**Tissue Engineering**
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**Lecture - 11**
**Self Assembly**

Good morning everyone. Today we will be talking about self-assembly and its application for tissue engineering scaffolds. Before we start with what self-assembly is, we will be looking at what is self-assembly is, what are the interactions that facilitate this self-assembly process, and how do we use them for our tissue engineering applications.

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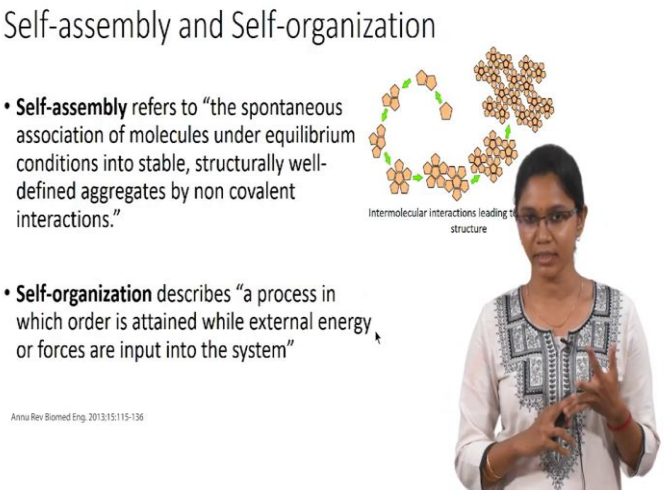


I will be giving you a definition of two terms, namely self-assembly, and self-organization. Self-Assembly is defined as the spontaneous associations of molecules under equilibrium condition into stable, structurally well-defined aggregates by non-covalent interactions. I hope everyone is familiar with the difference between covalent and non-covalent interactions. Today in self-assembly, we will be talking only about non-covalent interactions, but the definition of self-organization is necessary as these two terms are interchangeably used in the biological process.

There exists a minor difference between self-assembly and self-organization. Self-organization is defined as a process in which order is attained from a disordered state. It is similar to what self-assembly does, but with an input of external energy to maintain

the equilibrium, but in self-assembly, no energy is given to the system, it is a spontaneous process. Hence, self-organization facilitates other macromolecular systems, while in this lecture, we will be dealing only with self-assembly.

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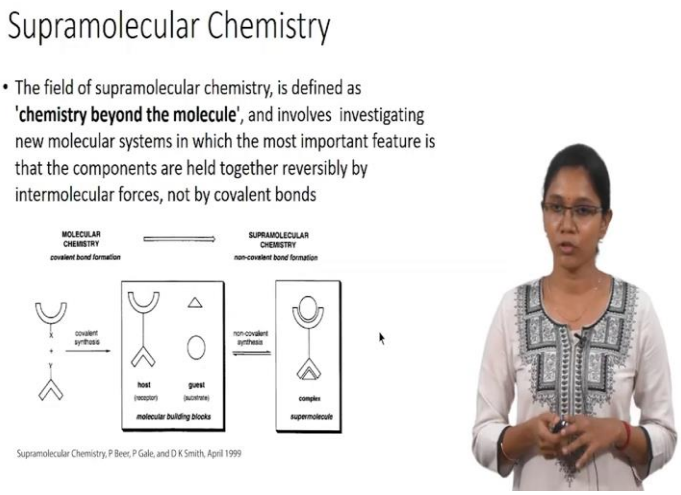


Self-assembly is a part of supramolecular chemistry. Today we will be learning how chemistry and biology are interrelated. Supramolecular chemistry is defined as "chemistry beyond the molecule." How are molecules formed? A molecule is formed when two or more atoms are covalently bonded. For example, C=O (C double bonded O) and the N and H atom forming $NH_2$ molecule. This process is chemistry. But what happens beyond the molecules?

When these molecules come together, what happens? What are the aggregates that are being formed? The pictorial representation in the slide depicts two molecules. In the early years, supramolecular chemistry involved mainly host-guest interactions. For example, taking one molecule to be the host and another to be the guest. When they combine, there is an interaction that exists in-between the molecules to form aggregates, which are termed as supramolecular aggregates. This process is called as supramolecular chemistry. Unlike the covalent bonds that are being formed within the atoms, here only non-covalent interactions come into play.

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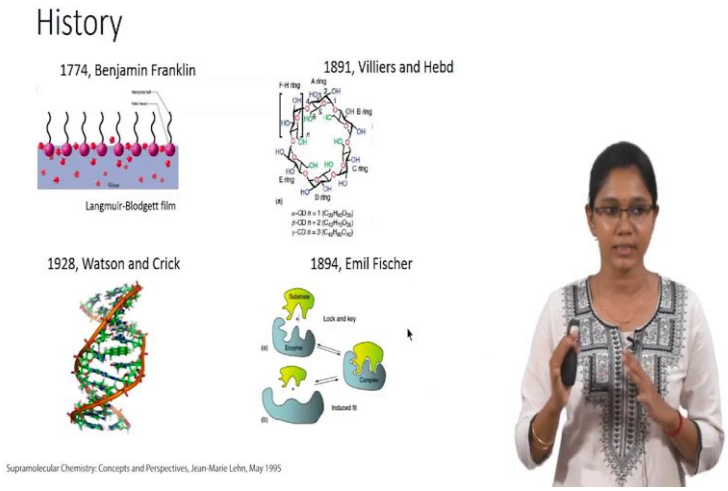
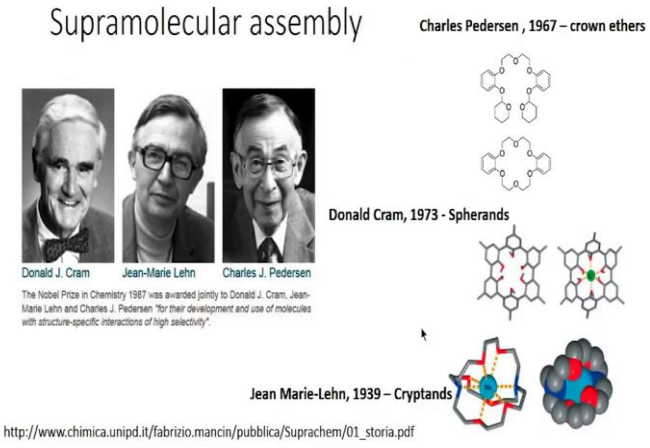



A brief history of how supramolecular chemistry developed dates back to 1774. I hope you are familiar with the LB film (Langmuir-Blodgett), which depicts monolayer adsorption. It explains how an amphiphile or oil arranges in water. This was discovered in 1774 by Benjamin Franklin. Another breakthrough in 1881 was the discovery of cyclodextrin molecules. The slide contains the picture of cyclodextrin found by Villiers and Hebd. These cyclodextrins are the most conventionally used systems for host-guest chemistry.

What is cyclodextrin? Most polymers that we encounter, for example, carbohydrate polymers are linear, but cyclodextrin was the first cyclic polymer that was found. These cyclins are of three different classes namely: alpha, beta, and gamma-cyclodextrin. The classification is based on the difference in the number of sugar units. Then, the other supramolecular principle is the substrate enzyme specificity that we see in the biological system. We have an enzyme and a substrate, which has high complementary binding between an enzyme and a substrate because of which they bind to form a product. This is also a part of the host-guest interaction.

