Pearl powder bio-coating and patterning by electrophoretic deposition

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Nacre forms the inner lustrous layer of many mollusk shells. It is also called the *mother of pearl* because pearls with the same compositions and structure are formed within the layer. Nacre has been the focus of many researchers mainly because of three reasons. First, nacre has the simple brick-wall-like structure (Fig. 1a) built from aragonite ($CaCO_3$) platelets and thus provides an ideal model to study biomineralization [1–4]. Second, nacre demonstrates high toughness and significant inelasticity which are outstanding for a material made of 95% ceramic [5–7]. Third, nacre powder as a bone substitute material has been reported to have better osteoinductivity (ability to induce bone formation) than hydroxyapatite, a bioceramic widely studied as a bone substitute material because of its great similarity to bone mineral [8–17]. Each year at least 550 tons of pearls are harvested [18], many of them (especially fresh water pearls) have too low quality to be processed into jewelry but have the same medical functions as nacre. The high potential of nacre as a bone substitute material therefore opens enormous opportunity to the pearl culture industry around the world. It is the purpose of this study to develop novel techniques of incorporating pearl powder into orthopedic implants.

So far, nacre as an implant material has been limited to either ground powder form, [9, 13–16], or the small intact pieces [11, 12]. Inspired by the studies of hydroxyapatite coating on hip implants to improve the bioactivity of inert metallic implants (titanium, cobalt–chromium alloy, etc.) [19–21], the author proposes to use pearl powder as a biocoating to improve the bioactivity and osteoinductivity of the orthopedic implants. As a first step, this pilot study sought to develop a technique of coating pearl powder onto titanium substrate.

In order to preserve the proteins inside the pearl powder, we narrowed down the coating technique to electrophoretic deposition (EPD) at room temperature. EPD has been widely used to make ceramic (including hydroxyapatite) coatings [22–25]. Charged ceramic colloidal particles are driven under an external electrical field to the electrode where they form a thin coating [22]. A key step in EPD is the preparation of stable colloidal suspension in which the ceramic particles are charged (preferably positive charge). Following a similar procedure of preparing hydroxyapatite suspension [26], pearl powders (freshwater pearls, TongRenTang, Beijing) were ground to <1 μm size and were ultrasonically mixed in ethanol to make a 5 wt% suspension. Unlike hydroxyapatite, the pearl powder suspension would stabilize only when $\sim$5 vol% of acetic acid was added to ethanol. Although there have been no reports on the isoelectric point of pearl powder in ethanol, the zeta potential of nacre power in aqueous solution was found to be slightly negative at pH 7.5 and higher [27]. The addition of acetic acid was thus necessary to create a slightly acidic environment in the suspension to induce positive charges to the powder. Two parallel electrodes of 20 mm $\times$ 20 mm in size, one platinum as anode, and a polished titanium alloy plate (Ti-6Al-4V) as cathode, were immersed in pearl suspension and 12 mm apart. A dc electric field of 5–100 V/cm was then applied across the electrodes. After 30 s to 10 min, the positively charged particles would be driven to the titanium cathode surface and formed a uniform layer of pearl coating.

Fig. 1b is a scanning electron micrograph of the pearl powder coating on titanium. The average coating thickness was about 5 μm at 100 V/cm and 5 min. The coating shown in Fig. 1b is dense, and the individual particles in the coating have irregular shapes, as compared with the platelet shape of the crystals before grinding and EPD coating (Fig. 1a). To confirm the crystal structure, the coating was scratched off the substrate and examined with an X-ray diffractometer. The diffraction pattern is identical to that of pearl powder (Fig. 2), indicating that the preparation of suspension and the EPD process did not change the crystal structure.

One interesting observation was that the pearl powder coating was much more difficult to manually scratch off the titanium substrate than other EPD coatings such as hydroxyapatite (HA). Further quantitative scratch tests on the scratch resistance or adhesion strength of pearl powder coating were then carried out on an MTS Nano Indenter XP (Nano Instrument, Oak Ridge, TN) equipped with high loading capability. A spherical sapphire indenter of 0.3 mm in diameter was used to reduce the errors caused by relatively rough surface. Standard ramping load scratch tests (10 for each specimen) were carried out on the coated titanium specimens over a 1000 μm length at the rate of 10 μm/s. During each scratch test, the applied load was linearly increased from an initial 0.1 mN to the maximum of 300, 500, and 700 mN. Two types of hydroxyapatite coatings were also tested for comparison. The first type of hydroxyapatite coating (HA-EPD) on titanium was made by the same electrophoretic deposition as pearl powder, following the detailed parameters reported earlier [26]. The second type of hydroxyapatite coating was prepared by biomimetic method [28]. Briefly, polished titanium specimens were immersed in a simulated body

