

Electron Microscopy Documents The Microorganisms' Biodestructive Action On Polyurethane And The Production, Internalization And Vesicular Trafficking Of Nanoparticles



ABSTRACT

Prostheses in the oral cavity are constantly attacked by microorganisms. Bacteria and fungi colonize these surfaces concurring in the material's biodestruction; the corrosive action generates debris of different size, with particles ranging from few micrometers to

Not paragraph

Transmission Electron Microscope (TEM), Scanning Transmission Electron Microscope (STEM) and Focused Ion Beam/Scanning Electron Microscope (FIB/SEM) used in this study show that bacteria (*S. aureus*) and fungi (*C. albicans*) are able to adhere to the prostheses' surfaces (polyurethane) and operate a biodestructive process. Electron images document the damages on the polymeric surfaces and the formation of debris. Polyurethane nanoparticles can be detected not only outside the bacterial cells but even in cells, surrounded by membrane vesicles; this work ascertains that the uptake process occurs through endocytosis, and outlines that the cytoskeleton is implicated both in the nanoparticles internalization and in the vesicular trafficking within the bacterial cell.

not paragraph

Polyurethane nanoparticles we studied are not engineered, have unexpected characteristics and reactivity; moreover being surrounded by vesicles within bacterial cells they raise a new problem in toxicology, since this represents a new way through which nanoparticles may gain access to the body (driven by bacteria-host cells interactions), elude the immune system reaction to xenobiotic elements and provoke pathologies.

Keywords: *S. aureus*, nanoparticles, polyurethane, endocytosis, cytoskeleton, membrane vesicles, (nano)toxicology, Electron Microscopy

1. INTRODUCTION

The risk of infection associated with polymeric materials used in most medical fields, as stomatological and orthopaedical prostheses or catheters, is common whenever biomaterials are attacked by microorganisms [1-4]. Our focus in this work is on bacteria (*S. aureus*) and fungi (*C. albicans*) permanently present in the oral cavity [5] that colonize plastic surfaces such as polyurethane (PU) prostheses, form a biofilm and concur to the polymeric material's destruction (biodestruction) [6-10].

When cultures of microorganisms and polymeric material are incubated for a long time (weeks) the critical augmentation of the bacterial or fungal mass causes nutrients depletion [11-13], therefore microbes are forced to use plastic materials as an alternative source of nourishment [7, 14, 15]. This is the starting point of the formation of a biofilm, a mucoid

26 matrix with several physiochemical microenvironments in which bacteria and fungi act as a
27 community [9, 16].

28 The initial interactions that take place between bacteria or fungi and the plastic material'
29 surfaces are nonspecific and driven by hydrophobic, electrostatic and van der Waals forces.
30 Despite the many existing models trying to describe the bacterial adhesion, the degree of
31 hydrophobicity of the staphylococcal cell surface and that of the matching material' surface
32 are generally considered highly important for the first attachment [12, 17-22].

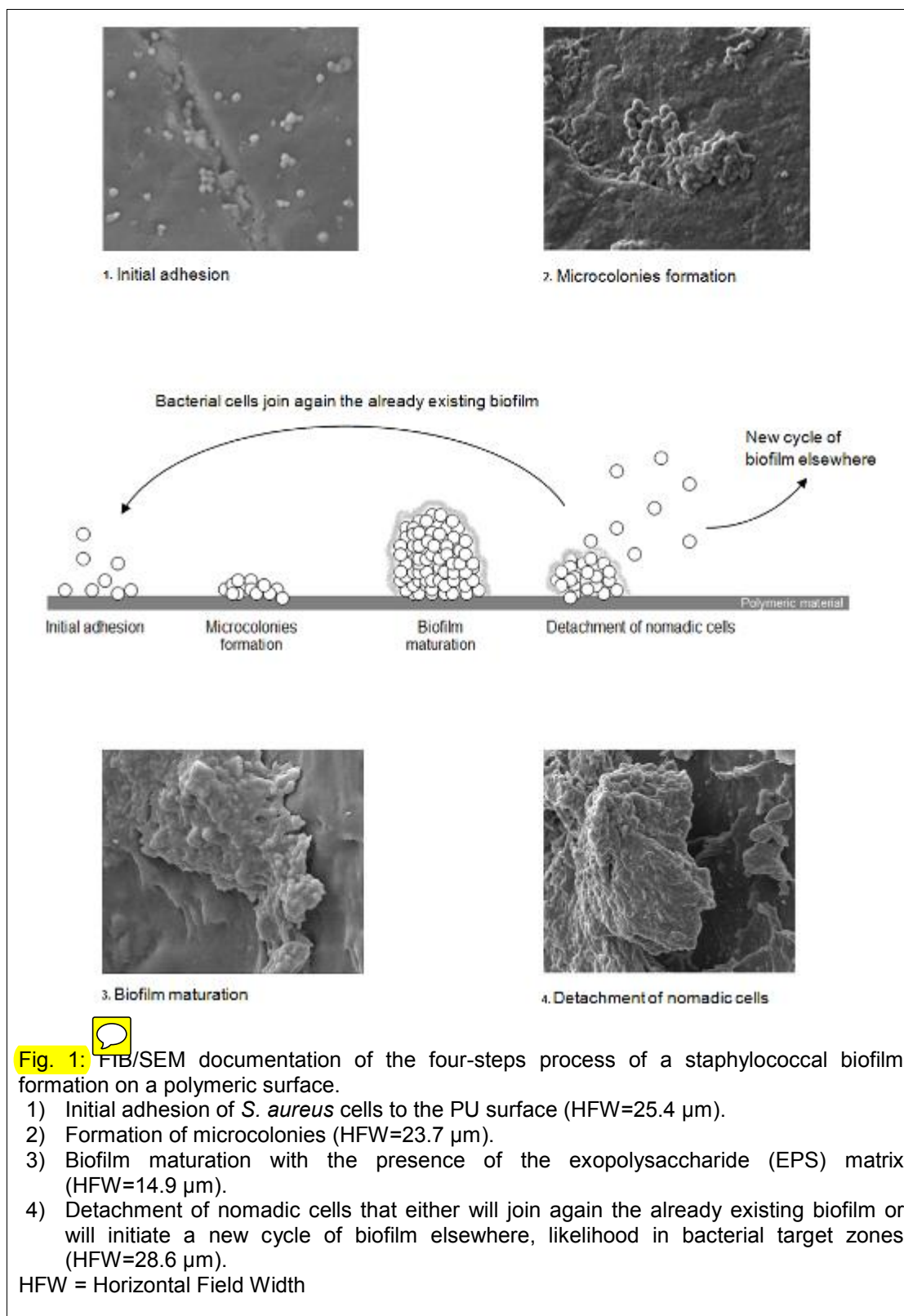
33 After this step cells aggregate, accumulate in multiple layers and form microcolonies. It
34 follows biofilm maturation and afterwards planktonic cells can detach from the biofilm,
35 disperse and start forming a new biofilm elsewhere (Fig. 1), likelihood in bacterial target
36 zones [23]. In fact microbial biofilms are a breeding-ground for pathogen agents, acting as
37 "niduses" of acute infection, and provide a huge metabolic advantage over the planktonic
38 mode of growth [9, 24, 25].

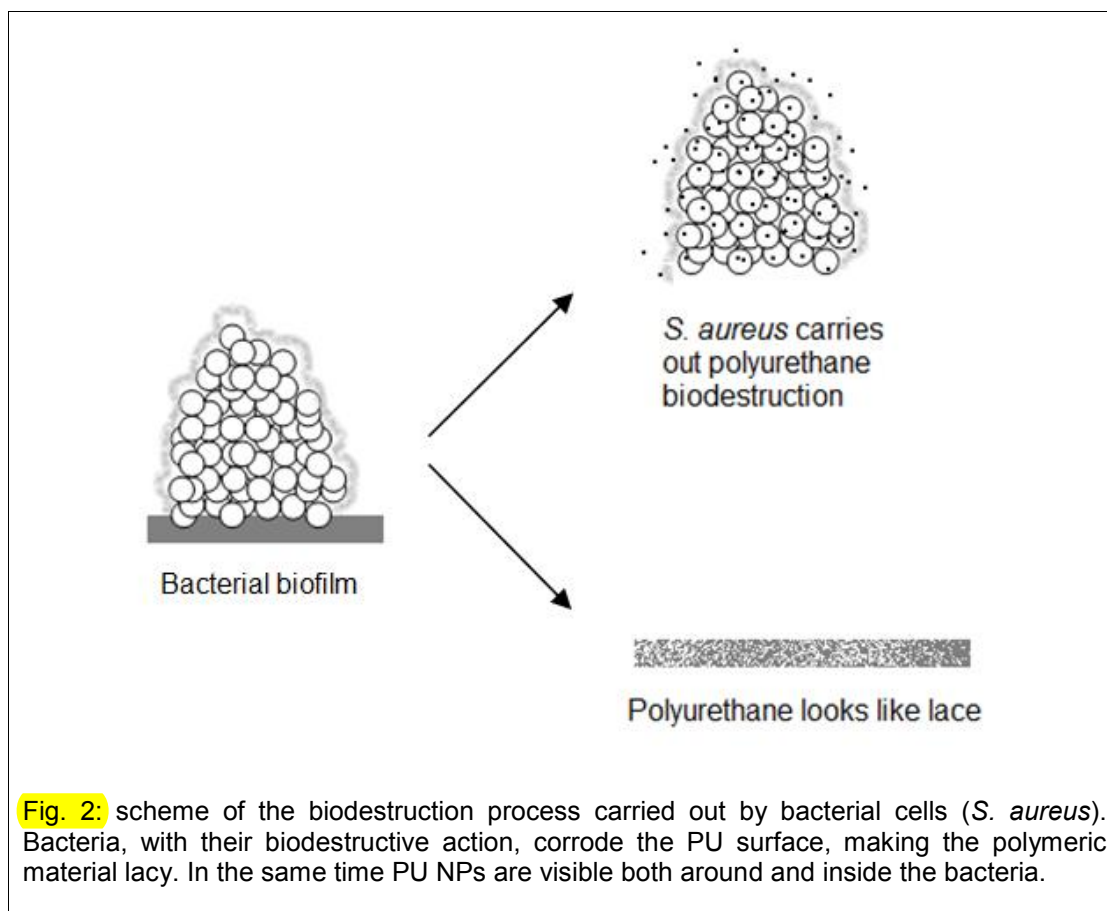
39 Bacterial or fungal biofilms have a corrosive effect on plastic surfaces, indeed microbes
40 manage to eat parts of the polymer away and the plastic surface appears seriously
41 damaged, almost lacy (Fig. 2). The microorganisms' action on PU generates debris of
42 different size, with particles ranging from few micrometers to nanometers [7, 8].

