Development of a novel matrix for the local controlled delivery of hirudin, an anti-thrombotic peptide

김 대 덕*

Center for Bioengineering and Department of Chemical Engineering
University of Washington, Seattle, WA 98195, USA

Introduction

Hirudin is a strong anti-thrombotic peptide from leeches (*Hirudo medicinalis*), and 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]. This report presents the 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 glow discharge plasma deposition (GDPD) technique.

GDPD is a novel technology used to obtain surfaces that are resistant to protein adsorption and cellular attachment [3], or to create a diffusional barrier film on a polymer matrix loaded with biologically active agent [4]. 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-hydroxyethylmethacrylate (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 matrix were studied.

Experimental Methods

Materials

Naive hirudin from leeches, 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 regent grade and used as purchased.

Preparation of polymer matrix

The mixed protein solution of hirudin and pore former (d-mannitol or BSA) was

lyophilized, sieved to fractionate various particle sizes (< 63, 63-90, 90-125 mm). 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 Teflon tray, and dried in a 60 °C oven for 24 hours. Matrices were trimmed to 1 cm^2, then stored at 4 °C until used.

HEMA plasma deposition

The capacitatively coupled RF plasma deposition reactor has been previously described [5]. 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 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 release rate. 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) containing 0.01% (w/v) gentamicin 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.

Surface morphology of matrix

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

Results and Discussion

Effect of Pore Former on the Release of Hirudin

Figure 1 shows the effect of d-mannitol, 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 than BSA, which might be related with the water solubility of pore former and requires further investigation.

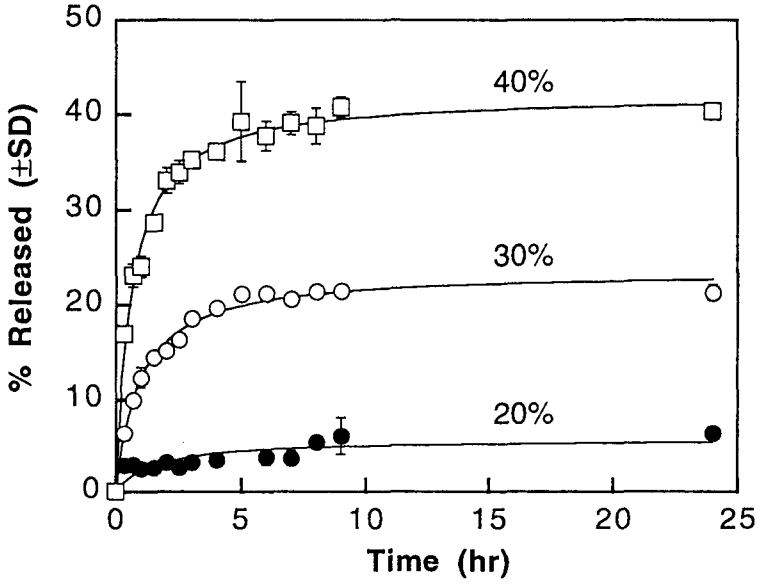

Figure 1. The effect of the weight fraction of d-mannitol (particle size; <90 μm) on the release of hirudin from BioSpan matrix.

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 crosslinked network to a dense, highly crosslinked glassy polymer.

ESCA study shows that more fragmentation of HEMA was observed when coating 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 consistent with increased crosslinking in the film.

