Accepted Manuscript

Title: Albumin biofunctionalization to minimize the *Staphylococcus aureus* adhesion on solid substrates

Authors: María Laura Martín, Valeria Pfaffen, Laura E. Valenti, Carla E. Giacomelli

Please cite this article as: María Laura Martín, Valeria Pfaffen, Laura E.Valenti, Carla E.Giacomelli, Albumin biofunctionalization to minimize the Staphylococcus aureus adhesion on solid substrates, Colloids and Surfaces B: Biointerfaces https://doi.org/10.1016/j.colsurfb.2018.04.006

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Albumin biofunctionalization to minimize the *Staphylococcus aureus* adhesion on solid substrates

María Laura Martín[1,2], Valeria Pfaffen[1,2], Laura E. Valenti[1,2]* and Carla E. Giacomelli[1,2]*

[1]. Universidad Nacional de Córdoba. Facultad de Ciencias Químicas. Departamento de Fisicoquímica.

[2]. CONICET. Instituto de Investigaciones en Fisicoquímica de Córdoba (INFIQC). Ciudad Universitaria, X5000HUA, Córdoba, Argentina.
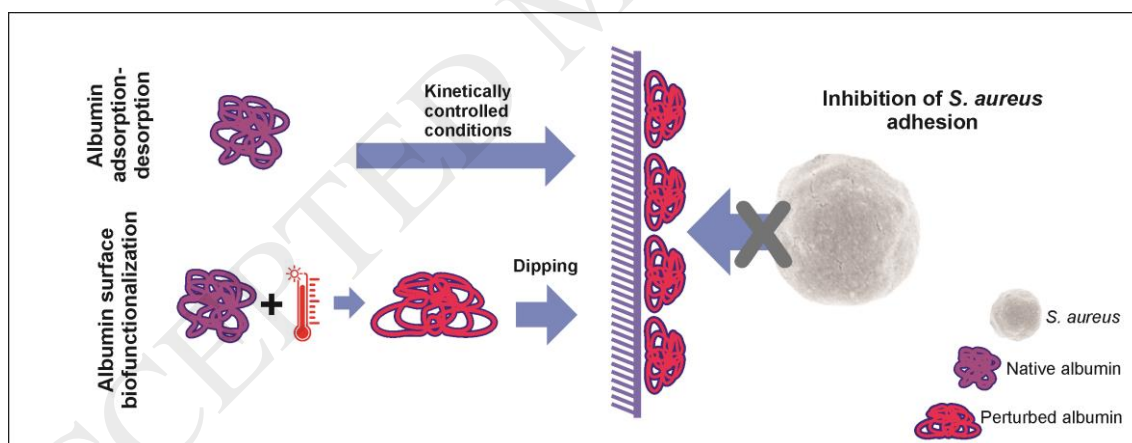
*Corresponding authors:

Laura E. Valenti: valenti@fcq.unc.edu.ar

Carla E. Giacomelli: giacomel@fcq.unc.edu.ar

Statistical Summary: 4970 words and 8 figures (figures 1 and 5 count as 2)

Graphical abstract

Inhibiting *S. aureus* adhesion from a factors-responses analysis of albumin-adsorption.

Highlights

- Factorial design of experiments to study the albumin adsorption-desorption process
- Bacterial adhesion correlation with the degree of albumin relaxation on the substrate
- Biofunctionalization with thermal treated albumin inhibits bacterial adhesion
- Novel and simple biofunctionalization strategy to prevent medical devices infections

Abstract

Staphylococcus aureus has become the most common opportunistic microorganism related to nosocomial infections due to the bacteria capacity to form biofilms on biomedical devices and implants. Since bacterial adhesion is the first step in this pathogenesis, it is evident that inhibiting such a process will reduce the opportunity for bacterial colonization on the devices. This work is aimed at optimizing a surface biofunctionalization strategy to inhibit the adhesion of *S. aureus* on solid substrates. The first part of the work deals with the albumin adsorption-desorption process, studied by a factorial design of experiments to explore a wide range of experimental factors (protein concentration, pH, flow rate and adsorption time) and responses (initial adsorption rate, adsorbed amount, desorbed extent) for hydrophilic and hydrophobic substrates, with a reduced number of experiments. This approach allows the simultaneous evaluation of the factors affecting the albumin adsorption-desorption process to find a qualitative correlation with the amount of alive *S. aureus* adhered on albumin biofunctionalized substrates. The results of this work point to a relationship between bacterial adhesion and the degree of albumin relaxation on the solid substrate. In fact, the inhibition of bacterial adhesion on albumin biofunctionalized substrates is due to the surface perturbation on the native structure of the protein. On this base, a biofunctionalization strategy was designed using a solution of thermally treated albumin molecules (higher β-sheet or unordered secondary structure elements) to biofunctionalize solid substrates by dipping. With these albumin biofunctionalized substrates *S. aureus* adhesion was minimized.

1-Introduction

Staphylococcus aureus has become the most common opportunistic microorganism related to nosocomial infections in the past years [1]. *Staphylococcus aureus* pathogenicity is fundamentally due to the bacteria capacity to form biofilms on biomedical devices and implants [2-4]. Nosocomial infections are acquired by patients during their stay in a hospital and other healthcare facilities causing a prolonged hospital stay, an increment in the morbidity and mortality rate as well as a general increase in the antimicrobial resistance. In the last years, many attempts have been made to prevent medical device-associated infections, like improving aseptic techniques, controlling the environment sterility, and perioperative antibiotic prophylaxis [5-9]. Since bacterial adhesion is the first step in the pathogenesis of foreign-body-related infections, it is evident that inhibiting such a process will reduce the opportunity for bacterial colonization to the device surface [10]. This approach to prevent bacterial adhesion on the surface of biomedical devices, is also promising as it would eliminate the need of using any other technique to avoid microbial colonization [11, 12].

Two different strategies have been developed to use materials that resist infections in the fabrication of biomedical devices and implants [13, 14]: (1) surface modification of the solid substrate to confer anti-adhesive properties, (2) material doping with anti-microbial agents. The first strategy was extensively exploited by the adsorption of hydrophilic polymeric brushes, based on poly(ethylene glycol) or poly(ethylene oxide), on different substrates, which inhibits protein adsorption and bacterial adhesion because of the large exclusion volume effect [15-17]. Further, methacrylate co-polymers bearing phosphonic or phosphonate groups were proposed to improve the ability of titanium substrates to inhibit bacterial adhesion [18]. On the other hand, a biomimetic design based on the crystallization on solid substrates of the two main lipid components of the nanostructured cicada and dragonfly wings has been intended to kill bacteria [19]. Other biomimetic design that relies on the microtopographic features of lotus leaves and shark skins (surface roughness, hydrophobicity and charge), has been used to provide solid substrates with antiadhesive properties [20]. The second strategy is mainly based on the local action of anti-microbial agents on the surface of solid substrates. Polysaccharides films were loaded with gentamicin, such released antibiotic leads to an excellent antibacterial action against *S. aureus* and *E. coli.* [21]. Besides antibiotics, chitosan has been widely applied to coat materials in order to decrease the number of attached viable bacteria [22, 23]. Metal nano-antimicrobials such as silver, copper and its complexes and zinc oxide, have been also developed to create engineered nanostructures with improved antifouling performances [24-

28]. In a similar way, antimicrobial peptides appeared as therapeutically useful antimicrobial agents [29, 30] that was combined with a biomolecular linker onto titanium surfaces [31].