Next, Watson and the Crick model, that is the DNA, which forms the basis of the genetics. DNA is a double helix model. It comprises of two parallel chains running across, held together by the hydrogen bonding. Hydrogen bonding is a non-covalent interaction that keeps the DNA intact. (Refer Slide Time: 05:05)




Three eminent scientists (Donald J Cram, Jean- Marie Lehn, and Charles J. Pearson) were awarded the Nobel prize for supramolecular chemistry. Caged like molecules namely the crown ethers, spherands, and the cryptands caged were discovered. These organic molecules were one of the first ligand-metal complexes/ host-guest systems that were synthesized. These discoveries laid the foundation for the field of supramolecular chemistry, after which people started noticing what supramolecular chemistry is or how we can utilize them for various applications.

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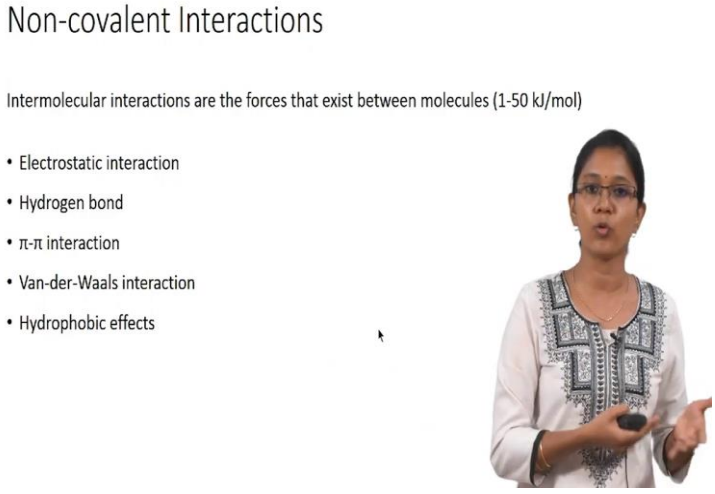

Before we learn the application of self-assembly or supramolecular chemistry in tissue engineering, we need to understand the interactions and how they differ from the covalent bonding. The five major interactions that we will be discussing are the electrostatic interactions, the hydrogen bonding, the π-π interactions, van der Waals interaction, and the hydrophobic effects.

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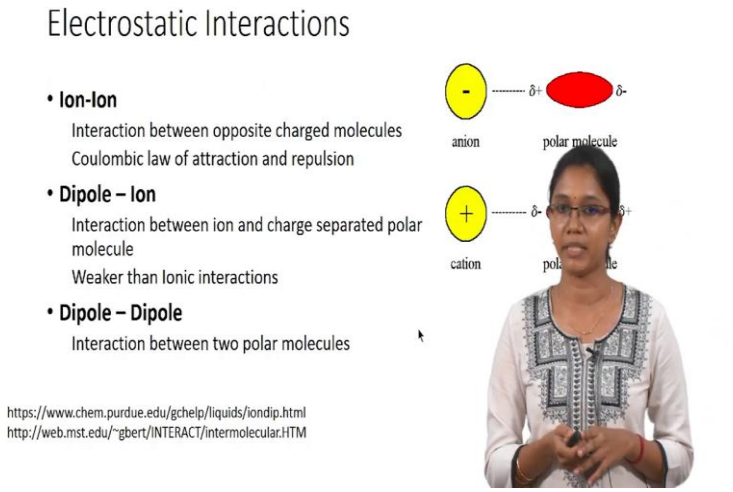


**Electrostatic interactions:** The non-covalent interactions do not require high energy, unlike the covalent bonds. For instance, the covalent bonds will need 500 Kilojoules/mol of energy. Non-covalent interactions are never termed as bonds, and we always define them to be interactions. There is very little energy involved in all these chemical processes, and they are mostly reversible. They can be reversed by any stimuli bringing them to its native state.

Ion-ion interaction is a part of electrostatic interactions. Ions can be positive or negative in charge, known as a cation or anion, respectively. When there exists an interaction between two differently charged molecules, it is termed as ion-ion interaction or ionic interaction.

Next, what is a dipole-ion interaction? As we are familiar with the charge-separated species in polar molecules that form a dipole, which generates a partial positive charge and a partial negative charge within the molecule. The charges are immobilized in different regions in a molecule and then combine or interact with a neighboring ion or a

dipole, leading to an interaction. This is called as dipole-ion interaction or a dipole-dipole interaction.

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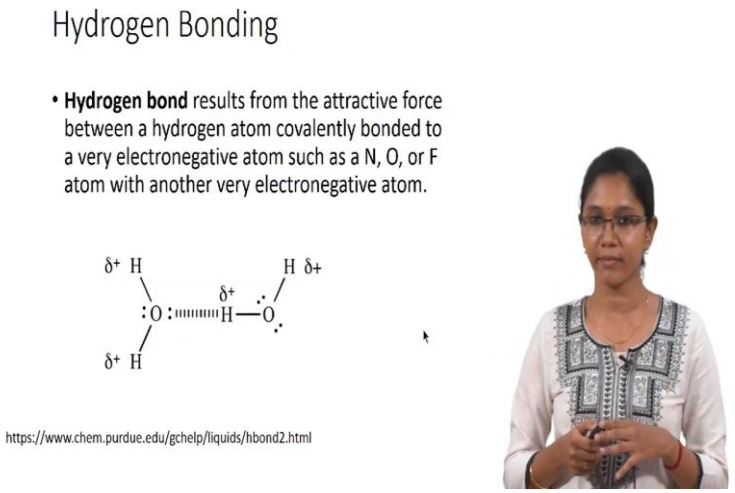


**Hydrogen Bonding:** Hydrogen bonding is one of the most important non-covalent interactions in supramolecular chemistry. Mainly for tissue engineering applications, where biological molecules are involved, consisting of an amide backbone or NH group that is involved in the hydrogen bonding.

Let us first understand what is a hydrogen bonding how to identify the bonding. It results from the attractive force between a hydrogen atom covalently bonded to a very electronegative atom such as nitrogen, oxygen, or fluorine with another very electronegative atom. Taking water molecule as an example to explain hydrogen bonding. In the slides, two water molecules, one to your left and the other to your right are displayed. There is a partial bond that is depicted in dotted lines between an O and H atom. The oxygen, which has a lone pair of electrons, is the highly electronegative atom, and then the hydrogen has a partial positive charge forming a hydrogen bond.

Next, in hydrogen bonding, we need to identify what is a hydrogen bond donor and what is a hydrogen bond acceptor. The hydrogen bond donors are always hydrogen, which is linked to the electronegative atom. In our example, the right-sided hydrogen, which is bound to an electronegative atom is the hydrogen bond donor. Whereas, the most electronegative atom is the hydrogen bond acceptor.

Hence, in any hydrogen bonding that you come across, try to identify the H-bond donor and the acceptor. This is important because any hydrogen atom will not facilitate hydrogen bonding. Only when the hydrogen is linked to an electronegative atom, it can facilitate a hydrogen bonding. A CH bond will not form hydrogen bonding, and only an OH group can form hydrogen bonding.

