METHODS ARTICLE

Electrochemically Preadsorbed Collagen Promotes Adult Human Mesenchymal Stem Cell Adhesion

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The present article reports on the effect of electric potential on the adsorption of collagen type I (the most abundant component of the organic phase of bone) onto optically transparent carbon electrodes (OTCE) and its mediation on subsequent adhesion of adult, human, mesenchymal stem cells (hMSCs). For this purpose, adsorption of collagen type I was investigated as a function of the protein concentration (0.01, 0.1, and 0.25 mg/mL) and applied potential (open circuit potential [OCP; control], +400, +800, and +1500 mV). The resulting substrate surfaces were characterized using spectroscopic ellipsometry, atomic force microscopy, and cyclic voltammetry. Adsorption of collagen type I onto OTCE was affected by the potential applied to the sorbent surface and the concentration of protein. The higher the applied potential and protein concentration, the higher the adsorbed amount (Γ_{collagen}). It was also observed that the application of potential values higher than +800 mV resulted in the oxidation of the adsorbed protein. Subsequent adhesion of hMSCs on the OTCEs (precoated with the collagen type I films) under standard cell culture conditions for 2 h was affected by the extent of collagen preadsorbed onto the OTCE substrates. Specifically, enhanced hMSCs adhesion was observed when the Γ_{collagen} was the highest. When the collagen type I was oxidized (under applied potential equal to +1500 mV), however, 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.

Introduction

PROTEINS ADSORBED ON MATERIAL surfaces mediate and modulate subsequent interactions of anchorage-dependent cells (i.e., those present in most mammalian tissues).^{1–3} Because cell adhesion and functions pertinent to new tissue formation are thus affected, understanding and controlling adsorption, distribution, and conformation of proteins on material surfaces before subsequent cell interaction are critical for the success of tissue engineering^{4,5} and regeneration applications, however, this remains (at best) partially understood. Aiming to address this gap in knowledge, the present study utilized interdisciplinary approaches, novel laboratory setups, cellular models, as well as biochemical assays to examine the electrochemical preadsorption of collagen type I (the predominant protein in the organic phase of bone) on optically transparent carbon electrodes (OTCE) and its subsequent effects on adult human mesenchymal stem cell (hMSC) adhesion, the first and most important function of anchorage-dependent cells, which is required for cell survival and subsequent functions pertinent to new tissue formation. The present research was

also motivated by the following research contributions related to the effect of electric current stimulation on (1) cellular- and molecular-level functions of osteoblasts^{6,7} and of adult hMSCs^{8,*} pertinent to new bone tissue formation and (2) adsorption of proteins onto nanostructured carbon films^{9–12} by our respective laboratories. The combined research interests and approaches provided a unique opportunity to address aspects of the underlying mechanism(s) involved when stem cells interact with (and function on) substrates under electric stimulation.

Experimental Design

Reagents and solutions

All aqueous solutions were prepared using 18 M Ω ·cm water (NANOpure DIamond, Barnstead, Dubuque, IA) and

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analytical reagent grade chemicals. Rat tail collagen type I (3 mg/mL) was purchased from Life Technologies Corporation (New York, NY). Glacial acetic acid was acquired from EM Science (Gibbstown, NJ). Unless otherwise stated, the collagen adsorption experiments were performed using a 0.10 mg/mL collagen solution prepared in 20 mmol/L acetic acid (pH=3.2). Sodium hydroxide and sodium phosphate monobasic anhydrous were obtained from Fisher Scientific (Fair Lawn, NJ). The pH of different solutions was adjusted using 1 mol/L NaOH and measured using a glass electrode/digital pH meter (Orion 420A+, Thermo; Waltham, MA).

