Some Background Concepts

ADSORBED PROTEINS **ON BIOMATERIALS**

- The replacement of injured or diseased tissues with devices made from **materials that are not of biologic origin** is the central approach in current biomaterials science and clinical practice due to the fact that **these materials are not attacked by the immune system,** unlike donor tissues or organs.

- **This fundamental difference arises from the presence of immunologically recognizable biologic motifs on donor tissue, and their absence on synthetic materials.**

- Nonetheless**, there are other types of biological responses to implanted biomaterials** that often impair their usefulness, including the **clotting of blood and the foreign-body reaction**. Clearly, the body does recognize and respond to biomaterials.

- **The basis for these reactions is the adsorption of adhesion proteins to the surface of the biomaterials that are recognized by the integrin receptors present on most cells.**

- The adsorption of adhesion proteins to the biomaterial converts it into a biologically recognizable material.

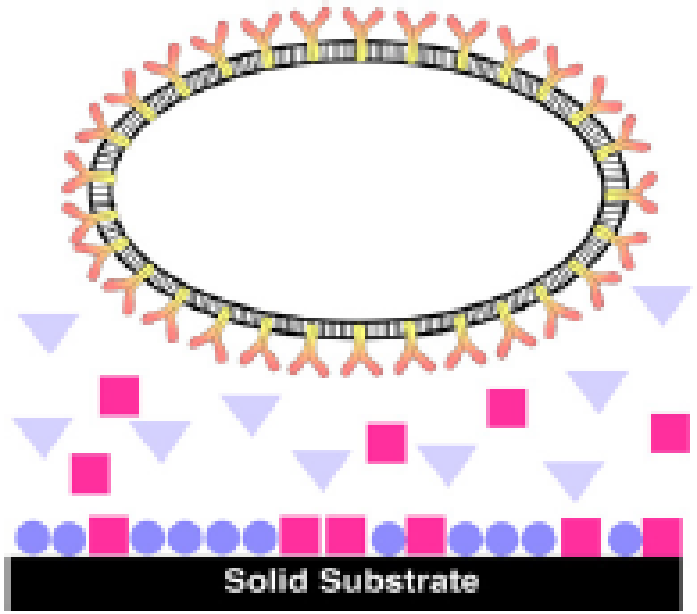

FIGURE II.1.2.1 Cell interactions with foreign surfaces are mediated by integrin receptors with adsorbed adhesion proteins that sometimes change their biological activity when they adsorb. The cell is shown as a circular space with a bilayer membrane in which the adhesion receptor protein molecules (the slingshot-shaped objects) are partly embedded. The proteins in the extracellular fluid are represented by circles, squares, and triangles. The receptor proteins recognize and cause the cell to adhere to only the surface bound form of one protein, the one represented by a solid blue circle. The bulk phase of this same adhesion protein is represented by a blue triangle, indicating that the solution and solid phase forms of this same protein have a different biological activity. The figure is schematic and not to scale.

EXAMPLES OF THE EFFECTS OF ADHESION PROTEINS ON CELLULAR INTERACTIONS WITH MATERIALS

➢ The Effects of Preadsorption with Purified Adhesion Proteins

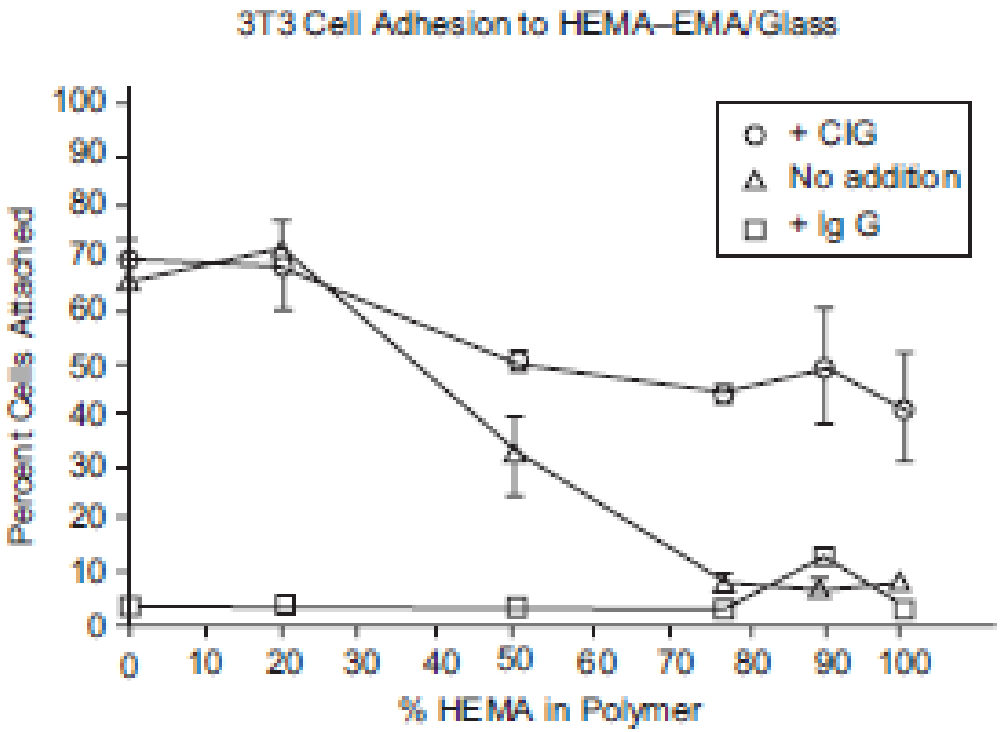

FIGURE II.1.2.2 3T3 cell adhesion to HEMA–EMA copolymers varying from hydrophilic (HEMA-rich) to hydrophobic (EMA-rich): effect of no adsorbed protein, preadsorption with fibronectin (designated CIG in the figure) or preadsorption with immunoglobulin G (IgG). The data is from the author's laboratory.

