Tissue Engineering
- Motta -

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Contents

Chapter 1

General course information

1.1 Textbooks

- Scaffold for Tissue Engineering: Biological Design, Materials, and Fabrication
- Introduction to Tissue material interactions (PDF)
- Selected chapters from : Principles of Regenerative Medicine (prof. Atala) (PDF)
- Selected chapters from: Adult wound healing (Yannis), and Extreme Tissue Engineering
- Selected scientific papers

1.2 Assessment

- *TODO Still trying to define exactly*

1.3 Topics

- Concept of therapeutic device, transplant or implant?
- From tissue substitution to tissue regeneration: Introduction to tissue engineering
- Biocompatibility: concept evolution, mechanism, new approaches
- Structure, function, of ECM, role in cell activity, Cell/ECM interactions
- Wound healing: repair, regeneration, scar tissue formation
- Foreign body reaction: immuno, inflammatory, blood coagulation, complement system ECM as a model for scaffold design: engineering biomimetic scaffolds
- Polymers, biopolymers, hydrogels, fabrication methods
- Material/biological system interactions
- Strategies to control scaffold vascularization

- Strategies in TE: from top down to bottom up approach
- Organ printing and cell encapsulation
- TE applied to 3D in vitro models and lab-on-chip: drug screen, cancer studies, personalized medicine.
- In vitro-in vivo evaluations: bioreactors
- Lectures in lab: practical demonstration on scaffold fabrication

Chapter 2

Basics of tissue engineering

2.1 Definition of tissue engineering

At first, **tissue engineering** was defined as *"A combination of principles and methods of life sciences with that of engineering, to develop materials and methods to repair damaged or diseased tissues, and to create entire tissue/organs replacements"* (1980s). This definition was soon outdated since it did not consider a fundamental difference:

- **Repair** is the act of closing the wound, restoring the macroscopic structure; the tissue formed during repair is often different from the starting tissue (scar tissue for instance), and thus the different properties undermine the function of the repaired area due to the different properties.
- **Regeneration** is also a way of closing a wound, but the structure is restored using cells of the same type as the starting one, therefore maintaining the functionality of the area.

Tissue engineering in fact aims at regenerating the structure and functionality of the district, not merely closing the wound.

A following definition puts the focus on the regeneration aspect, underlining the necessity to understand the mechanism guiding regeneration: *"The applications of principles and methods of engineering and life sciences, to obtain a fundamental understanding of structural and functional relationships in novel and pathological mammalian tissues, and the development of biological substitutes to restore, maintain or improve tissue function"*(late 1980s).

Nowadays, tissue engineering could be defined as *"a biomedical engineering discipline that uses a combination of cells, engineering, materials, methods and suitable biochemical and physicochemical factors to restore, maintain, improve or replace different types of biological tissues."*

Tissue engineering holds promise of producing healthy organs for transplant by using patient cells (or immuno compatible cells). Tissue engineering can be combined with gene therapy, therefore including the correction of incurable genetic defects (such as sickle cell anemia).

2.2 History of tissue engineering

Tissue engineering is born from the concept that interaction between cells and extracellular matrix is important for understanding the structural and functional relationship of these components. The first experiment on tissue engineering was conducted by W.T. Green, who tried to regenerate bone using chondrocytes (since in the physiological process, bone is obtained by calcification of cartilage);

he managed to obtain bone formation in nude mice, thus he concluded that with the advent of innovative biocompatible materials it would be possible to generate new tissue by seeding viable cells onto appropriately configured scaffolds. Later on, Langer and Vacanti created artificial scaffolds for cell-delivery, rather than using natural derived scaffolds which are difficult to replicate. The use of artificial matrices specifically designed for the system allowed to obtain reproducibility and high-quality. (*Skipped other experiments and history of tissue engineering*)

2.3 The need for tissue engineering

Tissue/organ transplant is a heavily limited solution for tissue/organ failure; some of the main limitations being:

- Donor-recipient compatibility: it is almost impossible to find a fully compatible donor (all major histocompatibility complexes matching with the recipient) and the use of non-fully compatible organs/tissues requires the recipient to undergo immunosuppresive therapy (generally chronically) to avoid rejection of the transplant.
- Rejection risk: rejection can occur regardless of compatibility and immunosuppressive therapy, therefore this risk can never be avoided completely.
- Organ/tissue scarcity: even not considering compatibility, the amount of organs/tissues that can be donated is very scarce, since most of them come from car accident victims or relatives.

