

Tailoring Cell-Biomaterial Interfaces: High-Aspect-Ratio Nanostructured Surfaces

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Purpose of these lectures

The aim of these two guest lectures is to introduce you to state-of-the-art ongoing research in the area of cell-biomaterial interactions. These are techniques that broadly still laboratory-based but are slowly being translated into clinical environments. Trying to understand their underlying mechanisms helps us design better biomaterials.

The two lectures are distinct, the common link is that they present different ways of modifying the cell-biomaterial interface. The first lecture considers how the topography of a material can be modified to create an extreme nanostructured environment for interfacing with cells and tissue. The second lecture considers how using a class of polymeric materials that have unusual electronic and ionic properties can be used to modulate a bioelectronic interface with cells and tissue.

As both topics are under active research, these lectures are based on the best available information at the time of writing. Where there is academic debate, I will try to highlight that. With time the understanding and knowledge in this area will improve, and some of the unknowns presented here may be resolved (possibly by you, if you continue in research).

Further reading

For your interest, a further reading list is provided below to give you direct links to the underlying academic literature supporting these lectures. There is no textbook, because we don't know enough yet to write one.

Please send any comments or corrections to: stuart.higgins@imperial.ac.uk



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Executive summary

- High-aspect-ratio nanostructured surfaces use extreme geometries (on the length scale of cells and cellular machinery) to introduce topographical stimuli into a material
- These stimuli have been used in a range of applications, from delivering drugs and other molecules into the intracellular environment, to improving the electrical contact of electrodes to electrically-excitabile cells
- There are multiple methods for engineering high-aspect-ratio nanostructured surfaces, based predominantly on cleanroom-based microfabrication techniques
- The interaction of high-aspect-ratio nanostructures with cells is more nuanced than simply penetrating through cell membranes, which typically only occurs when additional energy or force is applied to the system
- The biological mechanisms for the interaction of cells with high-aspect-ratio nanostructures remains an area of active research
- The primary research challenges in the field relate to reliably understanding fundamental mechanisms for cell-nanostructure interactions and the engineering challenging of incorporating these structures into a wider range of systems

Glossary

- **Nanostructure**
 - Loosely defined term that refers to structures with nanoscale dimensions, frequently encompassing systems with structures with both micro- and nanoscale patterning
- **High-aspect-ratio**
 - In this context, structures where the height-to-diameter ratio is > 10
- **Nanoneedles / nanowires / nanopillars**
 - Synonyms used by different research groups for high-aspect-ratio nanostructured surfaces
- **Intracellular access**
 - Gaining permanent or temporary access to the cell cytoplasm through penetration of the cell membrane
- **Photolithography**
 - A microfabrication technique that uses ultraviolet light projected through a mask (a glass or quartz plate that contains opaque and transparent regions) to selectively expose a photosensitive resin on the surface of a material
 - The exposed and un-exposed areas become less or more soluble (depending on the process used) allowing the soluble regions to be washed away, resulting in the mask pattern being transferred onto the material
- **Self-assembled lithography (also known as nanosphere lithography)**

- A microfabrication technique that takes advantage of the behaviour of small spheres to self-assemble and pack into regular arrays on the surface of a material
- Spheres on the surface act as nanoscale masks for subsequent processing causing the thinner edge regions to be etched at a faster rate than the thicker central regions, resulting in the transfer of a pattern.
- Nanoimprint lithography
 - A microfabrication technique that uses the combination of pressure and heat or ultraviolet light to bring together a stamp (also known as a shim) with a curable-polymeric material
 - The liquid polymer is forced around the stamp and cured, creating a negative mould of the original
 - This process can be repeated again using the negative mould to replicate positive versions of the original structure
- Subtractive manufacturing
 - Describes a range of manufacturing techniques that involve the removal of material from a surface, leaving behind the desired material
 - This is often used in conjunction with a patterning step, such as the lithographic techniques described above
 - Examples in microfabrication include wet etching, where acids are used to dissolve unprotected regions of a material, or dry etching, where a plasma is bombarded into unprotected regions to ionise and remove them
- Additive manufacturing
 - Describes a range of manufacturing techniques that involve the addition of material to a surface, in order to create a pattern (e.g. 3D printing)
 - In this context, examples include ion-beam deposition of metals into precise locations on a material
- Mechanotransduction
 - The process and mechanisms by which cells transmit and interpret mechanical forces from their microenvironment
- Electro- / opto- / chemo-poration
 - A range of techniques that induce the formation of temporary pores in a cellular membrane by applying an external perturbation (e.g. electric field, incident laser light, or membrane-disruptive chemical agent)

