Thin film techniques: the layer-by-layer self assembly technique

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Overview

- Thin films
- Thin film techniques
- Langmuir-Blodgett technique
- Chemical self-assembling
- Layer-by-Layer self-assembling
- Monolayer engineering technique

Thin films

Monomolecular based structures in which molecules are used as elementary building blocks to develop self-assembled films of predefined geometry and function.

Thin films techniques aim to control the order at the molecular level and thus should be considered potential techniques for the construction of nanostructures.

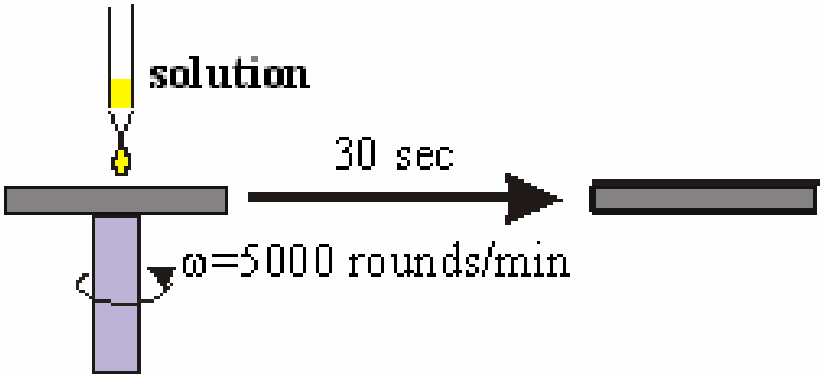
Thin film techniques

The methods for the assembly of thin films with various degrees of molecular order and stability include: spin coating and solution casting, polyion layer-by-layer self assembly, chemical self assembly and Langmuir-Blodgett technique.

The optimal combination of molecular order and stability of films determines the practical usefulness of these techiques.

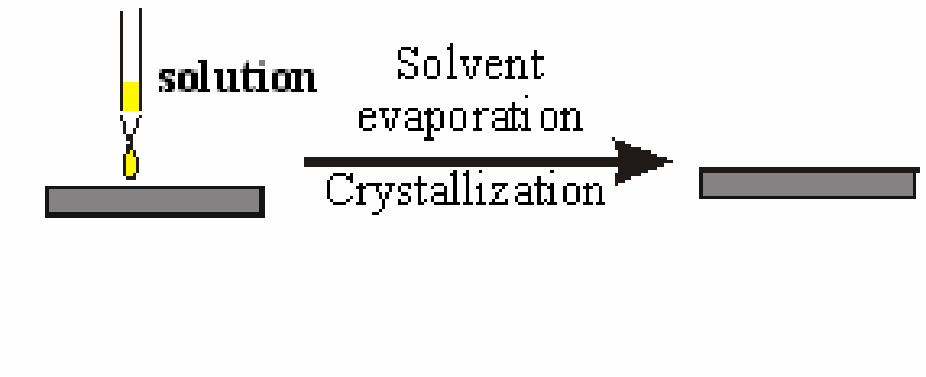
Spin coating

Spin coating is a method for application of thin, uniform films to flat substrates. An excess amount of polymer solution is placed on the substrate. The substrate is then rotated at high speed in order to spread the fluid by centrifugal force. Rotation is continued for some time, with fluid being spun off the edges of the substrate, until the desired film thickness is achieved. The solvent is usually volatile, providing for its simultaneous evaporation.



Solution Casting

It is based on the applying a drop of solution onto a planar surface and letting the solvent evaporate.



Langmuir-Blodgett technique

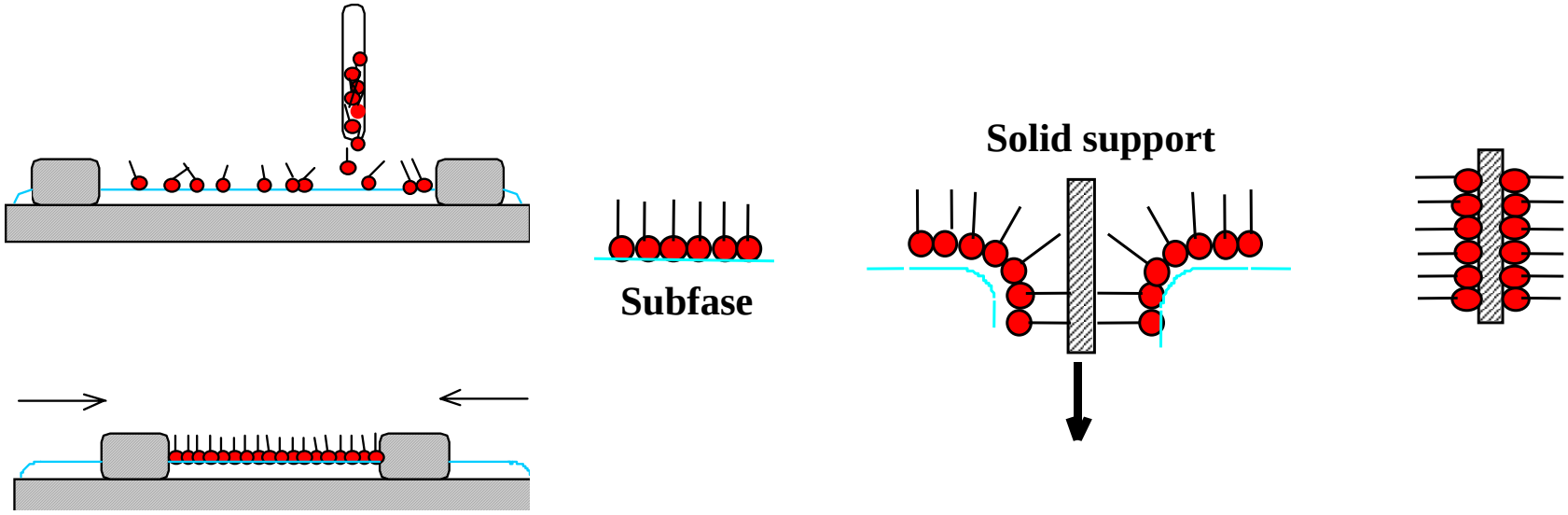
In 1920, Langmuir published his first work on the study of two dimensional systems of molecular films at the gas-liquid interface.