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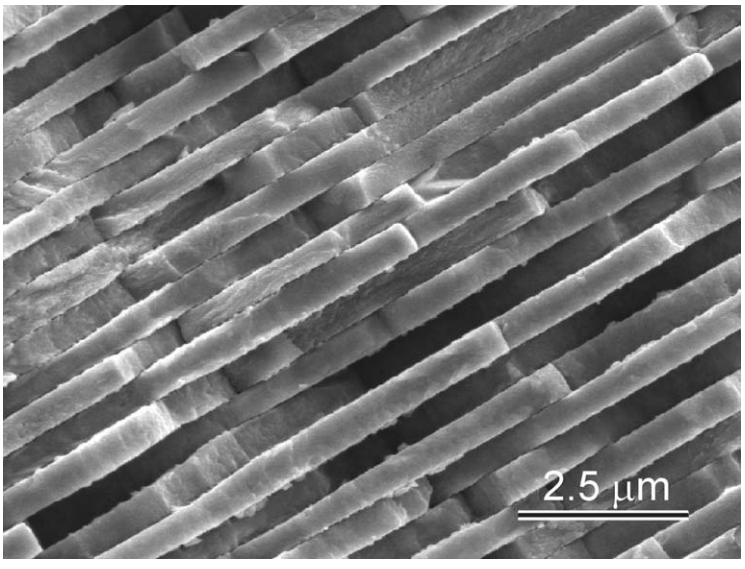
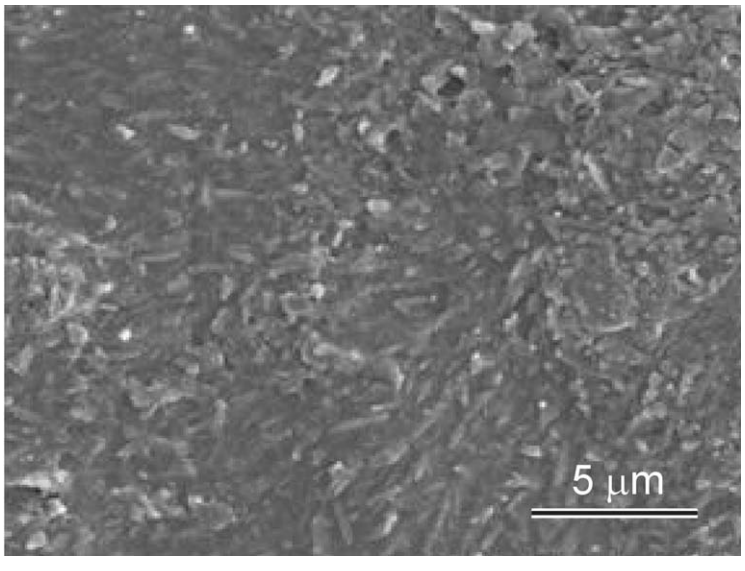


(a)



(b)

Figure 1 SEM micrographs of (a) fractured surface of a fresh water pearl, and (b) pearl powder coating on titanium substrate by electrophoretic deposition.

fluid solution for 1–2 days at 37 °C to form a porous biomimetic hydroxyapatite coating about 1.5 μm thick. The biomimetic apatite coating has been proposed as a biocoating at as-deposit state. A direct comparison between pearl powder coating and the hydroxyapatite biomimetic coating will therefore help assess the feasibility of the coating, since pearl coating can only be used in its as-deposit state. Any heat treatment techniques may cause possible decomposition of $CaCO_3$ and proteins.

Typical penetration depth–applied load curves and optical micrographs of the scratched lines are shown in Fig. 3. A cross examination of the scratched morphology and the scratching curves found three stages. At the first short stage, the indenter penetration depth increased quickly but smoothly with the load. The scratch lines were barely visible under optical microscope. In the second stage, the load exceeded the cohesion strength of the coating resulting in obvious fluctuation in the penetration depth–load curve. Scratch traces could be seen under the optical microscope (left side of the arrows in Fig. 3b). At the third stage, the load was high enough, and coating started to debond from the titanium substrate. This is reflected in the sudden increase in penetration depth (arrows in Fig. 3a,