For this reasons implanting artificial devices has grown more popular since:

- They are ready to use
- They immediately restore the function of the organ/tissue
- They can be personalyzed
- They do not cause rejection

Still, implants present many limitation:

- They require invasive surgery
- They can cause foreign body reaction
- They cannot replace completely the functions of the organ/tissue (limited performance)
- They have limited duration

2.3.1 Examples of implant devices

Some examples of implant devices are:

- **Hip joint prosthesis**: made of metallic alloys (mostly based on titanium) and ceramic (for the joint socket). It immediately restores the function of the joint but it requires very invasive procedures (long segment inside the femur) and overtime it sticks to the bone, making it really hard to substitute it. This last point is of little relevance if we consider older people, but it is a big problem for younger ones.

- **Vascular stent**: a stent is a cylindrical tool that is used in angioplastic procedures, meaning it prevents the stenosis (blockage) of (usually coronary) arteries. A flexible probe mounted with a stent, a balloon and some way to visualize the probe from outside the body (via ecography for example) is inserted in the femural artery; then the probe is navigated to the damaged region and the balloon inflates positioning the stent. This allows to restore blood flow to the miocardium without open heart surgery, even in an emergency setting such as in case of heart attack. The main problem is that the artery keeps contracting and expanding, therefore the stent can damage the vessel causing necrosis, proliferation of fibrotic tissue, inflammation; one way to reduce the problem is to use polymers rather than metallic alloys, since they are more flexible, but they are also less durable. Moreover stents are in contact with the blood and thus provide an abnormal surface that can start platelette aggregation and coagulation, creating blood clots; the use of slow release anticoagulant drugs stents helps with that aspect.

- **Artificial heart**: heart shaped device with valves. This device does not work as a pump since it cannot contract like miocardial fibres, therefore it require some form of auxiliary external pump. Just like with stents you can have abnormal coagulation on the surface of the device or due to turbolences in the blood flow.

- **Bone scaffolds**: they are three-dimensional biomaterial structures used for bone defect reconstruction. An ideal scaffold should have features such as improving cell adhesion, proliferation, osteogenic differentiation, vascularization, host integration and, where necessary, load bearing (drugs for instance). These design parameters should lead to specific scaffold properties, which include biocompatibility, porosity, micro and nano-scale structure, degradation rate, mechanical strength, and growth factor delivery, all of which dictate the biomaterial to be used or developed (*further explaination later on*).

2.4 Tissue engineering paradigma

Since artificial devices do not replace all the functions of a lost organ or tissue and often fail in the long term, a new approach stems from two considerations:

- Living tissues and organs can be routinely assembled and reliably integrated to the body to restore, replace or enhance tissue and organ functions.

- Biomaterials can interact with living tissue and influence cell function and response.

This approach is infact tissue engineering which is, using yet another definition for it, *"creation of a new tissue for therapeutic reconstruction of the human body, by the deliberate and controlled stimulation of selected target cells, through a systematic combination of molecular and mechanical signals"*. Basically you are not creating ex novo a tissue/organ to implant, but you are inducing some biological mechanisms that helps the body heal itself; this idea is more generally represented by the term **regenerative medicine**, which includes not only tissue engineering but also cell therapy and gene therapy. The main actors involved in this process are:

- **Cells**: generally stem cells derived from the patient (to avoid rejection).

- **Scaffold**: which is the structure used to induce and guide the growth of the cells; tissue engineering focusses mostly on material, surface and properties of this component.

- **Time**: required in order to induce and obtain regeneration in unnatural conditions.

2.4.1 Scaffold characteristics

The main characteristics of a scaffold that must be considered are:

- **Mechanical properties**: ability to withstand mechanical stress (elasticity, compressibility...).
- **Morphology**: shape, size, structure...
- **Physical properties**: behaviour when temperature, pH and other aspects of the environment change.
- **Histology**: type of tissue it has to replace.
- **Porosity**: permeability of the structure to different elements; it must allow the cells of interest to grow and penetrate into the structure while keeping outsione unwanted cell types.
- **Water content**: the structure must contain enough water to allow nutrients to reach all the cells.
- **Surface**: the surface of the scaffold must be functionalized with molecules that are recognized by surface receptors of the target cells, thus inducing gene activation and some form of response (adhesion, expression upregulation/downregulation...).

