**Tissue engineering triad**

*This lecture describes the components of the extracellular matrix and their influence on the cell properties and functions.*

### 1 Scaffolds

One of the key components of the tissue engineering triad is the ‘Scaffold’. Why is a scaffold necessary? Let us consider a common example. A building under construction requires scaffolding of metal and wooden structures to support it until the binding cement and mortar sets in to a hard mass that can support the building without external aid. Similarly, a group of cells require scaffolds that will hold them together until they can establish contacts with each other as well as produce their own matrix. Thus, scaffolds are temporary supports that will mimic the function of native scaffolds of tissues found in the biological system. What is the nature of this natural scaffolding material? Let us understand the structure and functions of the native scaffold in order to design appropriate synthetic scaffolds that can provide the same microenvironment to the cells as found in native tissues.

### 2 Extracellular matrix

Apart from the components of the immune system, the cells present in tissues do not exhibit migration to long distances. Then, where are they adhered? Well, each cell is found adhered to a basement membrane also known as the ‘extracellular matrix’ (ECM). The extracellular matrix not only acts as a substrate for adhesion of cells but also has a major influence on the growth and functioning of the cells. The ECM has the following functions in biological systems:

- Acts as a substrate to support the adhesion of cells
- Provides tensile strength to the cells so that it can maintain the multilayer of cells without disintegrating
- Provides contact guidance to the cells so that they grow to the right size and align themselves in the correct orientation
- Enables diffusion of nutrients to support the growth and survival of cells
- The three-dimensional architecture of the ECM facilitates establishment of cell-cell contacts as well as cell infiltration
- Acts as a medium to transfer signals in the form of soluble factors or mechanical stimuli to the cell

Thus the ECM influences all cell-fate processes. What is the ECM composed of? The ECM is a complex system with an intricate network of structural and functional biomolecules. The chief components of the ECM are:

- Collagen
Fibrous, structural and elastic proteins
- Proteoglycans or glycosaminoglycans (GAGs)
- Hyaluronic acid
- Soluble factors

Among these, collagen and the structural fibrous proteins contribute to the tensile strength of the ECM and constitute the insoluble fraction of the ECM. The glycosaminoglycans and hyaluronic acid are hydrated easily and form a gel that contributes to the compressive strength of the ECM. Thus the ECM can be visualized as a complex three-dimensional structure that consists of fibrous protein network distributed throughout a hydrogel. Figure 1 depicts the structure of an ECM.

![Polysaccharide molecules](image)

**Fig. 1: Pictorial representation of Extracellular Matrix (ECM)**

The composition of the ECM constituents is different for different type of tissues. This indicates that each tissue type prefers a different microenvironment and this knowledge will be invaluable in the design of biomimetic scaffolds for each tissue type. The major constituent of the ECM is collagen.

**Did you know?**
- Collagen is the most abundant protein found in the animal kingdom!
- Collagen is stronger than steel of the same weight!
- Every third residue in collagen is glycine. If this position is altered, then a strong triple helix will not be formed leading to abnormalities like the ‘brittle-bone disease’ or *osteogenesis imperfecta*
Collagen mainly consists of glycine, proline and hydroxyproline. The most common motif found in collagen is Gly-Pro-X where Gly is glycine, Pro is proline and X is any other amino acid. Three polypeptide chains twist to form a triple helix that is referred as ‘pro-collagen’. The pro-collagen molecules are stabilized through hydrogen bonding. These molecules then undergo self-assembly form bundles comprising procollagen arranged side-by-side. This self-assembled structure is now referred to as a ‘collagen fibril’. Collagen also has hydroxylysine residues that interact with lysine or N-terminus to form aldol links thereby resulting in stabilization of the fibril structure. (An aldol link is formed between amino groups and carbonyl groups). Each fibril is about 50 nm in diameter and can extend to several microns in length. The fibrils have excellent tensile strength. The fibrils then assemble into bundles forming the collagen fiber that has a diameter about 300-500 nm. Figure 2 shows the different stages leading to the formation of collagen fibers.

