Polímeros
ISSN 1678-5169 (Online)

https://doi.org/10.1590/0104-1428.20250067

ORIGINAL ARTICLE

Surface properties of stainless steel coated with plasma-modified gelatin films

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Abstract

This study investigates the surface modification of gelatin films coated on stainless steel using radio-frequency plasma treatment, with a particular emphasis on O_2-plasma. Gelatin films cross-linked with glutaraldehyde were subjected to radio frequency plasma modification using N_2, O_2, and Ar atmospheres. Results showed that O_2-plasma was most effective, reducing the water contact angle from 97.3° to 24.9° after 600 seconds due to the introduction of hydrophilic functional groups (–OH, –COOH, –$CONH_2$). Short O_2-plasma treatments (10-30 s) significantly decreased bovine serum albumin (BSA) adsorption from 452.5 to 334.4 μg/L ($P < 10^{-4}$), indicating improved anti-protein adsorption behavior. However, treatments exceeding 60 seconds caused surface cracking and increased BSA adsorption due to higher roughness. The study concludes that controlled short-term O_2-plasma modification effectively enhances the surface performance of gelatin-coated stainless steel for biomedical applications.

Keywords: *gelatin, plasma, surface modification, bovine serum albumin.*

Data Availability: All data supporting the findings of this study are available from the corresponding author upon request.

How to cite: Chang, S.-H., & Tseng, C.-Y. (2026). Surface properties of stainless steel coated with plasma-modified gelatin films. *Polímeros: Ciência e Tecnologia*, *36*(1), e20260005. https://doi.org/10.1590/0104-1428.20250067

1. Introduction

For decades, metallic biomaterials such as stainless steel, titanium and titanium alloys, cobalt-based alloys, and TiNi shape memory alloys have been extensively utilized in surgical prosthetics and orthotics[1-3]. These materials are generally characterized by their excellent mechanical properties and adequate biocompatibility, making them suitable for various medical and surgical applications. Stainless steel is commonly employed for orthopedic implants, such as in joint replacement and bone fracture fixation, mainly due to its low cost[4-6]. A key drawback, however, is the corrosion that occurs upon interaction with living tissues. This process compromises the protective chromium oxide layer, leading to the release of metallic ions (Ni^{2+}, Cr^{3+}, and Fe^{3+}) into the body. These released ions are potential hazards that can cause local and systemic adverse effects, contribute to prosthetic loosening, and interfere with the essential proliferation and differentiation balance observed in osteoblastic human alveolar bone cell cultures. Therefore, a significant challenge when these metallic biomaterials are exposed to physiological environments is their inherent inadequate corrosion resistance and the potential for undesirable leaching of metallic ions, which may restrict their long-term clinical viability. To overcome these limitations, the surfaces of these biomaterials are routinely modified or protected by various biomedical coatings, including ceramics, bioactive glass, functional coatings, and various polymers[7-15]. Gelatin is a highly versatile biomaterial, and it is frequently applied in coatings across diverse fields, notably within biomedicine. Its biocompatibility, biodegradability, and unique capacity to mimic the extracellular matrix render it exceptionally well-suited for tissue engineering and drug delivery. Therefore, gelatin is also a potential candidate for surface protection materials for metallic implants.

A substantial body of research has consistently demonstrated the significant potential of gelatin and its composite derivatives for various biomedical applications[16-18]. For instance, Tytgat et al.[19] developed a novel norbornene-functionalized gelatin combined with thiolated gelatin to create hydrogel scaffolds via additive manufacturing for adipose tissue engineering. These photo-click scaffolds, incorporating a cell-interactive crosslinker, formed a homogeneous network through step-growth polymerization. Benchmarked against methacrylamide-modified gelatin, these scaffolds exhibited superior physicochemical properties, supported high cell viability and proliferation, and demonstrated enhanced adipogenic differentiation, thus proving promising for tissue reconstruction. Ghorbani et al.[20] developed polycaprolactone (PCL) scaffolds, which were freeze-cast and subjected to oxygen plasma modification to facilitate gelatin grafting. This surface modification significantly enhanced the scaffolds' hydrophilicity and biodegradation while improving cell attachment and viability. The modified scaffolds also effectively supported filopodia formation. These findings collectively suggest that gelatin-grafted, oxygen plasma-modified PCL scaffolds hold considerable promise for wound healing applications. Wattanavijitkul et al.[21] fabricated poly(vinyl