From these premises we get the so called **tissue engineering paradigma**, which is the basic flowchart that most tissue engineering procedures follow. In general, the main steps are:

- **Collecting and isolating host cells of interest**: the type of cells needed for the procedure depends on the damage site; according to the cells needed (generally stem cells, sometimes primary cells) a biopsy is performed on an adequate tissue (blood, skin...). Moreover, since tissue are generally heterogeneous, some steps are required to isolate the cells of interest from the others. The fact that donor and recipient coincide, there are no compatibility issues.
- **Seeding cells on a scaffold**: the cells are then seeded on a scaffold made of some biomaterial which is biocompatible for the application at hand (*biocompatibility characteristics are discussed later on*). This scaffold must provide adequate conditions for cell growth and proliferation, such as the presence of growth factors and cytokines.
- **Cell stimulation in a bioreactor**: The seeded scaffold is then placed into a static or dinamic bioreactor which induces and stimulate cell growth and proliferation. This bioreactor must be able to provide all the stimuli needed for cell differentiation and organization according to the desired final result (this may include mechanical stress for instance, needed for miocardial differentiation, or type of surface, since the differentiation of chondrocytes depends on the form they assume due to adhesion).
- **Re-implantation**: the fully prepared and populated construct is then implanted into the damaged area, where it will integrate itself with the surrounding tissues.

This approach has some major downsides, namely:

- It is very **labour intensive** and **time consuming** since it takes time for the cells to proliferate and populate the scaffold, moreover the growth conditions depend on the cell type of interest (which can be difficult to define, since the whole physiological environment must be taken into account, therefore angiogenesis, immune system, vacularization, lymphatic system and much more).

- Given the production time, this approach is **not ready to use**, therefore it cannot be used in an emergency setting.
- The amount of time and labour needed for the production implies **high cost**.

In some cases, it is possible to simplify the procedure by implanting the scaffold immediately after it was populated with cells in the damaged area; this is called **in-situ regeneration**. In situ regeneration requires way less preparation time (almost ready to use) and uses the body of the recipient as a bioreactor, therefore reducing labour (you already have all the machinery required), need for bioreactor setup and overall costs. Notice that model animals cannot be used as bioreactors, since the mechanical properties of the tissues are not comparable (vertebrae of pigs and sheeps are built to support different weights compared to the human ones), the use of primates is strictly regulated by law and no animal is perfectly compatible with humans (therefore you risk rejection). The in-situ regeneration approach is not always applicable and just like the slower version it has a lot of room for improvement, for instance the development of more functional and durable polymers to use as scaffolds. Another problem for both strategies is the need of starting material, generally stem cells: the patient may not have enough excess tissue for an autologous transplant for instance. One way to mitigate this problem would be to save part of the umbilical cord to extract staminal cells, but in Italy this is forbidden by law (*as of writing the notes*).

2.5 Tissue engineering approaches

There are two different **approaches** for tissue engineering:

- **Top-down approach** (traditional approach): cells are harvested from the donor, cultured and modified if needed. They are then seeded on a porous scaffold that during cell proliferation is slowly degraded by the cells and replaced by extra cellular matrix (ECM). The engineered tissue is then implanted into the patient. The main advantage of this procedure is that it is possible to produce mechanical stress, which is needed for the differentiation on certain types of cells.
- **Bottom-up approach** (modular approach): Some fundamental elements, such as cell sheets, cell aggregates, cell laden modules and bioink (3D printer ink containing cells) are used to construct a 3D module assembly, which can then be implanted into the patient. This modular building process allows to create very complex structures, with gradients and without a scaffold. Since a temporary gelatinous matrix is used, the module assembly lacks the rigidity provided by a scaffold, therefore it does not easily maintain the mechanical stress. Furthermore, when 3D printing, many other aspects have to be taken into account, like the permeability of the matrix to nutrients, the sensibility of the cells to the stress due to the extrusion from the needle and others.

Chapter 3

Biocompatibility

Chapter 4

ECM

Chapter 5

Inflammation

Chapter 6

Biorecognition

6.1 Molecular Biorecognition

Tissue engineering and regenerative medicine require an intimate understanding of the native ECM, together with the complexity of cell and tissue biology. For therapeutic applications, we should mimic the basic structure of ECM using a variety of synthetic or naturally derived materials and fabrication methods. The scaffold can be seen as a 3D growth environment:

- basic structural properties of ECM
- molecular cues to control bio responses
- protease sensitive site for enabling migration
- locally delivery of soluble factors for tissue remodelling stimulation

6.1.1 Functions of the ECM

- aids in locomotion
- transmits and distributes mechanical loads
- prevents premature mechanical failure
- partitions cells and tissues into functional units (scaffold architecture)
- acts as a scaffold that define tissue and organ architecture
- acts as a storage and dissipative devices for elastic energy
- acts as substrates for cell adhesion, growth and differentiation

These functions are defined by composition, structure, mechanical properties and repair response, which depend on tissue type, physiopathology, mechanical forces, damage and healing process.

Figure 6.1:the first example is a normal situation, good substrate. In an aged person we have a loss of elasticity. In the case of a wound, we observe a coagulation cascade and scar tissue formation - which is characterized by a compact tissue. The temporary material (crust) is used as scaffold.