This technique enables to form highly ordered monomolecular amphiphilic films at the air-water interface and to subsequently transfer them onto the surface of a solid support.

Monolayer formation and deposition

A solution of molecules in an organic solvent is spread at the air-water interface, their hydrophilic heads groups will be immersed in the water while the hydrophobic chains will be exposed to air.

After the speading amphiphilc molecules are in the form of a monolayer, this means that the thickness of the layer is one molecule only. This monolayer can be compressed with movable barriers and then transferred to the surface of a solid support.



LB drawbacks

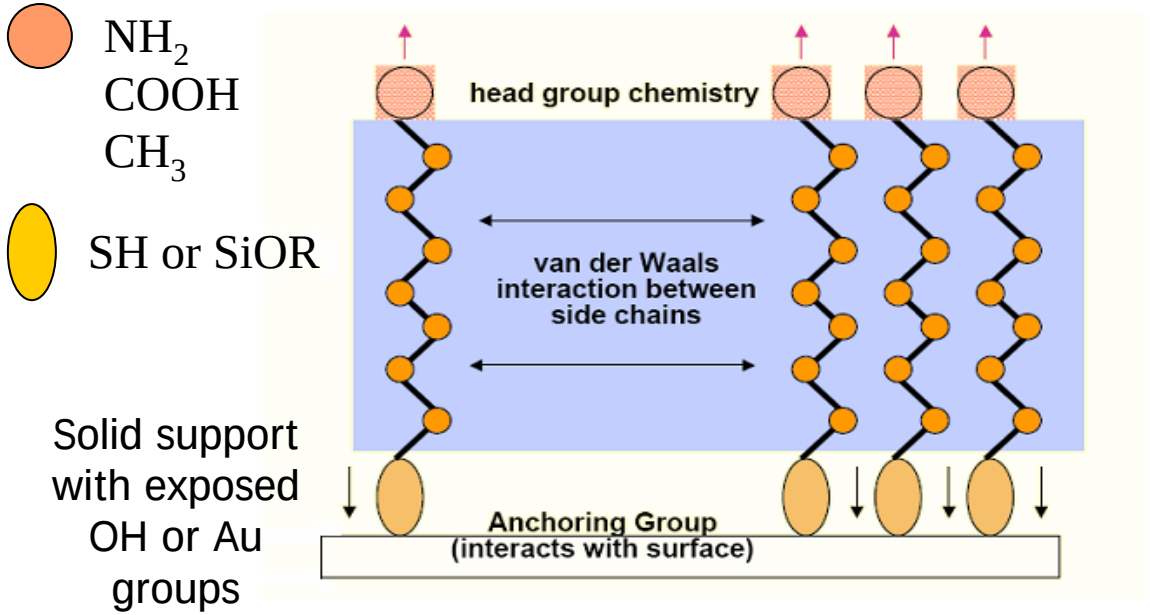
Every year hundreds of papers on Langmuir-Blodgett (LB) films appear.

However the technique does not have at the moment any industrial applications, because only small and flat substrates can be covered by LB-film

Chemical Self-Assembly

In 1980, a self assembled (SA) monolayer of octadecyltrichlorosilane was introduced as a possible alternative to the LB system.

This technique is based on the activation of silicon or gold surfaces by means of thiol or silane compounds.



SA drawbacks

This technique is limited to few substrates (silicon or gold) and the monolayer properties are affected by the substrate quality.

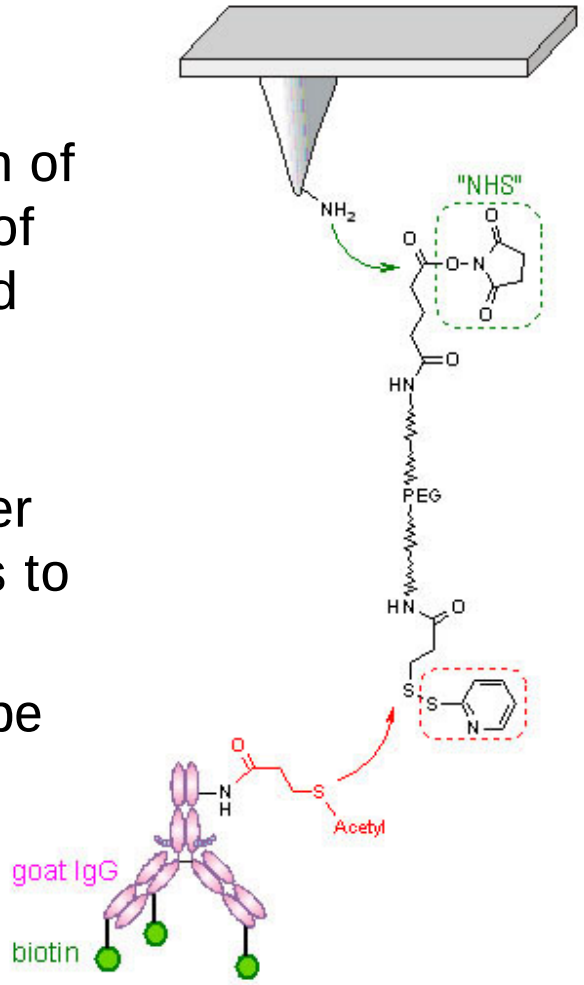
Moreover SA monolayers are unstable over time because of oxidation processes.

SA application to Atomic Force Microscopy

This technique has been used for the functionalization of silicon AFM tips for the subsequent immobilization of thiolated biomolecules to be used in receptor-ligand recognition mapping.

Specifically we have employed a NHS-PEG-PDP linker (pyridyl dithio-PEG succinimidylpropionate) which has to be reacted to amino-functionalised tips.