These two strategies can be also addressed by surface biofunctionalization that emerges as the process by which biomolecules are incorporated to solid substrates to repel bacteria (in the first case) or kill them (in the second one). Meanwhile, protein biofunctionalization of solid substrates to repel bacterial adhesion has been scarcely explored in the literature. Recently, it was reported that surface coating with fungal proteins (hydrophobins) reduced biofilm formation of different strains of *S. epidermidis* on polystyrene surfaces [32, 33]. It is generally assumed that the strategies of surface modification that minimize protein adsorption, are also capable of inhibiting bacterial adhesion [34, 35]. This work is aimed at optimizing a surface biofunctionalization strategy to inhibit the adhesion of *S. aureus* on solid substrates. With such a purpose, albumin biofunctionalized silica substrates were used as model systems to correlate the surface properties of any material with both the protein adsorption process and the bacterial adhesion behavior (see supplementary information, Scheme 1-SI). On the one hand, albumin is not only the most abundant plasma protein by far [36], but also a nontoxic, biocompatible and biodegradable [27] biopolymer for surface biofunctionalization. On the other hand, the surface properties of silica substrates, such as hydrophobicity and charge density, can be easily modified to account for their effect on the protein adsorption and bacterial adhesion processes. The first part of this work deals with the albumin adsorption-desorption process on hydrophilic and hydrophobic substrates, studied by a factorial design of experiments to explore a wide range of experimental factors (protein concentration, pH, flow rate and adsorption time) with a reduced number of experiments. Hence, the factorial design of experiments allows the simultaneous evaluation of the effect that several factors have on the optimization of the protein adsorption-desorption process related to bacterial adhesion. Although the adsorption-desorption process of albumin has been extensively reported in the literature [37-39], it has not been studied by factorial design of experiments in order to explore the relevant factors to optimize the experimental conditions that minimize bacterial adhesion. In this way, the outcome of the experimental design can be related to the adhesion of *S. aureus* on solid substrates with previously adsorbed albumin. This relationship represents the basis for the second part of the work in which a simple and novel biofunctionalization strategy was proposed to minimize bacterial adhesion based on the response given by the best combination of experimental factors (see supplementary information, Scheme 1-SI).

2- Materials and methods

2.1-Materials

2.1.1-Reagents

Bovine serum albumin (A-4503) was purchased from Sigma (Saint Louis, MO). This sample was characterized in several works [39, 40]. Bovine and human serum albumin share 76% sequence homology [41]. Glutaraldehyde, chloroform, NaCl, $NaH_2PO_4.H_2O$, ethanol, dextrose and K_2HPO_4 were obtained from J.T. Baker, (3-Aminopropyl) trymethoxysilane (APTMS) from Fluka, acetone and dimethyldichlorosilane from Carlo Erba, casein acid peptone, soy peptone and agar from Britania and LIVE/DEAD BacLight Bacterial Viability Kit (L-13152) from Invitrogen. All reagents were of analytical grade and used without further purification. Aqueous solutions were prepared in 18 MΩ/cm resistance water (Milli-Q, Millipore; Billerica, MA). Phosphate buffer saline (PBS) consists of 5 mM NaH_2PO_4 solution and 150 mM NaCl solution. The pH adjustment was performed by adding KOH (Baker) or HCl (Baker) solutions and the pH measurements were carried out with a glass electrode and a digital pH meter (Mettler Toledo–Seven Compact). Unless noted, all experiments were performed at room temperature (22±2 °C).

2.1.2-Substrates

Silicon wafers (100 mm, Silicon Valley Microelectronics Inc.; Santa Clara, CA) were oxidized for 1 h at 1000 °C in order to obtain a silica layer of about 100 nm thick, which was verified by ellipsometry. Such a thickness is essential to achieve high sensitivity in reflectometry experiments [42]. The wafer was then cut in strips (1 cm × 4 cm) following the crystallographic plane. Prior to each experiment, the silica strips were cleaned with boiling piranha solution (2:1 $H_2SO_4:H_2O_2$) and rinsed thoroughly with deionized water (Caution! Piranha solution is a powerful oxidizing agent that reacts violently with organic compounds; it should be handled with extreme care).

The surface hydrophobicity was modified by dipping the substrate in a 5% dimethyldichlorosilane solution in chloroform for 30 minutes. The contact angle was measured using the static sessile drop method and it was analyzed with low bond axisymmetric drop shape analysis (LBADSA) [43]. The contact angle was (43±8)° before the modification, while it was (94±2)° for the hydrophobic substrate (uncertainties were calculated as 95% confidence interval of three repeats). Further, the surface charge of the substrates was modified by

incorporating amino groups through a self-assembled monolayer (SAM), formed in a 1% (3-aminopropyl)trymethoxysilane (APTMS) solution in ethanol for 2 hours [44].

2.1.3-Bacterial strains, culture medium and stain

S. aureus (ATCC 25923) was kindly provided by Dr. Claudia Sola (CIBICI-CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina). The culture media were Tryptone Soy Agar (TSA) and Tryptone Soy Broth (TSB). The bacteria zeta potential changed from (-19.8±0.3) mV in PBS pH 7.3 to (-13.7±0.3) mV in TSB. LIVE/DEAD BacLight Bacterial Viability Kit (L-13152) was employed to visualize the adhered bacteria to the solid substrates by Fluorescence confocal microscopy as well as to discriminate between live and dead bacteria. The kit utilizes a mixture of the nucleic acid stain SYTO® 9 (green-fluorescent) and propidium iodide (red-fluorescent), thus, bacteria with intact cell membranes stain fluorescent green, whereas bacteria with damaged membranes stain fluorescent red [45, 46]. The final concentration of each dye was 0.0006 mM SYTO 9 stain and 0.003 mM propidium iodide.

2.2-Methods

2.2.1- Albumin adsorption-desorption process

The albumin adsorption-desorption process was studied by reflectometry (AKZO Research Laboratories, Arnhem) as reported elsewhere [37, 42, 47]. The reflectometer was equipped with a stagnation point flow setup, previously described [48, 49]. In such a condition, the protein transport rate J_0 (mg/m^{2}s) towards the surface depends on the geometry of the cell, the diffusion coefficient of the molecule, the flow rate and the concentration in solution, as described by the following equation [42, 48]:

$$J_0 = 0.53(D^2 \alpha\, F_r\, R^{-4})^{1/3}\, C_p = kC_p \tag{1}$$

where D is the diffusion coefficient of albumin (6 × 10^{-11} m^2/s), α is a dimensionless flow intensity parameter which is constant for a given cell geometry and flow rate (3.5), F_r is the flow rate which was controlled by a peristaltic pump, R is the radius of the circular hole through which the solution enters the cell (0.9 × 10^{-3} m) and C_p the protein concentration in mg/m^3. The calculated value for k was 3.85 × 10^{-6} m/s (F_r = 0.02 mL/s) or 6.59 × 10^{-6} m/s (F_r = 0.10 mL/s).

The factorial design of experiments to explore the albumin adsorption-desorption process included different factors depending on the surface properties of the substrates. The factors evaluated for the hydrophilic substrates were: pH (4.8, the isoelectric point of albumin, and

7.3, the pH of the physiological medium), F_r (0.02 mL/s and 0.10 mL/s), C_p (0.005 mg/mL and 0.100 mg/mL albumin) and adsorption time (50 s and 300 s). On the other hand, with the hydrophobic substrates the pH was fixed at 7.3 and the other factors were the same ones. As a consequence, 2^4 and 2^3 factorial designs were used for hydrophilic and hydrophobic substrates, respectively. The evaluated responses were the initial adsorption rate (IAR), the albumin adsorbed amount at a given adsorption time (Γ) and the desorption percentage (see supplementary information, Table 1-SI). Common statistical tools, such as analysis of variance (ANOVA), F-test and Student's t test, were used to find out the significant factors which affect the responses of the adsorption-desorption process. The STATISTICA software was used to design and randomize experiments and analyze modeled responses. The level of significance was given as values of the probability (p-value) less than 0.05.

Briefly, the adsorption-desorption kinetic experiments were conducted using a peristaltic pump (0.02 mL/s or 0.10 mL/s) as follows: from 0 to 200 s, PBS (pH 4.8 or 7.3) was introduced into the cell to establish a stable baseline, from 200 s up to 250 s or 500 s (depending on the adsorption time of the experiment) the flow was switched from PBS to the albumin solution (prepared in PBS) at either 0.005 mg/mL or 0.100 mg/mL. After that, for the next 300 s, the flow was switched back to PBS in order to analyze the desorption process by dilution.

