

## SUPPLEMENTARY MATERIALS

# Effect of Local Topography on Cell Division of *Staphylococcus* spp.

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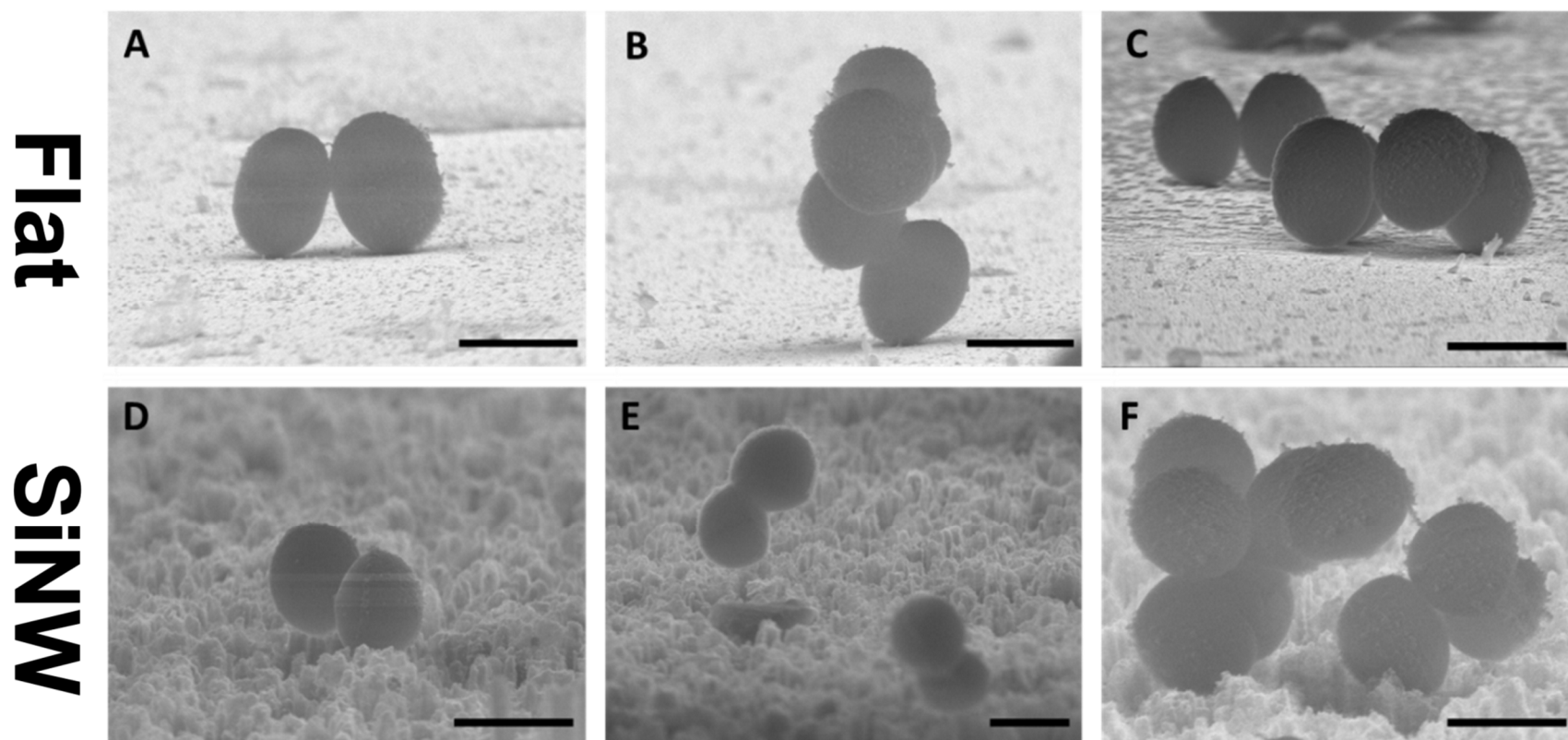
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S1 Additional SEM Images of *Staphylococcus* spp. on Flat Si and SiNWs Surfaces