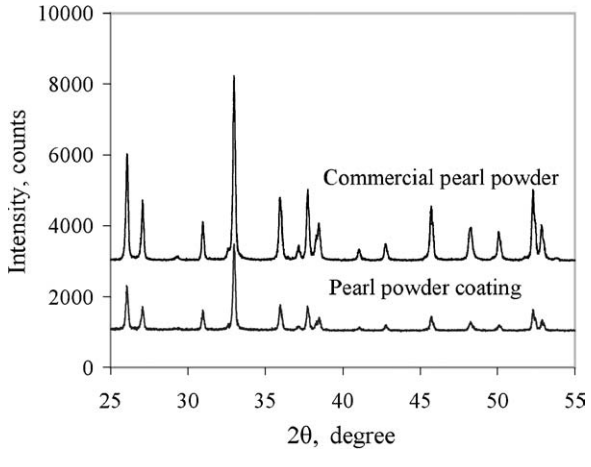


Figure 2 X-ray diffraction patterns of pearl powder and pearl coating.

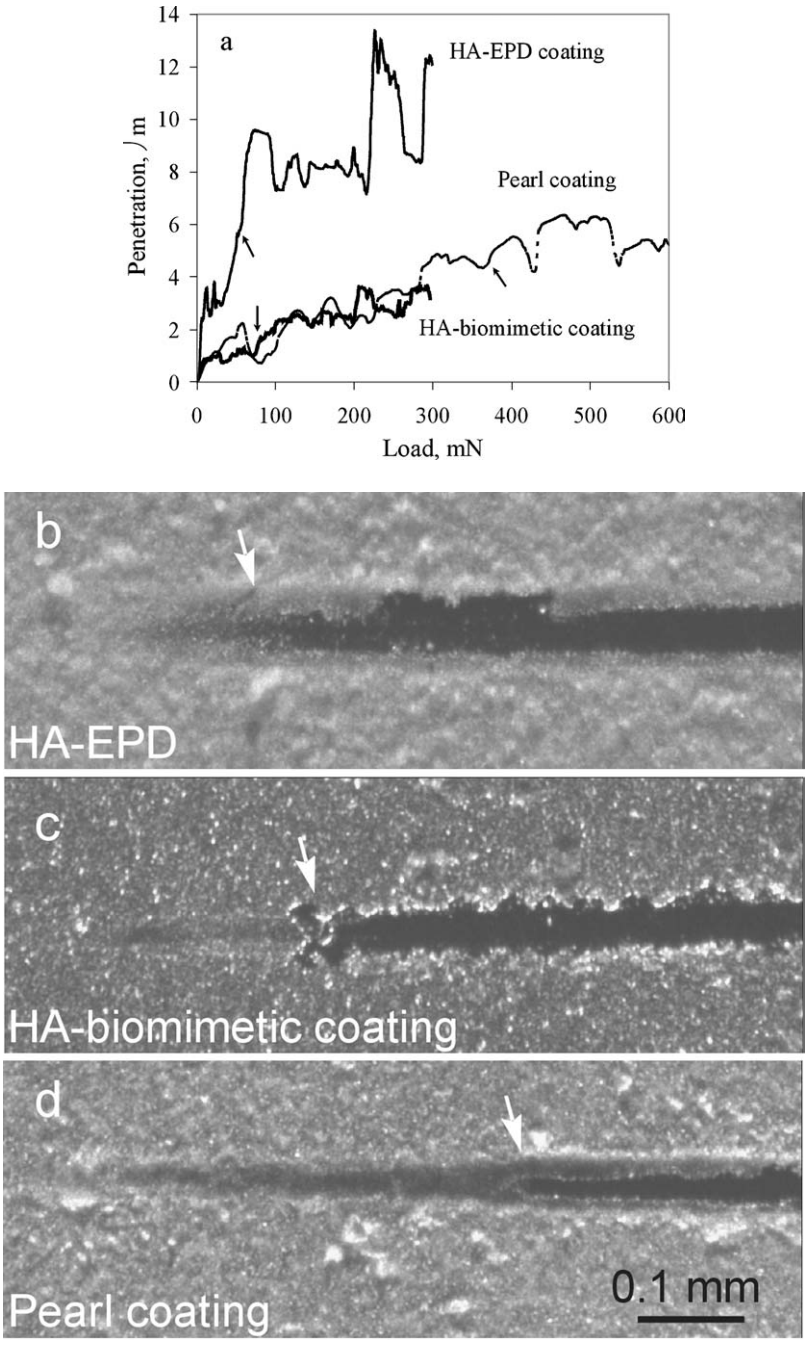


Figure 3 Scratch test results of pearl powder coating, hydroxyapatite coatings by electrophoretic deposition (HA-EPD) and biomimetic deposition (HA-Biomimetic). (a) Penetration depth–normal load curves as measured by a 0.3 mm spherical tip. (b, c and d) Optical micrographs of scratched coatings. The tip scratched from left to right. Arrows indicate the beginning of coating debonding.