Thiolated biomolecules (containing SH groups) can be directly coupled to NHS-PEG-PDP modified tips.



Receptor – ligand recognition

Biotin/Avidin interactions have been investigated on mica using a PicoPlus system by Molecular Imaging.

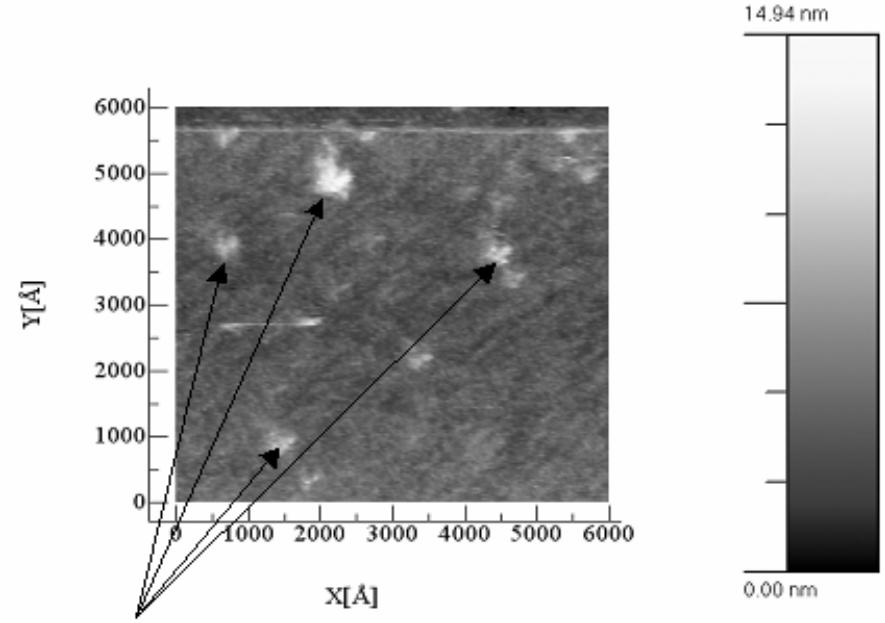
A monolayer of avidin has been electrostatically adsorbed to mica.

A simultaneous topography and recognition imaging technique named PicoTrecTM has been used to analyse the surface of mica treated with avidin using biotin modified AFM tips.

In order to assess the specificity of the binding events of biotin have been injected in the liquid cell while carrying out the AFM analysis.

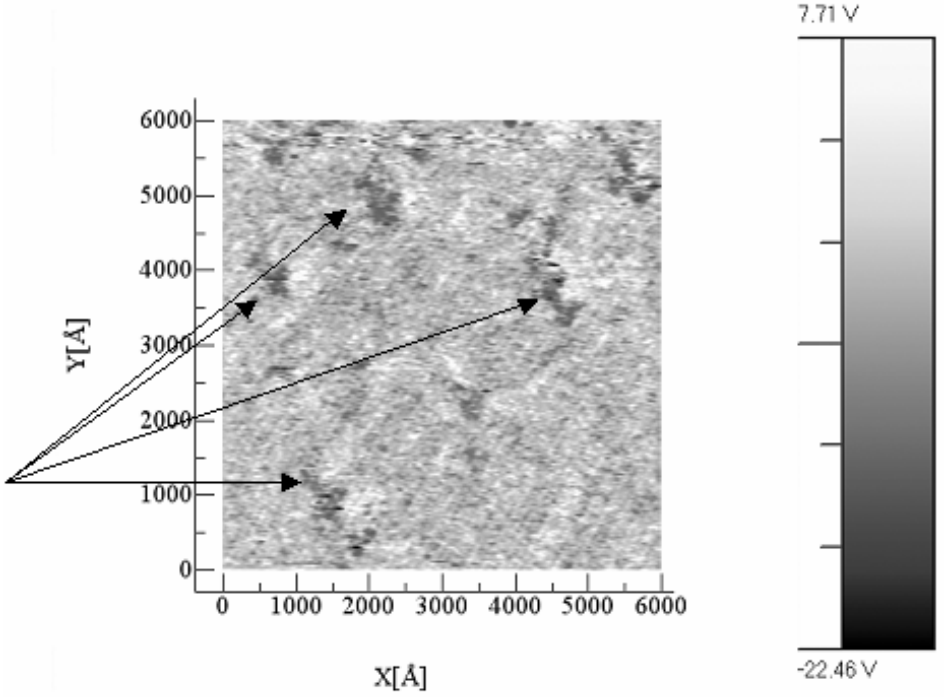
This work is supported by the EU project TASNANO (Tools and Technologies for the Analysis and Synthesis of Nanostructures – Contract Number: 516865)

Results I



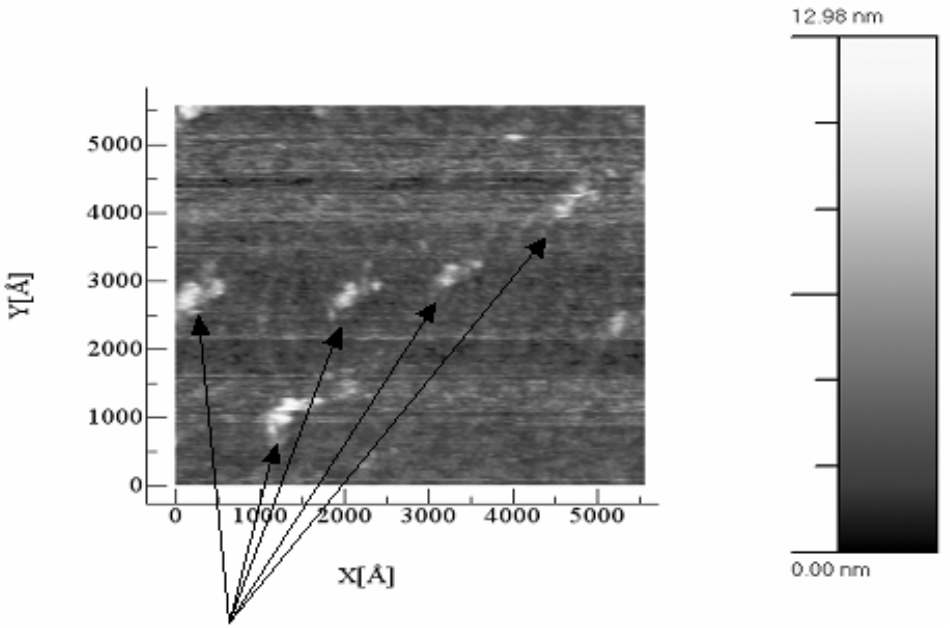
Topography image: avidin electrostatically adsorbed to mica