2.2.2-Bacterial adhesion

S. aureus was grown overnight at 37 °C in TSA and resuspended in TSB in order to reach a 1×10^8 CFU/mL bacteria concentration by measuring the OD_{600} [50]. Appropriate controls were performed to quantify the number of viable bacteria in each bacterial suspension previous to each experiment. Later, the bacterial suspension was centrifuged at 8500 rpm for 5 minutes and the cells were resuspended in PBS pH 7.3, a procedure which was conducted twice [50, 51]. Control experiments to check the bacteria viability were performed in PBS during the adhesion time. This medium was selected in order to minimize the interference of the adsorption of the culture media components on the substrates during incubation with the bacteria. Then, the substrates were incubated in the bacterial suspension in vertical position at 37 °C for 40 minutes. A duplicate series of experiments were carried out in each case. Afterwards, the substrates were washed with 0.9% NaCl to remove non-adhered bacteria, stained with 200 μL of dye solution, as previously detailed, and incubated in the dark for 15 minutes. The visualization of the stained samples was performed in an Olympus FV300 Confocal Fluorescence microscope [50, 52]. For the analysis of *S. aureus* adhesion, 8 images of each duplicate for each condition (n=16) were analyzed by quantifying dead (red) and alive (green) bacteria. The bacterial adhesion was evaluated through total bacteria/μm^2 and alive

bacteria percentage. For each case, the results are expressed as a confidence interval with a level of 95%. Statistical analysis was performed using one-way ANOVA to evaluate the differences between experimental conditions. A $p < 0.05$ was considered statistically significant.

For the visualization of adhered bacteria by SEM (FE-SEM Carl Zeiss Sigma), the adhered bacteria were fixed with 2.5% glutaraldehyde in PBS pH 7.3 for 1 h. After three washes of 10 minutes with PBS, the cells were dehydrated in solutions of increasing acetone concentration (25–100%) for 10 minutes each. Samples were critical-point dried with liquid CO_2 as the transition fluid and then coated with gold [53].

2.2.3-Albumin surface biofunctionalization

Surface biofunctionalization was performed through the physical adsorption of partially denatured albumin by thermal treatment on hydrophilic substrates at pH 7.3. For the thermal treatment, 0.100 mg/mL albumin solution was heated up to 65 °C for 1 or 18 hours. The secondary structure of albumin was studied by circular dichroism (CD) on a JASCO J-810 spectropolarimeter using a 0.1 cm quartz cuvette. The spectra were scanned between 190 and 260 nm with 0.2 nm resolution; 16 scans were accumulated with a scan rate of 100 nm/min and a time constant of 0.125 s. For the quantitative analysis of the spectra, the algorithm CDSSTR [54] was employed from the web server DICHROWEB [55]. For CD experiments, NaCl was replaced by Na_2SO_4 (Baker) in PBS [56].

The hydrophilic substrates were biofunctionalized with thermal-treated albumin (0.100 mg/mL) by dipping them for 1 h ($\Gamma \approx 0.5$ mg/m^2). Bacterial adhesion on these biofunctionalized substrates was studied as previously described.

3-Results and discussion

3.1. Correlating albumin adsorption and *S. aureus* adhesion

3.1.1 Factorial design of experiments of the adsorption-desorption process

In order to study the albumin adsorption-desorption process on hydrophilic and hydrophobic substrates, a wide range of experimental factors was employed to analyze a group of responses using a factorial design of experiments (see supplementary information, Scheme 1-SI and Table 1-SI). The protein adsorption process comprises different stages that depend on both the surface properties of the substrate (mostly charge and hydrophobicity)

and the nature of the biomolecule [57, 58]. The relative rates of each one of these steps determine the final adsorption amount and the conformation of the proteins at the interface in such a way that this state may be either thermodynamic or kinetically controlled. In this scenario the experimental factors that control the biofunctionalization process on a particular substrate can be traced from different variables such as pH, C_p, F_r and the adsorption time (supplementary information, Table 1-SI), which are related to the kinetics of the process, the strength and type of the protein-substrate interactions and the degree of surface coverage. Similarly, the experimental responses to establish the features of the substrate biofunctionalization are associated with the IAR, Γ and the extent of desorption upon dilution [57, 58] (supplementary information, Table 1-SI). In figure 1, the three evaluated responses (IAR, Γ and the desorption percentage), are shown for all the combinations of the experimental factors addressed: red symbols represent hydrophilic substrates at pH 7.3 (solid) and 4.8 (patterned) whereas green symbols stand for the adsorption on hydrophobic substrates at pH 7.3, measured at the two levels of C_p, F_r and adsorption times. It is important to note that the combination of surface properties and pH values gives a general picture on the interplay between electrostatic and hydrophobic interactions ruling the albumin adsorption-desorption process. In the first place, the values of IAR are lower than the protein transport rate (J_0) given by equation (1) for all the studied factors (see supplementary information, Table 2-SI), evidencing a delay for the adsorption when the protein molecules arrive to the surface. As a consequence, the interaction between albumin molecules and the substrates (hydrophilic as well as hydrophobic) controls the rate of the adsorption process rather than the transport from the solution.

The statistical analysis (ANOVA) of the results provided in figure 1, clearly shows that all of the experimental factors strongly affect the albumin adsorption on hydrophilic substrate whereas on the hydrophobic one, only C_p play a significant role on IAR and the adsorption time on both Γ and the desorption percentage (see supplementary information, Figures 1-SI and 2-SI and Table 3-SI). The level of C_p is particularly important at the initial stage of the adsorption, reinforcing the idea that albumin attachment rules the process, driven by either electrostatic or hydrophobic interactions. Electrostatic attachment causes slower initial adsorption steps on hydrophilic substrates that also depend on F_r. On the other hand, the dependence of Γ on the two levels of the adsorption time of albumin on both substrates indicates a slower step after the first attachment, surely due to some degree of protein spreading driven by either hydrophobic or electrostatic interactions [58]. An interesting result at pH 4.8 is that observed at the high level of C_p and the low level of adsorption time (red-patterned diamond), in

9

which Γ depends on F_r, in contrast to the same condition at the high level of adsorption time (red-patterned pentagon). This factors-response dependence suggests that longer residence time is required to optimize the contact points between albumin and the hydrophilic substrates under favorable electrostatic interactions. Finally, it is generally assumed [57, 58] that a reduction in the desorption percentage is due to stronger protein-substrate interactions that in turns are due to the relaxation of the adsorbed proteins. This effect is clearly observed on the hydrophilic substrate because increasing the adsorption time leads to lower desorption percentages for the combinations of the two levels of C_p and F_r. Thus, the relaxation process is conditioned by a tradeoff between C_p and F_r. Moreover, the dependence of this response with the adsorption time implies that attachment and relaxation occurs at the same timescale on hydrophilic and hydrophobic substrates. All together these responses indicate that in order to correlate the protein adsorption process with the bacterial adhesion it is mandatory to know the amount of adsorbed albumin remaining after desorption upon dilution as well as the degree of relaxation of the biomolecules after attachment.

Figure 2 shows the adsorbed amount remaining after desorption upon dilution (Γ_{rem}) as a function of Γ measured at all the experimental factors (see the supplementary information to focus on the lowest values, figure 3-SI). The trend indicates the presence of two zones: a constant Γ_{rem} at around 0.04 mg/m^2 up to Γ = 0.20 mg/m^2 followed by a linear relationship with a 0.91 slope (R^2=0.98). The first zone is mostly observed at pH 7.3 on hydrophilic substrates, where from higher Γ values (0.04-0.20 mg/m^2) only 0.04 mg/m^2 are maintained after desorption upon dilution. Hence, there are at least two populations, a loosely attached one and a strongly adsorbed one. Obviously, this Γ_{rem} is a consequence of relaxed adsorbed albumin molecules attached with several contact points to the substrate. The second zone highlights that the desorption process is almost absent in most of the evaluated experimental factors. This fact is related to the strength of the protein-substrate interactions driven by electrostatics or hydrophobic interactions and/or the lateral interactions between adsorbed proteins [57, 58].

3.1.2-Bacterial adhesion

Before discussing the effect of adsorbed albumin on the adhesion process of *S. aureus*, the surface properties of the bare substrates were evaluated. Figure 3 A and B shows the effect of the surface charge (negative [59] and positive) and hydrophobicity on bacterial adhesion, expressed as the alive bacteria percentage and total bacteria/μm^2. The statistical analysis indicates that the surface properties do not have any significant effect on the adhesion process

10

of *S. aureus*, suggesting that the interactions involved in the adhesion process are very diverse (electrostatic, hydrophobic, etc.).

