

# The cell adhesion practical



The extracellular matrix consists of many polymeric proteins and polysaccharides that are assembled into an organised meshwork. The extracellular matrix in our own bodies can be found as connective tissues such as bone and tendon. In animals the extracellular matrix forms a structure called the basal lamina. Basal lamina is a thin, tough, flexible sheet of matrix molecules and is essential in underpinning all of the epithelia. The basal lamina separates the cells and epithelia from the surrounding connective tissue and forms a connection between them. A Basal lamina determines the cell polarity, promotes cell survival, proliferation, and serves as a direct route for cell migration. The basal lamina in the extracellular matrix of animals consists of two main classes of extracellular macromolecules: fibrous proteins known as glycoproteins and glycosaminoglycans which are polysaccharide chains covalently linked to core proteins to form proteoglycans. The matrix applies powerful influences on the cells. These influences are applied mainly through transmembrane cell adhesion proteins that act as matrix receptors. These matrix receptors have a main role in epithelial cells and mediating their interactions with the basal lamina. Many types of molecules can function as matrix receptors. The main receptors on animal cells for binding most extracellular matrix proteins are the integrins. Integrins are a large family of heterodimeric proteins that exist as both alpha and beta heterodimers. Many matrix proteins in vertebrates have multiple integrins. 5 can bind to Laminin and 9 types of  $\beta$  subunits and 24 of  $\alpha$  subunits are formed from human integrins.  $\beta$ 1 subunits form dimers with 12  $\alpha$  subunits. These subunits are found on all vertebrates and an example of this integrin is the  $\alpha$ 6 $\beta$ 1 Laminin receptor. Fibrinogen is bound by the  $\beta$ 3 integrins. These integrins are found on many cells such as

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blood platelets. Platelets interact with fibrinogen during blood clotting. It is known for  $\alpha_2\beta_1$  to bind to collagens,  $\alpha_5\beta_1$  binds to fibronectin, and  $\alpha_6\beta_1$  binds to laminins. The binding of a matrix component to an integrin can send a message to the inner part of the cell, and this causes the interior part of the cell to send a signal back to the matrix. The extracellular matrix of connective tissues plays an important role in many multicellular organisms. The connective tissue in the matrix is constructed from glycosaminoglycan polysaccharides which form proteoglycans and fibrous proteins such as collagen. The collagen fibres strengthen and help organise the matrix together. The fibrous proteins give the matrix the flexibility. Collagens are secreted in large quantities by the connective tissue cells. A main feature of a collagen molecule is its triple helical structure. The fibrous proteins give the matrix strength and form structures to which the cells can be anchored using glycoproteins such as Laminin and fibronectin that have many binding sites for integrins on the cell surface. Laminin is the first extracellular protein synthesised in a developing embryo. It is made up of three polypeptide chains held together by disulphide bonds. The extracellular matrix also contains noncollagen proteins with multiple domains and specific binding sites for receptors on the surface of cells. These proteins organise the matrix and help cells attach to the matrix.

The extracellular matrix components are degraded by proteolytic enzymes called proteases that act close to the cells that produce them. These proteases belong to two general classes: matrix metalloproteases and serine proteases. Matrix metalloproteases depend on bound  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$  for activity. The matrix metalloproteases and serine proteases cooperate to

degrade the matrix proteins collagen, Laminin, and fibrinogen. The adhesion assay to be used in this experiment involves using trypsin. Trypsin is an enzyme used to remove the living C6 glioma cells from the confluent dish. Gilal cells are important cells in the brain. The experiment will use rat tumour cells. The media will be grown in calf serum. The Calf serum contains growth factors which are used for cells to divide and also contains the ECM components such as fibrinogen. Trypsin is dissolved in a solution that contains a divalent cation chelatum. This will interfere with the calcium and magnesium cations that are important for cell-cell interaction and cell matrix interactions. The cells will be put in suspension to see how they attach to different ECM components and then placed in the two top lanes of a 96 well dish. The first lane will contain Laminin, collagen I, and fibrinogen. The 2nd lane is the control. When matrix components are placed on well they will stick but not all stick this is when BSA is used in second lane. BSA is a protein added to other ECMs to block the sites that the 3 different ECM components haven't stuck to. (1)(2)(3)(4)(8)(9) Cell adhesion assays are useful in many aspects of cell physiology and the mechanism of the adhesive response itself. Cell Adhesion Molecules (CAMs) are proteins located on the cell surface that are involved with the extracellular matrix (ECM) in the process called cell adhesion. Cell adhesion molecules have been identified from epithelial cells and studies demonstrate the role of extracellular matrix proteins, fibronectin and Laminin in cell attachment to matrix. These proteins contain specific domains which interact with other matrix components such as collagen. Cell adhesion is a complex process that involves molecular interactions and receptor ligand binding. Adhesion assays measure the contacts between a cell and extracellular adhesion proteins and also provide

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information about the cellular events. Cell adhesion assays are used to test the ability of a cell line to adhere to a substrate(4)(5).

