# MME 4506 Biomaterials

Modification of biomaterial surfaces

# Surface Modification Possibilities

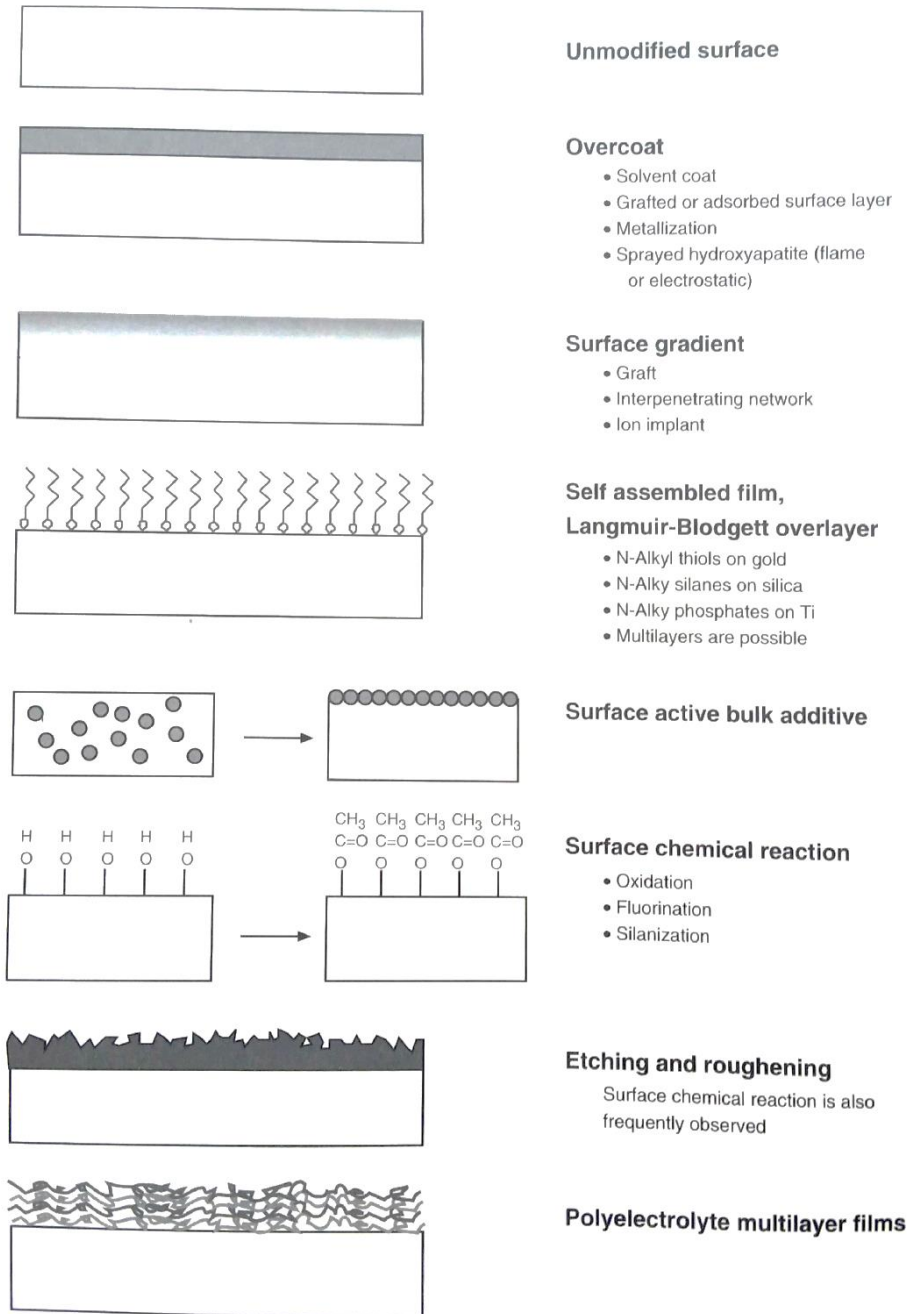
Surfaces of biomaterials are modified in order to influence the biointeraction while the key physical properties of the material are retained.

Surface modifications alter surface properties:

- Hydrophobicity
- Charge
- Topography

The three categories of surface modification are

1. Chemically or physically altering the atoms, compounds or molecules in the existing surface by chemical reactions, etching, mechanical roughening
2. Overcoating the existing surface with a material having a different composition by coating, grafting, thin film deposition.
3. Immobilizing biomolecules that assist in immunomodulation



The modified zone at the surface of the material should be as thin as possible. Modified surface layers that are too thick can change the mechanical and functional properties of the material.

Thick coatings are also more subject to delamination and cracking.

Ideally alteration of only the outermost molecular layer (0.3-1 nm) should be sufficient.
However thicker films than this are necessary in practice since it is difficult to ensure that the original surface is uniformly covered .

Extremely thin layers may be subject to surface reversal and mechanical erosion.

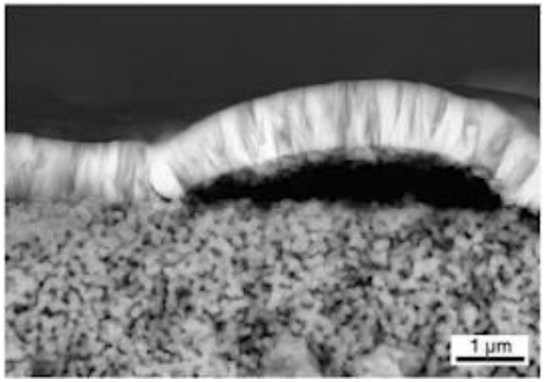
Some coatings like PEG protein resistant layers require a minimum thickness to function that is related to the molecular weight of chains.

In general minimum thickness needed for uniformity, durability, and functionality is determined experimentally for each system.

The surface-modified layer should be resistant to delamination and cracking.

This is achieved by

- covalently bonding the modified region to the substrate
- intermixing the components of the substrate and the surface film at an interphase zone like an interpenetrating network
- applying a compatibilizing layer at the interface
- incorporating appropriate functional groups for strong intermolecular adhesion between a substrate and an overlayer



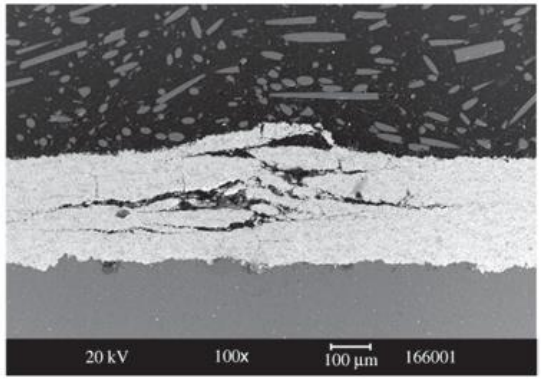




**Figure 1.** SEM cross-section images for the coating built up with 30 torch passes before immersion in aerated and unstirred 3.5% NaCl solution.

Surface rearrangement can occur in metallic and organic materials by the driving force for minimization of interfacial energy.

Sufficient atomic or molecular mobility must exist for the surface changes to occur in observable periods of time.

Surface chemistries and structures can switch because of diffusion or translation of surface atoms or molecules in response to the external environment.

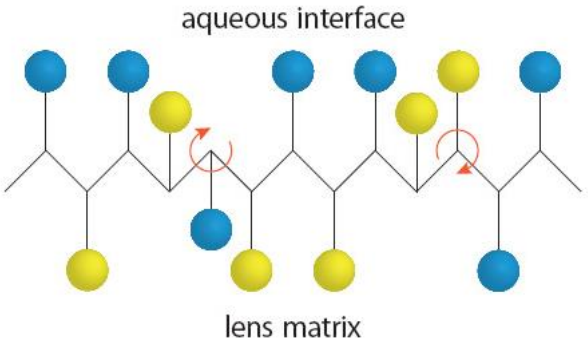



**Figure 4. A moist environment attracts hydrophilic groups.**

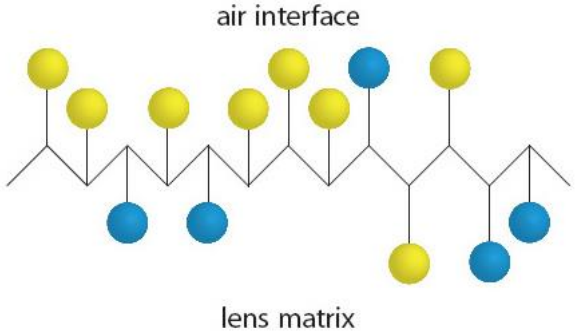
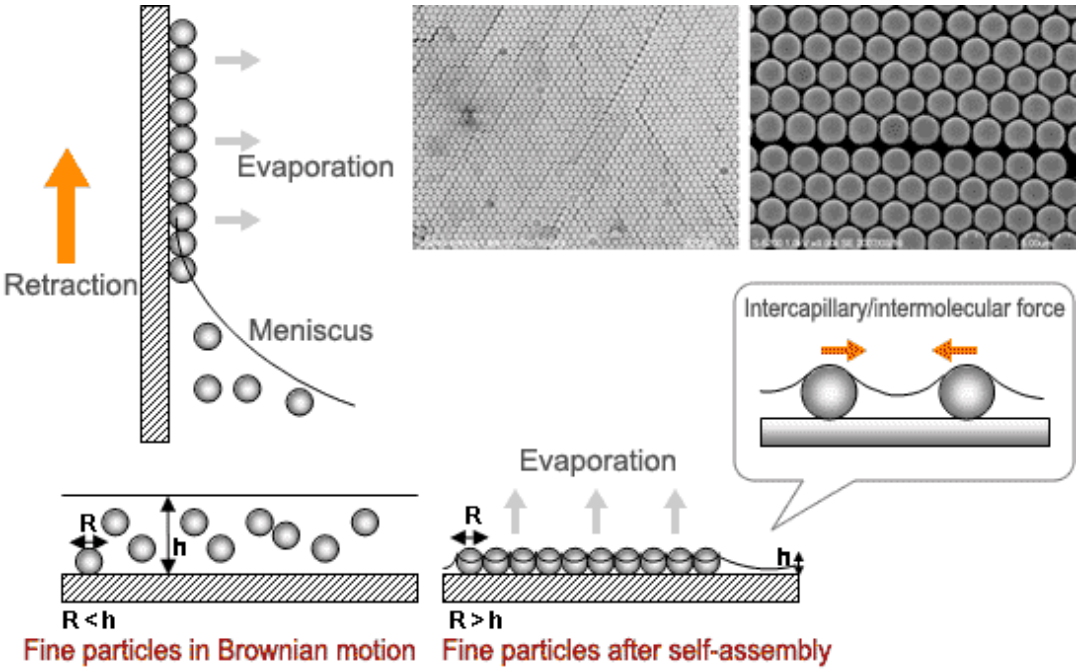



**Figure 5. A dry environment stabilizes hydrophobic groups, causing them to predominate on the lens surface.** FIGURES

A newly formed surface chemistry can migrate from the surface into the bulk or molecules from the bulk can diffuse to cover the surface.

Surface reversal must be prevented or inhibited by cross-linking, sterically blocking the ability of surface structures to move, incorporating a rigid, impermeable layer between the bulk material and surface modification.

Common modification techniques
Noncovalent coatings
Solvent coating



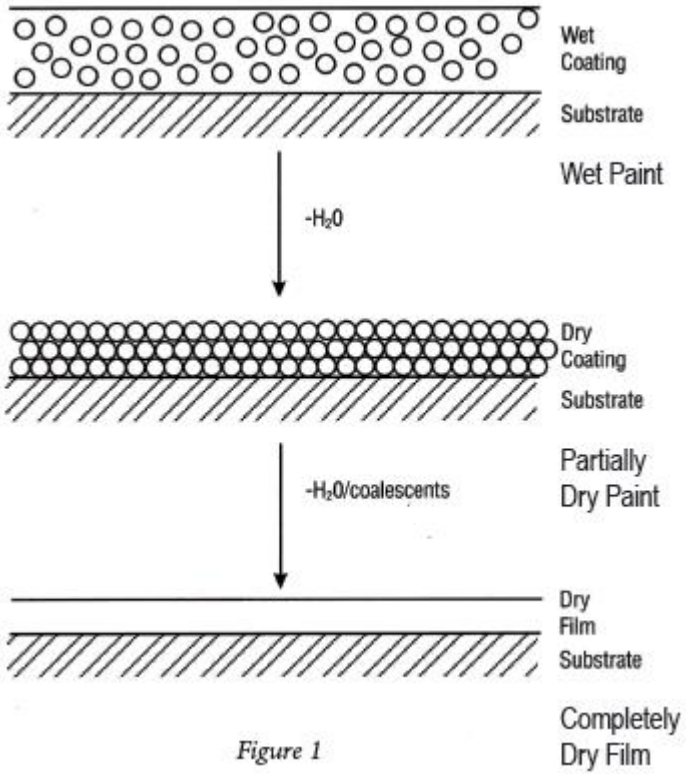
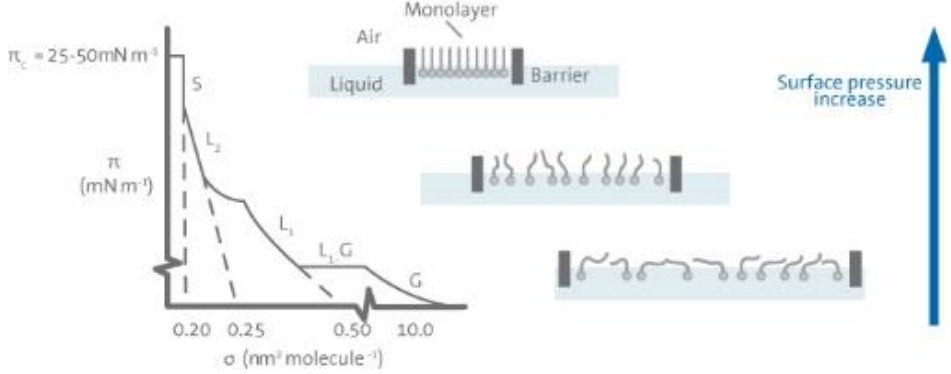
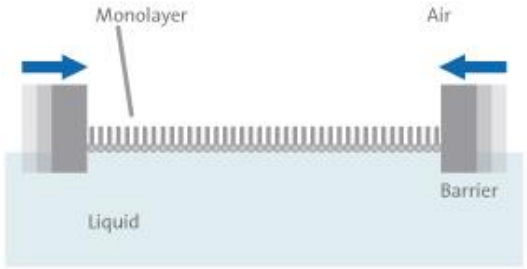




*Figure 1*

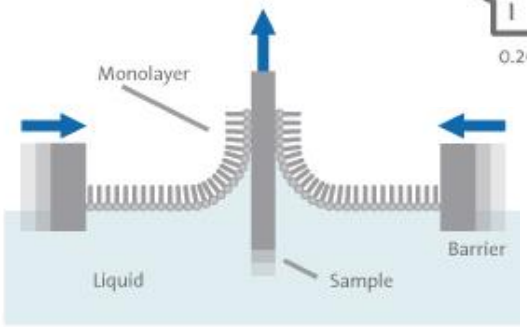
Noncovalent coatings
Langmuir-Blodgett films




