Chapter 4. Visualisation of the Bacterial Interaction with Smooth Titanium Substrata
4.1. Overview

The interactions that take place between bacterial cells and the surfaces onto which they often attach are a function of the characteristics of both the bacterial cell and the substrate surface. The attributes of both of these objects can be categorized in terms of their surface physical structure and surface chemistry. The chemical control of bacterial cell attachments has been widely studied (Fletcher and Loeb, 1979, Jacobs et al., 2007, Statz et al., 2008, Mauclaire et al., 2010, Oliveira et al., 2011). The physical structure of the surface, such as the surface topography, plays a vital role in the surface-cell interactions. It has been receiving much more attention more recently, even though the process of adhesion remains ambiguous (Ivanova et al., 2010, Fadeeva et al., 2011).

The experimental work conducted by Webb et al. (2013) aimed to characterise the surface architecture of three sub-nanometrically smooth titanium (Ti) surfaces. It was important to identify the roughness parameters that were most directly correlated to bacterial attachment of *S. aureus* and *P. aeruginosa*, on sub-nanometrically smooth Ti substrata. Ti was chosen as the substratum because of its application as one of the main materials used in the manufacture of biomedical implants. This work was an extension of previous work (Ivanova et al., 2011b) where it was shown that sub-nanometrically smooth, or molecularly smooth Ti, exhibits different morphology-dependent effects on cell adhesion. The actual dynamic process of the bacterial cell-surface interaction, however, remained unclear; only hypothetical assumptions were able to be made regarding the adhesion process.

In terms of the Scenario-based visualisation applicability to recreate these interactions, this first stage was the development of a scenario based on hypothetical assumptions regarding the adhesion process. This required an effective display of the Ti surface topographical data as 3D models at the nano- and sub-nanometric scale. Additionally it was necessary to construct basic dynamic motions of 3D cells models, representing the two different types of bacterial interactions with the surfaces.
It was assumed that both species of bacteria behaved differently when they came in a contact with the topographical features of the substrate surface. In general, when *S. aureus* cells reach the surface, they undergo an initial contact followed by a small amount of movement. In doing so, they lose their velocity and adhere onto the surface topographical features of the surface. On the other hand, the *P. aeruginosa* cells would slide off the surface after the initial contact. As explained in Chapter 3, one of the reasons for this behaviour lies in the membrane properties of the cells. *S. aureus* are Gram positive bacteria, and as such possess a more rigid membrane structure. *P. aeruginosa*, on the other hand, are Gram negative bacteria that have a membrane structure that is more flexible. Considering the differences in the flexibility of the membrane, it was also important to highlight this difference in the dynamic simulation.

4.2. Data collection

The surface preparation and AFM scan described in this section were performed by Dr. Hayden Webb and members on Nano-biotech group at Swinburne University of Technology.

Three different titanium substrate surfaces were used in this study, their differences being only in their surface topographies. They contained either sub-nanometric, nanometric and micro-/nano-structures on their surfaces. In order to create accurate 3D models, AFM surfaces topographic data files were used. A scanning probe microscope (Solver P7LS, NT-MDT) was used to obtain images of the surface morphology and to quantitatively measure and analyse the surface roughness of metallic surfaces on the nanometre scale (Truong et al., 2010).

