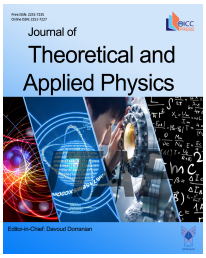

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# A mini review on cell interactions with biological metamaterials

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**Invited Review**

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**Abstract:**

The mini review summarizes the interactions between biological cells and high aspect ratio nanostructures termed as biological metamaterials, while focusing on what happens at the fundamental level. The questions pertaining to the interfacial phenomenon producing modes of interaction, what instigates them, what are the favourable conditions for controlled interaction, and how are they influenced by the nanostructure geometry are answered constructively. Recent but widely distributed cell-nanostructure interaction modelling techniques ranging from elastic theory models to molecular dynamics simulations are weaved together to extract out the possible ways of optimizing the interaction. High impact of the nanostructure design, its sharpness, spacing, aspect ratio, and the membrane properties are observed on the biological response.



**Keywords:** Biological metamaterials; Cell-nanostructure interaction; Biointerface; Elastic theory; Conditions for controlled interaction

## 1. Introduction

Biological metamaterial is a term usually given to a class of nanomaterials with a high aspect ratio [1–11] that interact with biological cells resulting in an unusual biological response. This response when optimized can lead to an improvement in the efficiency of processes with applications in drug delivery, control of specific group of cells, etc. Various methods ranging from basic seeding to centrifugation have been coined for realizing the most effective cell-nanostructure (nanoneedle) interaction [7–16]. More importantly, what happens next, i.e., the pointed nanostructures penetrate into the cells, or the cells engulf around the nanoneedles, is a subject of interest, and inferences have been made on the way in which this interaction will take place based on experimental studies [13, 15, 17–23]. More importantly, what are the preferred conditions for nanostructure penetration into the cell has been an important yet debatable topic. Various attempts have been made to model the interaction guided by the key parameters including the membrane mechanical properties, structure of individual and array of nanostructures, and many more [24–33]. Computationally expensive yet reliable elastic theory models have touched this problem at a deeper level by considering the free energy balance at the cell-nanostructure interface. Interesting results concerning with the threshold parameters for the nanostructure dimensions and its chemical properties have been found which also agree experimentally. Furthermore, a detailed study addresses how favourable penetration can be facilitated while considering the rupturing of the cell membrane [33]. The present review aims to systematically collect all these ideas at one place while uncovering the limitations of the techniques discussed and hints on what stands for the future.

### 1.1 What are biological metamaterials?

A metamaterial is typically an arrangement of repeated elements that showcases properties that do not occur naturally. These properties are a result of playing with the geometrical quantities of the structure that gives one the control of altering the overall properties of the metamaterial. Following on the same lines, it might seem that biological metamaterials exhibit a similar nature and maybe they are made from a biological material. However, biological metamaterial is a parent term that covers nano dimensional materials having a high aspect ratio i.e., a very thin tip and relatively