To study the effect of adsorbed albumin on the bacterial adhesion, three conditions of the wide range of the explored combination of the experimental factors were chosen from the results shown in Figure 2. Since the three selected conditions represent the actual amount of adsorbed albumin remaining after desorption upon dilution as well as the extent of protein relaxation, the effect of low (red circle), medium (green triangle) and high (patterned-red square) Γ_{rem} on the adhesion process provides with a first relationship between these processes (Figure 4). Further, this selection also represents all the factors studied in the factorial design of experiments previously discussed; hence, low Γ_{rem} (0.04±0.02 mg/m^2) was taken from the hydrophilic substrate at pH 7.3 (C_p = 0.100 mg/mL, F_r = 0.02 mL/s and 50 s adsorption time), medium Γ_{rem} (0.92±0.09 mg/m^2) was selected from the hydrophobic substrate at pH 7.3 (C_p = 0.005 mg/mL, F_r = 0.10 mL/s and 300 s adsorption time) and high Γ_{rem} (2.93±0.01 mg/m^2) was chosen from the hydrophilic substrate at pH 4.8 (C_p = 0.100 mg/mL, F_r = 0.02 mL/s and 300 s adsorption time). Figure 4 A and B show the *S. aureus* adhesion on the biofunctionalized substrates at these three conditions, expressed as the percentage of variation of alive bacteria percentage and total bacteria/μm^2 related to the bare substrates (mean value from all the results shown in figure 3). Therefore, positive results indicate more alive bacteria on the biofunctionalized substrates compared to the bare ones whereas negative results stand for the inhibition of *S. aureus* adhesion. Therefore, albumin adsorbed at the conditions represented by the lowest Γ_{rem} on the hydrophilic substrate at pH 7.3 correlates with the minimum adhesion of alive bacteria. The observed differences on the different substrates highlight the influence of the protein adsorbed amount and the biomolecule relaxation extent on the bacterial adhesion. These results clearly indicate that bacterial adhesion can be modulated by changing the biofunctionalization features.

Considering the experimental condition that leads to a minimal bacterial adhesion as a reference, variations in the experimental factors involved in albumin biofunctionalization were introduced to explore whether it was possible to improve the response towards *S. aureus* adhesion. The experimental factors involved in albumin adsorption were slightly modified to hydrophobic substrates (green circle) or to longer adsorption times (red square) as indicated in figure 4 A and B. None of them result in better responses: either a higher number of total adhered bacteria or alive bacteria are reached in comparison with the previous biofunctionalization condition. However, the values of Γ_{rem} in these cases are about 0.7 mg/m^2 indicating that Γ_{rem} could be a good parameter to correlate the albumin adsorption process

11

and *S. aureus* adhesion. This behavior is further corroborated by just reducing C_p (red triangle) on the hydrophilic substrate (Γ_{rem}= 0.04±0.02 mg/m^2). Finally, these same conditions on the hydrophobic substrate (green triangle) do not give a low Γ_{rem} value (0.21±0.04 mg/m^2) nor improve bacterial adhesion. This piece of evidence indicates that albumin Γ_{rem} modulates *S. aureus* which may be due to the low amount of adsorbed protein and/or to the relaxation state reached by the protein population that cannot be removed by dilution. This fact suggests a qualitative correlation between albumin relaxation state on the solid substrate and the amount of alive adhered bacteria.

Figure 5 shows the images obtained from fluorescence confocal and scanning electron microscopies of adhered *S. aureus* on biofunctionalized hydrophilic substrates. The first one was used to follow alive (green) and dead (red) bacteria whereas electron microscopy (SEM) was employed to explore the differences in bacterial morphology between dead and alive cells. Figure 5 A shows the positive control experiment that was performed on the hydrophilic substrate biofunctionalized with albumin (C_p= 0.100 mg/mL) at pH 7.3 during 50 seconds. Such a condition results in a high amount of alive adhered bacteria (83±5%) after 90 minutes of adhesion. In the SEM image, the adhered bacteria are visualized as symmetrical diplococci cells corresponding to alive *S. aureus*. On the other hand, in the negative control experiment (Figure 5 B) bacteria adhesion was performed overnight at room temperature in order to induce the bacterial death. Fluorescence confocal microscopy images show that most of the cells are dead and from SEM image, it can be observed disturbances at bacterial surface which suggest the outflow of cellular content. Finally, the substrate biofunctionalization that results in the minimal bacterial adhesion is included in the images of Figure 5 C. More dead bacteria are visualized in the fluorescence confocal microscopy images, as well as changes in the cell morphology-like in SEM images.

3.2- Albumin surface biofunctionalization

The results discussed in the previous section point to a relationship between bacterial adhesion and the degree of albumin relaxation on the solid substrate. The assumption underlying these results is that the inhibition of bacterial adhesion on albumin biofunctionalized substrates is due to the perturbation on the native structure of albumin induced by the optimization of the protein-substrate interactions. On this basis, albumin surface biofunctionalization strategy was designed using partially disturbed albumin molecules to minimize bacterial adhesion on solid substrates (see supplementary information, Scheme 1-SI). To address this issue, a thermal treatment of native albumin in solution was first explored;

afterwards, this solution was employed for substrate biofunctionalization with albumin. It is well known that the extent of α-helical structure of albumin sharply decreases with rise of temperature beyond 30 °C [60] and that the transition temperature to a perturbed structure is about 57 °C [61]. Consequently, native albumin in solution was thermally treated at 65 °C for two incubation periods (1 and 18 h). After thermal treatment, the secondary structure of albumin molecules shows some loss in the helix content as judged from the changes of the CD bands at 195 and 222 nm (see supplementary information, Figure 4-SI and Table 4-SI). Treatment for 1 hour leads to an increase in the β-sheet element whereas the treatment during 18 h shows a marked secondary structure loss together with arising of the unordered structure [55, 61].

Figure 6 A and B compares the bacterial adhesion on albumin biofunctionalized substrates (alive bacteria percentage and total bacteria/μm^2 respect to the bare substrate) using different strategies: a) native albumin adsorbed on hydrophilic substrate at pH 7.3 (C_p = 0.100 mg/mL, F_r = 0.02 mL/s, 50 s adsorption time and low Γ_{rem}) as previously discussed (Figure 4, red circle), b) partially denatured albumin (1 h thermal-treatment) adsorbed on hydrophilic substrate at pH 7.3 (C_p = 0.100 mg/mL dipping for 1 hour) and c) partially denatured albumin (18 h thermal-treatment) adsorbed on hydrophilic substrate at pH 7.3 (C_p = 0.100 mg/mL dipping for 1 hour). One-way ANOVA established that there were no significant differences between the variations of the alive bacteria percentage (Figure 6 A). However, the surface biofunctionalization with thermally treated albumin improves the response regarding the total amount of adhered bacteria (Figure 6 B). These results indicate, on the one hand, that undoubtedly the inhibition of bacterial adhesion on albumin biofunctionalized substrates is due to the perturbation on the native structure of albumin (see supplementary information, Figure 5-SI). On the other hand, the simple strategy proposed to minimize bacterial adhesion properly works and it could be generalized to different solid substrates.

4. Conclusions

The adhesion process of *S. aureus* strongly depends on the presence of adsorbed albumin molecules on the solid substrates. In fact, there is a clear relationship between albumin relaxation state on the substrate and the amount of alive adhered bacteria. The structural changes that albumin molecule suffers during surface relaxation leads to the minimization of the bacterial adhesion. Consequently, bacterial adhesion can be modulated by changing the biofunctionalization features, particularly the structure of the protein molecules. As a matter

of fact, biofunctionalized solid substrates with thermally treated albumin inhibits the adhesion of alive *S. aureus*. In the long term, the results of this work point to a novel strategy to minimize the risk of medical device-associated infections involving the biofunctionalization of solid substrates with partially denatured albumin.

Acknowledgments

References

[1]   M.P.D. Deege, D.L. Paterson, Reducing the Development of Antibiotic Resistance in Critical Care Units, Curr. Pharm.Biotechnol*. 12 (2011) 2062-2069.

[2]   T.J. Foster, J.A. Geoghegan, V.K. Ganesh, M. Höök, Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*, Nat. Rev. Microbiol. 12 (2014) 49-62.

[3]   J. Hernandez-Montelongo, E.G. Lucchesi, I. Gonzalez, W.A.A. Macedo, V.F. Nascimento, A.M. Moraes, M.M. Beppu, M.A. Cotta,Hyaluronan/chitosan nanofilms assembled layer-by-layer and their antibacterial effect: A study using *Staphylococcus aureus* and *Pseudomonas aeruginosa*, Colloids Surf., B. 141 (2016) 499-506.

[4]   H.P. Loveday, J.A. Wilson, K. Kerr, R. Pitchers, J.T. Walker, J. Browne,Association between healthcare water systems and *Pseudomonas aeruginosa* infections: A rapid systematic review, J. Hosp. Infect. 86 (2014) 7-15.

