of, the matrix.

Since the release of hirudin from the matrix increased with higher loading and larger particle size of pore former, BioSpan® matrix with 40% pore former (<90μm particle size) was used for HEMA plasma coating and further studies. As shown in Figure 3, more fragmentation of HEMA monomer was observed when higher power of GDPD was applied, which suggests higher cross-linking in the film. Theoretically, higher RF power and lower pressure will cause denser cross-linkage, and longer duration of plasma deposition will result in thicker coating (6). Thus, it would be possible to control the release rate of drug in the polymer matrix by changing the coating conditions, such as pressure, RF power, and duration of coating. The release rate of hirudin was significantly reduced after HEMA plasma coating (Figure 4). In SEM study with BioSpan® matrices, typical channel formation was observed after 24 hours of release study, which suggested that pore former was leached out together with hirudin during the release study (Figure 5). Although dramatic reduction of release rate was not observed with these conditions (40Watts, 150 mTorr, 10min), it is clear that HEMA plasma deposition worked as a diffusional barrier. Therefore, these results demonstrated the feasibility of using HEMA plasma coating by GDPD technique for controlling the release rate of hirudin from the matrix. Further research is under way in this laboratory for better control of release rate by improving the GDPD plasma coating technique.

Despite the use of aspirin and intravenous heparin during the procedure, there is a 6 to 8% incidence of acute coronary occlusion, occurring either intraprocedurally or within 24 hours after balloon dilation (10). Also, 30 to 50% of patients with successfully treated coronary lesion will develop recurrent stenosis (restenosis) over the ensuring 3–6 months (11). Systemic administration of pharmaceuticals to inhibit restenosis often resulted in lower arterial wall drug concentrations than would be achieved with regional administration via a pharmaceutic implant (12). Considering the relatively small amount of space on the stent structure and implatable device for cardiovascular disorders, a compound with strong activity, such as hirudin, should be incorporated in polymers or coated stents. On a weight basis, hirudin is a three to five times stronger anticoagulant than heparin (13). Moreover, since hirudin selectively and irreversibly inhibits thrombin, this new antithrombotic peptide offers promise in the treatment of venous and arterial disorders, including deep venous thrombosis, restenosis after coronary angioplasty, and the management of acute ischemic syndromes related to coronary artery disease (14). Prolonged local drug delivery using drug-containing pharmaceutic stents or related implants seems to be a more effective approach to prevent cardiovascular disorder, while avoiding systemic side effects.

The blood compatibility of any material will be crucial in designing implantable polymeric systems. Novel biomaterials designed in this study will release a controlled dose of hirudin into the near-wall region, thus should be more blood compatible because they inhibit coagulation in the region it is likely to occur, namely at the blood-polymer interface.

CONCLUSIONS

The release rate and the total amount of hirudin released from BioSpan® matrix could be controlled by changing the weight fraction and particle size of pore former. The plasma treatment of hirudin-BioSpan® matrices produced a thin, highly cross-linked film which limits the release of hirudin incorporated in a polyurethane matrix. The plasma coating served as a diffusional barrier, and should also work to control the release kinetics of hirudin from the matrix by changing the various coating conditions, such as pressure, RF power, and duration of the plasma deposition. Thus, this kind of new process can be used for the local controlled delivery of hirudin to treat various cardiovascular disorders, and also for enhancing the blood compatibility of biomaterials.

ACKNOWLEDGMENTS

The authors would like to gratefully acknowledge the assistance of Drs. V. Labhasetwar and R. Levy (University of Michigan) in the initial phase of this project. Financial support for this work was provided by a grant from the National Institutes of Health (Grant No. HL 51260).

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