There are about 26 different types of collagen that have been identified. These are designated as type I, II, III, IV etc. Not all types of collagen form fibers. Some of them exist in non-fibrous forms. The different classes of collagen are listed below:

- Fibril-forming collagen: Types I, II, III, V
- Fibril-associated collagen with interrupted triple helices (FACIT): Types IX, XII, XIV
- Basement membrane collagen: Type IV
- Anchoring fibril: Type VII
- Microfibrillar collagen: Type VI
- Hexagonal network-forming collagens: Types VIII, X
- Transmembrane collagens: Types XIII, XVII

Fig. 2: Different stages of collagen formation
• Multiplexins: Types XV, XVI, XVIII

Among the various types of collagen, Types I, II and III are most abundant and Type I has been most widely investigated. These collagen types exist in different forms such as basket weave, parallel fibers, orthogonal lattices etc. Why should there be so many forms of collagen? The different structures adopted by collagen can expose different regions of the molecule and hence present different peptide motifs for binding with receptors or cytokines. This can result in different types of signals that can be produced as a result of such interactions.

The other structural proteins that are present in the ECM are fibronectin, elastin, laminin, vitonecin, fibrillin etc. These contribute to the mechanical strength of the ECM as well as serve as adhesion points for binding of cell surface receptors. Apart from proteins, glycosaminoglycans are an important class of molecules found in the ECM. These are carbohydrate molecules that are generally associated with a protein entity. The GAGs generally consist of repeating units of a sulphated glycosamine and/or auronic acid moiety. The common GAGs found in the ECM are chondroitin sulphate, heparan sulphate, dermatan sulphate, keratan sulphate and hyaluronic acid. The structures of these components are shown in Figure 3.

Fig. 3: Different types of glycosaminoglycans (GAGs) in extracellular matrix
These GAGs, with the exception of hyaluronic acid, form proteoglycans through association with proteins. The glycans are added to the proteins through post-translational modifications. The different matrix proteoglycans that are found in the ECM are aggregans, perlecans, versicans, biglycans, fibromodulins, and decorins. Figure 4 shows the different proteoglycans seen in the ECM. In addition, some of these proteoglycans are also found associated with cell membranes. These membrane-associated proteoglycans include syndecans, glypicans, and thrombomodulins.

![Fig. 4: Different types of proteoglycans in extracellular matrix](image)

What is the major role of these proteoglycans? All these molecules are charged due to the presence of sulphate groups. The number of sulphate groups per molecule can vary. In addition, the uronic acid moieties in the GAGs also have a negative charge due to the presence of carboxylic acid groups. The chain length of the GAGs associated with the proteins is yet another variable that will dictate the number of charges in a proteoglycan. These highly anionic groups result in an influx of polar water molecules and counter ions, leading to formation of a highly hydrated matrix. This hydrated matrix provides compressive strength and volume to the ECM. The core proteins in the proteoglycans can be associated with the same or different GAGs as shown in Figure 5.

![Fig. 5: Organization of glycosaminoglycans to the core proteoglycans in extracellular matrix](image)
The hyaluronic acid does not contain any sulphate groups. It is the most abundant GAG in the ECM and has very high molecular weight. It is also known as ‘hyaluronan’ and colloquially referred to as ‘goo’. It increases the viscosity of the system and is found in abundance in the synovial fluid where it functions as a lubricant. Hyaluronan is also said to have a role in cell migration and adhesion. The proteoglycans also play a role in transport of hormones, soluble factors, regulate cell movement, proliferation and differentiation as well as are said to restrict the movement of microorganisms.

How is the ECM formed? The cells produce the ECM components! The degradation of ECM components are also brought about by the cells using special enzymes known as matrix metalloproteinases (MMPs). The cells periodically modify the composition as well as the topography of the ECM in response to the environment. This process is referred to as ‘matrix remodeling’. This process has a lot of significance when one designs a biomimetic scaffold.