Recognition events appear as dark areas, and correlate with the position of avidin molecules in the topography image



Biotin / Avidin recognition events

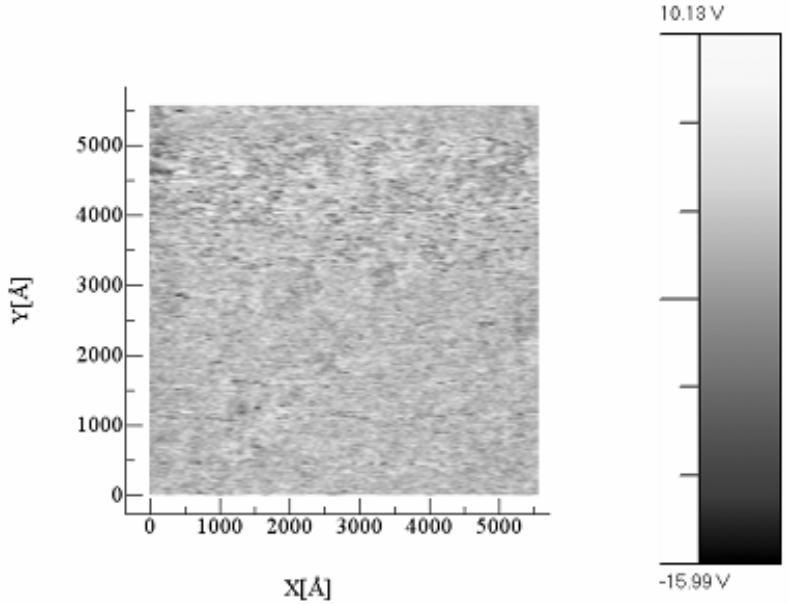
Results II



Topography image: avidin electrostatically adsorbed to mica

The recognition image disappears when avidin-biotin interactions are blocked by free biotin

Blocking of specific interactions with free biotin



SA monolayers have been found to be a suitable functionalization strategy for the modification of AFM tips to be used in the characterization of specific interactions.

Layer-by-Layer Self Assembly

The sequential adsorption of oppositely charged colloids was reported for the first time in a seminal paper in 1966 by Iler.

The electrostatic self-assembly was subsequently "rediscovered" in the nineties and extended to the preparation of more complicated multilayers.

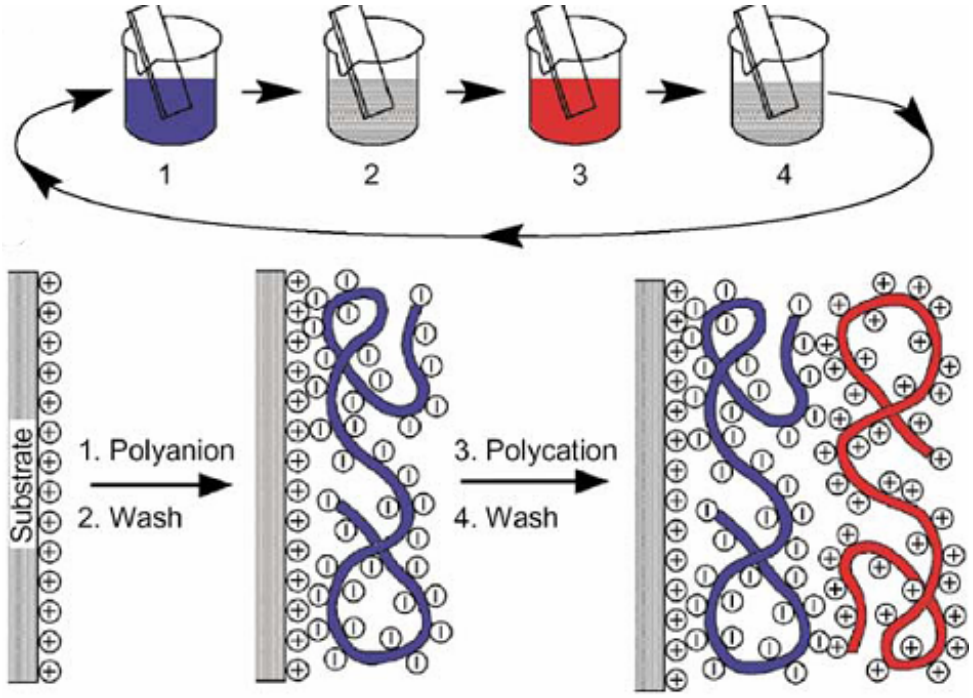
Layer-by-Layer Self Assembly

This technique makes use of the alternate adsorption of oppositely charged macromolecules to build up multilayered structures.

- Definite knowledge of their molecular composition.
- Predetermined thickness ranging from 5 to 1000 nm.
- Precision 1 nm.
- Insoluble in buffer solutions.

These films have a lower molecular order than LB or SA films but they have the advantage of high strength, easy preparation and possibility to be deposited on supports of any shape and dimension.