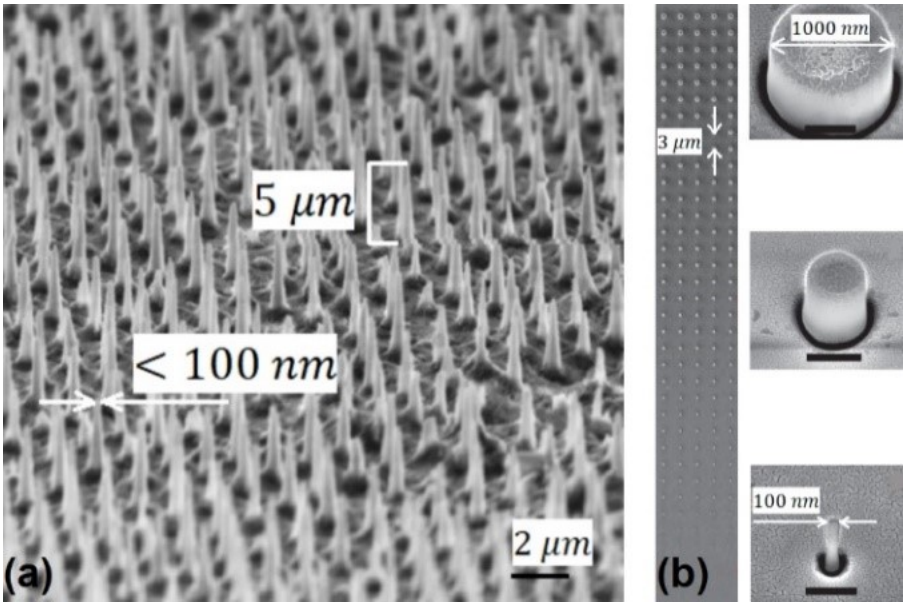


**Figure 1.** SEM images of an array of biological metamaterials. (a) A uniform assembly of identical nanopillars of height 5 $\mu$m each. Scale bar is 2 $\mu$m. (Modified from [11]). (b) Arrangement of nanopillars with decreasing diameter from top to bottom (1000 nm to 100 nm). Scale bar is 10 $\mu$m for the array and 400 nm for zoomed in images. (Modified from [12]).

long length [1–5]. As per literature pertaining to biological nanostructures, aspect ratio greater than or equal to 10:1 is considered high [6–8]. Even plasmonic metallic [9] and dielectric nanostructures [10] with the above-mentioned aspect ratio are placed in this class. An array of biological nanomaterials (nanopillars) is shown in Fig. 1. Meanwhile, Fig. 1(b) depicts such an array with varying tip widths along with the zoomed in images of each nanopillar.

Now, the term biological relates to the fact that these nanomaterials are considered for their interactions with the cells in a living organism. More importantly, these interactions can lead to not so normally observed biological response of the cells. The ability to guide this response resulting in improved efficiency in applications such as drug delivery, control of cancerous cells, etc. makes this a subject of interest. But before diving deeper into how these interactions take place, it is first crucial to comment upon how to bring the cells and nanostructures in contact.

### 1.2 How to make cells and nanostructures interact?

In the past decade, several interesting methods have been proposed to make the cells and nanostructures come in contact with each other [13–17]. As expected, the most obvious way is to place the cells on the array of nanostructures [15] or place the nanostructures on a layer of cells [13] as depicted in Fig. 2(a). In both the scenarios, gravity has a crucial role to play; however, they are not the most efficient. An unintuitive yet commendable idea of placing the nanostructures at an angle with the horizontal was suggested by Kim et al. [17] who used Gallium nitride (GaN) for fabricating the nanostructures ascribed to its high mechanical strength (Fig. 2(b)). Considering the forces acting at the microscopic interface, it turned out that the use of this method makes it relatively easier to insert the desired element via the nanostructure inside the cell. Moreover, the resulting damage at the cell membrane is also smaller as compared to other methods. Along with this, the experimental procedure of adding a foreign element to the cell becomes easier due to a change in the angle of insertion improving the alignment of the pipette with the nanostructure.

One interesting technique used specially to augment the insertion forces on the cells is to exploit the centrifugal force [14, 16] (Fig. 2(c)). As soon as the cells come in contact with the nanostructures, the arrangement is made to rotate such that the force at the interface is controlled based on the angular velocity. Zhu et al. employed this idea to quantify the improvement in efficiency over the non-centrifugal or conventional methods [14]. They considered a matrix of diamond nanostructures termed as nanoneedles and a layer of cell was held in contact with the matrix followed by centrifugation. Firstly, they were interested to know whether this method has any added advantage over the conventional technique to transport foreign molecules into the cells. For comparison, centrifugation and incubation were run together with an aim to transfer fluorescent molecules inside the cells. As a result, the fluorescence intensities that implied the quantity of fluorescent molecules were merely 10% in the case of incubation whereas around 60% in case of centrifugation, clearly depicting improved transport. Secondly, it was concluded that the transport is relatively faster. To prove the same, cisplatin was added to the cell layer, and it was found that within 30 seconds, there was a drop of more than 30% in the number of healthy cells when centrifugation was used which was even doubled when centrifuged for 300 seconds. Meanwhile, in the conventional case, almost all cells were intact showing negligible reduction in cell viability. These observations translated into an inference that centrifugation is an efficient intracellular delivery method.