[5]   J.-M. Yin, Z.-T. Liu, S.-C. Zhao, Y.-J. Guo, Diagnosis, management, and prevention of prosthetic joint infections, Front. Biosci., Landmark Ed. 18 (2013) 1349-1357.

[6]   C. J. Crnich, P. Drinka, Medical Device-Associated Infections in the Long-Term Care Setting, Infect. Dis. Clin. N. Am. 26 (2012) 143-164.

[7]   J. Marschall, L.A. Mermel, M. Fakih, L.R. Hadaway, A. Kallen, A.R. Marie Pettis, M.E. Rupp, T. Sandor, L.L. Maragakis, D.S. Yokoe, L.A. Mermel, M. Fakih, L. Hadaway, A. Kallen, A. Marie Pettis, T. Sandora, L.L. Maragakis, D.S. Yokoe, Strategies to Prevent Central Line–Associated Bloodstream Infections in Acute Care Hospitals: 2014 Update.Infect. Control Hosp. Epidemiol. 35 (7) (2014) 753-771.

[8]   N.P. O'Grady, M. Alexander, L.A. Burns, E.P. Dellinger, J. Garland, S.O. Heard, P.A. Lipsett, H. Masur, L.A. Mermel, M.L. Pearson, I.I. Raad, A.G. Randolph, M. E. Rupp, S. Saint,Guidelines for the Prevention of Intravascular Catheter-related Infections, Clin. Infect. Dis. 52 (9) (2011) 162-193.

[9]   P. Vergidis, R. Patel,Novel Approaches to the Diagnosis, Prevention, and Treatment of Medical Device-Associated Infections, Infect. Dis. Clin. N. Am. 26 (2012) 173-186.

[10] D. Campoccia, L. Montanaro, C.R. Arciola,A review of the biomaterials technologies for infection-resistant surfaces*, Biomaterials 34 (2013) 8533-8554.

[11] I. Uçkay,P. Hoffmeyer, D. Lew, D. Pittet,Prevention of surgical site infections in orthopaedic surgery and bone trauma: state-of-the-art update, J. Hosp. Infect. 84 (2013) 5-12.

[12] M. Ramasamy, J. Lee,Recent nanotechnology approaches for prevention and treatment of biofilm-associated infections on medical devices, BioMed Res. Int. 2016 (2016) 1-17.

[13] C.R. Arciola, D. Campoccia, P. Speziale, L. Montanaro, J.W. Costerton,Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials, Biomaterials. 33 (2012) 5967-5982.

[14] S. Wu, X. Liu, A. Yeung, K.W.K. Yeung, R.Y.T. Kao, G. Wu, T. Hu, Z. Xu, P.K. Chu,Plasma-modified biomaterials for self-antimicrobial applications, ACS Appl. Mater. Interfaces 3 (2011) 2851-2860.

[15] L.C. Xu, C.A. Siedlecki, Protein adsorption, platelet adhesion, and bacterial adhesion to polyethylene-glycol-textured polyurethane biomaterial surfaces, J. Biomed. Mater. Res. Part B 105 (3) (2017) 668-678.

[16] P. Kingshott, J. Wei, D. Bagge-Ravn, N. Gadegaard, L. Gram,Covalent attachment of poly(ethylene glycol) to surfaces, critical for reducing bacterial adhesion, Langmuir 19 (2003) 6912-6921.

[17] A. Roosjen, H.C. van der Mei, H.J. Busscher, W. Norde, Microbial adhesion to poly(ethylene oxide) brushes: Influence of polymer chain length and temperature, Langmuir 20 (2004) 10949-10955.

[18] T. Huang, H. Liu, P. Liu, P. Liu, L. Li, J. Shen,Zwitterionic copolymers bearing phosphonate or phosphonic motifs as novel metal-anchorable anti-fouling coatings, J. Mater. Chem. B 5 (27) (2017) 5380-5389.

[19] E.P. Ivanova, S.H. Nguyen, Y. Guo, V.A. Baulin, H.K. Webb, V.K. Truong, J.V. Wandiyanto, C.J. Garvey, P.J. Mahon, D.E. Mainwaring,R.J. Crawford, Bactericidal activity of self-assembled palmitic and stearic fatty acid crystals on highly ordered pyrolytic graphite, Acta Biomater. 59 (2017) 148-157.

[20] V.B. Damodaran, N.S. Murthy,Bio-inspired strategies for designing antifouling biomaterials, Biomater. Res. 20 (2016) 18-29.

[21] S. Kondaveeti, T.C. Damato, A.M. Carmona-Ribeiro, M.R. Sierakowski, D.F.S. Petri, Sustainable hydroxypropyl methylcellulose/xyloglucan/gentamicin films with antimicrobial properties, Carbohydr. Polym. 165 (2017) 285-293.

[22] Z. Ma, A. Garrido-Maestu, K.C. Jeong, Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles as antimicrobial agents: A review, Carbohydr. Polym. 176 (2017) 257-265.

[23] F. Kara, E.A. Aksoy, Z. Yuksekdag, N. Hasirci, S. Aksoy, Synthesis and surface modification of polyurethanes with chitosan for antibacterial properties, Carbohydr. Polym. 112 (2014) 39-47.

[24] M.C. Sportelli,R.A. Picca, N. Cioffi, in D.A. Phoenix, F. Harris, S. Dennison (Eds.), Novel Antimicrobial Agents And Strategies, Wiley-VCH, Weinheim, 2015, Chapter 8.

[25] X. Zhang, J. Li, X. Wang, Y. Wang, R. Hang, X. Huang, B. Tang, P.K. Chu, Effects of copper nanoparticles in porous TiO_2 coatings on bacterial resistance and cytocompatibility of osteoblasts and endothelial cells, Mater. Sci. Eng. C 82 (2018) 110-120.

[26] J. Karbowniczek, L. Cordero-Arias, S. Virtanen, S.K. Misra, E. Valsami-Jones, L. Tuchscherr, B. Rutkowski, K. Górecki, P. Bała, A. Czyrska-Filemonowicz, A.R. Boccaccini,Electrophoretic deposition of organic/inorganic composite coatings containing ZnO nanoparticles exhibiting antibacterial properties, Mater. Sci. Eng. C 77 (2017) 780-789.

[27] L.E. Valenti, C. E. Giacomelli, Unaffected features of BSA stabilized Ag nanoparticles after storage and reconstitution in biological relevant media, Colloids Surf. B 132 (2015) 71-77.

[28] L. Rizzello, R. Cingolani, P.P. Pompa, Nanotechnology tools for antibacterials materials, Nanomedicine. 8 (5) (2013) 807-821.

[29] S. Dennison, F. Harris, D. Phoenix, in D.A. Phoenix, F. Harris, S. Dennison (Eds.), Novel Antimicrobial Agents And Strategies, Wiley-VCH, Weinheim, 2015, Chapter 4.

[30] M. Hassan, M. Kjos, I.F. Nes, D.B. Diep, F. Lotfipour,in D.A. Phoenix, F. Harris, S. Dennison (Eds.), Novel Antimicrobial Agents And Strategies, Wiley-VCH, Weinheim, 2015, Chapter 5.

[31] H. Geng, Y. Yuan, A. Adayi, X. Zhang, X. Song, L. Gong, X. Zhang,P. Gao, Engineered chimeric peptides with antimicrobial and titanium-binding functions to inhibit biofilm formation on Ti implants, Mater. Sci. Eng., C. 82 (2018) 141-154.

[32] R.Y. Zhang, D.D. Hu, J.G. Gu, J.X. Zhang, P.H. Goodwin, Q.X. Hu, Purification of a novel hydrophobin PN1 involved in antibacterial activity from an edible mushroom *Pleurotus nebrodensis*, Eur. J. Plant Pathol. 143 (4) (2015) 823-831.

[33] M. Artini, P. Cicatiello, A. Ricciardelli, R. Papa, L. Selan, P. Dardano, M. Tilotta, G. Vrenna, M.L. Tutino, P. Giardina,E. Parrilli,Hydrophobin coating prevents *Staphylococcus epidermidis* biofilm formation on different surfaces, Biofouling 33 (7) (2017) 601-611.