alcohol)/gelatin (PVA/gelatin) hydrogel films, cross-linked with glutaraldehyde, to coat vancomycin-loaded titania nanotubes (TNTs). This aimed to enhance biocompatibility and achieve controlled vancomycin release. The coated TNTs demonstrated slower vancomycin release compared to uncoated TNTs. Furthermore, they promoted osteogenesis, evidenced by increased alkaline phosphatase activity and calcium accumulation, and exhibited antimicrobial efficacy against *E. coli* and *S. aureus*. These findings highlight the potential for efficient drug delivery and controlled release in biomedical implant applications.

Gelatin's surface is frequently modified for medical applications to overcome its inherent limitations and tailor its properties for specific biomedical functions. Common surface modification techniques include grafting, chemical cross-linking, nanoparticle coatings, and plasma modification[22-24]. Among these, plasma surface modification stands out as an economical and effective method for enhancing biomaterial films' biocompatibility, biofunctionality, and hydrophilicity[25-29]. Plasma is recognized as the fourth state of matter, formed by energizing a gas. This energy input causes excitation and subsequent partial or complete ionization of the gas molecules. The resulting plasma is a complex mix of ions, free radicals, reactive species, unstable dynamic molecules, and radiation of varying wavelengths emitted as excited molecules stabilize. Consequently, plasma processes have become a promising alternative to conventional coating and grafting methods for the surface modification of polymeric membrane materials, offering significant advantages such as fast reaction times, waste-free operation, and high versatility[30]. Several studies report that plasma modifications effectively enhance the surface properties of gelatin for biomedical applications. For example, Mozaffari et al.[31] introduced novel argon and argon–oxygen plasma treatments for electrospun tannic acid-crosslinked gelatin nanofibers, specifically targeting tissue engineering applications. Their research indicated that plasma treatment increased surface roughness and introduced new chemical groups on the nanofibers. Notably, argon–oxygen plasma significantly enhanced hydrophilicity, which fostered greater fibroblast cell adhesion and viability. Hesari et al.[32] fabricated porous polyurethane (TPU) scaffolds using freeze-drying and subsequently grafted gelatin onto them via oxygen plasma treatment. This modification significantly enhanced the scaffolds' wettability and hydrolytic biodegradation. Although a slight decrease in mechanical properties was observed, the authors suggested that this method successfully created an activated surface for gelatin grafting, thereby achieving optimal features for neo-tissue formation. Despite these advancements in modifying gelatin on other substrates, the effects of plasma modifications directly on gelatin films' intrinsic properties remain largely unexplored. Therefore, this study aims to investigate how plasma surface modification influences the surface properties of gelatin films.

2. Materials and Methods

2.1 Preparation and surface modification of gelatin films

The stainless steel used in this study was acquired from Soonglee Metals Inc., Taiwan. Its surface underwent progressive grinding with abrasive paper, followed by polishing with 0.3 μm Al_2O_3 powder. The stainless steel specimens were then precisely cut into dimensions of 20 × 30 × 1 mm using a low-speed diamond saw. To ensure cleanliness, the stainless steel samples were sequentially cleaned with isopropanol and deionized water to remove any residual organic matter. Subsequently, these cleaned samples were immersed in piranha solution (a 3:1 v/v mixture of concentrated sulfuric acid (95%) and hydrogen peroxide solution (35%)) for 15 minutes. This pretreatment aimed to increase the density of hydroxyl functional groups on the sample surface. The gelatin solution was prepared by thoroughly mixing 0.5 g of gelatin and 25 mL of 85% formic acid. This mixture was stirred at 300 rpm at room temperature until the gelatin was completely dissolved. Following dissolution, 0.5 mL of 25% glutaraldehyde was added as a cross-linking agent. The solution was allowed to react for 30 minutes, then left to stand to remove any air bubbles. For coating, the pretreated stainless steel specimens were placed in a glass culture dish, and the prepared gelatin solution was poured over them. The dishes were then placed in a vacuum cabinet at room temperature for 24 hours to facilitate the formation of the gelatin-coated stainless steel specimens, which were subsequently used for characterization. All chemical reagents, including gelatin, formic acid, and glutaraldehyde, were purchased from Echo Chemical Co. Ltd., Taiwan.