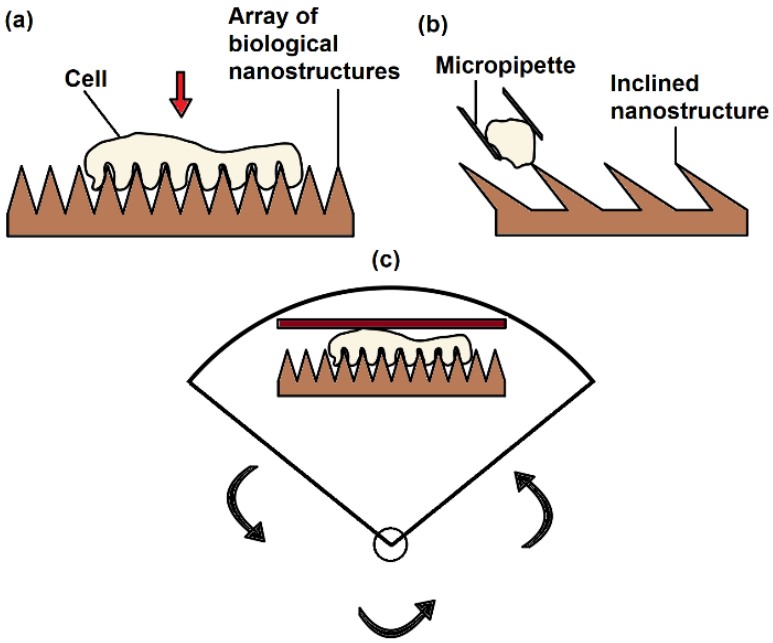


**Figure 2.** Three major methods of making cells and nanostructures interact. (a) Seeding cell on nanostructure (b) Micro pipetting cell on inclined nanostructure (c) Centrifugation.

## 2. What happens after the cell and nanostructure come in contact?

Once the contact has been established, it is exciting to figure out how the cell will react to the intruding array of nanostructures. Intuition hints that the sharp nanoneedles might tear apart the cell membrane to deliver the desired drug; however, this is not always observed. Instead, it depends on several conditions such as membrane stiffness, diameter of individual nanostructures, their distribution, etc., if such penetration also termed as spontaneous penetration will occur or not which makes it highly debatable [20, 21]. Generally, cells showcase the ability to wrap around the sharp nanoneedles without damaging their membrane, i.e., cells can engulf [13, 15, 22, 23]. A step further, atomic force microscopy was employed alongside with finite element simulations to cover how to cells react and change their environment due to the presence of a nanomaterial [24].

### 2.1 Is spontaneous penetration a reality?

To clarify the above stated dilemma, Xie et al. dived further into this and the dynamic nature of the interface between the nanoneedles and the cells was examined via experimental studies [20]. Using scanning electron microscopy (SEM), the process and the interface were imaged at different time stamps, as shown in Fig. 3. Initially, the spherical cell is placed on the top of the array and no contact between the cell and the surface on which nanoneedles are placed i.e., substrate is established until the end of 5 minutes. However, slowly some portions of the cell, especially the outer regions start touching the substrate and the spherical cell kind of melts on the array ($t = 30$ min). At the end of 3 hrs, much of the cell surface covers the array, and peaks can be observed in the SEM image that depict the nanoneedles. As seen in Fig. 3(a), at $t = 180$ min, the cell is wrapping around the needles and this process is known as engulfing. Finally, at the end of 24 hrs or 1440 min, a tent like structure is formed with the nanoneedles engulfed by the cells across the length of the cell including the ends.

However, it was still not clear whether these nanoneedles penetrated inside the cells or not. To check the same, a method known as cobalt ion delivery assay was employed [26]. In short, if the nanoneedles penetrated it would result in the diffusion of the cobalt ions into the cell, leading to fluorescent spots at those locations. Following this, the percentage penetration proportional to the ratio of the number of fluorescent spots and the total number of nanoneedles in a unit area was calculated. This percentage rises abruptly in the initial half followed by negligible changes. At the end of 24 hrs, only about 6% of the nanoneedles penetrate the cell which clearly shows the supremacy of engulfment. Like the findings discussed above, a focused study on the interface of a bacteria and an array of nanostructures deals with the forces due to which the spontaneous penetration is taking place [25]. However, to improve the spontaneous penetration, researchers came up with unique design such as drilling a hole in the nanoneedle to make it act like a nano dimensional straw [21]. Their design made a way for direct transport of molecules into the cells through the hollow cavity in the nanoneedle. Moreover, fundamental methods such as the application of electric field or using laser pulses to enhance the permeability of the cell membrane, known as electroporation [26, 27] and optoporation [28], respectively, have been proved to slightly improve the penetration of the nanoneedles.