Five parameters were used for the characterisation of the surfaces: average roughness (*Ra*), root mean square (RMS) roughness (*Rq*), maximum height (*Rmax*), skewness (*Rskw*) and kurtosis (*Rkur*) (Webb et al., 2011a, Webb et al., 2011b, Ivanova et al., 2012). The parameters provide detailed information regarding the surface roughness and provide a numerical assessment of the smoothness or roughness of the surface. All of the roughness data are an average of four scans from the 10 µm × 10 µm scanning areas.
Titanium surfaces on the sub-nanometre roughness scale were represented by the titanium thin films of 12 nm thickness (henceforth referred to as 12 nm films) that were fabricated on the pre-cleaned silicon wafers using a Kurt J Lesker CMS - 18 magnetron sputtering thin film deposition system (Ivanova et al., 2010). This approach allowed the controlled atomic deposition of titanium for the purposes of producing metallic thin films possessing sub-nanosopic and nanoscopic surface roughness (Wang et al., 2008). The surfaces of the 12 nm films are remarkably smooth on the sub-nanometer scale, i.e., \(Ra\) of 0.20 nm and \(Rq\) of 0.24 nm on the 10 \(\mu\)m \(\times\) 10 \(\mu\)m scanning areas (Ivanova et al., 2011a). Nano-structured titanium surfaces were fabricated using an equal channel angular pressing (ECAP) procedure, which enhances the strength of bulk metallic materials, by the formation of a sub-micron or nano-scale grain structure (Truong et al., 2010).

4.3. Production workflow

At this phase of the Scenario-based visualisation, the newly developed tool for 3D surface visualisation in Maya was not available. Therefore, Avizo was utilised in order to complete the semi-automated step from the third stage of the methodology presented in Chapter 3. The 3D models of the surface topography were imported as an .obj file format, which was exported from external application Avizo. The interactions between bacterial models and the surfaces were created using the Dynamic menu functions in Maya.

The surfaces were made as passive bodies. In order to highlight the variation in surface topology across the substrate surface, animations containing both true-scale and exaggerated scaling were developed. A scaling of 25 units along the axis perpendicular to the surface (the Y-axis in Maya world coordinates) was found to be suitable, but the selection of axis to achieve this result was found to be somewhat arbitrary. In order to avoid non-proportional scaling of the bacterial shapes, the spherical and rod-shaped bacterial cells were animated as transparent objects.
The data of two scanning areas (1 µm × 1 µm and 10 µm × 10 µm) for each type of a surface were used for the visualisation process. Even though, the scans were performed on the same samples, the data containing the 1 µm × 1 µm surface height was not part of the 10 µm × 10 µm scanning area. In order to show the topography and capture the essence of the “surface magnification” from the 10 µm × 10 µm to 1 µm × 1 µm scanning areas, Maya’s texturing options were utilised in order to achieve the magnification effect. A ramp texture was assigned to the surfaces representing the colour map of the surface height values (Fig. 4.1). The ramp texture was made with five colours each spaced equally with linear method selected. Because the two surfaces of different scales are separate models, in order to make them appear as a single object, a blend colour option was used. This allows two different textures to be connected in single shading node, enabling a transformation from one texture to another. Therefore, while the camera is in the focus showing the 10 µm × 10 µm surface, the 1 µm × 1 µm surface would blend in with the colour map of that of the 10 µm × 10 µm scanning area. When the camera reaches the 1 µm × 1 µm area, the colour blend option would switch to the 1 µm × 1 µm area original colour map.
Figure 4.1. Three-dimensional representations of the surface topographies of three sub-nanometrically smooth Ti samples, as imaged by atomic force microscopy on 1 μm × 1 μm (left) and 10 μm × 10 μm (right) scanning areas.
4.3.1. *S. aureus* interaction with titanium surfaces

When depicting the mechanisms taking place when a bacterial cell approaches the surface, the main goal was to depict the approach and interaction that takes place between a single cell and the surface. The bacterial cell objects, made as active bodies, where initially positioned 1000 units above the surfaces, before they were influenced by any dynamic force field. A gravity field was assigned to each of the bacterial cell types, allowing the appearance of a more natural cell motion as they came into contact with the substrate surfaces. By default, every dynamic object was created as a rigid body, which was sufficient to adequately represent the rigidity of *S. aureus* cells. The attributes of the rigid body, were adjusted in order for the 3D models of *S. aureus* cell to behave according to the hypothetical assumptions regarding the *S. aureus* behaviour when comes in contact with a surface. In this case, the main attribute to be adjusted was the extent to which the cells would bounce on the surface after they came into contact. Setting this attribute to zero prevented the model cell from bouncing off the surface when it came into contact. All the other attributes were set using Maya’s default values.

