Environmentally Friendly Sterilization and Enhancement of Cellulose Using RF Plasma Process

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(Received: 06. Sep. 2025, Accepted: 27. Dec. 2025, Published online: 31. Dec 2025)

Cellulose-based materials are widely used in wound care due to their biocompatibility, biodegradability, and fluid-handling capacity. While chemical functionalisation is commonly employed to impart antimicrobial activity, the role of physical surface modification in regulating bacterial adhesion remains less explored. In this study, low-pressure radiofrequency (RF) oxygen plasma was used as a dry and environmentally friendly approach to modify the surface of medical-grade cellulose without altering its bulk properties. Plasma treatment was performed in both glow and afterglow regions, enabling controlled exposure to reactive oxygen species. Surface modification resulted in pronounced nanoscale roughening and fissured topography of cellulose microfibers, as observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM). Plasma-induced oxidation of the cellulose surface, characterised previously by X-ray photoelectron spectroscopy (XPS), accompanied the morphological changes. Bacterial adhesion experiments using a non-pathogenic Escherichia coli model strain revealed significantly enhanced bacterial attachment on plasma-treated cellulose compared to untreated controls, with the strongest effect observed for glow-region treatments. The increased adhesion is attributed to the combined effects of surface roughness amplification and plasma-induced chemical functionalisation, which together increase the effective contact area between bacteria and the substrate. Rather than aiming to inhibit bacterial attachment, this work explores a physico-mechanical design concept in which surface topography is intentionally modified to favour bacterial binding to the dressing material itself. The observed behaviour is interpreted qualitatively using concepts from membrane mechanics as a phenomenological framework, without invoking a quantitative predictive model. While the present study does not assess net bacterial load reduction in wound environments, it establishes a materials-level basis for a "capture-and-remove" hypothesis, whereby preferential bacterial adhesion to a removable dressing could contribute to microbial load management during dressing changes. These findings highlight the potential of plasma surface engineering as a versatile tool for tailoring the bio interface of cellulose-based biomedical materials.

(DOI: https://doi.org/10.31281/87jpcc23)

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I. Introduction

Cellulose, derived from botanical sources or synthesised by microbial processes, has emerged as a cornerstone material in modern regenerative medicine [1]. Its unparalleled biocompatibility, biodegradability, and exceptional fluid management capabilities have established it as an ideal platform for advanced wound dressings, surgical meshes, and tissue engineering scaffolds [2-4]. While the chemical functionalisation of cellulose to impart antimicrobial, anti-

inflammatory, or pro-angiogenic properties on nano cellulose has been extensively explored [5-6], the profound influence of its physical and topographical surface characteristics on biological outcomes is increasingly recognised as a critical design parameter. Nano cellulose increases the available surface area by forming nanoscale fibrils [7], thereby generating a highly developed fibrillar network. In contrast, in our system the large surface area is achieved not through fibril formation, but by direct modification of the substrate surface using plasma treatment, which induces high surface roughness.

The surface of a biomaterial serves as the primary interface for all host and microbial interactions [8]. In wound care, this interface must perform a dual role, integrating with and supporting regenerating tissue while defending against pathogenic colonization [9]. Traditional strategies often focus on creating anti-adhesive or eluting surfaces to suppress bacterial colonization [8]. However, this work hypothesizes an alternative paradigm: the intentional and strategic engineering of surface topography to actively guide microbial behaviour for therapeutic benefit. At the forefront of this physical surface manipulation is low-pressure plasma treatment as a versatile, dry, and eco-friendly technology. Plasma modification uniquely allows the simultaneous sterilization of materials and the precise alteration of surface morphology and energy without affecting bulk properties [10-13]. Through controlled etching, it can transform a smooth cellulose fiber into a complex, nano- and micro-rough landscape with significantly increased surface area [14].