[34] P. Moazzam, A. Razmjou, M. Golabi, D. Shokri, A. Landarani-Isfahani, Investigating the BSA protein adsorption and bacterial adhesion of Al-alloy surfaces after creating a hierarchical (micro/nano) superhydrophobic structure, J. Biomed. Mater. Res. A. 104 (9) (2016) 2220-2233.

[35] K.D. Park, Y.S. Kim, D.K. Han, Y.H. Kim, E.H.B. Lee, H. Suh,K.S. Choi, Bacterial adhesion on PEG modified polyurethane surfaces, Biomaterials 19 (1998) 851-859.

[36] F. Kratz, B. Elsadek, Clinical impact of serum proteins on drug delivery, J. Controlled Release. 161 (2012) 429-445.

[37] L.E. Valenti, P.A. Fiorito, C.D. García, C. E. Giacomelli, The adsorption-desorption process of bovine serum albumin on carbon nanotubes, J. Colloid Interface Sci. 307 (2007) 349-356.

[38] W. Norde, C.E. Giacomelli, BSA structural changes during homomolecular exchange between the adsorbed and the dissolved states, J. Biotechnol. 79 (2000) 259-268.

[39] C.E. Giacomelli, M.J. Avena, C.P. De Pauli, Adsorption of Bovine Serum Albumin onto TiO_2 Particles, J. Colloid Interface Sci. 188 (1997) 387-395.

[40] C.E. Giacomelli, M.J. Esplandiú, P.I. Ortiz, M.J. Avena, C.P. De Pauli, Ellipsometric Study of Bovine Serum Albumin Adsorbed onto Ti/TiO_2 Electrodes, J. Colloid Interface Sci. 218 (1999) 404-411.

[41] B.X. Huang, H.Y. Kim, C. Dass, Probing three-dimensional structure of bovine serum albumin by chemical cross-linking and mass spectrometry, J. Am. Soc. Mass Spectrom. 15 (8) (2004) 1237-1247.

[42] J.C. Dijt, M.A.C. Stuart, G.J. Fleer, Reflectometry as a tool for adsorption studies, Adv. Colloid Interface Sci. 50 (1994) 79-101.

[43] A.F. Stalder, T. Melchior, M. Müller, D. Sage, T. Blu, M. Unser,Low-bond axisymmetric drop shape analysis for surface tension and contact angle measurements of sessile drops, Colloids Surf., A. 364 (2010) 72-81.

[44] L.E. Valenti, V.L. Martins, E. Herrera, R.M. Torresi,C.E. Giacomelli, Ni(II)-modified solid substrates as a platform to adsorb His-tag proteins, J. Mater. Chem. B. 1 (38) (2013) 4921-4931.

[45] Molecular Probes, 2004. LIVE/DEAD BacLight Bacterial Viability Kits. Product Information.

[46] A. Pascaud, S. Amellal, M.L. Soulas,G. Soulas, A fluorescence-based assay for measuring the viable cell concentration of mixed microbial communities in soil, J. Microbiol. Meth. 76 (2008) 81-87.

[47] L.E. Valenti, A.M. Smania, C.P. De Pauli, C.E. Giacomelli, Driving forces for the adsorption of a His-tag Chagas antigen. A rational approach to design bio-functional surfaces, Colloids Surf., B. 112 (2013) 294-301.

[48] J.C. Dijt, M.A.C. Stuart, J.E. Hofman, G.J. Fleer, Kinetics of polymer adsorption in stagnation point flow, Colloids Surf. 51 (1990) 141-158.

[49] L. Wågberg,I. Nygren, The use of stagnation point adsorption reflectometry to study molecular interactions relevant to papermaking chemistry, Colloids Surf., A. 159 (1999) 3-15.

[50] J.D. Patel, M. Ebert, R. Ward,J.M. Anderson, *S. epidermidis* biofilm formation: Effects of biomaterial surface chemistry and serum proteins, J. Biomed. Mater. Res., Part A. 80A (3) (2007) 742-751.

[51] B. Fang, S. Gon, M. Park, K.N. Kumar, V.M. Rotello, K. Nusslein, M.M. Santore, Bacterial adhesion on hybrid cationic nanoparticle-polymer brush surfaces: Ionic strength tunes capture from monovalent to multivalent binding, Colloids Surf., B. 87 (2011) 109-115.

[52] C.Y. Flores, A.G. Miñán, C.A. Grillo, R.C. Salvarezza, C. Vericat, P.L. Schilardi, Citrate-capped silver nanoparticles showing good bactericidal effect against both planktonic and sessile bacteria and a low cytotoxicity to osteoblastic cells, ACS Appl. Mater. Interfaces. 5 (2013) 3149-3159.

[53] J. Bruzaud, J. Tarrade, A. Coudreuse, A. Canette, J.M. Herry, E. Taffin de Givenchy, T. Darmanin, F. Guittard, M. Guilbaud, M.N. Bellon-Fontaine, Flagella but not type IV pili are involved in the initial adhesion of *Pseudomonas aeruginosa* PAO1 to hydrophobic or superhydrophobic surfaces, Colloids Surf., B. 131 (2015) 59-66.

[54] W.C. Johnson, Analyzing protein circular dichroism spectra for accurate secondary structures, Proteins: Struct., Funct., Genet. 35 (3) (1999) 307-312.

[55] S.M. Kelly, T.J. Jess,N.C. Price, How to study proteins by circular dichroism, Biochim. Biophys. Acta, Proteins Proteomics. 1751 (2005) 119-139.

[56] N.J. Greenfield, Using circular dichroism spectra to estimate protein secondary structure, Nat Protoc. 1 (6) (2006) 2876-2890.

[57] W. Norde, Colloids and Intefaces in Life Sciences, Marcel Dekker, New York, 2003, Chapter 15.

[58] W. Norde, J. Buijs, H. Lyklema, in J. Lyklema (Ed.), Fundamentals of Interface and Colloid Science, Vol. 5, Elsevier, The Netherlands, 2005, Chapter 3.

[59] M. Kosmulski, Compilation of PZC and IEP of sparingly soluble metal oxides and hydroxides from literature, Adv. Colloid Interface Sci. 152 (2009) 14-25.

[60] Y. Moriyama, Y. Kawasaka,K. Takeda, Protective effect of small amounts of sodium dodecyl sulfate on the helical structure of bovine serum albumin in thermal denaturation, J. Colloid Interface Sci. 257 (2003) 41-46.

[61] C.E. Giacomelli, W. Norde, The Adsorption-Desorption Cycle. Reversibility of the BSA-Silica System, J. Colloid Interface Sci. 233 (2001) 234-240.

Figures Captions

Figure 1.
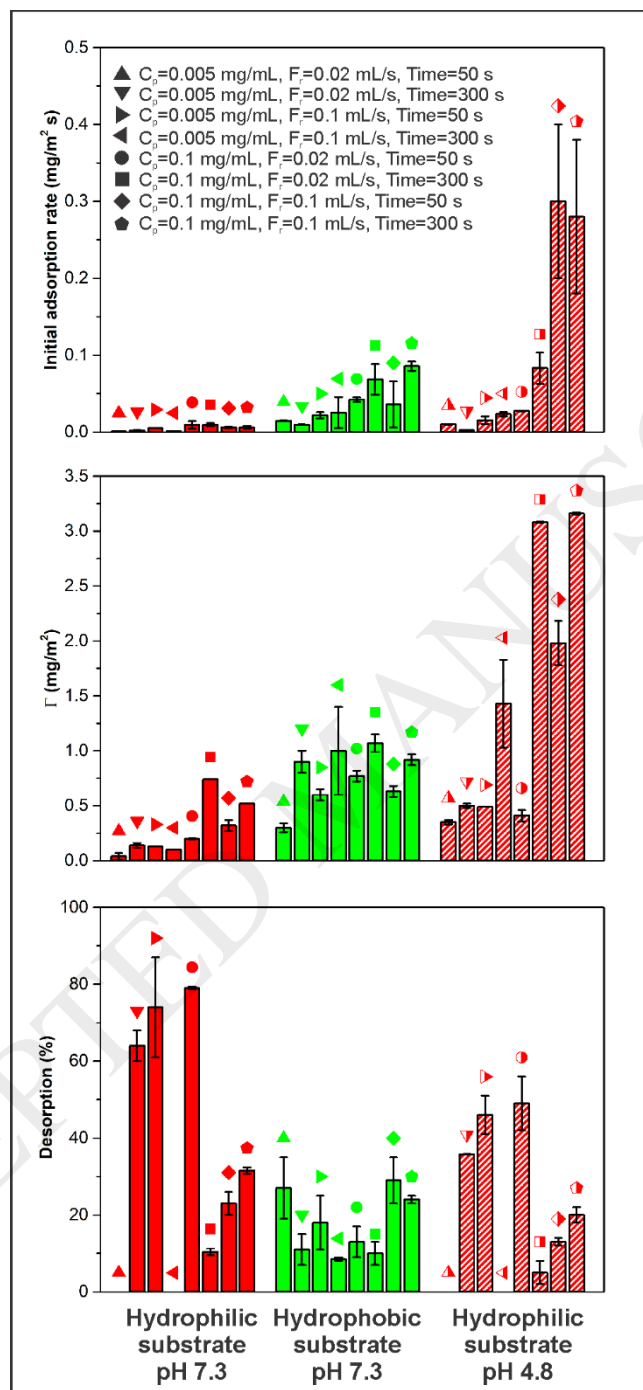
Figure 1. Initial adsorption rate (IAR), adsorbed amount (Γ) and desorption percentage for all the combinations of the experimental factors evaluated for the albumin adsorption-desorption process:(red) hydrophilic substrates at pH (solid) 7.3 (patterned) 4.8 and (green) hydrophobic substrate at pH 7.3. The shape of the symbols represents the combination of the studied factors, C_p, F_r and adsorption time, respectively: ($\blacktriangle$) 0.005 mg/mL-0.02 mL/s-50 s, ($\blacktriangledown$) 0.005