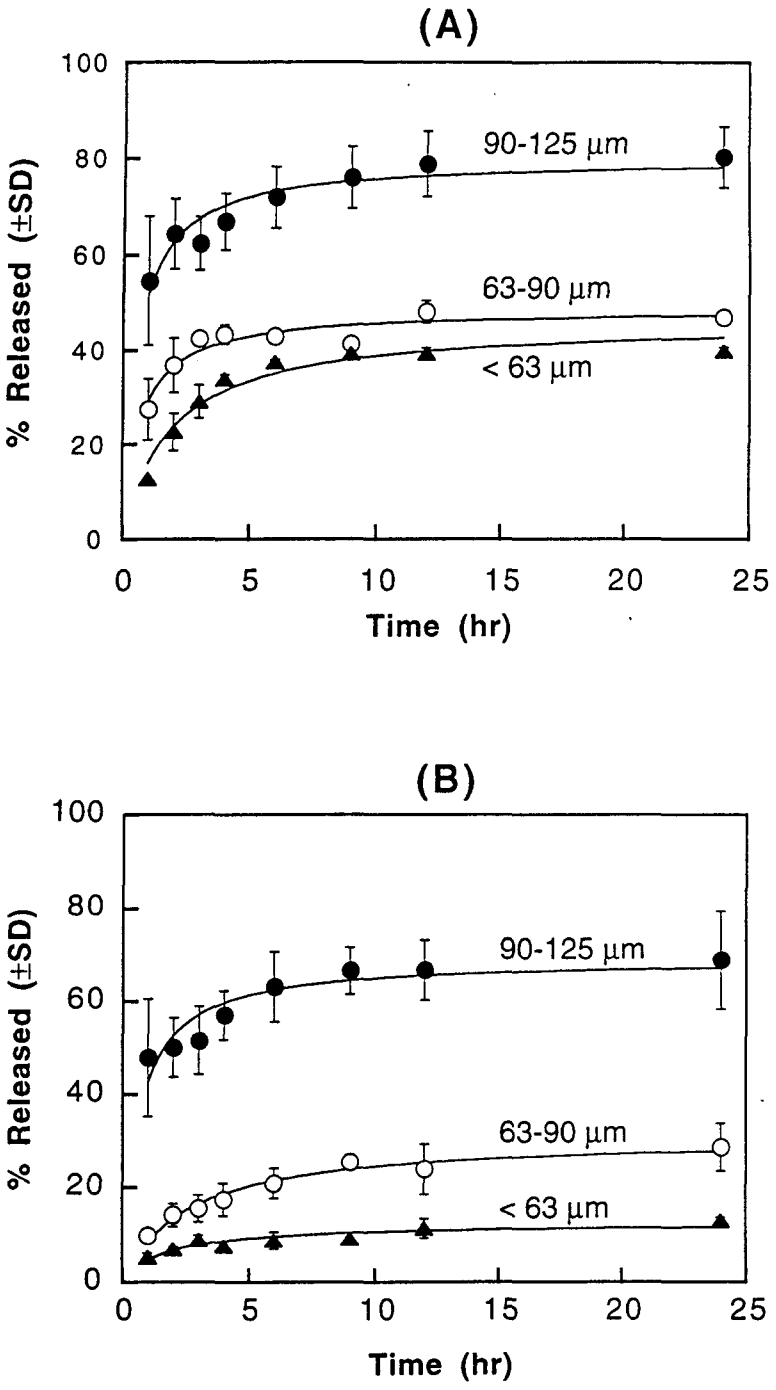

Figure 2. The effect of the particle size of pore former on the release of hirudin from BioSpan matrix when 40% (w/w) of (A) d-mannitol or (B) BSA was used as a pore former.

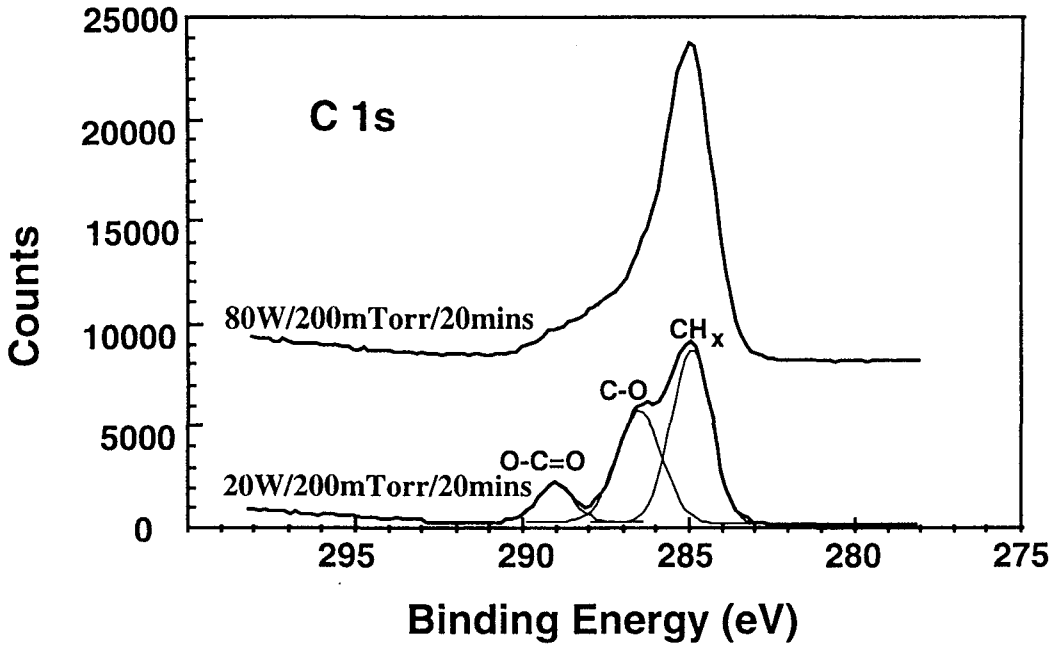

Figure 3. Comparison of ESCA study of HEMA plasma deposited on the surface of BioSpan matrix for 20 min with low (20W) or high (80W) radio-frequency power at 200 mTorr.