In this article, we present an investigation into the effects of plasma-induced physical modification on the biological performance of cellulose-based wound dressings. Contrary to the conventional pursuit of ultra-smooth, bacteria-repellent surfaces, our experimental analysis reveals a compelling and unexpected finding: Plasma treatment, by increasing surface roughness and area, consistently enhances the initial adhesion and binding of bacteria (*Escherichia coli*, strain *DH5α*) to cellulose matrices. Rather than viewing this enhanced adhesion as a detrimental outcome, we propose its exploitation as a novel "capture-and-remove" therapeutic mechanism. We demonstrate that plasma-treated, rough cellulose gauze acts as a highly effective substrate, outcompeting the vulnerable wound bed for bacterial colonization. Subsequent removal of the dressing then facilitates the physical debridement of a bacteria load, offering a powerful method for microbial contamination reduction in heavily exuding wounds, such as burns [15]. Furthermore, we underscore that the plasma process itself serves as a final, efficient sterilization step prior to material packaging, ensuring microbial safety and creating a stable, bioactive surface ready for clinical application, which is a very well known procedure [16, 17].

This work shifts the focus from purely chemical warfare against bacteria to a physico-mechanical strategy [18], highlighting how the deliberate design of cellulose's physical landscape, achieved through plasma engineering, can unlock a new function: transforming a passive wound cover into an active decontamination device. We detail the characterization of the modified surfaces, describing bacterial adhesion dynamics, and discuss the critical parameters for optimizing this approach for safe and effective use in advanced wound management.

II. Experimental

Cellulose was studied experimentally using a small plasma reactor. The discharge chamber was of cylindrical shape with inner diameter 36 mm and length 250 mm and was connected to the afterglow chamber through a glass tube of inner diameter 5 mm in order to assure surface neutralization of charged particles. Both discharge and afterglow chambers were made from borosilicate glass. A copper coil was wrapped around the discharge chamber and connected to a radiofrequency generator with a nominal power of 700 W and a standard industrial frequency of 27.12 MHz. The afterglow chamber was pumped continuously during experiments using a Leybold fore-pump with an ultimate pressure of 0.1 Pa. Oxygen was leaked continuously into the discharge chamber through a manually adjustable needle valve. The pressure in discharge chamber was estimated with a Pirani gauge and was about 200 Pa while in the afterglow chamber it was about 100 Pa. Plasma parameters were estimated with electrical and catalytic probes as well as by optical emission spectroscopy. Both hydrogen and oxygen atomic lines were observed in spectra indicating the presence of excited atoms with excitation energy exceeding 10 eV. The density of charged particles was of the order of 10^{16} m^{-3} and the electron temperature was close to 2.5 eV (40000 K) [14], [18] . The density of neutral oxygen atoms in the discharge chamber was $7x10^{21}$ m^{-3} while in the

afterglow chamber at the position of substrates it was $4x10^{20}$ m^{-3} under reactor-level conditions. Local exposure at curved fibers may vary; sample-level dosimetry and etch-rate calibration were documented to ensure reproducible surface modification. The reported atom densities characterize reactor-level conditions at the positions indicated. Local atom flux and etch rate at the fiber surface, and exposure uniformity across sample positions, were not directly measured in this study and will be mapped to support reproducibility in further experiments.

We have been using highly reactive RF oxygen plasma created in a vacuum chamber, shown in the schematic image of different regions in Fig. 1. At the sample cellulose fibre position in the glow region in the glass tube, the treatment times were varied from 1 to 60 s where we could observe destruction of the cellulose material. At the position in the afterglow region in the glass tube, the treatment time was adjusted from 10 to 500 s, where no degradation of the cellulose was observed. In the glass tube presented on Fig.1 positions of samples treated in plasma glow or in the afterglow region are shown. Our experimental samples of medical grade cellulose membranes were incubated in *E. coli* bacterial solution after plasma glow and afterglow treatment. 0.1 g of cellulose samples, which were inserted into a 70 mL container and incubated with 1.0 mL of liquid culture containing 1-2 x 10^6 colony forming units (CFU) of bacteria. The bacterial concentration was determined using two complementary methods: UV-VIS spectroscopy, which measures the cloudiness of the liquid to estimate the total number of bacteria, and agar plate counting, which involves growing the bacteria in a Petri dish to count the actual number of living, viable cells. Incubation was set at 37 °C for 2 h. Treated and untreated samples of cellulose fibres where washed 3 times each with 20 ml of MQ water to remove unattached bacteria from the surface. Samples were after cleaning fixed with glutaraldehyde and dried with ethanol to observe attached bacteria.