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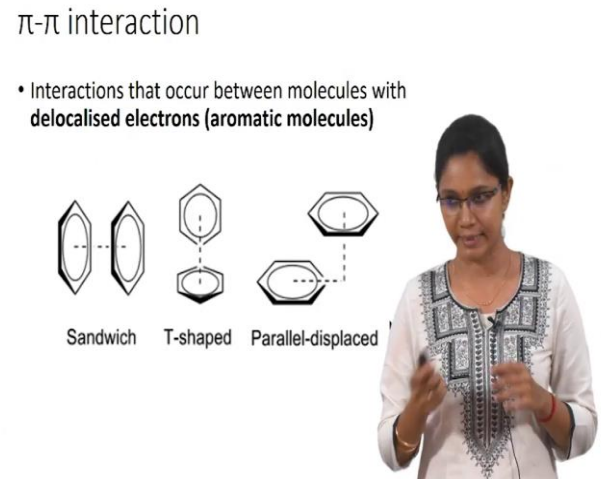


**π-π interactions:** π-π interactions exist in aromatic molecules. The π electrons are delocalized within the electron cloud in the aromatic systems. Hence, when these aromatic molecules come close to each other, they tend to form a stacking assembly.

π-π interaction is commonly found in aromatic systems, such as a benzene unit and also in phenylalanine. There exists a difference in the arrangement of the stacking namely the face to face π-π stacking or the T-shaped packing, which is a face and an edge packing and then the parallel displacement packing.

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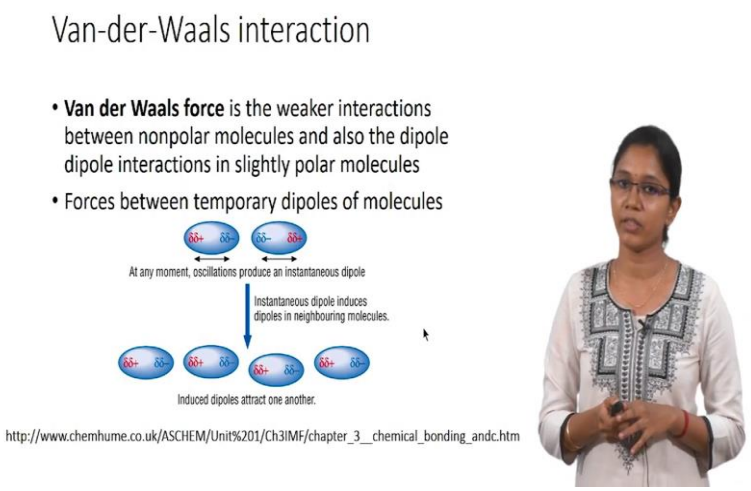


**van der Waals interactions**: The weakest interactions among all the non-covalent interaction. This is also called the London dispersion forces, where people debate on whether both are similar or they have the same property. van der Waals interactions exist between non-polar molecules. We know that charge-separated species exist in polar molecules. However, non-polar molecules and inert molecules such as helium may have temporary dipoles or oscillating dipoles. These non-polar molecules in a medium form a temporary dipole. These temporary dipoles are always oscillating, which has a weak partial positive charge or a partial negative charge.

When such molecules with negligible oscillating temporary dipoles come together, they tend to oscillate or arrange in a specific pattern. The negligible partial positive or a partial negative charge in the non-polar molecule starts oscillating in a similar pattern and form a self-assembled array, which is termed as Van der Waal interaction. By order of energy involved the van der Waals interaction is the least among all the non-covalent interactions.

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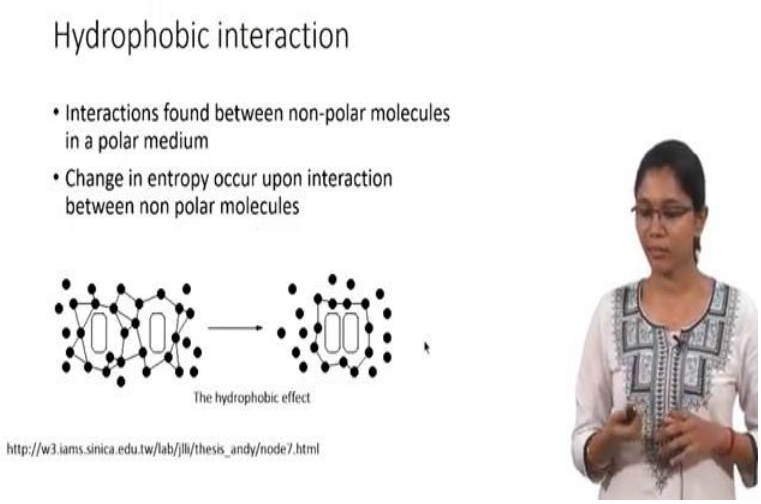


**Hydrophobic interactions**: Interaction found in the hydrophobic cavity of the proteins. Hydrophobic interactions arise when a non-polar molecule is put in water or an aqueous medium. For example, similar to a situation where you are into a very unfavorable condition. You tend to become very closed; you do not open up. This is what happens even when a hydrophobic molecule, which does not like water is dropped inside water. The molecules tend to minimize their energy and interaction with the surrounding. The water surrounding the molecule form a cage around the hydrophobic molecules. This causes a rise in the entropy of the surrounding system, and the hydrophobic molecules bury deep inside the cavity. Hydrophobic interactions help in protein folding processes, in micelles and bilayer assembly where hydrophobic molecules interact with water.

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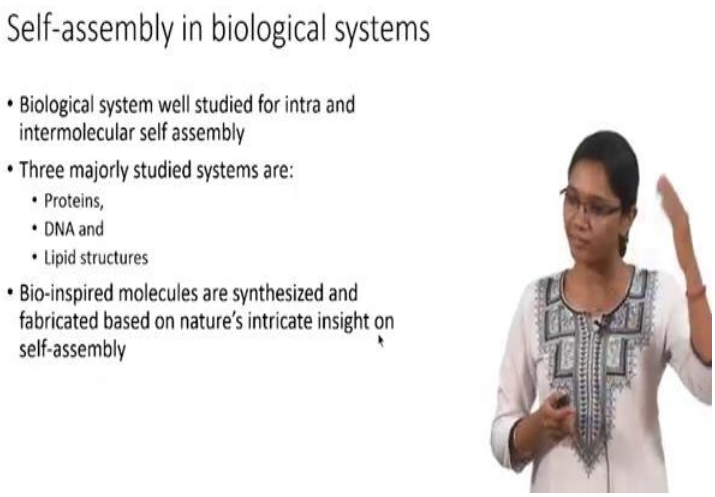


As we have discussed the different non-covalent interactions that form supramolecular chemistry. Further, let us discuss how they drive our life. There are three important structures within the biological system that is well characterized and studied for the non-covalent interactions. The proteins, DNA, and the lipid structures.

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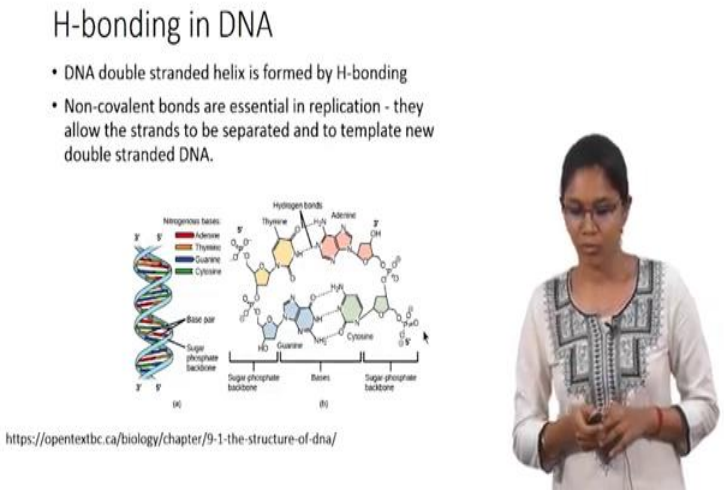


The first topic is the hydrogen bonding in DNA. When you look at the DNA structure, we know there is a DNA double-stranded helix, as I have already told before. There are phosphate groups, sugars, and a nucleoside base. Hydrogen bonding is the important aspect holding the DNA double helix intact, forming an interaction between the bases. There are four nitrogenous bases, namely adenine, thymine, guanine, and cytosine in the

DNA nucleotide. The hydrogen bond exists between the Adenine (A) and Thymine (T), and Guanine (G) and Cytosine (C). Does anyone know why it is always between A-T and G-C? Why not the other way?

