Problem 1

A) From lecture notes on 10/4 and the Chap. 6 handout we know that the shear stress these platelets experience at the surface of the chamber is dependent on the fluid’s viscosity, the flow rate, and the dimensions of the channel (Eq. 6-8). Therefore, we can use the data in the table to calculate the shear stress as follows:

\[ \tau = -\mu \frac{dv_y}{dx} \bigg|_{x=a} = \frac{3 \mu Q}{2a^2 b} \]

\[ Q = 0.6 \frac{L}{\text{min}} \times \frac{1000 \text{ml}}{1 \text{L}} \times \frac{1 \text{cm}^3}{1 \text{ml}} \times \frac{1 \text{m}^3}{1 \times 10^6 \text{cm}^3} \times \frac{1 \text{min}}{60 \text{sec}} = 1 \times 10^{-5} \frac{m^3}{\text{sec}} \]

\[ \tau = \frac{3 \times (2.7 \times 10^{-3} \frac{N \cdot \text{sec}}{m^2}) \times (1 \times 10^{-5} \frac{m^3}{\text{sec}})}{2 \times (2 \times 10^{-3} \frac{m^3}{m^2}) \times (0.02 \text{m})} \]

\[ \tau = 0.51 \frac{N}{m^2} = 0.51 \text{Pa} \times \frac{10 \text{ dynes}}{1 \text{ Pa} \cdot \text{cm}^2} = 5.1 \frac{\text{ dynes}}{\text{ cm}^2} \]

Repeat this calculation for the other volumetric flow rates:

\[ Q = 0.9 \frac{L}{\text{min}} \Rightarrow \tau = 7.6 \frac{\text{ dynes}}{\text{ cm}^2} \]

\[ Q = 1.2 \frac{L}{\text{min}} \Rightarrow \tau = 10.1 \frac{\text{ dynes}}{\text{ cm}^2} \]

\[ Q = 1.5 \frac{L}{\text{min}} \Rightarrow \tau = 12.7 \frac{\text{ dynes}}{\text{ cm}^2} \]

\[ Q = 1.8 \frac{L}{\text{min}} \Rightarrow \tau = 15.2 \frac{\text{ dynes}}{\text{ cm}^2} \]
Platelets are small, disk-shaped, anuclear cells that are formed in the bone marrow and circulate throughout the bloodstream. By adhering and aggregating to injured vascular surfaces, platelets help prevent blood loss. When a blood vessel incurs trauma, blood comes into contact with exposed subendothelial structures such as collagen. Circulating platelets adhere to these structures. This contact initiates a series of catalytic events in which a proenzyme clotting factor is changed into its enzymatic form which then activates the next proenzyme in the chain. This cascade of events result in thrombin formation, which activates the adhered platelets. Activated platelets are associated with a conformational change in the $\alpha_{IIb}\beta_3$ integrin found on the platelet surface. As discussed in lecture (9/30) this conformational change involves the integrin going from an inactive “bent” conformation to an active “rod-like” conformation. In the rod-like conformation, the $\alpha_{IIb}\beta_3$ integrin can bind fibrinogen, a soluble plasma protein. Aggregation of these and other circulating factors ultimately form a fibrin clot that acts as a “patch” to prevent further blood loss and allows the damaged area to heal.

Looking at the curves for P1 and P2 in part A), the platelets in P2 are not adhering to the fibrinogen at the surface even at rather low flow rates. Knowing that the $\alpha_{IIb}\beta_3$ integrin is the major molecule on the platelet surface responsible for binding fibrinogen one would hypothesize that the $\alpha_{IIb}\beta_3$ integrin is not functioning properly on the platelets in the P2 sample.
C) **Experiment #1: Recombinant DNA & Monoclonal Abs**