Starting the simulation, the cell shapes would start to move towards the surface model, influenced by the gravity field. The moment the bacteria model came in contact with the surface, it collided with the surface and start moving along the surface. This motion mainly depended on the nature of the surface topography. For example, if the bacterial model reached a section of the surface where there were high bumpy features, the dynamic active object (the cell 3D model) would “detect” the feature and cause the cell to react accordingly. If there was a hole on the surface, the model cell would roll into it, and if there was a high bump into which the cell came into contact, then the model cell would stop moving, or change direction after making contact. However, because the surface and bacterial cell models are true to scale, these dynamic motions are hardly noticeable, because the surface features appear very smooth and the cell model cannot recognize the features on sub-nanometric scale. After the desired dynamic motion was achieved, the simulation was stored as keys for each frame with the “Bake simulation” tool (Fig. 4.2a).
With all the keys set, the simulation could be played at any desired frame speed without affecting the models. Additional adjustments could be made to the keys as well. Generally, the model’s motion along the surface was at a constant speed, which was not sufficient to represent the required bacterial attachment process, where the bacterium loses its velocity and stops moving. This was achieved by selecting the last few keys and setting them apart for few frames, each key further then the previous one (Fig. 4.2b).

![Figure 4.2](image)

**Figure 4.2.** *Bake simulation* application. A key (red line) is stored for each frame containing the dynamic simulation values (a). By setting apart the last few keys the motion of the 3D object will become slower (b).

### 4.3.2. *P. aeruginosa* interaction with titanium surfaces

The simulation of the attachment of *P. aeruginosa* cells to the surface involved similar process that had been previously performed using the *S. aureus* bacterial model. However, as previously discussed, the models of *P. aeruginosa* cells would be expected to react differently when they came into contact with the surface. Three identical polygonal models of *P. aeruginosa* cells were made as active bodies and were positioned 1000 units above the surface. The difference here was that a soft body option was applied to the *P. aeruginosa* cells, since their membrane was more flexible than...
that of their *S. aureus* counterparts. The original 3D object was hidden when a soft body was created, because Maya created an exact copy of the shape of the object, constructed from a particle system. The visibility of the original object was a matter of choice, and could be changed at any time during the animation/simulation process.

Initially, the particle weightings were all set to the maximum level. This value could be changed with the **Paint soft body weight tool**, which allowed the weighting values of the individual particles to be increased or decreased as desired. When the tool was selected, the model appeared in a grey scale mode. The brush tool was used to slightly decrease the weighting on the top and the bottom of the 3D model. These parts of the model would be more flexible when they interacted with any geometry, in this case the surface. When the simulation reached the required dynamic behaviour (which was determined according to hypothetical assumptions, the membrane fluctuations) the bake simulation option was applied. Based on the assumed behaviour of the bacteria, the *P. aeruginosa* cells, would not be expected to remain on the surface after their initial interaction, but rather slide off, with the sliding motion being animated by manual key framing of the position of the 3D polygonal model and not via the soft body particle system. When any transformation was applied to the original 3D object, the particles would follow.

### 4.3.3. Image and movie production

For the development of the final movie, additional animations were developed such as the camera motion, where the camera path was animated in order to capture the interactions between the cells and the two surfaces. The final 3D animations were rendered using the mental ray plugin. The images were exported in TARGA format with the production quality pre-set, numbered sequentially. The final movies were realised in Adobe Premier, where additional information was added, such the colour maps and colour maps values, surface titles, surface dimensions, etc. The movies were exported in MPEG file format. The images were finalised in Adobe Photoshop.
4.4. Summary

The final movie sequences (DVD-Videos 1-6) show the nano- and sub-nanometric surfaces structure of the ECAP and 12 nm thin film titanium samples. They also show a representation of the two different species of pathogenic bacterial cells interacting with the surfaces (Fig. 4.3). It is acknowledged that the dynamic behaviour of the bacteria model was not based on experimental data sets, however the surface topography was based on a data set obtained from the AFM. However, the 3D models of the cells do move according to the surface topography, which can be considered as first steps towards depicting the accurate dynamic motion of the cells on the surface. If any additional information was available regarding the dynamic motion of the bacterial cells or their membrane structure, it would have been possible to also incorporate these features in the visualisation process.