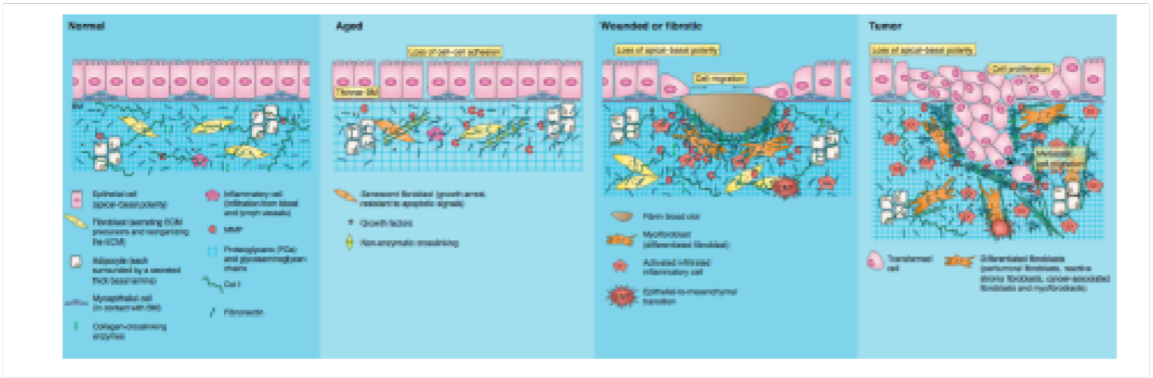

Figure 6.1: ECM functionalization

We can have healing by repair or regeneration. There is a higher rigidity in the case of temporary material. In the case of a tumour, we have an abnormal growth and migration inside - which they should not be present. The migration is caused by fibers not organising to close the hole.

6.1.2 Surface chemistry: biorecognition in ECM

Cell biology is governed by a complex series of interactions with the ECM. It is not enough to promote proliferation, we should control the adhesion involving the integrins (connected with few genes). We need a pool of molecules, since biological functions are orchestrated by a symphony of signals. Communication between cells and the cellular matrix is very relevant.

A **biomaterial** is a substance that has been engineered to take a form which, alone or as a part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure in human or veterinary medicine. The aim is to recreate a basic environment before inducing regeneration. We need to upregulate proliferation at the beginning to create a population, then stop it for reaching ECM formation and achieving a therapeutic impact. Cells can interact moving molecules through the gap junctions or through integrins; there are cells that must be very close e.g. skin, myocardium, in other cases integrins are mandatory. The ECM should also provide security for travelling molecules, as molecules should not be degraded and must be kept in the active conformation. Nutritional status is the bottle neck of tissue engineering, because in big wounds it is difficult to provide nutrition - as we need angiogenesis in order to have it. Hydrogels are cool because they can provide a lot of these requirements and can be combined to provide also mechanical support.

The cell language is based on a mapping (micro- and nano-patterning) of biopolymers (dynamic ECM) shapes to their complementary binding. Chemical process during cell activity:

- Chemical reactions (irreversible): provide free energy (ATP $\rightarrow$ ADP)
- Biopolymer (ECM) shape changes (reversible): provide control over chemical reactions

6.1.3 Biocompatible materials: foundation ideas

In order to design suitable materials, we should learn the biological pathways that lead to normal healing and reconstruction. Secondly, it is required to develop bio recognition surfaces that turn these pathways on and off. It is necessary to know specific affinities for the key molecules found in healing wounds that are associated with vascularised healing and regeneration (triggering local "unnatural" local healing). If biomolecules are immobilised at the surface, they must be in the correct orientation and conformation. Porosity should be engineered to induce vascularisation and less fibrotic tissue. Furthermore, the modulus matching of scaffold-biomaterial should match with their intended use (modulus mismatch should exacerbate the FBRx). Lastly, the engineered scaffold should be able to degrade into non-reactive substances at predetermined degradation rates to serve as a temporary guide for healing.

6.2 Cell interactions

Cells interact with the environment thanks to soluble factors, extracellular matrix and receptors. Signals form ECM and neighbouring cells:

- gene expression regulation leads to adult stem cell differentiation [into the lineage of interest e.g. osteoblasts in bone]
- tissue specific differentiation
- survival of primary cells
- Interaction with apoptosis: under external stimuli the cells may go to apoptosis. This may be useful in case of chemotherapy