# *S. aureus*

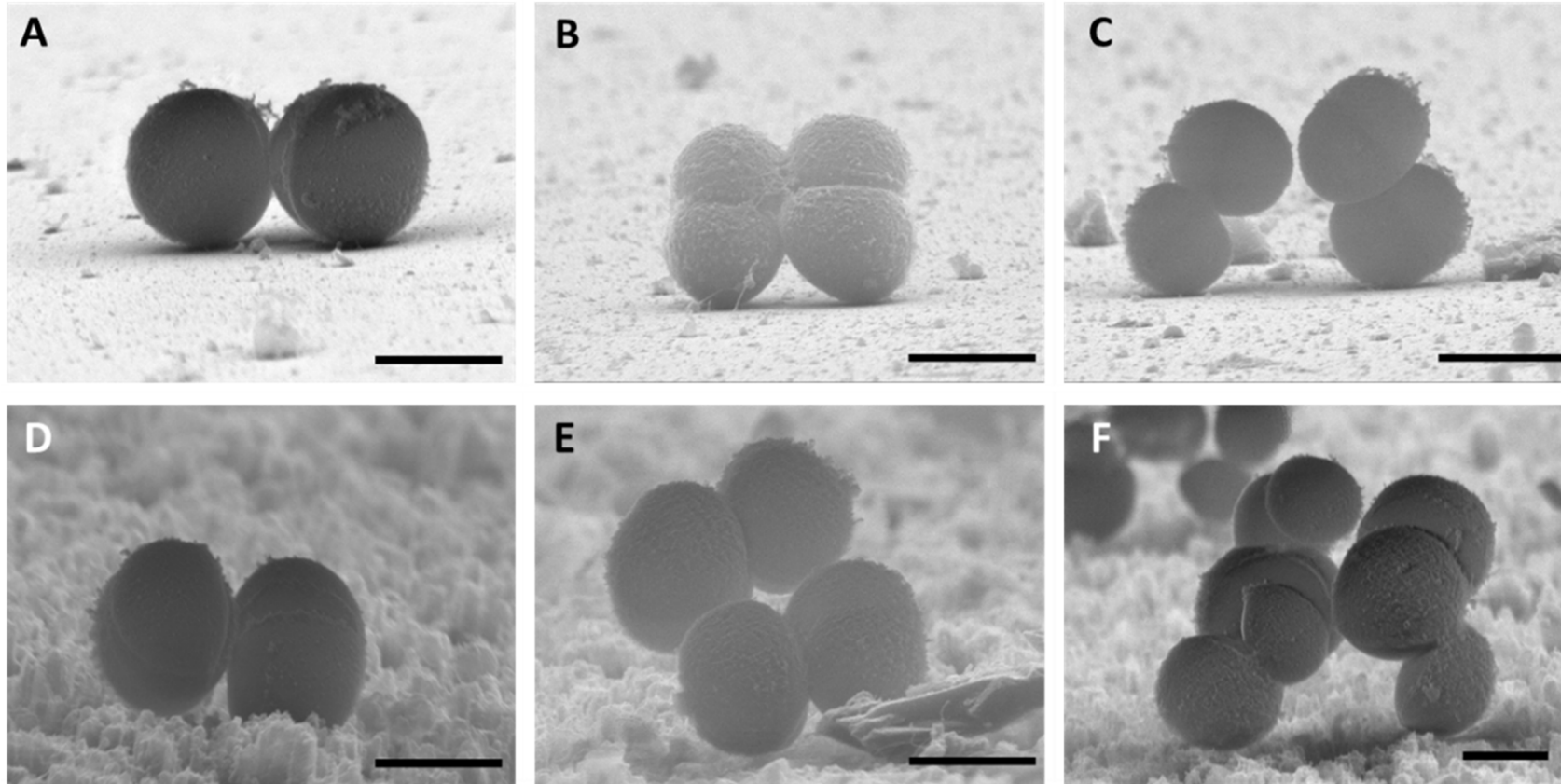


**Figure S1.** Representative SEM images of *S. aureus* on flat Si (A, B, C) and SiNWs (D, E, F) surfaces. Scale bars are 1 μm.

# *S. aureus*

Flat

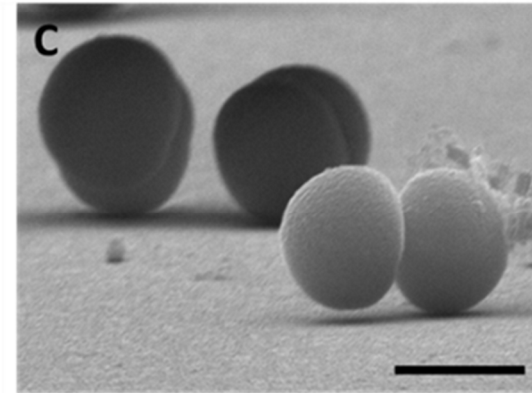
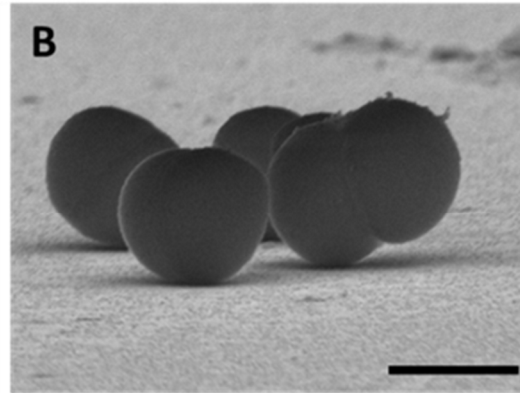
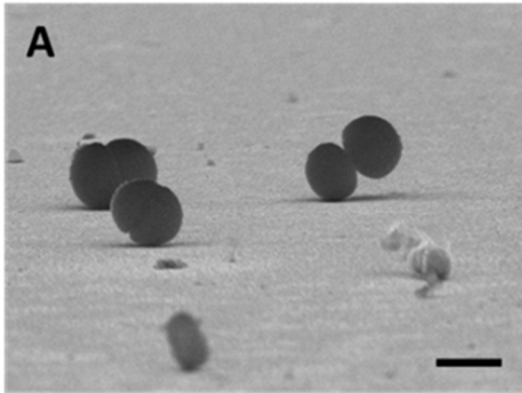
SiNW