Some of the gelatin-coated stainless steel specimens underwent further surface modification via plasma treatment. The plasma modification was carried out using a radio frequency (RF) plasma system, which consisted of a Pyrex bell jar reactor. The RF generator's frequency was set to 13.56 MHz. Within the reactor, the cathode was connected to the high-potential end of the RF generator, while the anode was grounded. The gelatin-coated specimens were positioned on the anode electrode. The distance between the cathode and anode was maintained at 100 mm. Before gas introduction, the system was evacuated to a base pressure of 10^{-3} Torr or below. High-purity gases (99.9%) of argon, oxygen, and nitrogen were then separately introduced into the chamber as working gases. The working pressure was adjusted to a stable 0.125 Torr with a flow rate of 10 sccm for each gas. The plasma modification process itself was performed at a constant power of 50 W, with treatment durations varying from 10 to 600 sec.

2.2 Characterization

The wettability properties of the plasma-modified gelatin film surfaces were assessed using the sessile drop method with a contact angle instrument (FTA125, First Ten Ångstroms, USA). The measuring range of the water contact angle meter is 0° to 180°, the experimental accuracy is 0.01°, and the minimum scale for quantitative titration is 0.002 mL. Nine measurements were recorded at various locations on the film; the maximum and minimum values were omitted to calculate the average contact angle. The functional groups present on the plasma-modified gelatin films were identified using an attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectrometer (Spectrum 100, PerkinElmer). The spectral range of the ATR-FTIR spectrometer is 7,800 to 350 cm^{-1}, the wavelength resolution is 0.5 cm^{-1}, and the wavelength accuracy is 0.1 cm^{-1}. Each specimen was measured in the range of 4000–6500 cm^{-1} using 16 scans at a resolution of 4 cm^{-1}. Surface

morphology observations of the plasma-modified gelatin films were performed using a scanning electron microscope (SEM) (5136 MM, Tescan Instruments). The amount of adsorbed protein on the surface of plasma-modified gelatin films was quantified using the bicinchoninic acid (BCA) protein assay. Bovine serum albumin (BSA), purchased from Bio Basic Inc., served as the protein for the BCA assay. Phosphate buffer solution (PBS) and sodium dodecyl sulfate (SDS), used for protein adsorption analysis, were acquired from UniRegion and Sigma-Aldrich, respectively. The optical density (OD) values from the protein adsorption analysis were measured at 562 nm using a spectrophotometer (GENESYS 20, Thermo Scientific), with PBS solution as the blank cuvette. The spectral bandwidth of the spectrometer is less than 8 nm, the wavelength accuracy is 2 nm. The average OD value was calculated based on nine measurements, omitting the maximum and minimum values.

3. Results and Discussions

3.1 Wettability properties

Figure 1 presents the water contact angles (WCAs) of stainless steel, stainless steel coated with unmodified gelatin film, and those coated with plasma-modified gelatin films using N_2, O_2, and Ar atmospheres for 600 sec. The bare stainless steel and the unmodified gelatin-coated samples exhibited hydrophobic surfaces, with WCAs of approximately 94.3 ± 1.9° and 97.3 ± 2.3°, respectively. While gelatin is generally considered hydrophilic due to its abundance of carboxyl, amino, hydroxyl, and amide functional groups, the hydrophobic nature of the unmodified gelatin film observed in this study is attributed to the inclusion of glutaraldehyde during the cross-linking process, which alters the film's surface hydrophilicity. Plasma modification significantly reduced the WCA of the gelatin surface from above 90° to approximately 34.7 ± 1.7° (N_2-plasma), 24.9 ± 2.5° (O_2-plasma), and 45.2 ± 1.0° (Ar-plasma) after 600 sec of treatment. This indicates that plasma modification with all tested atmospheres rendered the gelatin surface hydrophilic. Notably, O_2-plasma modification demonstrated a more pronounced effect than N_2 or Ar because of its relatively higher reactivity. Consequently, subsequent experiments will focus exclusively on the impact of O_2-plasma modification on the surface properties of the gelatin films.