- **An example of the effect of fibronectin adsorption is shown in Figure II.1.2.2, which also contrasts it with the effects of the non-adhesive protein immunoglobulin G.**
- As shown in the figure, the adhesion of the fibroblast-like 3T3 cells to a series of polymers and copolymers of 2-hydroxyethyl methacrylate (HEMA) and ethyl methacrylate (EMA) not previously adsorbed with protein (and without proteins in the cell suspension) varies, being much less on the hydrophilic polyHEMA-rich surfaces than on the hydrophobic polyEMA-rich surfaces.
- **These data are an example of direct or non-specific cell adhesion in which the cells adhere directly to the surfaces, rather than to adsorbed proteins.**

➢ Depletion Studies

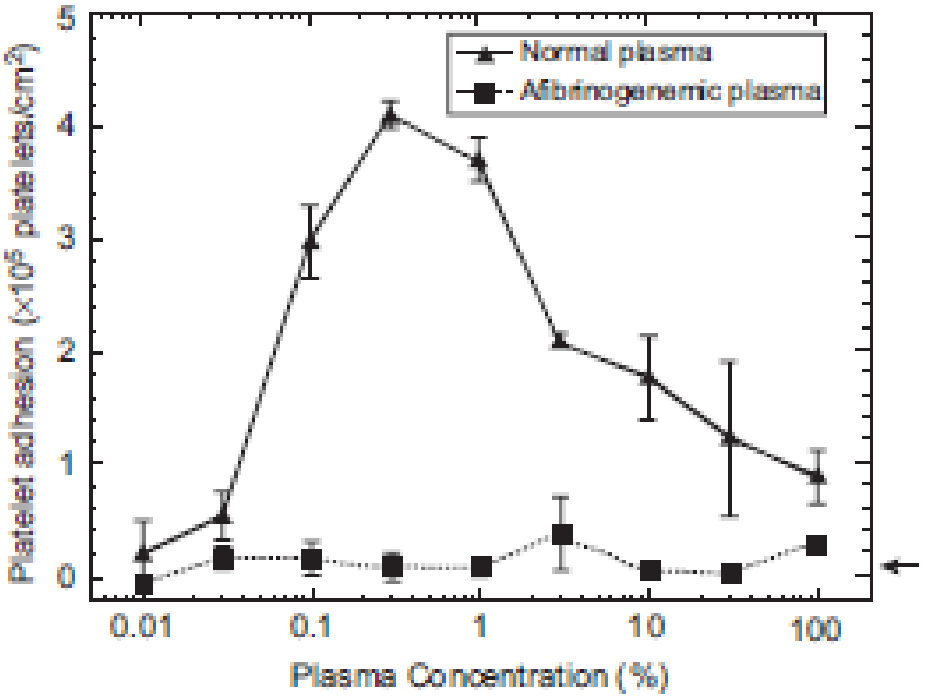

FIGURE II.1.2.3 Platelet adhesion to Immulon I® preadsorbed with normal plasma (triangles) or afibrinogenemic plasma (squares). The solid line represents the platelet adhesion to Immulon I® preadsorbed with a series of dilutions of normal plasma, whereas the dotted line represents the platelet adhesion to Immulon I® preadsorbed with a series of dilutions of afibrinogenemic plasma. The arrow at the lower right corner indicates platelet adhesion to Immulon I® preadsorbed with 2% BSA only (BSA is bovine serum albumin). (Source: Figure 4 in Tsai and Horbett, 1999. Copyright permission received.)

- Platelet adhesion to surfaces preadsorbed with plasma deficient in fibrinogen is much less than to the same surface preadsorbed with normal plasma

➢ Inhibition of Receptor Activity with Antibodies

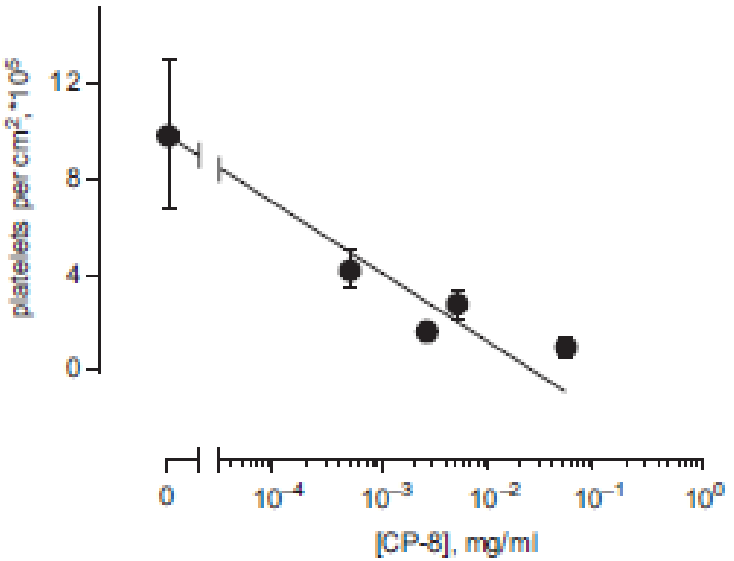

FIGURE II.1.2.4 Effect of anti-IIb/IIIa antibody on platelet adhesion to Biomer preadsorbed with plasma. Adhesion of platelets incubated in monoclonal antibody CP-8 (monovalent Fab' fragment directed against the glycoprotein (GP) IIb/IIIa complex) to Biomer. Substrates were contacted with 1.0% plasma for 2 hours, then with washed, antibody-treated platelets for 2 hours. (From: Chinn J.A., Horbett T.A., Ratner B.D. (1991). Baboon Fibrinogen Adsorption and Platelet Adhesion to Polymeric Materials. *Thromb Haemost*, 65, 608–17.)

- Platelet receptor-mediated interactions appear to be the primary mechanism of platelet interaction *in vivo* with certain vascular grafts, because platelet deposition is largely inhibited by antibodies to the **glycopro IIb/ IIIa receptor, the major integrin on the surface of platelets.**

- *In vitro* platelet adhesion to surfaces preadsorbed with blood plasma is also inhibited by **anti-glycoprotein IIb/IIIa** in a dose-dependent manner

THE ADSORPTION BEHAVIOR OF PROTEINS AT SOLID–LIQUID INTERFACES

- an experiment that is performed to demonstrate the adsorption of proteins to surfaces

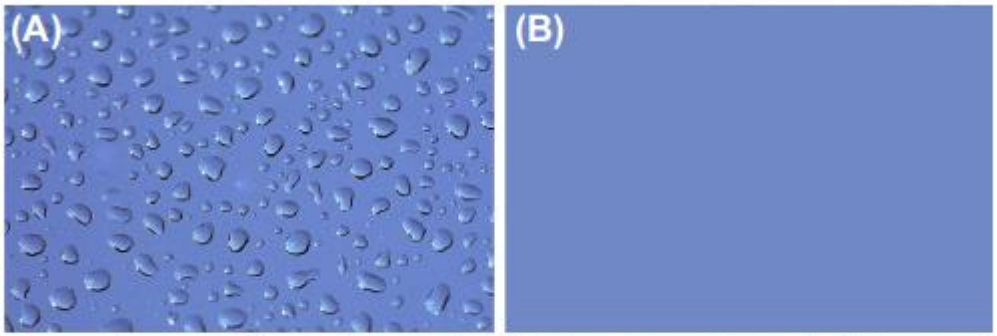

FIGURE II.1.2.5 The conversion of non-wettable polystyrene surface (A) into one completely wettable by water (B) is due to the adsorption of proteins (simulated images based on actual observations).