43 Current literature mainly talk about engineered nanoparticles (NPs) in cell cultures or NPs
44 entering organisms via inhalation, ingestion or via skin absorption [26-28]; moreover
45 functionalized NPs injection for drug delivery in medical applications is a field rapidly
46 developing [29]. In this work we face a new type of "non-engineered" NPs generated from
47 the prostheses' biodestruction. Our interest focuses on debris of about 20 nm, which are
48 more readily absorbed than bigger particles [30]. At these sizes NPs present peculiar
49 features: being able to cross biological membranes they are more likely to react with cells
50 and enter bacteria (and fungi). The NPs biocompatible surface properties depend both on
51 the charges carried by the NPs themselves [26], and on the possible shielding from the
52 elements dispersed in the medium (bio-molecules) [31]. Nanoscaled materials can display
53 unexpected and unusual toxicity as compared with bulk material [26, 28, 30, 32-35].

54 In this work we prove that Transmission Electron Microscope (TEM), Scanning Transmission
55 Electron Microscope (STEM) and Focused Ion Beam/Scanning Electron Microscope
56 (FIB/SEM) are useful techniques to document the formation of bacterial and fungal biofilm on
57 plastic materials (Fig. 1), the damages that biofilms provoke on PU, the consequent
58 biodestruction of the polymer, resulting in the generation of NPs (Fig. 2), the presence of
59 NPs outside and inside the bacterial cells, many of them being included in membrane
60 vesicles (MVs), and the transitory lysis of the bacterial membrane; the observed dynamics of
61 NPs (which electrochemical characteristics are still unknown) to approach and enter
62 bacterial cells suggest how the uptake process works and highlight the NPs/MVs spatial
63 organization.

64 Combining our *in vitro* results with knowledge from literature, we hypothesize what could
65 happen *in vivo*, in the event of a bacterial biofilm infection of a dental prosthesis (PU).
66 Nomadic cells detached from a mature biofilm to start a new cycle of biofilm elsewhere
67 represent a high risk since they have PU NPs within themselves. Nomadic cells, guided by
68 their bacterial infectiousness, could transport the PU NPs to target zones, causing
69 biomaterial-centered infections [36] in the human body. Eventually the nanoscaled material
70 would be more likely to travel from bacteria to their target human cells, and the NPs' ways
71 and chances to enter various organs would be increased, constituting an augmented
72 toxicological risk [26, 28, 32-35, 37].





74

75

76 2. MATERIAL AND METHODS

77

78 Samples of PU (Dentalur Russia, Russian Federation) with various types of surfaces were
 79 analyzed. As described in refs. 7 and 8 the culture of *S. aureus* was isolated from a patient
 80 with a periodontal disease and was incubated with PU slices of different roughness. Control
 81 samples were a PU slice with a non-sawed surface in broth and a broth with *S. aureus* with
 82 no PU.

83 PU samples and bacteria were incubated from 1 to 45 days at 37 °C. After centrifugation
 84 (6000 rpm for 10 mins) the bacterial pellet was placed on a thin silicon substrate for the
 85 FIB/SEM observation or prepared for the TEM/STEM analysis. The preparation of biological
 86 specimens for scanning or transmission electron microscopy consisted in primary fixation,
 87 washing, and secondary fixation. At this point the sample used for the FIB/SEM observation
 88 was dried, mounted on an aluminum stub and coated with a 5-nm-thick gold layer in a SPI-
 89 Module Sputter/Carbon Coater System (SPI Inc., USA). Instead the sample observed with
 90 the TEM underwent infiltration with transition solvent and resin, embedding, curing and
 91 cutting.

92 In samples preparation chemical-physical methods of dehydration were not applied in
93 accordance with the traditional techniques, because standard drying operations led to
94 structural changes of both the biofilm and the cells [38].

95 Samples were treated with copper sulphate and stained with Alcian Blue in order to have a
96 better view of the exopolysaccharide (EPS) matrix of the biofilm [39].

97 Generally TEM is the most appropriate technique to investigate the characteristics of
98 nanoscaled particles [40]; in our type of investigation, instead, the combined use of TEM,
99 STEM and FIB/SEM is essential, since the user is allowed to freely search the surface of
100 interest and to obtain images with a large range of magnification.

101 TEM and STEM images were obtained with a Tecnai F20 X-TWIN microscope (FEI
102 Company, USA) equipped with a 200 kV FEG column and a CCD detector. Bright Field (BF),
103 Dark Field (DF) and High Angle Annular Dark Field (HAADF) images have been collected to
104 improve contrast and resolution [41], and to gain more information about PU NPs' size and
105 their relative position compared to cell structures.

106 A set of detectors collected the transmitted electrons, according to the scattering angle
107 influenced by density, thickness and atomic mass. STEM BF images contain information
108 from all the electrons passed through the sample; STEM DF images are formed from
109 specific diffracted beams that give orientation contrast [42]. Subtle features hidden in BF
110 images are visible in high-contrasted and less intense DF images.

111 A third detector collected the electrons scattered at angles typically higher than 50 mrad
112 (electrons derived from thermal diffuse scattering) to form HAADF images. With this last
113 method the specimens' denser/heavier areas appear brighter allowing the extraction of
114 information from what was usually interpreted as "background intensity" [41].

115 Comparing transmission techniques, STEM images had several advantages over the TEM
116 ones. First of all the resolution in STEM is a $\sqrt{2}$ factor better than in TEM; moreover in
117 conventional TEM imaging an objective aperture is used to select the transmitted or
118 scattered beam in order to form BF or DF images respectively. In STEM imaging the
119 transmitted or scattered beam is selected in an equivalent way using on-axis or annular
120 detectors rather than apertures, since an annular detector collects more electrons than an
121 aperture. Finally, as no lenses are used to form STEM DF images, they are less noisy than
122 TEM DF ones.

123 Dual beam FIB/SEM Quanta 200 3D (FEI Company, USA) was used to observe specimens
124 in both high and low vacuum, mostly at 5 kV electron beam acceleration. The beam
125 penetration is related to the acceleration of the electrons: at 5 kV electron beam acceleration
126 it is possible to get better images because of the lower signal contribution of the sample's
127 sub-surface. The operator has less problems concerning the charge, and images have better
128 details of superficial and subsuperficial parts [38, 43-48].

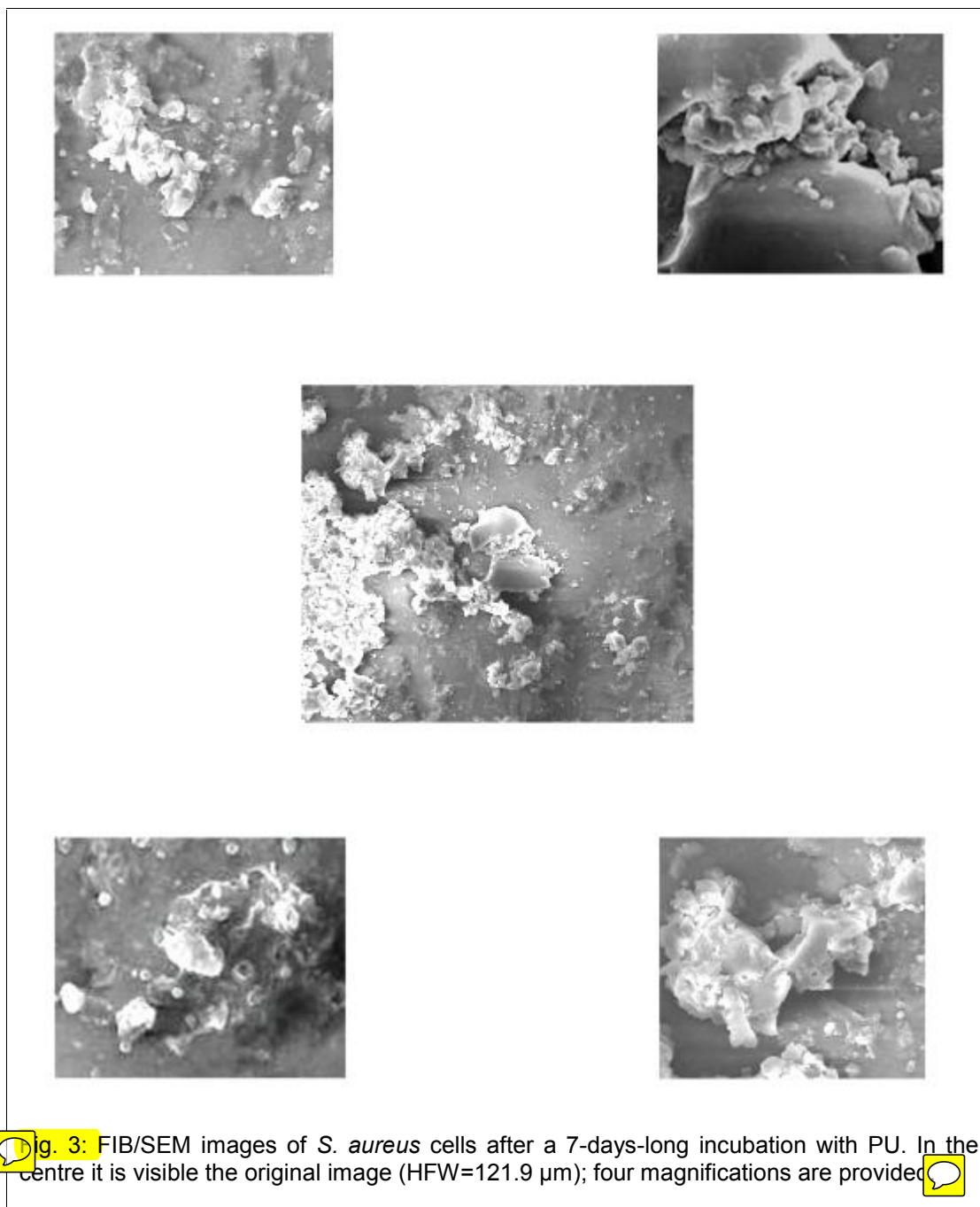
129

130 3. RESULTS

131

132 Smooth and rough surfaces of PU were incubated with  Bacillus subtilis. After a 24 hours-long
133 incubation on the smooth PU surface there were just single bacterial cells and/or
134 microcolonies, whereas the processes of colonization and adhesion on the rough surface
135 progressed much faster [7, 8]. In Fig. 3 it is shown the process of biofilm formation after a 7-

136 days long incubation. After 45 days of incubation all the PU surfaces (both smooth and
137 rough) were thoroughly covered by biofilm (Fig. 4).