LANGMUIR FILM

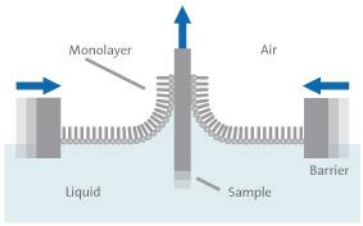



LANGMUIR-BLODGETT DEPOSITION

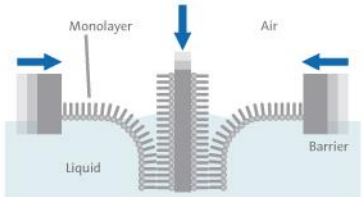



MULTIPLE DEPOSITIONS

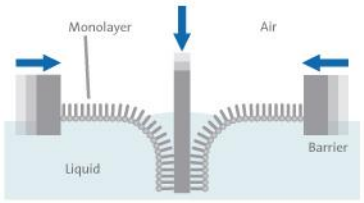
LB DEPOSITION ON A HYDROPHILIC SURFACE



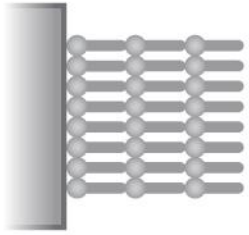

LB DEPOSITION ON A HYDROPHILIC SURFACE - 2ND LAYER



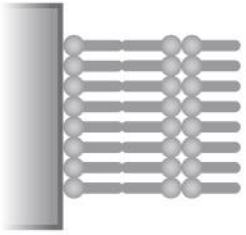

LB DEPOSITION ON A HYDROPHOBIC SURFACE



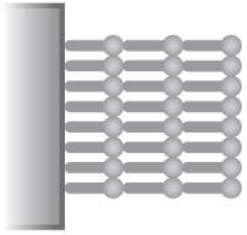

Z-TYPE ON A HYDROPHOBIC SURFACE



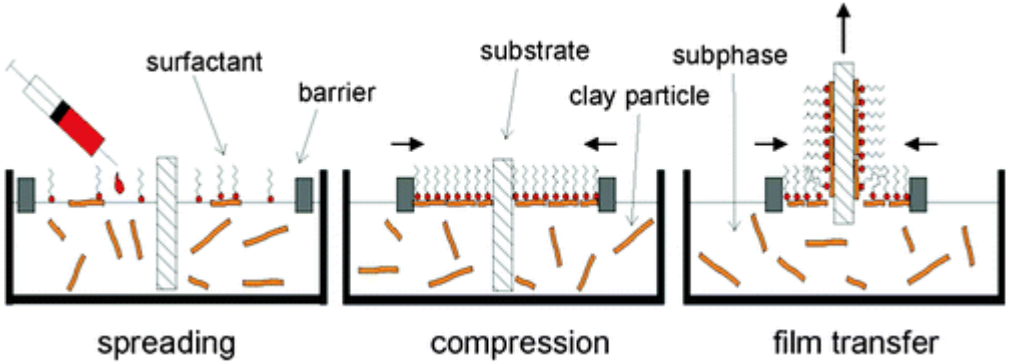
Y-TYPE ON A HYDROPHOBIC SURFACE



X-TYPE ON A HYDROPHOBIC SURFACE



Noncovalent coatings
Langmuir-Blodgett films



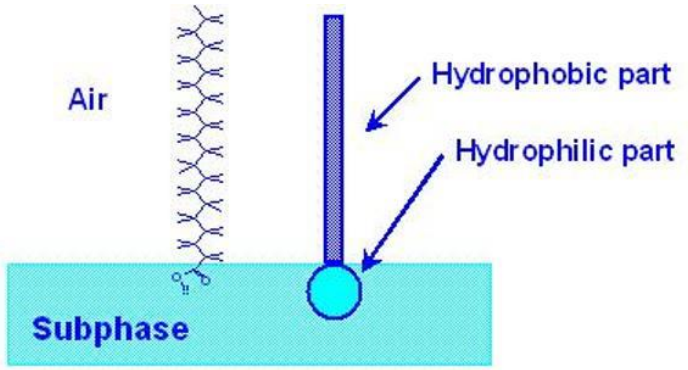




Figure 1 Amphipathic molecule (arachidic acid).

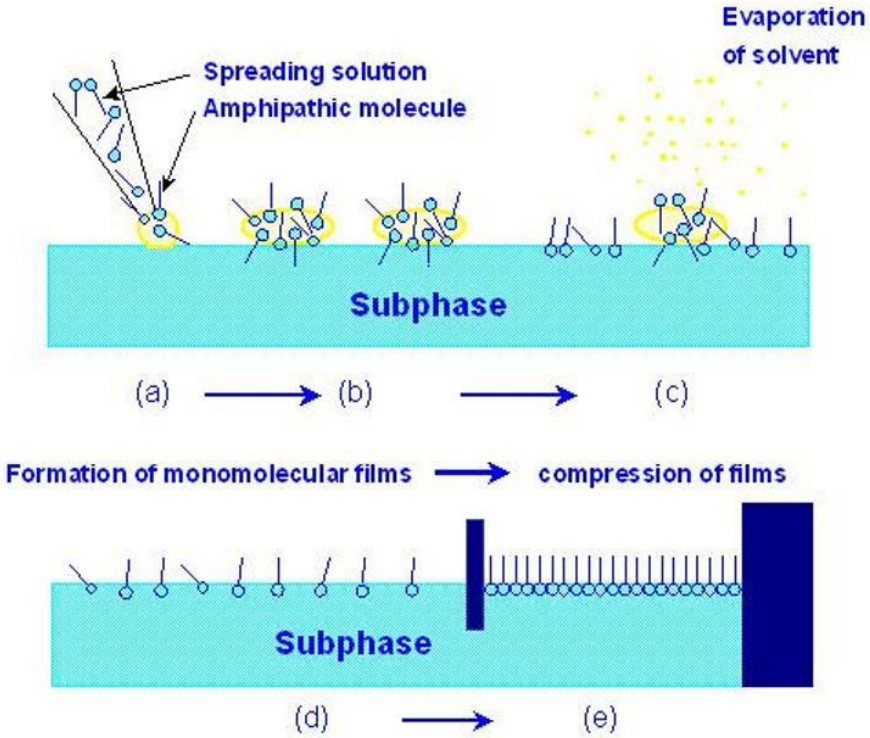
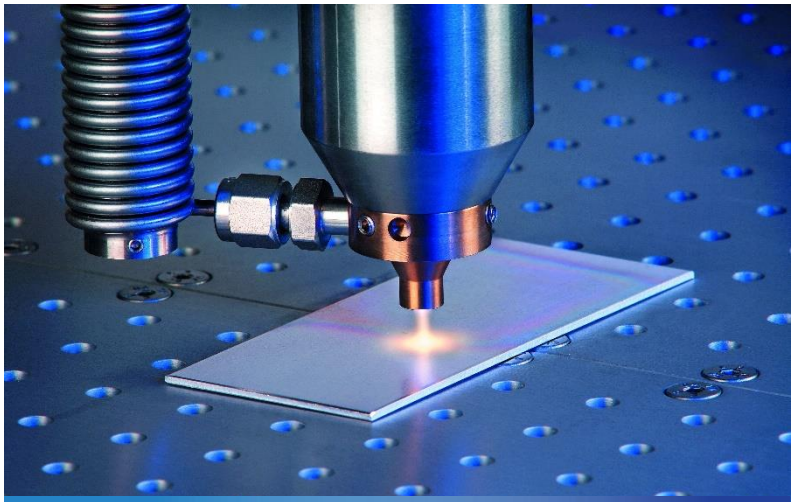
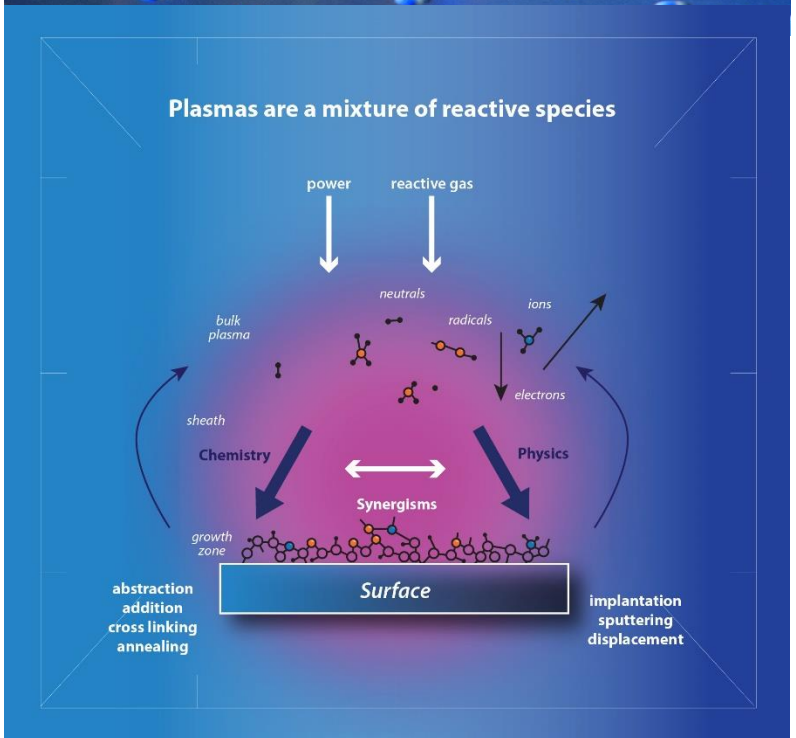
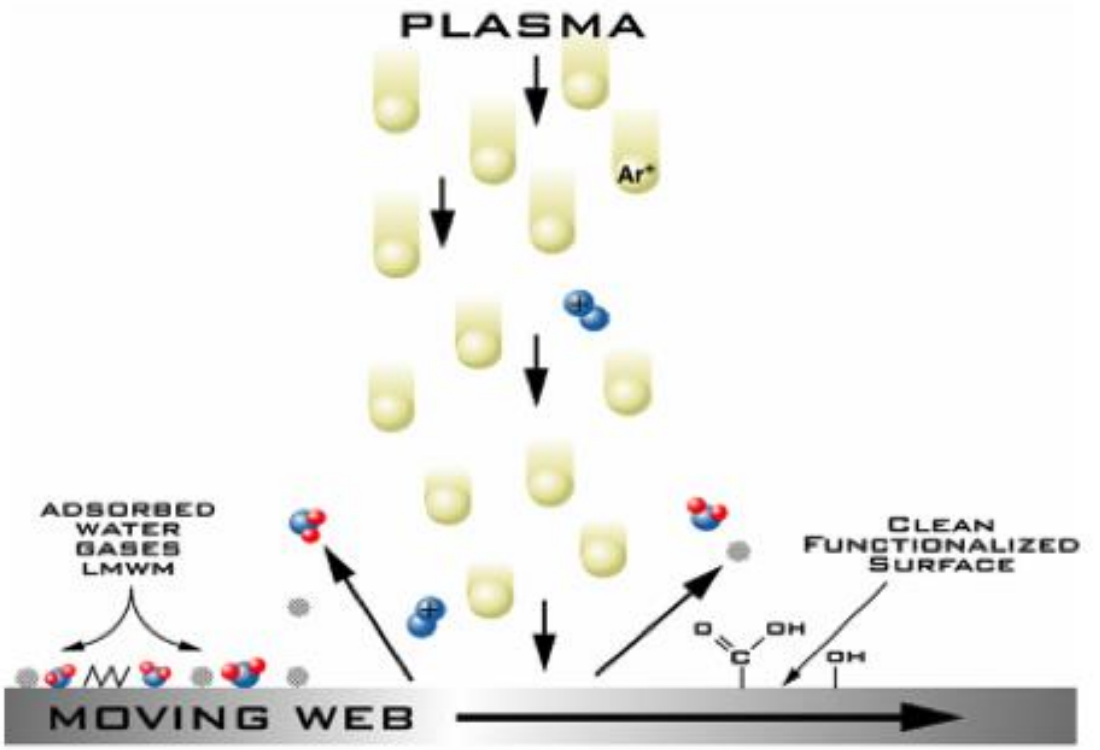


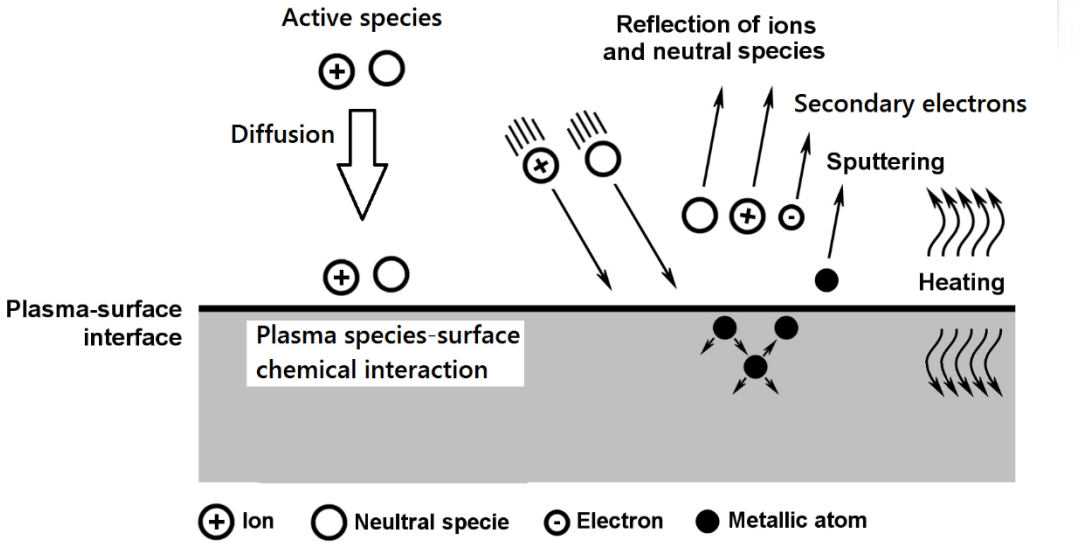

Figure 2 Formation of monomolecular films.