The effect of plasma treatment of cellulose materials was compared to untreated materials in terms of surface modification and bacteria adhesion by inspecting the sample fibres with a scanning electron microscope (SEM) Fig. 2 and atomic force microscope (AFM) Fig. 3. We used the high-resolution FEG-SEM 7600F from JEOL. Images were taken at 1,000x, 2,500x, 5,000x and 10,000x magnification using 5 kV accelerating voltage. The samples were sputter-covered with Au/Pd, and the cover was 3 nm thick. Low energy electron images (LEI) are presented in this paper. Images of cellulose fibres were obtained with an AFM (Solver PRO, NT-MDT) in the tapping mode in air at room temperature. The surfaces were analysed with standard Si cantilever with a force constant of 10 N/m and at resonance frequency of 170 kHz. The scanning rate was around 1.0 Hz.

III. Results

III.a). Cellulose Fibres Surface Morphology

The employed non-equilibrium RF plasma contained sufficiently high energetic particles to break C−C and C−O bonds within the cellulose polymer chain. The chemical influence of plasma on the surface of the cellulose fibres was investigated in a previous study [18] . There, high-resolution XPS C 1s spectra were used to resolve the surface chemical states and their quantity. In the untreated samples, the dominant contribution arose from C–C/C–H bonds, while oxygen-containing components (C–O, O–C–O, O=C–O) were less abundant. After plasma exposure, a progressive decrease in the C–C component was observed, accompanied by a clear increase in the C–O signal with treatment time, reflecting efficient surface oxidation. The most pronounced rise in C–O content occurred after 30 s of oxygen plasma treatment. The O–C–O contribution nearly doubled after 10 s of plasma exposure, and, although diminished somewhat at longer treatment times, it remained higher than in the untreated samples across all plasma conditions. A similar trend was seen for the O=C–O component, which also increased upon plasma treatment and stayed above the initial level, indicating the formation and persistence of more highly oxidized carbon species on the plasma modified surface. Afterglow (indirect) plasma generally produces a lower degree of surface oxidation and thus a lower density of newly formed polar oxygen containing groups than a glow (direct) plasma at comparable treatment times. This is reported in an article using wheat seed samples [19], where this manifests as a smaller XPS increase in O at.% for afterglow treated samples compared with glow treated samples, indicating weaker oxidative functionalization and, by extension, a lower affinity for oxidation driven surface interactions.

AFM analysis (Fig. 2) revealed a observable increase in cellulose surface roughness following plasma exposure. In the glow region with high density of neutral oxygen atoms aggressive

etching led to a rapid increase in roughness. While prolonged exposure (>60 s) caused structural degradation of the fibers, shorter treatments created a highly fissured topography. Conversely, treatment in the afterglow region with lower neutral oxygen atom density allowed for surface functionalization and milder etching without compromising the mechanical integrity of the cellulose.

The SEM analysis (Fig. 3) clearly illustrates the profound impact of plasma treatment on both the surface morphology of cellulose fibers and the resulting bacterial behavior. The untreated cellulose fibers exhibit a relatively smooth surface with minimal bacterial attachment, serving as a baseline for the study. Samples treated in the afterglow region show a slight increase in surface roughness compared to the untreated cellulose.

III.b) Qualitative Interpretation of Surface–Bacteria Interactions

This mild topographical modification corresponds to a noticeable increase in bacterial binding, indicating that even subtle changes in surface texture enhance the initial interaction between the fiber and the bacteria. The most significant changes are observed in the fibers treated within the plasma glow region, which exhibit the highest degree of surface roughness. These samples demonstrate the densest bacterial colonization, where cells are not only attached to the complex surface landscape but also appear to bind to one another, forming multi-layered attachments.

This visual evidence supports the "capture-and-remove" hypothesis. The intense roughening created by the glow plasma creates an environment that bacteria strongly prefer, effectively "locking" them onto the dressing material. The observation of bacteria remaining attached even after washing steps suggests a stable, high-affinity bond driven by the mechanical compatibility between the bacterial membrane and the modified surface topography.