**Student**: Because the interactions would not be stable

**Reply**: Ok, what interactions?

**Student**: Hydrogen bonding.

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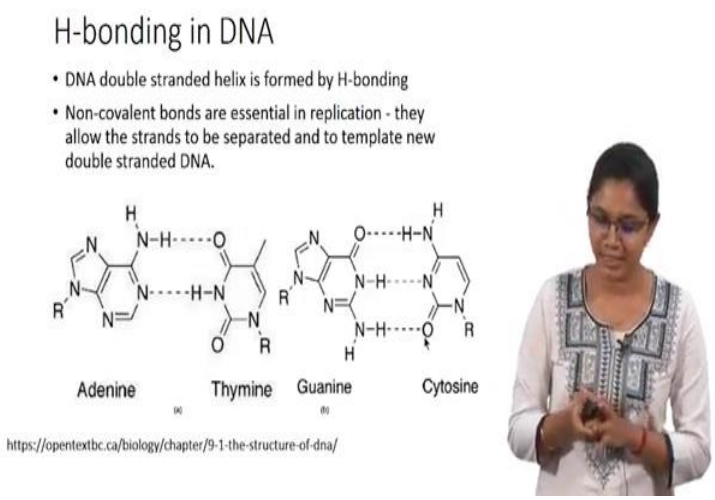


Let us take an example of the DNA hydrogen bonding. Adenine and thymine and then guanine and cytosine. If we intend to link them the other way, say adenine and cytosine, guanine, and thymine, how many hydrogen bonds each one will have?

**Student**: Two

Which has two?

Student: Both.

Does everyone agree with this answer?

**Student**: Even in that, where hydrogen bonds it would not be, it might not be planar.

But the hydrogen bonding is more of a distance-related phenomenon.

Student: Yes.

Taking adenine and cytosine as an example, adenine has one donor and one acceptor system and then whereas, in cytosine we have one donor and two acceptors. Hence, when adenine and cytosine are to be linked, it will have only one hydrogen bond and thymine and guanine will have two. Thus, this is the reason there exists a complementary binding between A and T, G, and C, showing that hydrogen bonding plays a significant role in keeping the double helix strand in place. Also, there exists intramolecular or intermolecular hydrogen bonding, and care should be taken while designing molecules for self-assembly. Moreover, non-covalent interactions are distance-dependent. The distance between the molecules can either lead to repulsion or an attraction. Hence, the distance between the molecules will help in facilitating the self-assembly process.

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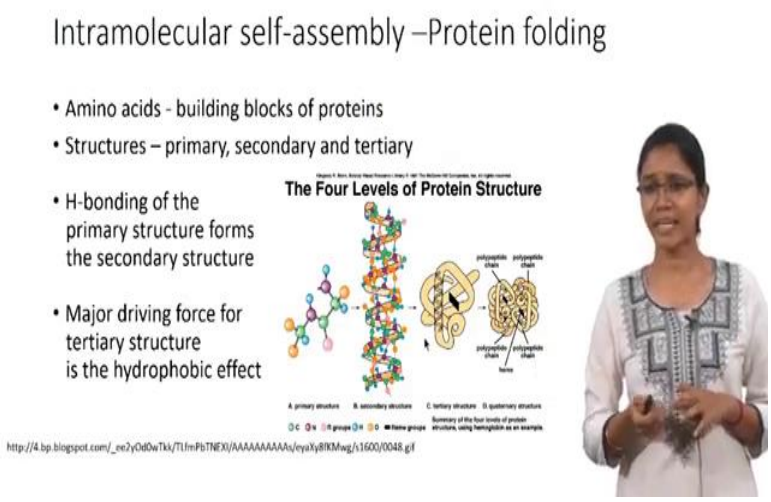


The next biomolecular interaction is protein folding. Protein folding is mostly an intramolecular self-assembly process. Amino acids are the primary structure of the proteins, which are covalently bonded. The amino acids form an amide bond to make a peptide sequence. Next, what happens in the secondary structure? In the secondary structures, the primary driving force is the hydrogen bonding. Hydrogen bonding helps in the formation of alpha-helix and beta sheets. Further, the tertiary structure involves protein folding and intramolecular hydrogen bonding.

What happens when a protein is put into an aqueous environment? The hydrophobic regions bury inside, minimizing the exposure of the hydrophobic cavities to the protein exterior. So, lysine residues or cationic/ anionic amino acid residues are exposed to the outside. Hence, this is how a protein folds with the help of hydrophobic interactions. In quaternary structures, intermolecular hydrogen bonding occurs where two or more polypeptides interact. All proteins do not have a quaternary structure.

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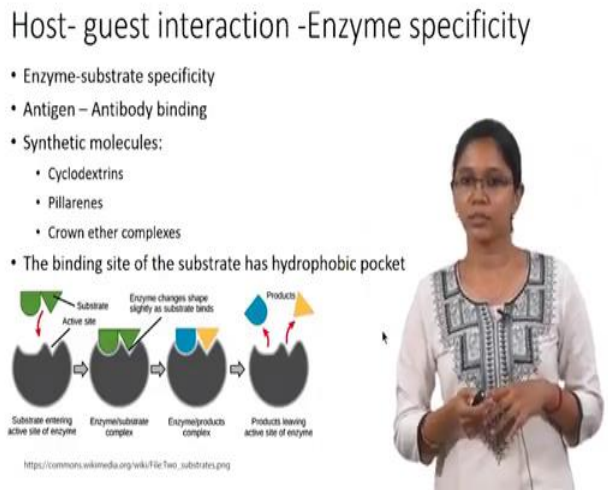


The host-guest chemistry is one of the important self-assembly mechanisms. Parallel to enzyme catalysis reactions, cyclodextrins, pillarene, and other host-guest systems came into supramolecular chemistry. There exists a high specificity for an enzyme-substrate complex similar to an antigen-antibody complex. The binding substrate has specific pockets or sites for which you have to design molecules accordingly to facilitate interaction for the product formation.