Covalent coatings
Plasma treatment

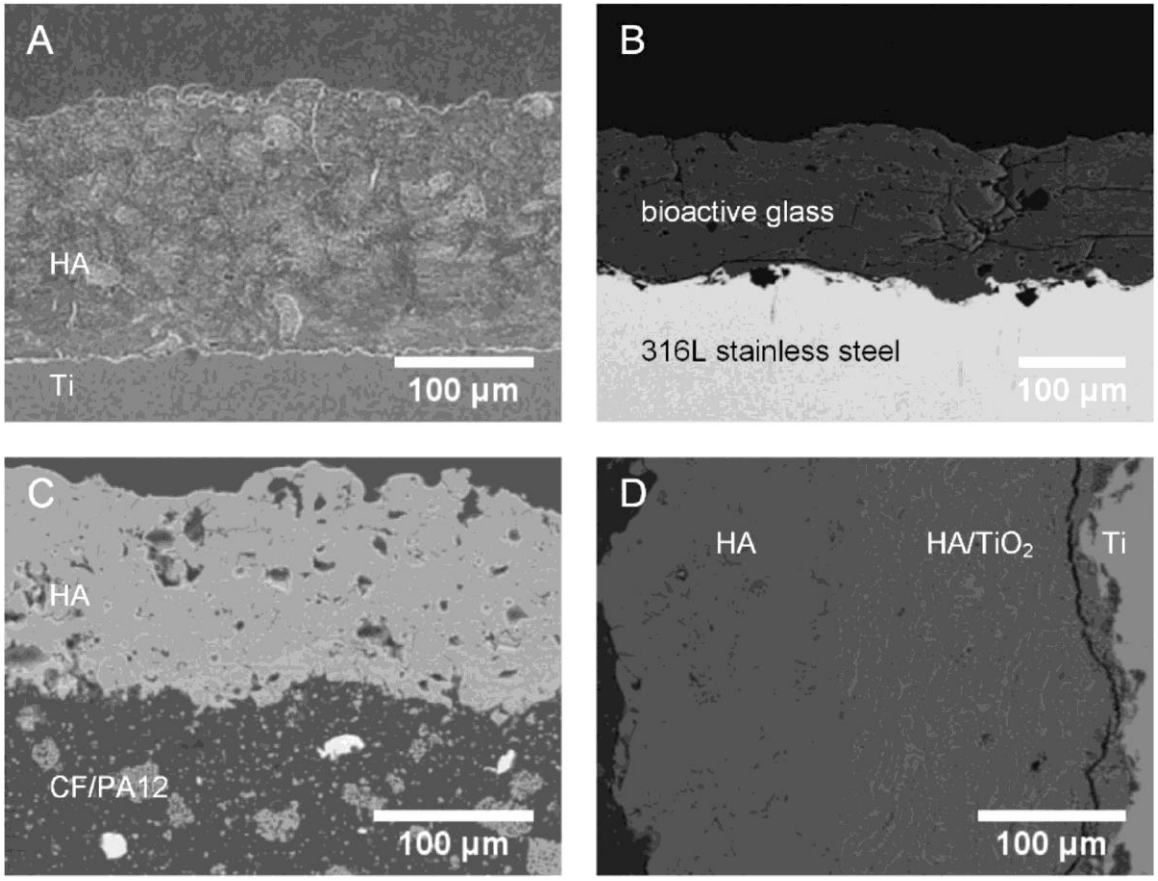




Covalent coatings
Plasma spray

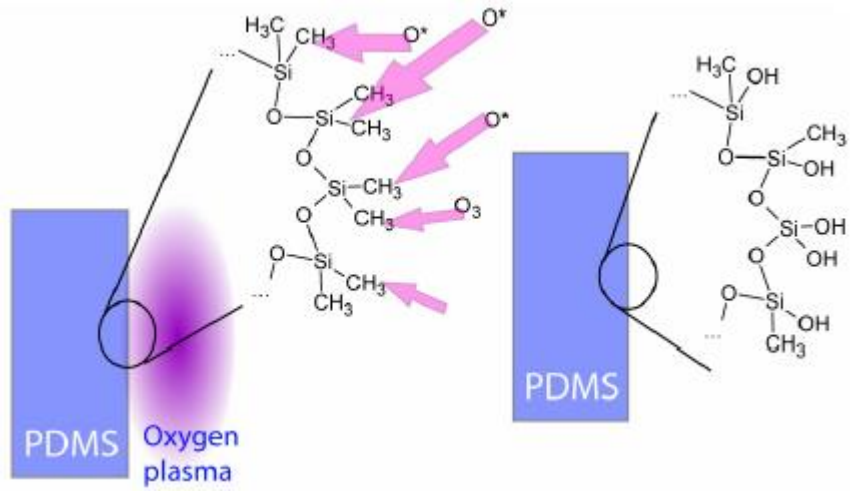


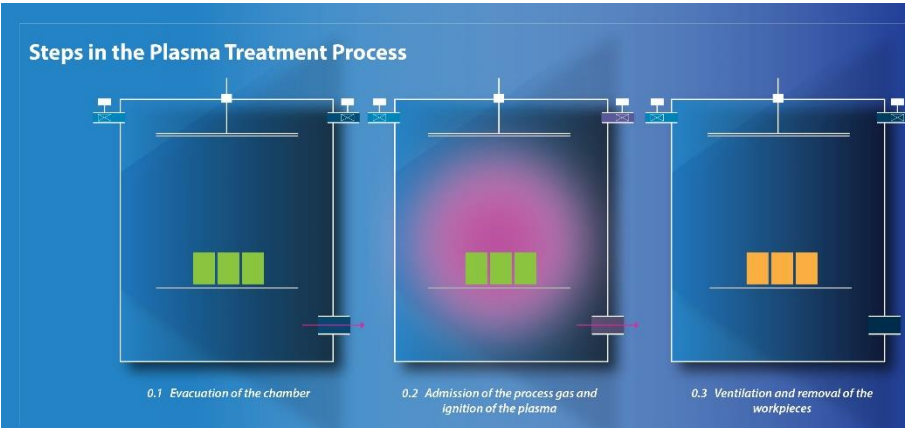
Covalent coatings

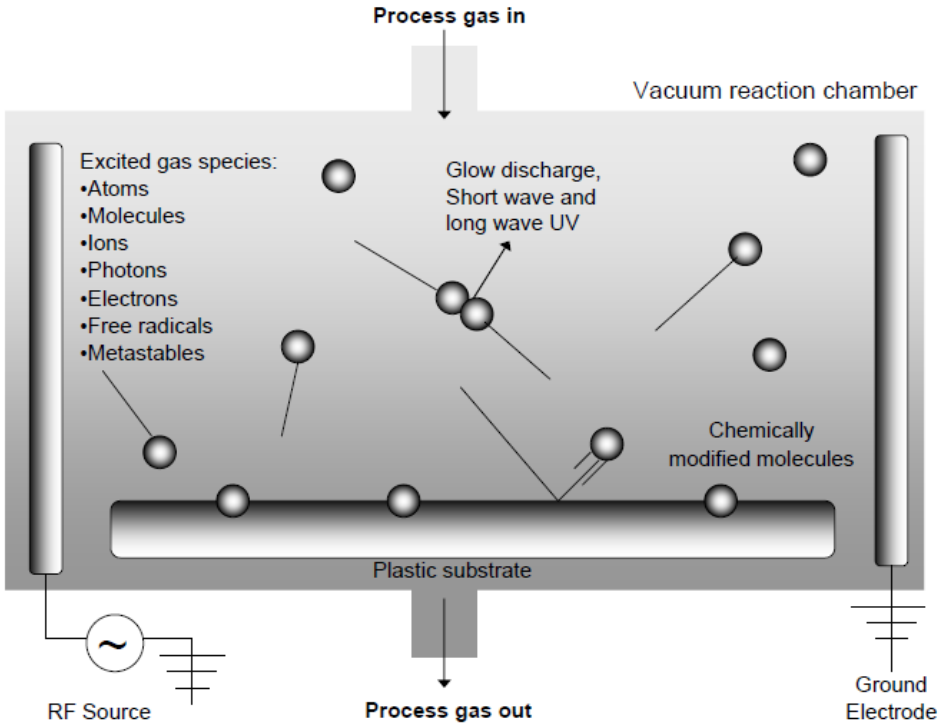
Plasma treatment in chamber

Plasma polymerization is a process that ionizes monomer gas into plasma state and induces radical polymerization to create polymer coating on a substrate, so as to enhance corrosion resistance of metallic biomaterials or improve biocompatibility and bioactivity of relatively inert materials

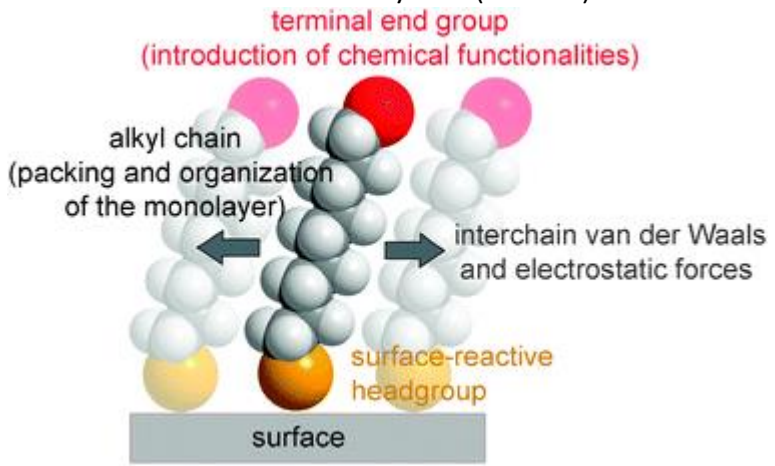
Oxygen plasma is useful to create –OH groups and clean organic contamination

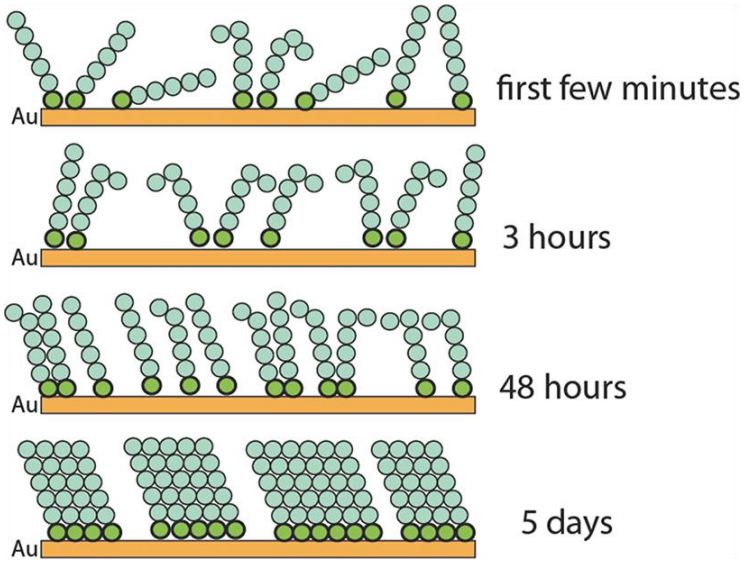


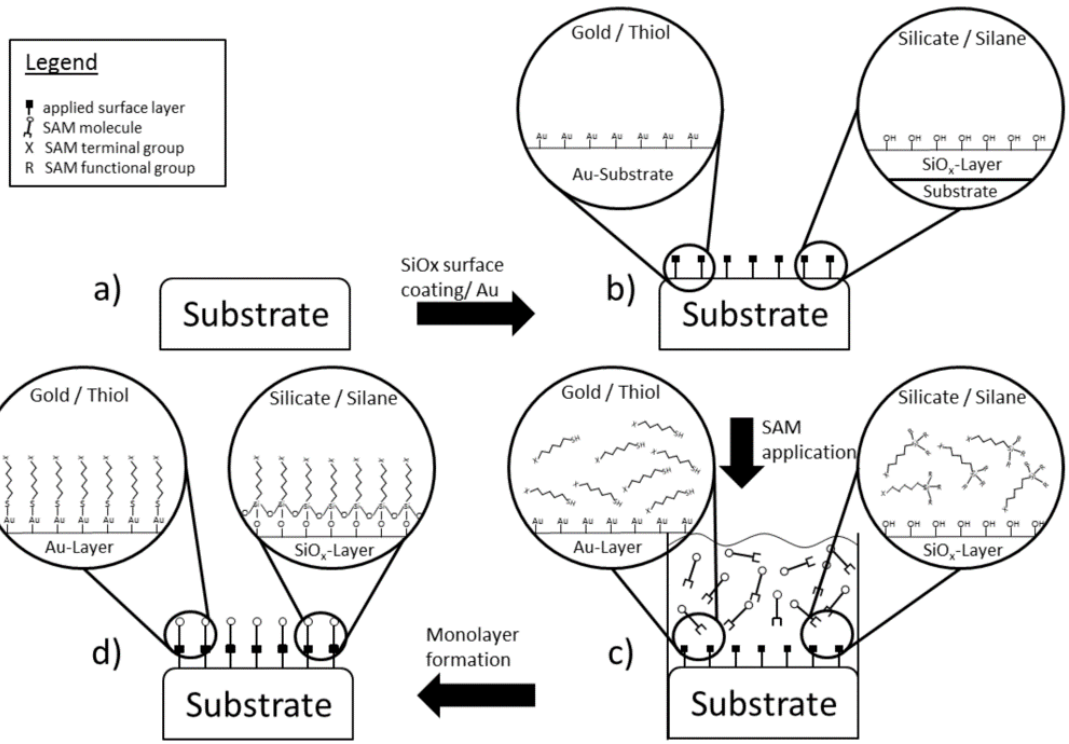



Covalent coatings
Self-assembled monolayers (SAMs)



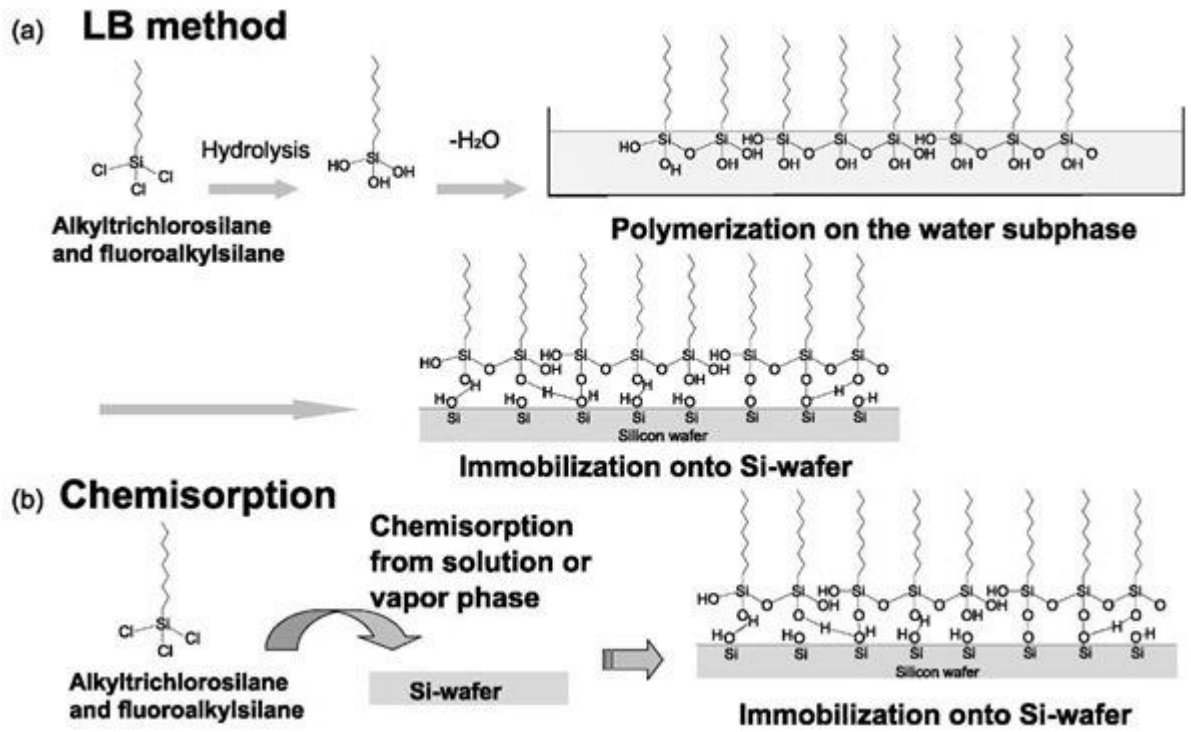



Covalent coatings

Self-assembled monolayers (SAMs)