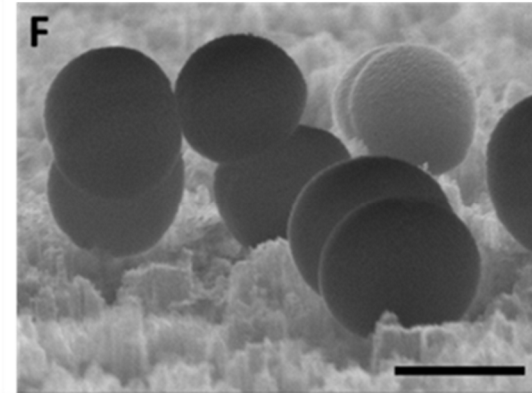
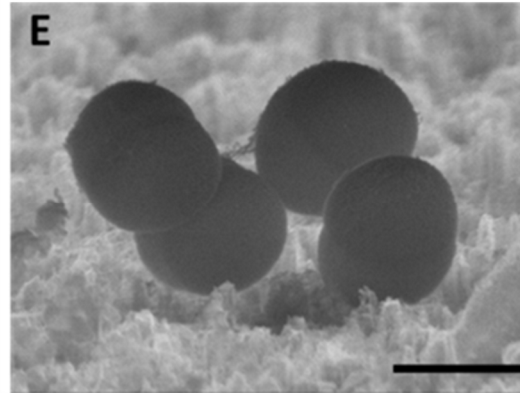
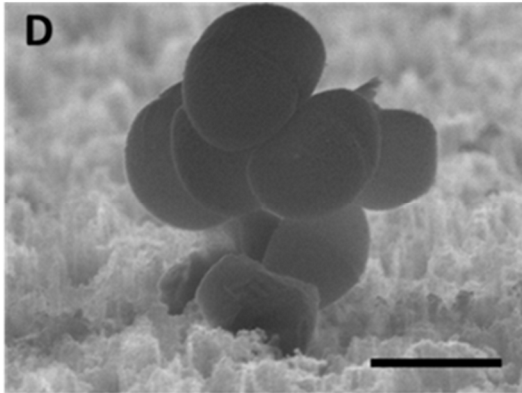
**Figure S2.** Representative SEM images of *S. aureus* on flat Si (A, B, C) and SiNWs (D, E, F) surfaces. Scale bars are 1 μm.