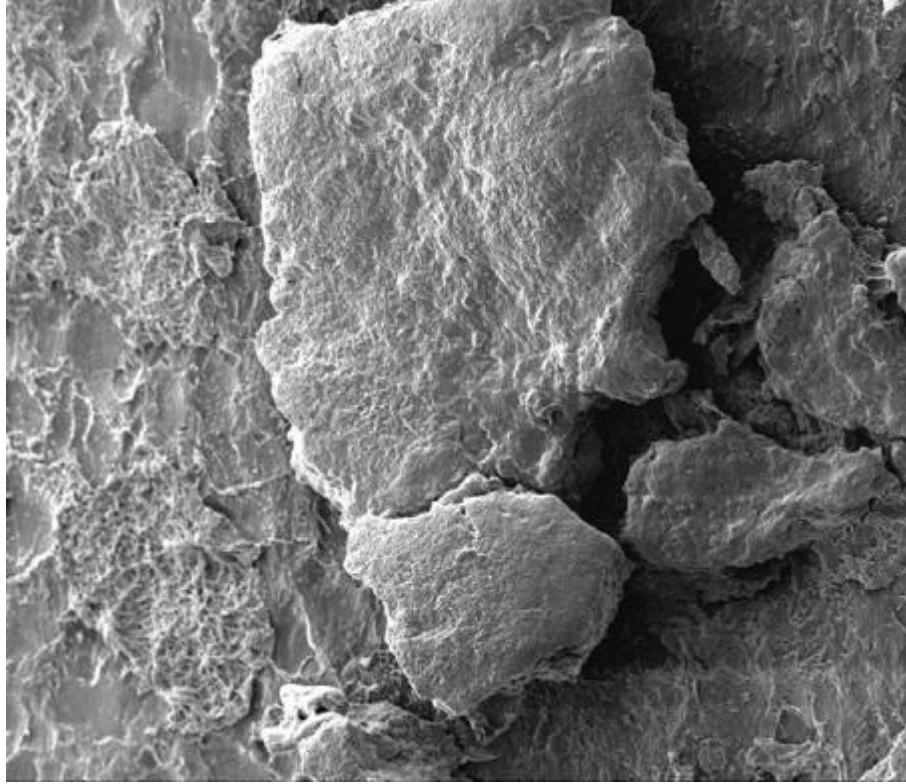


Fig. 4: FIB/SEM image of a fragment of a mature staphylococcal biofilm on PU after a 45 days-long incubation (HFW=249 μm).

139

140 Due to the coccal biodestructive action, damages appeared on the PU (Fig. 5), the plastic
 141 surfaces looked like lace [7, 8], and micro- and nano-debris detached from the polymer were
 142 detected on the exopolysaccharide (EPS) matrix (Fig. 6).

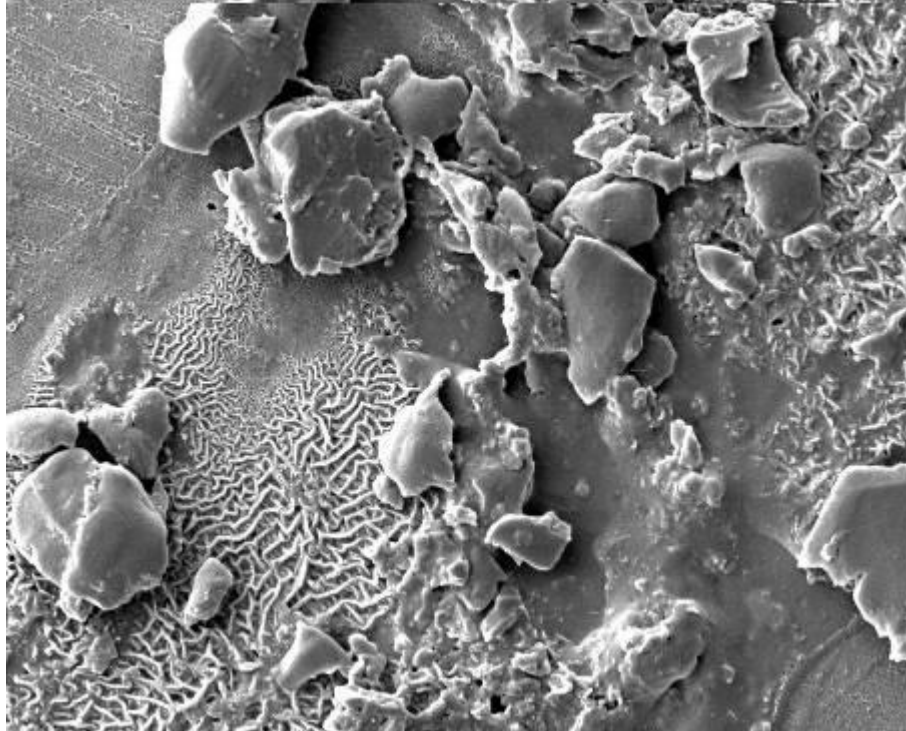


Fig. 5: FIB/SEM image of the evolution of the damage on the PU surface due to the staphylococcal biofilm after 3.5 months of incubation. (HFW=51.8 μm)

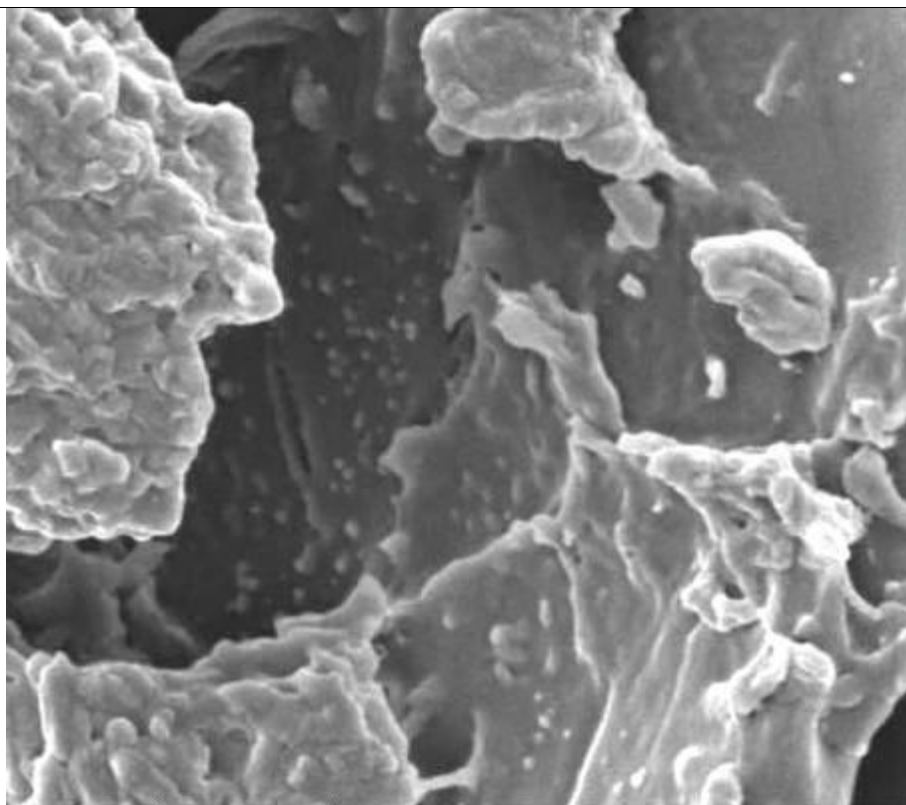


Fig. 6: FIB/SEM image of micro- and nano-debris detached from the PU surface, detected on the exopolysaccharide (EPS) matrix (HFW=19.5 μm).

144

145 Samples were observed with transmission microscopy, and TEM and STEM images were
 146 acquired. TEM BF image (Fig. 7) shows a *S. aureus* cell with PU NPs inside the cell within
 147 vesicles, on the cell wall not surrounded by membranous structures, and in the external
 148 environment.

149 The same sample of Fig. 7 was observed in the same transmission microscope using the
 150 STEM technique, and in Fig. 8 it is shown the resultant HAADF image. PU NPs have higher
 151 electron density than the cell biological components, so they appear darker than the
 152 surrounding medium in BF images (Fig. 7) and brighter in HAADF ones (Fig. 8).

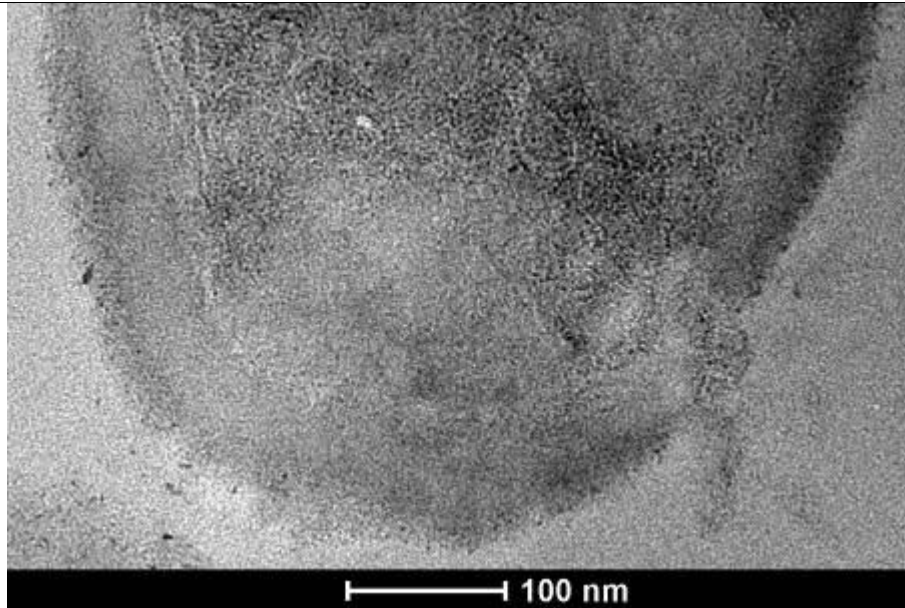


Fig. 7: TEM BF image of a *S. aureus* cell with PU particles (dark particles) detectable in the proximity of the cell wall, on the cell wall, and inside the bacterium within vesicles. Details of the ruffle and lysis of the plasma membrane are visible.

