Three-dimensional visualization of nanostructured surfaces and bacterial attachment using Autodesk® Maya®

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There has been a growing interest in understanding the ways in which bacteria interact with nano-structured surfaces. As a result, there is a need for innovative approaches to enable researchers to visualize the biological processes taking place, despite the fact that it is not possible to directly observe these processes. We present a novel approach for the three-dimensional visualization of bacterial interactions with nano-structured surfaces using the software package Autodesk Maya. Our approach comprises a semi-automated stage, where actual surface topographic parameters, obtained using an atomic force microscope, are imported into Maya via a custom Python script, followed by a 'creative stage', where the bacterial cells and their interactions with the surfaces are visualized using available experimental data. The 'Dynamics' and 'nDynamics' capabilities of the Maya software allowed the construction and visualization of plausible interaction scenarios. This capability provides a practical aid to knowledge discovery, assists in the dissemination of research results, and provides an opportunity for an improved public understanding. We validated our approach by graphically depicting the interactions between the two bacteria being used for modeling purposes, Staphylococcus aureus and Pseudomonas aeruginosa, with different titanium substrate surfaces that are routinely used in the production of biomedical devices.

Titanium and titanium alloys are biomaterials that are widely used in biomedical applications. They represent substrata upon which bacteria can attach, and successfully colonize, forming biofilms. Bacterial colonization of biomaterial surfaces and their organization in complex microbial aggregates have received considerable attention over the past few decades. As a result, intensive research has been focused on modifying the surfaces of these materials at the nano-scale and/or creating a nano-structured surface architecture, often by mimicking naturally occurring surfaces, in order to restrict and eliminate bacterial attachment. Despite numerous efforts to create bacterial-resistant surfaces, there is still a lack of understanding of the initial processes taking place when bacterial cells first interact with nano-structured surfaces. It is difficult to obtain this understanding, mainly because of challenges associated with the small dimensions of the objects under investigation, and the difficulty in capturing images during continuous real-time processes.

Three-dimensional (3D) visualizations and computer-generated animations may, in part, fill this gap in understanding by providing researchers with the ability to effectively display surface topographical data, together with possible animated scenarios, to describe the bacterial cell—surface interactions that are taking place. 3D visualization has had a great impact in many fields, including nanotechnology and biomedical research, where objects under investigation can be visualized on a micron- and nano-metric scale. The aim of this work was to develop and evaluate a practical approach for the 3D visualization of nano- and microscopic objects and their interactions with surfaces using the 3D animation software package Autodesk Maya. Visualization of the different topographic surface architectures of several metallic surfaces was developed using data files that were generated using Atomic Force Microscopy (AFM), and the bacterial cell-surface interaction scenarios developed using Maya’s Dynamics capabilities. The resulting 3D visualizations allow an increased insight into the bacterial attachment processes that are taking place. The resulting animations are informative, and greatly enhance the ability to visualize the interaction, which assists in the dissemination of research to both scientific and public audiences, and perhaps provide additional motivation for future developments in the area of direct imaging of bacterial attachment onto nano-structured surfaces.
Results

Dynamic, three-dimensional visualizations of the interactions between two bacterial species (S. aureus and P. aeruginosa) and three different types of titanium surfaces that represent characteristic examples of micron-, nano- and sub-nano-metric-scale surface topographies have been developed. The 3D visualizations were constructed using Maya and based on an approach that consists of two stages, an initial semi-automated stage, followed by a subsequent ‘creative’ stage. (i) During the first stage, the AFM data (in comma-separated, CSV, format) were imported into Maya using a custom developed Python script. A color map was applied to the surface, based on the minimum and maximum data values. Scaling of the height of the surface features was maintained in order to emphasize the topographic features. (ii) In the second, creative stage, Maya’s geometric modeling and texturing capabilities were employed to create realistic bacterial images and animations. Dynamic properties were associated with the bacterial models, allowing them to interact with simple force fields. Importantly, using experimental data pertaining to the substrates ensured the scientific accuracy of the visualizations was retained. This was not possible, however, for the modeled bacteria. In the future, as improved analytic or numerical models of surface interactions become available, additional Maya Embedded Language (MEL) or Python scripts may be utilized to further increase the scientific accuracy of the resulting animations. The key features of the two steps of the visualization process are as follows.