Figure 2 illustrates the WCAs of unmodified and O_2-plasma modified gelatin films treated for various time intervals. The WCA of the gelatin film significantly decreased from approximately 97.3 ± 2.3° to 47.2 ± 2.8° after only 10 sec of O_2-plasma modification. This notable reduction demonstrates that even a short-term O_2-plasma treatment can effectively enhance the hydrophilicity of the gelatin film surface. Subsequently, the WCA gradually decreased with increasing O_2-plasma modification time, reaching a minimum of 24.9 ± 2.5° after 600 sec treatment.

3.2 ATR- FTIR analysis

Figure 3 presents the ATR-FTIR spectra of stainless steel specimens coated with O_2-plasma modified gelatin films treated for various time intervals. As shown in Figure 3, the unmodified gelatin film exhibits characteristic absorption bands. A broad peak observed between approximately 3000–3500 cm^{-1} is attributed to the overlapping stretching vibrations of the –NH and –OH groups. The band at approximately 2942 cm^{-1} corresponds to the stretching vibration of the –CH group. Furthermore, the characteristic absorption band at 1631 cm^{-1} is assigned to the C=O stretching of the primary amide (Amide I), while the band at 1538 cm^{-1} corresponds to the –NH bending vibration and –NO and –CN stretching of the secondary amide (Amide II). The peak at approximately 1450 cm^{-1} is indicative of the –C=N stretching of the aldimine formed due to gelatin cross-linking. Finally, the absorption band at approximately 1080 cm^{-1} is assigned to the –CO stretching vibration.

Figure 3 further illustrates that the characteristic peaks corresponding to oxygen-containing and nitrogen-containing functional groups became more pronounced following O2-plasma modification. This enhancement is attributed to the reaction between active plasma species and atoms on the gelatin film surface, leading to new oxygen-containing functional groups forming during modification. Additionally, free radicals generated on the gelatin surface during plasma treatment may react with atmospheric nitrogen, forming various nitrogen-containing functional groups. Consequently,

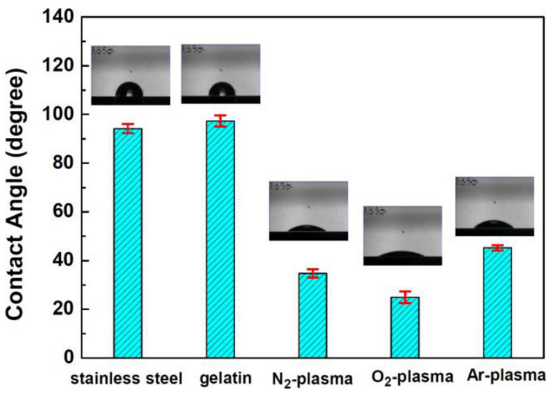

Figure 1. Water contact angle of bare stainless steel, stainless steel coated with unmodified gelatin film, and those coated with plasma-modified gelatin films using N_2, O_2, and Ar atmospheres for 600 sec.

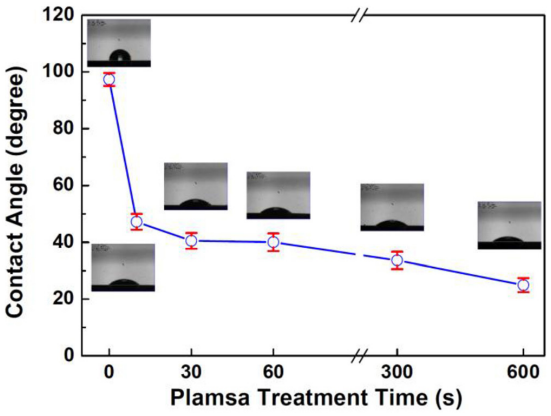

Figure 2. Water contact angle of stainless steel specimens coated with O_2-plasma modified gelatin films as a function of plasma treatment duration.

the observed increase in hydrophilic functional groups such as –OH, –COOH, and –$CONH_2$ is directly responsible for the improved hydrophilicity of the gelatin film surface after plasma modification

3.3 SEM imaging

Figures 4a-f present SEM images (100× magnification) showing the surface morphology of gelatin films modified by O_2-plasma for 0 (unmodified), 10, 30, 60, 300, and 600 sec, respectively. Figure 4a shows that the unmodified gelatin film exhibits a smooth and homogeneous surface morphology. Figures 4b-d reveal that this smooth and homogeneous surface remains intact when the O_2-plasma modification duration is less than 60 sec. However, Figure 4e depicts the initial appearance of fine cracks on the gelatin surface after 300 sec O_2-plasma modification, as indicated by the arrows. With extended treatment to 600 sec, Figure 4f clearly shows more pronounced crack propagation (indicated by arrows). This observation suggests that prolonged O_2-plasma modification time leads to the deterioration of the surface integrity of the gelatin films.