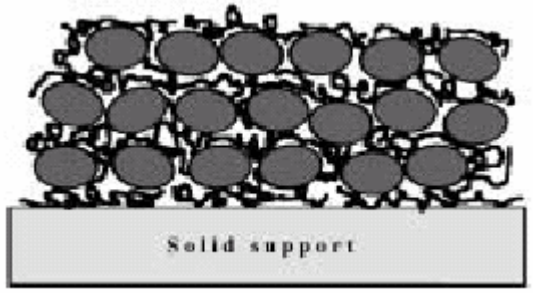
Assembly procedure



A positive solid support, is immersed into a solution of an anionic polyelectrolyte for the adsorption of a monolayer, and then it is rinsed. Then the support is immersed into a solution of a cationic polyelectrolyte for the adsorption of a monolayer, then it is rinsed.

Layer constituents

- Synthetic polyelectrolytes
- Inorganic nanoparticles
- Lipids
- Ceramics
- <u>Biomolecules</u>



Schematic rappresentation of the protein-polyion multilayer

Protein/polyion multilayers

- Complex biofunctional architectures
- Enhanced functional stability

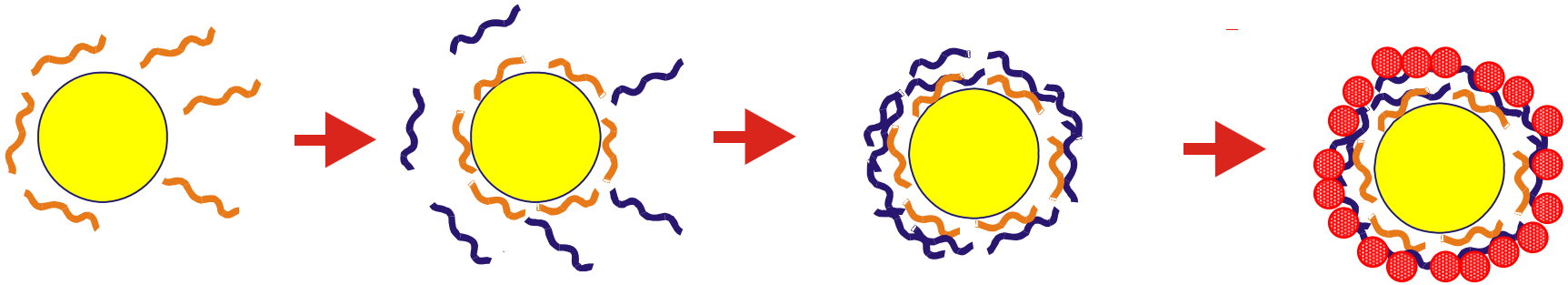
Kinetics of polyion adsorption

For the time-dependent control of adsorption and monitoring of the assembly in situ, the quartz crystal microbalance method (QCM) is used as illustrated by Dr Viitala.

The kinetics of the adsorption process could be delineated by the QCM-technique, which is indispensable for establishing proper assembly conditions (e.g., a saturation adsorption time).

Bionanoparticles: shell assembly on micro/nanocores

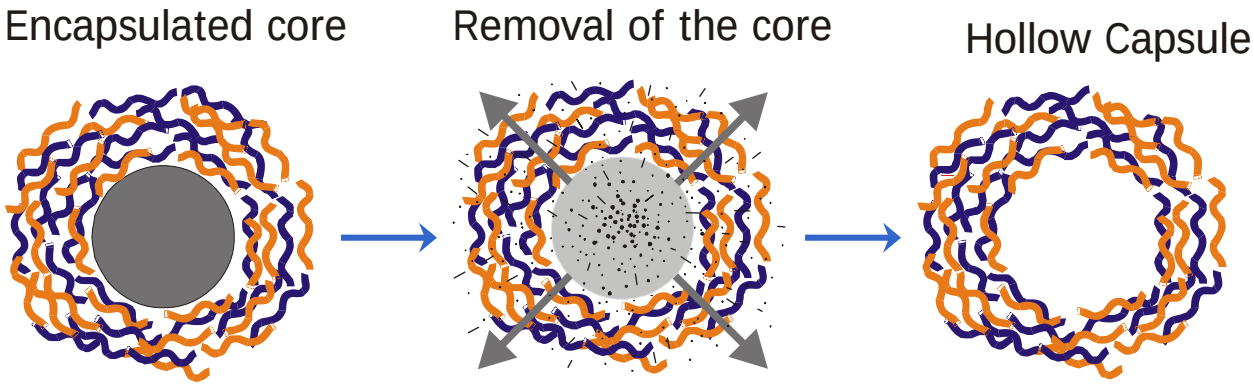
The assembly process for a solid support may be extended to an assembly on porous carriers (e.g. membranes, porous beads and fibers) or on the surface of charged micro-or nanocores.



Protein shell assembly on a latex sphere (d = 20-500nm) for the creation of complex catalytic colloids.