### 2.2 Engulfment: how and where?

With this, it was clear that engulfment is a more favourable process. However, now the question is where and how much

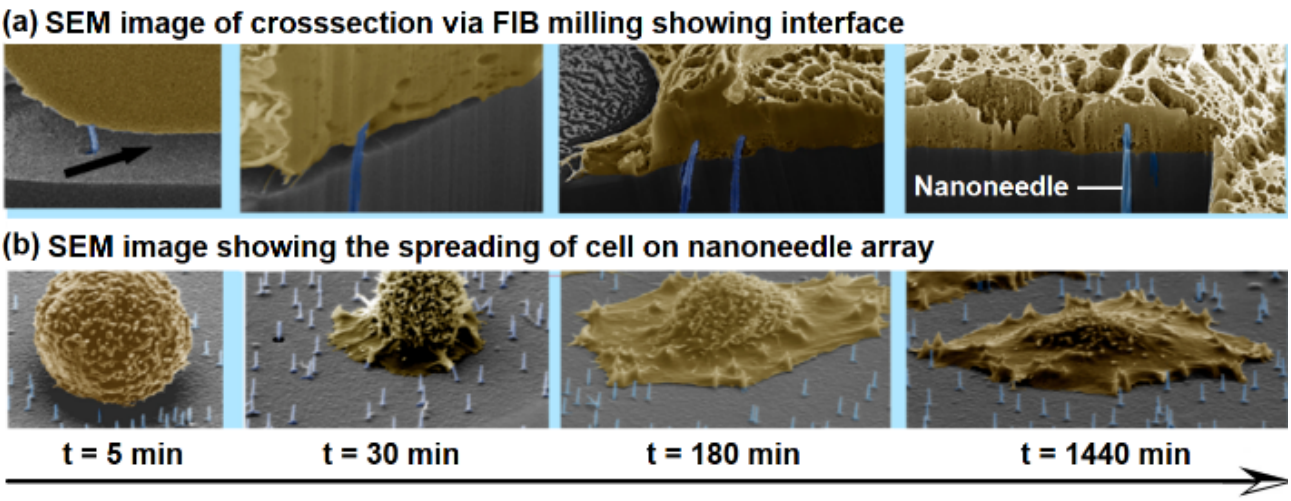


**Figure 3.** SEM images of the cell-nanoneedle interaction process at four time stamps. (a) Cross-section revealed using FIB milling showing the engulfment process. Scale bar is 2 $\mu$m. (b) Spreading of cell on the nanoneedle array. Scale bar is 5 $\mu$m. (Modified from [20]).

the engulfment takes place on the surface of the cell. For exploring the same, Santoro et al. positioned the nanoneedles at different locations on a planar surface such that three sections were created [15]. These were labelled as centre, middle and the edge, and were defined as the concentric elliptical regions as one moved radially away from the centre of the cell. Using fluorescent microscopy, the cells were observed and with the help of SEM, different regions were revealed and differentiated, as shown in Fig. 4. The area surrounded by the black ellipse denotes the central section (Fig. 4(a)) and stretching to the red ellipse, the middle section is formed (Fig. 4(b)). Finally, the region bounded between the blue and the red ellipses covers the edge of the cell (Fig. 4(c)).

The authors observed that percentage of the engulfing activity rises as we move towards the edge from the centre, approaching as high as 40% engulfment in the edge section. Therefore, along with knowing that penetration is not a favourable activity, it was also found that engulfment is preferred at the edge, and nanostructures in this region have a higher chance of experiencing the same.