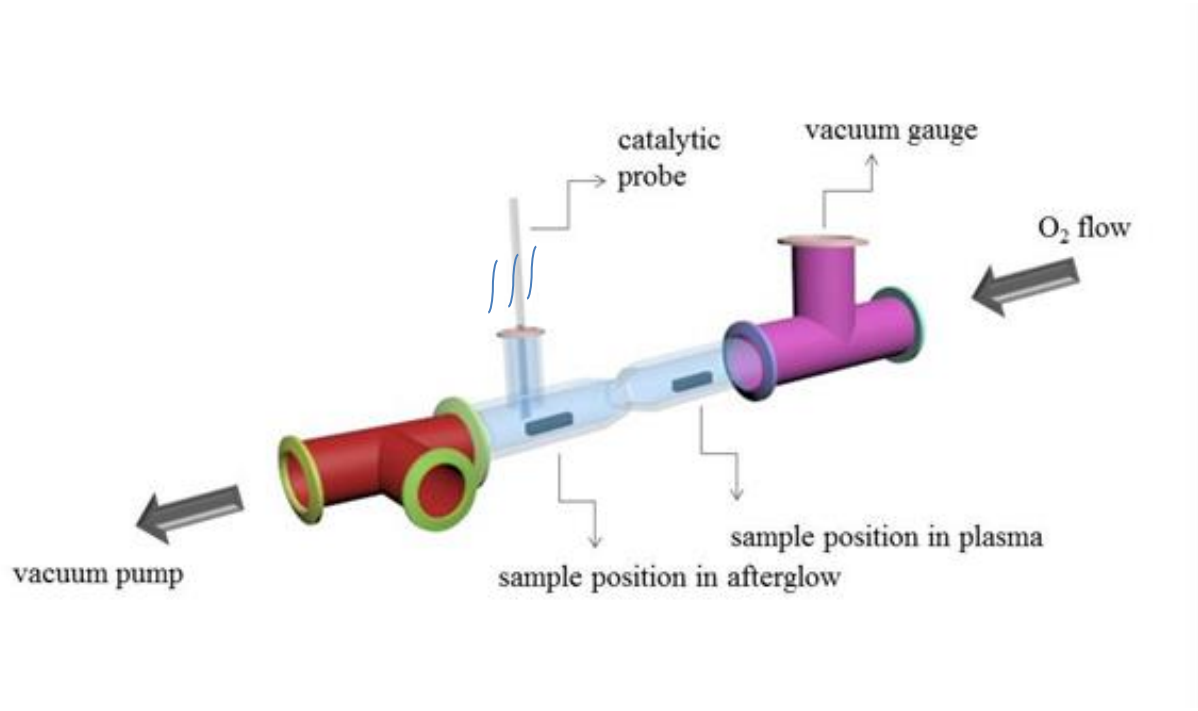

Figure 1: The experimental setup for treatment of samples with neutral oxygen atoms. Components: vacuum pump, experimental chamber, discharge chamber (sample position), sample position in afterglow, vacuum gauge, catalytic probe, inlet valve, oxygen flask.

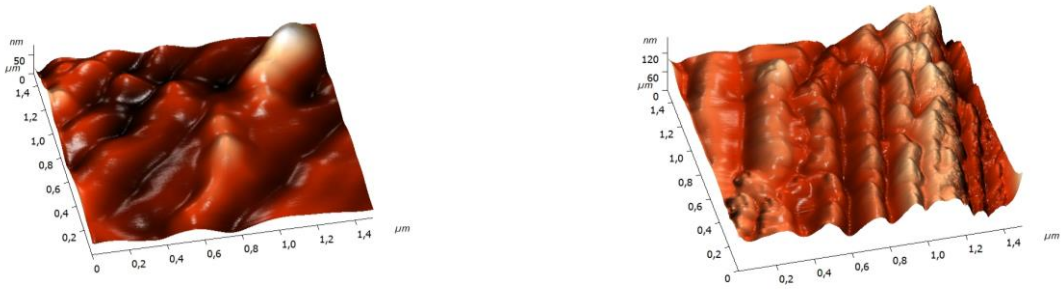

Figure 2: Atomic force microscopy (AFM) topographs of cellulose fibers. **(A)** Untreated fibers exhibit smooth morphology with a height scale of 0–50 nm. **(B)** Glow plasma-treated fibers (30 s exposure) display significantly increased nano roughness, with a height scale extended to 0–120 nm. The plasma treatment induces pronounced fissured topography and elevated RMS roughness compared to the untreated controls.