SAMs are basically covalently bonded Langmuir films that are produced without the need for a subphase



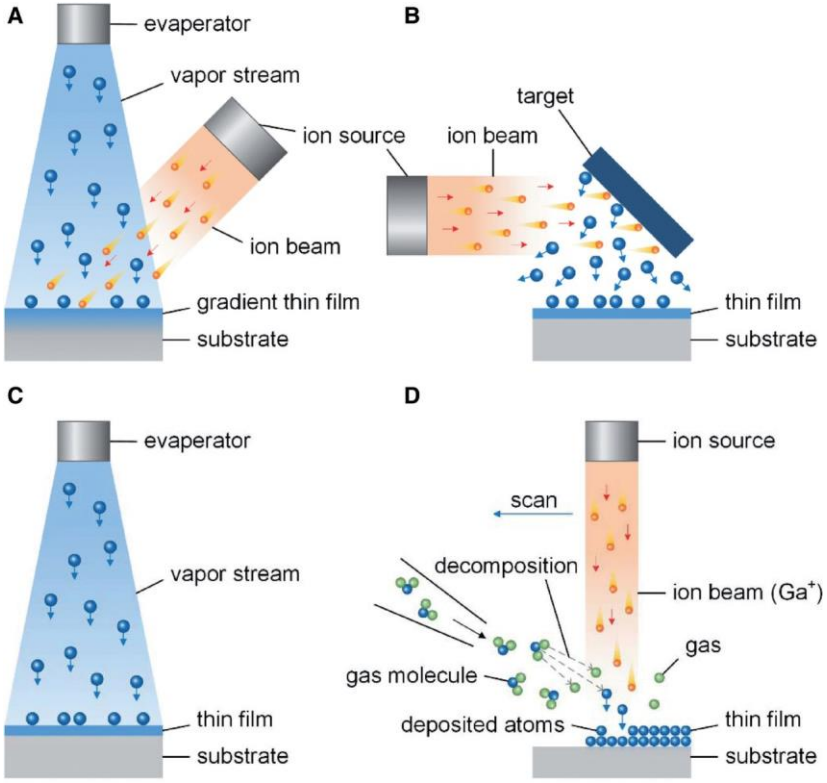
Covalent coatings

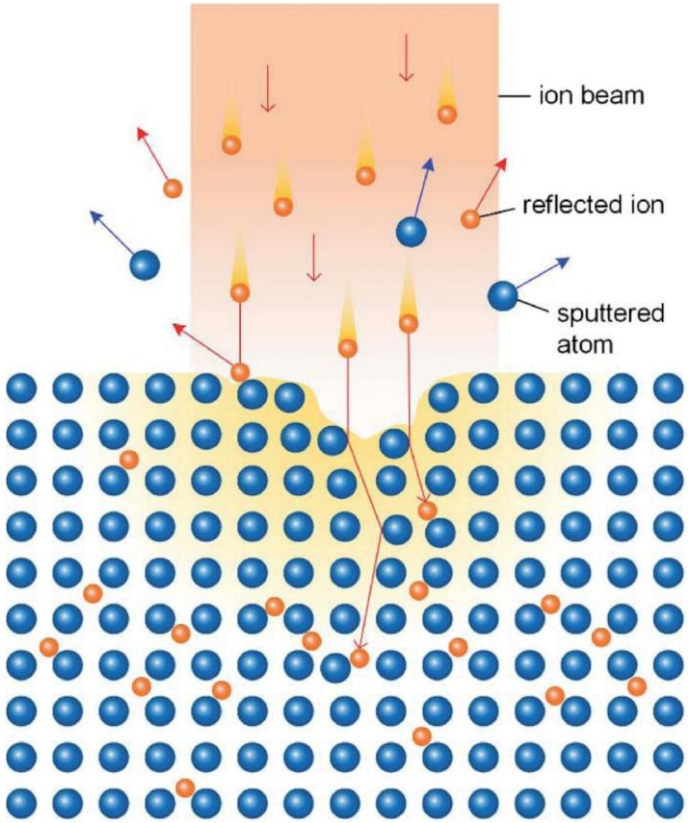
Ion beam assisted deposition (direct)

Ion implantation is a physical surface modification process that injects accelerated high-energy ions into the surface of a material to modify its physicochemical and biological properties

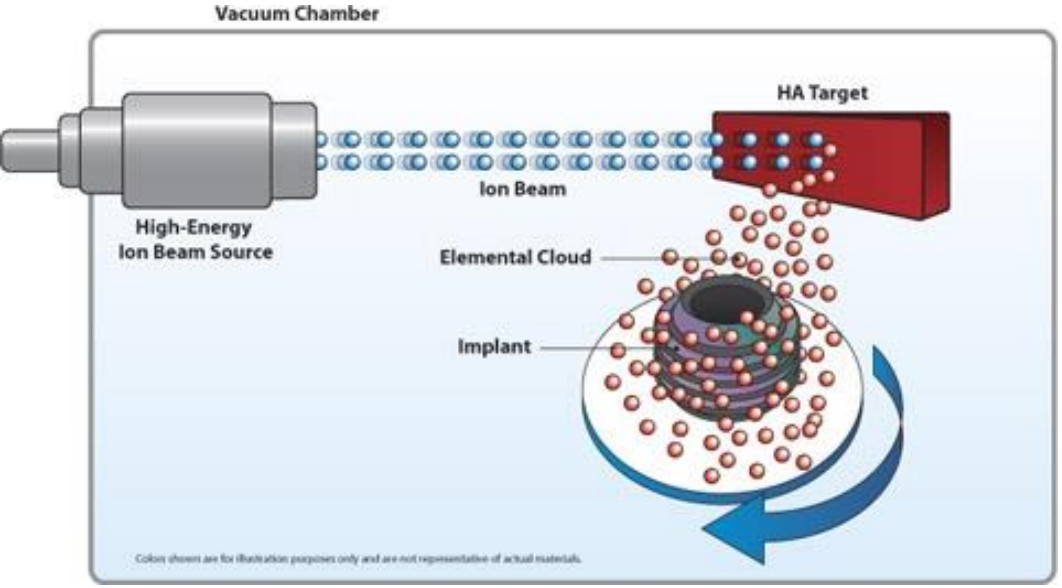
Implanted ions dispersed within a certain depth of substrate surface without forming a new layer, avoiding drawbacks (e.g. cracking and detachment) of traditional coatings;

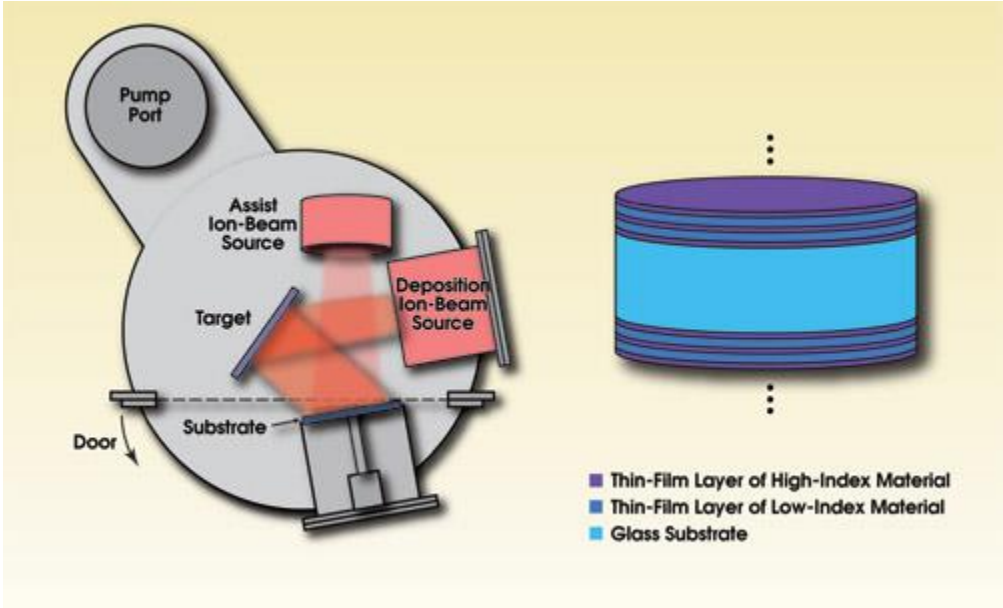
Low operating temperature (sometimes at room temperature) does not affect the substrate material.



Covalent coatings
Ion beam assisted deposition (indirect)

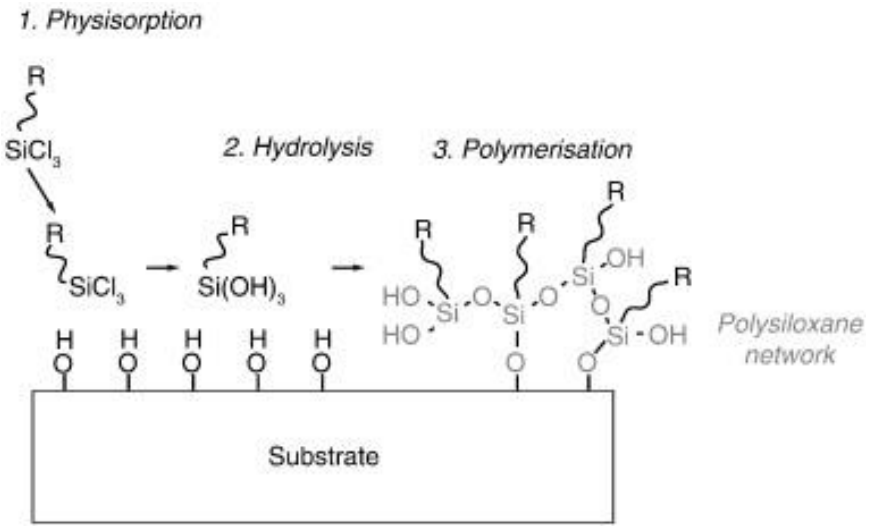


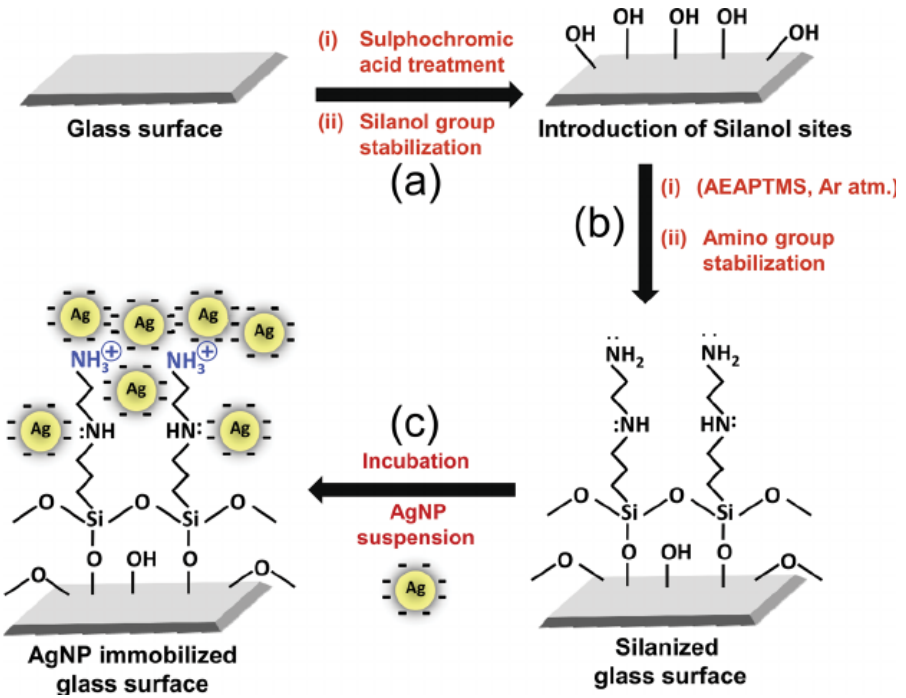



Covalent grafting

Grafting techniques do not create isolation layer between the material and surrounding organisms, like coatings so that many advantageous properties of the substrates are still useful for surrounding organisms after treatment

Silanization - a low-cost and effective covalent coating method to modify material surface that are rich in hydroxyl groups