## 3. What if we could model the interaction? Is it a roadmap to optimization?

Numerous methods have been proposed to provide a computational overview of the interaction; however, the question is that did all these studies also result in a guided framework for uncovering the preferred conditions and threshold values of interface parameters required for desired interaction? More importantly, were they real enough to match experimentally, leading to a reliable model that could be used for designing the nanostructures with successful applications? We will focus our survey to the ones that address these areas.

### 3.1 A model to start with

Majorly, the elastic theory models that utilise the balance of forces are continuum based which treat the membrane such that it can be characterized based on its mechanical properties such as young's modulus [27, 28]. Xie et al. proposed a model based on the experimental observations of cell-nanoneedle interface [27]. They considered free wrapping of the cell on the nanoneedles based on the net gravitational force balanced against the hydrostatic pressure

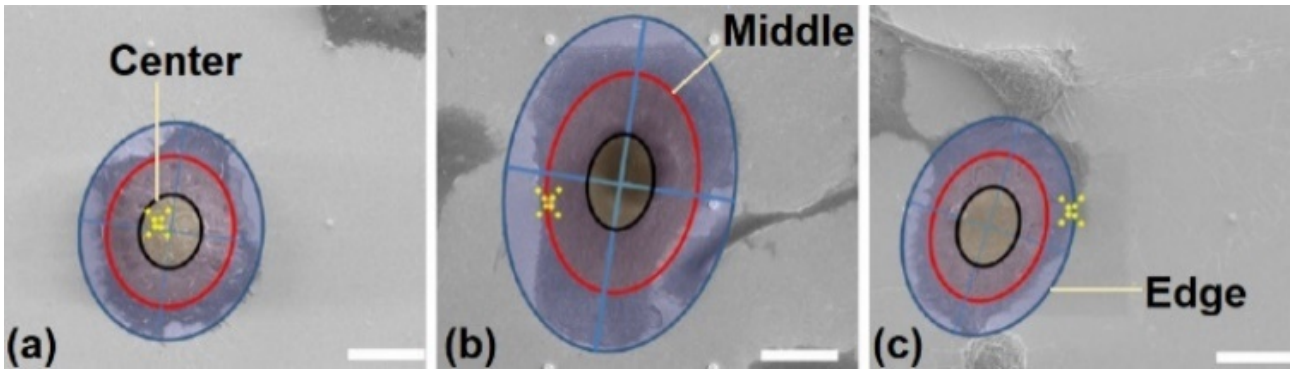


**Figure 4.** SEM images of cells engulfing the nanoneedles creating three regions of interest (a) Center (b) Middle (c) Edge. The yellow marks depict the position of the nanoneedles. Scale bar is 15 $\mu$m. (Modified from [15]).

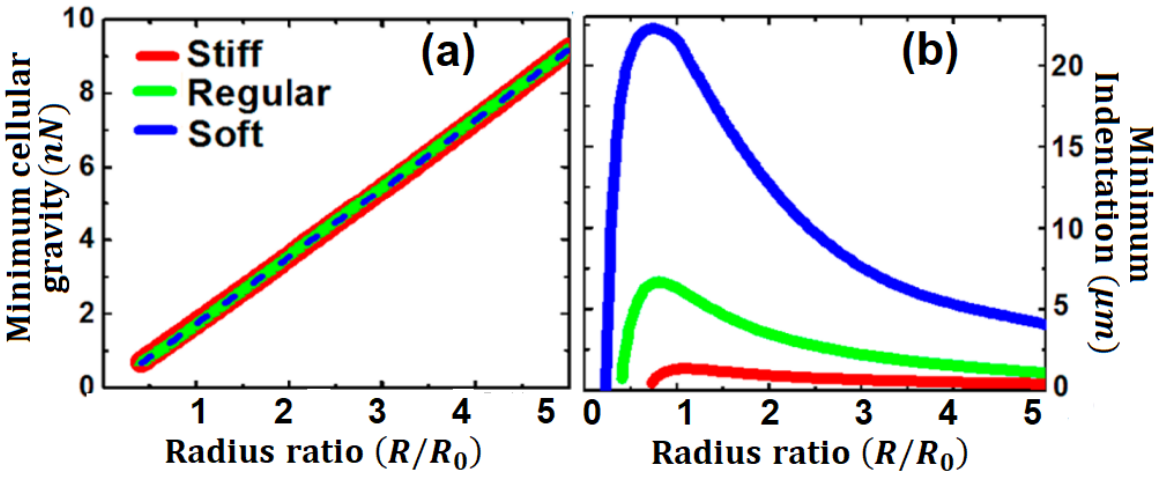


**Figure 5.** Influence of a single nanoneedle radius ratio ($R/R_0$) with $R_0 = 50$ nm on (a) Minimum cellular gravity (b) Minimum indentation required for penetration. (Modified from [27]).