153

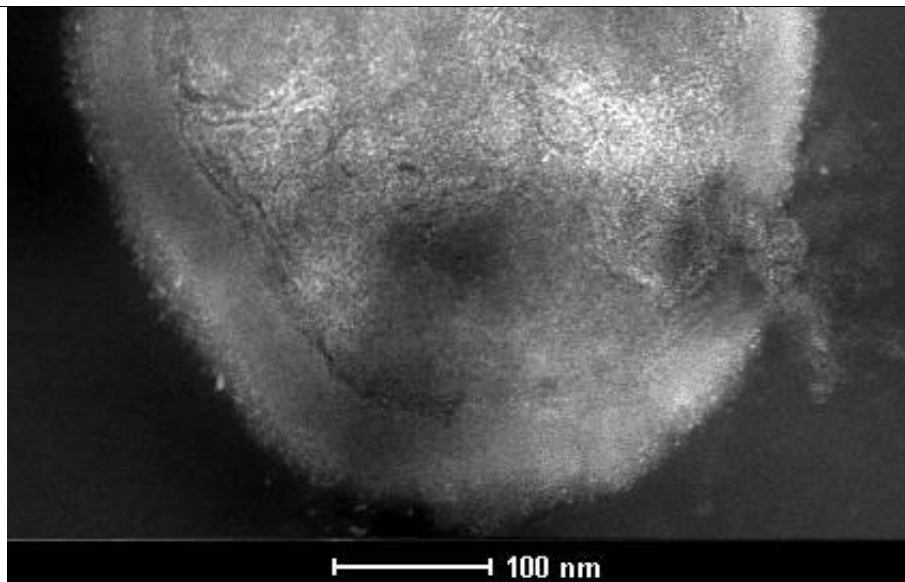


Fig. 8: STEM HAADF image of the same sample of Fig. 7. This microscopy technique gives a better view of PU particles, since they appear brighter than the surrounding medium.

154

155 Analyzing Figs. 7 and 8 we recreated a scheme of the visible vesicles (Fig. 9) and their
 156 position within the bacterial cell. It is evident that vesicles follow a pre-established linear
 157 route that runs across the bacterium. In the next section it will be discussed the relation
 158 between this result and the existence of bacterial cytoskeletal structures, important not only
 159 as support for the cell and during the division phases of the cell cycle, but even as dynamic
 160 structures for vesicular trafficking.

161 From Figs. 7 and 8 it is also possible to see that *S. aureus* is invaded by vesicles carrying
 162 one or more NPs (a magnification of a detail of Fig. 7 is provided in Fig. 10). Moreover PU
 163 NPs do not aggregate during the uptake process and maintain their individuality even when
 164 more than one NP is inside the vesicle. Electron images can be of help in the toxicological
 165 investigations, since we can have an idea of the dose of NPs taken up by a single cell in an
 166 *in vitro* assay. This situation, represented even in Fig. 9, opens discussions about NPs'
 167 physical features and debates about whether the biodestruction-generated nature of NPs
 168 could have any relations with the non-aggregated state on PU NPs [49].

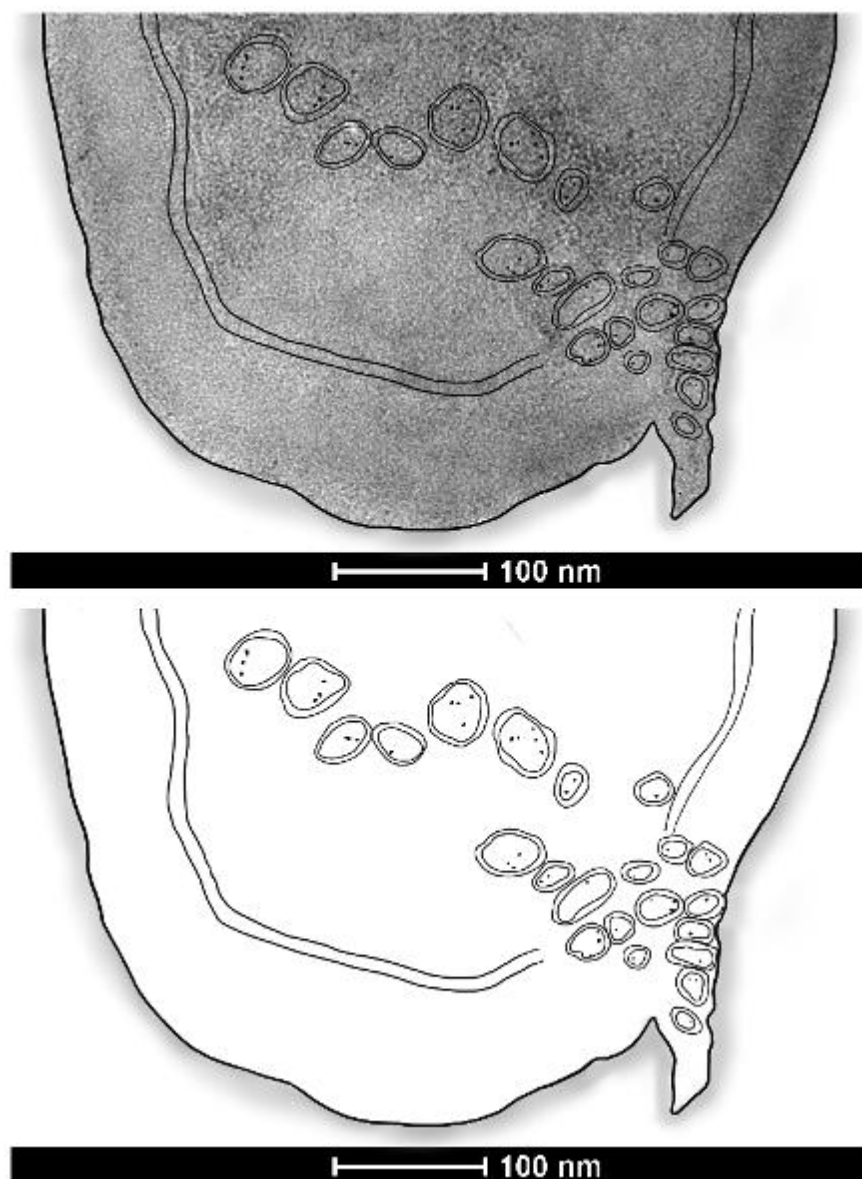
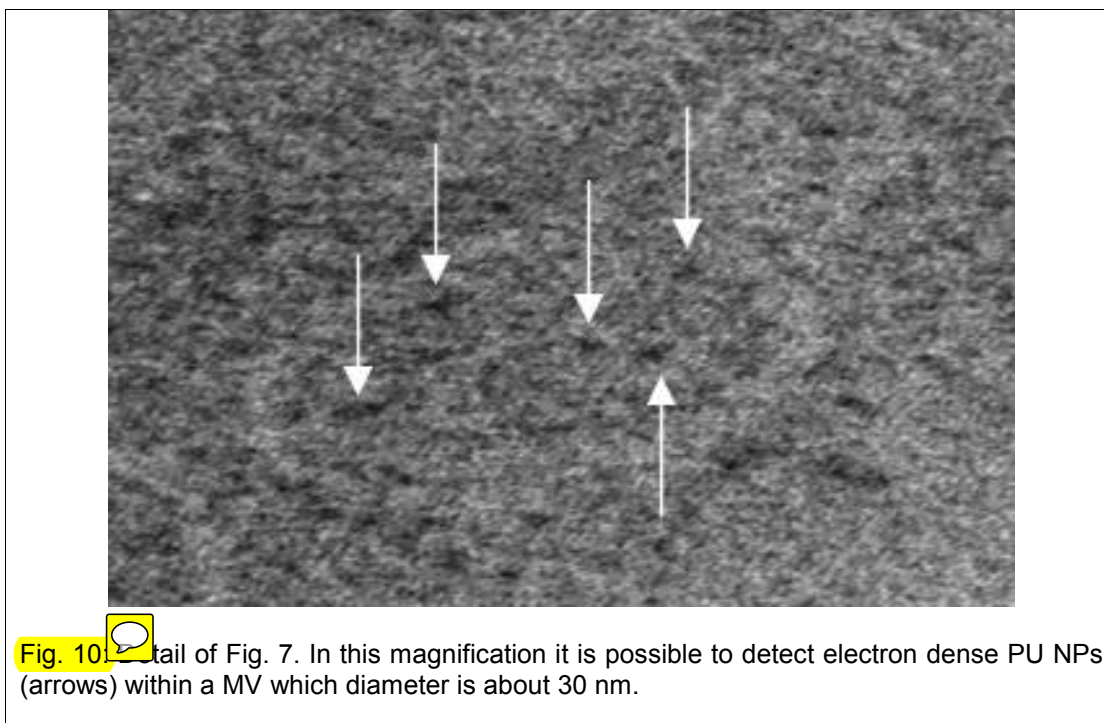


Fig. 9: representation of the same sample of Figs. 7 and 8. Electron images (Figs. 7 and 8) could not be analyzed automatically using a software [50, 51], so the analysis and the schematization were conducted integrating manual and automated procedures.

This scheme highlights the vesicles' spatial distribution within the bacterial cell: vesicles seem to follow a linear route that runs across the bacterium. In this picture it is also schematized that not all the vesicles carry the equal number of NPs, in fact one or more NPs per vesicle are visible (a detail of a MV loaded with electron dense PU NPs is provided in Fig. 10).