Surface visualization. During the file import process, the Python script reads each row of the CSV data file, parsing it into a set of equally spaced curves created based on the surface height values from the file (a). Each curve consists of control vertices (CVs) to which values can be applied for each dimension (X, Y and Z) (b). By selecting all the curves and the ‘Create Surface’ command from the script interface (c), a polygonal surface can be created (d). A planar UV map is then assigned to the surface geometry, in order to apply the ramp texture that is used to obtain the color map values of the surface (e). Autodesk screen shots reprinted with the permission of Autodesk, Inc.
Development of the 3D model for bacterial cells. The models of the bacterial cells, *S. aureus* (spherical) and *P. aeruginosa* (cylindrical shape with round caps) were created as polygonal models using Maya’s standard modeling tools (Fig. 2a and 2b). A 2D bump texture was assigned to allow a more detailed representation of the bacterial shape, whilst maintaining the underlying 3D model of the cells at lower resolution (Fig. 2c). This allows the work to be simplified and avoids the generation of complex geometrical structures until they are rendered in the final stages of the visualization process.

Dynamic interactions in the visualization process. Maya’s Dynamics function supports the construction of active or passive, rigid or soft bodies, and particle and fluid effects, while simulating their interactions within different force fields such as gravity. Passive bodies in Maya’s environment are bodies that can interact with other objects, but these bodies are not influenced by the interaction. For example, when a sphere (which is, in this instance, an active body) is bouncing on a plane, the plane will be passive and it will not move when the sphere contacts the surface of the plane. When a soft body is assigned to a 3D geometry, Maya creates a corresponding particle object. The combination of the particle and the geometry of the object define the soft body. When a force field affects the particle system, the vertices of the 3D object will move in response to the changes in the particle position. This affords a level of flexibility to the 3D geometry that would be very difficult to model on a frame-by-frame basis. The amount of flexibility is controlled by adjusting the ‘weighting’ assigned to the particle. This can range from 0 (flexible) to 1 (rigid) (c). For the *P. aeruginosa* cells, the value of 0.75 was assigned in order to approximate the membrane fluctuations that would take place on the bacterial surface, however this number is arbitrarily assigned.

Figure 2 | 3D models of bacterial cells. The round shaped bacteria *S. aureus* and rod shaped *P. aeruginosa* cells were created using Maya’s modeling tools. A cube is used as a base mesh for the development of the models (a). The ‘smooth’ and ‘extrude’ tool were used for the further modeling of the bacterial cell shapes (b). A 2D bump texture was then assigned in order to create bacteria with a more realistic appearance (c) and at the same time to preserve a lower quality mesh. Autodesk screen shots reprinted with the permission of Autodesk, Inc.

Figure 3 | Dynamic objects. The *S. aureus* cells were created as rigid dynamic bodies (a), meaning that the cell does not deform when interacting with the substrate surface. The membrane flexibility and fluctuation of *P. aeruginosa* cells was achieved by assigning a soft dynamic body to the 3D polygonal mesh (b). When a soft body is created, a particle system (b, right) is assigned to every vertex of the polygonal mesh (b, left). The changes made to the particle positions by a force field, or another object, affects the corresponding vertex of the 3D model, resulting in a flexible object. This flexibility can be control by adjusting the ‘weighting’ assigned to the particles, ranging from 0 (flexible) to 1 (rigid) (c). For the *P. aeruginosa* cells, the value of 0.75 was assigned in order to approximate the membrane fluctuations that would take place on the bacterial surface, however this number is arbitrarily assigned.
Previous studies have shown that both species of bacteria interact
cells were created as Dynamic soft bodies (Fig. 3b), with
aeruginosa
representation of the cell membranes was created by assigning the
surfaces, and the interactions between the bacteria and the titanium
surfaces were created using Maya’s Dynamic functions. The dynamic
representation of the cell membranes was created by assigning the
Dynamic objects to the 3D polygonal model of the bacterial shape. S. aureus
cells were created as Dynamic rigid bodies (Fig. 3a) while P. aeruginosa
cells were created as Dynamic soft bodies (Fig. 3b), with
the goal of recreating a realistic approximation of the membrane
fluctuations experienced by the less rigid P. aeruginosa cell walls.
Previous studies have shown that both species of bacteria interact
differently with the two surface types
. The reasons for the
difference in interaction have been reported elsewhere
. In order to
highlight the variation in surface architecture, animations
containing both the actual dimensions and an 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 the scaling value 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
ability to have full control over the attributes of individual elements
within a scene is a feature common to many animation and modeling
packages, however this functionality is not routinely included in
domain-specific scientific visualization software. The annotated
screenshots of the Videos S1 and S2 can be found in the
Supplementary Figures S1 and S2, available online.