Despite this drawback, the surface topographical data that was derived from AFM was not imported directly in Maya, but it did confirm that Maya could support a file format (.obj), which is a common available format in many 3D applications. The movies demonstrated the applicability of the tools that Maya provides, for the visualisation of dynamic interactions. The ability to have full control over the attributes of individual elements within a scene is a feature common to many animation and modelling packages, however this functionality is usually not included in domain-specific scientific visualisation software.

These animations show simple dynamic behaviour and motion of the 3D models. In the following chapters, a more advanced dynamic system (nDyanamic) will be incorporated in order to provide the ability to develop more complex dynamic simulations of the bacterial cell- surface interactions.
Figure 4.3. *S. aureus* and *P. aeruginosa* cells interacting with three different subnanometrically smooth Ti surfaces. Images are screenshots extracted from the Videos presented in this chapter. In these animations, *S. aureus* and *P. aeruginosa* cells first fall onto a 10 µm × 10 µm surface, and upon contact can be seen to roll/slide slightly for several seconds before settling on the surface. The camera then rotates 360° and zooms in to show a higher resolution, 1 µm × 1 µm section of the same surface. The surfaces were produced based on real AFM data. The cells are also briefly made transparent and the surface features are extruded to better demonstrate the surface topography.
Chapter 5. Visualisation of *P. aeruginosa* Cell Interaction with Cicada Wing Surface
5.1. Overview

The surface structures of natural surfaces, such as lotus leaves (*Nelumbo nucifera*) (Barthlott and Neinhuis, 1997), shark skin (e.g., the Mako shark, *Isurus oxyrinchus*) (Jung and Bhushan, 2009), and the feet of geckos (fam. Gekkonidae) (Bhushan, 2007), along with the superhydrophobic surfaces of insect wings such as dragonflies (Song et al., 2007), butterflies (Smentkowski et al., 2006) and cicada (*Psaltoda claripennis*) wings (Sun et al., 2009), have been found to possess superhydrophobic and self-cleaning properties (Burgess et al., 2003, Schumacher et al., 2007, Yang et al., 2013).

It has been recently discovered that cicada (*Psaltoda claripennis*) wing surfaces were bactericidal against Gram negative bacterial cells including *P. aeruginosa* (Ivanova et al., 2012). *P. aeruginosa* cell interactions with nano-pillars array on the surface resulted in affective rapturing of the cells within approximately 300 seconds (5 minutes) (Fig. 5.1). It was shown that in the first 220 seconds, the cell remained on the surfaces and within next 100 seconds was sunken inside the nanopillars. It was further shown that the cell membrane was not able to withstand the pressure and actually between the pillars.

*Figure 5.1.* Time frame of *P. aeruginosa* sinking on cicada wing nanopillars. Adopted from (Ivanova et al., 2012).
The event of bacterial cell rupture described above has been visualised. The scenario-based workflow was employed to understand and inspect the process of the cell-surface interactions. Based on the experimental data and developed hypothesis, a 3D interpretation has been recreated to visualise the dynamic interactions using Maya’s nDynamic system.

5.2. Data collection

The surface preparation and AFM scan described in this section were performed by Dr. Jafar Hasan and members on Nano-biotech group at Swinburne University of Technology.