Substrates

Silica wafers layered with thin films (Si/SiO₂/OTCE) were prepared following the procedure described in previous publications.^{9,13} Briefly, standard <111> silicon wafers (Si/SiO₂; Sumco, Phoenix, AZ) were first scored using a computer-controlled engraver (Gravograph IS400; Gravotech, Duluth, GA). The process defined pieces of 1 cm in width and 3–5 cm in length, which were then manually cut and cleaned in piranha solution (30% hydrogen peroxide and 70% sulfuric acid) at 90°C for 30 min. After thorough rinsing with distilled water, the substrates were immersed and stored in ultrapure water and thus maintained until use in experiments. Subsequently, the clean wafers were dried at 80°C for 30 min. A thin layer of photoresist (AZ P4330-RS; AZ Electronic Materials USA Corp., Somerville, NJ) was spread onto each silicon wafer using a spin coater (Model WS-400-6NPP; Laurell, North Wales, PA). The photoresist-coated silica wafers were then heated at 110°C for 60 s in a convection oven (to evaporate the solvent) and finally transferred to a tube furnace (Thermolyne F21135; Barnstead International, Dubuque, IA) for pyrolysis. The carbonization step began by flushing the system with forming gas (95% Ar/5% H₂, v/v) at 1 L/min for 5 min. Next, the temperature of the furnace was increased to 1000°C at a rate of 20°C/min.¹⁴ After pyrolysis for 1 h, the system was allowed to cool down to room temperature. Finally, the samples were stored in a Petri dish for a minimum of 3 days (to complete spontaneous material surface oxidation).

Spectroscopic ellipsometry

Protein adsorption experiments were performed using a variable angle spectroscopic ellipsometer (WVASE; J.A. Woollam Co., Lincoln, NE) following a procedure described elsewhere.^{15–18} The spectroscopic ellipsometry (SE) fundamental is the measurement of 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. The intensity ratio of R_p and R_s can be related to the ellipsometric angles (Ψ and Δ) using Equation 1:

$$\tan(\Psi)e^{i\Delta} = \frac{R_p}{R_s} \quad (1)$$

The amplitude ratio (Ψ) and phase difference (Δ) collected as a function of wavelength and time were fitted by using the WVASE software package (J.A. Woollam Co.). The mean square error (MSE; calculated by a built-in function in WVASE) was used to measure the difference between the experimental and model-generated data. MSE values lower

than 15 were considered acceptable and in agreement with published reports.^{15,16} The optical model used in this study to interpret the raw ellipsometric data was developed in the Garcia laboratory and introduced in earlier publications.⁹ The ellipsometric model describes the microstructure of the samples in terms of the refractive index (n), extinction coefficient (k), and thickness (d) of the various layers, which form the resultant substrate. In consequence, five uniaxial layers with optical axes parallel to the substrate surface were considered in the optical model. Because the experiments were performed in aqueous media, the optical properties of water were also considered in the model. First, the dielectric functions of Si (bulk, $d=1$ mm) and SiO₂ ($d=2.1 \pm 0.5$ nm) were used to describe the optical behavior of silica wafers. The optical constants of carbon^{13,19} were used to define the ellipsometric response of the OTCE ($d=19.6 \pm 0.7$ nm). Second, a void layer taking into consideration the nanobubbles^{20–23} formed on the hydrophobic and rough surface of the OTCE was incorporated to improve the accuracy of the optical model. Finally, the protein layer adsorbed on the OTCE was described successfully using a Cauchy parametrization model, where $A=1.465$, $B=0.01$, and $C=0$ are computer calculated, fitting parameters describing the relationship between the refractive index and the wavelength of the incident beam.

Dynamic protein adsorption

Dynamic adsorption experiments were performed using a modified electrochemical “cell”¹⁵ (J.A. Woollam Co.) mounted directly on the vertical base of the ellipsometer, with an incident angle of 70°. Before adsorption of collagen on the substrates of interest in the present study, the thickness of the OTCE was measured by placing the substrate in the ellipsometry “cell” and performing a spectroscopic scan from 300 to 1000 nm (with 10 nm steps) using 20 mmol/L acetic acid solution as the ambient medium. Next, each collagen adsorption experiment began by recording a baseline of the bare OTCE at open circuit potential (OCP; the potential at which no current flows through the ellipsometry “cell”) while the solution was pumped inside the “cell” at a rate of 1 mL/min. After 20 min of baseline recording, the collagen solution was injected; a protein monolayer was allowed to adsorb on the OTCE at the OCP (approximately +200 mV). An initial fast protein adsorption process that gradually slowed down was always observed. When constant ellipsometric signal readings were obtained (stable adsorbed amount), the selected potential (+400, +800, or +1500 mV) was applied and held until the end of the experiment. The change of potential was performed using a CHI812B Electrochemical Analyzer (CH Instrument, Inc., Austin, TX) and a silver/silver chloride (Ag/AgCl/KCl_{sat}) and a platinum wire as the reference and counter electrode, respectively. Finally, a spectroscopic scan was performed to obtain the thickness of the adsorbed collagen layer under potential conditions. The procedure described in this section provided the data needed to calculate the thickness of both the OTCE and the collagen film (which had accumulated as a result of the protein adsorption process).

