SELF-ASSEMBLED SMART NANOCOATING AND COLLOIDAL MATERIALS FOR BIOMEDICAL APPLICATION

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Introduction

Creation of novel and smart materials is one of the driving forces for the evolution of coating and encapsulation technologies. A challenge of materials engineering is the controlled assembly of purposefully designed molecules, or ensembles of molecules, into nano- and micrometer-scale structures to provide increasingly precise control at molecular levels, over the structure, properties and function of materials. Layer-by-layer (LbL) self-assembly technique provides opportunities to create such advanced materials since its universal character does not impose any restriction on the type of polyelectrolyte. New functionalities were imposed on multilayered assemblies by introducing novel structures and/or incorporating functional materials. Liposomal drug formulations offer the advantage of reducing drug toxicities, but the insufficient morphological stability of traditional liposomes may limit their applications. Hence, highly stable anticoagulant coating was constructed by LbL self-assembly of heparin and diazoresin incombination of in situ photocrosslinking. A novel super-stable and freestanding hybrid nanoliposome (partially ceramic- or silica-coated liposome) was fabricated using self-assembly and sol gel strategy to overcome general problems associated with current liposome technology.

Experimental

Materials

An alanine-based cationic lipid having a $(CH_3CH_2O)_3Si$- group on the quaternized ammonium nitrogen was synthesized according to the reported method [1]. Heparin (Hep, MW 13 500-15 000) was obtained from CalBio Chem. Span 60 and Tween 80 were purchased from Sigma.

Methods

7 ml of aqueous solution is added to 3.4 mg of cerasome forming lipid. The suspension is then agitated using vortex mixer, followed by sonication with a probe-type sonicator at 30 W for 3 min in the pulse mode at >23ºC.

Highly stable anticoagulant coating between a material surface and blood using a defined multilayer assembly of Alg and Hep was constructed by Layer-by-layer (LbL) technique in combination with photoreaction in situ.

PFC-containing microbubbles (ST68-PFC) were prepared by sonication based on surfactants (Span 60 and Tween 80) [2]. Subsequently, the resulted ST68-PFC microbubbles were coated using oppositely charged polyelectrolytes by microbubble-templated LbL self-assembly technique via electrostatic interaction and its effects were evaluated in contrast imaging on normal rabbit's liver parenchyma.

Results and Discussion

A novel stabilized hemocompatible coating was engineered by consecutive alternating adsorption of heparin (Hep) and a photosensitive cross-linker, *p*-diazonium diphenyl amine polymer (PA) onto a biomedical surface via electrostatic interaction in combination with photoreaction in situ. Photo-cross-linking resulted in higher stability. Chromogenic assays for heparin activity proved definitively that anticoagulation activity really comes from surface-bound heparin in multilayer film, not from solution-phase free heparin that has leaked from multilayer film (Fig. 1). The uncoated and $(PA/Hep)_8$-coated biomedical devices showed relatively strong platelet adhesion. On the contrary, no sign of any cellular matter was seen on the $(PA/Alg/PA/Hep)_4$ surface. It is believed that the

phenomenon of interlayer diffusion resulted in blended structures, hence, the enhanced wettability and antifouling properties after the incorporation of alginate layers.

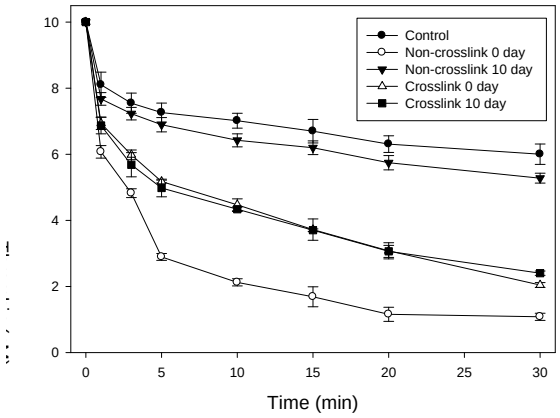

Fig. 1 Thrombin inactivation by AT III in the presence of multilayer of $(PA/Hep)_5$ before and after photocrosslinking.

The surface of the resulting ST68-PFC microbubbles was modified by microbubble-templated LbL self-assembly technique via electrostatic interaction. Confocal microscopic images indicate that the microbubbles are polydisperse, they are sphere and no aggregation is observed.

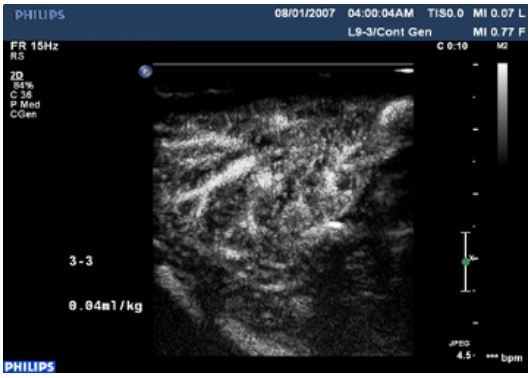

Fig. 2 2D ultrasonography of rabbit liver postinjection of ST68-PFC microbubbles coated with poly (allylamine) hydrochloride and poly (sodium styrene sulfonate).

In vivo measurement results showed that the LbL coated microbubbles enhanced effectively ultrasonic imaging of vessel and parenchyma of the liver (Fig. 2). The life sign of the rabbit is smooth in the whole contrast process. There is no arrhythmia and untoward effect happened.

An alanine-based cationic lipid having a $(CH_3CH_2O)_3SiCH_2CH_2CH_2$ group on the quaternized ammonium nitrogen was synthesized according to the reported method. Then, the obtained lipid was dispersed in water by agitation and subsequent ultrasonication, resulting in the formation of a liposome which self-rigidifies via in situ sol-gel processes on the surface. The formation of siloxane bonds on the cerasome surface was proved by Fourier transform infrared(FT-IR) spectroscopy. The AFM micrograph (Fig. 3) showed the presence of cerasomes with an average diameter of ca. 60 nm. The resulting cerasome (partially ceramic- or silica-coated liposome) could maintain its initial spherical structure and no disruption was observed even in dry environment. The high stability results from the siloxane network formed on the vesicular surface and the hydrogen-belt domain formed among the connecter units in the liposomal membrane.

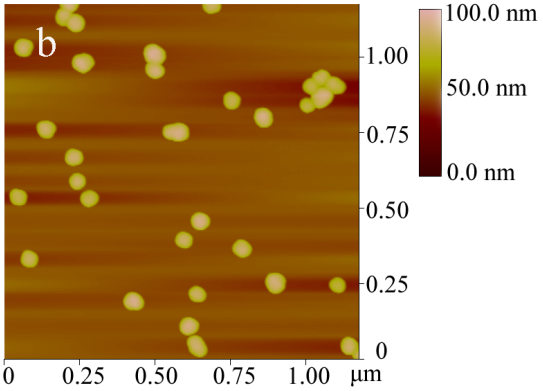

Fig. 3 AFM images of cerasomes.

Conclusion

There is greater advantage in utilizing cross-linked alginate/heparin surfaces rather than merely the heparin surface for improving blood-compatible devices. The multilayer coated microbubble enhances greatly the imaging of rabbit's liver parenchyma effectively. The stability of the cerasome was much superior than that of conventional liposomes.

References

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2. Basude R., Duckworth J. W., Wheatley M. A. Influence of envoromental conditions on a new surfactant-based contrast agent: ST68. Ultrasound in Med & Biol, 26 (2000) 621-628.