Isolate cells that normally do not adhere to fibrinogen. One could prove the inability to bind fibrinogen by flowing these cells through the parallel plate flow chamber and verifying that none of the cells are binding to the surface. Transfect these cells that do not normally express the $\alpha_{\text{thb}}\beta_3$ integrin with a vector carrying the DNA code for the $\alpha_{\text{thb}}\beta_3$ integrin. Repeat the parallel plate flow chamber experiment. If these cells are now able to adhere to the fibrinogen coated surface then one could conclude that the $\alpha_{\text{thb}}\beta_3$ integrin is responsible for the cells’ ability to bind fibrinogen. Now add monoclonal antibodies, raised against the active site on either the $\alpha$ or $\beta$ chain of the $\alpha_{\text{thb}}\beta_3$ integrin, to a sample of these cells and repeat the parallel plate flow chamber experiment. If the cells’ ability to bind fibrinogen is absent then one could conclude that the $\alpha_{\text{thb}}\beta_3$ integrin is indeed responsible for binding fibrinogen and that platelets from the P2 sample must have a non-functional $\alpha_{\text{thb}}\beta_3$ integrin expressed on their surfaces.

**Experiment #2: Antibody tag & flow cytometry**

Isolate platelets from Patient 1 (P1) and expose them to thrombin in order to activate them. Add a monoclonal Ab (with a fluorescent tag) against the active site in the $\alpha_{\text{thb}}\beta_3$ integrin. Run these platelets through a flow cytometer to distinguish the number of platelets that have functional $\alpha_{\text{thb}}\beta_3$ integrins on their surfaces. Flow cytometry is a process in which measurement of the physical and/or chemical characteristics of a cell are made while cells pass in single file through the detector in a fluid stream. In this case the detector would measure the fluorescence of the monoclonal Abs attached to the cell surface. Repeat this experiment with platelets from Patient 2 (P2). If significantly fewer platelets are identified in the flow cytometer one could infer that there are fewer $\alpha_{\text{thb}}\beta_3$ integrins in the active conformation to bind the fibrinogen than in the P1 sample.

**Experiment #3: Liposome Fusion**

Isolate wildtype $\alpha_{\text{thb}}\beta_3$ integrin by chromatography on a column containing a specific monoclonal antibody against the $\alpha_{\text{thb}}\beta_3$ integrin. Incorporate these integrins into liposomes made of pure phospholipids. Fuse these liposomes to the platelets from the P2 sample. By fusing liposomes containing functioning $\alpha_{\text{thb}}\beta_3$ integrins to these platelets unable to bind fibrinogen, one should be able to restore the platelets’ ability to bind fibrinogen. Repeat the parallel plate flow chamber experiment. If these altered platelets adhere to fibrinogen on the surface in a manner similar to the platelets from the P1 sample then one can conclude that the $\alpha_{\text{thb}}\beta_3$ integrin is the major molecule responsible for binding platelets to fibrinogen.
Problem 2

The band in the lane for Patient 1 is the same as the band in the + Control lane which means that Patient 1 has the wildtype version of the $\alpha 5$(IV) chain. However, the problem states that all patients have Alport Syndrome which means that Patient 1 has mutant versions of the $\alpha 3$ (IV) or $\alpha 4$ (IV) chain. Remember, this Western blot only used a primary AB against the $\alpha 5$ (IV) chain. Therefore, this blot can not tell us which chain ($\alpha 3$ (IV) or $\alpha 4$(IV)) is mutated.

The band in the lane for Patient 2 has a lower band (~70kDa) than the band in the lane for the + Control which indicates that it is a truncated version of the wildtype $\alpha 5$ (IV) chain. There are several ways that a mutation could result in a truncated protein. One example would be that a point mutation in the gene sequence caused the codon to indicate a STOP signal during protein translation instead of the normal amino acid in the wildtype sequence. This early STOP signal would result in a truncated version of the wildtype protein. Since the problem states that all the patients have Alport Syndrome, the truncated version of the $\alpha 5$(IV) chain must not be functional.

The upper band in the lane for Patient 3 corresponds to the normal $\alpha 5$ (IV) chain. The lower band represents a mutant form of the $\alpha 5$ (IV) chain. One explanation for these bands is based on the patient being female. As a female, Patient 3 has two chromosomes and therefore, in theory, should have two copies of the gene for the $\alpha 5$ (IV) chain. However, if we recall the rule of random X-chromosome inactivation in mammals that states that no matter how many X chromosomes are present, there is only one transcriptionally active X chromosome in each somatic cell. Therefore, from the sample of kidney cells taken from Patient 3 some have the inactivated X chromosome carrying the wildtype copy of the gene while other cells in the sample have the inactivated X chromosome carrying the mutant copy of the gene for the $\alpha 5$ (IV) chain. Therefore, in the Western Blot the primary Ab would recognize two bands, one corresponding to the mutant protein and the other corresponding to the wildtype protein.