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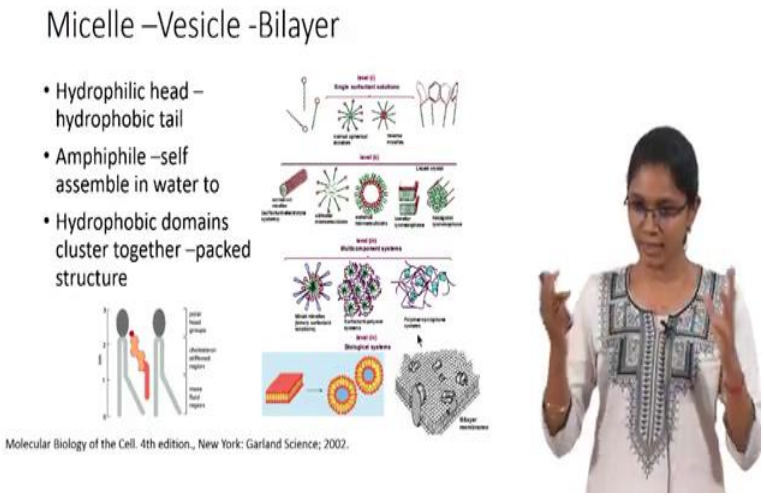


Next is the lipid bilayer. It is similar to a monolayer assembly. When a molecule consisting of a hydrophilic head and hydrophobic tail is put in water, they start to self-assemble. Either they form a micelle, or they form a bilayer. A bilayer is formed when two hydrophobic tails come together and start packing in ordered assembly. This bilayer can further extend to form a vesicle. Our eukaryotic cell membrane is highly composed of phospholipids (amphiphile in nature) along with cholesterol. The cholesterol also has a similar structure and shape and thereby controlling the inflow of small molecules and facilitating hydrophobic interactions with small molecules hence, the phospholipids, along with the cholesterol help in the intracellular flow of small molecules, and is also one of the reason that hydrophobic molecules tend to bind more effectively to the cell membrane. Small molecules are known to enter the cell through the diffusion process.

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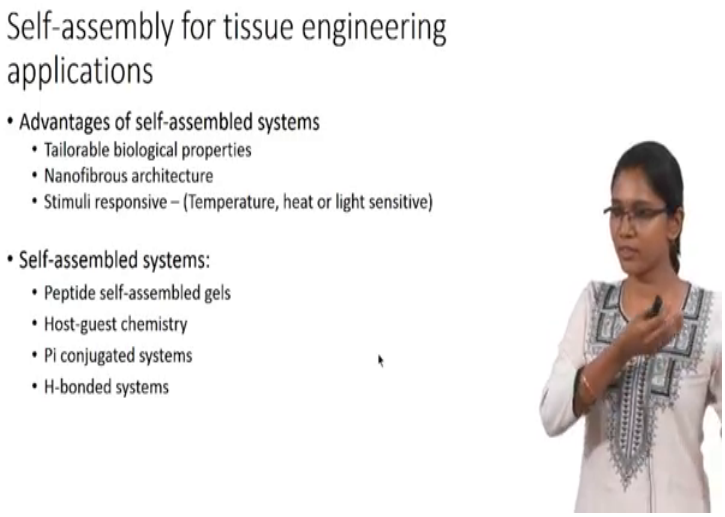


Now we understand the importance of non-covalent interactions in our life and day to day activities. The significance of non-covalent interaction for self-assembly is due to its stimuli sensitive property. To form a covalent bond, there is a lot of energy involved. But a non-covalent interaction is stimuli responsive. Temperature, heat, or any pH can lead to an alteration in the assembly, giving rise to reversibility. We also have known the biological process that is governed by non-covalent interactions, which we try to mimic for tissue engineering. These self-assembled or self-aggregates form stable structures, and the structures are mostly in the nano regime. Even if not a part of the nano regime, they have a nanostructure, say a fiber that has a width in the nanometre regime. This property can be tailored for several applications.

Further, we will discuss the self-assembled systems used for tissue engineering applications. 1) peptide self-assembled system, 2) host-guest chemistry, 3) pi-conjugated systems, and 4) hydrogen-bonded systems. They are some of the well-studied interactions for tissue engineering. There also exist other interactions that are being explored.

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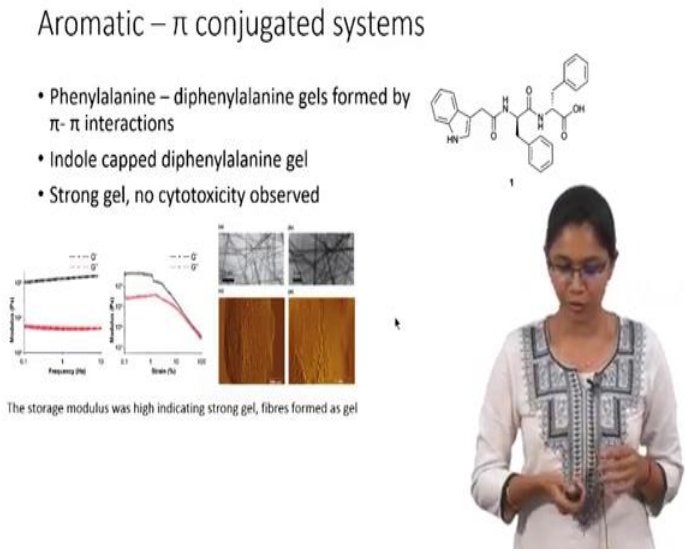


The first one we will discuss is an aromatic-pi conjugated system. The research was published in Chemical communications journal. It details the use of f-moc conjugated diphenylalanine as a hydrogel. It is a small molecule that self-assembles in water to form a hydrogel. The formed gel is visualized by an inverted method. For instance, a solution that does not flow is termed as a gel. Phenylalanine is known to form a gel, and, in this study, they have conjugated an indole moiety to the amino acid. They intend to see if the aromaticity of the indole moiety increases the self-assembly property, its stability, and also the cytotoxicity due to an aromatic addition. The initial characterization was performed, and the rheology properties of the storage modulus and the loss modulus were measured. Rheology explains the viscoelastic property of the gel. When the G' (storage modulus) is higher than the G" (loss modulus), it means the gel is more elastic in nature. Moreover, the gels were very highly stable because of the indole moiety.

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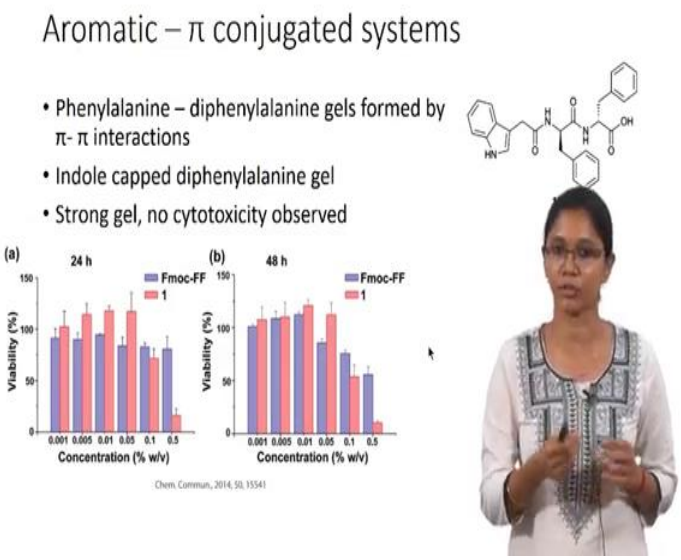


Further, they have performed the cytotoxicity tests to know how the viability of the cells. They observed that only at very high concentrations of the indole capped fmoc-diphenylalanine there is a reduction in the viability of the cells, indicating its biological-applicability as a self-assembled hydrogel.