inside the cell and the buoyancy force. Actually, as the cell, which is considered to be spherical continues to engulf, the penetration only becomes possible if the needle is able to pass through the cell membrane which consists of the lipid bilayer and the cytoskeleton. Interestingly, the lipid bilayer in between the stiff cytoskeleton and the nanoneedle experiences strong compression which creates tension within the bilayer and when the tension reaches a sufficient value for rupture, the needle penetrates. The membrane tension is variable due to the dynamic change in area of membrane and depends on the bending tension.

The results from this model show that using a typical nanoneedle i.e., with radius $R_0 = 50$ nm, it is impossible for gravity to solely make the needle penetrate. Moreover, taller nanoneedles turn out to be a requisite for softer cells as they wrap around easily. However, needles with smaller radii $R$ are a solution. Fig. 5 shows the influence of the nanoneedle radius on the minimum net gravity and the minimum indentation required for penetration. One clear observation is that low indentation is enough, and it is easier to penetrate in a stiffer cell as the area of contact between the cell membrane and the needle is smallest relatively. The same was verified via atomic force microscopic images of nanoneedle penetration in an animal cell [29].

There is a linear relation between minimum gravity and needle radius (Fig. 5(a)) which is expected but the nature of variation of minimum indentation with the radius is interesting. It rises gradually with the falling radius, touches a maximum value followed by an abrupt drop. This is due to the reason that for higher radius, the change in membrane

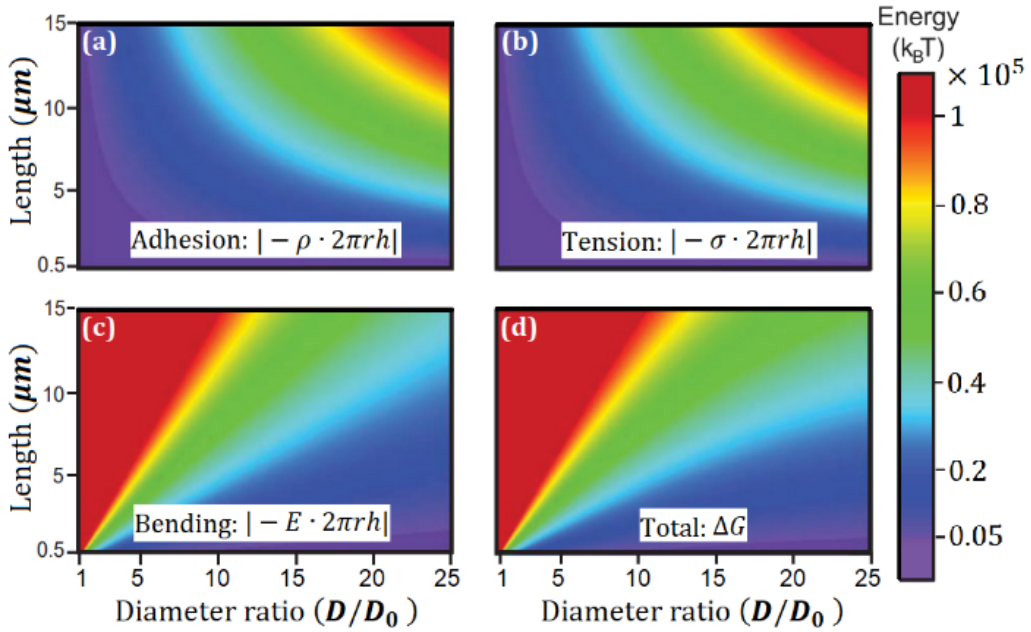


**Figure 6.** Contour plots depicting the variation of free energy due to (a) Adhesion (b) Tension (c) bending components and (d) total free energy as a function of nanostructures length ($h$) and diameter ratio ($2r/D_0$) where $2r = D$ and $D_0 = 20$ nm. $E$ is the modulus of bending. (Modified from [30]).

area has a significant role but as the radius gets smaller, the area change becomes negligible, and the bending term dominates, leading to smaller indentation distance. Finally, if radius drops below 10 nm, then almost no external force is required for penetration.