The methods for measuring cell adhesion can be divided into two types. In the first type of methods, cell adhesion is analysed under static conditions. Static assays are used widely to assess the adhesion of different types of cells such as fibroblasts and epithelial cells. This assay describes the cells ability to adhere. This method is simple to perform and provides assessment of the adhesiveness of cells to a defined extracellular matrix substrate for example Laminin. However, static assay methods poorly stimulate adhesion that occurs in blood. Therefore, a second method is used to measure the cell adhesion using flow chambers. The use of flow chambers enables the researcher to stimulate blood flow and to reconstruct cell systems in the presence of shear. Flow chambers are available commercially and most commonly used to study leukocyte adhesion, either with endothelial cells or to substrates purified ligands. The aim of this practical is to perform a cell adhesion assay with 3 different ECM components and to investigate the ability of extracellular components to adhere to c6 glioma cells. (4)(5)

## **Results**

In the practical there was an experimental error this is due to mixing with serum. Therefore only B9-B12 was used along with other colleague's results.

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chelatum. This will interfere with the calcium, magnesium cations that are important for cell-cell interaction and cell matrix interactions. Cells are put in suspension to see how cells attach to different ECM components.

## **Discussion**

From the results it is shown a hierarchy of ECM components can be concluded. Matrix 1 concludes to be fibrinogen; this is because it is the least to adhere with C6 glioma cells. Asano et al,(2004) stated that ' C6 cells adhered a little more than twice as well to type I collagen as to Laminin' this can be clearly shown on the bar chart as the two greater bars (matrix 2 and 3) indicating fibrinogen as the least. Results for both individual and model show C6 glioma cells adhered well to both type I collagen and laminin-1 but C6 glioma cells did not adhere well to fibrinogen. A reason to why the C6 glioma cells did not adhere could be that they are derived from glial cells in the rat brain. Cells derived from glial cells are unlikely to adhere with fibrinogen and therefore unlikely to express integrins which interact with fibrinogen and this can be seen clearly in both model data and own data. A number of integrins require activation to bind their ligand and anchor the cell to the ECM. For example alphaIIb beta 3 integrin from platelets cannot bind to fibrinogen unless the platelets are activated by binding collagen or thrombin in a forming clot. The activation leads to changes in the integrin which are linked to the cytoskeleton remodelling. In both the model data and own matrix 3 shows to have the greatest absorbance and adherence. This is because collagen is the most abundant protein and most of the extracellular matrix is made up of collagen. Collagen shows the most adherences to the cells this is because collagen contains more integrin receptors than the other

ECM components. Trypsin breaks down the ECM properties and therefore cells appear separated under the microscope. A reason to a higher adherence to collagen could be trypsin breaks down the collagen components faster than fibrinogen. A low adherence is shown for fibrinogen this is because fibrinogen in the brain is low. Glioma cells originate from the brain and therefore they would not encounter with fibrinogen so the integrins only bind to collagen and Laminin.(3)(2) Another experiment in determining which integrins are important in cell adhesion to the ECM component is using antibodies. Antibodies identify the functions of the ECM receptors in cell attachment and cell migration by attaching to the alpha and beta and are also used to block adhesion to ECM . Blocking the function of the beta subunit inhibited cell adhesion to Laminin and fibronectin. These results were expected because a beta polypeptide can associate with several alphas to form receptors for collagen and Laminin. In the late 80s functionally blocking antibodies were used on  $\alpha 2$  integrins to block adhesion to collagens and  $\beta 1$  integrins blocked the adhesion to several ligands. 2 Rat monoclonal antibodies have been used against integrins. Monoclonal antibody (mAb) 13 recognizes the integrin class 1 beta polypeptide and monoclonal antibody 16 recognizes the fibronectin receptor. These monoclonal antibodies were used to test the inhibitory activities in cell adhesion, spreading, and migration using w138 human fibroblasts. It was shown mAb 13 inhibited the attachment and spreading of w138 cells on fibronectin and Laminin substrates. It was shown Laminin mediated adhesion and was sensitive to mAb 13 compared to mAb 16 which showed cell attachment to fibronectin but not Laminin. This shows this receptor is not involved in Laminin cell adhesion. (7)

The  $\alpha1\beta1$  integrin can bind to collagen types I, II, III, and V but favours other subtypes such as type IV and XIII. This is why  $\alpha2\beta1$  integrin is the major receptor for type 1 collagen. An experiment in determining the collagen receptors is to see the effect of collagenase-cleavage of type I collagen on  $\alpha2\beta1$  integrin-mediated. Collagenase-3 cleavage of type 1 collagen has shown to have an effect on  $\alpha2\beta1$  integrin. An isolated  $\alpha2\beta1$  integrin and  $\alpha2$  integrin A- domain were found to bind native collagen and native k fragment. It was shown integrins were lost after heat denaturation of the collagen fragments and shown human fibrosarcoma cells (HT1080) adhered to type 1 collagen and k fragment at 37°C. HT1080 cells cultured on type 1 collagen and collagen fragments expressed high in  $\alpha2\beta1$  integrin on cell surface and very little to  $\alpha\nu\beta3$  integrin detected by immunolocalization. This experiment shows that  $\alpha2\beta1$  integrin binding to type 1 collagen is dependent on the maintenance of the collagen triple helical conformation. (6)

In conclusion integrins are important adhesion molecules involved in cell-cell-matrix adhesions.