Electrophoretic Deposition of Hyaluronic Acid and Composite Films for Biomedical Applications

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Hyaluronic acid (HYH) is a natural biopolymer, which has tremendous potential for various biomedical applications. Electrophoretic deposition (EPD) methods have been developed for the fabrication of HYH films and composites. New methods for the immobilization of drugs and proteins have been utilized for the fabrication of organic composites. Electrophoretic deposition enabled the fabrication of organic-inorganic composites containing bioceramics and bioglass in the HYH matrix. It was shown that the deposition yield, microstructure, and composition of the films can be controlled. Potential applications of EPD for the surface modification of biomedical implants and fabrication of biosensors are highlighted.

INTRODUCTION

New electrochemical methods have attracted increasing attention for the surface modification of biomedical implants. Electrochemical deposition of thin films and coatings offers the advantages of simple and low cost equipment, high purity of the deposited materials, and the possibility of deposition on substrates of complex shape. Many investigations were focused on electrophoretic deposition (EPD) of hydroxyapatite (HA) and other bioceramics on metallic substrates.\(^1\)\(^2\) \(^3\) Hydroxyapatite is an important material for biomedical implants, as its chemical composition is similar to that of the mineral phase of bone tissue. A complicating factor in the fabrication of ceramic films by EPD is sintering shrinkage, which usually results in deposit cracking. Moreover, it is important to avoid decomposition of HA and degradation of substrates during sintering at elevated temperatures. These problems can be addressed by the development of composite films. The combination of polymeric and inorganic phases is a common feature of various natural materials, including bone, which can be considered as a composite, comprising collagen and HA crystals. Recently it was discovered that polysaccharides form interfaces between organic and inorganic components in bones and govern the crystallization of HA nanoparticles.\(^4\) This discovery has generated a new wave of interest in the fabrication of bone substitute materials containing natural polysaccharides. Moreover, polysaccharides are becoming increasingly important for the surface modification of biomedical implants.

Hyaluronic acid (HYH) is a naturally occurring anionic polysaccharide consisting of alternating units of glucuronic acid and N-acetylgalcosamine.\(^5\) HYH presents at high concentrations in skin, joints, and cornea.\(^6\) HYH is a bioactive material that has been associated with several cellular processes, including angiogenesis and the regulation of inflammation.\(^7\) In living tissue HYH usually combines with proteins to control various functions of the tissue.\(^8\) HYH coatings formed on surfaces of various implant materials provided improved biocompatibility and gliding resistance, reduced platelet adhesion and promoted new bone formation.\(^9\)\(^12\) HYH has been utilized for the fabrication of drug eluting coatings for cardiovascular stents.\(^13\)

Recent studies showed the feasibility of electrophoresis of HYH and composite HYH-HA films.\(^14\)\(^15\) The method offers the advantages of room temperature processing and paves the way for the fabrication of novel composite coatings containing other functional materials. The goal of this investigation was the development of electrochemical strategies for the EPD of composite
coatings containing other bioactive ceramics, bioglass, heparin and salicylate drugs, and bovine serum albumin (BSA) protein in the HYH matrix.

See the sidebar for details on the electrodeposition of HYH films.

**COMPOSITE MATERIALS CONTAINING DRUGS AND BSA PROTEIN**

In this investigation heparin and salicylate were utilized as model drugs for the fabrication of composite films by EPD. Heparin is widely used as an anticoagulant due to its ability to accelerate the rate at which antithrombin inhibits serine proteases in the blood coagulation cascade, especially thrombin. However, the performance of heparin may vary significantly depending on many factors, such as the method of immobilization and the amount of heparin. Salicylate is a commonly used non-steroidal anti-inflammatory drug. Significant interest has been generated in the development of composite materials with controlled release of heparin and salicylate, which were immobilized in the polymer matrix.

Heparin and salicylate are negatively charged in aqueous solutions. Therefore, anodic EPD has been utilized for the fabrication of composite films containing heparin and salicylate in a HYH matrix. Composite films were prepared from 0.5–3 g L⁻¹ HYNa solutions containing 0.5–2 g L⁻¹ heparin or salicylate. Figure Ab and c shows typical cross sections of the composite films prepared from aqueous solutions. The films were relatively dense and adhered well to stainless steel, platinum, titanium and graphite substrates. The SEM studies of the HYH-heparin and HYH-salicylate films prepared at different experimental conditions indicated that film thickness can be varied in the range of 0–20 μm by the variation of deposition time in the range of 0–10 min. and electric field in the range of 3–10 V cm⁻¹.

BSA has been utilized as a model protein for the development of electrochemical methods for the incorporation of proteins into the HYH matrix. It should be noted that BSA contains cationic and anionic groups. The total charge of BSA depends on pH and represents the sum of charges of all ion sable groups. It is known that BSA exhibits an isoelectric point at pH 5 and it is negatively charged at physiological pH. Electric field provided electrophoretic motion of anionic BSA in aqueous solutions at pH 7.4 towards the anode and resulted in accumulation of the BSA macromolecules at the anode surface. However, no anodic deposition was observed. This can be attributed to the pH decrease at the anode due to Reaction 2 and reversal of the BSA charge from negative to positive upon passing through the isoelectric point at the anode surface. It is suggested that the charge reversal resulted in BSA-electrode electrostatic repulsion, which prevented film formation. However, composite films were obtained from the 0.5–3 g L⁻¹ HYNa solutions containing 0.1–1.7 g L⁻¹ BSA.

Deposition experiments performed from 3 g L⁻¹ HYNa solutions at electric field of 12 V cm⁻¹ showed that the deposition rate of pure HYH was 0.15 mg cm⁻² min⁻¹. However, the deposition yield increased drastically after the addition of BSA to the HYNa solutions. The increase in BSA concentration from 0 to 1.7 g L⁻¹ in the 3 g L⁻¹ HYNa solutions resulted in increas-

**ELECTRODEPOSITION OF HYH FILMS**

Electrophoretic deposition of HYH was performed from sodium hyaluronate (HYNa) solutions in water or mixed ethanol-water solvent. It was suggested that the dissociation of sodium hyaluronate (HYNa) resulted in the formation of anionic HY⁻ species:

\[
\text{HYNa} \rightarrow \text{HY}^- + \text{Na}^+
\]

Electric field provided electrophoretic motion of the anionic HY⁻ species towards the anode surface, where the pH decreased owing to the electrochemical decomposition of water:

\[
2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4e^-
\]

The charge compensation in the low pH region at the electrode surface resulted in the formation of hyaluronic acid films (HYH):

\[
\text{HY}^- + \text{H}^+ \rightarrow \text{HYH}
\]

Figure Aa shows a typical cross section of HYH film prepared from aqueous 3 g L⁻¹ HYNa solution. Films thickness was varied in the range of 0–10 μm by the variation of the electric field in the range of 0–7 V cm⁻¹ and deposition time in the range of 0–10 min. EPD resulted in the formation of uniform and adherent films on various conductive substrates, such as stainless steel, Ti and Ti alloys, graphite and platinized silicon wafers. The use of mixed ethanol-water (70% ethanol) solvent offers the advantage of reduced gas evolution attributed to the electrochemical decomposition of water. In this case, higher electric fields can be applied, which are beneficial for the fabrication of composite materials, containing bioceramics and bioglass in the HYH matrix. On the other hand, composite materials containing drugs, proteins and enzymes must be prepared from aqueous solutions in order to avoid reactions of these materials with ethanol.

Figure A, SEM images of cross sections of (a) HYH, (b) HYH-salicylic acid, (c) HYH-heparin, and (d) HYH–BSA films, F–film, S–substrate.