Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Mechanical spectroscopy of retina explants at the protein level employing nanostructured scaffolds

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Quantification of protein adsorption by SDS-PAGE method

We employed densitometric analysis of proteins on sodium dodecyl sulfate - polyacrylamide gel electrophoresis[1] (SDS-PAGE) to study adsorption of horse serum albumin on the nanotube scaffolds. Scaffolds were soaked in horse serum containing medium for overnight at 37 °C. Subsequently, scaffolds were washed twice with 5 ml PBS for 10 minutes and incubated with 200 µl 2x Laemmli sample buffer at 90 °C for 5 minutes. The eluted protein was then quantified by densitometry after separating by 10 % SDS-PAGE and Coomassie staining (Figure S1). Here, proteins were separated according to their electrophoretic mobility based on molecular weight (Figure S1).

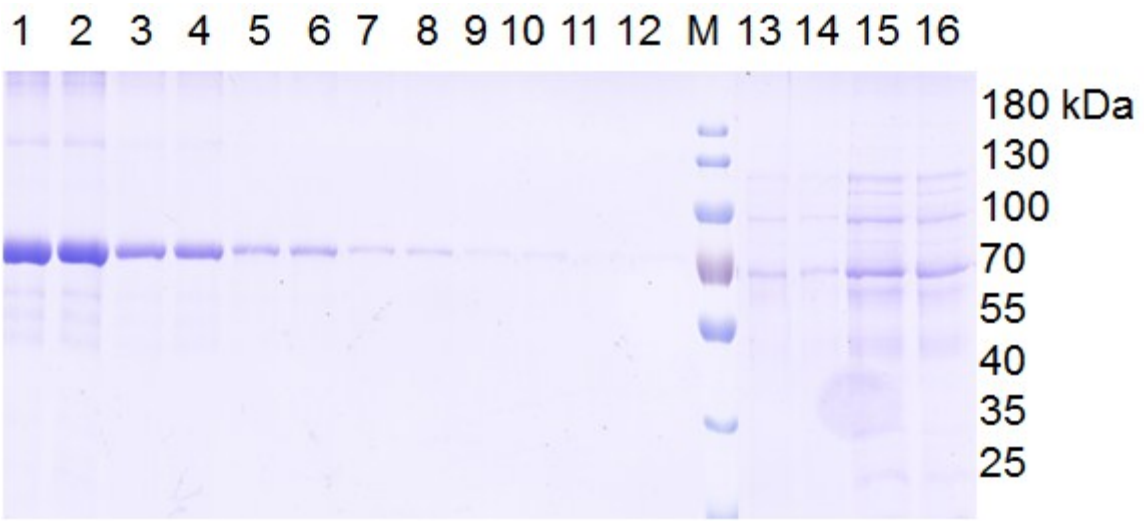

Fig. S1 SDS-PAGE pattern of the serum proteins on 10 % SDS-PAGE. Here a dilution of 3.33 µg (lane1-2), 1 µg (lane 3-4), 0.33 µg (lane 5-6), 0.1 µg (lane 7-8), 0.03 µg (9-10) and 0.01 µg (lane 11-12) of BSA standard protein, as well as 5 µl (lane 13-14) and 20 µl (lane 15-16) of eluted horse serum protein of TiO_2 scaffolds is shown. The molecular weight of the protein standard (M) is depicted on the right hand side.

For densitometry analysis, an image (16 bit gray scale 400 dpi) of the stained gel was captured, stored (by using Epson perfection V750 Pro scanner) and imported in TINA 2.09

software (Raytest, Straubenhardt, Germany). Regions of interest were defined for each band and intensities within these regions were determined and compared with a bovine serum albumin (BSA) standard dilution curve. The BSA standard dilution curve was employed as reference because BSA and the used horse serum albumin have very similar amino acid sequences and almost the same molecule weight.

As a result, from densitometry measurements we determined a horse serum albumin density of 4.54 ± 0.71 µg/cm² on the nanotube scaffolds which corresponds to 3.98 x 10^{13} molecules/cm^2.

Finite element calculations on a nanotube cell including proteins

We employed finite element calculations on structural mechanics within the framework of linear elasticity to study the mechanical properties of the nanotube layer after protein adsorption. Here COMSOL Multiphysics® was used as described in the Experimental section. We set up a TiO_2 model cell of a hexagonal nanotube structure in which the nanotube geometry was taken from electron microscopy evaluations (nanotube diameter: 71 nm, wall thickness: 6 nm). The nanotube length does not influence the mechanical properties of the reed when an uniaxial stress is applied parallel to the nanotube surface as shown in Figure S2. A 100 nm long fragment of the full nanotube was found to be sufficient to correctly account for the mechanics of the full tube - and was consequently chosen to keep computational costs moderate. For the TiO_2 nanotubes composed of TiO_2 a Young's modulus of 230 GPa Poisson ratio of 0.28 were employed, respectively.

