Chitosan

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MDPI

Layer by layer electrophoretic deposition of chitosan and hyaluronic acid for and applications in regenerative medicine.

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INTRODUCTION & AIM

The use of natural polysaccharides like hyaluronic acid (HA) and chitosan (CHI) in the production of films offers significant advantages, including excellent biocompatibility and bioactivity, making them ideal for regenerative medicine and tissue engineering.

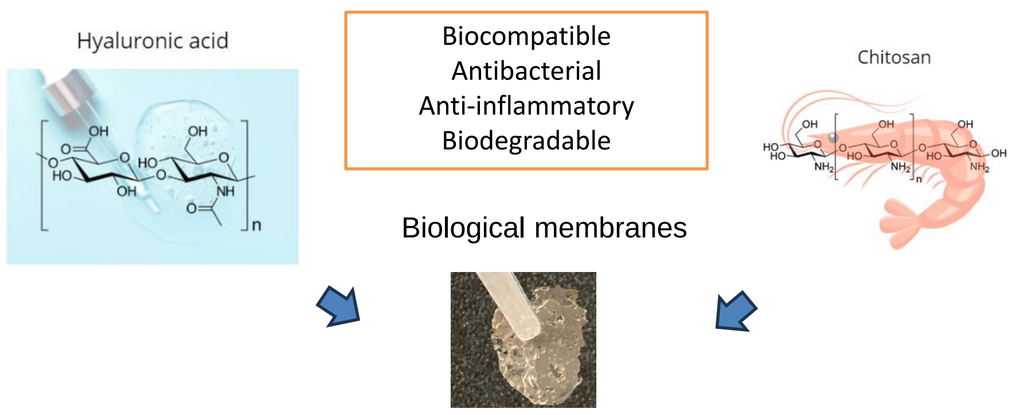


Figure 1a. Properties of of HA and CH

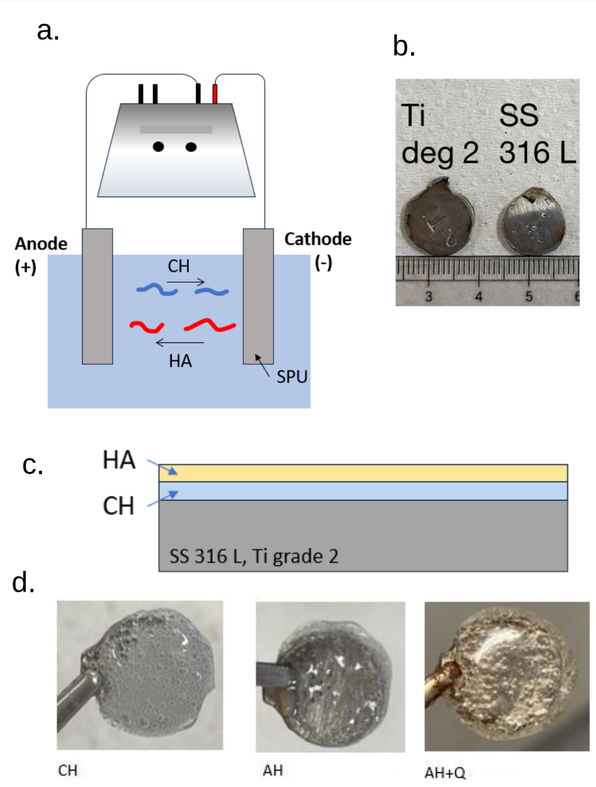
Electrophoretic deposition (EPD) is an important technique for deposition of materials and composites from colloidal suspensions or solutions of biopolymers. The deposition of the EPD of multilayer films, containing individual layers of controlled thickness and composition is of interest in the biomedical field. Moreover, EPD allows for the fabrication of films of graded composition and controlled morphology.

The objective of this research is to engineer multilayer films of chitosan and hyaluronic acid by means of EPD.

METHODS

Sample preparation and EPD:

1. Dissolve CH for a concentration of 2% w/v in water with 2% acetic acid v/v for 24 h.
2. Dissolve HA in 70/30 ethanol/water for a concentration of 10 mg/ml during 24 h.
3. Deposition time of 10 min with 7 V
4. Perform sequential EPD first with CH/HA and then HA/CH.