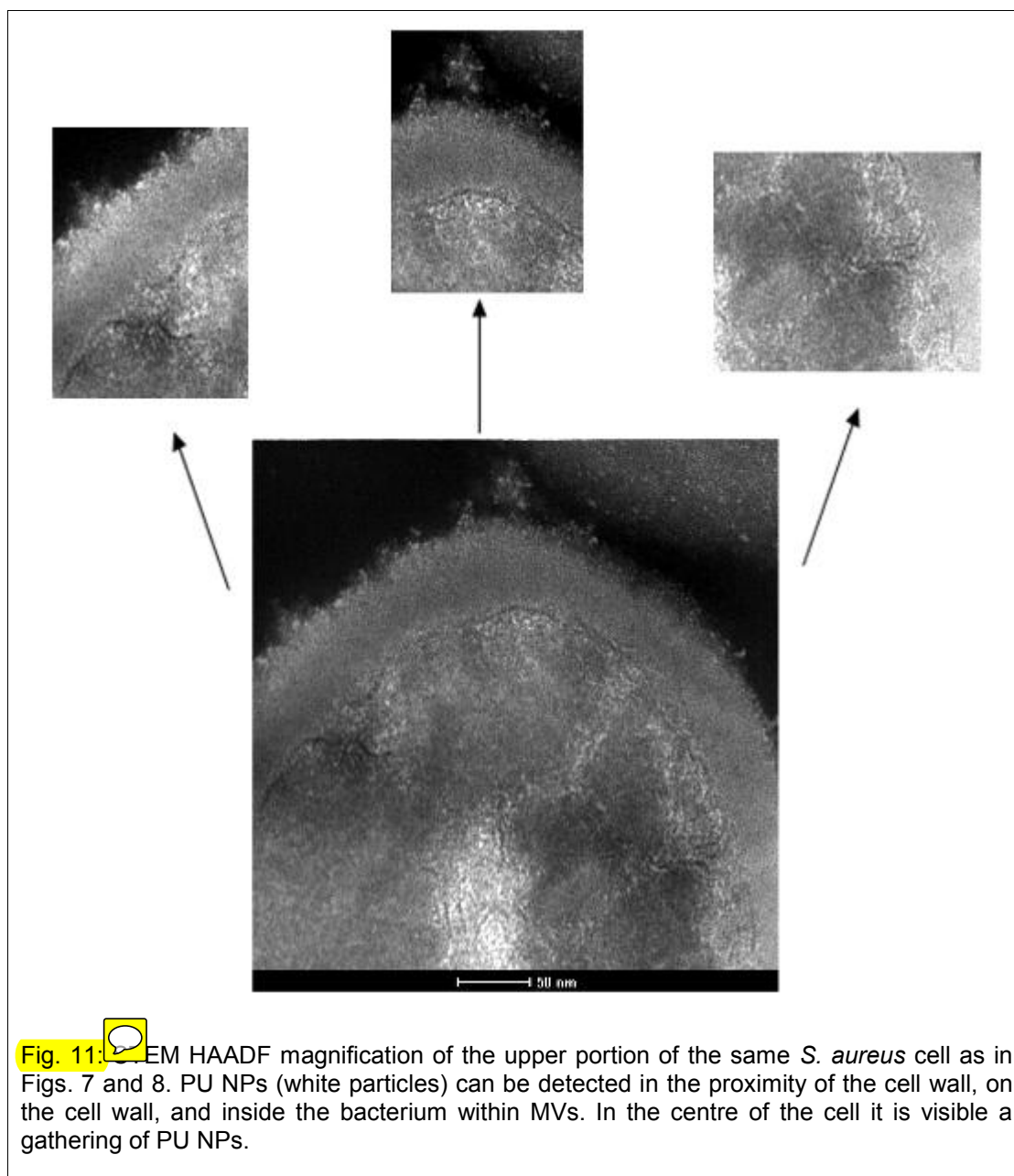


170

171 In order to verify our findings, we analyzed the upper part of the same *S. aureus* cell as in
 172 Figs. 7 and 8. We obtained the same run down:

- 173
- 174 • PU NPs detectable in the proximity of the cell wall, on the cell wall, and inside the
 bacterium within MVs (Fig. 11);
 - 175 • a course of PU NPs running across the bacterium (Fig. 12);
 - 176 • a gathering of PU NPs in the centre of the bacterial cell (Figs. 11 and 12).

177



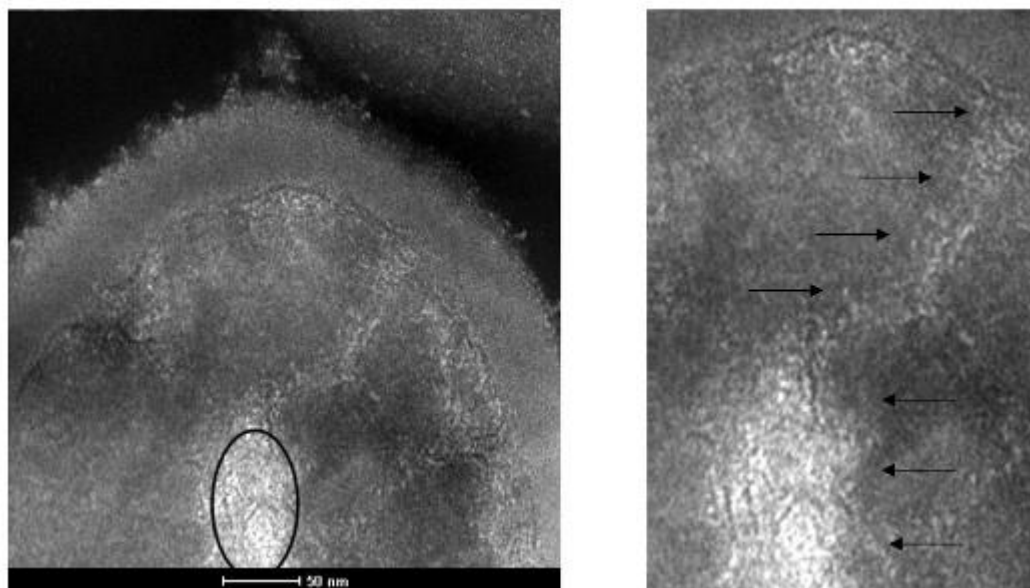


Fig. 1. TEM HAADF magnification of the upper portion of the same *S. aureus* cell as in Figs. 7 and 8. In the left image it is possible to detect PU NPs in the centre of the cell (circle) within MVs; in the right image it is shown the course of PU NPs running across the bacterium (from the outside to the centre of the cell).

179

180 4. DISCUSSION

181

182 Electron and ion correlated microscopies (FIB/SEM, TEM and STEM) are important to prove
183 NPs generation after the plastic material's biodestruction carried out by bacteria (*S. aureus*);
184 similar results can be expected also for fungi, since *Candida* biofilms are able to colonize
185 polymeric surfaces [52, 53], and can result in the biodestruction of the PU (Fig. 13).

186 Electron images prove the presence of bacterial biofilm on PU surfaces (Figs. 1, 3, 4 and 5).
187 The FIB/SEM subsuperficial analysis of the PU surface allows to view the increment of
188 existing defects and the formation of new ones on the polymer. The plastic surface looks like
189 lace [7], and PU particles (Fig. 6) ranging from micrometers to nanometers can be detected
190 in the cellular environment, revealing the biodestructive potential of *S. aureus* [8].

191 *In vitro* experiments show that nanosized particles detached from the PU surface can be
192 internalized into the bacterial cells (Figs. 7-12) [7, 8, 54]. It is remarkable that the presence
193 of PU NPs does not compromise the bacterial viability, in fact ultrastructural data prove that
194 the internalization of NPs does not prevent cell fission [7]. Bulk PU is non-toxic [10], but it is
195 not possible to say the same thing about PU NPs. NPs behavior and effects depend on their
196 morphology, size, surface characteristics, inner structure and reactivity. They can remain
197 free or group together, according to the attractive or repulsive interaction forces between
198 them [33, 49, 55-57]. The possible resulting aggregation strongly affects the NPs
199 internalization dynamics. The ability of NPs to move inside the body sets a great threat when
200 associated with the potential hazard of the nanomaterial. Further studies are necessary in
201 order to understand whether the particles' size and/or their bioaccumulation potential are
202 linked to any pathogenicity factor [32, 58-60].

203 It is not known how NPs interact with the bacterium, but surely the NPs vicinity to the
204 microorganism brings into play forces of electromagnetic origin. In literature researchers talk
205 about a protein cover that could envelop NPs, called protein corona [61, 62]; it has been
206 shown that the NP itself, which can be positively or negatively charged as well as neutral, is
207 able to affect the composition and maturation of the protein corona, attracting bio-molecules
208 dispersed in the environment [31, 63-66]. We are not able to see the effective presence of
209 the corona in our images, but we must keep in mind that this is another element that
210 modifies the NP charge [31, 67-73] and its approach to the bacterium. The bacterial
211 membrane has a very high electric field up to 10^9 V/m [74-76], therefore the closeness of PU
212 NPs, with or without the protein corona, to the bacterial cell can induce electrical dipoles in
213 NPs and consequently determine their movement [55, 57].

214 From Figs. 7, 8, 11 and 12 it is evident that NPs in the proximity of bacterial cells do not
215 aggregate. This could be related to the biodestruction-generated nature of the NPs that put
216 them in a "non-engineered" class, but we cannot forget to take into account all the elements
217 (protein corona) that likely can play a key role interfering with bacteria [49, 77-79].

218 Polymeric NPs are internalized by the bacterial cell through endocytosis, a general term for
219 uptake processes. Endocytosis, including pinocytosis and phagocytosis, describes the
220 internalization of fluids, solutes, macromolecules and particles through the invagination of
221 the plasma membrane and the scission of MVs which envelop the external material.
222 Vesicles, freely moving or actively transported along or by cellular structures, can assume
223 different names (early endosome, late endosome, lysosome, endolysosome, exosome) [80-
224 83]. TEM and STEM images show that NPs within the bacterium are surrounded by a MV
225 which diameter is about 30 nm (Figs. 7, 8, 10-12).

~~226 Endocytosis is a well known process in the eukaryotic domain [84], but debate is intense
227 about prokaryotes [85]. Since the mechanisms of endocytosis and the roles of the different
228 molecules involved are complicated, it is necessary a general view of how endocytosis
229 works for eukaryotes and prokaryotes. The cytoskeleton has an important role in the
230 endocytic process of both eukaryotes and bacteria [86], not only during the internalization of
231 molecules and particles, but also in the vesicle trafficking within the cell. Cytoskeletal
232 structures have been studied in the budding yeast *S. cerevisiae*. Studies on the eukaryotic
233 microorganism have been useful to clarify the complex structure of cytoskeleton, the role of
234 molecular homologues of actin, tubulin and intermediate filament, the involvement of the
235 actin homologue in internalization and post internalization events, and the sorting of
236 endocytosed material into the recycling vacuolar pathways [87].~~

~~237 Bacteria were thought to lack cytoskeletal filaments, but over the past few years bacterial
238 homologues of eukaryotic microtubules, actin filaments and intermediate filaments have
239 been observed in prokaryotes [86, 88-94], and studies confirmed that bacteria not only
240 possess cytoskeletal structures but even the cytoskeleton was a prokaryotic invention [91].
241 Bacterial homologues of tubulin (FtsZ) and actin (MreB, ParM and FtsA) not only resemble
242 their eukaryotic counterparts structurally, but also show similar functional characteristics. As
243 eukaryotes use endocytic processes mainly based on actin regulation, so bacteria have
244 actin-like molecules responsible for the preliminary steps of endocytosis (membrane
245 curvature, invagination and vesicles formation) [95-101] and for the internal trafficking of
246 vesicles. The actin-like molecule found in *S. aureus* is FtsA; MreB genes are found
247 exclusively in non-spherical cell types [97, 98, 100, 102, 103].~~

248 In the electron images (Figs. 7, 8, 11 and 12) it is possible to observe a lot of vesicles, all
249 loaded with one or more NPs, arranged along linear paths that connect the whole cell. These
250 linear routes seem to indicate the actual existence of bacterial internal structures, important

as support for the cell. ~~Cytoskeleton and in particular microtubules are considered the dynamic spatio-temporal structures supporting vesicle trafficking [104]. Therefore electron microscopy, pointing out the vesicles' spatial distribution, provides us a direct proof of the presence of cytoskeletal structures within the bacterial cell. In literature papers support the existence of the cytoskeleton showing images of cytoskeletal structures obtained with fluorescence microscopy [95, 99, 105]; preparing samples using different preparation techniques and observing them with electron microscopes, one can see cytoskeletal structures in many different types of bacterial and eukaryotic cells [106-108].~~

From *in vitro* experiments it is shown that the microorganisms' biodestructive action leads to the generation of NPs from PU prostheses (Figs. 1-6), and snapshots of the internalization and vesicular trafficking of PU NPs within bacterial cells are provided (Figs. 7-12). Extrapolating *in vitro* results, this study suggests some hypotheses regarding NPs-cell interactions in *in vivo* dynamics and the implications in the toxicological field.