The interaction between cells and matrix can be compared to a chemical reaction, where reagents can give rise to a reaction in a specific condition. Reactants are the cell + matrix, the specific condition is the biorecognition, we need integrins [without the plus ("+") there is no reaction. All the reactants must communicate with each other. Integrins are important for response]. In order to achieve regeneration, we need to activate a number of functionalities, which should be listed according to time. The scaffold is a reactant, it is added in the bioreaction. Of course we also have mechanical stresses involved in the reaction. We need to take into account the control system, which should not be downregulated e.g. activate tissue regrowth and activate control system to stop when the tissue volume is enough.
How does the body respond to the material? Cells are in suspension in the culture, which contains serum (proteins). The surface first absorbs a coating of ions and proteins/lipids, which are then recognized and bound by cells. Which kind of proteins will be absorbed by the implant? The quality of proteins depends on the chemical/mechanical properties of the scaffold and the context e.g. blood, skin, brain... For sure the first protein absorbed will be taken by plasma when we have bleeding. Depending on the proteins selected, the cells will adhere when the integrins of the cells can find some ligands among the proteins absorbed. We only have cell adhesion when we have biorecognition of the scaffold.

Surfaces must be designed in order to control protein absorption. Depending on the phenotype, we have different groups of integrins and also integrins specific for different genes. The aim of TE is to promote cell adhesion through specific integrins needed to achieve regeneration. Scaffolds and cells are not isolated, the empty spaces are filled by the ECM (providing proteins and water). This

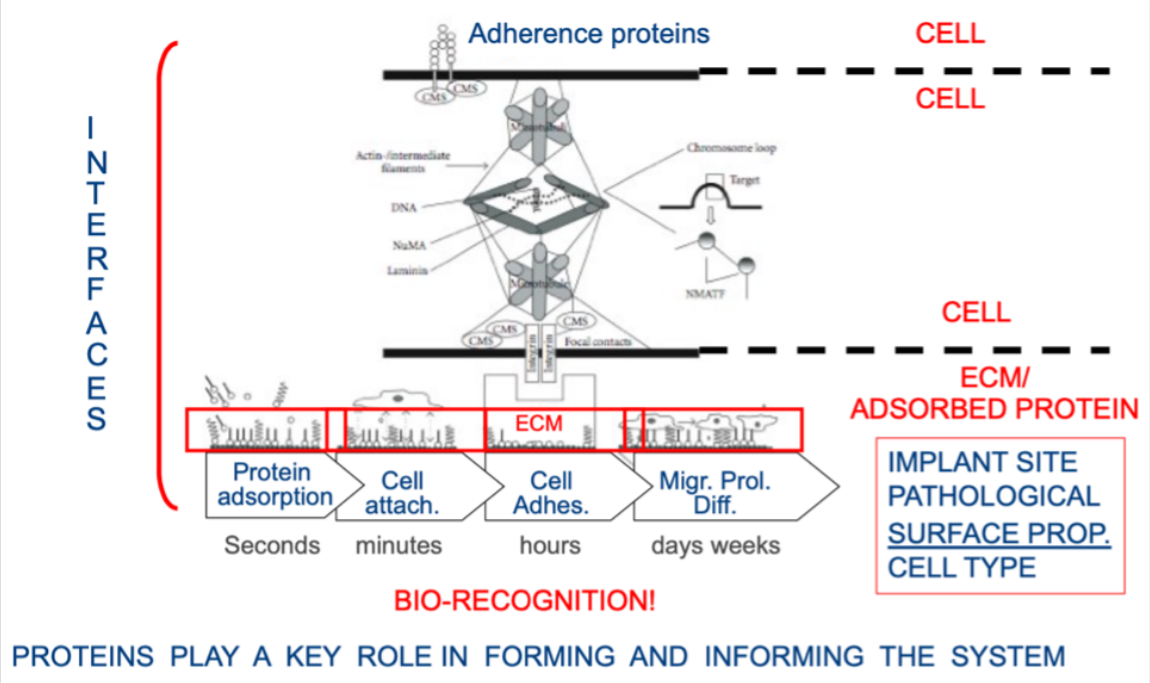

Figure 6.2

first step is crucial, depending on this we will reach failure or success. Therefore, the surface of the scaffold is really relevant. We can change the chemical properties, functionalize with new chemical groups, modify morphology to drive the interaction between scaffold and cells. The interaction between the implant and biological systems is a dynamic process. The biomaterial is characterized by specific properties. If we incubate the material in a single protein solution, where the protein is in active conformation. Depending on the surface properties, the biomaterial will absorb the proteins, which will remain active, or we will witness deactivation / degradation / modification.

