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Tomás E. Benavidez
Clemson University

Marissa E. Wechsler
Clemson University

Madeleine M. Farrer
Clemson University

Rena Bizios
Clemson University

Carlos D. Garcia
Clemson University

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Electrochemically-Preadsorbed Collagen Promotes Adult Human Mesenchymal Stem Cell Adhesion on Carbon Nanostructured Substrates

Tomás E. Benavidez, Marissa E. Wechsler, Madeleine M. Farrer, Rena Bizios, and Carlos D. Garcia
Department of Chemistry, Clemson University | cdgarci@clemson.edu

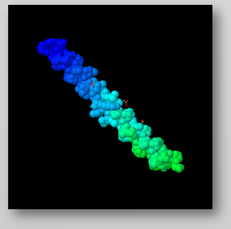
Abstract

The effect of electric potential on the adsorption of collagen type I onto optically transparent carbon electrodes (OTCE) and its mediation on subsequent adhesion of adult, human, mesenchymal stem cells (hMSCs) is described. Adsorption was investigated as a function of the protein concentration and applied potential. The resulting substrate surfaces were characterized using spectroscopic ellipsometry (SE), atomic force microscopy (AFM), and cyclic voltammetry (CV). While the higher applied potential and protein concentration, the higher the adsorbed amount, the application of potential values higher than +800 mV resulted in the oxidation of the adsorbed protein. Subsequent adhesion of hMSCs on the substrates under standard cell culture conditions was also affected by the potential applied and when the collagen type I was oxidized (under applied potential > +800 mV), hMSCs adhesion was decreased. These results provide the first correlation between the effects of electric potential on protein adsorption and subsequent modulation of anchorage-dependent cell adhesion.

Selected Protein

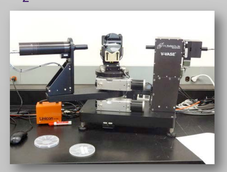
Collagen Type I was selected for these experiments

- Major structural protein, forming molecular cables that strengthen the tendons and vast, resilient sheets that support the skin and internal organs, bones and teeth
- Relatively simple protein composed of three chains, wound together in a tight triple helix
- Molecular dimensions: 1.5 nm x 1.5 nm x 300 nm
- Rod-like structure



Experimental Design

- Substrates were fabricated by pyrolysis of photoresist (AZ P4330-RS; AZ Electronic Materials USA Corp.; Somerville, NJ), spin-coated over Si/SiO_2 wafers
- Protein adsorption experiments were performed using a variable angle spectroscopic ellipsometer (WVASE; J.A. Woollam Co.; Lincoln, NE) by following the change in the reflectance and phase difference between the parallel (R_P) and perpendicular (R_S) components of a polarized light beam upon reflection from a surface.



- Adult Human Mesenchymal Stem Cells (hMSCs) were cultured under standard conditions in mesenchymal stem cell growth medium consisting of mesenchymal stem cell basal-medium supplemented with serum, L-glutamine, and gentamicin/amphotericin-B (passage number 3-5 were used for the experiments).
- hMSCs were seeded at 2500 cells·cm^{-2} on the surface of each substrate sample, allowed to adhere for 2 hours, fixed in situ, and stained using DAPI.

Mechanism and Results

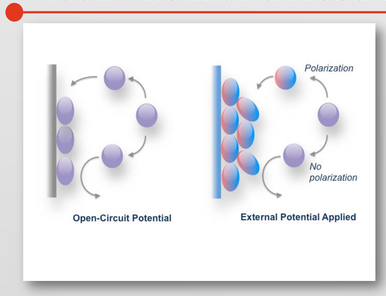


Figure 1: Proposed mechanism for the accumulation of proteins under electrical stimulation

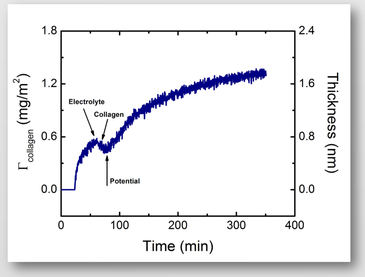


Figure 2: Dynamic adsorption experiment of collagen performed using a solution of 0.1 mg·mL^{-1} collagen in 20 mmol·L^{-1} acetic acid solution (pH = 3.2) at a flow rate of 1 mL·min^{-1}.

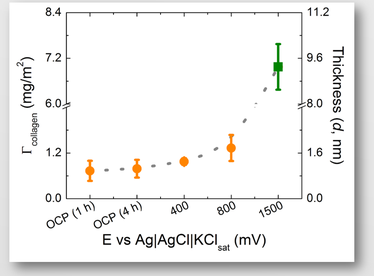


Figure 3: Effect of applied potential on the adsorbed amount (Γ) and thickness of collagen type I film on OTCE. The experiments were performed using 0.1 mg·mL^{-1} collagen in 20 mmol·L^{-1} acetic acid solution at pH = 3.2. The experimental data were obtained by either SE (●) or AFM (■), respectively. Note that both "y" axes have a break region in the range 2.4-6.0 mg·m^{-2} ($\Gamma_{collagen}$) and 3.2-8.0 nm (d).