# *S. epidermidis*

Flat



SiNW



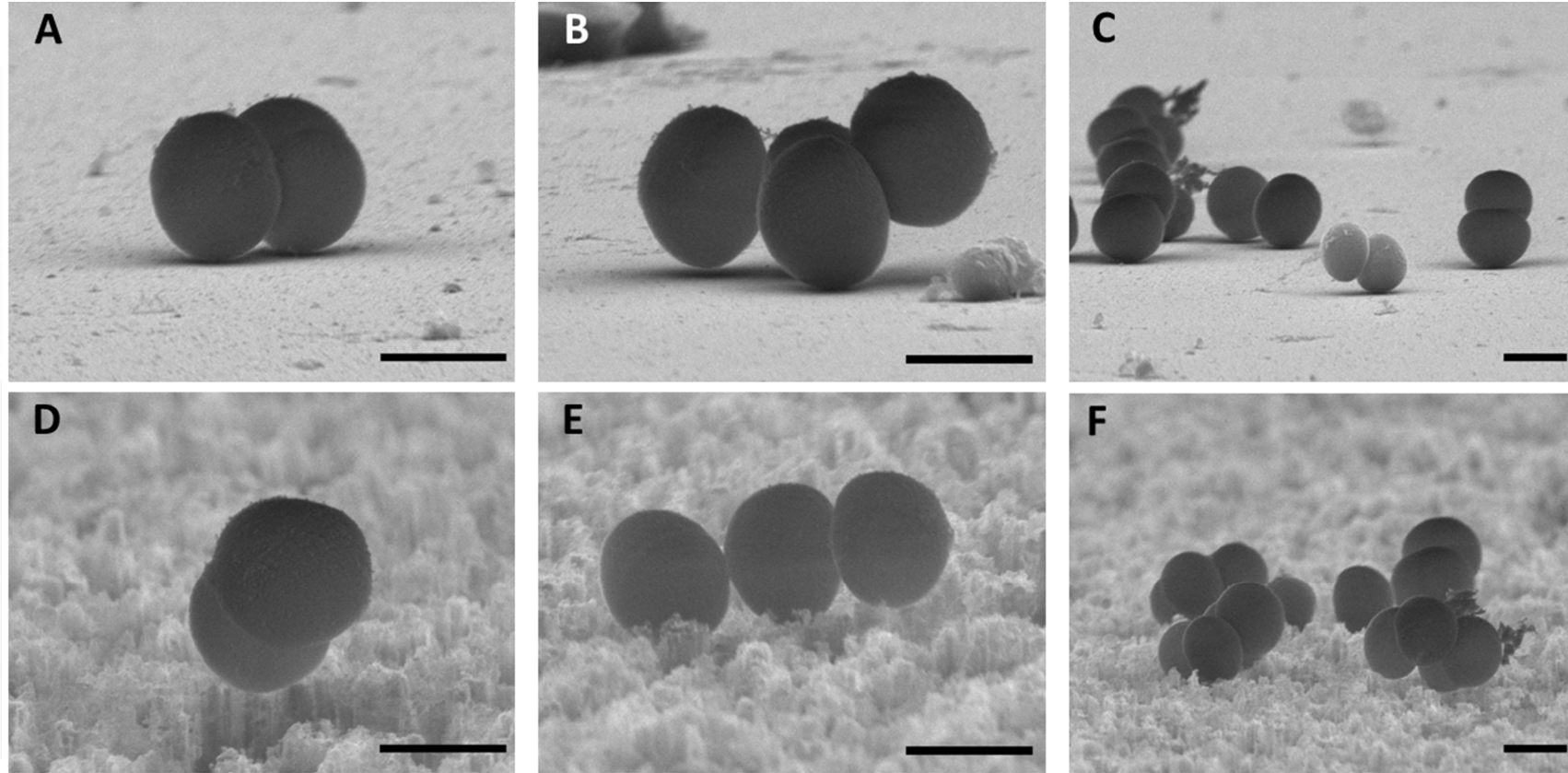
**Figure S3.** Representative SEM images of *S. epidermidis* on flat Si (A, B, C) and SiNWs (D, E, F) surfaces. Scale bars are 1  $\mu\text{m}$ .



# *S. epidermidis*

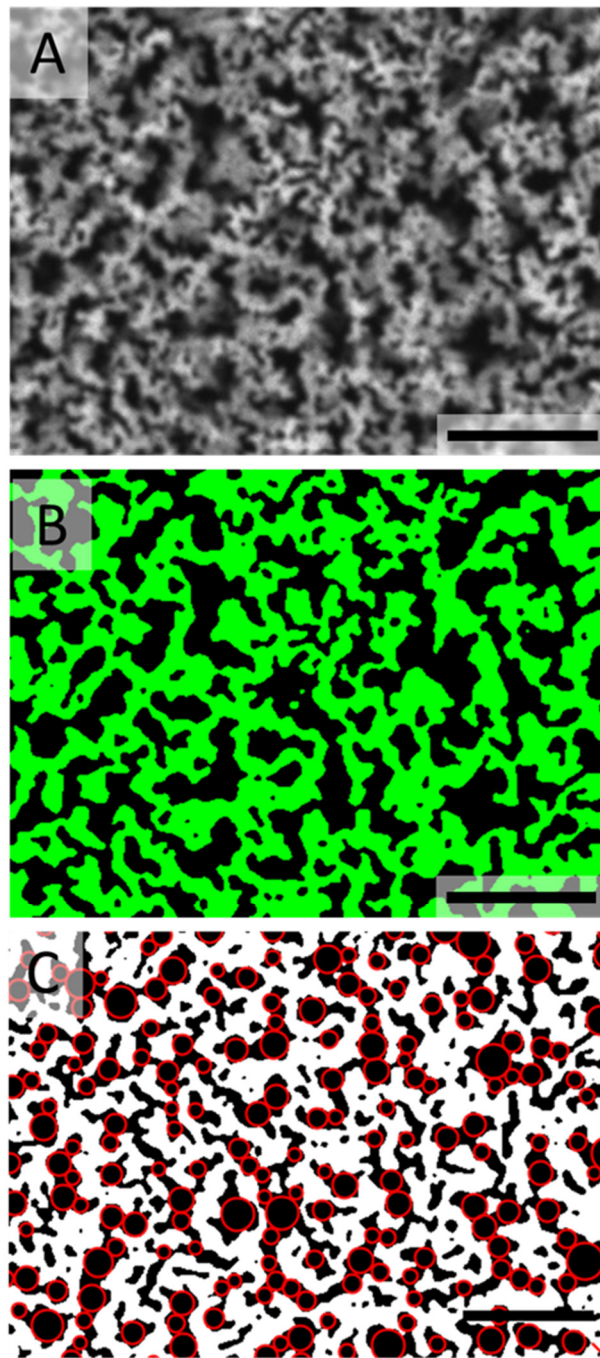
Flat

SiNW



**Figure S4.** Representative SEM images of *S.epidermidis* on flat Si (A, B, C) and SiNWs (D, E, F) surfaces. Scale bars are 1 μm