Atomic force microscopy

Atomic force microscopy (AFM) was used to evaluate the topography of the collagen-coated OTCE substrates and to

confirm the presence of the protein film obtained at the chosen applied potentials. Experiments were performed using a Veeco diMultimode Nanoscope V scanning probe microscope operating in tapping and noncontact modes.

Adult hMSCs and cell culture

hMSCs, which have the potential to differentiate into osteoblasts,²⁴ were obtained commercially (Lonza Walkersville, Inc., Walkersville, MD). These cells, characterized by the vendor, were used in the present study without any further characterization. For passaging, the hMSCs were treated with trypsin/EDTA obtained from, and according to protocols provided by, the vendor (Lonza Walkersville, Inc.). These cells were cultured under standard cell culture conditions (i.e., a sterile, humidified, 37°C, 5% CO₂/95% air environment) in mesenchymal stem cell growth medium (consisting of mesenchymal stem cell basal-medium supplemented with serum, L-glutamine, and gentamicin/amphotericin-B). The concentrations of all supplements in this medium were considered proprietary information and not disclosed by the vendor (Lonza Walkersville, Inc.). Adult, hMSCs of passage number 3–5 were used for the experiments.

Adhesion of hMSCs on OTCE

Rat tail collagen type I (0.1 mg/mL in 20 mM acetic acid; pH 3.2) was adsorbed on the surface of OTCE under +400, +800, or +1500 mV, at room temperature, for 3 h (as described in the Dynamic protein adsorption section). Subsequently, hMSCs (in hMSC basal medium without serum) were seeded at 2500 cells/cm² on the surface of each substrate sample and allowed to adhere in a humidified, 37°C, 5% CO₂/95% air environment for 2 h. At the prescribed time, the adherent hMSCs were fixed *in situ* and their nuclei were stained using 4', 6-diamidino-2-phenylindole (DAPI). The stained hMSCs (mononuclear cells) were visualized using fluorescent microscopy (358 nm excitation; 461 nm emission). Adherent hMSCs on five, separate, randomly chosen fields per substrate sample were photographed, manually counted *in situ*, averaged, and reported as "cells per cm² substrate surface area." The data of hMSC adhesion on OTCE substrates pretreated with electrochemically adsorbed collagen type I were compared to results obtained from the respective controls. Controls were hMSCs seeded in parallel on (1) OTCE substrates without exposure to the electric potential, (2) OTCE substrates with preadsorbed protein without exposure to the electric potential (OCP), and (3) tissue culture polystyrene (nonconductive substrate); these controls were seeded with adult, hMSCs, maintained under similar experimental conditions, and analyzed using the aforementioned techniques. All hMSC adhesion experiments were run in duplicates per substrate type tested and repeated at three separate intervals.

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the results of the adhesion of hMSCs onto the substrates of interest for the present study. A nonparametric ANOVA, specifically the Kruskal–Wallis test, was used to compare the adhesion of hMSCs on OTCE precoated with collagen at the different electric potential conditions tested with the designated controls. For this analysis, the Infostat/

Profesional version 1.1 statistical software package (School of Agronomic Sciences, National University of Cordoba, Argentina) was used. Pertinent results are reported as average $\pm$ standard error of the mean. $p < 0.05$ was considered statistically significant.