mg/mL-0.02 mL/s-300 s, (▶) 0.005 mg/mL-0.10 mL/s-50 s, (◀) 0.005 mg/mL-0.10 mL/s-300 s, (●) 0.100 mg/mL-0.02 mL/s-50 s, (■) 0.100 mg/mL-0.02 mL/s-300 s, (◆) 0.100 mg/mL-0.10 mL/s-50, (⬠) 0.100 mg/mL-0.10 mL/s-300 s. The error bars represent the standard deviation calculated from duplicate experiments for each combination of the factors.
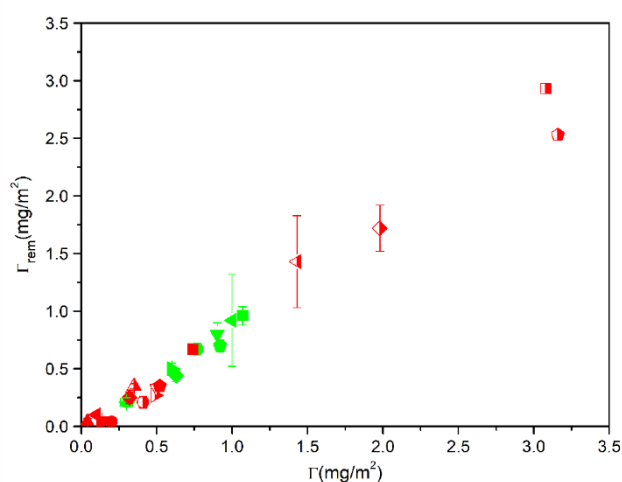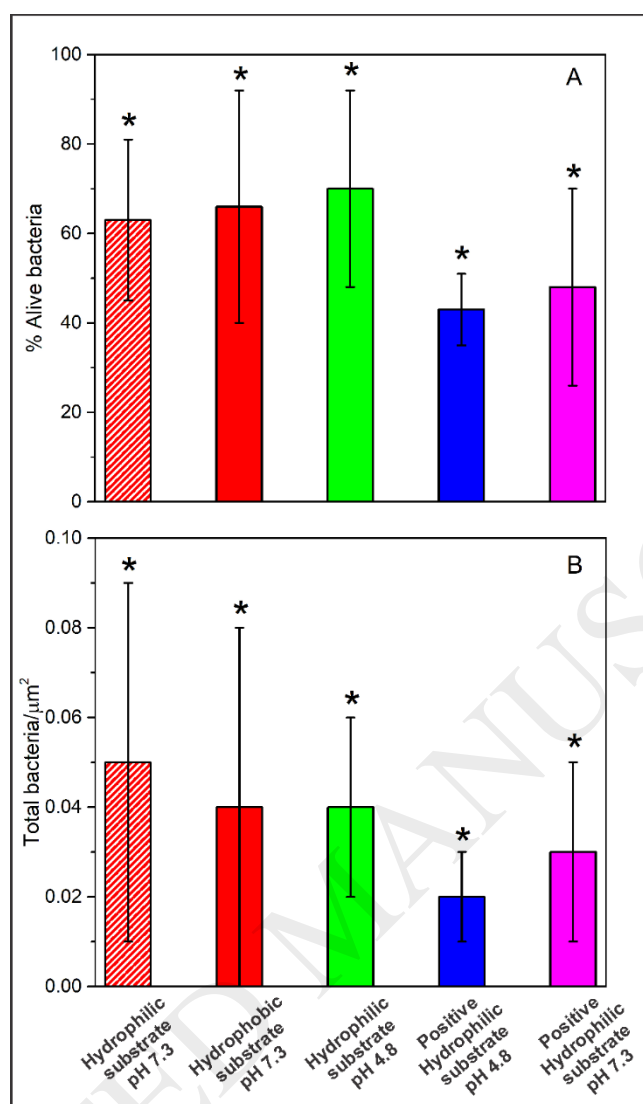
Figure 2.

Figure 2. Albumin adsorbed amount remaining after desorption (Γ_{rem}) as a function of albumin adsorbed amount (Γ) at different experimental factors: (red) hydrophilic substrates at pH (solid) 7.3 (patterned) 4.8 and (green) hydrophobic substrate at pH 7.3. The shape of the symbols represents the combination of Cp, Fr and adsorption time, respectively: (▲) 0.005 mg/mL-0.02 mL/s-50 s (▼) 0.005 mg/mL-0.02 mL/s-300 s (▶) 0.005 mg/mL-0.10 mL/s-50 s (◀) 0.005 mg/mL-0.10 mL/s-300 s (●) 0.100 mg/mL-0.02 mL/s-50 s (■) 0.100 mg/mL-0.02 mL/s-300 s (◆) 0.100 mg/mL-0.10 mL/s-50 s (⬠) 0.100 mg/mL-0.10 mL/s-300 s. The error bars represent standard deviation calculated from duplicate experiments for each combination of the factors.
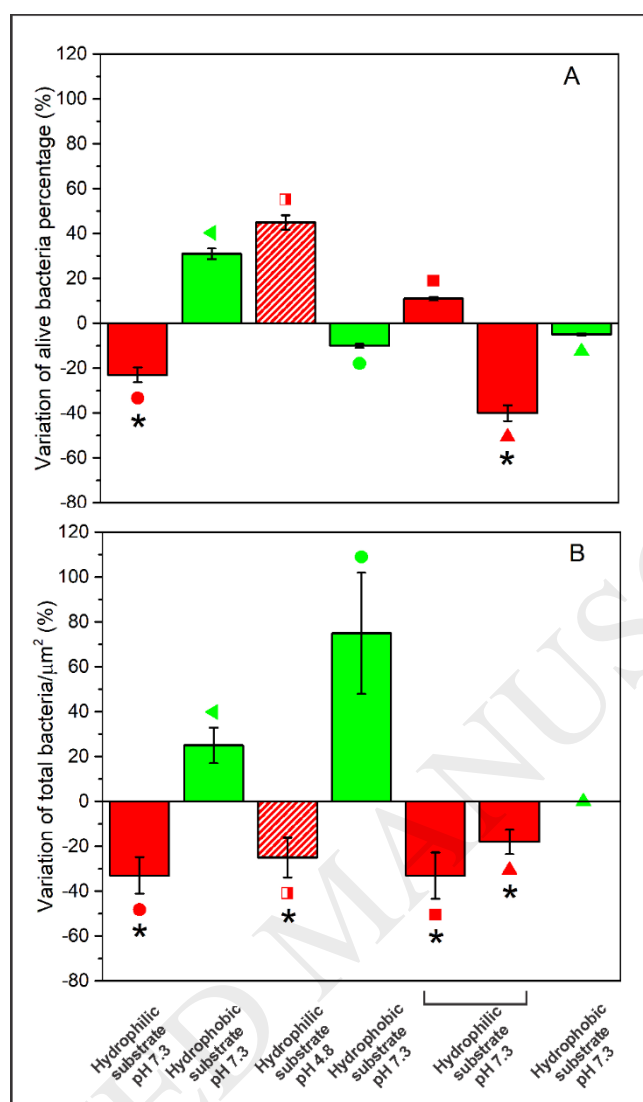
Figure 3.