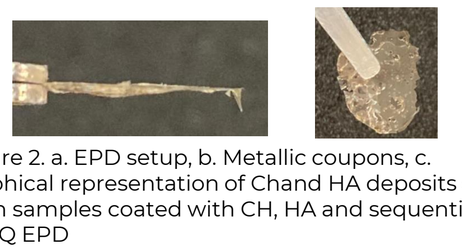


Figure 2. a. EPD setup, b. Metallic coupons, c. graphical representation of Chand HA deposits d. fresh samples coated with CH, HA and sequential HA+Q EPD

HA has carboxyl groups (-COOH) that, under physiological or alkaline conditions, dissociate and give it a **negative charge**. This charge allows the HA to move towards the positive electrode (anode) in an electric field during electrophoretic deposition, allowing the formation of uniform and controllable coatings on metal surfaces such as titanium or stainless steel, used in biomedical implants (Luo et al., 2023; Ma et al., 2010).

On the other hand, the presence of amino groups (-NH2) in the CH structure of **CH** gives it a **positive charge** under **acidic conditions**, which facilitates its interaction with polyanions such as AH. Unlike AH, Ch is a cationic polymer which migrates towards the cathode in a EPD setting. Its solubility is high in acidic solutions or low pH ((Clavijo et al., 2016)).

The EPD coating forms hydrogels of the polymers around the surfaces (see Figure 2d). After drying, a thin layer adhered to the metal is formed (see figure 2e).

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RESULTS & DISCUSSION

Gravimetric studies of the samples reveal that chitosan is deposited with a higher surface density than HA under the same conditions. Similarly, sequential deposits of Q acid + HA also show a higher density compared to samples in which hyaluronic acid is deposited first.

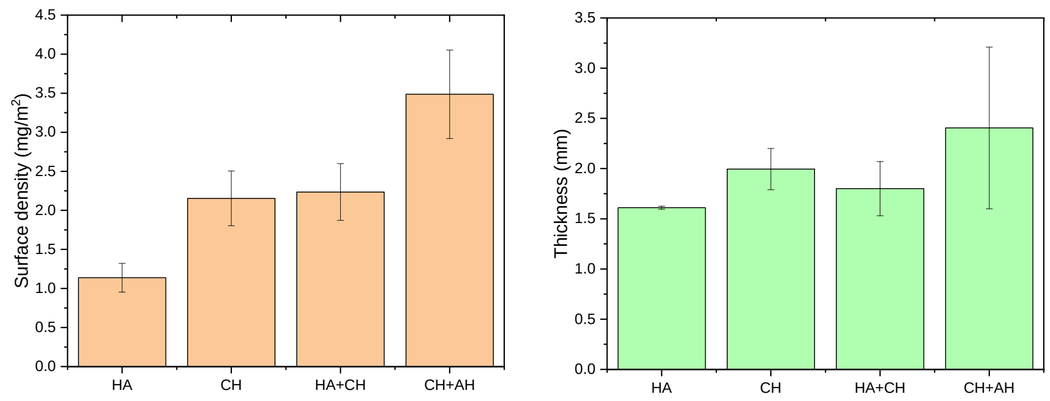

Figure 3. a.Surface density grpahs and b Thickness of EPD layers.

SEM micrographs show the formation of cracks in the chitosan surface which occurs during the escape of air bubbles formed during EPD. HA samples show wrinkles, resulting from the breaking up of fiber aggregates, presumably occurring during drying.