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. Previously, it was reported that the release of echistatin was also significantly reduced after HEMA plasma treatment on the matrix to a degree which depended upon deposition parameters [4].

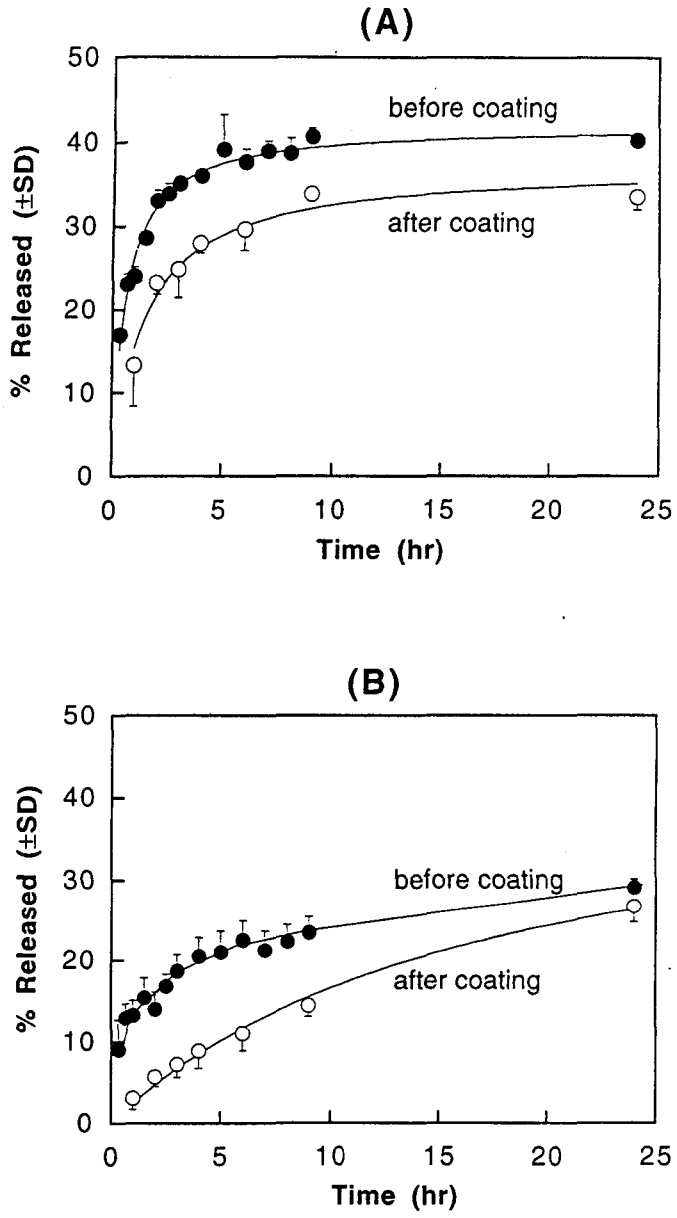

Figure 4. The effect of HEMA plasma coating (40W/150mTorr/10min) on the release of hirudin from BioSpan matrix when 40% (w/w) of (A) d-mannitol or (B) BSA was used as pore former (particle size; 90 m).

Conclusion

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 crosslinked 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, radio-frequency power, and duration of the plasma deposition.

References

[1] Markwardt, Haemostas., 21 (suppl 1), 11 (1991).

[2] Levy et al, J. Controlled Release, 36, 137 (1995).

[3] Lopez et al, J. Biomed. Mater. Res., 26, 415 (1992).

[4] Takeno et al, Proc. Intern. Symp. Control. Rel. Bioact. Mater., 22, 147 (1995).

[5] Haque & Ratner, J. Appl. Polym. Sci., 32, 4369 (1986).

Acknowledgements

The author would like to gratefully acknowledge the assistance of 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 Institute of Health (NIH, # HL 51260).

* 현재: 부산대학교 약학대학 약학과