These results are a first step towards the understanding of the modelling of cell-nanostructure interface and uncover important conclusions relating to the nanoneedle design. However, this model is relatively simple and considers that the membrane properties do not vary whereas in reality due to the biological processes inside the membrane it exhibits other modes of relaxation which should be considered for a more advanced model.

### 3.2 A different perspective for modelling

All this hinted that to critically understand the interaction it is rather useful to understand the balance of free energy of the membrane. The cell interface with nanostructures arrays (CINA) model coined by Buch-Månson et al. [30] considers the cell as a uniform and soft shell, and defines the change in free energy $\Delta G$ because of change in the adhesion of cell with the substrate, change in surface tension, and bending, expressed as:

$$\Delta G = \sigma \Delta S - \rho A_{contact} + \Delta G_b \qquad (1)$$

where, $\sigma$ is the surface tension, $\Delta S$ is the change in surface area of the cell, $\rho$ is the specific adhesion energy per unit area, $A_{contact}$ is the area of contact, and $\Delta G_b$ is the bending energy term. Accordingly, two states are defined, one being the 'top' state which names the situation when the cell rests on the top of the array, while the 'bottom' state refers to the case when the cell has completely engulfed with the array of nanostructures. As a result, the impact of the nanostructure length and radius on $\Delta G$ and its components was plotted, as shown in Fig. 6.

Interestingly, the adhesion energy and surface tension components are very close in nature and as per (1), $\Delta G_b$ dominates and $\Delta G$ is majorly governed by the same. However, this is the energy barrier that needs to be crossed to reach the bottom state, and is therefore it proves that this is not a favourable process to behold. Moving a step ahead, the influence of the array packing density was also investigated and as a conclusion this model could predict the way in which the cell is going to settle, a value addition to the present models. To advocate the reliable use of model, these results were experimentally validated and found to be in good agreement [31, 32].