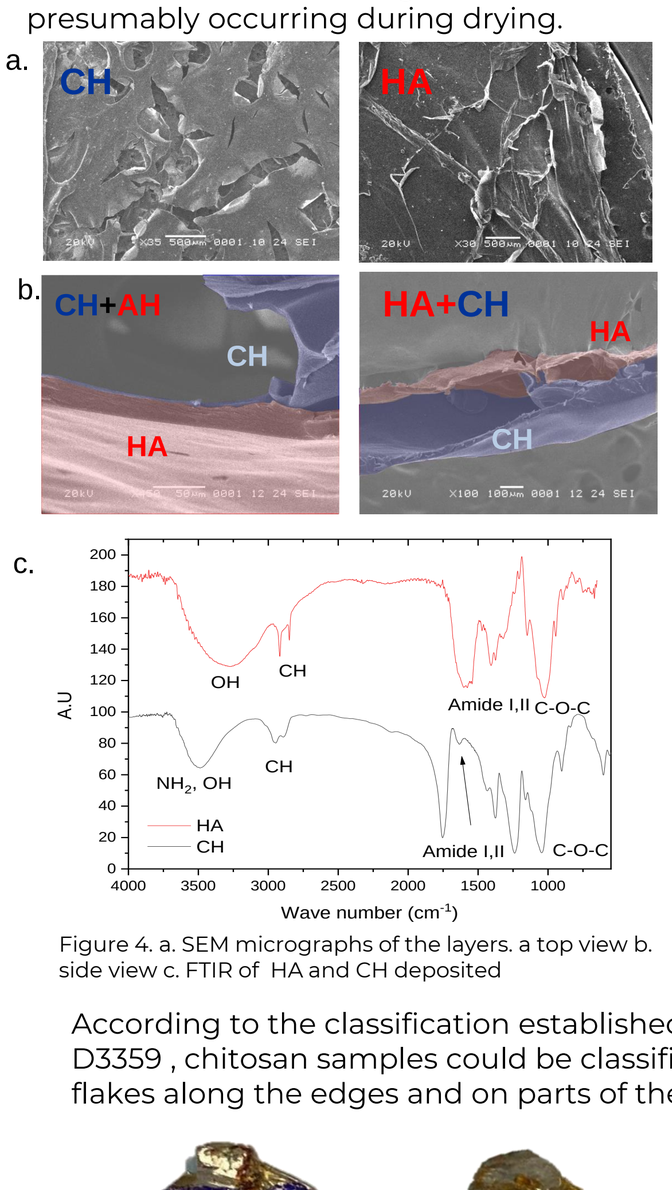

Figure 4. a. SEM micrographs of the layers. a top view b. side view c. FTIR of HA and CH deposited

A cross section of the samples is needed to observe whether a sharp layer-by-layer coating is obtained or whether the layers are intertwined. SEM pictures reveal the presence of two layers, where one of the layers (chitosan, in blue) appears to be porous in comparison to the hyaluronic acid layer (red) (Fig 4b). Likewise, the thickness of the layers is different, with the HA layer being more compact, which is consistent with the fact that the porosity of the chitosan layer increases the volume of the layer.

FTIR measurements were performed for Q and HA films obtained after EPD see figure 4c. For both CH and HA, the identified bands are in agreement with literature reports (Manju *et al 2011* and Gieroba *et al 2020*). Meaning that EPD does no compromise the structure of the biopolymers.

According to the classification established by the standard norm ASTM D3359 , chitosan samples could be classified as 2B, since the coating has flakes along the edges and on parts of the squares.

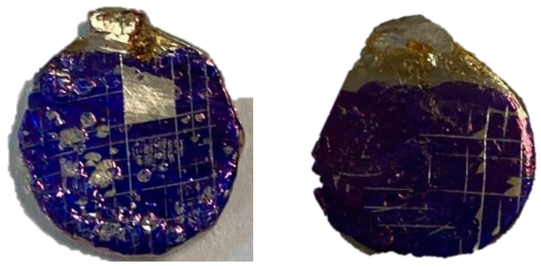

Figure 2. a. Adhesion test of chitosan and HA, samples were tinted with to enhance the visual contrast.

The affected area is ~15 to 35 % of the square. While hyaluronic acid coatings could be classified as 3B surfaces since the small flakes of the coating are separated along the edges and at the intersections of the cuts. The affected area is 5 to 15 % of the square.

CONCLUSIONS

- The EPD deposition was achieved for CH and HA
- The structure of individual layers of CH show cracks on the surface, caused by gas bubbles produced during EPD
- Individual layers of HA are more homogeneous.
- The sequential deposition of CH+HA and AH+CH shows the formation of two distinct layers, linked in some positions and separated due to bubble formation, mainly due to CH deposition.
- Further studies with different solvent composition are needed to limit the bubble formation (mixes of water /ethanol)
- It is of interest to study the formation of layers in presence of a crosslinker