- **in part (A),** water droplets sprayed on the surface of an unused polystyrene cell culture dish are easily visible because **they bead up**. If a cell were placed on a polystyrene dish instead of the water droplet, it would also encounter a very non-wettable surface.

- **in Part (B) ,** figure illustrates the results of spraying water droplets on the surface of a polystyrene dish that had first been exposed to a protein solution for a short time, and then rinsed extensively with water.

    - As illustrated, no water droplets can be seen on this surface, reflecting the fact that in this case the added drop of water completely spread out over the surface of the preadsorbed dish. **This happens because the water in part (B) was not able to interact with the polystyrene surface, because the surface had become coated with a layer of the hydrophilic protein adsorbate.**

    - Similarly**, cells that come into contact** with surfaces adsorbed with proteins do not directly “see” the substrate, but instead they interact with the intervening protein adsorbate.

➢ Rapid Adsorption Kinetics and Irreversibility

- The time course of adsorption of lysozyme on silica measured with a high speed, automated ellipsometer capable of very rapid measurements

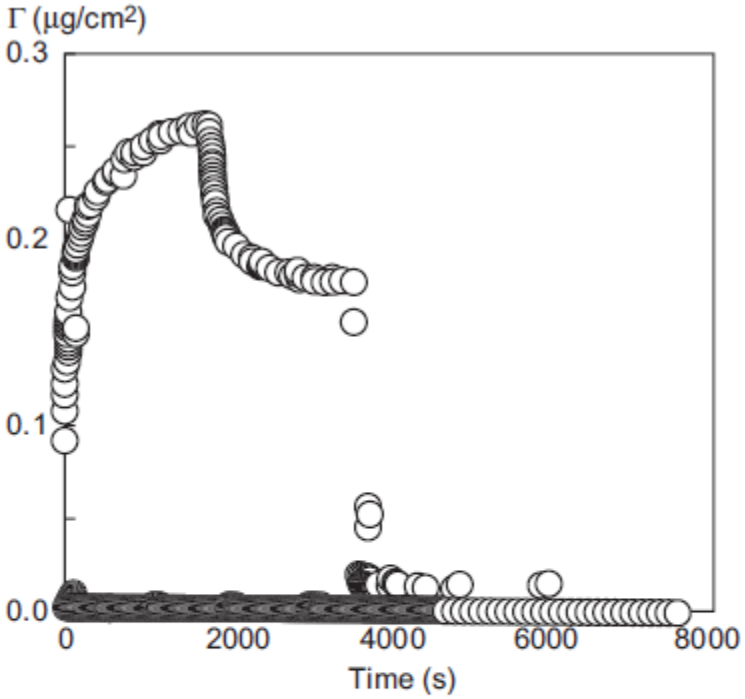

FIGURE II.1.2.6 The adsorption kinetics of lysozyme to a silica surface as studied with ellipsometry. The adsorbed amount versus time for adsorption of lysozyme to silica followed by buffer rinsing after 1800 seconds, addition of surfactant (sodium dodecyl sulfate) after 3600 seconds, and a final rinse with buffer after 5400 seconds (open circles). Adsorption from a mixture of the protein and surfactant for 1800 seconds followed by rinsing is also included (closed circles). The experiments were carried out at 25°C in 0.01 M phosphate buffer, 0.15 M NaCl, pH 7. (Reprinted with permission from: Arnebrandt and Wahlgen, 1995. Copyright © 1995 American Chemical Society.)

- **At the earliest measurement time**, less than a second into the study, the adsorption has reached almost half of the steady-state value.
- **At 2000 seconds**, the protein solution was replaced with a buffer, resulting in some removal of loosely bound protein, but the **adsorption stabilizes and would have remained at this value for much longer than shown, due to the tight, irreversible binding.**
- **At 4000 seconds**, a solution of the detergent sodium dodecyl sulfate (SDS) was infused, leading to complete removal of the protein.
- **Thus, this experiment illustrates the rapid adsorption of proteins. It also illustrates that most of the adsorbed protein is irreversibly bound, as indicated by the fact that washing the surface with buffer does not remove the protein.** The adsorbed protein is only removed when a strong surfactant (SDS in this example) is used. All these features are characteristic of protein adsorption to solid surfaces.

➢ The Monolayer Model

- The existence of a close packed monolayer of adsorbed protein is suggested by studies with single protein solutions, in which a saturation effect can often be observed in the adsorption isotherm