## S2 Topographic Analysis of SiNWs Surfaces



**Figure S5** Representative sequence of imaging processing of SEM data for SiNWs surfaces: A) Original SEM image; B) Binary mask generated by ImageJ's *Default* threshold; C) pore size analysis using largest inscribed circle fitting, (reference to <https://github.com/BIOP/ijp-max-inscribed-circles>; accessed on 15 January 2022). Scale bar is 2  $\mu\text{m}$ .

**Table S1** Statistical descriptors for the weighted distributions of pore size  $r_{\text{pore}}$ , fraction of free cell area  $f_{\text{growth}}$ , average vertical offset  $\Delta z_{\text{average}}$ , and critical angle  $\theta_{\text{max}}$  for the SiNWs surfaces. On this table: “sem” stands for “standard error of the mean” and QD for “quartile deviation”. All the distributions are weighted by the area of the pore (i.e. weighting factor =  $\pi(r_{\text{pore}})^2$ )

|                             | Mean   | sem    | Median | QD    | Q25    | Q75    |
|-----------------------------|--------|--------|--------|-------|--------|--------|
| $r_{\text{pore}}$           | 153 nm | 0.4 nm | 142 nm | 27 nm | 122 nm | 176 nm |
| $f_{\text{growth}}$         | 0.937  | 0.003  | 0.950  | 0.02  | 0.925  | 0.961  |
| $\Delta z_{\text{average}}$ | 755 nm | 0.3 nm | 747 nm | 20 nm | 731 nm | 772 nm |
| $\Delta z_{\text{min}}$     | 306nm  | 0.8nm  | 284nm  | 54nm  | 244nm  | 352nm  |

### S3 Geometric Formulations

In this section we will discuss some considerations that allowed us to calculate the different topographical parameters determining cell growth and micro-colony architecture as a function of the local topography. On the basis of purely geometrical considerations, we calculated the topographically hindered area of a quasi-spherical cell of radius  $R_{cell}$ , interacting with a topographic cavity of size  $r_{pore}$  as follows:

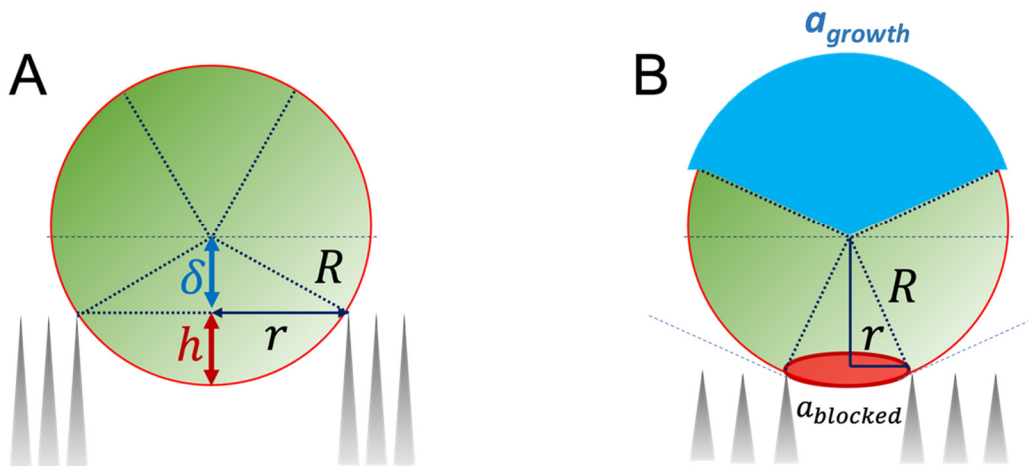
$$a_{blocked} = 2\pi \cdot R_{cell} \cdot h \quad (S1)$$

where  $h$  is defined in Figure S6, and the following geometrical relationships apply:

$$R_{cell} = h + \delta \quad (S2)$$

$$(R_{cell})^2 = \delta^2 + (r_{pore})^2 \quad (S3)$$

$$h = R_{cell} - \sqrt{(R_{cell})^2 - (r_{pore})^2} \quad (S4)$$



**Figure S6.** Geometric considerations used for the calculation of  $f_{growth}$ .

Subsequently, we defined the fraction of blocked area in the lower cell hemisphere:

$$f_{blocked} = \frac{a_{blocked}}{2\pi \cdot (R_{cell})^2} \quad (S5)$$

And using Equations (S2)-(S4) we obtained:

$$f_{growth} = \sqrt{1 - \frac{(r_{pore})^2}{(R_{cell})^2}} \quad (S6)$$

From here, we obtained the fraction of free area of the cell  $f_{growth}$ , assuming