The third movie sequence (see Supplementary Video S3, available
online) involved the interaction of S. aureus bacterial cells with a
titanium surface that has been modified with laser treatment so that
it mimics the surface architecture of a Lotus leaf. The titanium
surfaces were created as passive collision objects. As is the case with
passive bodies that are manipulated using the Dynamic menu, these
objects can interact with other nDynamic objects without being
affected by that interaction. The S. aureus cell-like 3D polygonal
meshes were made as nCloth objects, allowing a better representation
of the deformation of the shape of a spherical cell as it came into
contact with the substrate surface. After adjusting the Nucleus’s
attributes until the required interaction processes were realized
(e.g., by increasing the number of substeps in Maya’s nDynamic
simulations, causing a greater number of calculations to be made
per frame), multiple nCaches were saved for each nDynamic object.
The nCaching option allowed the nDynamic calculations to be stored
and played back when needed without the nDynamic objects being
active. The nCache file can also be used to scale the overall duration
of the simulation within the Maya timeline, as well as selecting the
time at which the simulation should start (using the Trax Editor). An
additional simulation of this process was created using Maya’s
nParticle system, which was used to recreate the process of secretion
of the extracellular polymeric substances (EPS) by the cells attaching
to the surface (Fig. 4a–c). The EPS were recreated by assigning an
nParticles system to each bacterial model. An emitter, a node that
generates the particles, was set to emit the particles from the object
mesh. After adjusting some of the attributes of the nParticle (e.g., the
shape attributes such as Particle Size, Collisions, Output Mesh,
Dynamic Properties), the final simulation was saved using the
nCaching option. Finally, these particles were converted into a poly-
gonal geometry for later rendering into the final animation sequence.

The final adjustment of the nCloth attributes resulted in represent-
ing the distinguishing dynamic motion of the cells, together with
their moving membrane behavior. An example of the functionality
that was available using the nCloth simulation functionality can be
seen in the process where the bacteria cells slide over the surface,
which is a function of the membrane flexibility and the extent to
which the membrane sticks to the substrate surface, resulting in
the final attachment and grouping of cells (Fig. 4d and 4e). This
animation was based on the interpretation of bacterial cell interac-
tions with Lotus leaf-like titanium surfaces. It reflects the typical
number of bacterial cells that are able to attach onto the surfaces
during the first thirty minutes of interaction, as has been reported
elsewhere
. The annotated screenshots of Video S3 can be found in the
Supplementary Figure S3, available online.

Discussion

The proposed approach comprises two main stages: the first is a semi-
automated stage, where the data pertaining to the actual surface topo-
graphy are loaded into Maya. This is followed by a ‘creative stage’,
where the interaction between the bacterial cells and surface is mod-
eled using Maya’s Dynamic systems. The key difference between these
two stages is that with the former, the surfaces are visualized using a
set of topographical parameters that are obtained directly using atomic
force microscopy. The development of the bacterial cell models and
their interactions with the surfaces (‘creative stage’), however, was part
of a design process that took into account the size of the cells, their
geometry and scaled proportionally to match the size of the surface
models. The nDynamic interactions were then developed, and based
upon a careful analysis of the scanning electron microscopy (SEM)
and confocal scanning electron microscopy (CLSM) micrographs.
Despite the fact that the micrographs were not directly processed
using Maya, as was the case for the surface topographical data
obtained from the AFM scans, the visual information contained in
the SEM and optical images was utilized for the development of the
dynamic interaction that was imagined to have taken place between
the cells and the substrate surface. This included the time frame of the
attachment and/or the number and pattern of the cells that attached to
the surface over a given period of time.

An additional distinction between the stages is their degree of
influence into the eventual visualization of the processes taking place
when a cell comes in contact with the substrate surface. For example,
in the initial semi-automated stage, most of the procedures require no
human intervention. The creative stage, however, requires a degree of
subjective interpretation in order to visualize the process of bacterial
cell–surface interactions. As demonstrated in this study, utilizing 3D
animation software packages such as Maya for the creation of scient-
fic animations provide the opportunity to visualize the data and to
propose data-informed research hypotheses. For example, the Avizo
Standard software (http://www.vsg3d.com/avizo/standard) has been
used to display the topographical surface features obtained from
AFM analysis data
. Whilst this software can be used to describe
the qualitative and quantitative characteristics of surfaces, Avizo does
not currently provide the necessary functionality to allow the anima-
tion or simulation of bacterial interactions with surfaces.