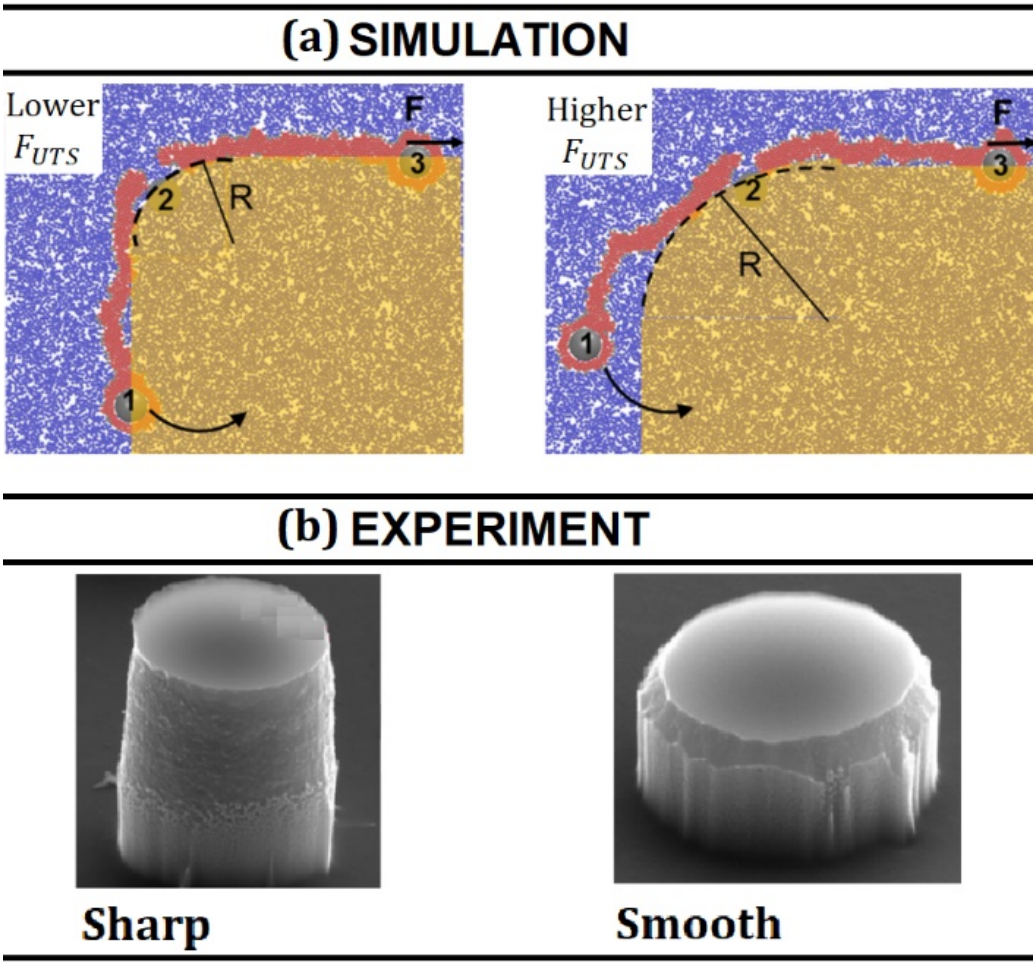


**Figure 7.** Impact of edge sharpness on penetration. (a) 2D MD simulation using 3-cylinder geometry to obtain the relationship between $F_{UTS}$ and $R$. (b) SEM images of a sharp and long, and a smooth and short nanoneedle for experimental testing of percentage penetration. Scale bar is 1 $\mu$m. (Modified from [33]).

### 3.3 Exploring deeper with molecular based simulations

Despite the agreements and the reliability of the above continuum models, they are still unable to incorporate the disruption of the cell membrane when the abrupt shift from the side wall to the top of the nanostructure takes place. For addressing the same, molecular dynamics (MD) simulations that directly hit at the molecular level are a way out. Capozza et al. found that the edge of the nanoneedles guides the local curvature of the membrane in contact and how the membrane reacts to this is interesting [33]. Using a two-dimensional coarse-grained MD model and employing the three-cylinder simulation geometry, the authors were interested in finding out the relationship between the curvature (through radius of curvature $R$) and the ultimate tensile strength ($F_{UTS}$) leading to rupture of the membrane, as shown in Fig. 7(a).
One clear observation was that a higher radius of curvature ($R$) corresponded to a higher $F_{UTS}$ ($\sim$ 25 pN for greater $R$ and $\sim$ 7 pN for smaller $R$). The obtained relationship showed that only a small force is required to rupture the membrane; however, the edge should be sharp i.e., small $R$. To further check the validity of the results, the impact of edge sharpness on the penetration was experimentally estimated. As depicted in Fig. 7(b), separate arrays of two nanoneedles with same radii but different heights and edge sharpness were used to culture cells. The result showcased a much higher percentage of penetration in case of sharp-edged needles, a result matching with their simulations.

## 4. Conclusions and future directions

Thanks to the combination of experimental studies and simulations, it is possible to optimize the nanostructure array geometry to facilitate the molecule transfer into the cell. The ability to predict the cell settling state for fabricating nanostructures for desired cell settling is interesting and all these ideas can be exploited for building an efficient drug delivery framework. However, there are still some areas that need to be explored further such as:
• The present studies lack considering other interaction processes possible at the interface that might have a role to play in disturbing the membrane rupture.
• Moreover, the natural techniques such as self-relaxation of the membrane to release the stress needs to be explored.
• The cell membrane is a biological material and exhibits self-repair as soon as the membrane is damaged. Adding this aspect to the models will help understand the dynamic nature of molecules transport and when and how it stops based on the repair events.
• Finally, the simulations to date have considered total membrane rupture ascribed to the 2D nature of modelling. An ambitious goal could be to model the effect of geometrical parameters of the nanostructures and the array on the 3D membrane rupture.

**Authors Contributions**
Not applicable

**Availability of data and materials**
There is no data associated with this study.

**Conflict of Interests**
The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



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