Hollow nanocapsules

Preparation

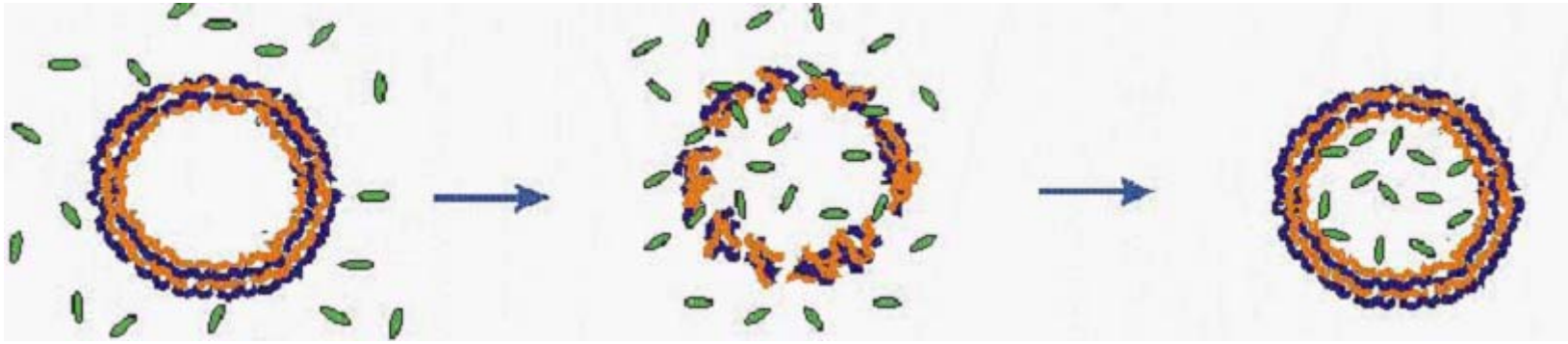


Core: e.g. melamine formamide particles d=100nm

Controlled encapsulation of macromolecules

Nanocapsules may potentially be loaded via diffusion, where nanocapsules are suspended in a solution with molecules of interest and concentration gradients drive movement of molecules to the interior of capsules. In addition to loading by diffusion, two additional methods will be used as possibilities to control permeability of nanocapsule walls after formation. These include changing the pH and dielectric constant of the solvent.

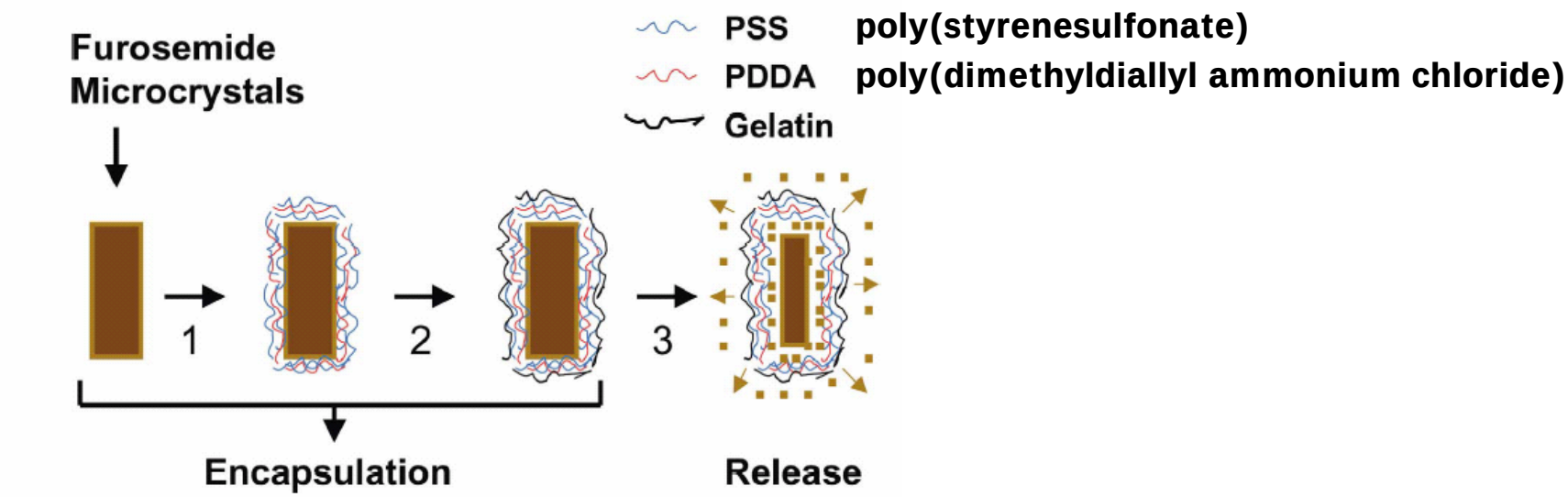
Loading



in water closed ethanol/water 1:1, open in water encapsulated

Encapsulation of drug microcrystals for sustained release purposes

In step 1, precursor layers of (PSS/PDDA) are assembled onto positively charged furosemide microcrystals. In step 2, (PSS/ gelatin) layers are added. The assembly is done at pH of 4 because the low solubility of furosemide at this pH ensures that the microcrystal shapes and sizes are not altered. In step 3, drug release in aqueous solution at pH7.4 (blood) is monitored



Ai H. et al, Journal of Controlled Release, vol. 86, pp. 59 – 68, 2003

Biocompatible LbL films for tissue engineering

Cells are sensitive both to the micro/nano-topographic and chemical features of their surrounding environment

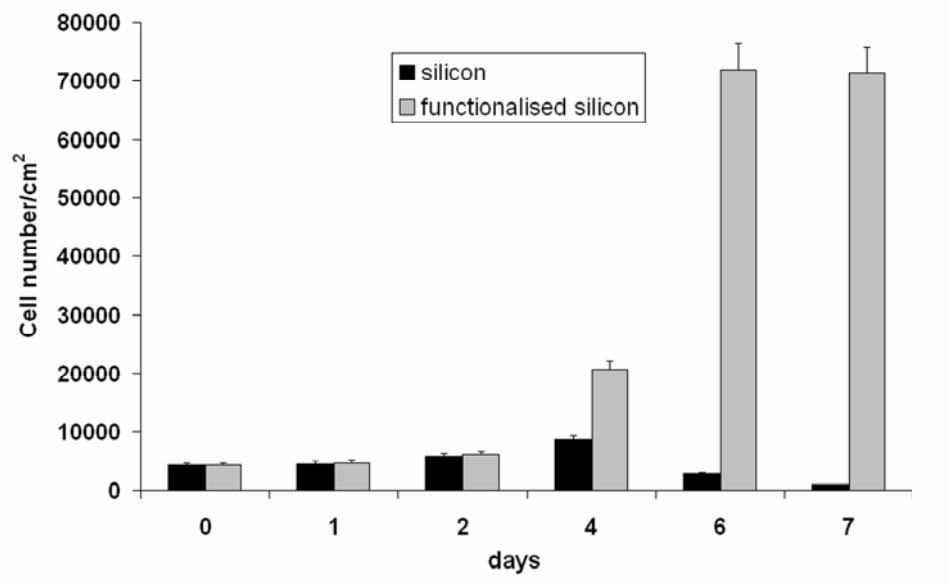
The engineering of surface properties of biomaterials is critical to develop bioactive devices with which to elicit appropriate cellular responses.