Lecture notes

1. High-aspect-ratio nanostructured surfaces use extreme geometries (on the length scale of cells and cellular machinery) to introduce topographical stimuli into a material¹
 - a. Cells are sensitive to their microenvironment and change behaviour when exposed to different material cues²
 - b. A high-aspect-ratio nanostructured surface is a material that has been patterned with an array of nanoscopic spikes
 - i. Heights vary between 1 – 10 μm , with a base diameter of roughly $< 1 \mu\text{m}$
 - ii. The height-to-diameter ratio is the aspect-ratio, typically $> 1:10$
 - iii. Ratios of $> 1:10$ are considered 'high' (this is not a strict definition) in part because manufacturing higher ratios becomes increasingly challenging
 - iv. Nanostructures may be tapered to a point (e.g. $< 50 \text{ nm}$ tip diameter)
 - v. The spacing between nanostructures typically varies between 0.1 – 10 μm , either in a regular array or spaced stochastically (i.e. a semi-random statistical distribution created using some physical/chemical process)
 - c. High-aspect-ratio nanostructures resemble microneedles and have conceptual similarities however they are an order of magnitude smaller and general treated as a distinct field of research
 - d. Many researchers refer to these materials by different names, for example: nanoneedles, nanopillars, nanostraws, nanowires
 - e. The spacing and geometry of the structures are on the same length scales as the cell and cellular machinery
 - i. Multiple nanostructures impinge on a single cell
 - ii. Surfaces can disrupt the cell membrane
 - iii. Surfaces can alter the shape of the cell
 - iv. Surfaces can modify a cell's ability to form cellular adhesions
2. High-aspect-ratio nanostructured surfaces have a wide-range of potential applications
 - a. Surfaces can be used to aid the delivery of molecules into cells and tissues
 - i. Example: enhancing gastrointestinal drug delivery using nanostructured pill³ – for improving uptake of oral drugs
 - ii. Example: delivering plasmids into mouse muscle tissue, resulting in better distributed blood vessel formation than single injection alone⁴ – for improved regeneration of tissue/implant acceptance
 - b. Surfaces can be used to promote stem cell differentiation
 - i. Example: texturing the surfaces to promote osteogenesis and inhibit bacterial growth⁵ – for reducing orthopaedic implant failure
 - c. Surfaces can be used for intracellular extraction
 - i. Example: hollow nanostructures can be used to momentarily extract small volumes from cells without killing them⁶ – for drug screening

- d. Adding high-aspect-ratio nanostructures to electrodes can improve electrical coupling with cells
 - i. Combined with electroporation, nanostructures penetrate the membrane, resulting in intracellular sensing⁷ - for improving electrode interfaces
 - ii. Nanostructured surfaces can interface many cells at once and are a pathway to overcoming limitations of patch-clamp approaches
3. There are multiple methods for engineering high-aspect-ratio nanostructured surfaces¹
- a. A common approach is to pattern the target material with an array of 2D dots
 - i. Photolithography and electron-beam lithography allow precise control over location of each nanostructure, but are resource intensive
 - ii. Self-assembled lithography is less resource intensive but has much less control over location and dimension of nanostructures
 - iii. Subtractive manufacturing uses dry or wet etching techniques to remove material in regions not masked by the initial pattern
 - iv. Additive manufacturing uses techniques such as the vapor-liquid-solid growth mechanism, to catalyse the growth of nanostructures up from the surface
 - v. Processes are normally carried out in cleanroom laboratories (laboratories with controlled levels air particulates that might otherwise disturb micro- and nanoscale processes)
 - b. Replication techniques allow resource expensive master samples to be replicated into other materials
 - i. Roll-to-roll nanoimprint lithography is an example of how nanostructures can be rapidly manufactured on a large scale⁸
 - c. Many alternative fabrication approaches exist, but are relatively unexplored
4. The interaction of high-aspect-ratio nanostructures with cells is more nuanced than simply penetrating through cell membranes¹
- a. Originally assumed that nanostructures would spontaneously penetrate the cell membrane, under the influence of gravity alone
 - b. The cell membrane is extremely dynamic, fluid environment, resulting in a number of different interaction scenarios
 - i. The cell membrane can engulf the nanostructures
 - ii. Under the influence of gravity alone, cells will rarely be spontaneously penetrated by the nanostructures
 - c. Introducing force and energy into the system can increase the degree of membrane penetration
 - i. Increasing the applied mechanical force, or vibrating the nanostructures can increase penetration
 - ii. Nanostructures can be combined with other membrane poration techniques, e.g. electroporation, optoporation or chemical poration

- d. Penetration is not a requirement for many applications, such as guiding cell morphology or influencing mechanotransduction
5. The biological mechanisms for the interaction of cells with high-aspect-ratio nanostructures remains an area of active research
- a. Evidence that nanostructures might disrupt focal adhesions, protein complexes that link the material, cell membrane, cytoskeleton and nucleus⁹
 - i. The absence of forces transmitted to the nucleus affects the activation of transcription factors, altering gene expression
 - b. Nanostructured surfaces might trigger cell processes such as endocytosis¹⁰
 - c. Theoretical modelling of the cell-biomaterial interface is challenging
 - i. Continuum models treat the membrane as a continuous sheet, at the expense of discrete molecular interactions known to be present
 - ii. Molecular simulations capture better discrete interactions, but are too computationally expensive to run on areas larger than single structure interactions
 - iii. Modelling to date supports experiment observations that geometry (height, tip size, spacing) and material surface-energy all play an important role in determining how a cell will interact with the surface
6. The primary research challenges in the field relate to reliably understanding fundamental mechanisms for cell-nanostructure interactions and the engineering challenging of incorporating these structures into a wider range of systems¹
- a. The field has not reached the stage where generalised structure-property relationships can be drawn
 - i. Different cell types respond differently to the same surface, reflecting the complexity of the challenge
 - ii. Techniques such as image-based cell profiling can help capture the complexity and subtle nuances in cellular response, helping to understand how subtle geometry changes influence a wide-range of cellular parameters¹¹
 - b. Most engineering approaches use hard, brittle materials that are non-ideal for many applications
 - i. Moving towards flexible materials may aid this process
 - ii. New approaches required to help transfer patterns onto a wider range of biomedical/bioengineered constructs.

Further reading

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