Since PU NPs we are taking into account derive from the PU biodestruction carried out by microbes and therefore are not engineered, the nanomaterial can present unexpected physical and structural characteristics and follow unexplored pathways. Bacteria (*S. aureus*) and fungi (*C. albicans*) studied in this work are microorganisms permanently present in the oral cavity as commensals [5]; sometimes they can be highly pathogenic, especially *S. aureus* have plenty of virulence factors, including a great ability to evade host immune defenses and to develop resistance against the most used antibiotics [109-112].

Driven by intrinsic infectious potential, bacteria loaded with PU NPs could infect numerous human host tissues provoking pathologies [113-115], releasing, in the same time, PU NPs in human host cells. Therefore NPs surrounded by vesicles within bacteria represent a new way through which PU NPs may gain access to human organs with the risk of bioaccumulation [32]. NPs could be discharged through exocytosis, and therefore exit the bacterial cell without the vesicle (little is known about the presence or the absences of the protein corona), or they could be released in consequence of the bacterial death and exit the bacterium enveloped in the vesicle [116]. Moreover TEM and STEM images show the presence of NPs stuck on the bacterial cell wall and not yet internalized (Figs. 7, 8, 11 and 12). Once the bacterium attacks the host cell, these NPs could access it with no vesicle at all [116].

The probability to find NPs surrounded or not by vesicles in human host cells [117] raises an important issues in the toxicological field since bacterial cells can act as targeted vectors of possible toxic material and could release in the body highly reactive PU NPs, eluding the immune system reaction to xenobiotic material [109-112, 116, 118].

Biofilm formation and PU biodestruction are not unique for *S. aureus*. In literature authors talk about the synergy of fungi and bacteria in the biofilm formation, and the clinical implications that these complex interactions would have in immunocompromised hosts [119]. For instance, denture-associated biofilms contain multiple microbial species and include *S. aureus*, *E. coli* and fungi in the genus *Candida*, most notably *C. albicans*. The aetiology of denture stomatitis is multifactorial with *Candida* infections, trauma and poor dental hygiene playing an important role; moreover plaque may serve as a protected reservoir for *C. albicans* [9].

Interactions between *Candida* and *S. aureus* are involved in several functions such as coaggregation, growth stimulation or inhibition, and production of changes in candidal adherence [9].

297 Fig. 13 shows that *C. albicans* has an interaction with PU similar to that of *S. aureus*, and an
 298 increment of the biodestructive capacity is expected by the mutual work of both *S. aureus*
 299 and *C. albicans*.

300

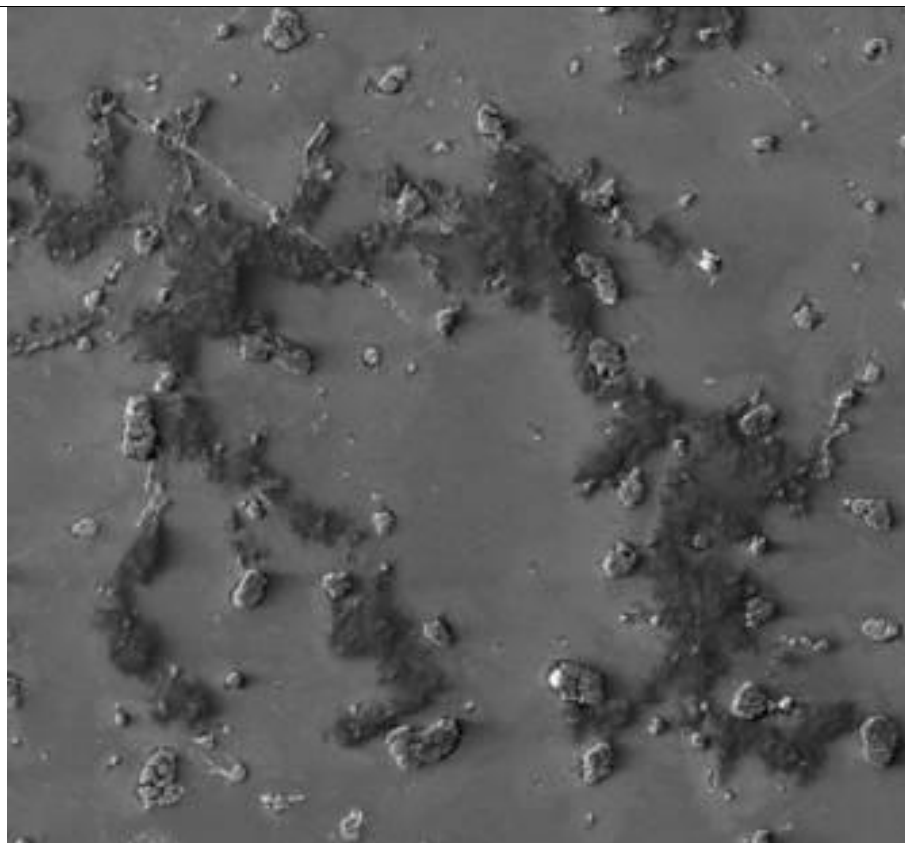


Fig. 13: FIB/SEM image of the colonization of a PU surface carried out by *C. albicans* (HFW=894 μm).

301

302 5. CONCLUSION

303

304 Electron microscopy techniques are promising in the investigation of bacterial and fungal PU
 305 **biodestruction**, and in the observation of the consequently generated PU NPs. Although
 306 microscopy cannot cover all the investigating parameters, it is an effective method to study
 307 how NPs approach cells, which chemical or physical interactions lead to the plasma
 308 membrane invagination, and whether several materials with different electric characteristics
 309 carry to diversified interactions with cells, resulting in supraventricular spatial organization.

310 This study contributes to raise some important issues which need to be further investigated:
 311 the bacterial cytoskeletal activities, the role of the cytoskeleton in endo- and exo-cytic
 312 processes, and the transport of PU NPs within vesicles from the medium to bacteria and
 313 then to infected host cells, with related toxicological issues

314 Transposing the situation seen *in vitro* to *in vivo* host organisms remains a challenge [120]
315 and more research needs to be done in order to understand the NPs' behavior and their
316 bioaccumulation potential in living systems at different structural levels (cells, tissues,
317 organs), giving contributes to studies in the toxicological field [78].

318

319

REFERENCES

- 320 1. Marrie TJ, Nelligan J, Costerton JW. A scanning and transmission electron microscopic
321 study of an infected endocardial pacemaker lead. *Circulation*. 1982;66:1339-1341.
- 322 2. Ramage G, Martínez JP, López-Ribot JL. *Candida* biofilms on implanted biomaterials: a
323 clinically significant problem. *FEMS Yeast Res*. 2006;6:979-986.
- 324 3. Schaudinn C, Gorur A, Keller D, Sedghizadeh PP, Costerton JW. Periodontitis: An
325 archetypical biofilm disease. *J Am Dent Assoc*. 2009;140:978-986.
- 326 4. Teterycz D, Ferry T, Lew D, Stern R, Assal M, Hoffmeyer P *et al*. Outcome of orthopedic
327 implant infections due to different staphylococci. *Int J Infect Dis*. 2010;14:e913-e918.
- 328 5. Martins CAP, Koga-Ito CY, Jorge AOC. Presence of *Staphylococcus* spp. and *Candida*
329 spp. in the human oral cavity. *Brazilian Journal of Microbiology*. 2002;33:236-240.
- 330 6. Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW. Biofilm formation in
331 *Staphylococcus* implant infections. A review of molecular mechanisms and implications for
332 biofilm-resistant materials. *Biomaterials*. 2012;33:5967-5982.
- 333 7. Didenko LV, Avtandilov GA, Shevlyagina NV, Smirnova TA, Lebedenko IY, Tatti F *et al*.
334 Biodestruction of polyurethane by *Staphylococcus aureus* (an investigation by SEM, TEM
335 and FIB). In: Méndez-Vilas A, editor. *Current Microscopy Contributions to Advances in*
336 *Science and Technology*. Formatex Research Center. 2012;1:323-334.
- 337 8. Curia R, Milani M, Didenko LV, Avtandilov GA, Shevlyagina NV, Smirnova TA. Beyond the
338 biodestruction of polyurethane: *S. aureus* uptake of nanoparticles is a challenge for
339 toxicology. In: Méndez-Vilas A, editor. *Microscopy: advances in scientific research and*
340 *education*. Formatex Research Center. 2014;1:16-23.
- 341 9. Ghannoum MA, O'Toole GA, editors. *Microbial Biofilms*. Amer. Society for Microbiology
342 Press. Washington DC, USA. 2004.
- 343 10. Howard GT. Microbial biodegradation of polyurethane. In: *Recent Developments in*
344 *Polymer Recycling*. Transworld Research Network. Kerala, India. 2011;215-238.
- 345 11. Franson TR, Sheth NK, Menon L, Sohnle PG. Persistent In Vitro Survival of Coagulase-
346 Negative Staphylococci Adherent to Intravascular Catheters in the Absence of Conventional
347 Nutrients. *J Clin Microbiol*. 1986;24:559-561.
- 348 12. Lopez-Lopez G, Pascual A, Perea EJ. Effect of plastic catheter material on bacterial
349 adherence and viability. *J Med Microbiol*. 1991;34:349-353.
- 350 13. Peters G, Locci R, Pulverer G. Adherence and growth of coagulase-negative
351 staphylococci on surfaces of intravenous catheters. *J Infect Dis*. 1982;146:479-482.
- 352 14. Amara AA, Moawad H. PHAC synthases and PHA depolymerases: the enzymes that
353 produce and degrade plastic. *IJUM Engineering Journal*. 2011;12:21-37.
- 354 15. Lenz RW, Marchessault RH. Bacterial Polyesters: Biosynthesis, Biodegradable Plastics
355 and Biotechnology. *Biomacromolecules*. 2005;6:1-8.
- 356 16. Vert M, Doi Y, Hellwich KH, Hess M, Hodge P, Kubisa P *et al*. Terminology for biorelated
357 polymers and applications (IUPAC Recommendations 2012). *Pure Appl Chem*. 2012;84:377-
358 410.
- 359 17. Dunne WM. Bacterial Adhesion: Seen Any Good Biofilms Lately? *Clin Microbiol Rev*.
360 2002;15:155-166.
- 361 18. Gross M, Cramton SE, Götz F, Peschel A. Key Role of Teichoic Acid Net Charge in
362 *Staphylococcus aureus* Colonization of Artificial Surfaces. *Infect Immun*. 2001;69:3423-
363 3426.
- 364 19. Hwang G, Kang S, El-Din MG, Liu Y. Impact of conditioning films on the initial adhesion
365 of *Burkholderia cepacia*. *Colloids and Surf B Biointerface*. 2012;91:181-188.