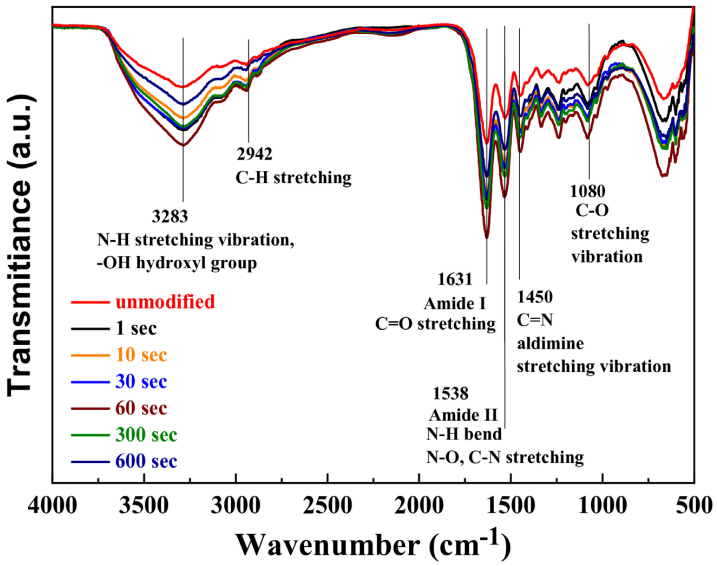

Figure 3. The ATR-FTIR spectra of stainless steel specimens coated with O_2-plasma modified gelatin films treated for various time intervals.

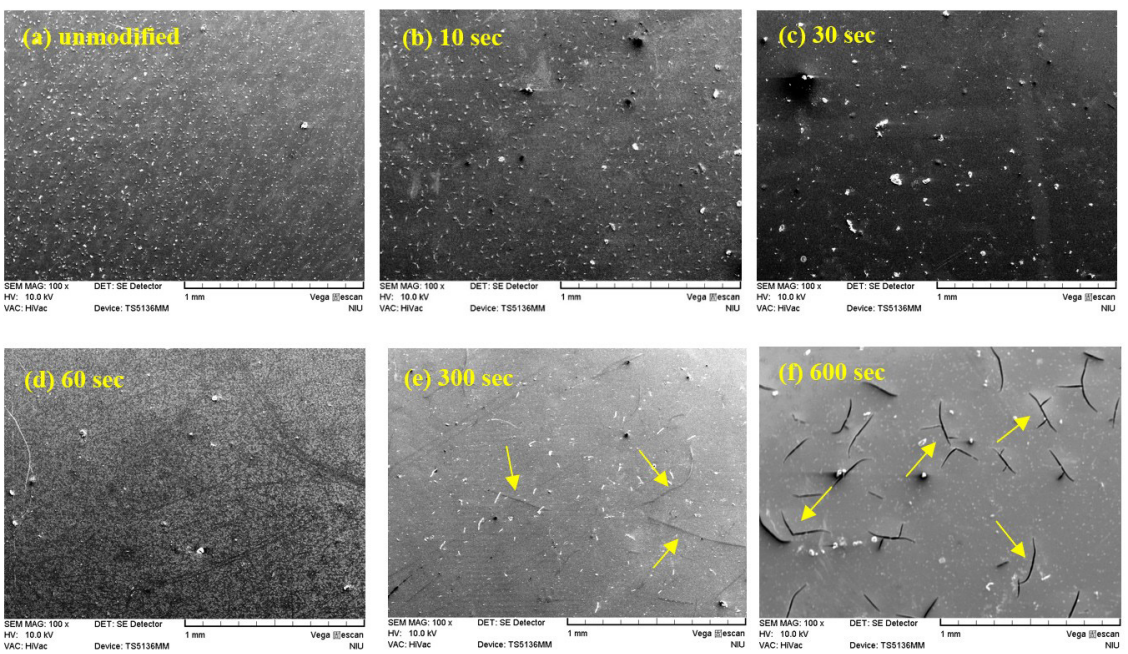

Figure 4. SEM images (100×) of gelatin film plasma-modified for (a) 0 sec (unmodified); (b) 10 sec; (c) 30 sec; (d) 60 sec; (e) 300 sec; (f) 600 sec.