AFM scans were performed using an Innova microscope (Veeco, Bruker, USA) in tapping mode. Phosphorus doped silicon probes (MPP-31120-10, Veeco, Bruker) with a spring constant of 0.9 N/m, tip radius of curvature of 8 nm and a resonance frequency of ~20 kHz were utilized for surface imaging. Scanning was carried out perpendicular to the axis of the cantilever at 1 Hz. Scans were performed on at least ten areas of each of five samples. The resulting topographical data were processed with first order horizontal and vertical levelling before performing roughness analysis. Determination of the surface roughness parameters was carried out using the instrument software (SPM Lab Analysis v.7.11, Veeco, Bruker). Point force spectroscopy experiments were carried out on a JPK Instruments NanoWizard II AFM in buffered solution at room temperature with a Mikromash CSC37 cantilever; spring constant 0.35 N/m. The tip was positioned on top of a cell in contact with the wing surface, and the piezo movement required to maintain constant force between the cell and the tip monitored.
5.3. Production workflow

The preliminary AFM scan of the wing surface was not sufficient to recreate the 3D model of the cicada wing nano-pillars with the accurate dimension of the pillars shape due to the complexity of the wings surface structure (Fig 5.2a). An accurate numerical model was developed based on the calculations of the dimensions of the cicada wing nano-pillars. The model was used for 3D reconstruction of the pillars shape and final animation of the cell-surface interaction (Fig 5.2b) (Pogodin et al., 2013). The visualisation of *P. aeruginosa* cell dynamic interaction with the surface was realised with the nDynamic menu sets. The surface data values were imported into Maya with the Python tool and visualised as 3D polygonal geometry. The model was made as passive collision object.

![Figure 5.2](image)

*Figure 5.2.* Three-dimensional surface models of cicada wing nano-pillars. An AFM scan of the wing’s nano-pillars did not produce accurate data of the pillars structure (a). A “perfect model” was developed and visualised (b) based on the calculation of the dimensions of the pillars.
The 3D polygonal model of the *P. aeruginosa* cell shape was created as an nCloth object. The values of the nCloth attributes had to be adjusted so that the 3D object would have enough flexibility to deform when it comes in a contact with the surface. Maya provides presets for the nCloth shapes. In general, these presets were predefined attributes to simulate the nCloth dynamic behaviour according to some real world object’s structures (e.g. water, concrete, lava, leather, etc.). The presets were useful to gain the understanding of how these attributes work and what type of control they offer.

For the purpose of the *P. aeruginosa* cell visualisation the “lava” preset was used as a starting point of attributes adjustments. This preset set the attributes values closest to the required specifications. However, additional adjustments had to be made in order to achieve the dynamic behaviour of the cell according the “mental image”. The main attributes adjusted were: Stretch resistance, Rigidity, Deform resistance, and Pressure (see Appendix 5.1 for the values). When the desired nCloth attribute values were set, the attributes values can be then saved as a new preset. This will preserve the defined values of the nCloth. Therefore, those values could always be applied, if needed, to new nCloth objects in future dynamic visualisations.

Two main nDynamic simulations were developed. The first simulation represented the process of bacterial cell adhesion on the surface and the rapture of the cell membrane. The nCloth cell shape was position above the surface 300 units. The gravity field pulled the bacterial cell towards the surface. When the 3D model reached the surface and the collision takes place, the bacterial cell shape started to deform according to the surface structure (in this case the pillars) (Fig. 5.3a).

In order to achieve the cell rapture, an nConstraint - Tearable surface option was assigned. Edges of the 3D model were selected from the parts that were sinking between the pillars. When the Tearable surface option was applied, it allowed for the two pieces of the model that share the selected edge to split. This option has its own sets of attributes that allow full control over the transformation (in this case the split). The “Glue strength” attribute sets the value that controlled the tearing. This attribute was key framed (animated) to change from full strength to none (Fig. 5.3b).
At this stage, the first simulation was completed. The nCache option was applied in order to save the simulation on a hard drive. The initial state was applied to the nCloth object. This option ‘remembers’ the deformations and the position of the nCloth object. This was necessary step in order to start the second simulation from the end of the first one, and not from the actual beginning. This step saves time and processing power.