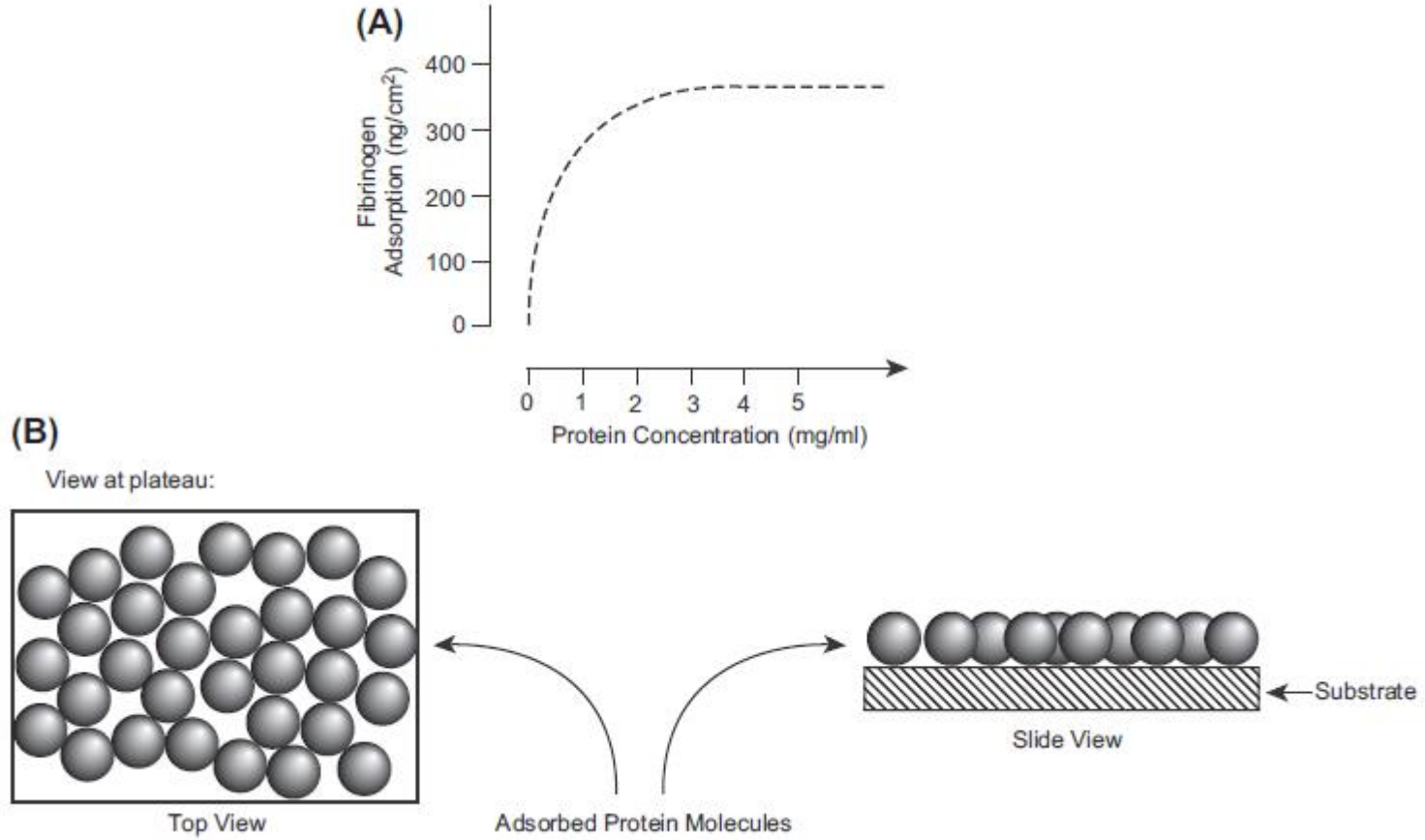

FIGURE II.1.2.8 Adsorption isotherms (A) and the monolayer concept (B).

- Adsorption to surfaces exposed to different concentrations of protein until steady-state adsorption is achieved (2 hours or more) increases steeply at low bulk phase concentrations, but typically reaches a plateau or saturation value at higher bulk concentrations. This behavior is called a Langmuir isotherm.

➢ Competitive Adsorption of Proteins to Surfaces from Protein Mixtures

- **The competitive phenomena underlying differential enrichment from multi-protein mixtures are most clearly illustrated in binary mixtures of proteins**

- The curves in Figure represent the typical outcome of **binary mixture studies for three different conditions.**

- when a radiolabeled protein such as fibrinogen ("A" in the figure) is mixed with various amounts of an unlabeled protein such as albumin ("B" in the figure), the adsorption of fibrinogen ("A") always declines when sufficiently high amounts of albumin ("B") are present.

- However, the amount of competing protein needed to inhibit the adsorption of the labeled protein is different in each curve. **This is meant to illustrate that, for a given pair of competing proteins, the competition curves will be different if the surfaces they are competing for are different**.

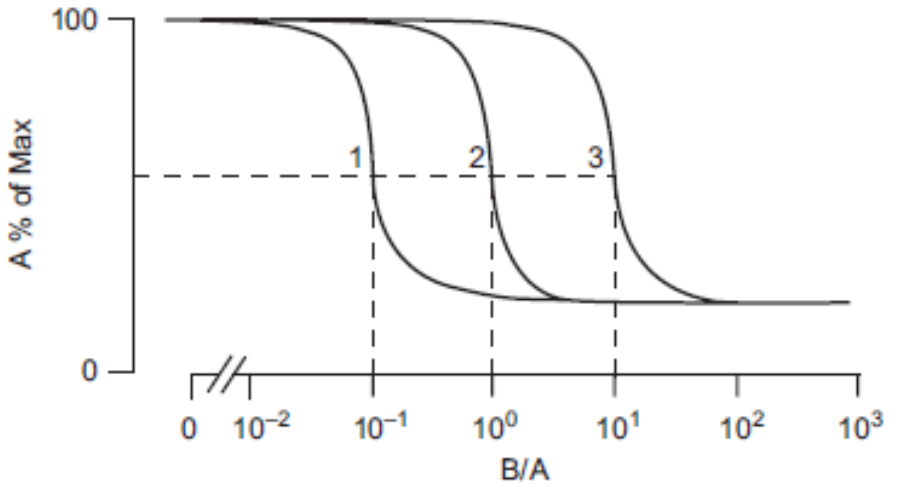

FIGURE II.1.2.9 Competitive adsorption of two proteins from a mixture. (From: Horbett, 1993.)

- An experimental example of surface chemistry-dependent selective adsorption of proteins from a complex protein mixture

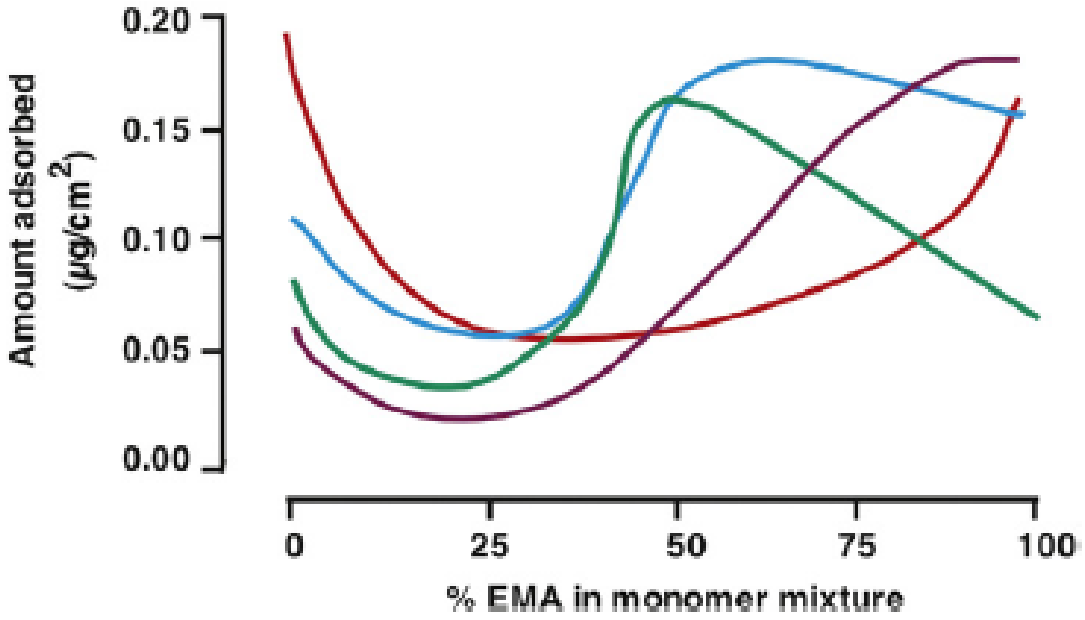

FIGURE II.1.2.10 Differential affinity of proteins to a series of polymers: adsorption of Fg (green line); IgG (purple line); Alb (red line); and Hb (blue line) from plasma to hydroxyethyl methacrylate – ethyl methacrylate copolymers. (Redrawn version of a figure from: Horbett, 1981.)