$f_{blocked} + f_{growth} = 1$ , and leading to:

$$f_{growth} = \sqrt{1 - \frac{(r_{pore})^2}{(R_{cell})^2}} \quad (S7)$$

This result implies that on a flat surface, where there are not topographic cavities (i.e.  $r_{pore} = 0$ ), the fraction of topographically blocked surface is  $f_{blocked} \approx 0$  and therefore  $f_{growth} \approx 1$  and no growth inhibition caused by a chemically inert surface can be predicted. Conversely, in the presence of a topographic feature of finite size, like those of SiNWs surfaces,  $f_{growth} < 1$  and the surface may display some bacteriostatic effect due to hindrance of growth when the 1<sup>st</sup> generation division septum lays within the blocked fraction of the cell surface. The extreme case  $f_{growth} \approx 0$  may occur when the size of the topographic feature is similar to the size of the cell (i.e.  $r_{pore} \approx R_{cell}$ ), however for our vertically aligned SiNWs arrays, this event was found to be unlikely due to the specific pore size distribution of the topography, showing pore sizes smaller than the characteristic size of *Staphylococcus* cells.

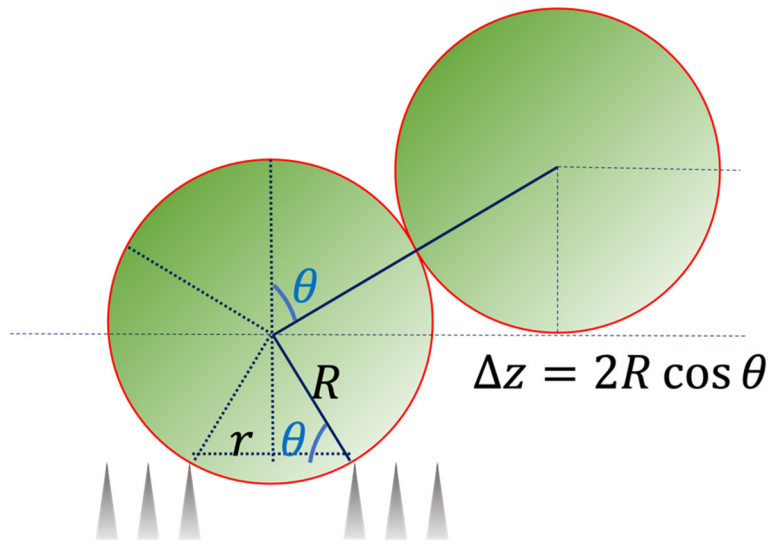
The hindered area on the cell surface reduced also the angular range where cell growth and colony development can occur. The maximum azimuthal angle  $\theta_{max}$  at which a 1st generation daughter cell can growth was determined by the expression:

$$\theta_{max} = \arccos\left(\frac{r_{pore}}{R_{cell}}\right) \quad (S8)$$

This expression led to the trivial solution  $\theta_{max} \approx \pi/2$  for a flat surface (i.e.  $r_{pore}=0$ ), suggesting that the 1<sup>st</sup> generation cells can lay perpendicular to the surface normal and therefore completely horizontal growth is allowed, in agreement with experimental observations of *Staphylococcus* cells on flat Si (main text Figure2, and Figure S1 A,B and Figure S2 B,C). On the other hand, in the presence of a topographic feature of finite size, like on SiNWs surfaces,  $\theta_{max} < \pi/2$  due to the effect of the topographic

hindrance, forcing the 1<sup>st</sup> generation daughter cells to grow further away from the surface plane (Figure S1 E,F and Figure S2 D,E). Another way of representing this effect is by calculating the displacement in the vertical axis, perpendicular to the surface plane, induced by the angular constrain. For any azimuthal angle  $\theta$ , the displacement in the vertical axis  $\Delta z$  can be calculated according to Figure S7 as:

$$\Delta z = 2 \cdot R_{cell} \cdot \cos \theta \quad (S9)$$



**Figure S7** Geometrical consideration used for the calculation of the vertical displacement  $\Delta z$  of a daughter cell as a function of the azimuthal angle  $\theta$ .