3.4 Bicinchoninic acid (BCA) protein assay

The protein adsorption properties of O_2-plasma modified gelatin films were determined using a BCA protein assay. All unmodified and O_2-plasma modified gelatin films were first rinsed with PBS three times. Each rinsed film was immersed in 5 mL of BSA solution at 37 °C for 24 hours, followed by another rinse with PBS. Subsequently, each film was incubated in 2 mL of SDS solution for 24 hours to desorb the adsorbed protein. A 0.1 mL aliquot from each resulting solution was mixed with 1 mL of BCA solution in a cuvette, and its OD was measured using a spectrophotometer. The concentration of BSA adsorbed onto the films was calculated from these OD values using a pre-established BSA concentration standard curve.

Figure 5 presents the concentrations of adsorbed BSA on the surfaces of the unmodified and O_2-plasma modified gelatin films in the BCA protein assay. The unmodified gelatin film exhibited a BSA adsorption concentration of approximately 452.5 ± 22.8 μg/L. Notably, after just 10 sec of O_2-plasma modification, the BSA concentration on the gelatin film's surface significantly decreased to 394.8 ± 19.5 μg/L. This marked reduction indicates plasma modification effectively enhances the film's anticoagulant properties. Further increasing the O_2-plasma modification time initially led to a continued decrease in adsorbed BSA, reaching approximately 334.4 ± 19.0 μg/L after 30 sec of O_2-plasma modification. However, for O_2-plasma modification times extending from 60 to 600 s, the concentration of adhered BSA on the surface of the modified gelatin films showed a slight increase, rising from 421.0 ± 36.4 to 442.2 ± 24.5 μg/L.

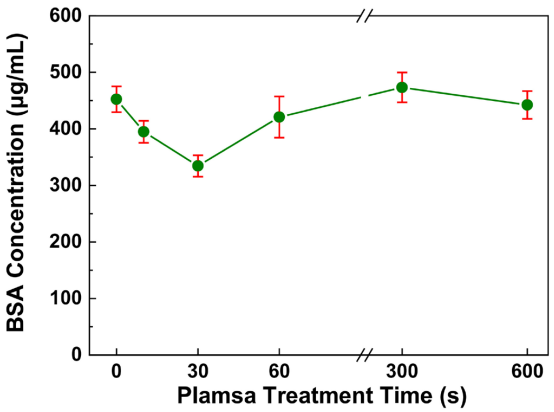

Figure 5. BSA adsorption concentrations of stainless steel specimens coated with O_2-plasma modified gelatin films as a function of plasma treatment duration.

Table 1 lists the 95% confidence interval values and the P-values of the BSA adsorption concentrations of stainless steel specimens coated with O_2-plasma modified gelatin films estimated from Figure 5. According to the significance analysis, the P-values calculated from the BSA adhesion concentration results were approximately 2.9×10^{-4} and 1.5×10^{-6} after O_2-plasma modification for 10 and 30 sec, respectively. This indicates that the decrease in the BSA adhesion concentration upon 10 and 30 sec O_2-plasma modifications was highly significant (P□0.05). Accordingly, we concluded that both 10 and 30 sec O_2-plasma modification effectively improved the protein adsorption property of the gelatin film. On the other hand, the P-values calculated from the BSA adhesion concentration results were approximately 0.0808, 0.1420, and 0.4314 after O_2-plasma modification for 60, 300, and 600 sec, respectively. This suggests no statistically significant difference between the unmodified gelatin film and the prolonged O_2-plasma modified gelatin films.