MOLECULAR SPREADING EVENTS: CONFORMATIONAL AND BIOLOGICAL CHANGES IN ADSORBED PROTEINS

- **Proteins that adsorb to solid surfaces can undergo conformational changes, because of the relatively low structural stability of proteins and the tendency to unfold to allow further bond formation with the surface.**

- Conformational changes can be detected with many types of physicochemical methods, and also by measuring changes in the biological activity of the adsorbed proteins.

➢ **Physicochemical Studies of Conformational Changes :** Comparison of the adsorptive behavior of different proteins to their molecular properties indicates that **less stable proteins are more adsorptive.**

➢ **Changes in Biological Properties of Adsorbed Proteins:** While physicochemical studies sometimes suggest complete denaturation of adsorbed proteins**, most probes for biological activity suggest the changes are more limited.** Thus, enzymes retain at least some of their activity in the adsorbed state, especially when the surfaces are more fully loaded with enzyme.

- **Fibrinogen undergoes a time-dependent transition after its adsorption to a surface that results in reduced platelet and antibody binding to the adsorbed fibrinogen**

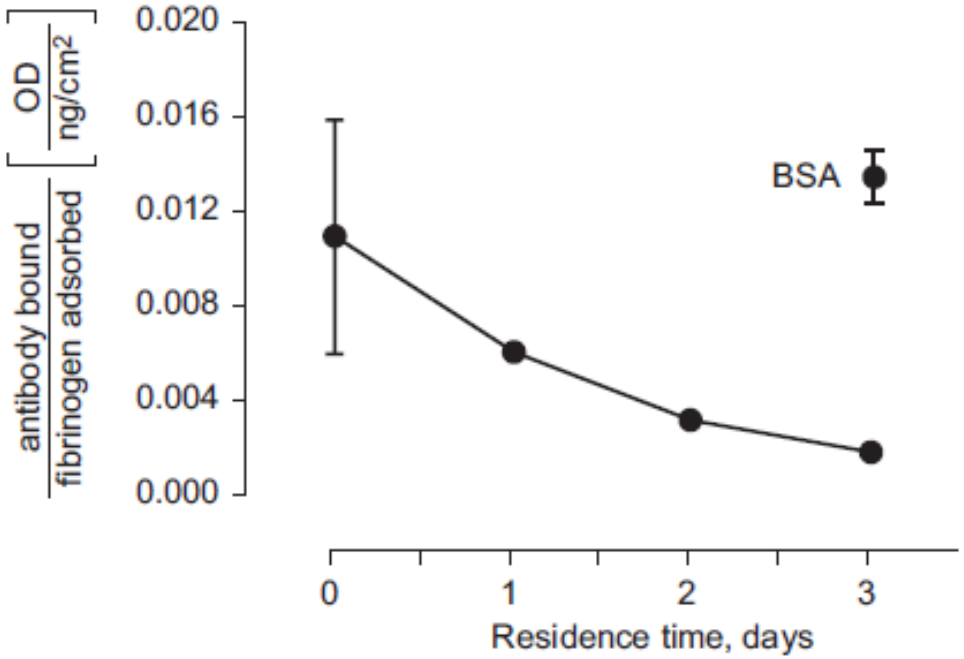

FIGURE II.1.2.12 Transitions in adsorbed fibrinogen. The effect of three-day residence in buffer or buffered albumin solution upon anti-fibrinogen binding to fibrinogen adsorbed from dilute plasma to Biomer polyurethane is shown. BSA: bovine serum albumin. (From: Fig. 3A in Chinn et al., 1992.)

CELLS AND SURFACES *IN VITRO*

- **Tissue culture is a general term for the harvest of cells, tissues or organs, and their subsequent growth or maintenance in an artificial environment.**

- Mammalian cells cultured *in vitro* have the same basic requirements as cells growing within an organism.

- *In vitro*, blood is replaced by the culture media, which bathes the cells and provides an energy source (glucose), essential nutrients (salts and amino acids), proteins and hormones (from added serum), and a buffer (to maintain pH balance).

- During culture, the byproducts of cellular metabolism are released into the media as its constituents are depleted.

- Since the culture media is not continually circulated and purified, it must be changed regularly to maintain optimal conditions for cell function.

- These culture conditions are also favorable for the growth of **unwanted organisms such as fungi and bacteria.** Since there is no immune system to control infection *in vitro*, anti-fungal and anti-bacterial agents can be added to the media on a prophylactic basis.

- To further reduce the likelihood of contamination by microorganisms, **cells are manipulated under a laminar flow hood using strict aseptic (sterile) techniques**. Laminar hoods control and direct filtered air to facilitate a sterile working environment by reducing the contact of air-borne bacteria and particulates with the culture dish and hood surfaces.

- Cells in media are contained in culture dishes or flasks and incubated in a temperature-controlled (37°C) and humidified (95%) chamber.

- The exchange of gas at the media surface acts like the lungs to maintain the gas balance necessary for metabolism. CO_2 is usually added to the incubator at a low concentration (5%). The CO_2 interacts with the bicarbonate buffer in the media to help maintain a pH of 7.0–7.4.

- Buffering counteracts pH changes in the media as it accumulates waste from cellular activity. Phenol red is commonly added as an indicator to monitor pH, and a change in the color of the media is often a sign of poor culture conditions.

- Since the culture dishes are not sealed they are prone to evaporation.

- To reduce evaporation, and prevent a subsequent change in media concentration, a water dish (with an anti-fungal/anti-bacterial agent) is usually placed in the bottom of the incubator to maintain a high level of ambient humidity.

- **Cells for *in vitro* work may be obtained from tissue (primary culture) or from cell lines.**

➢ Primary Culture

- **Cells for primary culture are obtained by surgical dissection of living tissues.** Cells can be obtained by passing media through the marrow cavities of the long bones, by collecting cells or by enzymatic digestion of tissue that contains cells. Enzymatic digestion of small pieces of tissue immersed in collagenase at 37°C breaks down the surrounding ECM and releases entrapped cells.

➢ Cell Lines

- **Cell lines refer to cells that can be passaged many times without loss of their phenotype**. Physiologically, these cells can divide repeatedly, without shortening of their telomeres
- Cell lines differ from other cells in that they have escaped the Hayflick limit and are *immortalized* (Hayflick, 1985). Examples of a handful of commonly used cell lines, from a large number of existing cell lines, are presented in

TABLE II.1.3.1 Examples of Some Commonly Used Cell Lines

Cell Line	Organism	Tissue of Origin	Further Cell Information
3T3	Mouse	Embryonic fibroblast	Fibroblasts
AML-12	Mouse	Liver	Liver cells
HeLa	Human	Cervical cancer	Epithelium (first cell line reported)
HUVEC	Human	Umbilical cord vein	Endothelium (stem cells)
MC3T3-E1	Mouse	Calvarial fibroblast	Differentiate to osteoblast

- Cell lines can be obtained from nonprofit organizations such as the American Type Culture Collection (ATCC), the European Collection of Cell Cultures (ECACC), and the Coriell Institute for Medical Research (CIMR).