- 366 20. Satriano C, Messina GML, Carnazza S, Guglielmino S, Marletta G. Bacterial adhesion
367 onto nanopatterned polymer surfaces. *Materials Science and Engineering C*. 2006;26:942-
368 946.
- 369 21. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics
370 and/or surface topography on biofilm development. *Clin Oral Implants Res*. 2006;17:68-81.
- 371 22. Yoda I, Koseki H, Tomita M, Shida T, Horiuchi H, Sakoda H *et al*. Effect of surface
372 roughness of biomaterials on *Staphylococcus epidermidis* adhesion. *BMC Microbiol*.
373 2014;14:234.
- 374 23. Costerton W, Veeh R, Shirtliff M, Pasmore M, Post C, Ehrlich G. The application of
375 biofilm science to the study and control of chronic bacterial infections. *J Clin Invest*.
376 2003;112:1466-1477.
- 377 24. Cosford RE. PANDAS (Pediatric Autoimmune Neuropsychiatric Disease Associated with
378 Streptococcus) in Autism? A Case History. *Electronic Journal of Applied Psychology: Innovations in Autism*. 2009;5:39-48.
- 380 25. Costerton JW, Stewart PS, Greenberg EP. Bacterial Biofilms: A Common Cause of
381 Persistent Infections. *Science*. 1999;284:1318-1322.
- 382 26. Moore MN. Do nanoparticles present ecotoxicological risks for the health of the aquatic
383 environment? *Environ Int*. 2006;32:967-976.
- 384 27. Nowack B, Bucheli TD. Occurrence, behavior and effects of nanoparticles in the
385 environment. *Environ Pollut*. 2007;150:5-22.
- 386 28. Sanvicens N, Marco MP. Multifunctional nanoparticles – properties and prospects for
387 their use in human medicine. *Trends Biotechnol*. 2008;26:425-433.
- 388 29. Hu CMJ, Fang RH, Luk BT, Chen KNH, Carpenter C, Gao W *et al*. Marker-of-self
389 functionalization of nanoscale particles through a top-down cellular membrane coating
390 approach. *Nanoscale*. 2013;5:2664-2668.
- 391 30. De Jong WH, Borm PJA. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine*. 2008;3:133-149.
- 393 31. Docter D, Distler U, Storck W, Kuharev J, Wünsch D, Hahlbrock A *et al*. Quantitative
394 profiling of the protein coronas that form around nanoparticles. *Nat Protoc*. 2014;9:2030-
395 2044.
- 396 32. Bystrzejewska-Piotrowska G, Golimowski J, Urban PL. Nanoparticles: Their potential
397 toxicity, waste and environmental management. *Waste Manag*. 2009;29:2587-2595.
- 398 33. Editorial Nature Nanotechnology. The dose makes the poison. *Nat Nanotechnol*.
399 2011;6:329.
- 400 34. Nowack B. The behavior and effects of nanoparticles in the environment. *Environ Pollut*.
401 2009;157:1063-1064.
- 402 35. Oberdörster G. Safety assessment for nanotechnology and nanomedicine: concepts of
403 nanotoxicology. *J Intern Med*. 2009;267:89-105.
- 404 36. Gristina AG. Biomaterial-Centered Infection: Microbial Adhesion Versus Tissue
405 Integration. *Science*. 1987;237:1588-1595.
- 406 37. Aiken GR, Hsu-Kim H, Ryan JN. Influence of Dissolved Organic Matter on the
407 Environmental Fate of Metals, Nanoparticles, and Colloids. *Environ Sci Technol*.
408 2011;45:3196-3201.
- 409 38. Milani M, Drobne D, Tatti F. How to study biological samples by FIB/SEM? In: Méndez-
410 Vilas A, Díaz J, editors. *Modern Research and Educational Topics in Microscopy*. Formatex
411 Research Center. 2007;787-794.
- 412 39. Bosak S, Pletikapić G, Hozic A, Svetličić V, Sarno D, Viličić D. A Novel Type of Colony
413 Formation in Marine Planktonic Diatoms Revealed by Atomic Force Microscopy. *PLoS One*.
414 2012;7:e44851.
- 415 40. Utsunomiya S, Kogawa M, Kamiishi E, Ewing RC. Scanning Transmission Electron
416 Microscopy and Related Techniques for Research on Actinide and Radionuclide
417 Nanomaterials. In: Kalmykov SN, Denecke MA, editors. *Actinide Nanoparticle Research*.
418 Springer. 2011;33-62.

- 419 41. Utsunomiya S, Ewing RC. Application of High-Angle Annular Dark Field Scanning
420 Transmission Electron Microscopy, Scanning Transmission Electron Microscopy-Energy
421 Dispersive X-ray Spectrometry, and Energy-Filtered Transmission Electron Microscopy to
422 the Characterization of Nanoparticles in the Environment. *Environ Sci Technol*. 2003;37:786-
423 791.
- 424 42. Nellist PD, Pennycook SJ. The Principles and Interpretation of Annular Dark-Field Z-
425 Contrast Imaging. In: *Advances in Imaging and Electron Physics*. Academic Press.
426 2000;113:147-203.
- 427 43. Butler JH, Joy DC, Bradley GF, Krause SJ. Low-voltage scanning electron microscopy of
428 polymers. *Polymer*. 1995;36:1781-1790.
- 429 44. Candia Carnevali D, Milani M, editors. *Electron and ion microscopy and*
430 *micromanipulation: common principles and advanced methods in applied sciences*.
431 *Proceedings of Summer School 2008 & 2009*. Miriam, Società Editrice Esculapio. Bologna,
432 Italy. 2010.
- 433 45. Joy DC, Joy CS. Low Voltage Scanning Electron Microscopy. *Micron*. 1996;27:247-263.
- 434 46. Kizilyaprak C, Daraspe J, Humbel BM. Focused ion beam scanning electron microscopy
435 in biology. *J Microsc*. 2014;254:109-114.
- 436 47. Liu J. High-Resolution and Low-Voltage FE-SEM Imaging and Microanalysis in Materials
437 Characterization. *Materials characterization*. 2000;44:353-363.
- 438 48. Milani M, Drobne D. Focused Ion Beam Manipulation and Ultramicroscopy of
439 Unprepared Cells. *Scanning*. 2006;28:148-154.
- 440 49. Warheit DB. How Meaningful are the Results of Nanotoxicity Studies in the Absence of
441 Adequate Material Characterization? *Toxicological Sciences*. 2008;101:183-185.
- 442 50. Didenko LV, Shevlyagina NV, Curia R, Erega A, Milani M. Electron Microscopy Furthers
443 the Investigation of Bacteria-Nanoparticles Interactions Subcellular Dynamics. *Global*
444 *Journal of Medical Research: D Radiology, Diagnostic Imaging and Instrumentation*.
445 2014;14:13-23.
- 446 51. Postek MT, Vladár AE. Does Your SEM Really Tell the Truth? - How Would You Know?
447 Part 1. *Scanning*. 2013;35:355-361.
- 448 52. Douglas LJ. *Candida* biofilms and their role in infection. *Trends Microbiol*. 2003;11:30-36.
- 449 53. Hawser SP, Douglas LJ. Biofilm Formation by *Candida* Species on the Surface of
450 Catheter Materials In Vitro. *Infect Immun*. 1994;62:915-921.
- 451 54. Didenko LV, Avtandilov GA, Shevlyagina NV, Shustrova NM, Smirnova TA, Lebedenko
452 IY et al. Nanoparticles Production and Inclusion in *S. aureus* Incubated with Polyurethane:
453 An Electron Microscopy Analysis. *Open Journal of Medical Imaging*. 2013;3:69-73.
- 454 55. Palomino D, Stoll S. Fulvic acids concentration and pH influence on the stability of
455 hematite nanoparticles in aquatic systems. *J Nanopart Res*. 2013;15:1428.
- 456 56. Revell PA. The biological effects of nanoparticles. *Nanotechnol Percept*. 2006;2:283-
457 298.
- 458 57. Verma A, Stellacci F. Effect of Surface Properties on Nanoparticle–Cell Interactions.
459 *Small*. 2010;6:12-21.
- 460 58. Borm PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K *et al*. The
461 potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol.
462 2006;3:11.
- 463 59. Buzzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: Sources and toxicity.
464 *Biointerphases*. 2007;2:MR17-MR71.
- 465 60. Hansen T, Clermont G, Alves A, Eloy R, Brochhausen C, Boutrand JP *et al*. Biological
466 tolerance of different materials in bulk and nanoparticulate form in a rat model: sarcoma
467 development by nanoparticles. *J R Soc Interface*. 2006;3:767-775.
- 468 61. Del Pino P, Pelaz B, Zhang Q, Maffre P, Nienhaus GU, Parak WJ. Protein corona
469 formation around nanoparticles – from the past to the future. *Mater Horiz*. 2014;1:301-313.