Figure 5 and Table 1 illustrate that short-term O_2-plasma modification significantly reduces the quantity of BSA protein adsorbed on the gelatin film surface. This phenomenon can be attributed primarily to the enhanced hydrophilicity of the gelatin film surface after plasma treatment. Proteins typically exhibit a higher affinity for hydrophobic surfaces, as non-polar environments can destabilize proteins. This promotes conformational reorientations, leading to stronger protein-protein and protein-surface interactions[33,34]. Additionally, BSA is rich in carboxylic acid groups, and the abundant carboxylic acid groups introduced on the plasma-modified gelatin surface may generate charge-charge repulsion, further inhibiting BSA adhesion. However, an unexpected trend was observed for prolonged plasma treatment: gelatin films modified for more than 60 sec exhibited a higher concentration of adsorbed BSA than films treated for 30 sec, despite their superior hydrophilicity. This counterintuitive result stems from the formation of cracks on the film surface after prolonged O_2-plasma modification, as demonstrated in Figure 4. These surface cracks increase the surface roughness, leading to a higher concentration of BSA protein adsorption. Therefore, this study demonstrates that while O_2-plasma modification significantly improves the hydrophilicity and reduces protein adsorption of gelatin, whereas precise control over the plasma treatment duration is crucial to avoid surface deterioration.

4. Conclusions

This study demonstrated the potential of O_2-plasma modified gelatin films for various biomedical applications, particularly when coated on stainless steel. A central finding

Table 1. The 95% confidence interval values and the P-values of the BSA adsorption concentrations of stainless steel specimens coated with O_2-plasma modified gelatin films estimated from Figure 5.

O_2-plasma modifications	95% Confidence Interval	P-value
0 sec (unmodified)	452.5 ± 21.1	-
10 sec	394.8 ± 18.0	2.9×10^{-4}
30 sec	334.4 ± 17.6	1.5×10^{-6}
60 sec	421.0 ± 33.7	0.0808
300 sec	473.3 ± 24.5	0.1420
600 sec	442.2 ± 22.7	0.4314

was the successful conversion of initially hydrophobic gelatin films into hydrophilic surfaces through O_2-plasma modification. This enhanced hydrophilicity, characterized by a substantial reduction in water contact angles, was attributed to the increased presence of hydrophilic functional groups such as –OH, –COOH, and –$CONH_2$ on the film surface. Short-term O_2-plasma treatments (10-30 sec) effectively inhibited BSA adsorption on the gelatin films. This beneficial effect is primarily linked to the improved hydrophilicity, as proteins generally exhibit lower affinity for hydrophilic surfaces. However, the research also revealed a critical aspect: prolonged O_2-plasma modification (exceeding 60 sec) led to surface deterioration, specifically the formation of cracks and increased roughness. This surface damage, despite continued hydrophilicity, counterintuitively increased in BSA adsorption. Therefore, while O_2-plasma modification is a promising technique to enhance the surface properties and anti-protein adsorption capabilities of gelatin films, precise control over treatment duration is paramount to optimize performance and prevent detrimental surface changes.

5. Author's Contribution

- **Conceptualization** – Shih-Hang Chang.
- **Data curation** – Shih-Hang Chang; Chun-Yi Tseng.
- **Formal analysis** – Shih-Hang Chang; Chun-Yi Tseng.
- **Funding acquisition** - Shih-Hang Chang.
- **Investigation** – Shih-Hang Chang; Chun-Yi Tseng.
- **Methodology** – Shih-Hang Chang; Chun-Yi Tseng.
- **Project administration** – Shih-Hang Chang.
- **Resources** – Shih-Hang Chang.
- **Software** – NA.
- **Supervision** – Shih-Hang Chang.
- **Validation** – NA.
- **Visualization** – Shih-Hang Chang; Chun-Yi Tseng.
- **Writing – original draft** – Chun-Yi Tseng.
- **Writing – review & editing** – Shih-Hang Chang.

6. Acknowledgements

The authors gratefully acknowledge the financial support for this research provided by the National Science and Technology Council (NSTC), Taiwan, under Grant NSTC 112-2221-E-197-014-MY2.

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Received: Aug. 20, 2025
Revised: Oct. 13, 2025
Accepted: Nov. 06, 2025

Editor-in-Chief: Sebastião V. Canevarolo