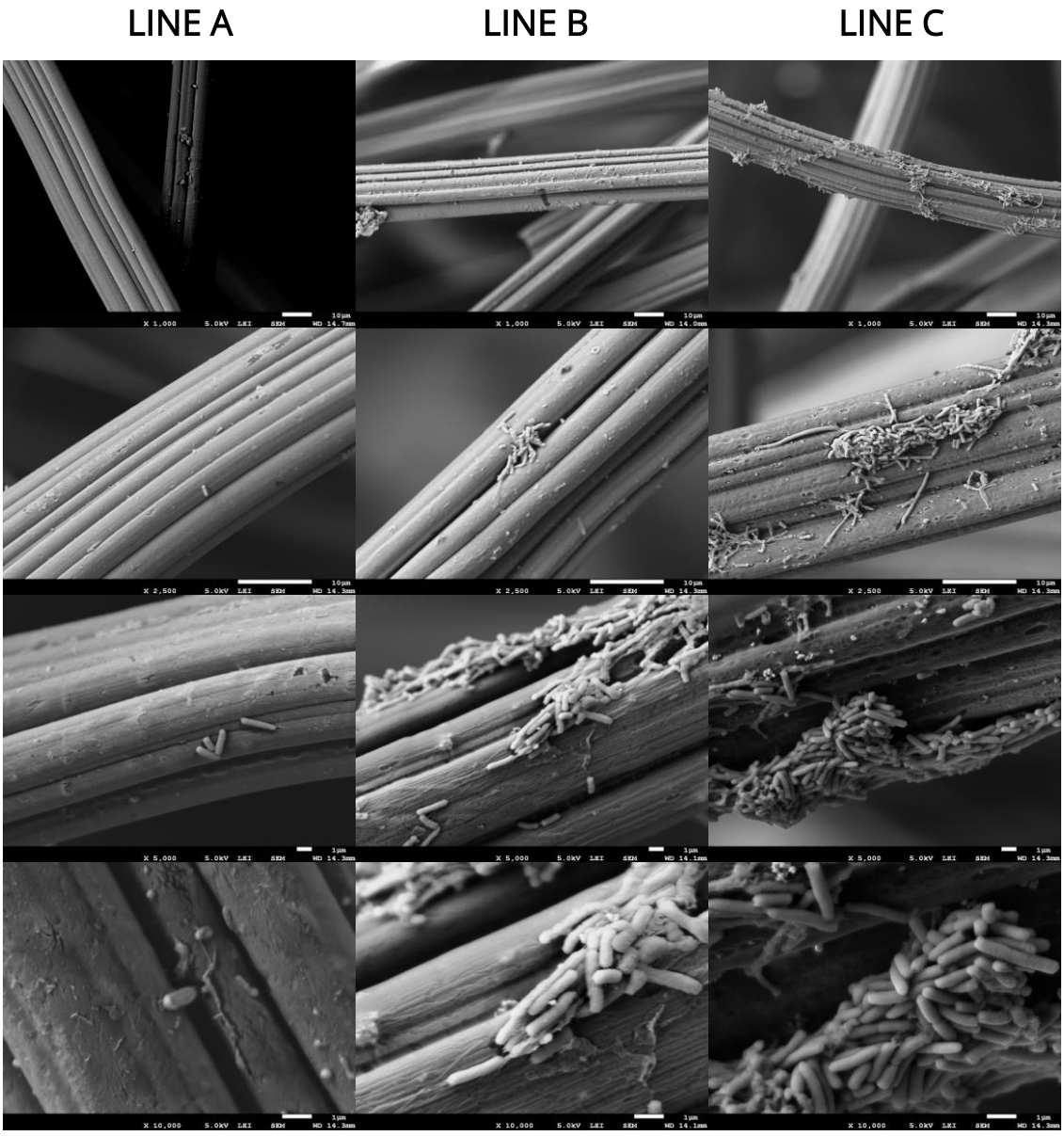

Figure 3: Scanning electron microscopy (SEM) images of cellulose fibers incubated in *E. coli* suspension for 2 hours. **(A)** Untreated fibers show minimal bacterial attachment. **(B)** Afterglow plasma-treated fibers (500 s exposure) exhibit moderate bacterial colonization. **(C)** Glow plasma-treated fibers (60 s exposure) demonstrate the highest bacterial density with extensive surface coverage. Both plasma treatments significantly enhance adhesion compared to untreated controls, with glow conditions yielding substantially more attachments than afterglow.