Covalent grafting
Silanization and other grafting methods

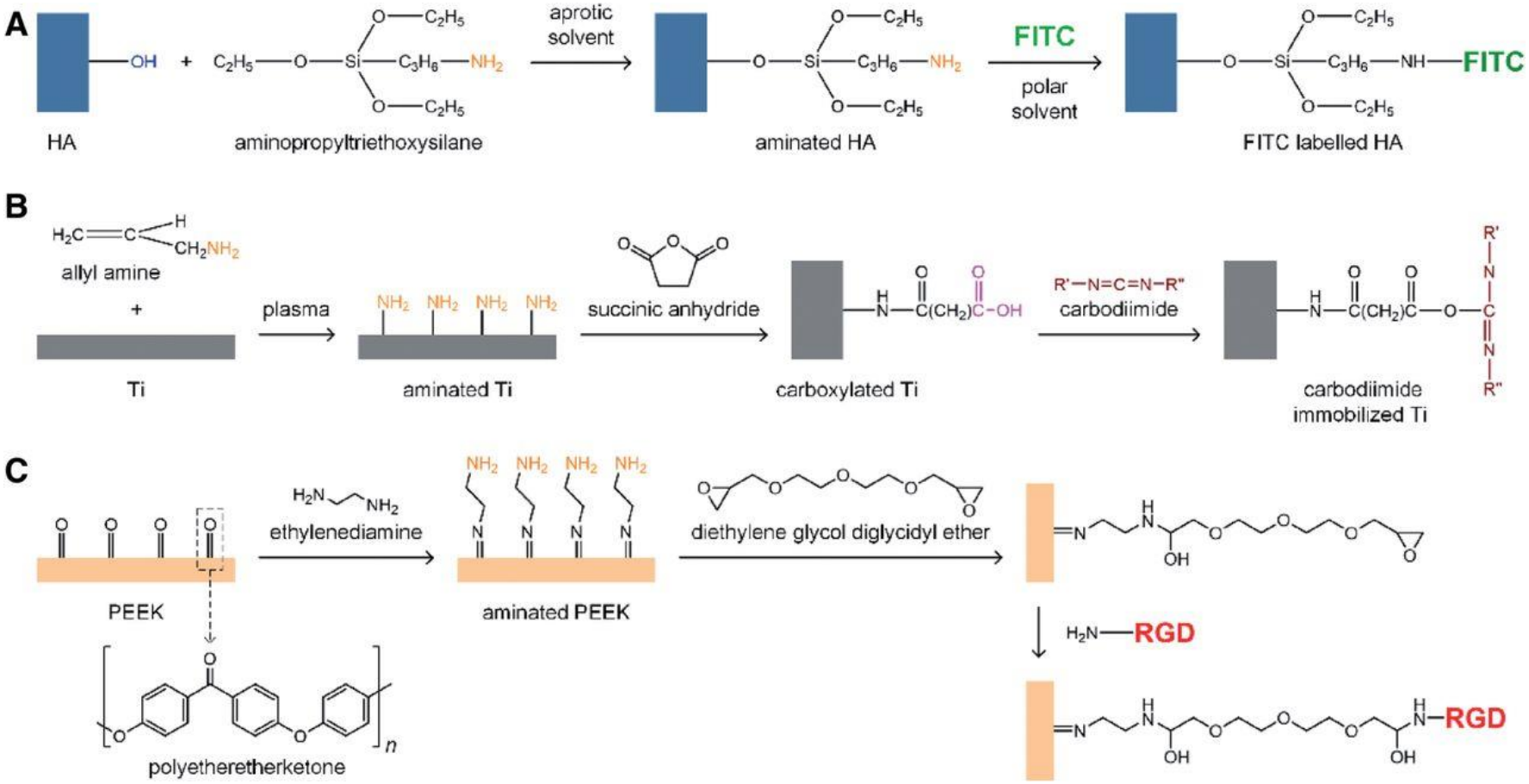
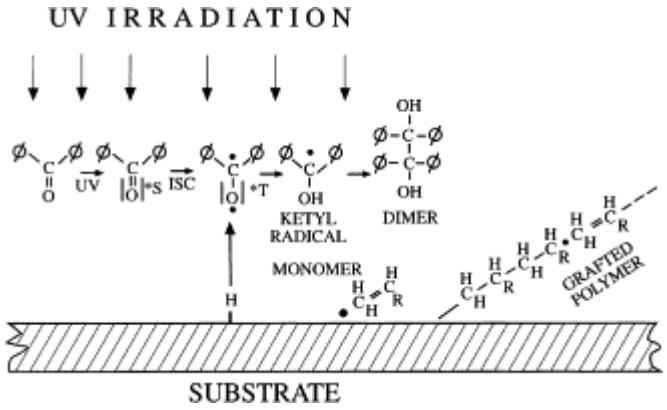
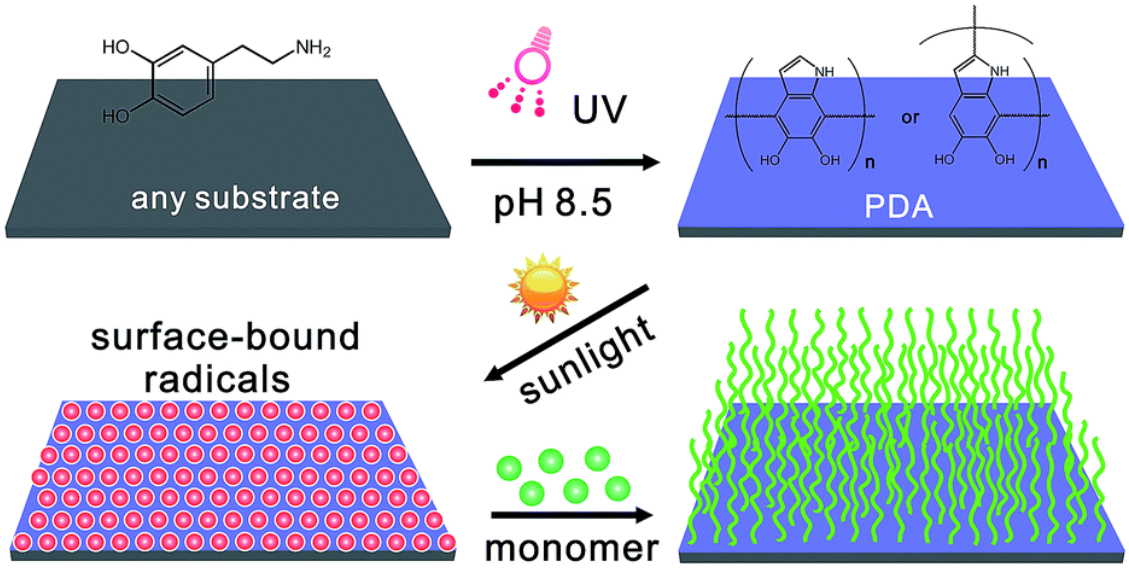


Photo grafting

Chemical grafting is useful to obtain stable surface modifications for biomaterials with active groups (e.g. –OH, –COOH and –NH2) exposed to the surface with high chemical reactivity for the grafting.
It is difficult to perform chemical grafting on the surface of bioinert materials, since there are only a few or no active groups exposed to their molecular surface.

In order to conduct grafting on the surface of these biomaterials, extra energy must be introduced to the grafting reaction.
Radiation breaks chemical bonds on material surface to be grafted, and form free radicals. The reactive surface will be then exposed to monomers to initiate surface graft polymerization



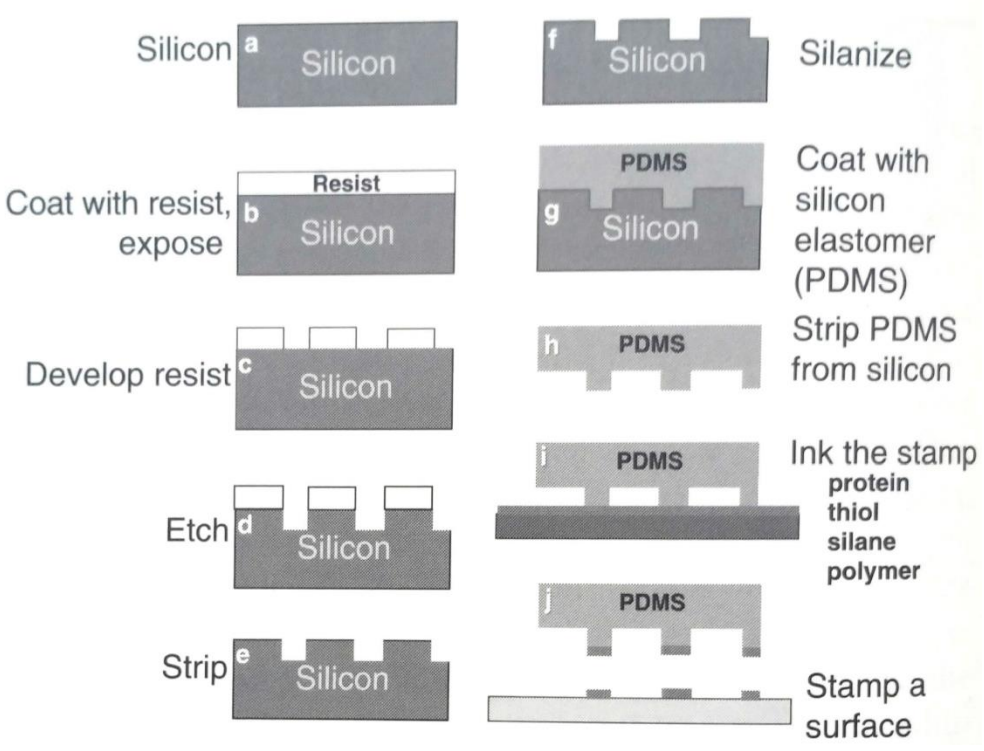



All of the mentioned surface modification methods can be applied both as a uniform surface treatment or as patterns on the surface with length scales of millimeters, microns or nanometers.

Proteins and cells are deposited in surface patterns and textures in order to control bioreactions

Photolithographic techniques that were developed for microelectronics have been applied to patterning of biomaterial surfaces when used together with surface modification techniques. For example, plasma-deposited films patterned using a  photoresist lift-off method

Microcontact printing is a newer and simpler method that utilizes a rubber stamp made of the pattern which is desired on the biomaterial surface. It can be inked with thiols to stamp gold, silanes to stamp silicon, proteins or polymers to stamp many types of surfaces

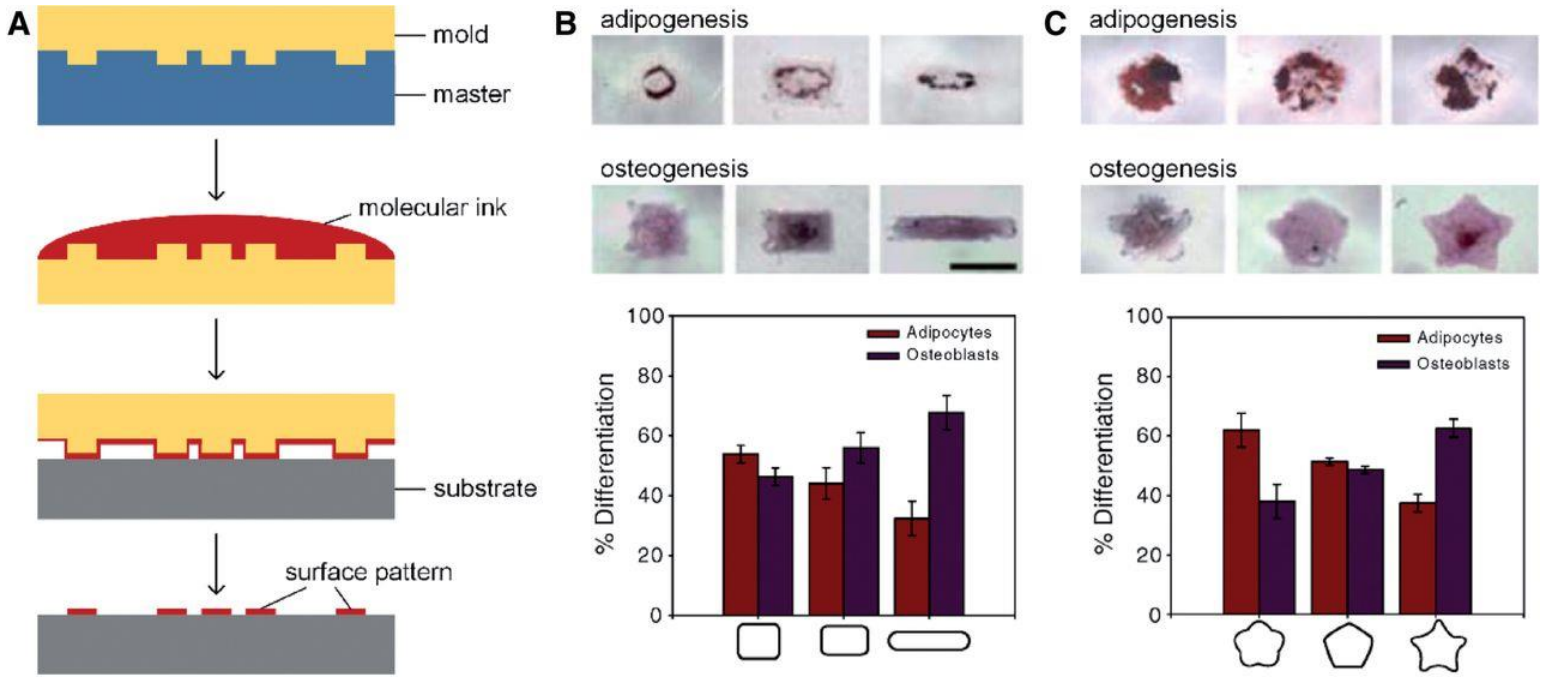


Scanning probe lithography

SPL is a direct-write method that moves a micro- or nano-stylus on material surface to mechanically 'write' patterns

The tip of an atomic force microscope is used to create patterns by directly writing on the surface using a variety of molecular inks (solutions of molecules)

Microcontact printing is a similar method that uses a template with patterns to replicate patterns on a substrate



Surface texturing

Photolithography

Due to the optical diffraction of the focused light beam, the resolution of the pattern created by photolithography is restricted to about half of the wavelength of the light source, typically several hundred nanometers which is much smaller than cell sizes

Steps

(i) prepare a clean and flat substrate;

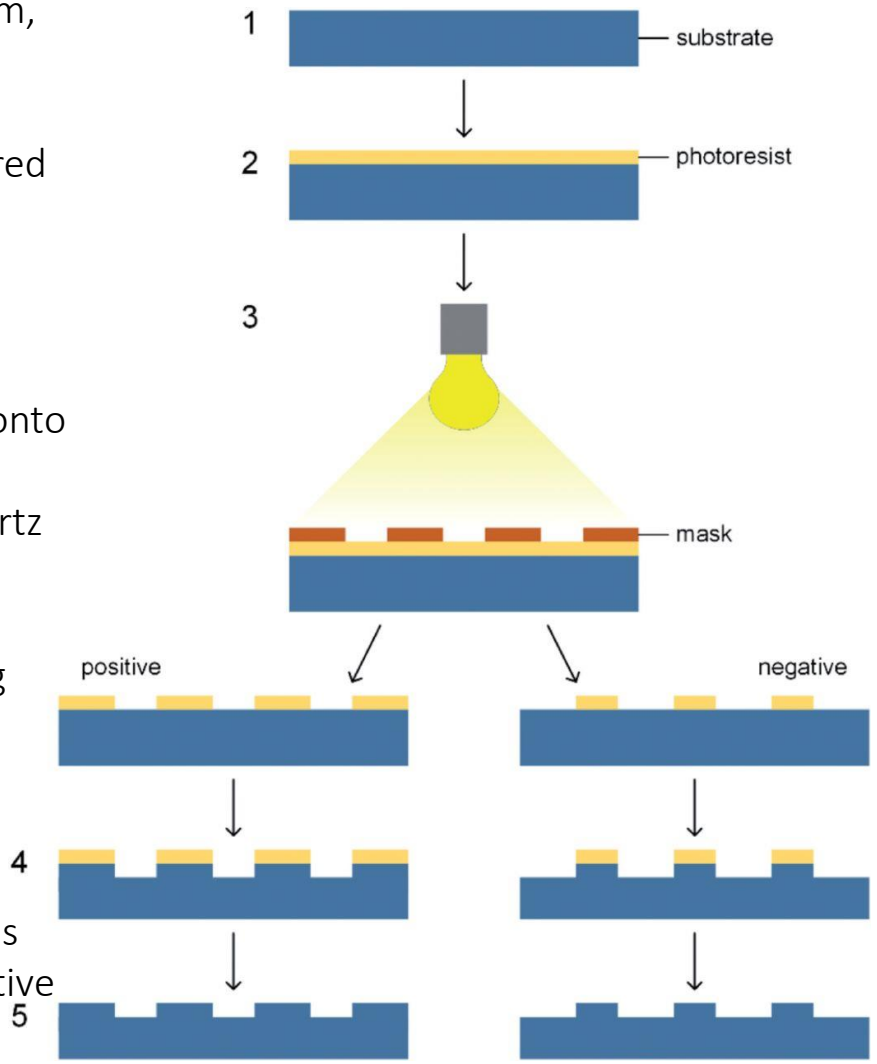
(ii) coat a light sensitive polymer (called photoresist) onto the substrate;

(iii) expose the photoresist under a mask (usually quartz or metal) to form a desired pattern;

(iv) transfer the pattern to the substrate by an etching process (development process)

(v) remove the photoresist.