There are no bands in the lane for Patient 4. One explanation could be that a mutation in the gene coding for the $\alpha 5$ (IV) chain or the promoter for the gene resulted in no protein being successfully produced. There could also be the situation where the mutation produced an $\alpha 5$ (IV) chain that does not contain the epitope for the primary Ab used in the Western.
Problem 3

For a tumor cell to move from its site of origin it would have to be deficient in molecules that mediate cell-cell adhesion. Since the problem states that this tumor cell is epithelial nature one can conclude that E-cadherin, a major molecule of cell-cell adhesion amongst epithelial cells, is most likely absent from its surface. To demonstrate that E-cadherin, is necessary you could make it non-functional and determine if the cells are still capable of adhering to one another. For example, one could take a monolayer of cultured epithelial cells, which normally express E-cadherin, and verify under a microscope that the cells adhere to one another. Add a monoclonal Ab that binds either the Ca$^{2+}$ binding site or the active site of the E-cadherin that binds other E-cadherins. If one observes that these epithelial cells are no longer attached to one another this proves that E-cadherin is necessary for cell-cell adhesion. To demonstrate that E-cadherin is sufficient one could use a cell line that normally does not express cadherins and tends to adhere poorly to each another. Transfect these cells with the gene for E-cadherin so now these cells will express E-cadherin. If one observes that these cells now adhere to each other then this proves that E-cadherin is sufficient for cell-cell adhesion.
Problem 4

A) From the problem statement we know that individual 2 (I2) has a non-functioning enzyme that is necessary to synthesize heparan sulfate. The band in the lane for I2 is lower than the band in the + Control lane indicating that proteoglycan recognized by the primary Ab in the I2 lane has a lower molecular weight than the wildtype heparan sulfate proteoglycan. From this info one could deduce that the proteoglycans for I2 are missing the heparan sulfate chains. Recall that proteoglycans have multiple polysaccharide chains attached to a protein core. This mutant proteoglycan would be smaller than the wildtype version and therefore it would run farther in the gel which explains the lower band on the membrane.

B) Wound healing is the process by which tissue repair takes place. It involves the interaction of a complex cascade of cellular events that generate resurfacing, reconstitution, and restoration of the injured tissue. The process of wound repair differs little from one kind of tissue to another and is generally independent of the form of the injury. The healing process requires the action of various cells including epithelial, endothelial, inflammatory cells, platelets, and fibroblasts. Fibroblasts are recruited to the wound site and proliferate in response to several factors that include Fibroblast Growth Factor (FGF). At the site of injury fibroblasts secrete collagen, proteoglycans, fibronectin, proteases and other ECM components. From the Lodish text (Chap. 22) we know that normally FGF binds to the heparan sulfate chains in extracellular proteoglycans so that the extracellular matrix serves as a reservoir of FGF. Active FGF can be released from this reservoir by proteolysis of the proteoglycan core protein or by partial degradation of the heparan sulfate chains. FGF must be attached to a heparan sulfate chain in order to interact with the FGF receptor. Since individual 2 (I2) is unable to produce functional heparan sulfate chains the FGF will not be able to interact with its receptor on the fibroblast. Without this interaction, the recruitment and proliferation of the fibroblasts will be impaired which will hinder the extracellular matrix secretion necessary for successful tissue repair. Besides residing in the ECM, proteoglycans are also located on the surfaces of many cells. Syndecan is one of the most common proteoglycans on the plasma membrane. Syndecan contains heparan sulfate chains that function to bind to fibrous collagens and fibronectin located in the interstitial matrix surrounding the basal lamina. Therefore, proteoglycans such as syndecan help anchor cells to the matrix. The absence of the heparan sulfate chains in I2’s case would negatively affect the ability of cells migrating to the wound area to attach to the newly deposited matrix and hinder the overall healing of the wound.