Results and Discussion

Dynamic adsorption experiments, performed with the intention of investigating the effect of applied potential on collagen type I adsorption on OTCE, involved three different stages (specifically, the baseline, adsorption of a protein monolayer at OCP, and finally, the adsorption of a secondary protein layer under the application of the external potential). All experiments began by placing a bare OTCE substrate in contact with the background electrolyte (20 mmol/L acetic acid; pH = 3.2) and once the baseline was established (~ 20 min), collagen type I was allowed to adsorb on the OTCE at OCP (0.1 mg/mL collagen in 20 mmol/L acetic acid; pH = 3.2 for 40 min). The adsorbed protein layer had a thickness of 1.0 ± 0.3 nm (corresponding to an adsorbed amount, Γ_{collagen} , of 0.8 ± 0.2 mg/m²), values below the molecular dimensions of collagen ($1.5 \times 1.5 \times 300$ nm, rod-like structure).²⁵ These experimental results suggest that the adsorbed collagen molecules adopted a "side-on" orientation on the OTCE surface and that subsequent structural rearrangement of the collagen molecules upon adsorption led to the formation of an incomplete (average of $\sim 67\%$) monolayer of protein on the OTCE surface. After the amount of collagen adsorbed on the surface reached a constant value (typically, at 60 min), electrolyte was injected (to verify stability), and then the potential applied to the OTCE was changed from the OCP to +400, +800, or +1500 mV and maintained for 3 h while a solution containing collagen (at the selected concentration) impinged on the surface. A representative example of the experiment is shown in Figure 1.

As shown, the applied potential induced a secondary adsorption process, which was attributed to the induction of dipoles (polarization) in protein molecules when located in the

FIG. 1. Dynamic adsorption experiment of collagen performed using a solution of 0.1 mg/mL collagen in 20 mmol/L acetic acid solution (pH = 3.2) at a flow rate of 1 mL/min. The arrows indicate the specific time points when the designated experimental conditions were changed.

vicinity of the electrode surface.^{9,10} In all experiments, a fast initial process ($d\Gamma/dt_1$) that transitioned into a secondary one ($d\Gamma/dt_2$) was observed upon application of the external potential. These experiments also allowed for measurement of the adsorbed amount of collagen at the end of the experiment ($\Gamma_{\text{collagen } 300 \text{ min}}$). The following four sections describe the effect of the most significant variables in the adsorption of collagen under the effect of the external potential.

Effect of concentration on the adsorption of collagen

To evaluate the effect of collagen concentration on the protein adsorption process induced by the electric potential, dynamic adsorption experiments were performed. As previously described, a first layer of collagen was adsorbed (using 10 mmol/L acetic acid solution containing 0.1 mg/mL of protein at pH=3.2) at OCP, resulting in an average thickness of 1.0 ± 0.3 nm. Then, the background electrolyte and a solution containing collagen (at 0.01, 0.1, or 0.25 mg/mL) were sequentially injected at OCP, allowing the preadsorbed layer to stabilize before the external potential (+800 mV) was applied and maintained until the end of the collagen adsorption experiments. In all tested conditions, increased concentration of collagen resulted in increased accumulation on the OTCE substrates. These results are in agreement with previous literature reports^{9,10,26} and can be explained by considering the increased number of collagen type I molecules in the vicinity of the electrode surface. The highest initial adsorption rate ($d\Gamma/dt_1 = 0.018 \pm 0.002 \text{ mg/m}^2 \cdot \text{min}$) was observed when a solution containing 0.25 mg/mL of collagen type I was used. This value corresponds to a rate that was 22.5 and 1.6 times higher than the initial adsorption rate ($d\Gamma/dt_1$) obtained using the 0.01 and 0.10 mg/mL collagen solutions, respectively. As summarized in Table 1, the application of the electric potential (for 180 min) resulted in higher accumulation of collagen type I on the surface of the OTCE; the amount ($1.6 \pm 0.2 \text{ mg/m}^2$) obtained under these conditions greatly surpassed the value ($0.8 \pm 0.2 \text{ mg/m}^2$) obtained during the same time but without the external potential. To optimize the thickness of the adsorbed protein layer, the amount of protein, and the time required for each experiment, solutions containing 0.10 mg/mL were used for the remaining experiments.

TABLE 1. INITIAL ADSORPTION RATE ($d\Gamma/dt_1$), LINEAR APPROXIMATION OF THE SECOND ADSORPTION PROCESS ($d\Gamma/dt_2$, CALCULATED IN THE 200–300 MIN INTERVAL OF THE DYNAMIC ADSORPTION EXPERIMENTS), AND ADSORBED AMOUNT OF COLLAGEN TYPE I ($\Gamma_{\text{collagen } 300 \text{ min}}$) ONTO THE OTCE SUBSTRATE SURFACES AS A FUNCTION OF THE COLLAGEN CONCENTRATION UNDER THE APPLICATION OF +800 mV FOR 300 MIN

Collagen concentration (mg/mL)	$d\Gamma/dt_1$ ($\times 10^{-3} \text{ mg/m}^2 \cdot \text{min}$)	$d\Gamma/dt_2$ ($\times 10^{-3} \text{ mg/m}^2 \cdot \text{min}$)	$\Gamma_{\text{collagen } 300 \text{ min}}$ (mg/m ²)
0.01	0.8 ± 0.4	0.63 ± 0.03	0.8 ± 0.2
0.10	11 ± 2	1.77 ± 0.05	1.3 ± 0.3
0.25	18 ± 2	2.23 ± 0.04	1.6 ± 0.2

OTCE, optically transparent carbon electrodes.