1. Protein is absorbed with original conformation = still active
2. Denaturation during absorption = not active, switch off
3. Different conformation = different activity [dangerous, unexpected situation]
4. Degradation = no specific activity, negative response and inflammation [small pro-inflammatory peptides are released into the environment]

Having the protein active/inactive could be both positive or negative, it depends on what we need, what we want. The protein absorption mechanism is very dynamic, the absorbed proteins can also be released.
The protein substrate is important, we can change the material and see how it behaves. We could observe different interactions with the proteins according to the starting material.Biocompatibility is a two-way process, the host affects the implant and vice versa. Also depending on the domain we will have a different adhesion, we can find differential adhesion distribution.

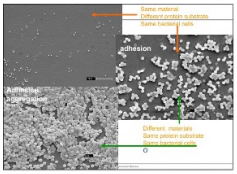

Figure 6.3

Figure 6.3: the aim was to produce a surface avoiding bacterial infection. The polymer is polyurethane, used for catheter production - antibacterial properties are really required. We see that the surface in contact with different protein substrates leads to different adhesion levels. When instead the protein substrate is equal with different materials, we see either adhesion or adhesion aggregation.

Figure 6.4: vascular graft that failed after 6 years of implantation. The implant failed because the

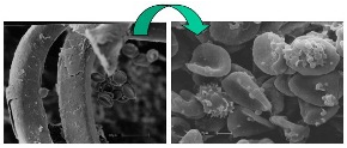

Figure 6.4

surface was completely destroyed. The bacterial cells (there was infection) adhered to the implant and this activated inflammatory response. Inflammatory cells digested the implant, made of a really strong polymer (same as parachutes). In addition, the implant started to detach in the circulation system + bacteria were able to attach to red blood cells. This is one of the few cases in which we do not want cell adhesion.

6.2.1 Molecular mechanism of cell adhesion

The orientation of ligands is critical for cell adhesion and biological function. The RGD sequence promotes cell adhesion and it is usually included in a long peptide – to avoid that RGD is involved in the link between cells, it would be invisible. Depending on the protocol parameters, we can achieve a nice or absent adhesion. The density is the same, the only difference is the orientation. For instance we could have a different density of signal, leading to a different outcome in adhesion. The density of signal is important for the function. Our aim is to reach an equilibrium, not too much or too little adhesion. In the second scenario the cell is able to move, good choice if cells are required to migrate into the scaffold. If instead we need a fast formation of a layer we can choose the first case. No absorption = no adhesion at all. This may be useful in some cases, e.g. in blood vessels to prevent thrombus, release of proteins from nanoparticles in cancer treatment. Cell adhesion is mainly controlled by the surface. How can we know whether the conformation and orientation is correct? We can employ cell sensors, screening.

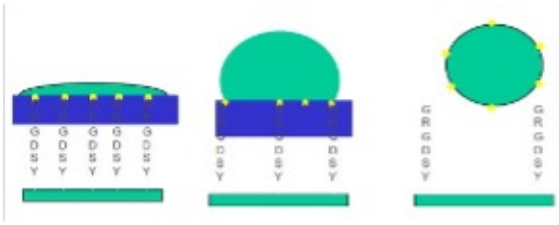

Figure 6.5

6.2.2 Cell adhesion

Cell adhesion is a tightly regulated and dynamic biological process. It is central to physiological and pathological processes and critical to biomedical and biotechnological applications. Adhesive interactions involve:

- anchorage (promotes migration, tissue organisation)
- signaling (promotes activation, survival, proliferation, differentiation)

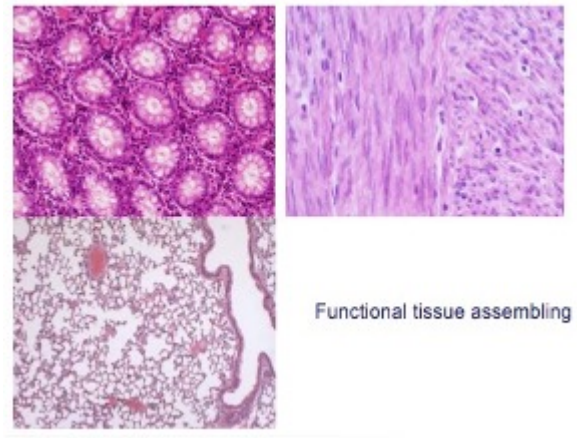

Figure 6.6

In figure 6.6 starting from top left we see the functional tissue assembling of glands (radial section), muscles, and lung/alveolar tissue. In the case of muscles, functional tissue should support mechanical stress. In glands we have cells forming very well polarized tubes for transport, which are called capillary tubes. For lung tissue we have blood vessels, we need to have a small surface and permeability for gas exchange. The air should move into the bloodstream, so the tissue should be very thin and adherent to the vessels. We have a low amount of ECM, the mechanical support is provided by cells themselves. The structure is function-dependent. Assembly is driven by ECM and adhesion patterning. The morphology should depend on the function. Thin layer of ECM in the basal lamina, collagen fibrils. This provides elasticity, the fibrils are not a bundle, but organise somehow.