The second simulation involved the sinking of the cell. To achieve the sinking effect, only one attribute was changed – the pressure. This attribute was set to zero, and represents the releasing of the internal structure (Fig. 5.3c). The nCache option was applied for the second simulations as well.
Figure 5.3. Three-dimensional representation of the modeled interactions between a rod-shaped cell and the wing surface. As the cell comes into contact and adsorbs onto the nanopillars (a), the outer layer begins to rupture in the regions between the pillars (b) and collapses onto the surface (c).
With the two simulation stored in nCache files, the next step was to combine the files and adjust the start of each simulation. In the Trax editor, the two nCache files were imported. The process of cell membrane rapture on cicada wing takes 300 sec to complete. However, the original time of the nDynamic simulations lasts about 100 frames (4 seconds with 24 frames per second). The advantage of this type of visualisation is that the time can be controlled and adjusted. The final movie had to represent the whole natural process in 1 minute (it required to expend the time-length of the nDynamic simulation to speed up the actual process of interaction). This was achieved by adjusting the length of the nCache files and the time when the simulation should start. The first simulation was set to start from the first frame. The second simulation cache (the sinking of the membrane) was adjusted to start form the end of the first simulation (Fig. 5.4).

![Figure 5.4. Trax editor. Two nCache files representing the two simulations of the cell-surface interaction were imported into the Trax editor, where the time of the simulations was adjusted.](image)

Upon the 3D visualisation stage completion, the final stage, the production of the actual movie, was applied. The animation was rendered with mental ray plug-in as a TARGA image sequence. The images were imported in Adobe Premier, from where the movie was exported in MPG4 format.
5.4. Summary

The movie (DVD-Video 5.1.) produced shows a basic representation of an interaction of a single *P. aeruginosa* bacterial cell and the cicada wing nano-pillars. It involves an advanced dynamic representation and deformation of the 3D cell objects compare to the dynamic simulation explained in Chapter 4. The dynamic behaviour was recreated by constant adjustment of the nCloth attribute until the final results were achieved.

At the time of the development of the animation, only hypothetical assumptions were available as a guide for the simulation process. A mathematical model presented in (Pogodin et al., 2013) contains calculations of the actual membrane rapture and could be implemented in the future simulation of accurate dynamic membrane rapture of the cells. One option would be to develop an algorithm in Maya based on the mathematical model that would take into account the dynamic deformation of the 3D models.
Chapter 6. Visualisation of the Interaction of \textit{S. aureus} with the Lotus-like Titanium Surface
6.1. Overview

Different species of plants such as taro leaves (*Colocasia esculenta*), cabbage leaves (*Brassica oleracea*) and lotus leaves (*Nelumbo nucifera*) possess characteristics, including self-cleaning, directed wetting, drag reduction and anti-reflection (Bhushan and Jung, 2010). It has been shown that the surface superhydrophobicity can be related to the existence of a hierarchical surface roughness (Koch et al., 2009). These nanostructured patterns have inspired researchers to, for example, modify biomaterials in an effort to create implants that are less susceptible to the formation of biofilms, and hence reduce the likelihood of implant-related infections. Recently, laser ablation was used for the fabrication of titanium surfaces that possessed surfaces that mimicked the surface structure of the lotus leaf (Fadeeva et al., 2011). The modified surface consisted of large grain-like convex features between 10 and 20 µm in size, which contained further nano-features, resulting in a surface that contained hierarchical roughness characteristics. One feature of the modified titanium surface was its ability to reduce the extent of bacterial attachment for some bacterial species (Truong et al., 2012).

The modified titanium surface, whilst being less attractive to some bacteria, still acted as a suitable substrate for other types of bacteria. The actual process of adhesion of the cells was unclear. It is believed that when cells come into contact with the lotus-like titanium surface, they slide off the main features of the Lotus-like Ti and mainly attach in the crevices between the features of the surface. The air bubble formation found on the structure provides more room for cell to attach, where an increase of the number of *S. aureus* cells have been noticed from couple of cells in the first minutes, continues increase throw-out the time (around 7 cells in the thirtieth minute). Based on the hypothetical assumption (“mental image”) the interactions were developed using accurate generated data of the surface and the nDynamics.