The photoresist can be either a positive one that areas exposed to the light beam can be dissolved or a negative one



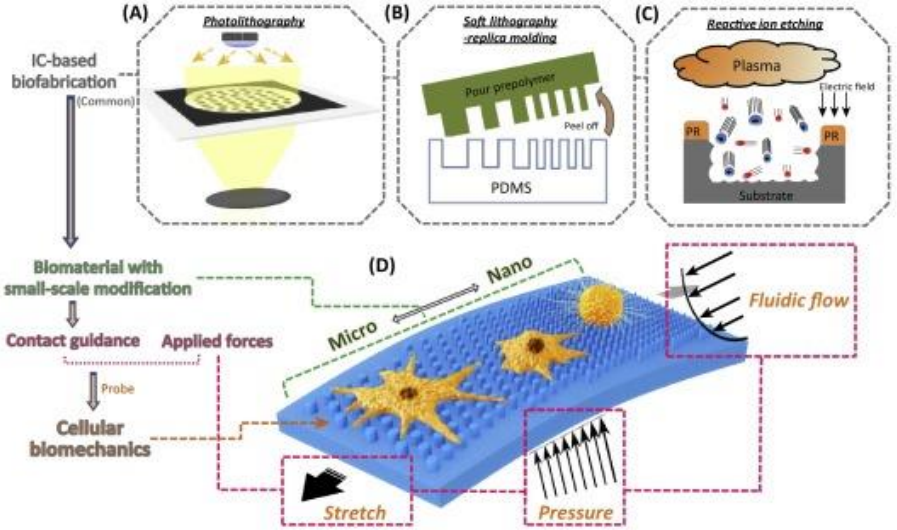
Surface irregularities on medical devices such as grooves, hills, pores, steps are expected to guide many types of cells and to aid tissue repair after injury.

Pores or surface roughness repeated periodically are termed texture.

Porous scaffolds used in vivo and in vitro for tissue engineering direct the response of cells by rough and porous surfaces, improving the following:

Organization of the cytoskeleton
Orientation of extracellular matrix
Amount of produced extracellular matrix
Angiogenesis

The exact cellular and molecular mechanisms underlying cellular and matrix orientation around surface irregularities are not yet clear.

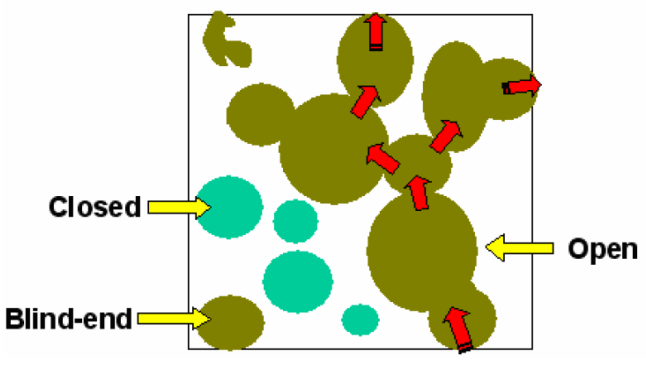


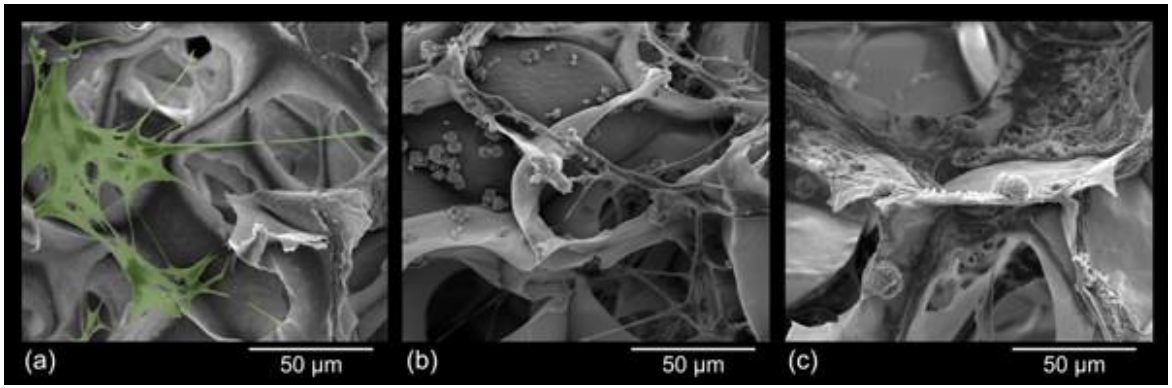
For many biomedical applications there is a need for porous implant materials

The specific requirement for a porous material used in bone growth is to have diameter in the optimum range of 75-250 micrometers. For ingrowth of fibrocartilegenous tissue the recommended pore size is in the range 200-300 micrometers. Other important requirements are pore interconnectivity, interconnection size and compressibility,

Porous biomaterials are used for artificial blood vessels, artificial skin, drug delivery, bone and cartilage reconstruction, periodontal repair and tissue engineering.

Porosity is generally considered as a microtexture based on the importance of this type of surface morphology for cell and tissue response.



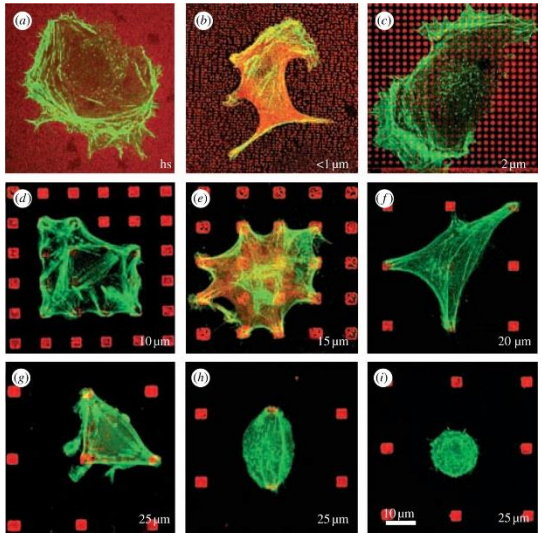
Contact guidance is the phenomenon that cells adapt and orient to the biomaterial surface microtopography.

Alignment and adhesion of cells to microgrooves with dimensions 1.65-8.96 micron in width and 0.69 micron in depth has been reported and explained due to the mechanical inflexibility of cytoskeletal components that prevents bending of “arms” of the cells over surfaces.

Cells want to achieve a biomechanical equilibrium condition with a resulting minimal net sum of forces. It is found that the anisotropic geometry of surface features establishes stress and shear-free planes that influence the position of cytoskeletal elements.

There are hypotheses relating cell contact site, deposited extracellular matrix and surface microtexture to cell response.

For example, a microtextured surface possesses local differences in surface free energy resulting in a specific deposition pattern of proteins.



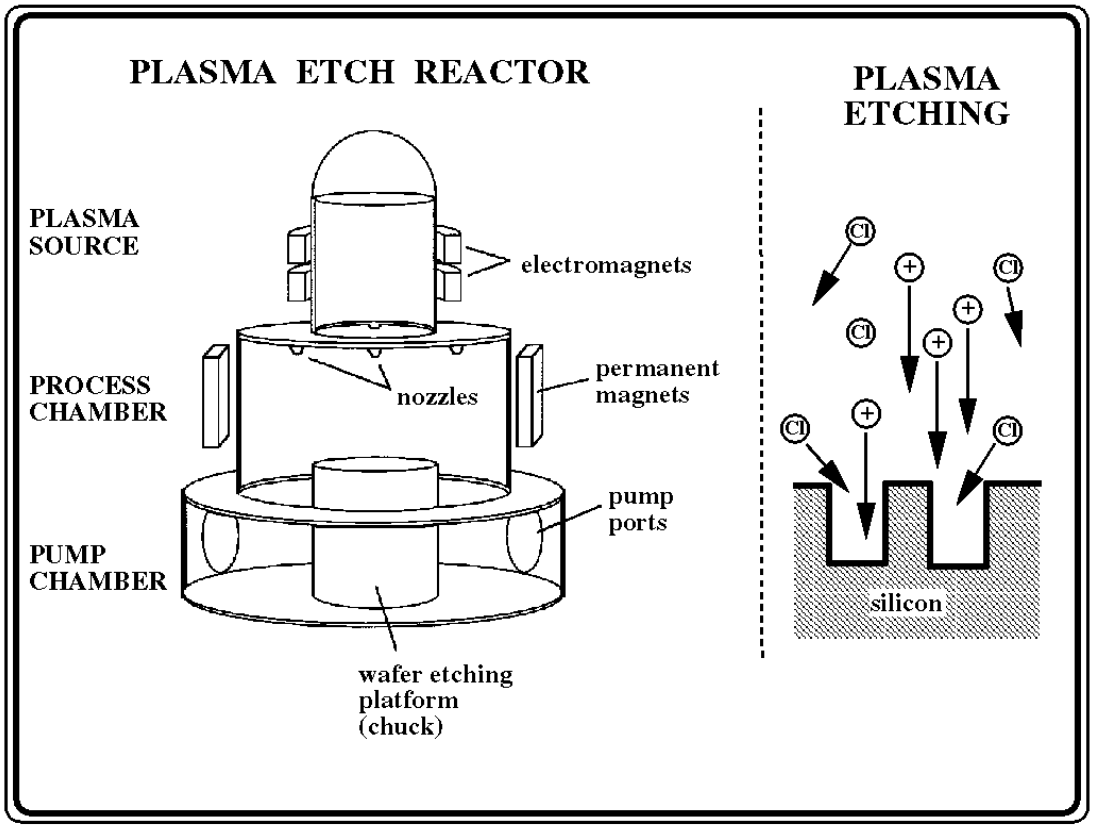

Fluorescence microscopy images showing the effect of substrate geometry on cell adhesion and spreading:
(a) homogeneous substrate (hs),
(b) (*b*) 0.1 mm2 dots, approximately 1 mm apart,
(*c*) 1mm2 dots, 2 mm apart,
(*d*) 9mm2 dots, 10 mm apart,
(*e*) 9mm2 dots, 15 mm apart,
(*f*) 9mm2 dots, 20 mm apart,
(*g–i*) 9mm2 dots, 25 mm apart

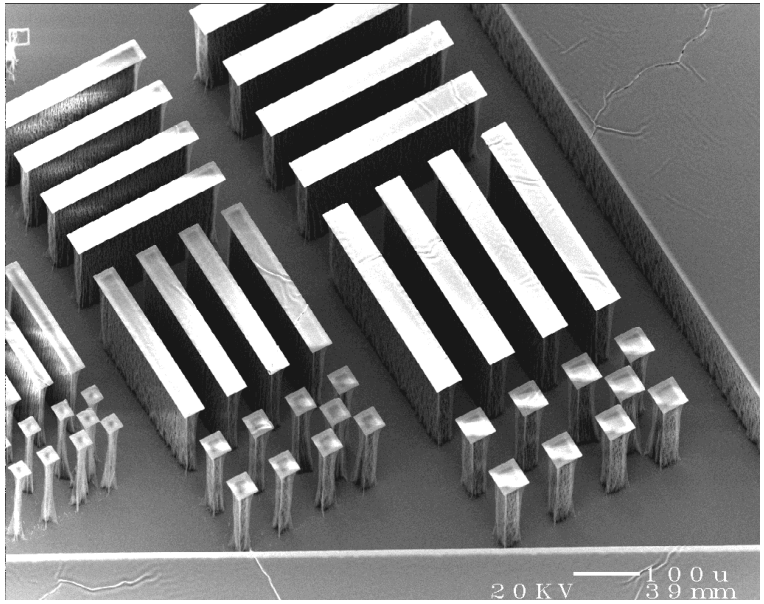
Surface patterning

Surface patterning techniques are widely used and are developing rapidly in the field of microelectronics. Recently advanced methods for surface patterning on biomaterials were partially derived from those in microelectronics industry.

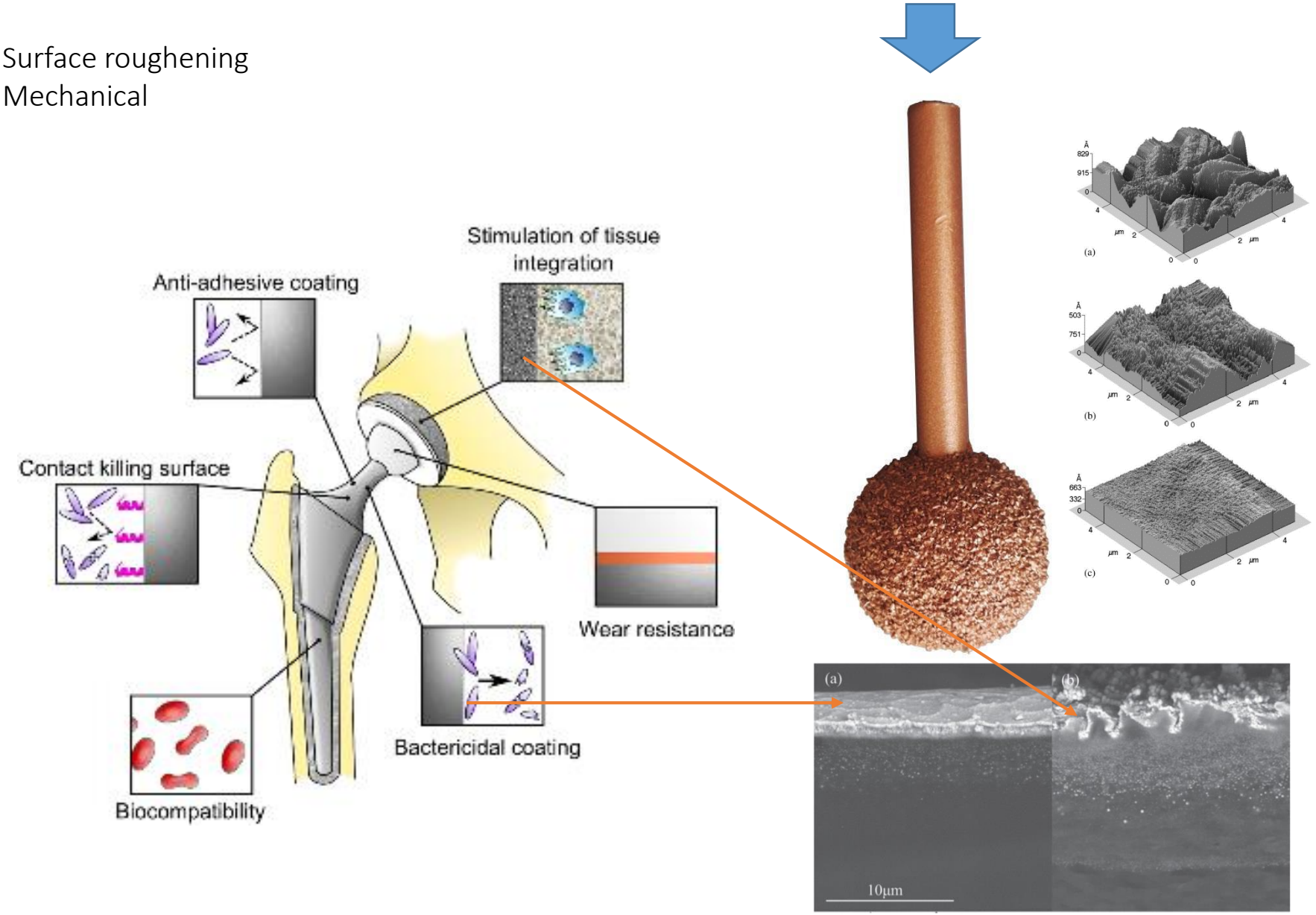
Etching

Plasma etching modifies a surface by shooting a high-speed stream of plasma onto the substrate. Plasma etching is helpful to improve surface activity for bioinert polymers, with less influence on surface topography than chemical etching process.