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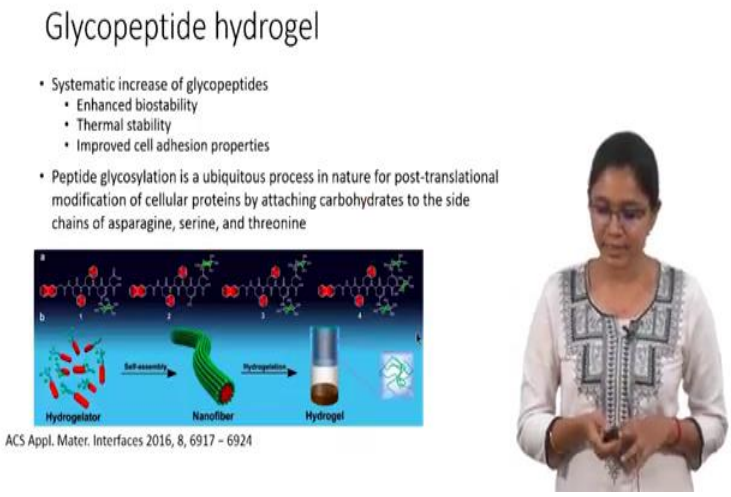


Another study explained here is from Applied Materials and Interfaces, where they explore five different molecules. They have a peptide molecule, and to this, they wanted to add a glucose moiety into the backbone and see the effect on thermal and biostability. The major disadvantage of peptides is their degradable nature. The amide bonds of the peptides are easily degraded by the enzymes that are present in our body. When you take

a self-assembled peptide system, it is degraded readily due to the enzymatic environment, but they are highly compatible with our body and regarded as native molecules. Therefore, to improve the thermal and the biostability, they have tried conjugating glucose molecule, which is known to have no cytotoxic effects.

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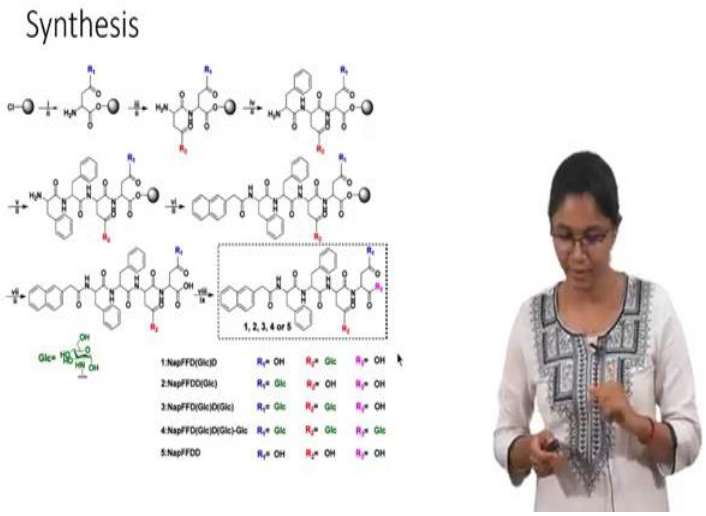


The picture on the slide depicts the five different compounds (labeled as 1, 2, 3, 4, 5) under study. Nap is a naphthalene molecule towards your left, followed by two phenylalanine rings that are conjugated. This is then linked to a glucose moiety in the aspartate region. The molecules are synthesized by varying the glucose units at the aspartate moiety. There are five different compounds modified with glucose units (1 or 2 in number). The $5^{th}$ compound will have no glucose residue, only aspartate, termed as the control. Therefore, there are four compounds with glucose subunits and the $5^{th}$ one with no glucose unit.

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## Thermal and Biostability

**Table 1. Summary of the Conditions and Properties of Supramolecular Hydrogels 1−5**

| sample | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| MGC (wt %) | 0.2 | 0.3 | 0.3 | 1.0 | 1.0 |
| pH | 5.5 | 5.5 | 7.0 | 7.4 | 4.5 |
| width of fibers (nm) | 13 ± 1 | 14 ± 2 | 12 ± 2 | 13 ± 3 | 22 ± 3 |
| critical strain (%) | 0.83 | 0.63 | 0.17 | 1.46 | 0.35 |
| G′ (kPa) | 4.7 | 16.0 | 13.8 | 0.086 | 11.5 |
| biostability[a] | 48.5 | 29.4 | 52.0 | 53.9 | 0 |
| $T_{gel-sol}$ (°C) | –[b] | 96 | –[b] | 67 | 53 |

[a]Compound remained (%) after 24 h. [b]The $T_{gel-sol}$ of hydrogels were more than 100 °C.

- Gel 5 was completely degraded in proteinase medium

The characterization results are summarized in a table indicating how the thermal stability and biostability are varied. MGC here stands for the minimum gelator concentration or weight %, i.e., the concentration at which the gel is formed. The table then indicates the pH at which the gel is formed, and the width of the nanofiber diameter. The strain % is the strain at which the gel becomes a solution. Then we have the biostability and the T gel in the table.

The biostability factor for the 5$^{th}$ compound is found to be 0. In this study, biostability is the monitoring of the remaining gel after 24 h incubation with an enzymatic fluid. In all 4 compounds, there is about 48.5 -29.4 % of gel remaining, whereas in the 5$^{th}$ compound, there is 0 % gel remaining indicating that the peptide hydrogel was completely degraded. This shows that the addition of glucose unit increases the biostability of the peptide molecule.

Then, T gel was monitored. It is the temperature at which the gel loses its stability. The T gel of the hydrogels conjugated to glucose units was found to be higher in comparison to the gel without the glucose unit. The 5$^{th}$ gel degrades at 53°C while the compounds with glucose have high thermal stability.

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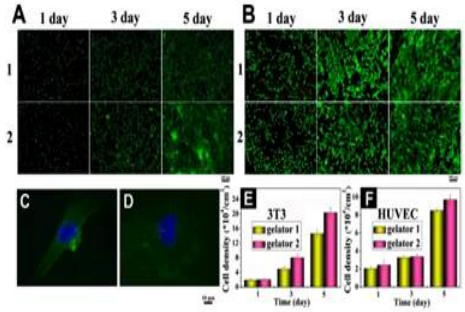


- Fluorescence images of live/dead assays of (A) NIH/3T3 fibroblast cells and (B) human umbilical vein endothelial cells (HUVEC) cultured on hydrogels 1 and 2 over the course of 5 days. Actin staining (Cand D)
- Cell densities of (E) NIH/3T3 cells and (F) HUVECs determined by cell counting with a hemocytometer over the course of a 5-day culture on hydrogels 1 and 2.

To further use the gels as scaffolds, they have used two gels, namely the 1st and 2nd gel, to perform cell culture experiments. They have used NIH/3T3 fibroblast cells and HUVEC cells. The figure caption A depicts the results for the fibroblast cell line, and B is for the HUVEC cells (endothelial), incubated over five days on the gels and monitored for proliferation. The green fluorescence that is observed is when the cells are stained with fluorescein diacetate, staining the cytoplasm green. C and D images depict actin staining.

Further, they have also calculated the cell density to correlate cell proliferation over a period of time. The results demonstrate that these peptide gels can be used effectively for tissue engineering applications with excellent cell adhesion proliferation properties.