Compare to the nDynamic simulation of the *P. aeruginosa* with cicada wing nano pillars, where only a single cell was simulated, the simulations of *S. aureus* interaction with lotus-like titanium surface required development of dynamic interaction of multiple cell shapes with the lotus-like Ti surface topography. Apart from that, additional task was to recreate the cellular production of the EPS. The animation included two time periods of bacterial attachment: the first and the thirtieth minute.
6.2. Data collection

The surface preparation and AFM scan described in this section were performed by Dr. Khanh Truong and members on Nano-biotech group at Swinburne University of Technology.

The Ti surface topographies were evaluated using an Innova atomic force microscope (Veeco, Bruker, USA). Three samples of each type of surface type were briefly scanned to evaluate the overall homogeneity of the surface and the topographical profiles were studied in detail at several different locations. Imaging was performed under tapping mode, utilising phosphorus doped silicon probes (MPP-31120-10, Veeco, Bruker) with a spring constant of 0.9 N m\(^{-1}\), a tip radius of curvature of 8 nm and a resonance frequency of ~20kHz. Scanning was carried out perpendicular to the axis of the cantilever at 1 Hz.

6.3. Production workflow

The csv file containing the surface topography was imported into Maya as 3D polygonal object with the tool developed with the Python script (Fig. 6.1).

![Figure 6.1. 3D surface representation of Lotus-like titanium.](image-url)
The surface model was made as passive collision object. The only attribute of the passive collision object adjusted was the friction. This will allow some resistance to the bacterial motion when the 3D modes of the cell start moving along the surface. The first animation that represents the first minute of the cellular attachment involved two bacterial cell shapes (Fig. 6.2a.). For the second animation (showing the thirtieth minute) additional five cell shapes were added (Fig. 6.2b.). Firstly was developed the nDynamic simulation of the cell-surface interaction. The second nDynamic simulation was the nParticle simulation, representing the EPS formation.

![Figure 6.2. S. aureus cell attachment on Lotus-like Ti. The animation of the S. aureus interaction with the Lotus-like surface was developed with the nDynamic tools and objects representing the first minute (a) and the thirtieth minute (b) of the bacterial interaction](image)
6.3.1. Bacterial cells simulation

Seven 3D polygonal models of the *S. aureus* cell shapes were created and positioned for 16 000 units above the micro features of the surface. The cell models were made as nCloth objects. The nCloth attributes adjustments were set as same as for the *P. aeruginosa* cell described in the chapter 5, with the nCloth “lava” preset. However in this case, the attributes of the nCloth were set to simulate more rigid dynamic behaviour by adjusting the values of the nCloth shape attributes. The dynamic properties where optimized by increasing the values of the stretch, compression and bend resistance attributes. In addition, the rigidity attribute value was also increased. The friction attribute was set to zero value. At this stage the simulation was played. Five of the cell nCloths were disabled and hidden, as for only couple of 3D objects were needed to create the first interaction representing the first minute of cells attachment.

The gravity field was again the driving force that was set to pull the bacterial cells towards the surface. The solver substeps attribute value was increased to achieve more calculations and with that more accurate interaction among the 3D objects. When a cell comes in the first contact with the surface, it will not stay on the top of micro features, but rather will start to slide down along the features. Because the friction attribute for the surface was increased, as the nCloth slides along the surface, the components of the nCloth (i.e. vertices) are trying to stick to the surface. Eventually, the cell models will reach the crevice of the surface, where their dynamic motion will start to decay. The moment the cell reaches the crevice it will start to produce the EPS (the process of EPS visualization is explained in the following section).