Surface roughening
Mechanical

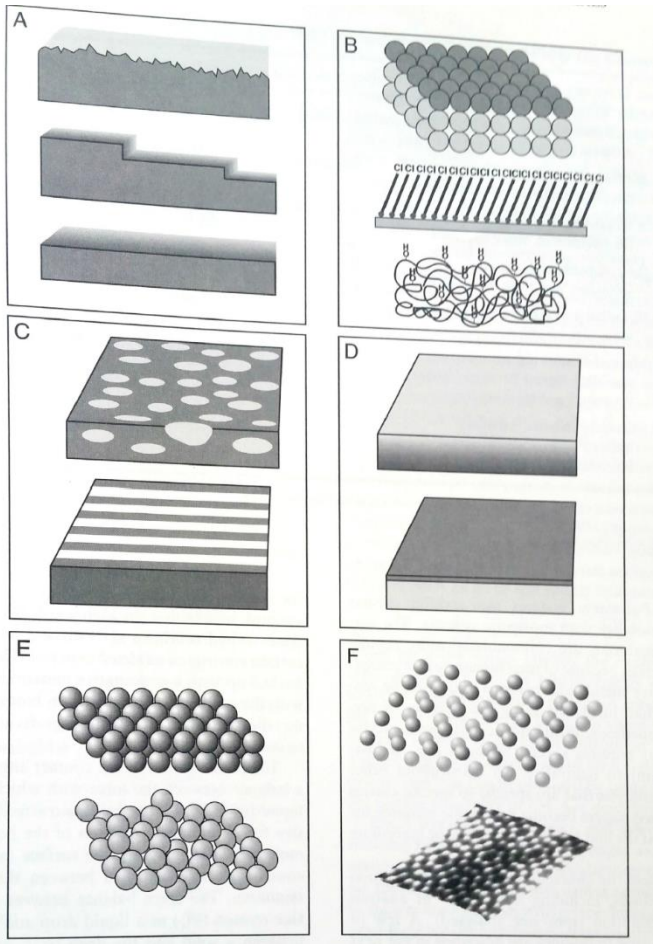


The surface modification reaction should be monitored by surface analysis techniques to ensure that the intended surface is formed.

The surface-modified region is thin and consists of small amounts of material.
Undesirable contamination can be introduced during modification reactions
The potential for surface reversal to occur during surface modification is also high.

Special surface analytical tools are necessary since conventional analytical methods are not sensitive enough to detect surface modifications:

Contact angle methods
Electron spectroscopy for chemical analysis
Secondary ion mass spectrometry
Scanning electron microscopy
Infrared spectroscopy
Scanning tunneling microscopy
Atomic force microscopy
Scanning probe microscopy



Some physicochemically surface modified biomaterials

To modify blood compatibility
- Octadecyl group attachment to surface for albumin affinity
- Plasma fluoropolymer deposition
- Chemically modified polystyrene for heparin-like activity

To influence cell adhesion and growth
- Oxidized polystyrene surface
- Plasma deposited acetone or methanol film
- Plasma fluoropolymer deposition to IOLs to reduce cell adhesion

To control protein adsorption
- Surfaces with grafted PEG to reduce adsorption
- Plasma treated ELISA dish surface to increase adsorption
- Surface cross-linked contact lens to reduce adsorption

To improve lubricity
- Plasma treatment
- Radiation grafting of hydrogels
- Interpenetrating polymeric networks

To improve wear and corrosion resistance
- Ion implantation
- Diamond deposition
- Anodization

To alter transport properties
- Polyelectrolyte grafting

To modify electrical characteristics
- Polyelectrolyte grafting
- Magnetron sputtering of titanium

Physical and Chemical Surface Modification Methods

| | Polymer | Metal | Ceramic | Glass |
|---|---|---|---|---|
| Noncovalent coatings | | | | |
| Solvent coating | ✓ | ✓ | ✓ | ✓ |
| Langmuir–Blodgett film deposition | ✓ | ✓ | ✓ | ✓ |
| Surface-active additives | ✓ | ✓ | ✓ | ✓ |
| Vapor deposition of carbons and metals[a] | ✓ | ✓ | ✓ | ✓ |
| Vapor deposition of parylene (*p*-xylylene) | ✓ | ✓ | ✓ | ✓ |
| Covalently attached coatings | | | | |
| Radiation grafting (electron accelerator and gamma) | ✓ | — | — | — |
| Photografting (UV and visible sources) | ✓ | — | — | ✓ |
| Plasma (gas discharge) (RF, microwave, acoustic) | ✓ | ✓ | ✓ | ✓ |
| Gas-phase deposition | | | | |
| • Ion beam sputtering | ✓ | ✓ | ✓ | ✓ |
| • Chemical vapor deposition (CVD) | — | ✓ | ✓ | ✓ |
| • Flame spray deposition | — | ✓ | ✓ | ✓ |
| Chemical grafting (e.g., ozonation + grafting) | ✓ | ✓ | ✓ | ✓ |
| Silanization | ✓ | ✓ | ✓ | ✓ |
| Biological modification (biomolecule immobilization) | ✓ | ✓ | ✓ | ✓ |
| Modifications of the original surface | | | | |
| Ion beam etching (e.g., argon, xenon) | ✓ | ✓ | ✓ | ✓ |
| Ion beam implantation (e.g., nitrogen) | — | ✓ | ✓ | ✓ |
| Plasma etching (e.g., nitrogen, argon, oxygen, water vapor) | ✓ | ✓ | ✓ | ✓ |
| Corona discharge (in air) | ✓ | ✓ | ✓ | ✓ |
| Ion exchange | ✓[b] | ✓ | ✓ | ✓ |
| UV irradiation | ✓ | ✓ | ✓ | ✓ |
| Chemical reaction | | | | |
| • Nonspecific oxidation (e.g., ozone) | ✓ | ✓ | ✓ | ✓ |
| • Functional group modifications (oxidation, reduction) | ✓ | — | — | — |
| • Addition reactions (e.g., acetylation, chlorination) | ✓ | — | — | — |
| Conversion coatings (phosphating, anodization) | — | ✓ | — | — |
| Mechanical roughening and polishing | ✓ | ✓ | ✓ | ✓ |

[a] Some covalent reaction may occur.

[b] For polymers with ionic groups.

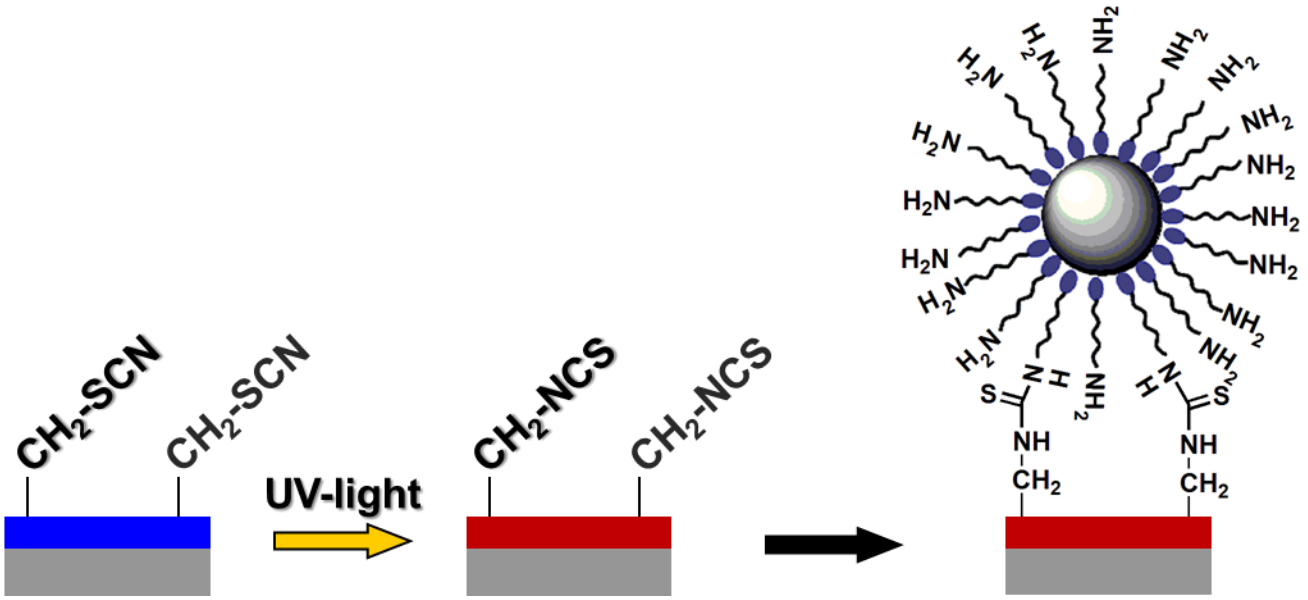
Immobilization of biomolecules

Immobilization of adhesive proteins or peptides to biomaterial is done

- To increase cell adhesion or direct cell migration
  - Fibronectin
- To prevent adhesion
  - Heparin
- To trap specific cells
  - Antibodies
- To direct cellular functions
  - Growth factors

Polymers are preferred in biological modification to other classes of biomaterials because:

- Their surfaces may already contain reactive groups or may be readily derivatized with reactive groups that can be used to covalently link biomolecules
- They may be fabricated in many forms including films, membranes, tubes, fibers, fabrics, particles, capsules and porous structures
- Their compositions vary widely and their molecular structures include homopolymers, random, alternating, block and graft copolymers




When surfaces of metals or inorganic glasses or ceramics are involved, biological functionality can be added via a chemically immobilized or physisorbed polymeric or surfactant adlayer in addition to techniques such as plasma gas discharge to deposit polymer compositions having functional groups

Many different biologically functional molecules can be chemically or physically immobilized on or within polymeric supports:

Proteins-peptides for bioreactors, bioseperation, biosensors, drug delivery, biocompatible surfaces
Enzymes, antibodies, antigens, cell adhesion molecules, blocking proteins

Saccharides
Sugars, oligosachharides, polysaccharides

Lipids for thrombo-resistant and albuminated surfaces
Fatty acids, phospholipids, glycolipids

Drugs for drug delivery systems, thrombo-resistant surfaces
Antithrombogenic agents, anticancer agents, antibiotics, contraceptives, drug antagonists

Ligands
Hormone receptors, cell surface receptors, avidin, biotin

Nucleic acids for DNA probes and gene therapy
Single or double stranded DNA, RNA

All kinds of cells for bioreactors, artificial organs, biosensors

Some of these solids become hydrogels when water swollen and biomolecules may be immobilized on the outer gel surface as well as within the swollen polymer gel network.

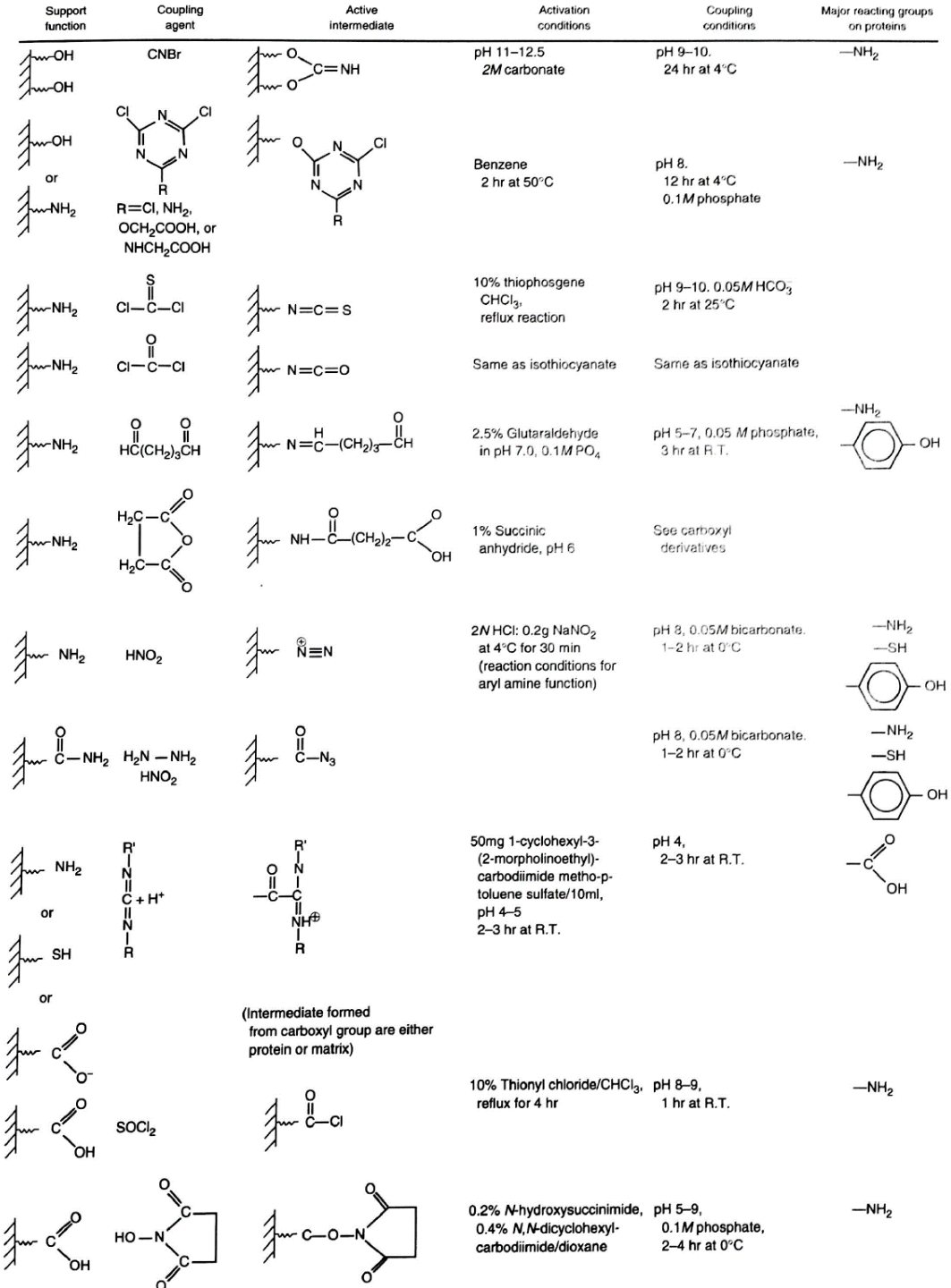
Immobilization of many types of proteins on polymer surface groups are possible

Especially OH, NH2, COOH groups are utilized

Especially OH, $NH_2$, COOH groups are utilized

OH group can bond with hydrophilic side chains

NH2 and COOH surface groups can bond with NH2 and COOH chains of aminoacids



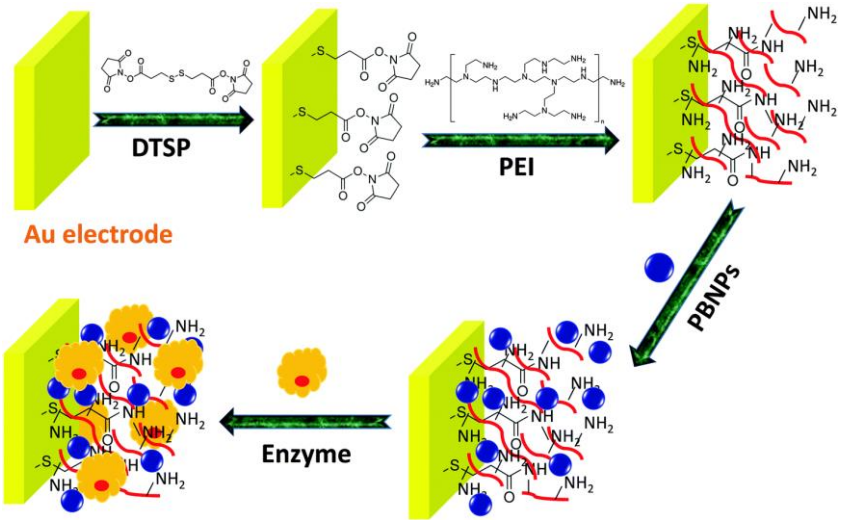
An inert polymer surface can be covalently bound by initially modifying the surface to provide reactive groups like -OH, -NH2, -COOH, -CH—CH2

Surface modification techniques including ionizing radiation, graft copolymerization, plasma gas discharge, photochemical grafting, ozone grafting, and chemical derivatization can be used.

