Supplementary Information

Simultaneous determination of the mechanical properties and turgor of a single bacterial cell using atomic force microscopy

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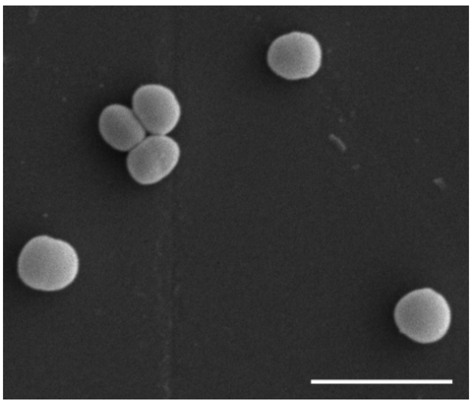
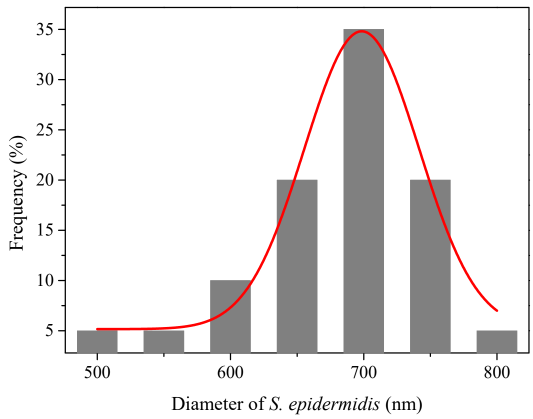
Scanning electron microscopy and live/dead bacteria fluorescence assays

For scanning electron imaging (SEM) using a TESCAN Vega LMU microscope, the bacterial cells on mica were dehydrated using critical point drying [1]. Prior to SEM imaging, the samples were fixed in 2 % glutaraldehyde in 3 M Sorenson's phosphate buffer overnight at 4 °C, and then transferred to a new plate and dehydrated through a series of ethanol solutions of 25 % (v/v), 50 %, 75 %, and 100 %, followed by critical point drying. After critical point drying, the samples were sputter-coated with 5 nm gold coating using a Polaron SEM coating unit. Fig. S1 shows a scanning electron image (SEM) of *S. epidermidis*. The averaged diameter of *S. epidermidis* is 700 nm, comparable to what was in our mathematical models.

Bacterial viability was assessed using SYTO9 and propidium acid nucleic acid stains. *S. epidermidis* cells were adhered to gelatin-coated mica pieces prepared by the same method as for the AFM experiments. The pieces were submerged in DI water, PBS, and 100 mM $CaCl_2$ solution for 4 hours. Viable and dead bacteria were fluorescently stained green or red, respectively. The images were captured using an inverted fluorescence microscope (BX61, Olympus) with a 20X objective lens. Fig. S1(c) displays *S. epidermidis* remained viable in these three environments after 4 hours.



(a)



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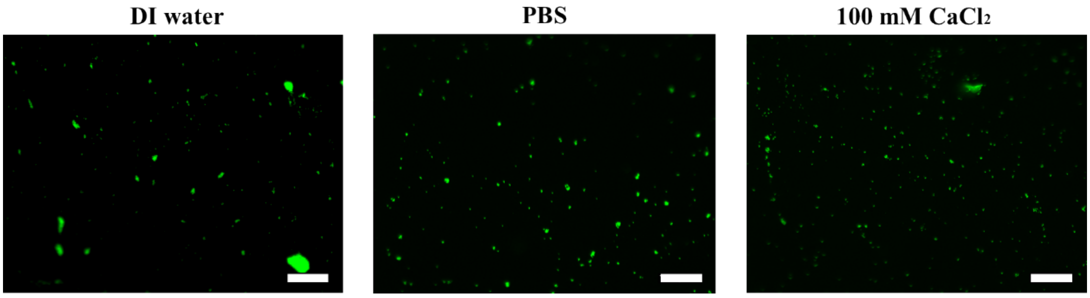
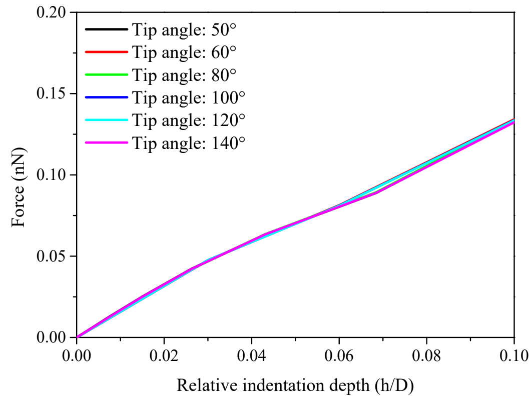