For two cells, an nCache file was created. An initial state option was set for the models. This allows for the first cell to will keep their position between the micro features, when the other cells start their dynamic motion. The next part of the simulation of additional cells attachment (the thirtieth minute) was achieved in the same way. The nCloths where enabled, and based on the same principal and attributes the bacterial cell models will start to interact with the surface and each other, forming the biofilm. With the desired interaction completed, additional nCache file was created for the rest of the bacterial cells models.
6.3.2. EPS simulation

With the nDynamic cell-surface simulation completed, the next goal was to recreate the EPS. As previously mentioned in this chapter, the EPS formation was achieved with nParticles system. There are few options to create nParticle shapes and the source they are emitted:

- nParticle tool (this tool allows to interactively create particles in the 3D viewport or on a 3D model). The amount and the positioning of the particles is based on the settings in the paint tool.
- Create a source from which the particles will be emitted (an emitter). Depending where the emitter is stationed, the nParticles will be produced from the same position.
- Emit particles from an object (an emitter will be assigned to the selected 3D model).
- Fill an object with nParticles (the amount of particles depends of the attributes of the fill object option).

The four options mentioned above are the default options included in Maya. Other method to create nParticles is to use scripting. For the purpose of recreating the EPS, for each 3D cell model an “emit particles from an object” option was applied (Fig. 6.3a). Both nParticle shape and the emitter, have their own sets of attributes. When an nParticle system is created, it will share the nucleus solver, which drives the nCloth objects as well. The interactions between nParticles and nCloth will only occur if they share the same nucleus solver.

The type of the emitter was set to “Surface” and the rate of the particles was set to be 1500 particles per second. The speed of the emission was increased. As for the nParticle shape node it self, the lifespan of the particles was set to be constant, which will allow to set a time value for how long the nParticles should exist (the value represents seconds rather then frames, in this case 1.5 seconds). For the radius of each particle system, the value was set by default. As for the collision, the self collide option was turned off, which will disable interactions among the nParticles. The bounce, friction and stickiness were set to zero.
In the dynamic properties tab, the dynamic weight and the conserve attributes were adjusted. The dynamic weight defines how strong will the interaction be, while the conserve defines the increasing of the velocity of the particles. Another important attribute that was adjusted was the time. The particles were set to start their dynamic behaviour from the frame when the cell 3D objects reach the caverns of the surface and start losing their velocity. At the designated frame the emitter will start to produce the particles. Once the nParticle simulation is satisfactory, it can be stored on an nCache file.

One of the best features of nParticles is their ability to be converted into a polygonal geometry. This cannot be achieved with the particles from the Dynamic menu. The conversion can provide effects and dynamic deformation of the polygonal geometry, that otherwise can be difficult or impossible to achieve. The nParticle shape node also contains a set of attributes (Output mesh) that will define the shape of the 3D geometry (Fig. 6.3b).

Figure 6.3. Three-dimensional EPS visualisation. The exo-polymeric substances (EPS) were created using an nParticle system (a), which was converted to a polygonal mesh (b).

103
6.3.3. Movie post-production

Even though, the simulations were developed in a single scene, the frames representing the two-time periods of interactions (the first and the thirtieth minute) the two simulations were rendered separately. This provides enough flexibility for the final editing of the movies. The images were rendered with mental ray plug-in in TARGA format and imported into Adobe Premier as two separate movie clips, which were assembled into a single sequence. Additional information was added, such as the dimensions of the surface, the colour map values and the time periods. The movies were exported into MPEG format.

6.4. Summary

The movie presented in this chapter (DVD-Video 6.1) depicts the interactions of the *S. aureus* cells with the 3D model of Lotus-like surface topography. The development of the animation followed the comparable principals of nDynamic simulation of 3D objects as previously explained in the Chapter 5. The motion path of the cell models is based on the surface topographical features. In other words, the cells motion was not animated manually, as for the dynamic forces and the surface topography were responsible for most of the movement of the cells objects. Beside the cell-surface interaction, the simulation involves interaction among few cell models. This determines the possibility for more complicated scene assembles in Maya, where interactions between multiple cells and a surface could be achieved. However, the models complexity, for example the mesh resolution, could be challenging for Maya to process in the dynamic simulations occurrences.