So how do the cells adhere to the ECM? The cells have special receptors on the cell surface that bind with specific motifs present in the ECM proteins thus anchoring the cell to the ECM. These cell surface receptors form a part of the family of cell adhesion molecules (CAMs). One of the major cell surface receptors present in cells is the ‘integrins’. Let us understand a few basic facts about this ‘cell-ECM binding glue’! Integrins are heterodimeric transmembrane proteins that are located in the cell membrane. What is a heterodimeric protein? A dimeric protein is one that consists of two polypeptide chains (‘di’: two; ‘mer’: unit). If both polypeptides have distinctly different sequences, the protein is referred to as ‘heterodimeric’ (‘hetero’: different). If they possess identical sequences, the protein is referred to as ‘homodimeric’ (‘homo’: same). Thus, integrins have two polypeptide chains that are referred as the alpha (α) and beta (β) subunits. Different isoforms of the α and β subunits have been identified. Thus many types of integrins with different combinations of the α and β subunits are available in nature. For example, the integrin α5β1 consists of the isoforms 5 and 3 of α and β subunits respectively. About 18 isoforms of the α and about 8 types of β subunits have been identified thus far. But, all possible combinations of integrins are not available. About 24 different integrins have been identified in mammalian systems. Among these, the β1 and α5 occur in many heterodimer combinations.

Now let us look at where the integrins are localized in the cell. The integrins are transmembrane proteins. This means that they have three distinct domains – extracellular, transmembrane and intracellular. The extracellular domain of integrin is the largest among the three and it projects into the ECM. It has calcium binding sites and also unique binding sites that recognize and associates with specific peptide sequences in the ECM proteins. The transmembrane domain spans the cell membrane and continues as a short intracellular domain that is associated with the
cytoskeleton. Thus it is evident that the integrins serve as a bridge between the ECM and the cytoskeleton of the cell. Figure 6 shows the representation of an integrin receptor.

The integrins bind to specific sequences that are present on the proteins present in the ECM. For example, the sequence RGD (arginine-glycine-aspartate) found in the ECM proteins collagen and fibronectin is recognized and bound by several types of integrins causing the cells to adhere with the ECM. Similarly, the LDV (leucine-aspartate-valine) sequence present in fibronectin is another binding motif for integrins. The peptide sequence SVVYGLR (Serine-valine-valine-tyrosine-glycine-leucine-arginine) present in osteopontin has also been implicated to be involved in integrin-matrix protein interactions.

So what happens when integrins bind with the ECM proteins? The most obvious reason would be anchoring of the cell to the ECM. In addition, the binding of integrin with a ligand triggers a complex pathway of signaling that elicits a specific cellular response. How? The intracellular domain of integrins is associated with ‘adaptor proteins’ like talin, vinculin, paxicillin, dystrophin etc. that connect the integrin with the actin network forming the cytoskeleton. Figure 7 depicts a cartoon representing the integrin-adaptor protein-cytoskeleton network.
The ligand binding causes a change in the conformation of the extracellular domain of integrin that is reflected in the intracellular domain also. This conformation change in turn alters the conformation of the adaptor proteins that activates associated kinases thereby triggering a cascade of biochemical signaling pathways. For example, focal adhesion kinase (FAK) and tyrosine kinases have been involved in the integrin-mediated signaling. How does the cell know which pathway is to be activated? Well, though the mechanism is largely unknown, it is believed that the type and number of ligands involved in the interaction could be a major determinant in deciding the pathways activated.

Alternately, the ligand binding can trigger clustering of the integrin receptors, which in turn can be transmitted to the actin filaments through the adaptor proteins. This can change the actin distribution through elongation or contractile forces. Such modifications can be reflected in the extension or contraction of the cells in a particular direction! Such types of cell-ECM adhesion are referred to as ‘focal adhesion complexes’ or simply ‘focal contacts’. Thus integrin-ligand binding involves three major events – recognition and binding to specific ligands, clustering of integrin-ligand receptors and finally transmission of mechanical stimuli to the cell leading to signaling pathways.
3 Reference

Source:
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