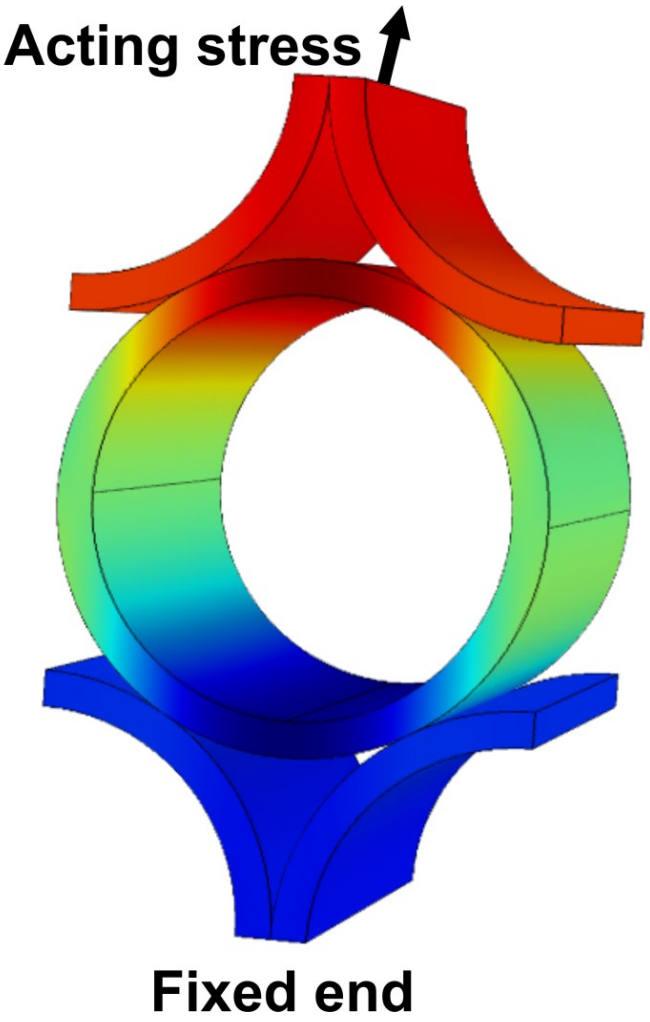

Fig. S2 Model cell of a hexagonal nanotube structure. The bottom side of the nanotube cell is fixed, while a force is acting on the upper side of the cell parallel to the surface (arrow). The color code marks the displacement field of the empty nanotubes upon loading with 100 MPa with strains ranging from -0.181 nm (blue) to 1.145 nm (red).

To model protein adsorption, all voids within the unit cell were filled with a material of unknown Young's modulus and a Poisson ratio of 0.49.[2] Subsequently, an uniaxial stress of 100 MPa was applied parallel to the nanotube surface (Figure S2). We would liketo point out that – except for a test run – we did not simulate stress dependency of the resulting strain due to our inherent framework of linear elasticity.

The Young's modulus of the nanotubes including protein filling was taken from sandwich beam calculations (see Results) with an obtained value of $E_{NT+Prot}$ = 37.3 GPa. How the Young's modulus of the entire nanotube layer including proteins depends on changes of the protein Young's modulus E_{Prot} is shown in Figure S3. It can be seen that without proteins, the Young's modulus of the empty nanotubes is E_{NT} = 2.1 GPA in excellent agreement with studies by Fischer and Mayr,[3] which corroborates our initial approach to model the reed with an effective modulus.

For an employed protein stiffness of 15 GPa, the determined modulus of the entire nanotube layer including proteins is reached. Thus, serum proteins attached to the nanotube walls comprise a modulus about 3.8 as high as the Young's modulus of the retina and show similar stiffness values as many different proteins (see e.g. ref. [2] and references herein).

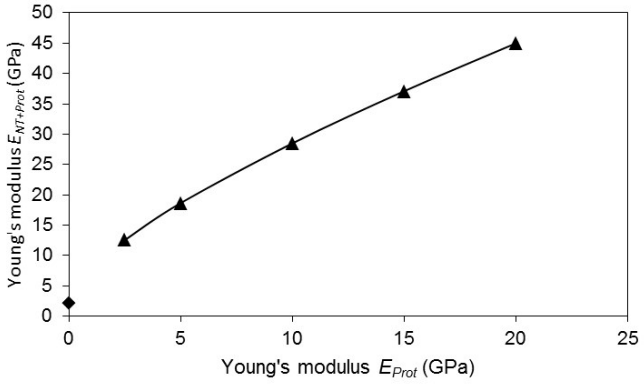

Fig. S3 Dependency of Young's modulus of the protein filled nanotubes on protein stiffness (▲), obtained from finite element calculations. For empty nanotubes without proteins a modulus of 2.1 GPa results (♦).

References

1. U. K. Laemmli, *Nature*, 1970, **227**, 680–685.
2. S. Perticaroli, J. D. Nickels, G. Ehlers, H. O'Neill, Q. Zhange and A. P. Sokolov, *Soft Matter*, 2013, **9**, 9548-9556.
3. K. Fischer and S. G. Mayr, *Advance Materials*, 2011, **23**, 3838-3841.