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Study of the effect of the number of bilayers of chitosan and hyaluronic acid adsorbed on metallic substrates on surface properties

Gabriela Pereira de Freitas[a], Estela Kerstner Baldin[a,b], Eduarda Baggio Paglia[a],

Hernandes Faustino de Carvalho[c], Marisa Masumi Beppu [a,*]

[a] *Faculdade de Engenharia Química, Universidade Estadual de Campinas, Campinas - SP, 13083-852, Brazil*
[b] *Centro de Tecnologia da Informação Renato Archer, Campinas - SP, 13069-901, Brazil*
[c] *Instituto de Biologia, Universidade Estadual de Campinas, Campinas – SP, 13083-865, Brazil*
[*] *Corresponding author: beppu@unicamp.br*

Abstract

Multilayer films composed of natural polyelectrolytes, specifically chitosan (CHI) and hyaluronic acid (HA), present significant potential for biotechnological applications, particularly in cancer detection and monitoring. The interaction between HA and the CD44 receptor, overexpressed on circulating tumor cells (CTCs), facilitates the adhesion of cancer cells, enabling the use of these films to capture and analyze CTCs in various diagnostic and therapeutic contexts. Cell adhesion on surfaces is influenced by substrate properties, such as surface characteristics, topography, and charge mobility, which are critical for the development of effective biomedical platforms and devices. This study investigated the impact of varying the number of CHI/HA bilayers on the topography and charge density of the films. Multilayer films were deposited on Ti6Al4V substrates using the layer-by-layer (LbL) technique. The results indicated that film adsorption with 3.5 bilayers helped to level out the grooves caused by substrate sanding, while increasing the number of bilayers led to increased roughness due to the formation of polymeric islands. Furthermore, surface potential measurements revealed that films with 3.5 and 20.5 bilayers likely had more free ammonium groups, while films with 15.5 bilayers had lower surface potential due to the greater interaction between the CHI and HA groups. These findings provide valuable information for controlling surface properties, benefiting the development of advanced biomedical devices, with potential applications in tissue engineering, drug delivery, and biosensors for early cancer detection and monitoring. This research advances the understanding of cellular interactions with engineered surfaces, paving the way for new and improved biomedical technologies.

Keywords: Topography; Surface potential; Cell adhesion; Layer-by-layer.

1. Introduction

Multilayer films composed of natural polyelectrolytes, specifically chitosan (CHI) and hyaluronic acid (HA), offer significant potential for biotechnological applications, particularly in cancer detection and monitoring. The interaction between HA and the CD44 receptor, which is overexpressed on circulating tumor cells (CTCs), facilitates cancer cell adhesion. This property allows these films to be used to capture and analyze CTCs in various diagnostic and therapeutic contexts.

Cell adhesion on surfaces is crucially influenced by substrate properties such as surface characteristics, topography and charge mobility. These factors play a crucial role in determining how cells interact and attach to surfaces, which is essential for developing effective biomedical devices and platforms.

Multilayer polyelectrolyte films can be easily deposited on surfaces of various shapes using the layer-by-layer (LbL) technique. Ultrathin polyelectrolyte multilayers are formed by alternating adsorption of oppositely charged polyelectrolytes in the presence of excess surface charge. This technique is widely used due to its advantages including low cost, simplicity, versatility, reproducibility and mild chemical conditions required.

In this study, we investigated how varying the number of CHI/HA bilayers affects the topography and charge density of the films. By controlling these parameters, we aim to develop more effective strategies to control cell adhesion. This could lead to significant advances in diverse biotechnological applications, including tissue engineering, drug delivery and development of biosensors for early detection and monitoring of cancer. Findings from this research could pave the way for new and improved biomedical technologies, advancing understanding of cellular interactions with engineered surfaces.

2. Experimental procedure

Multilayer films of CHI and HA were deposited by the layer-by-layer technique on Ti6Al4V substrates produced by additive manufacturing. Dense Ti6Al4V samples were mechanically prepared using silicon carbide (SiC) sandpaper up to #1200. They were then cleaned with alkaline detergent and subjected to surface cleaning in an ultrasonic bath containing acetone and ethanol. Alternate immersions were carried out in 0.1% (w/v) polyelectrolyte solutions for 10 minutes, followed by three rinsing steps, resulting in a deposition of 3.5, 15.5, and 20.5 bilayers, with the final layer composed of HA. For topographic characterization, analyses were carried out in dynamic force mode, generating images with dimensions of 5×5 μm². Surface potential was analyzed using Kelvin Probe Force Microscopy. All analyses were performed using an Atomic Force Microscope (Nanosurf AG, model FlexAxiom SLD, Liestal, Switzerland) with data analysis performed using Gwyddion software.