UNDERSTANDING CELL–SUBSTRATE INTERACTIONS

- Surface chemistry, topography, and elastic modulus (stiffness) of the substrate are all means to control and guide cell activity, and ultimately modulate tissue formation.
- Since most cells interact with a substrate (e.g., within the ECM) it is often desirable to control the interaction of cells with substrates *in vitro*.
- **Modulation of the culture surface by fabrication of "engineered surfaces" comprised of micro- and nanosized chemical and topographical patterns or coatings have been used to investigate cell behavior such as adhesion, morphology, migration, proliferation, cell–cell communication, gene expression, production of ECM, differentiation, and responsiveness to extracellular signaling.**

➢ Surfaces for Cell Culture

- Cell attachment is often characterized by a change in cell morphology.
- Adherent cells possess a "flattened" appearance, often with an irregular cell shape and the extension of cellular processes.
- Regular observation of cells *in vitro* is essential for successful culture as cell density, state, and contamination can all be readily assessed visually

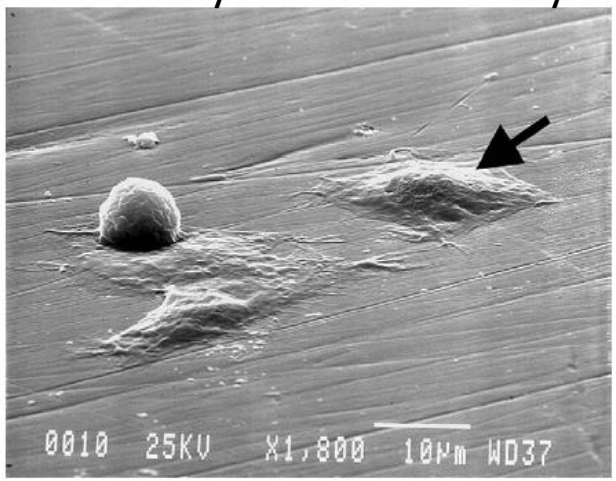

FIGURE II.1.3.1 Scanning electron microscopy image of MC3T3-E1 cells at various stages of adherence on polished titanium substrate demonstrating changes in cell morphology during attachment. Attached cells have a flattened morphology and form intimate contact with the substrate. Detached cells are round. SEM 1800X.

➢ Process of Cell Attachment *In Vitro*

- **Cell attachment is the initial step in a cascade of cell– biomaterial interactions**, and is important to cellular processes such as cell guidance, proliferation, and differentiation.

- *In vitro*, when hydrophilic surfaces like tissue culture polystyrene (TCP) are exposed to culture media containing serum, they are rapidly coated by a thin (~20 nm) layer comprised mainly of proteins that adsorb to the culture surface in a monolayer.

- The process of cell attachment to TCP (and many other materials) is indirect, since **cells do not bind directly to TCP but instead bind to the adsorbed protein monolayer.**

- **Cells make contact with, and anchor to, the adsorbed proteins at discrete peptide regions referred to as focal contacts.**

- **Cells possess heterodimeric transmembrane proteins composed of α and β subunits called *integrins.***

- Integrins are receptors that recognize and bind to specific anchoring proteins present on the conditioned TCP surface.

- **Integrins recognize and bind to specific ligands such as fibronectin, vitronectin, collagen, and laminin.**

- A common **peptide receptor for integrin is the RGD (arginine–glycine– aspartic acid**) sequence. For the majority of cells cultured *in vitro*, fibronectin and vitronectin are important for cell attachment to TCP.

TABLE II.1.3.2 Specific Peptide Sequences on Cell Anchoring Proteins

Peptide Sequence	Derived from ECM Protein	Conjugate Receptor	References
RGD	Fibronectin, laminin α-chain, collagen, vitronectin	Multiple integrins	(Ruoslahti and Pierschbacher, 1987; Massia and Hubbell, 1990a,b; Massia and Hubbell, 1991a,b; Ruoslahti, 1996)
YIGSR	Laminin β1-chain	$ß_1$ integrins	(Boateng et al., 2005; Weber et al., 2007; Weber and Anseth, 2008)
IKVAV	Laminin α-chain	LBP110	(Tashiro et al., 1989; Weber et al., 2007; Weber and Anseth, 2008)
REDV	Fibronectin	$α_4ß_1$ integrin	(Hubbell et al., 1991)
DGEA	Collagen type I	$α_2ß_1$ integrin	(Staatz et al., 1991; Weber et al., 2007; Weber and Anseth, 2008)
KQAGDV	Fibronectin γ-chain	$ß_3$ integrins	(Mann and West, 2002; Gobin and West, 2003)
VAPG	Elastin	Elastase receptor, $α_5ß_3$ integrin	(Mann and West, 2002; Gobin and West, 2003)

CELL RESPONSE TO SUBSTRATE CHEMISTRY

- At the macroscale, a variety of strategies can be employed to modify culture surface chemistry. **Most coating strategies aim to increase cell adhesion or to preferentially select for certain cell types.**

- Substrates have been coated in these ways with a v**ariety of organic and inorganic compounds,** such as collagen, fibronectin, gelatin, and poly-L-lysine.

- Culture surfaces have also been broadly **coated with specific adhesion-related peptides.** The peptide sequence arginine–glycine–aspartate (RGD) has been immobilized on a number of materials as a means of enhancing two-dimensional cell attachment.