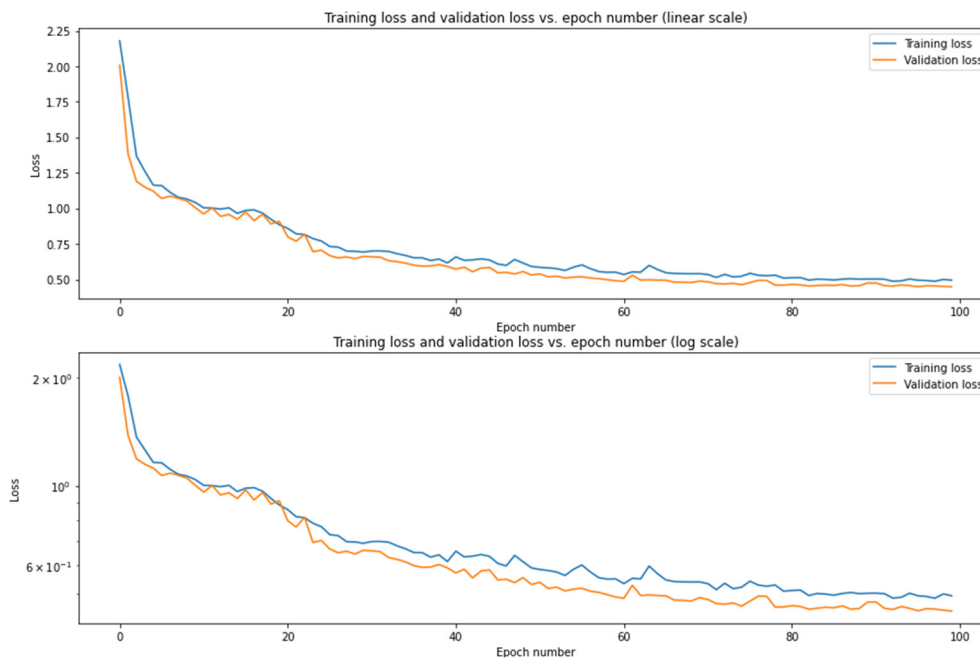
In the case of the flat surface,  $\theta$  took any value between 0 and  $\pi/2$ , and therefore  $\Delta z$  varied from  $2R_{cell}$  to zero. On SiNWs, as discussed above, the angular range is reduced, and therefore  $\Delta z$  is always greater than zero, reaching a minimum value for  $\theta = \theta_{max}$  at  $\Delta z_{min} = 2r_{pore}$ . Therefore, on a porous surface such as our SiNWs, the 1<sup>nd</sup> generation daughter cells will always be pushed away from the surface plane, having direct implications on the topological model discussed in the main text. Furthermore, we were able to calculate the average values of  $\Delta z$  by integration over the azimuth angle  $\theta$ :

$$\Delta z_{average} = 2 \cdot R_{cell} \cdot \frac{\int_0^{\theta_{max}} \cos \theta \, d\theta}{\int_0^{\theta_{max}} d\theta} \quad (S10)$$

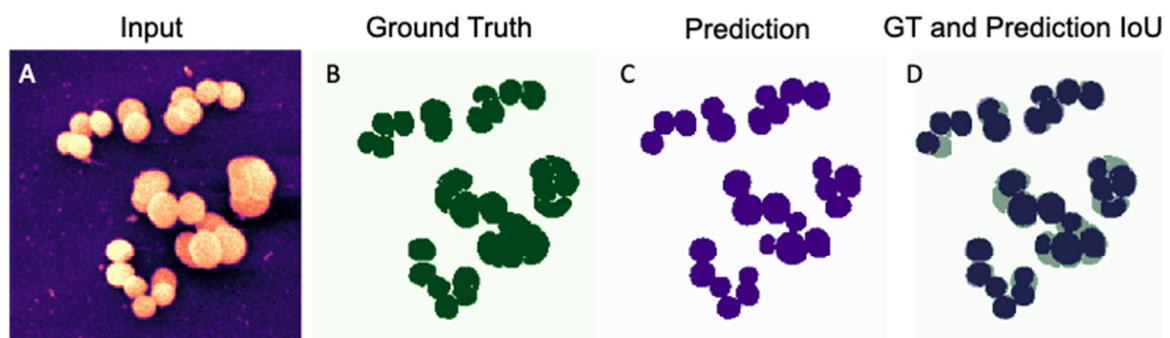
$$\Delta z_{average} = \frac{2 \cdot R_{cell} \cdot \sin(\theta_{max})}{\theta_{max}} \quad (S11)$$

Equations (S7), (S8) and (S11) were used to determine the probability density functions of the different topographical parameters as a function of the pore size distribution of SiNWs surfaces, weighting by the area of the pore to account for the fact that although bigger pores were less abundant, they displayed intrinsically a higher cross-section of interaction with *Staphylococcal* cells. These calculated probability density functions, including those of the surface pore size, are presented in the main text Figures 3D, 5E, and 6B, and some key statistical descriptors are reported in Supplementary Materials Table S1.

## S4 Morphological characterisation of *Staphylococcus* spp. micro-colonies on Flat Si and SiNWs surfaces



**Figure S8.** StarDist 2D model optimisation process measured as training and validation loss per number of training epochs.



| E | IoU       | Precision | Recall    | Accuracy  | F1        |
|---|-----------|-----------|-----------|-----------|-----------|
|   | 0.84±0.04 | 0.99±0.02 | 0.92±0.04 | 0.91±0.05 | 0.95±0.03 |

**Figure S9.** Quality control report of *S. aureus* StarDist2D segmentation model: A) Input image, B) ground truth target, C) predicted segmentation and D) ground truth and prediction intersection over union (IoU). E) Quality control metrics for *S. aureus* StarDist2D segmentation model.