Figure 3. (A) Percentage of alive and (B) total *S. aureus* adhered on bare substrates with different surface properties for 40 minutes: (red) hydrophilic substrates at pH (patterned) 4.8 and (solid) 7.3, (green) hydrophobic substrates at pH 7.3, (blue) positive hydrophilic substrates at pH 4.8 and (pink) 7.3. The error bars are 95% confidence interval and * represents values with $p > 0.05$.
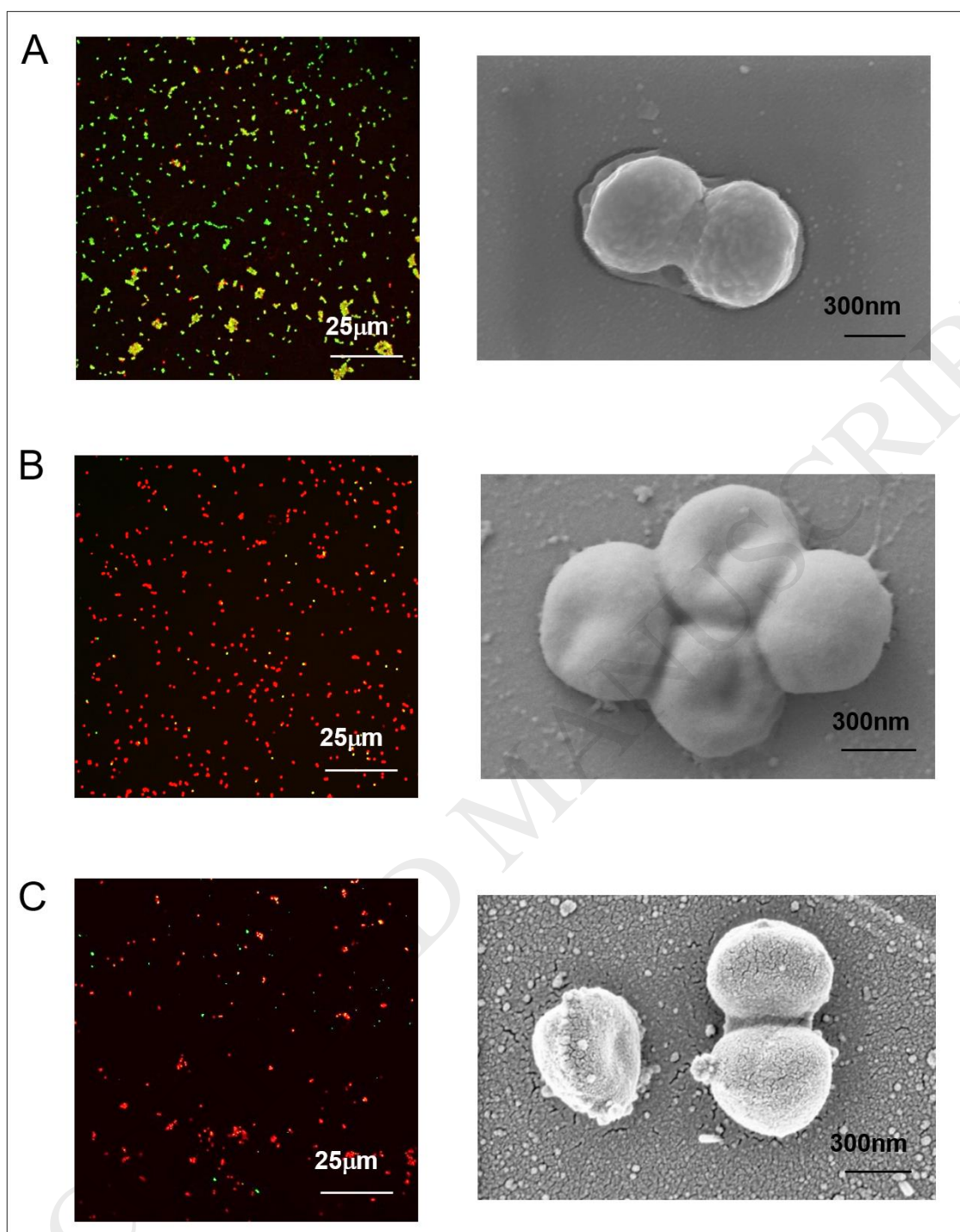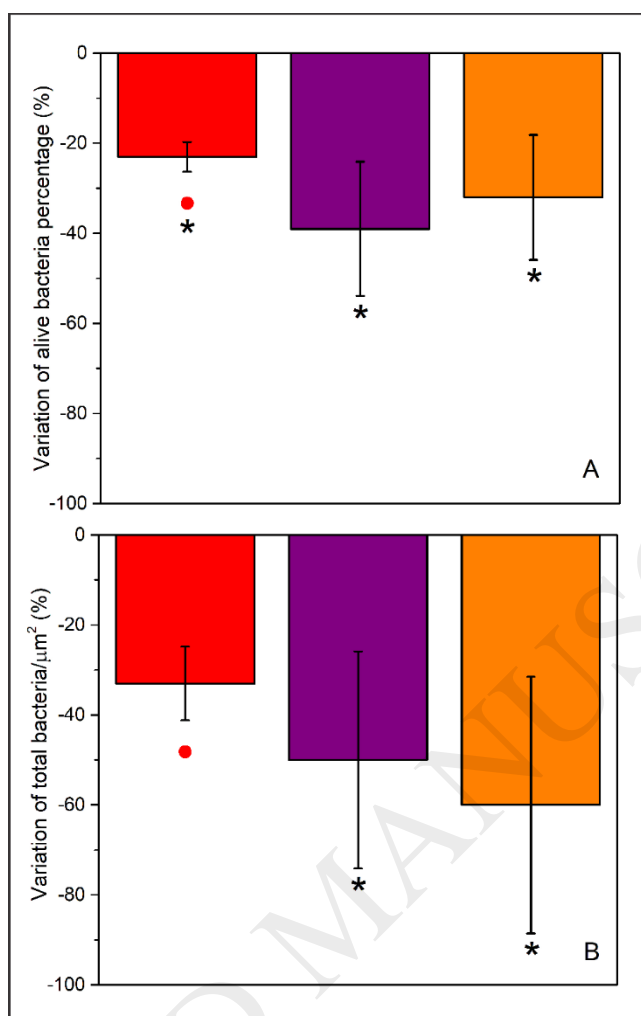
Figure 4.

Figure 4. Variation of the (A) percentage of alive and (B) total *S. aureus* on biofunctionalized solid substrates (related to bare ones) for 40 minutes: (red) hydrophilic substrates at pH (patterned) 4.8 and (solid) 7.3, (green) hydrophobic substrates at pH 7.3. The shape of the symbols represents the combination of Cp, Fr and adsorption time, respectively: (▲) 0.005 mg/mL-0.02 mL/s-50 s (◄) 0.005 mg/mL-0.10 mL/s-300 s (●) 0.100 mg/mL-0.02 mL/s-50 s (■) 0.100 mg/mL-0.02 mL/s-300 s. The error bars are 95% confidence interval and * represents values with $p > 0.05$.

Figure 5.

Figure 5. Fluorescence confocal and scanning electron microscopies images of adhered *S. aureus* on albumin biofunctionalized hydrophilic substrates at pH 7.3. (A) Positive and (B) negative controls. (C) Biofunctionalization conditions: C_p = 0.100 mg/mL, F_r = 0.02 mL/s, adsorption time = 50 s.

Figure 6.

Figure 6. Variation of the (A) percentage of alive and (B) total *S. aureus* on albumin biofunctionalized hydrophilic substrates at pH 7.3 (related to bare ones) for 40 minutes. Biofunctionalization conditions: (red) Native albumin at C_p = 0.100 mg/mL, F_r = 0.02 mL/s and 50 s, (purple) thermally treated albumin at 65 °C for 1 hour and 18 hours (orange) at C_p = 0.100 mg/mL and 1 h (dipping). The error bars are 95% confidence interval and * represents values with $p > 0.05$.