6.2.3 Cell-material interactions

Cells cannot adhere to synthetic surfaces, as there is no biorecognition (ECM performs better). Cell adhesion to synthetic and bio surfaces occurs through a specific receptor interaction with adhesion protein/motifs:

- proteins adsorbed from physiological fluids (fibronectin, vitronectin, fibrogen)
- ECM components present or deposited by cells (fibronectin, collagen, laminin)
- biospecific sequences engineered on surfaces (RGC,YIGSR) for biorecognition

Adhesion receptor families are cadherins, selecting, HSPG, integrins, Ig superfamily. Specific integrins act on specific receptors, so we must be precise. For instance, alpha5beta3 can recognize the ligands into fibronectin, collagen is recognize by alpha2beta1. FIG How to measure the adhesion strength of the cells? Experiment by Gallant and Garcia (2003). They provided two samples, performed centrifugation and measured cells attached and cells not adhered. In this way you can measure the difference in the strength of the adhesion.

Depending on the surface chemistry, we will have a different cell adhesion rate. According to the protein coating, e.g. either fibronectin or type I collagen, we will have different biological performance. A number of evaluation methods can give us an idea of the adherence strength.

Figure 6.7 design scaffold for neuron regeneration. Adhesion is necessary, but neurons also need to form connections with other neurons. RGD goal: neurite outgrowth, strategy: RGD- functionalization. They provided the material with fibrin, fibrin with low RGD and fibrin with high RGD. RGD is the classical adhesion peptide. We observe a different response: high density is too much,

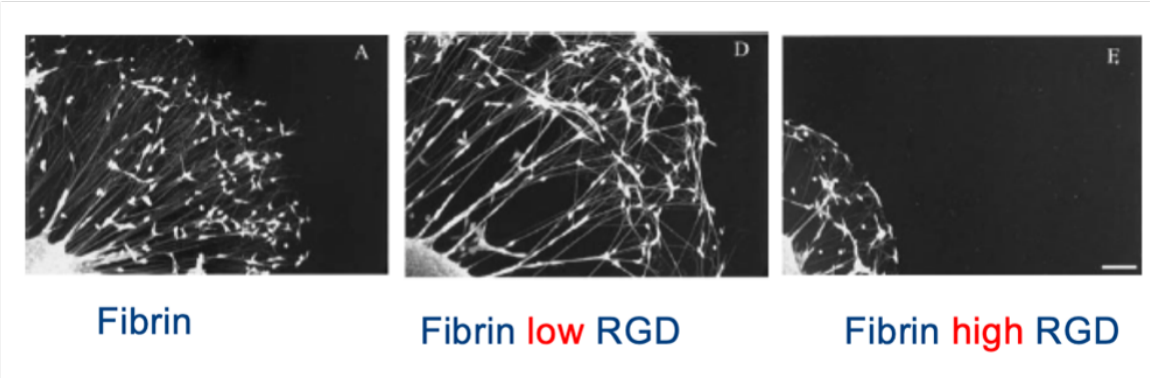

Figure 6.7

instead with a low amount of RGD we have a good outcome with a huge number of connections. When we increase RGD, the surface becomes more adhesive; this is mainly due to the fact that the cells are too sticky, but also because we must reproduce a biological environment. The amount of RGD should resemble the natural one. In fibronectin, the RGD content is 1 per chain, low amount. We have to move inside the natural range of pH, etc, ... We want natural conditions. The language of the cells cannot be changed, we have to adapt our language. Signal is the combination of the ligand and density.

Adhesion is completely different in 2D and 3D. Cell adhesion is characterized by three stages: attachment cell body, flattening and spreading, and organization of the actin skeleton with the formation of focal adhesion between cell and its substrate. The strength of adhesion becomes stronger with the length of time a cell is allowed to adhere to a substrate or another cell.

6.2.4 Cell organization

The cell's adhesive interactions with the surrounding ECM (number of adhesive motifs, distribution, density) and neighbouring cells define cell shape and organisation, controlling functionality. This environment regulates the cell survival, differentiation, proliferation and migration.

Chondrocytes are exposed to compressive forces, interstitial fluid flow and adhesive cues (cytokines) for cartilage maintenance. Chondrocytes are in a lacuna, they should behave like pillars and maintain their shape. Soluble and matrix-bound GFs and flow induced mechanical forces on blood vessel wall, endos after polarity, cell-cell contacts, and degrade the surrounding basement membrane and stromal ECM in order to migrate and form tubular sprouts. Adhesive and mechanical cues drive cell organization. Misregulation of the mechanism induces mechanical and structural changes in the ECM, and transformed epithelial cells migrate towards vasculature and eventually metastasize.