3. Results and discussion

The successful adsorption of polyelectrolytes onto the Ti6Al4V substrate using the LbL technique was confirmed by the observed changes in topography and roughness values, as shown in Figures 1 and 2, respectively, following the deposition of the CHI/HA bilayers.

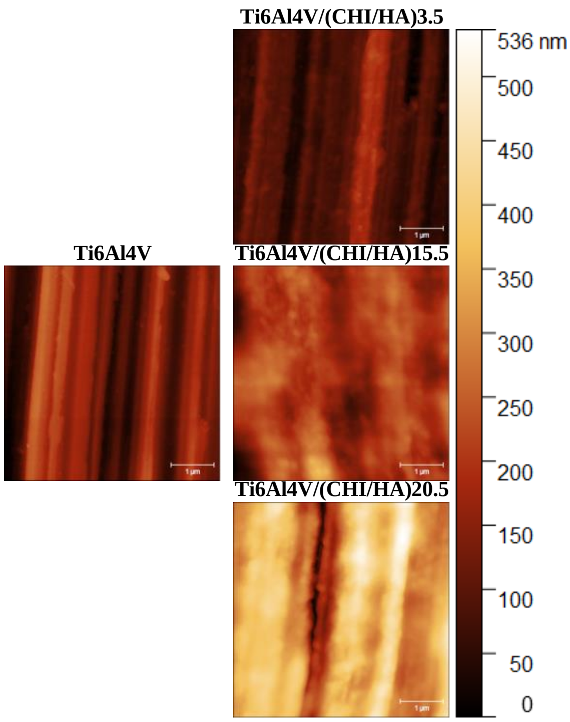

Fig. 1. AFM topography images of substrates obtained at 5×5 μm² analyzed area.

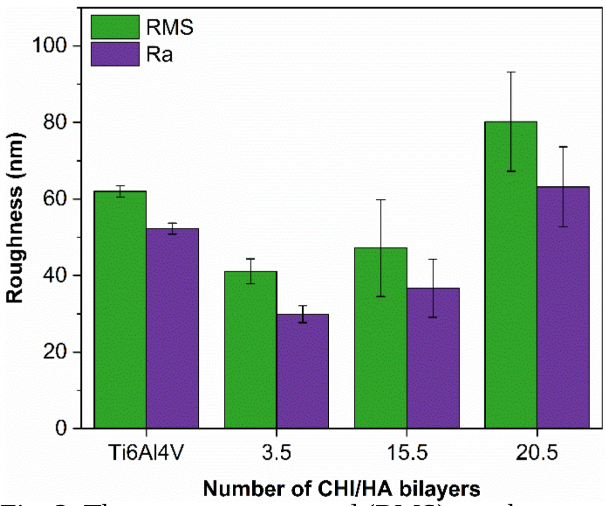

Fig. 2. The root-mean-squared (RMS) roughness and the roughness average (Ra) values.

The roughness values indicated that the adsorption of the film with 3.5 bilayers helped level the grooves caused by the substrate sanding process, reducing the Ra value from 52.26 nm to 29.88 nm and the RMS value from 62.00 nm to 41.12 nm. As the number of adsorbed bilayers increased, the roughness of the film also increased, with the highest RMS (80.22 nm) and Ra (63.15 nm) values observed for the film with 20.5 CHI/HA bilayers. This behavior is attributed to the formation of polymeric islands during polyelectrolyte adsorption, which merge and expand as the film grows, leading to variations in roughness. [3].

Substrate nanotopography, defined by nanometer-scale surface features, plays a pivotal role in governing cell adhesion. Cells adhere to substrates via physical and chemical interactions, where nanotopography influences cell orientation, morphology changes, and the contact area with the substrate [4]. Thus, optimizing substrate nanotopography becomes a crucial parameter in developing advanced biomedical devices.