**Table S2** Statistical descriptors for the distribution of morphological parameters of *S. aureus* colonies on Flat Si and SiNWs surfaces. On this table: “sem” stands for “standard error of the mean” and QD for “quartile deviation”.

|      |                                     | Mean              | sem                | Median            | QD                 | Q25               | Q75               |
|------|-------------------------------------|-------------------|--------------------|-------------------|--------------------|-------------------|-------------------|
| Flat | <i>N<sub>cells per colony</sub></i> | 12.0              | 0.1                | 8.0               | 6.0                | 4.0               | 16.0              |
|      | <i>Circularity</i>                  | 0.63              | 0.005              | 0.67              | 0.14               | 0.51              | 0.79              |
|      | <i>Feret's diameter</i>             | 3.2 $\mu\text{m}$ | 0.05 $\mu\text{m}$ | 2.7 $\mu\text{m}$ | 0.85 $\mu\text{m}$ | 2.1 $\mu\text{m}$ | 3.8 $\mu\text{m}$ |
|      |                                     | Mean              | sem                | Median            | QD                 | Q25               | Q75               |
| SiNW | <i>N<sub>cells per colony</sub></i> | 6.8               | 0.1                | 5.0               | 3.0                | 3.0               | 9.0               |
|      | <i>Circularity</i>                  | 0.68              | 0.005              | 0.74              | 0.12               | 0.56              | 0.81              |
|      | <i>Feret's diameter</i>             | 2.9 $\mu\text{m}$ | 0.03 $\mu\text{m}$ | 2.5 $\mu\text{m}$ | 0.65 $\mu\text{m}$ | 2.1 $\mu\text{m}$ | 3.4 $\mu\text{m}$ |

## S5 Imaging processing details

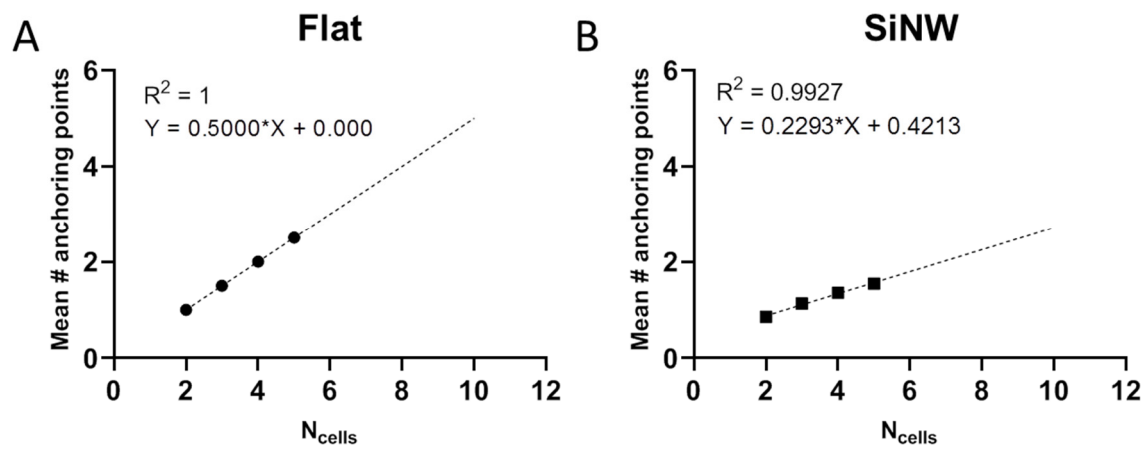
**Table S3.** Advanced parameter configuration used for *S. aureus* SEM segmentation model training.

| Parameter             | Value   |
|-----------------------|---------|
| number_of_epochs      | 100     |
| patch_size            | 176x176 |
| batch_size            | 2       |
| number_of_steps       | 20      |
| percentage_validation | 10      |
| n_rays                | 32      |
| grid_parameter        | 2       |
| initial_learning_rate | 0.0003  |

**Table S4.** Key Python packages used for *S. aureus* SEM segmentation model training.

| Python Package | Version   |
|----------------|-----------|
| Tensorflow     | v0.1.12   |
| Keras          | v2.3.1    |
| CSBDeep        | v0.6.1    |
| Numpy          | v1.19.5   |
| Cuda           | v11.0.221 |

# S6 Average Number of Anchoring Points as a Function of the Number of Cells.



**Figure S10.** Numerical fittings for the average number of anchoring points, calculated from the sub-family of allowed adjacency matrixes, as a function of the number of cells for: A) Flat Si; B) SNWs surface. Best fitting equation and regression coefficient are reported within the text inset.