An example:
we have developed biocompatible films for bone tissue engineering

Bone tissue engineering

- Biomimetic coatings containing fibronectin (FN), an adhesive glycoprotein of the extracellular matrix, have been assembled by means of the LbL technique.
- Three precursor bilayers of cationic poly(dimethyldiallyl ammonium chloride) (PDDA) and anionic poly(styrenesulfonate) (PSS) were deposited to impart an homogeneous and well-defined charge to the substrates.
- Subsequently cationic poly-L-lysine (PLL) was employed in alternation with FN.
- To evaluate the biocompatibility of the developed coatings MG63 human osteoblast-like cells were used.
- Cells were seeded onto functionalised and non functionalised silicon and Nichel/Titanium (NiTi) surfaces.
- Cell dispersion and morphology was evaluated on the surface of treated and non treated glass coverslips by phase contrast microscopy.

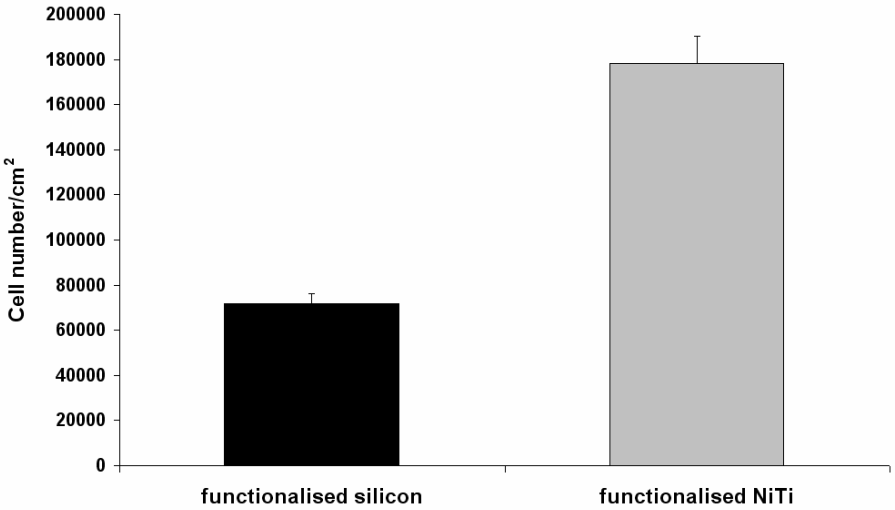
Results I



Amount of adherent cells on silicon over time

After 6 days of growth, a better response in terms of cell attachment and proliferation was detected on the surface of the functionalised NiTi substrates.

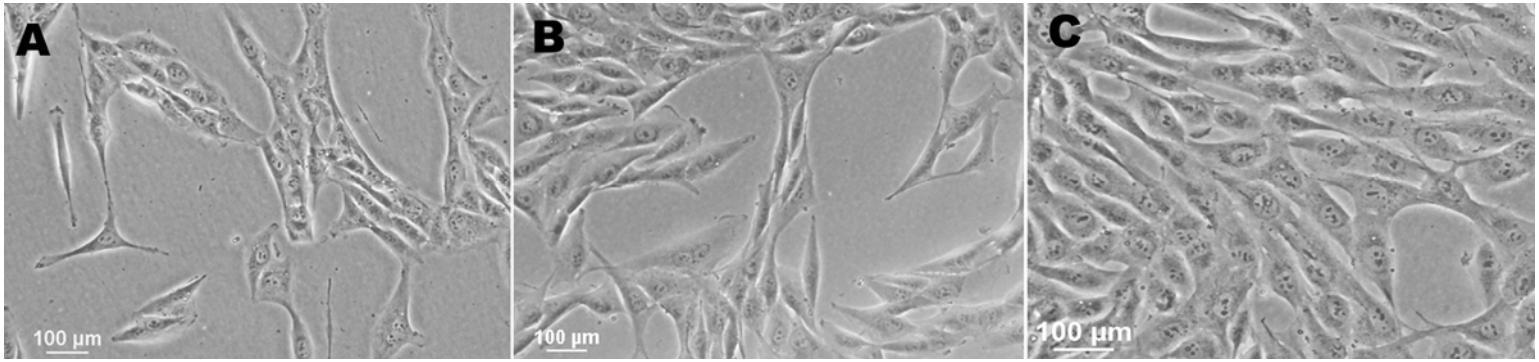
The amount of adherent cells estimated over time resulted to be higher on the functionalized materials than on the surface of their negative controls.



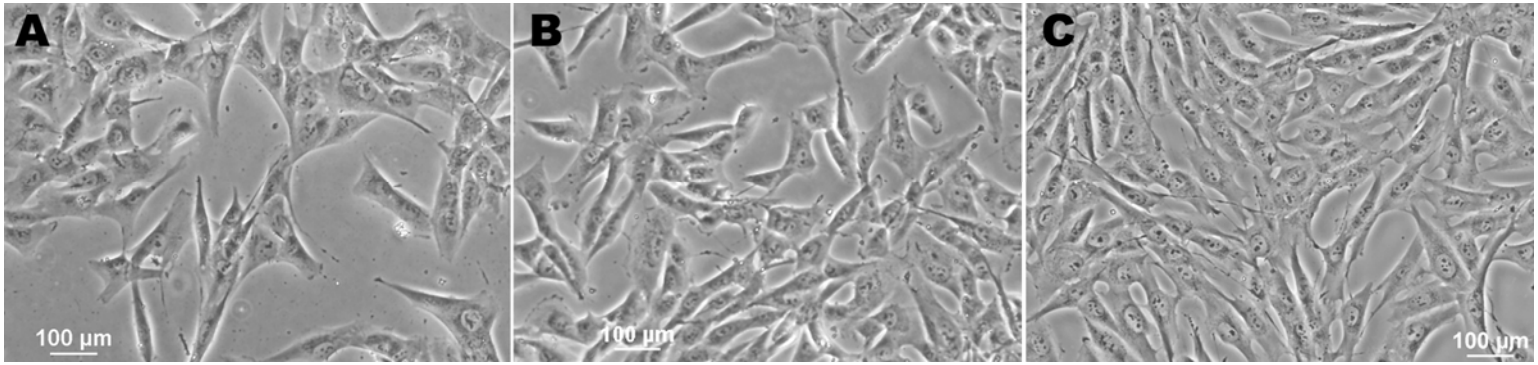
Amount of adherent cells estimated after 6 day on silicon and NiTi