Effect of applied potential on the adsorption of collagen

As previously described,^{9–11} potential applied to the OTCE is one of the most significant variables affecting the amount of collagen adsorbed. Again, a first layer of collagen was adsorbed on the OTCE (using 10 mmol/L acetic acid solution containing 0.1 mg/mL of protein at pH=3.2) at OCP, which was later followed by the secondary adsorption process where the external potential (+800 mV) was applied. As a summary, the amount and thickness of the adsorbed layer of collagen obtained at the end of the experiment are shown in Figure 2.

As shown in Figure 2, the thickness of the adsorbed layer of collagen obtained at OCP was similar to that obtained ($0.8 \pm 0.2 \text{ mg/m}^2$) when less than +400 mV was applied to the OTCE surface. The adsorbed protein thickness, however, was higher when the potential applied to the surface was greater than or equal to +800 mV. It is important to mention that, because the optical model did not fit the experimental results obtained at +1500 mV, AFM was used to determine the thickness of the adsorbed collagen layer by scratching the surface, a process that removed both the protein and carbon films from the silicon wafer substrate. As a result, a collagen thickness ($d = 9.3 \pm 0.8 \text{ nm}$) was calculated by subtracting the OTCE thickness (previously obtained using SE as $d_{\text{OTCE}} = 23.2 \pm 0.3 \text{ nm}$) from the thickness value obtained using AFM ($d_{\text{AFM}} = 32.5 \pm 0.5 \text{ nm}$). As shown in Figure 2, the experimental data provided evidence of a correlation between the Γ_{collagen} and the magnitude of the imposed applied potential; in other words, the higher the applied potential, the greater the increase in the Γ_{collagen} onto the OTCE. In agreement with previous reports,^{9,10} the adsorption of collagen observed in the present study was attributed to a combination of various parameters such as the polarizability of the collagen molecules, the probability of

FIG. 2. Effect of applied potential on the adsorbed amount (Γ) and thickness of the resulting collagen type I film onto the OTCE tested. The experiments were performed using 0.1 mg/mL collagen in 20 mmol/L acetic acid solution at pH=3.2. The experimental data were obtained by either spectroscopic ellipsometry (●) or atomic force microscopy (AFM) (■), respectively. Note that both y axes have a break region in the range 2.4–6.0 mg/m² (Γ_{collagen}) and 3.2–8.0 nm (d). OTCE, optically transparent carbon electrodes.

FIG. 3. AFM micrographs of (a) bare OTCE and (b) OTCE substrates modified with a layer of collagen at open circuit potential (c) at +800 mV and (d) at +1500 mV. The size of each micrograph is $1 \mu\text{m}^2$. Color images available online at www.liebertpub.com/tec

adsorption related to the protein concentration (as described in Table 1), as well as changes in the solvent-accessible surface area during the collagen adsorption process in the presence of an external electric potential.

In addition, to gain insight regarding the role of surface topography on the resulting collagen films, AFM micrographs were obtained immediately after the OTCE were coated with the collagen layer at the tested experimental conditions. Representative AFM images of the collagen-coated OTCE are shown in Figure 3.

The substrate topography was affected by the accumulation of collagen onto the OTCE. Subsequent data analysis provided evidence that different surface features were obtained as a consequence of the applied potential. On one hand, the bare OTCE substrate had a surface roughness of only 2.3 ± 0.3 nm. On the other hand, the adsorbed collagen films exhibited increased roughness (specifically, 2.6 ± 0.2 , 2.9 ± 0.3 , and 5.2 ± 0.5 nm) as the applied potential increased from +400 to +800, and to +1500 mV, respectively, during the collagen adsorption experiments. As a result, the SE and AFM techniques provided complementary information regarding the collagen films and were used to elucidate the physical characteristics of the resulting collagen-coated OTCE substrates.