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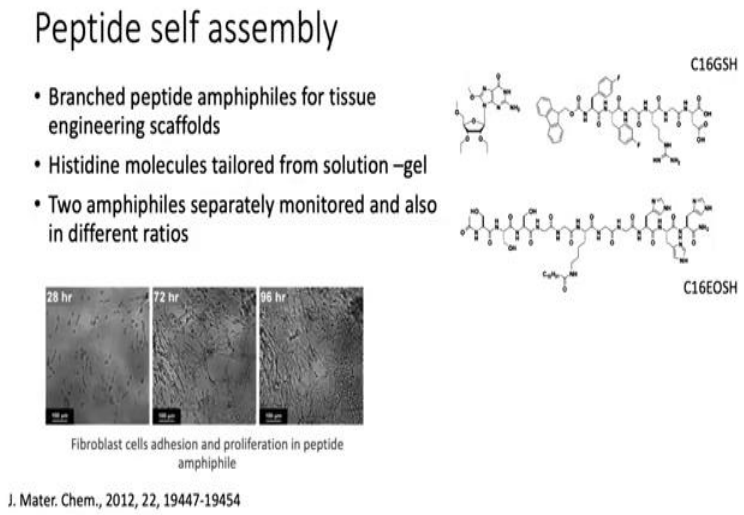


This is another peptide self-assembled system reported in JACS. In this study, two different peptide amphiphiles are characterized. In one of the molecules, histidine is conjugated to know the significance of histidine moiety. Also, both the amphiphiles were mixed in different ratios and characterized to see whether there is an effect on tissue culture. The microscope image depicts the incubation of fibroblast cells on the gel for 24, 72, and 96 h. A specific ratio of the peptide amphiphiles mixed at different concentrations displayed significant cell proliferation enhancement.

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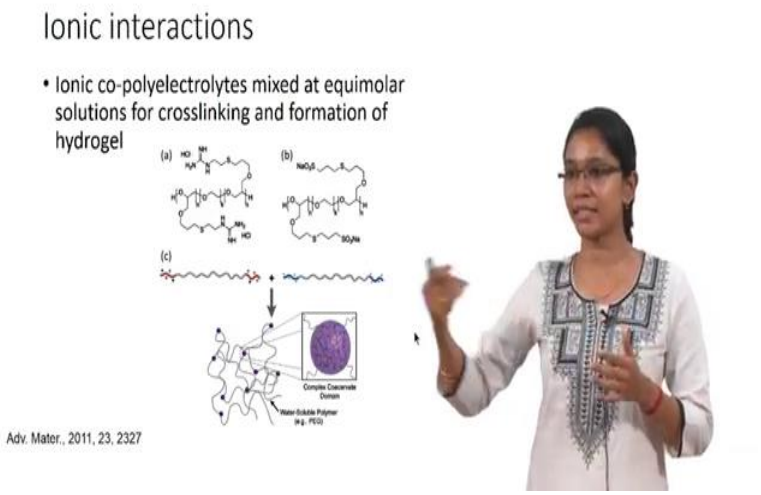


In the previous study, we discussed the role of hydrogen bonding interactions for gels. Besides, ionic interactions-based polymer gels are also studied for tissue engineering. In

this paper, an ionic co-polyelectrolyte polymer terminally modified is studied. A polyanionic and a polycationic polymer was utilized. When the two ionic polymers are mixed, they form a crosslinked gel. These are not covalently crosslinked gels but are intermolecular interactions. The purple color bubble (depicted in the picture) is the pictorial representation of the ionic interactions between the two polymers. Similar to the covalent bonds holding the molecules together, this is a non-covalent interaction keeping the gels intact.

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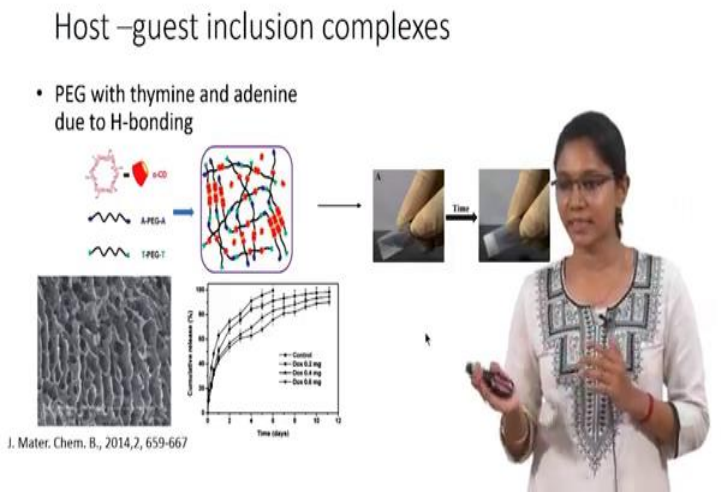


There are also host-guest inclusion complexes for tissue engineering. It is based on cyclodextrins. Cyclodextrins, as I have mentioned earlier, are cyclic glycans. In this paper, they have utilized two different supramolecular interactions. One, they have modified the polyethylene glycol (PEG) terminal with adenine and another PEG terminal to be thymine. As we already discussed, adenine and thymine have a nice complementary hydrogen bonding forming an A-T pair. What happens to the PEG molecules when they bind? They interact together and form hydrogen-bonded A-T pair forming an intermolecular interaction. These molecules then slide into the cyclodextrin core to further interact and form a stable gel. The formation of the gel is depicted in the picture. A polymeric solution of PEG is mixed with cyclodextrin to form a stable gel. The Scanning Electron Microscopy (SEM) image indicates the gel nature.

Further, doxorubicin, a cancer drug was loaded to the gel, and release kinetic was monitored. Moreover, they have also performed an *in vivo* study on rabbits to visualize the injectable effect of this gel on the rabbits and found to be successful.

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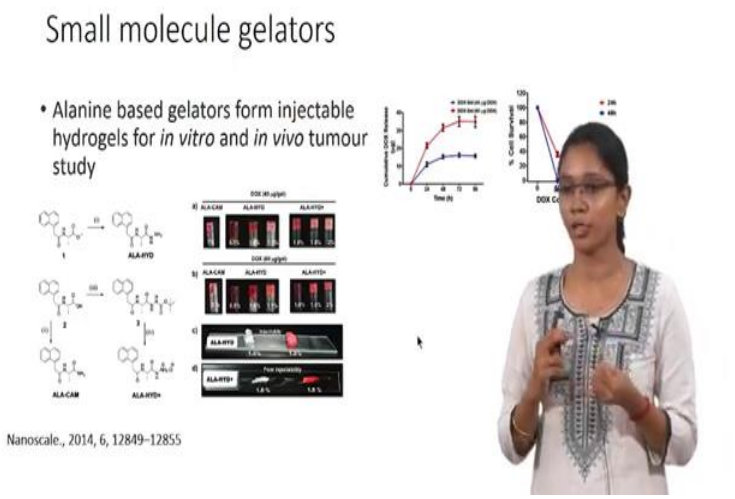


This example is of another small molecule gelator, where an alanine is the core molecule of the study. The exciting part of the paper is that they have made slight modifications to the terminal regions showing how small chemical modifications can alter the self-assembly pattern. The first molecule is alanine hydrazide ($NH\text{-}NH_2$); the second one is alanine-amide (CAM), which is $C{=}O\text{-}NH_2$ with an amide bond. The third molecule is the protonated form of a hydrazide ($HYD^+$). How are these small chemical modifications reflected in a self-assembly phenomenon? The amino terminated alanine (ALA-CAM) did not form a gel in any of the concentrations tested. Only the alanine hydrazide (ALA-HYD) and the protonated form of alanine hydrazide ($ALA\text{-}HYD^+$) formed gels at different weight percentages.

Further, the formed gels were tested as an injectable system. These molecular gels are not stable as a polymer gel and are termed as soft gels. Hence, it can be used as injectable gels. They checked the injectability of the gel and found that ALA-HYD displayed good injectable property in comparison to that of $ALA\text{-}HYD^+$. Hence, only ALA-HYD was taken for further drug loading and drug release studies. This tells us how small molecular differences have a significant impact on self-assembly.