62. Winzen S, Schoettler S, Baier G, Rosenauer C, Mailaender V, Landfester K *et al.* Complementary analysis of the hard and soft protein corona: sample preparation critically effects corona composition. *Nanoscale*. 2015;7:2992-3001.
63. Barrán-Berdón AL, Pozzi D, Caracciolo G, Capriotti AL, Caruso G, Cavaliere C *et al.* Time Evolution of Nanoparticle-Protein Corona in Human Plasma: Relevance for Targeted Drug Delivery. *Langmuir*. 2013;29:6485-6494.
64. Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc Natl Acad Sci U S A*. 2008;105:14265-14270.
65. Lynch I, Dawson KA. Protein-nanoparticle interactions. *Nanotoday*. 2008;3:40-47.
66. Xia XR, Monteiro-Riviere NA, Riviere JE. An index for characterization of nanomaterials in biological systems. *Nat Nanotechnol*. 2010;5:671-675.
67. Lesniak A, Fenaroli F, Monopoli MP, Åberg C, Dawson KA, Salvati A. Effects of the Presence or Absence of a Protein Corona on Silica Nanoparticle Uptake and Impact on Cells. *ACS Nano*. 2012;6:5845-5857.
68. Mahmoudi M, Lynch I, Ejtehadi MR, Monopoli MP, Baldelli Bombelli F, Laurent S. Protein-Nanoparticle Interactions: Opportunities and Challenges. *Chem Rev*. 2011;111:5610-5637.
69. Masserini M. Nanoparticles for Brain Drug Delivery. *ISRN Biochem*. 2013. Article ID 238428.
70. Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Baldelli Bombelli F *et al.* Physical-Chemical Aspects of Protein Corona: Relevance to *in Vitro* and *in Vivo* Biological Impacts of Nanoparticles. *J Am Chem Soc*. 2011;133:2525-2534.
71. Salvati A, Pitek AS, Monopoli MP, Prapainop K, Baldelli Bombelli F, Hristov DR *et al.* Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat Nanotechnol*. 2013;8:137-143.
72. Treuel L, Brandholt S, Maffre P, Wiegeler S, Shang L, Nienhaus GU. Impact of Protein Modification on the Protein Corona on Nanoparticles and Nanoparticle-Cell Interactions. *ACS Nano*. 2014;8:503-513.
73. Wang F, Yu L, Monopoli MP, Sandin P, Mahon E, Salvati A *et al.* The biomolecular corona is retained during nanoparticle uptake and protects the cells from the damage induced by cationic nanoparticles until degraded in the lysosomes. *Nanomedicine*. 2013;9:1159-1168.
74. Fröhlich H. The extraordinary dielectric properties of biological materials and the action of enzymes. *Proc Natl Acad Sci U S A*. 1975;72:4211-4215.
75. Del Giudice E, Doglia S, Milani M, Vitiello G. A quantum field theoretical approach to the collective behaviour of biological systems. *Nuclear Physics B*. 1985;251:375-400.
76. Pekker M, Shneider MN. The surface charge of a cell lipid membrane. 2014. Available: <http://arxiv.org/abs/1401.4707>.
77. Krug HF, Wick P. Nanotoxicology: An Interdisciplinary Challenge. *Angew Chem Int Ed Engl*. 2011;50:1260-1278.
78. Schrurs F, Lison D. Focusing the research efforts. *Nat Nanotechnol*. 2012;7:546-548.
79. Warheit DB. Debunking Some Misconceptions about Nanotoxicology. *Nano Lett*. 2010;10:4777-4782.
80. Cossart P, Helenius A. Endocytosis of Viruses and Bacteria. *Cold Spring Harb Perspect Biol*. 2014;6:a016972.
81. Huotari J, Helenius A. Endosome maturation. *EMBO J*. 2011;30:3481-3500.
82. Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. *Curr Opin Cell Biol*. 2014;29:116-125.
83. Wendler F, Bota-Rabassedas N, Franch-Marro X. Cancer becomes wasteful: emerging roles of exosomes in cell-fate determination. *J Extracell Vesicles*. 2013;2:22390.
84. Grant BD, Audhya A. The ins and outs of endocytic transport. *Nat Cell Biol*. 2005;7:1151-1154.

- 523 85. Lonhienne TGA, Sagulenko E, Webb RI, Lee KC, Franke J, Devos DP *et al.*
524 Endocytosis-like protein uptake in the bacterium *Gemmata obscuriglobus*. *Proc Natl Acad*
525 *Sci U S A*. 2010;107: 12883–12888.
- 526 86. van den Ent F, Amos L, Löwe J. Bacterial ancestry of actin and tubulin. *Curr Opin*
527 *Microbiol*. 2001;4:634-638.
- 528 87. Shaw JD, Cummings KB, Huyer G, Michaelis S, Wendland B. Yeast as a Model System
529 for Studying Endocytosis. *Exp Cell Res*. 2001;271:1-9.
- 530 88. Neimark HC. Extraction of an actin-like protein from the prokaryote *Mycoplasma*
531 *pneumoniae*. *Proc Natl Acad Sci U S A*. 1977;74:4041-4045.
- 532 89. Carballido-López R. The Bacterial Actin-Like Cytoskeleton. *Microbiol Mol Biol Rev*.
533 2006;70:888-909.
- 534 90. Erickson HP. Evolution in bacteria. *Nature*. 2001;413:30.
- 535 91. Graumann PL. Cytoskeletal Elements in Bacteria. *Annu Rev Microbiol*. 2007;61:589-618.
- 536 92. Shih YL, Rothfield L. The Bacterial Cytoskeleton. *Microbiol Mol Biol Rev*. 2006;70:729-
537 754.
- 538 93. van den Ent F, Amos LA, Löwe J. Prokaryotic origin of the actin cytoskeleton. *Nature*.
539 2001;413:39-44.
- 540 94. Wickstead B, Gull K. The evolution of the cytoskeleton. *J Cell Biol*. 2011;194:513-525.
- 541 95. Amos LA, van den Ent F, Löwe J. Structural/functional homology between the bacterial
542 and eukaryotic cytoskeletons. *Curr Opin Cell Biol*. 2004;16:24-31.
- 543 96. Cabeen MT, Jacobs-Wagner C. Skin and bones: the bacterial cytoskeleton, cell wall, and
544 cell morphogenesis. *J Cell Biol*. 2007;179:381-387.
- 545 97. Loose M, Mitchison TJ. The bacterial cell division proteins FtsA and FtsZ self-organize
546 into dynamic cytoskeletal patterns. *Nat Cell Biol*. 2014;16:38-46.
- 547 98. Löwe J, van den Ent F, Amos LA. Molecules of the bacterial cytoskeleton. *Annu Rev*
548 *Biophys Biomol Struct*. 2004;33:177-198.
- 549 99. Møller-Jensen J, Löwe J. Increasing complexity of the bacterial cytoskeleton. *Curr Opin*
550 *Cell Biol*. 2005;17:75-81.
- 551 100. Szwedziak P, Wang Q, Freund SMV, Löwe J. FtsA forms actin-like protofilaments.
552 *EMBO J*. 2012;31:2249-2260.
- 553 101. van den Ent F, Löwe J. Crystal structure of the cell division protein FtsA from
554 *Thermotoga maritima*. *EMBO J*. 2000;19:5300-5307.
- 555 102. Fujita J, Maeda Y, Nagao C, Tsuchiya Y, Miyazaki Y, Hirose M *et al.* Crystal structure
556 of FtsA from *Staphylococcus aureus*. *FEBS Lett*. 2014;588:1879-1885.
- 557 103. Yan K, Pearce KH, Payne DJ. A Conserved Residue at the Extreme C-Terminus of
558 FtsZ Is Critical for the FtsA-FtsZ Interaction in *Staphylococcus aureus*. *Biochem Biophys*
559 *Res Commun*. 2000;270:387-392.
- 560 104. Mashburn-Warren LM, Whiteley M. Special delivery: vesicle trafficking in prokaryotes.
561 *Mol Microbiol*. 2006;61:839-846.
- 562 105. Matteoni R, Kreis TE. Translocation and Clustering of Endosomes and Lysosomes
563 Depends on Microtubules. *J Cell Biol*. 1987;105:1253-1265.
- 564 106. Mayer F. Cytoskeletal Elements in Bacteria *Mycoplasma pneumoniae*,
565 *Thermoanaerobacterium* sp., and *Escherichia coli* as Revealed by Electron Microscopy. *J*
566 *Mol Microbiol Biotechnol*. 2006;11:228-243.
- 567 107. Milani M, Brundu C, Santisi G, Savoia C, Tatti F. Cell metal segregation and
568 ultramicroscopy techniques: towards mammalian liver pathologies microscopic
569 characterization. *International Journal of Transport Phenomena*. 2011;12:25-40.
- 570 108. Terada D, Hattori S, Honda T, Iitake M, Kobayashi H. Embossed-carving Processing of
571 Cytoskeletons of Cultured Cells by Using Focused Ion Beam Technology. *Microsc Res*
572 *Tech*. 2013;76:290-295.
- 573 109. Foster TJ. Immune evasion by Staphylococci. *Nat Rev Microbiol*. 2005;3:948-958.
- 574 110. Kim HK, Thammavongsa V, Schneewind O, Missiakas D. Recurrent infections and
575 immune evasion strategies of *Staphylococcus aureus*. *Curr Opin Microbiol*. 2012;15:92-99.

- 576 111. Rooijackers SH, van Kessel KP, van Strijp JA. Staphylococcal innate immune evasion.
577 Trends Microbiol. 2005;13:598-601.
- 578 112. Zecconi A, Scali F. *Staphylococcus aureus* virulence factors in evasion from innate
579 immune defenses in human and animal diseases. Immunol Lett. 2013;150:12-22.
- 580 113. Gordon RJ, Lowy FD. Pathogenesis of Methicillin-Resistant *Staphylococcus aureus*
581 Infection. Clin Infect Dis. 2008;46:S350-S359.
- 582 114. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med. 1998;339:520-532.
- 583 115. Lowy FD. Is *Staphylococcus aureus* an intracellular pathogen? Trends Microbiol.
584 2000;8: 341-343.
- 585 116. Curia R, Milani M, Didenko LV, Shevlyagina NV. Electron microscopy broadens the
586 horizons of toxicology: the role of nanoparticles vehiculated by bacteria. Current Topics in
587 Toxicology. 2013;9:93-98.
- 588 117. Van Bambeke F. Intracellular *S. aureus* what does it mean? Available: [http://www.gsk-](http://www.gsk-chair-infectious-diseases.be/Peschel-10-04/slides/Wednesday-13-10-04-Mini-Symposium/Vanbambeke-intracellular-S-aureus.pdf)
589 [chair-infectious-diseases.be/Peschel-10-04/slides/Wednesday-13-10-04-Mini-](http://www.gsk-chair-infectious-diseases.be/Peschel-10-04/slides/Wednesday-13-10-04-Mini-Symposium/Vanbambeke-intracellular-S-aureus.pdf)
590 [Symposium/Vanbambeke-intracellular-S-aureus.pdf](http://www.gsk-chair-infectious-diseases.be/Peschel-10-04/slides/Wednesday-13-10-04-Mini-Symposium/Vanbambeke-intracellular-S-aureus.pdf).
- 591 118. Editorial Nature Nanotechnology. Join the dialogue. Nat Nanotechnol. 2012;7:545.
- 592 119. Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: *Candida albicans*
593 and bacteria. FEMS Microbiol Lett. 2009;299:1-8.
- 594 120. Kettiger H, Schipanski A, Wick P, Huwyler J. Engineered nanomaterial uptake and
595 tissue distribution: from cell to organism. Int J Nanomedicine. 2013;8:3255-3269.

596 ABBREVIATIONS

597 **PU**: polyurethane; **NP**: nanoparticle; **MV**: membrane vesicle; **TEM**: Transmission Electron
598 Microscope; **STEM**: Scanning Transmission Electron Microscope; **FIB/SEM**: Focused Ion
599 Beam/Scanning Electron Microscope.
600
601