➢ Micrometer-Scale Chemical Patterns

- Using micropatterned fibronectin islands, Chen et al. reported that the area available for cell adhesion and the resultant cell shape governed whether individual cells grow or die

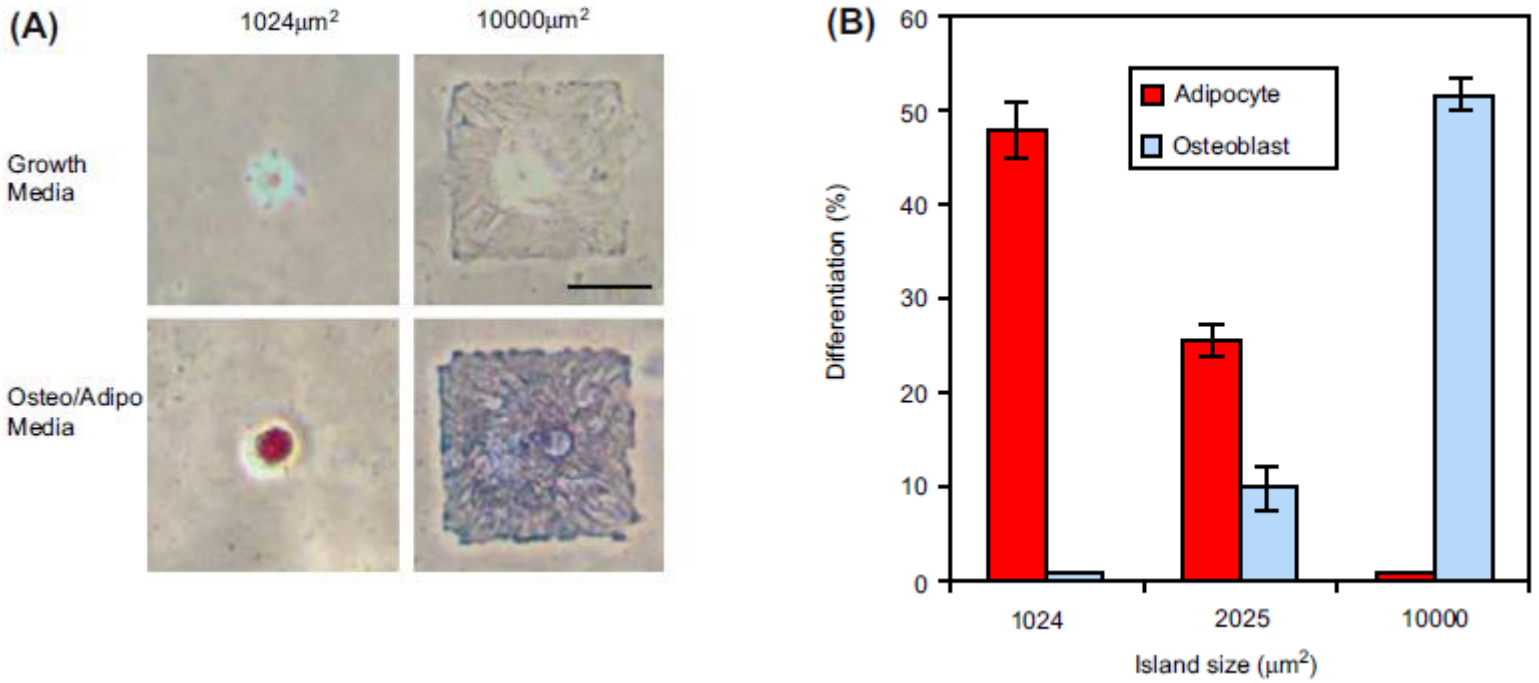

FIGURE II.1.3.2 Cell shape modulates hMSC differentiation: (A) Brightfield images of hMSCs plated onto small (1024 μm2) or large (10,000 μm2) fibronectin spots after 1 week. Fibronectin spots were patterned on a mixed SAM substrate by micro-contact printing. Large fibronectin spots supported osteogenesis (blue) while small fibronectin spots supported adipogenesis (red). Scale bar 50 μm. (B) Differentiation of hMSCs on 1024, 2025, or 10,000 μm2 islands after 1 week of culture. Small fibronectin spots resulted in adipogenesis, medium fibronectin spots supported both adipogenesis and osteogenesis, and large fibronectin spots resulted in osteogenesis. (Reprinted from McBeath et al. (2004). Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment, *Developmental Cell,* **6**(4), 483–495. Copyright (2004), with permission from Elsevier.)

➢ Nanometer-Scale Chemical Patterning

- Nanosized chemical patterns generally **do not direct cell shape** or orientation as a result of their extremely small size relative to the cell.
- However, surfaces with chemical features on the nanoscale do modulate cell functions such as adhesion, proliferation, migration, differentiation, and gene expression.

CELL RESPONSE TO SUBSTRATE TOPOGRAPHY

- **surface topography defines the specific morphological characteristics of a surface. Surface topography may be generally described as isotropic (uniformity in all directions) or anisotropic (uniformity in one direction).**

- In terms of cell activity, surface topography has been reported to affect proliferation, gene expression, cell adhesion, motility , alignment , differentiation , and matrix production .

- **In sensing and interacting with the topographical environment, evidence suggests that cells extend fine processes termed *filopodia.***

- While the specific mechanisms are poorly understood, changes in cell activity resulting from interaction with surface features have been linked to changes in cytoskeletal arrangement including actin filaments, nuclear shape, and ion channels.

➢ Micrometer-Scale Topography

- With microscale-sized features, cell alignment generally increases with increasing groove depth and decreasing groove spacing ; however, cell response to topography is highly dependent upon cell type