(c)

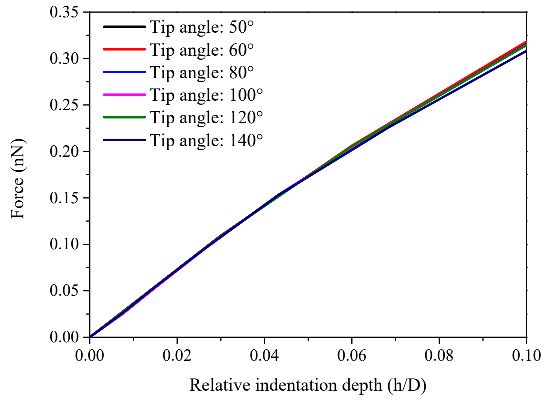
Fig. S1 (a) SEM picture of *S. epidermidis* cells for calibrating the cell diameter. Scale bar, 2 μm. (b) Distribution of the diameter of *S. epidermidis* cells. Red curve is Gaussian fitting. (c) Fluorescence images of *S. epidermidis* after immersing in different osmotic conditions for 4 hours. Scale bars: 50 μm.

Effects of sample thickness and tip angles on Reissner's model

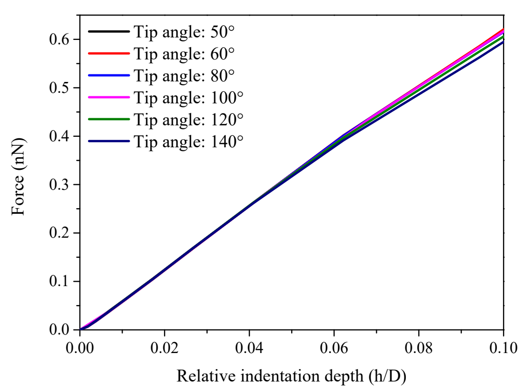
The Reissner's model is a theoretical model to describe the mechanical deformation of a hollow spherical shell structure under a concentrated point load based on elastic thin shell theory [2, 3]. However, in real measurements, the loads applied to the samples caused by the tips cannot be considered as a point load, therefore, Reissner's model must be modified under some specific loading conditions. In this study, we investigated the effects of the sample thickness and tip angles through FE models and suggest that the tip angle has no obvious influence in the mechanical behavior of the thin hollow spherical shell structures at a small deformation as shown in fig. S2(a)-(d). Specifically, according to fig. S2(e)-(f) the error of the force is over 10 % but less than 15 % for the samples with a thicker shell at a relative deformation of 10 %, which also indicates the importance of the thin shell assumptions. Therefore, an improved Reissner's model was developed in which an empirical parameter related to the thickness and tip angle ($a = f\ (t/D,\ \alpha)$) has been introduced in equations (8)-(9). Fig. S2(g) shows the relationship of the parameter *a* and the ratio of the thickness and diameter and the tip angle (*R-square* > 0.95).



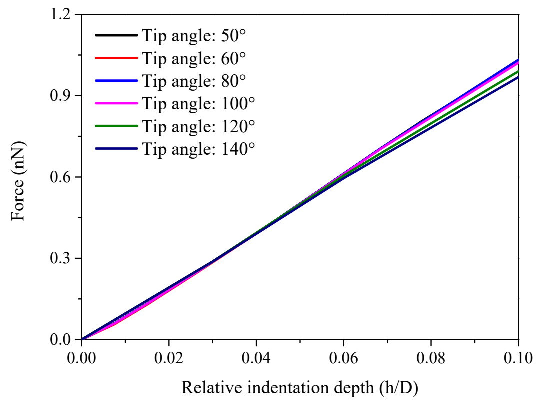
(a)