Physical modification of inert surfaces utilize hydrophobic interactions by attaching ligands to hydrophobic sequences.

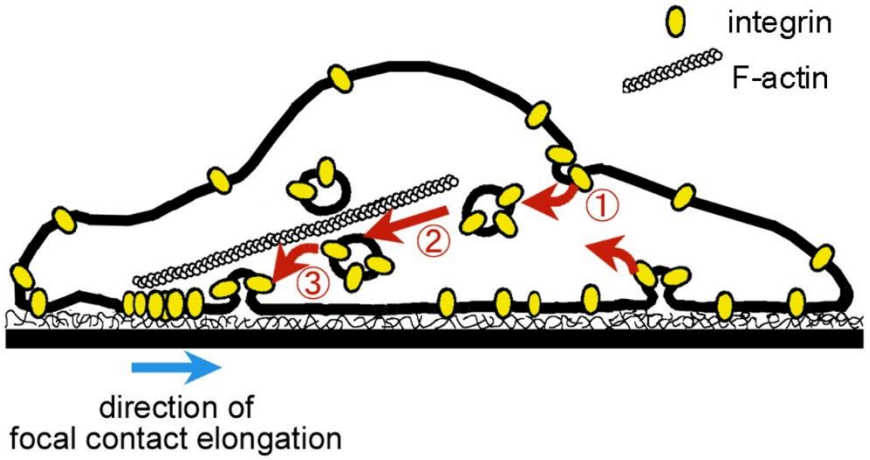
Inert surfaces whether polymeric, metal, or ceramic can also be functionalized by modification of a polymeric adlayer. Both physical and chemical immobilization methods can be used including

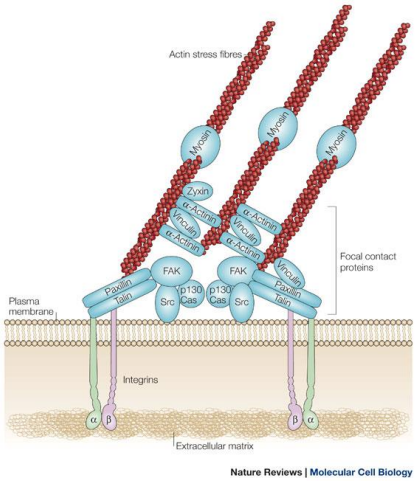
- Electrostatic interactions
- Hydrophobic interactions
- Specific chemical interactions such as that between gold and sulfur atoms, triethoxysilanes for metals or ceramics
- Plasma gas discharge to deposit polymeric amino groups



One of the most useful ways in immobilizing biomolecules is replacing the adsorbed protein layer with ligands for cell-surface  adhesion receptors by adsorbing or covalently grafting them.

Peptides have been grafted randomly over a substrate as well as in a clustered form. The latter has important advantage since cells normally cluster their integrin receptors into assemblies called focal contacts. Peptide clusters on the surface improve both adhesion strength and cell signaling.




Biomolecules can also be immobilized in order to control cellular functions.

For example immobilized polypeptide growth factor provides biological interaction with cells that signal specific cellular behavior including

- Support of liver-specific function in hepatocytes
- Induction of neurite extension in neurons
- Induction of angiogenesis
- Differentiation of mesenchymal stem cells into bone-forming osteoblasts

Also molecules that take part in enzymatic reactions can be immobilized at the surface.

In the case of a drug delivery system, the immobilized drug is expected to be released from the biomaterial while an immobilized enzyme or adhesion-promoting peptide in an artificial organ is designed to remain attached or entrapped for the duration of use.

Therefore immobilization can refer to both a transient or a long-term localization of the biomolecule on or within a support.

There are three major methods for immobilizing biomolecules

| Method: | Physical and electrostatic adsorption | Cross-linking (after physical adsorption) | Entrapment | Covalent binding |
|---|---|---|---|---|
| Ease: | High | Moderate | Moderate to low | Low |
| Loading level possible: | Low (unless high S/V) | Low (unless high S/V) | High | (depends on S/V and site density) |
| Leakage (loss): | Relatively high (sens. to ΔpH salts) | Relatively low | Low to none[a] | Low to none |
| Cost: | Low | Low to moderate | Moderate | High |

[a] Except for drug delivery systems.

Either physical or chemical immobilization can lead to permanent or long term retention.

If the polymer support is biodegradable, the chemically immobilized biomolecule may be released as the matrix erodes away.

The mechanisms of biomolecule immobilization methods:

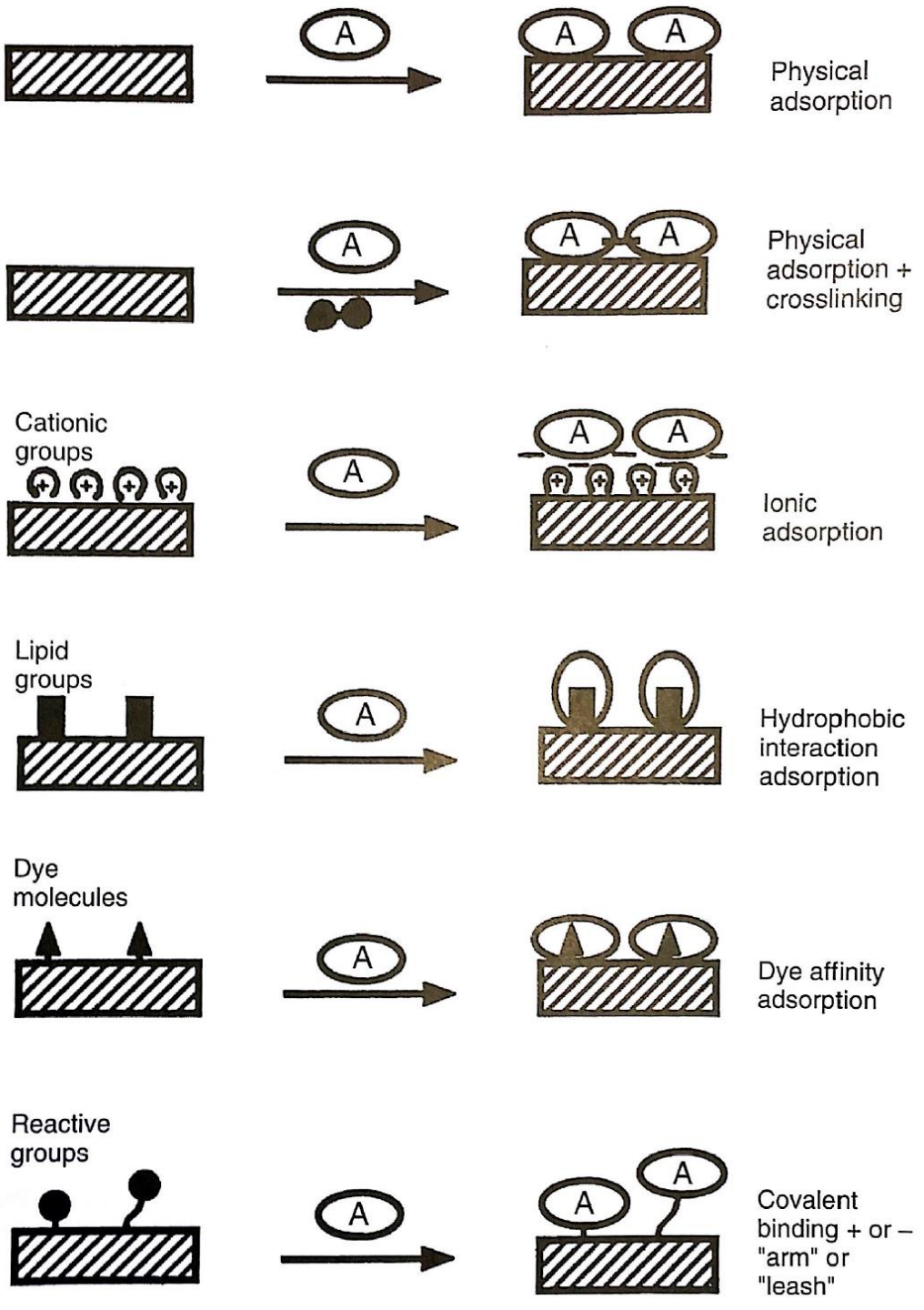
Physical adsorption
Van der Waals, electrostatic, affinity, adsorbed or cross-linked

Physical entrapment
Barrier systems, hydrogels, dispersed systems

Covalent attachment
Soluble polymer conjugates, solid surfaces, hydrogels

There are many different ways in which the same biomolecule can be immobilized to a polymeric support.

Heparin and albumin are the most common biomolecules that have been immobilized by a number of widely differing methods.



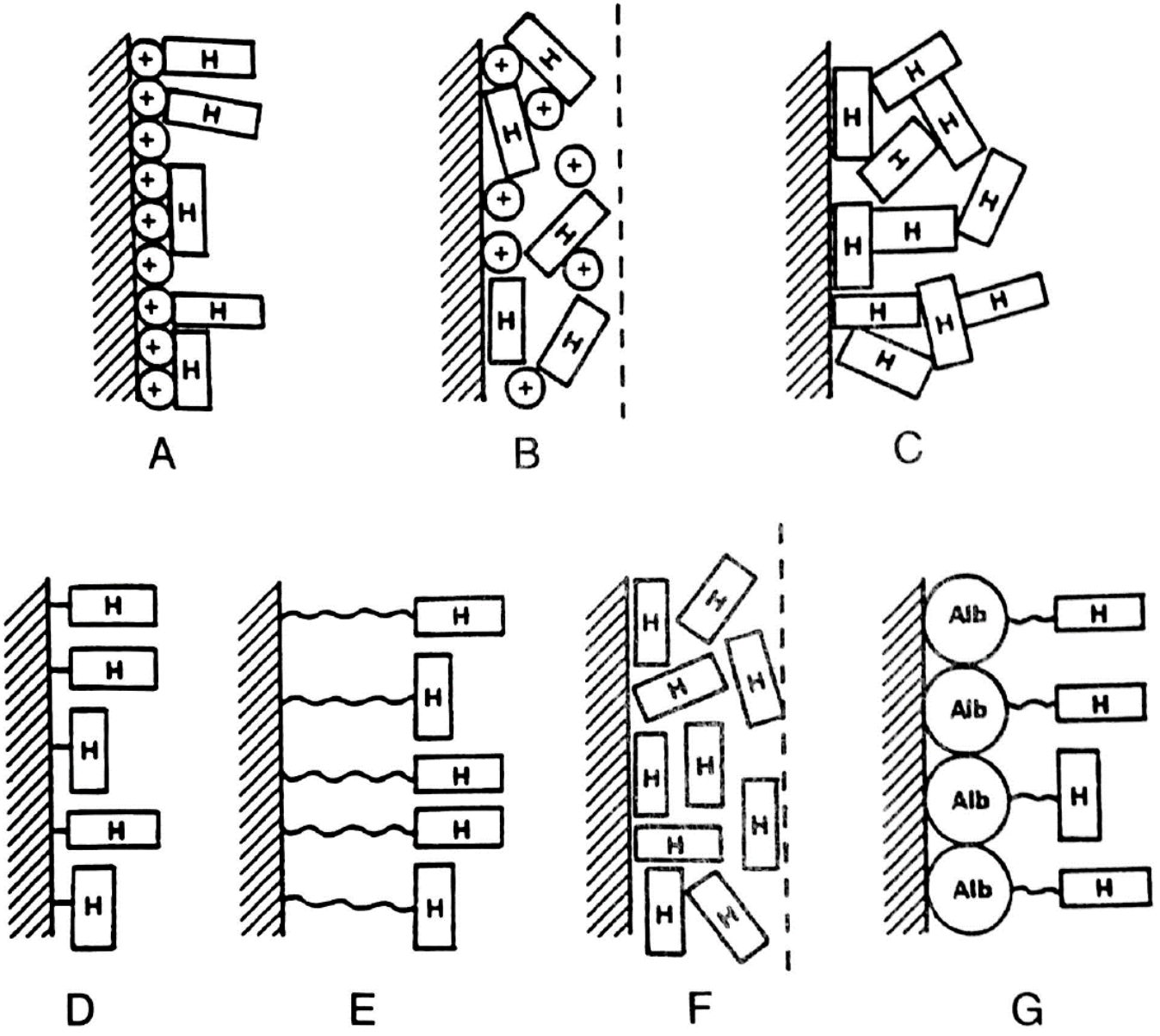



**FIG. 3.** Various methods for heparinization of surfaces: (A) heparin bound ionically on a positively charged surface; (B) heparin ionically complexed to a cationic polymer, physically coated on a surface; (C) heparin physically coated and self-cross-linked on a surface; (D) heparin covalently linked to a surface; (E) heparin covalently immobilized via spacer arms; (F) heparin dispersed into a hydrophobic polymer; (G) heparin–albumin conjugate immobilized on a surface

A large group of diverse methods have been developed for covalent binding of biomolecules to polymers

A chemically immobilized biomolecule may be attached by using a spacer group. Such arms can provide greater steric freedom, and greater specific activity for the biomolecule. The spacer arm may also be either hydrolytically or enzymatically degradable and will release the immobilized biomolecule as it degrades.