Electrochemical response of collagen adsorbed to OTCE

Conformation of protein molecules following adsorption on substrates is important because it mediates subsequent cell adhesion. Because the protein conformation (and conse-

quently the bioactivity) can be affected by oxidative processes,¹⁰ the electrochemical response of the collagen on OTCE substrates was investigated by cyclic voltammetry. Illustrative results of current density (j , $\mu\text{A}/\text{cm}^2$) as a function of potential applied to the OTCE are shown in Figure 4.

FIG. 4. Cyclic voltammograms of collagen on OTCE substrates after protein adsorption at +400, +800, 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 acetic acid solution (pH = 3.2).

An irreversible oxidation process was observed ($E_p = +800$ mV) after the collagen was adsorbed at either +400 or +800 mV. In contrast, no oxidation processes were observed when collagen was adsorbed at +1500 mV. It is important to note that the peak current (attributed to the irreversible oxidation of cysteine, tryptophan, and tyrosine present in the protein) decreased with the number of collagen molecules adsorbed at various (low to high) levels of the applied potential: the higher the potential, the higher the oxidative effect on the collagen molecules adsorbed onto the OTCE substrate. In agreement with the trends recently reported by the Garcia group regarding other proteins,¹⁰ the oxidation peak for collagen was only observed when the substrates were subjected to moderate potentials (less than or equal to +800 mV).

Adhesion of hMSCs onto collagen-coated OTCE substrates

To investigate the bioactivity of collagen preadsorbed onto OTCE substrates under different potentials, hMSC adhesion was examined. The hMSC remained viable under all tested conditions (data not shown).

As shown in Figure 5, similar hMSC adhesion was obtained on the substrate coated with collagen adsorbed at OCP and under the application of +400 mV (5540 ± 30 and 540 ± 50 cells/cm², respectively). These results may be attributed to the similar Γ_{collagen} accumulated under the aforementioned conditions (Fig. 2). In contrast, a significant ($p < 0.0001$) increase in cell adhesion was observed when collagen was preadsorbed on the OTCE at +800 mV (specifically, 840 ± 30 cells/cm²) compared to the observed decrease in cell adhesion when collagen was preadsorbed on the OTCE at +1500 mV (specifically, 14 ± 8 cells/cm²). To explain the difference in hMSCs adhesion results, it is im-

portant to consider not only the adsorbed amount of collagen type I (Fig. 2) but also the redox activity of the protein (Fig. 4). Loss of bioactivity can be attributed to either protein denaturation or conformation changes in the collagen structure as a result of the oxidation of electroactive amino acids (specifically, cysteine, tryptophan, and tyrosine) during the potential-assisted adsorption step.

Summary

The present study focused on the adsorption of collagen onto OTCEs induced by the application of an external potential and the effect that such preadsorbed protein layer had on hMSC adhesion. SE was used to investigate the dynamic adsorption of collagen subjected to different applied potentials (specifically, OCP, +400, +800, and +1500 mV) and both the thickness of the adsorbed collagen layer and the Γ_{collagen} were calculated. The experimental results provided evidence that the higher the applied potential, the higher the accumulation of collagen onto the substrate surfaces tested. Subsequent adhesion of hMSC was affected by the Γ_{collagen} (which depends on the magnitude of the applied potential). The hMSC adhesion density observed on the OTCE substrate preadsorbed with collagen at OCP and +400 mV was similar to the results obtained on the “bare” OTCE, but increased when the collagen was preadsorbed at +800 mV. The lowest adhesion of hMSC on preadsorbed 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. In conclusion, the experimental results demonstrated that adsorption of collagen on OTCE can be controlled by the application of electric potentials, which modulates subsequent hMSC adhesion.

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Disclosure Statement

No competing financial interests exist.

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FIG. 5. Effect of the experimental conditions used to obtain the collagen/OTCE substrates tested in the present study on the adhesion of human mesenchymal stem cells (hMSCs). hMSCs (2500 cells/cm²) in hMSC basal medium (without serum) were maintained in a humidified, 37°C , 5% $\text{CO}_2/95\%$ air environment for 2 h. A, B, and C indicate significant difference ($p < 0.05$) between the respective groups. n = number of experiments performed on separate occasions.

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