ECM-dependent regulators can be associated with 2D, 1D and 3D migration. In turns influence intracellular pathways that govern the migratory phenotype. 3D are characterized by pore size and interconnection (cross linking degree). Aligned fibers are randomly distributed with low density in the scaffold.

Adhesion and migration are controlled by the ECM composition, stiffness of the material and ligand density. While using fibers, something changes: by having a new parameter, aligned topography, we obtain different architectures. In the case of a mixed fibrous scaffold, we also have aligned/random, elastic behaviour, cross linking. Sending seed cells on the different structure, we will se diverse behaviour for orientation, migration, etc. Architecture can play a huge role.

The substrate contractility regulates 3D migration, regardless of pore size. Stiff fibers vs soft fibers: when the cell lands on stiff fiber it can adhere, but becomes very stable and not able to modify the body/migrate. Instead cells on soft fibers are able to move and form protrusions. Since cells in nature are connected to ECM fibers, when they move the ECM will also follow the contraction of the cell body. If the cell adheres to the soft fiber, the same natural movement can occur. When the substrate is too rigid contraction cannot occur, healing will be different. The scaffold should be soft enough to follow the reorganization needed by the cells.

Figure 6.8: two scaffolds prepared with the same polymer, the difference is in pore size and distribution. The scaffolds are built for bone regeneration; we should take into account the presence of collagen fiber in a continuous network. Osteoblasts should infiltrate the scaffold for building the network, porosity should allow this. Once the network is formed, we have the deposition of the hydroxy apatite on top of collagen fibers. Difference between P1 and P2 in terms of cell distribution: in P2 uniform, in P1 clusters (interconnection not complete). When we observe clusters of cells, we expect that the osteoblasts are not able to build a continuous network, due to tight collagen + no mineralization. When cells are not able to migrate, mineralization cannot occur. A continuous network is also required for capillary formation. The parameters can be fully controlled with scaffold

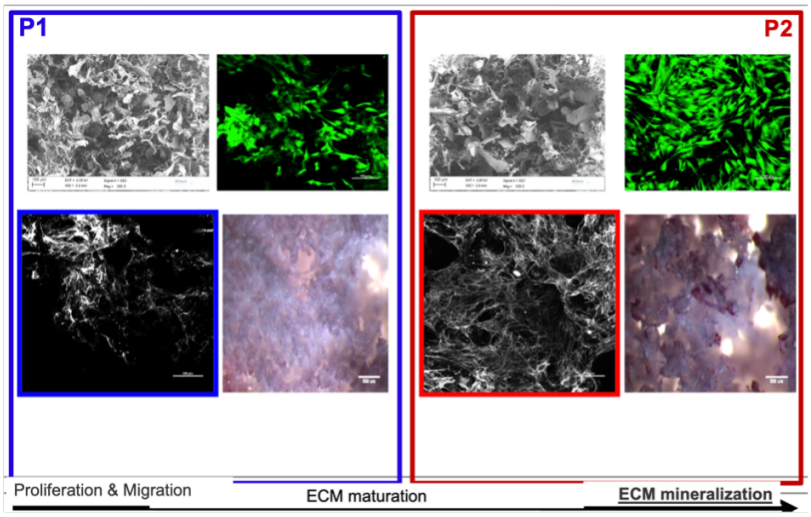

Figure 6.8

design. In order to improve the performance of P2 scaffold we could functionalize it with collagen or drug release systems.

The scaffold surface interacts with the cell through the ECM (biorecognition). Our aim is to reproduce in vitro a working environment, working for the cell and solving a specific task. Depending on the context, we can have the bioreaction: the cell recognizes the surface of the scaffold if it is functionalized or for protein absorption. We have to refer to the cell population we are interested in, we have specific integrins.

6.2.5 Methods for modulating receptor-ligand interactions

1. Natural ECM biomaterials: biologically relevant environment, but poor mechanical properties and inconsistent reproducibility
2. Whole ECM adsorption
3. Synthetic linear binding motif: surface functionalization. We need to define the density of the signal, the protein of interest , the stability (quantify the time for which the signal should remain, orientation, homogeneous or pattern distribution.
4. Spatially oriented binding motif
5. Nanopatterning with nanolithography: mix different molecules and patterns, as well as technologies
6. ECM-like biomaterials

???

Poly-lactic-acid (PLA): - FN-PLA: formulation - RGD-pla: performance Cells are stained in pink. Why using pLA alone If the goal is functionalizing? It was used to compare the efficacy of PLA and FN. Why fn works better when there is