Results II

Dispersion and morphology of cells cultured onto biomimetic coatings terminating with FN were very similar to those detected on the surface of standard tissue culture plates



Phase-contrast photomicrographs of MG-63 osteoblast-like cells seeded on standard tissue culture polystyrene multiwell plates after (A) 2 days, (B) 3 days and (C) 4 days of culturing.



Phase-contrast photomicrographs of MG-63 osteoblast-like cells seeded on glass coverslips functionalised with a FN-terminating film after (A) 2 days, (B) 3 days and (C) 4 days of culturing.

- The developed coatings resulted to be effective in improving cell response both on silicon and NiTi.
- These experimental evidences demonstrates the high potential of the assembled ultrathin films for the optimization of the surface properties of bone implants.

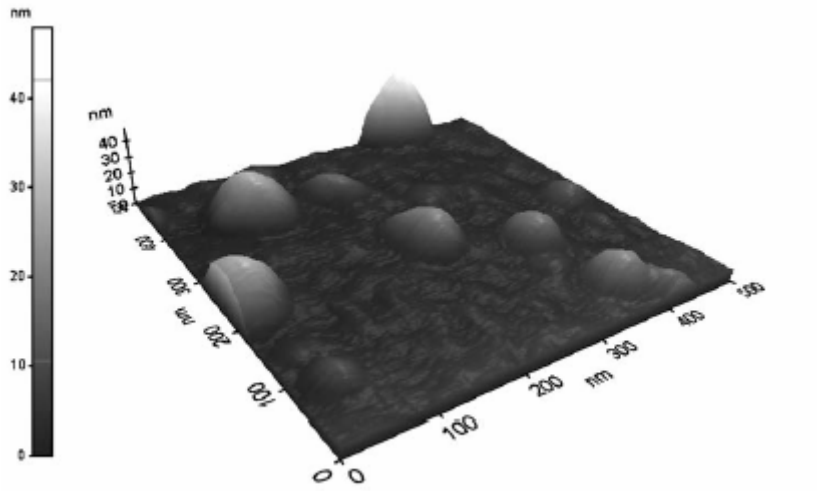
LBL films for biosensors development

Multilayer containing functional proteins can be assembled directly onto the surface of a trasducer for the setting up of a biosensor

Piezoelectric immunosensor for taxane monitoring

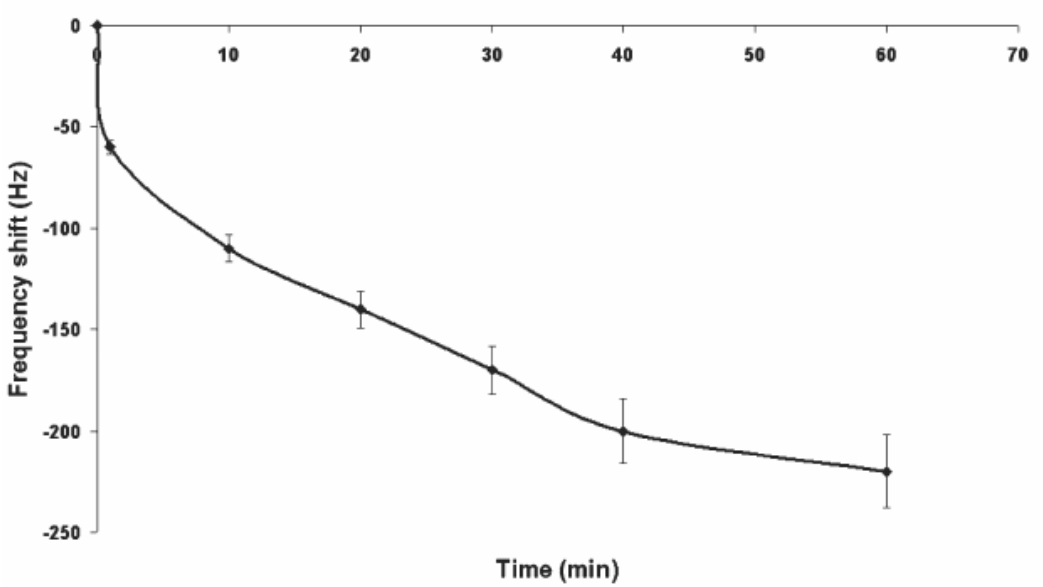
An antibody specific to the drug taxane has been immobilized be the LbL technique onto the surface of a piezoelectric quartz crystal.

Three precursor layers of cationic poly(dimethyldiallyl ammonium chloride) (PDDA) and anionic poly(styrenesulfonate) (PSS) were deposited to impart an homogeneous and well-defined charge to the substrates.



AFM image of the structure (PDDA/PSS)3/Antibody

Taxane monitoring by quartz crystal microbalance



QCM frequency–time profiles for the interaction of taxane with immobilized antibody t-taxane.

The immunological activity and specificity of the immobilized antibody against taxane was investigated.

The results obtained suggest that the functional characteristics of the immobilized antibody were preserved and permitted the evaluation of non specific interactions.

Our results show the feasibility of developing a piezoelectric immunosensor for paclitaxel detection.

Conclusions

Thin film techniques provide a simple method for the development of complex nanostructures using nanogram amount of material, giving a high level of control of the process, creating a biomimetic environment, thereby helping to stabilize biomolecules.