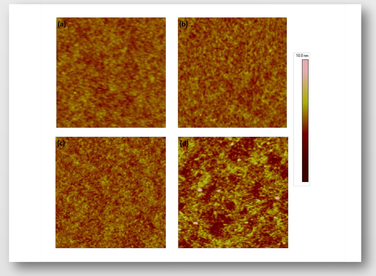


Figure 4: AFM micrographs of (a) bare OTCE, and (b) OTCE substrates modified with a layer of collagen at OCP, (c) at +800 mV, and (d) at +1500 mV. The size of each micrograph is 1 µm^2.

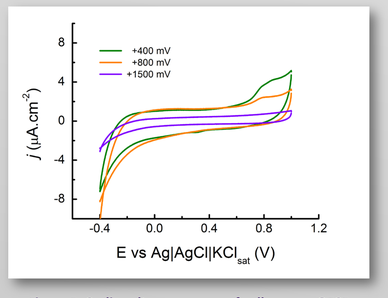


Figure 5: Cyclic voltammograms of collagen on OTCE substrates after protein adsorption at +400 mV, +800 mV, and +1500 mV. Experiments performed using OTCE modified with a layer of collagen deposited using a solution of 0.1 mg/mL collagen in 20 mmol·L^{-1} acetic acid solution (pH = 3.2).

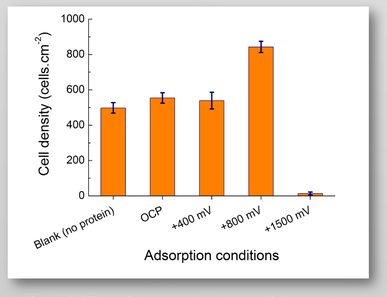


Figure 6: Effect of the experimental conditions used to obtain the collagen/OTCE substrates tested in the present study on the adhesion of hMSCs. hMSCs (2,500 cells·cm^{-2}) in hMSC basal medium (without serum) were maintained in a humidified, 37°C, 5% CO2/95% air environment for 2 hours.

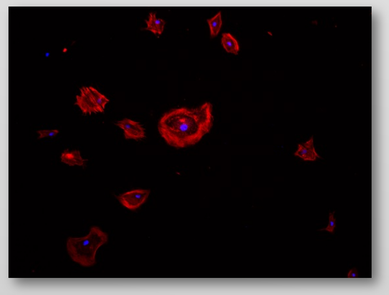


Figure 7: Fluorescence microscopy of hMSC. Cells fixed and observed using DAPI

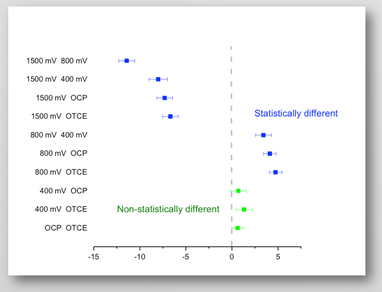


Figure 8: ANOVA analysis of cell adhesion on OTCE under electrical stimulation

Conclusions

- The higher the applied potential, the higher the accumulation of collagen onto the substrate surfaces tested.
- Subsequent adhesion of hMSC was affected by the $\Gamma_{collagen}$ (which depends on the magnitude of the applied potential). The hMSC adhesion density observed on the OTCE substrate pre-adsorbed with collagen at OCP and +400 mV was similar to the results obtained on the "bare" OTCE, but increased when the collagen was pre-adsorbed at +800 mV.
- The lowest adhesion of hMSC on pre-adsorbed collagen on OTCE substrates at +1500 mV can be attributed to irreversible electrochemical oxidation of the adsorbed protein. This oxidation may affect the epitopes on the protein structure recognized by cell membrane receptors during the adhesion of hMSC, rendering the cell adhesion mechanism(s) unattainable.

Acknowledgements

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[1] Spectroscopic and Electrochemical Characterization of Nanostructured Optically-Transparent Carbon Electrodes. T. E. Benavidez and C. D. Garcia. *Electrophoresis* **34** (2013) 1998 - 2006.
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[4] Adsorption of "Soft" and "Hard" Proteins onto OTCEs under the influence of an External Electric Field. T. E. Benavidez, D. Torrente, M. Marucho, and C. D. Garcia. *Langmuir* **31** (2015) 2455 – 2462.
[5] Electrochemically-Preadsorbed Collagen Promotes Adult Human Mesenchymal Stem Cell Adhesion. T. E. Benavidez, M. E. Wechsler, M. M. Farrer, Rena Bizios, and C. D. Garcia. *Tissue Engineering, Part C: Methods* **22** (2016) 69 - 75