Nanostructuring surfaces is an emergent strategy to endow materials with the ability to combat pathogenic bacteria. However, creating well-defined nano spike or nano fibrillar structures on curved polymeric substrates, such as gauzes and other microfibrous medical materials, remains highly challenging due to their complex geometry and intrinsic material limitations Approaches based on nano cellulose rely on the formation of nanoscale fibrils, which increase the available surface area through a dense fibrillar network, has been published by the Y. Hata group showing importance of surface structure on binding of bacteria [20]. However, our strategy does not involve the generation of nano spike or fibrillar architectures. Instead, a large effective surface area is achieved by plasma-induced modification of the polymer surface itself, where enhanced nanoscale roughness is introduced directly onto the randomly rough surface. This approach enables surface area increase without altering the macroscopic structure of the material or requiring the formation of discrete nano structures. Consistent with SEM evidence of enhanced bacterial adhesion to plasma-treated cellulose (Fig. 3), we hypothesize that preferential colonization of the dressing could enable physical bacteria load reduction upon removal of the gauze from a wound. However, this study does not quantify net reduction of wound-bed contamination or total bacterial load after dressing removal; system-level validation will be conducted in a co-culture assay in the future.

The experimental observation that plasma-treated cellulose exhibits enhanced bacterial binding can be qualitatively discussed using concepts from membrane mechanics. In earlier studies on nanostructured surfaces, it has been suggested that cell–surface interactions are influenced by a balance between membrane deformation and adhesion forces. This balance is often expressed using a dimensionless interaction parameter, referred to as the Interaction Index (Ic) [21].

We used a non-pathogenic laboratory strain of *E. coli* as a model organism, as the adhesion and surface interaction mechanisms probed here closely resemble those of clinically relevant pathogenic strains [22-24] involved in extraintestinal infections, including skin and soft tissue infections. For E. coli, the plasma treatment appears to create a surface geometry that is mechanically more compatible with the bacterial cell envelope and its appendages (such as pili or flagella), enabling closer contact with the surface compared to untreated cellulose.

III.c) Mechanical interlocking: A Physical Interpretation

We propose that the enhanced retention of bacteria on plasma-treated cellulose is driven by mechanical interlocking. Plasma treatment creates a "rough" landscape of nanoscale fissures that act as physical traps for bacteria. This interaction can be explained qualitatively through the Interaction Index (Ic), which balances the energy cost of a bacterium deforming its membrane to fit into a fissure against the energy gained from sticking to the surface. Our images suggest that plasma-treated samples fall into an "optimal" regime ($0<Ic<1$) where the attraction is strong enough to pull the bacteria into these surface gaps. In this state, the bacteria become physically "locked" within the topography, significantly slowing down their ability to detach. This creates a capture-and-remove effect: bacteria preferentially bind to the dressing's rough fibers rather than the wound, allowing them to be easily eliminated when the dressing is changed. While this interpretation is phenomenological and based on visual trends rather than new calculations, it aligns with established mechanical models of cell-surface compatibility.

IV. Conclusions

Low-pressure RF oxygen plasma treatment effectively modifies medical-grade cellulose by introducing nanoscale roughness that scales with treatment intensity. SEM analysis confirms a clear trend: untreated fibers remain smooth with minimal bacterial affinity, while afterglow plasma introduces slight roughness that increases binding. Glow plasma creates the highest degree of surface roughness, resulting in the densest bacterial colonization where cells firmly nest into the topography and to each other. This behavior is driven by mechanical interlocking, where the roughened landscape acts as a physical trap. Using the Interaction Index (Ic) framework, we interpret these results qualitatively: glow plasma creates an optimal environment ($0<Ic<1$) where adhesion energy outweighs the cost of membrane bending. This allows the *E. coli* envelope to integrate into surface features, supporting a "capture-and-remove" hypothesis where the dressing is predicted to serve as a more energetically favourable substrate for

bacterial attachment than the biological tissue of the wound bed.

Based on this framework, we predict that the bacterial population could be effectively displaced from the wound environment and localized within the fiber matrix. Consequently, the microbial load would be physically transferred from the wound site to the dressing, potentially enabling its mechanical elimination during dressing exchange. While our results establish a strong materials-level basis for this physical interlocking mechanism, further system-level studies are required to validate this predicted clinical efficacy.

V. Acknowledgment

The authors gratefully acknowledge the support of the RSF005 *SuharHeal* project conducted at the Jožef Stefan Institute and funding provided by the Slovenian Research Agency. The authors also acknowledge the program P2-0082.

VI. References

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