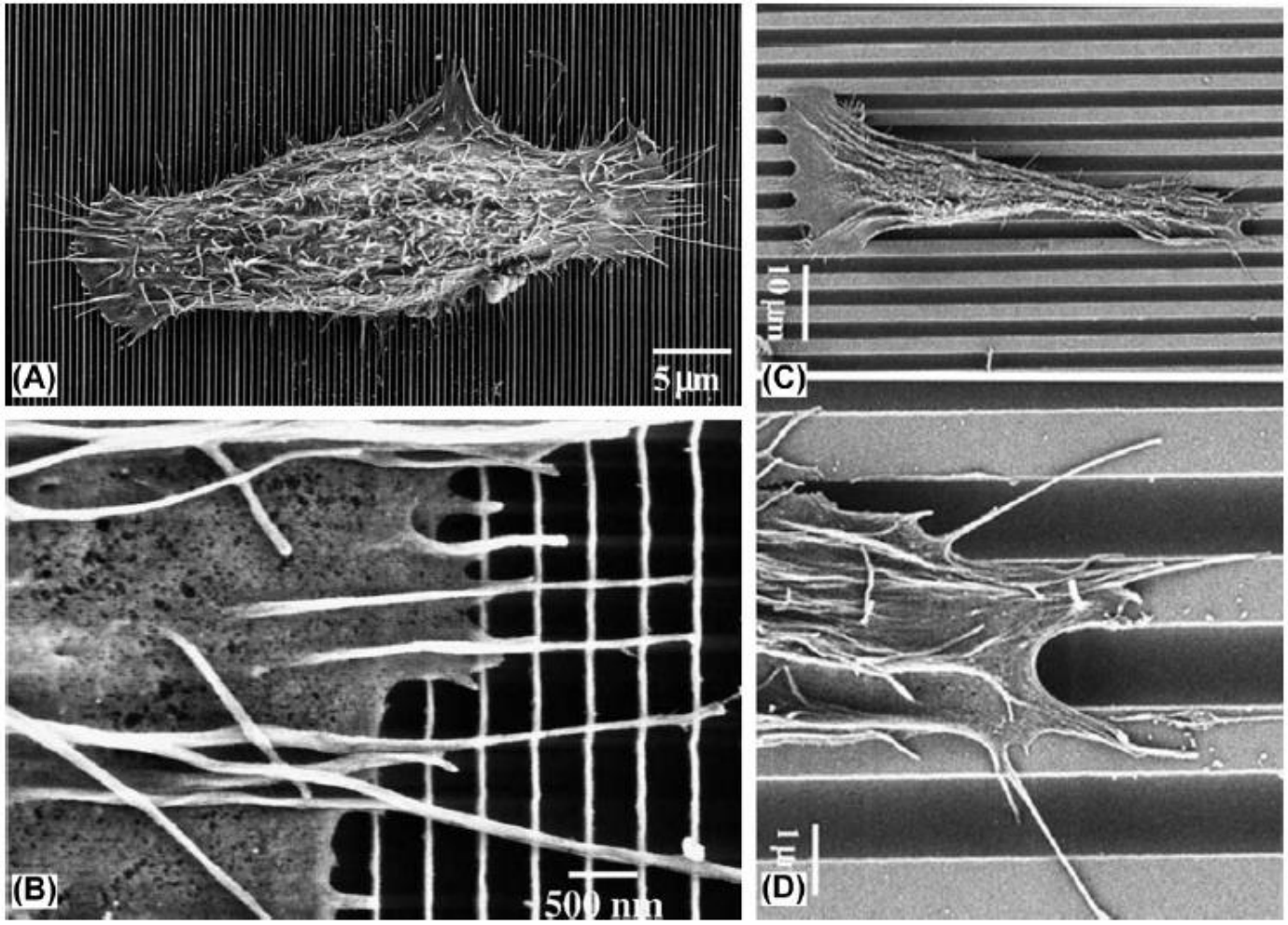

FIGURE II.1.3.4 Effect of micro and nano patterns on corneal epithelial cell alignment. (A) Cells were aligned perpendicularly to ridges that were 70 nm wide and 330 nm apart. (B) Expanded view of (A) showing filopodia also aligned perpendicularly to the patterns. (C) With increasing width and spacing (1900 nm ridge width, 2100 nm spacing), cells were aligned with the ridges; and (D) filopodia were guided by the topographic pattern. (Reprinted from Teixeira et al. (2006). The effect of environmental factors on the response of human corneal epithelial cells to nanoscale substrate topography. *Biomaterials*, **27**(21), 3945–3954. Copyright (2006), with permission from Elsevier.)

Nanometer-Scale Topography

- cells can sense and respond to features as small as 10–30 nm

- Compared to the microscale, repetition of similar features at the nanoscale has the greatest effect and provides the most predictable results regarding cell behavior.

- Proteomic-based studies have shown that cells respond to nm-sized pits and pores in irregular patterns, leading to **increased differentiation and matrix production by human osteoprogenitor cells.**

- Likewise, studies with surfaces fabricated by the arrangement of titanium nanotubes have demonstrated that the **nanotube diameter affects both hMSC differentiation and adhesion** (Oh et al., 2009).

    - **Smaller diameter nanotubes (30 nm) increased adhesion, while larger diameters (100 nm) increased differentiation.**

- **Topography at the nanoscale can also be used to effectively reduce cell adhesion.**

    - Kunzler et al. generated a constant gradient of nano-features (65 nm diameter and height) with the spacing as the only changing parameter along the gradient (Kunzler et al., 2007).

    - This study demonstrated that the **spacing or density of non-adhesive nanoscale features can disrupt cell adhesion, likely by restricting receptor–ligand interaction.**

CELL RESPONSE TO SUBSTRATE ELASTICITY

- **Cells respond to the physical or mechanical properties (stiffness) of the substrate**.

- **Stiffness** can be described as the resistance of a solid material to deformation, and is commonly defined by elastic modulus (*E*)

- **Hydrogels are well-suited to the study of cell substrate interactions** since their stiffness can be varied by controlling the water content of the gel, which is controlled by modifying the polymer concentration or the relative extent of cross-linking.

- Hydrogels can also be made from a very diverse variety of natural and synthetic materials, such as hyaluronic acid (HA), fibrin, alginate, agarose, chitosan, polyacrylamide, and PEG.

- **The stiffness of the substrate affects cell adhesion, spreading, and migration, especially the last two.**

- **In two-dimensional cultures, cells preferentially migrate towards surfaces of greater stiffness, a phenomenon referred to as mechanotaxis.**

    - In two-dimensional systems endothelial and fibroblast cells cultured on collagen-coated substrates that were classified as compliant (~5 × 10^3 Pa) or stiff (~70 × 10^3 Pa) demonstrated remarkably different behaviors.

    - **Cells cultured on compliant substrates** had reduced spreading, increased lamellipodia activity, and greater migration.

    - **Cells cultured on stiffer substrates** generally increased in proliferation; however, the specific effect seen is cell-type dependent .

    - There is substantial evidence that substrate stiffness also affects cell proliferation in two-dimensional as well as three-dimensional culture systems

CELL RESPONSE TO MECHANICAL DEFORMATION (STRAIN)

- Forces can be applied directly to adherent cells *in vitro* by either elongation or compression of their substrate (two-dimensional) or matrix (three-dimensional).

- With respect to cyclic tensile loads, in both two-dimensional and three-dimensional cultures, **cells predominantly align in the direction of the applied load and assume an elongated morphology.**

- Because of the loadbearing function and **well-known adaptation to load of the musculoskeletal system**, cells of this system have been evaluated on surfaces under conditions of tensile and compressive strain.

    - Toyoda et al. cultured **cells harvested from the anterior cruciate ligament (ACL) and synovium**. Cells were subjected to cyclic tensile load for 24 hours on culture plates with flexible rubber bases.

    - **For both cell types, tensile load increased cell alignment and elongation; however, tensile load only increased the production of collagen type I in cells derived from the ACL.**

- **fibroblasts subjected to cyclic tensile strain increased the formation of organized ECM**, increased collagen production, and increased metabolic activity compared to unloaded controls.

- **Bone** is another well-known loadbearing tissue, and the extent of mineralization and **bone mineral density is load dependent** .

    - *In vitro*, cyclic strain in both tensile and compressive loading enhances osteoblast mineralization. **Thus, strain appears to be an important stimulus for the generation of mineralized and tendon-like tissues *in vitro.***

- These findings have led to the **fabrication of bioreactors** that can apply physiologic loads to developing tissue to enhance the production and alignment of ECM in a number of tissues including cardiac , bone , cartilage , and tendon .