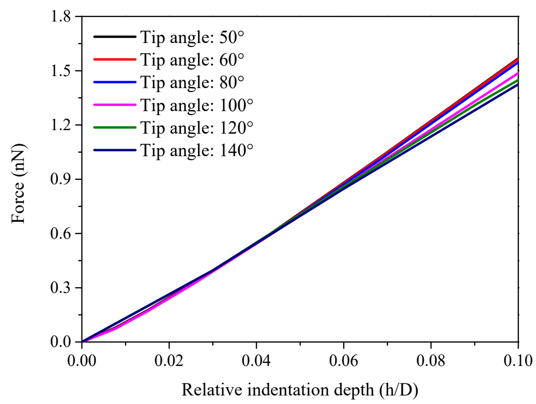
(b)



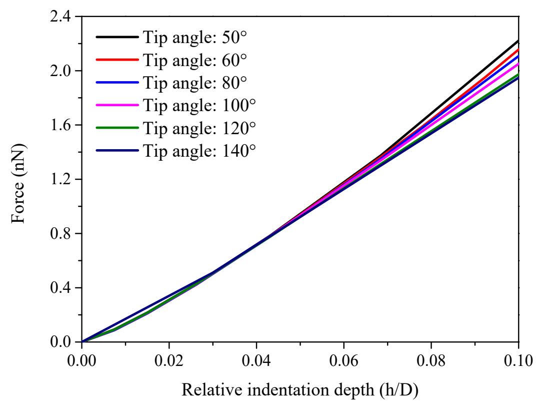
(c)



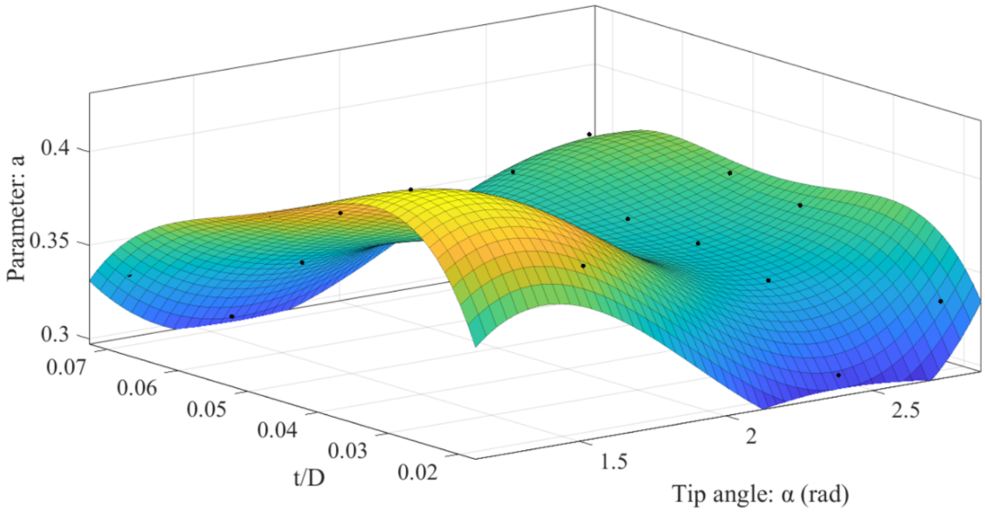
(d)



(e)



(f)



(g)

Fig. S2 Comparison of the FE results with different tip angles and ratios of the thickness and the diameter at small deformations. (a) 20 nm, (b) 30 nm, (c) 40 nm, (d) 50 nm, (e) 60 nm, (f) 70 nm, (g) The 3D plot of the introduced parameter *a* related to *t/D* and *α*.

References

1. G. M. Y Zhu, J Dawson, S Armstrong, G Wells, R Han, H Liu, W Vollmer, P Stoodley, NS Jakubovics, J. Chen, *ACS Applied Materials & Interfaces*, 2022, DOI: doi.org/10.1021/acsami.1c14533.
2. E. Reissner, *Journal of Mathematics and Physics*, 1946, **25**, 80-85.
3. E. Reissner, *Journal of Mathematics and Physics*, 1946, **25**, 279-300.