In addition to nanotopography, surface potential and charges influence cell adhesion. Surface charge determines the quantity, type, and degree of folding of absorbed proteins [5], affecting cell adhesion. A characteristic of all cell types is the negatively charged cell membrane. The electrical potential of cell membranes varies between −10 to −90 mV, depending on the type of cell [6]. Thus, due to electrostatic forces, positively charged surfaces favor cell adhesion, proliferation, and dissemination [7].

The surface potential was reduced with the adsorption of polyelectrolytes on the metallic substrate, as observed in Figures 3 and 4. As the surface potentials of the films are generated mainly by free ammonium groups (NH_3^+) from the outermost layers of CHI, films with 3.5 and 20.5 CHI/HA bilayers likely have more free ammonium groups. On the other hand, films with 15.5 bilayers haved lower surface potential compared to other films, probably due to the greater interaction between the ammonium groups of CHI and the carboxyl groups of HA.

When jointly considering topography and surface potential, the adsorption of 20.5 CHI/HA bilayers demonstrates a greater potential to increase cell adhesion. This is due to the ideal combination of increased surface roughness and favorable surface charge, which collectively promote stronger physical interactions between cells and the substrate. Consequently, the 20.5 bilayer configuration may be particularly advantageous for applications that require robust cell attachment, such as in tissue engineering and biosensor development.

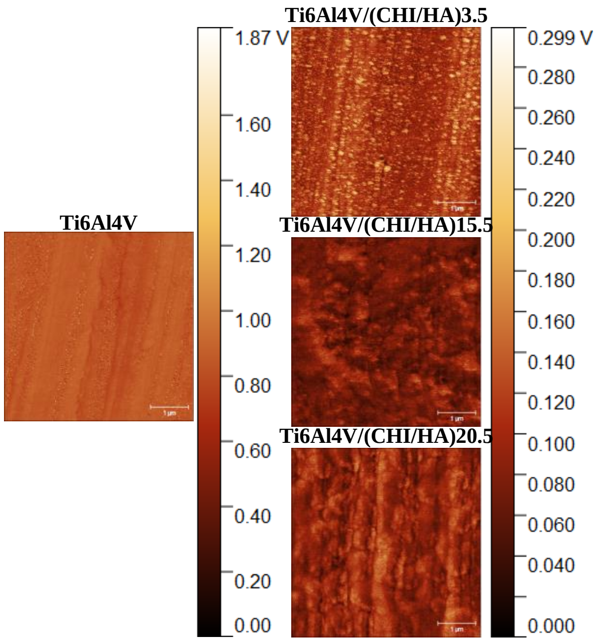

Fig. 3. Surface potential images of substrates obtained at 5×5 μm² analyzed area.

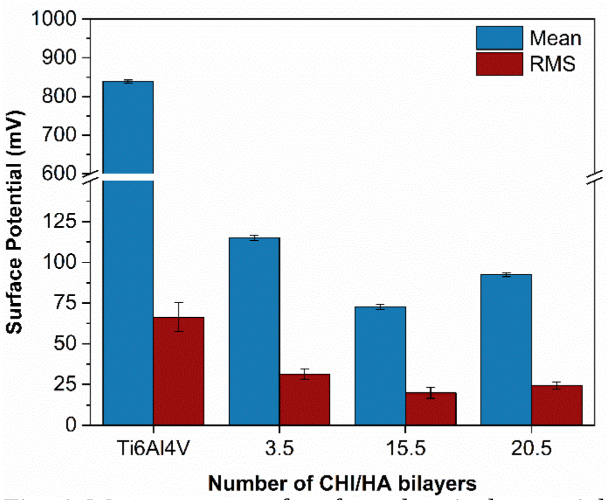

Fig. 4. Measurements of surface electrical potential of substrates.

4. Conclusion

This study demonstrated the ability to manipulate topography and surface charge properties through the controlled deposition of varying numbers of CHI/HA bilayers on Ti6Al4V substrates produced by additive manufacturing. These manipulations can significantly impact cell adhesion mechanisms. The findings offer valuable insights for developing advanced biomedical devices and platforms with potential applications in tissue engineering, drug delivery, and biosensors for early cancer detection and monitoring. This research advances the understanding of cellular interactions with engineered surfaces, paving the way for new and improved biomedical technologies.

Acknowledgements

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