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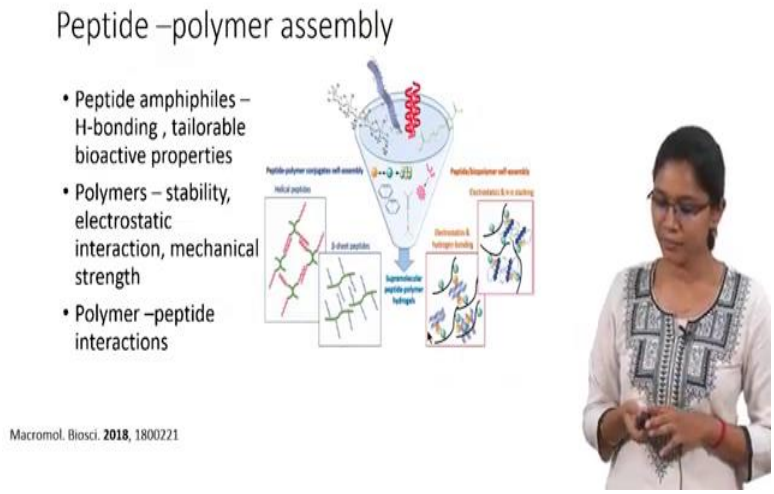


Here in this study, we have two different molecules coming into play, namely a peptide and a polymer. From our earlier examples, we know what is a peptide assembly, its advantages, and disadvantages.

Peptide amphiphiles are commonly used for their hydrogen-bonding interactions, and they also impart anionic or cationic interactions. They have tailorable bioactive properties. Polymers, on the other hand, are known for their stability because they are high molecular weight compounds. They impart the mechanical strength that is needed for a tissue engineering scaffold. Thus, in this study, a blend of a polymer and peptide is tried out to combine their properties. They can either be covalently or non-covalently bonded. However, in this study, only a blend of polymer and peptide with non-covalent interaction was carried out. The peptides undergo hydrogen bonding giving rise to helical peptides or beta sheets, while polymers have high strength and durability. They are combined to study their significance in tissue engineering.

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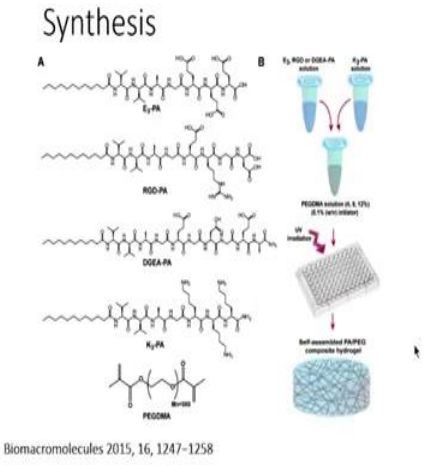


In this study, different peptide molecules were synthesized. It is a long peptide amphiphile with a hydrophobic chain towards one end labeled as $E_3PA$. Cell adhesion peptide RGD was conjugated to the second peptide amphiphile (RGD-PA). DGEA, another integrin that also has cell adhesion properties was conjugated to the peptide backbone for comparison (DEA-PA). The fourth compound was termed as K3-PA. The terminal functional groups of the peptide were changed to lysine residues having $NH_2$. These peptides were then blended to the polymers. The polymer is PEGDMA (PEG dimethyl acrylate), which is combined with peptides at different ratios.

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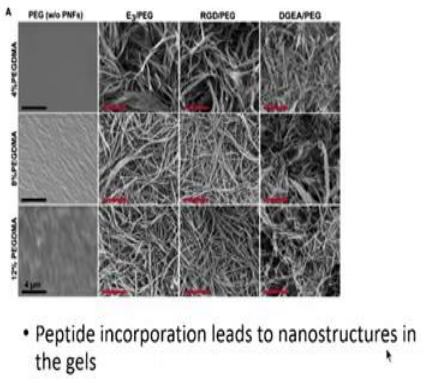


The blended gels were characterized by SEM. The first column in the image is of the PEG gel, which comprises only of the PEG polymers. This does not display any self-assembly or nanostructures. When the peptides are incorporated, what happens? The blending of peptides gives rise to helical or a nanofibrous structure in the gels due to the formation of molecular self-assembly. The peptides arrange to form a bilayer and then further self-assemble to form a long nanofibrous gel.

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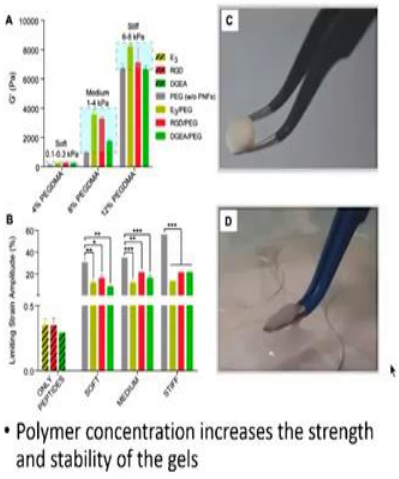


Next is to check the stability of the polymer-peptide gel. The main aim here was to have an enhanced mechanical strength by the addition of a polymer. They have PEGDMA at different percentages, namely 4 %, 8 %, and 12 % incorporated in the gels. The graph with crossed bars (slash lines) is the plain peptide gel. Upon addition of the polymer, there is an increase in G' transitioning from the soft gel to medium gel to a stiff gel. The gel stiffness from soft to medium to stiff is mainly attributed to the polymer component. This is because of the 4, 8, and 12% addition of the polymer. They have also monitored the difference in strain amplitude with a change in polymer concentration. But there was no significant difference observed in the strain amplitude.

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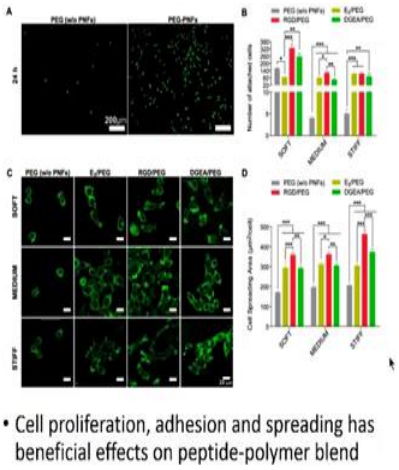


Further cell cultural experiments were performed. Here, the PEG molecules with and without the PNFs were monitored. PNFs are peptide nanofibers. The image C group the gels based on soft, medium, and stiff based on polymer wt %. In parallel, polymers without nanofibers, and with nanofibers with specific (RGD and DGEA) peptides were also grouped vertically. This is to identify whether the RGD peptide or the DGEA peptide has good cell adhesion properties.

Upon observation, it is found that RGD peptide has more cells attached in the soft gels medium and stiff gels. Then, also, in the cell spreading area, the RGD peptide is higher in comparison to that of the DGEA peptide. Among the two integrin proteins (RGD and DGEA) which help in cell adhesion, we say that RGD is effective than that of the DGEA peptide. This is the reason why researchers conjugate RGD peptides to polymers or gels to have good cell adhesion properties.

These are some of the examples that we have discussed in this lecture describing what self-assembly is and what supra-molecular interactions are. When we understand the basics of supramolecular interactions it will be helpful to design molecules for applications. Application or macromolecular aggregates depend upon the molecular interaction of how monomer assembles, and its with the surrounding environment. Thus, self-assembly and supramolecular interactions have a significant role in designing molecules for tissue engineering.

Thank you.