In the context of 3D visualization in Maya, the simulation of a
scientific process should not be mistaken with an actual scientific
simulation. If actual dynamic data were available, it would be possible
to accurately represent the actual forces that exist between the
bacteria and the nano-structured surfaces. Instead, the Dynamics
functionality of Maya is a useful alternative for the recreation of natural motions and collisions between objects in order to capture the essence of an interaction. Simulations in Maya originate from processes of interaction between objects that are affected by some forces, or each other, and therefore the calculations represent an interpretation of the changes taking place in the shape of an object. Simulations performed using Maya’s Dynamics and nDynamics functionalities do not currently support the option to directly import an object’s actual dynamic attributes. However, as has been shown in this study using the AFM data, it is possible to create scripts that could allow the importation of additional experimental, analytical or numerical data as new nodes into the software and to use these to create what is hoped to be more accurate and realistic dynamic interactions between the bacteria and the substrate surfaces. Developing and sharing scripts that can import scientific data directly into applications such as Maya could make these software packages more independent, easier to use, and more cost effective as an addition to existing domain-specific scientific visualization packages19,20,22–24. The visualization of this type of research data can be employed for different purposes, and can be used in the preparation of research publications and in the preparation of more graphic and interesting research presentations. The approach used in this study has the potential to open new avenues for experimental data analysis, including the possible further development and improvement of the tools currently available for the visualization of bacterial cell shapes and dynamic interactions within the Maya software.

**Methods**

**Software.** Autodesk Maya is one of the most commonly-used software packages [along with, for example, 3Ds Max (e.g., http://usa.autodesk.com/3ds-max/), Blender (http://www.blender.org/), Houdini (http://www.sidefx.com/), Lightwave (https://www.lightwave3d.com/), CINEMA4D (http://www.maxon.net/products/cinema-4d-prime/), etc.] for developing high-quality 3D animations with special effects. All of these packages were designed to take into account the needs of the entertainment industry, and are not designed to work using scientific data formats. Maya’s applicability for visualizing scientific research data has been previously recognized25–29. Maya was designed as a database for storing graphical information, which is deposited in objects called ‘nodes’. The nodes have properties (or attributes) that store information that pertains to their changeable characteristics, and data can flow between nodes. Maya’s graphical user interface (GUI) consists of over 900 commands, allowing users to create, modify and manipulate these nodes. Behind every GUI-accessible command is a script written in the Maya Embedded Language (MEL). MEL supports the customization of existing commands or the development of new commands to perform specific tasks that are not already part of Maya’s default menu set. Maya also provides application programming interfaces (APIs) for C++ and the Python programming language (http://www.python.org).

**Titanium surfaces.** The surfaces of three different titanium substrates, containing sub-nanometric, nanometric and micro-/nano-structured surface topographies were examined in this study. In order to create accurate 3D models, AFM surfaces topography 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 analyze the surface roughness of these metallic surfaces on the nanometer scale, as described elsewhere30. All of the roughness data presented are the average obtained from four separate scans of 10 μm × 10 μm areas. Five parameters were used for the characterization of surfaces: average roughness (R_a), root mean square (RMS) roughness (R_q), maximum height (R_max), skewness (R_skw) and kurtosis (R_kur)14,28,32. Titanium surfaces with sub-nanometer surface roughness were obtained via the deposition of 12 nm thickness titanium films on silicon wafers (henceforth referred to as 12 nm films). These films were fabricated using a Kurt J Lesker CMS – 18 magnetron sputtering thin film deposition system as previously described33. This approach allowed the controlled atomic deposition of titanium onto the substrates for the purposes of producing metallic thin films with sub-nanoscopic and nanoscopic surface roughness34. The surfaces of the 12 nm films were found to be remarkably smooth on the sub-nanometer scale, i.e., R_a of 0.20 nm and R_q of 0.24 nm on the 10 μm ×10 μm scanning areas34. The superhydrophobic titanium surfaces (with a
AFM data import. The raw data obtained using AFM were converted to a text file format using the free, open-source application Gwyddion (http://gwyddion.net/). The text files were then re-formatted as comma-separated value (CSV) files. The surface topography values from the CSV files were then imported into the Maya software package as 3D objects, using a custom Python script. Python, an open-source dynamic programming language, became part of Maya’s API in version 8.5. The script was written in Maya’s Script Editor, which simplifies the creation of interface components, such as a user window where the functions can be accessed from within the application menu. The surface models were visualized using a polygonal geometry consisting of 3D points (vertices) connected with lines (edges).

Rendering and post-production. The final animations were rendered using the mental ray plug-in (http://www.mentalimages.com/products/mental-ray) as a sequence of images in the TARGA format. To enable the greatest flexibility in future applications of the animations, the full-HD 1080p (1920 × 1080 pixels) resolution and the production quality preset were selected. Post-production was completed using Adobe Premiere CS5.5, where additional information was added to the movie sequences, such as titles and text overlays of color map values. The movies were then exported in an MPEG format.

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Author contributions

Conceived the project: E.P.I., E.P.L. Performed the experiments: V.B. Analyzed the data: C.J.F., E.P.I., V.B., R.J.C. Wrote the paper: V.B., C.J.F., E.P.I., R.J.C.

Additional information

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