determined based on microscope observations). The starting of coating debonding could be seen clearly under the microscope (marked by arrows in Fig. 3b). The applied loads at the beginning of debonding are the critical loads that reflect the adhesion strength of the coatings. The measured critical loads for coating debonding are 61.7 (±7.6), 75.0 (±17.2), and 376.0 (±52.6) mN for HA-EPD coating, HA Biomimetic coating, and pearl coating, respectively.

The critical load for pearl coating debonding is 5–6 times higher than HA-EPD coating and the biomimetic coating. Although scratch test could not provide the adhesion energy quantitatively, it is clinically relevant, since abrasion of the coating may happen during both surgery (installation abrasion) and *in vivo* service. The significantly higher critical load than hydroxyapatite biomimetic and EPD coatings indicates that pearl coating by EPD technique is mechanically robust as an orthopedic biocoating. The mechanism that pearl powder coating could achieve higher bonding strength is up to future study. It is hypothesized that the proteins (up to ∼5%) may act as superglue during EPD coating.

A latest design in bio-implant and biosensor processing is the patterning of bioactive materials on a substrate with the goal of organizing cells and tissues [29]. In a recent report, the author has demonstrated that regular array of hydroxyapatite dots could be deposited on metal substrates by electrophoretic deposition [26]. In this study, a similar procedure was employed to pattern pearl powder. Briefly, arrays of spherical gold dots

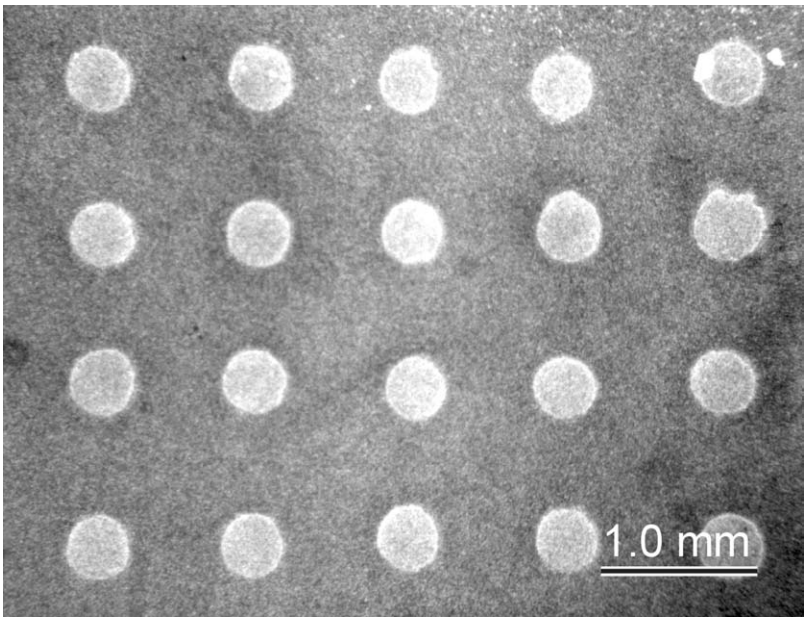


Figure 4 Optical micrograph showing arrays of pearl powder dots (white) patterned on a silicon substrate.

(each dot 3 nm thick and 500 μm in diameter) were vacuum-putter-coated on a silicon plate through a mask with arrays of holes. The patterned silicon plates were then used as cathodes in the electrophoretic deposition of pearl powder. Fig. 4 is the optical micrograph of the pearl powder patterned silicon surface. The pearl powder was preferentially coated on the spherical gold dots (white area), thus forming a distinct array pattern. The mechanism of the preferential deposition of pearl powder on gold as compared with silicon is probably due to the electrochemical properties of the substrates [26].

This study has demonstrated that pearl powder could be deposited on a metallic substrate both as a continuous layer and in patterned form by using electrophoretic deposition technique. The coating has higher critical load for debonding than biomimetically deposited porous calcium phosphate coating. Pearl coating can be directly used on orthopedic implants to introduce osteoinductivity. The patterned coating can also be used to study cell/substrate interactions or to organize cells into arrays for biosensor applications.

Acknowledgments

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