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82% of soldiers in battle suffer from traumatic limb injuries. Many of these injuries are large bone defects. Engineers at MIT are trying to create materials that mimic the function of the bone's natural healing processes. The structure and properties of these materials promote bone regeneration.

This video is part of the Structure-Function-Properties video series. The structure, function, and properties of a system are related and depend on the processes that define or create the system.

Hi, my name is Nisarg Shah, and I am a graduate student in Professor Paula Hammond's lab in the chemical engineering department at MIT. In the Hammond Lab, we design novel materials for tissue engineering, gene and drug delivery, and energy applications. My research in particular, focuses on developing and assembling different materials for bone tissue regeneration.

Before watching this video, you should be familiar with the concepts of pH and pKa, and with the common chemical functional groups.

After watching this video, you will be able to explain how the concepts of pH and pKa are useful in the design of materials via layer-by-layer assembly.

Many of the materials our lab develops are based on a technique called layer-by-layer assembly. Layer-by-layer assembly utilizes electrostatic interactions to create layers of chemical species of alternating charge. These chemical species could be anything – polymers, proteins, small molecules – the key is that they have the appropriate positive or negative charge to incorporate them into the film.

We assemble these films using a simple dip method. First, we take our substrate or surface that we wish to coat and bombard it with free radical oxygen in an instrument called a plasma cleaner. This not only cleans the surface of contaminants, but also leaves the surface with a negative charge.

Next, we take our substrate and dip it into a solution of positively charged molecules. The molecules in solution bind to the substrate because of Coulombic interactions.

This process is self-adsorption limited, meaning that once the newly adsorbed layer neutralizes the charged sites on the surface, additional molecules will not bind. This creates layers that are on the order of nanometers thick.

After a rinse step to remove any unbound components, the substrate is dipped into a solution of

negatively charged molecules. Interactions with the charges on the previous layer allow a new layer to be deposited.

Again, we would rinse to remove any unbound components. We can continue this process and dip our growing film into solutions of positively and negatively charged molecules, with rinse steps in between, building up our coating layer-by-layer. Many of the films I work with have on the order of 40 layers.

We use polymers in many of our layers. These long chain, high molecular weight molecules form more stable layers than small molecules. The long polymer chains from one layer weave through other layers creating an interlocking structure.

Also, because we use negatively and positively charged polymers in these layers, the polymers ionically crosslink, adding to the stability of the overall film.

Polymers whose repeating unit contains a charged group are called polyelectrolytes. Thus, many people refer to the films that we create as polyelectrolyte multilayers.

For every polymer or small molecule I incorporate into my layer-by-layer assembly, I have to be mindful of its pKa and select appropriate assembly conditions to ensure they have the desired charge.

For example, one of the polymers that I use in a layer-by-layer assembly is polyacrylic acid. You don't need to worry about its exact chemical structure. The important thing to note is the repeating carboxylic acid group.

Another polymer that I use in my layer-by-layer assembly contains repeating amine groups.

Let's say I'm creating my layer-by-layer assembly at a pH equal to 4. If the pKa of the carboxylic acid groups on our first polymer is approximately 4.5, and the pKa of the amine groups on our second polymer is approximately 6.5, what would the charge on each of these polymers be? Pause the video and take a moment to think about it.

At a pH of 4, some of the carboxylic acid groups on our first polymer would be deprotonated, resulting in a negative charge.

At the same pH, some of the amine groups on our second polymer pick up an extra hydrogen, resulting in a positive charge.

Because so many molecules could be viable candidates for incorporation into these films, they are being used in a wide variety of applications. My main interest is using these films in tissue engineering applications.

So, first I have to ask myself, what problem do I want to solve? Then, I have to ask myself how these multilayer films could be useful. What function do they need to have? What properties of the film would help to achieve that function? Taking all of this into account, how should I structure my film?

One of the problems I became particularly interested in is that of large bone defects. Large bone defects are large gaps in the bone that result from trauma.

Although bone is capable of regeneration, when there are large defects, some sort of intervention therapy is needed to bridge the defect for proper healing.

These intervention therapies typically involve taking bone tissue either from another location in the patient's body or from a deceased donor source and grafting it into the defect site.

While bone taken from the patient may be immune compatible and be more viable, this method has limitations. Tissue injury and trauma at the site of bone removal causes patients pain and long healing times.

Bone tissue from a deceased donor may cause an unfavorable immune response. The processes used to prepare these tissues for implantation may also compromise their mechanical properties.

Our lab had an idea for a possible solution to this problem. We asked ourselves, can we design a scaffold to bridge these large defects that will stimulate the growth of new bone tissue? The idea was to create a rigid, porous scaffold coated with a multilayer film. The multilayer film would deliver biological molecules that would stimulate the growth of bone both on the surface and throughout the scaffold. Over time, the scaffold would slowly degrade, leaving the new bone tissue behind.

In selecting components for both the scaffold and these films, we looked to the biological process of wound healing for inspiration. The idea was that if we could mimic the wound healing process in bone, we could potentially regenerate tissue that is mechanically and chemically identical to native bone tissue.

The bulk of our scaffold consisted of a polymer that would slowly degrade when placed in the body. This polymer was mixed with calcium phosphate, a significant component of native bone. Our thought was that calcium phosphate would promote the attachment, growth, and migration of cells that produce bone tissue within the scaffold.

We then used layer-by-layer assembly to create a multilayered film on the surface of the scaffold. The film contained layers of polymers and layers of biological molecules that we wanted to release into the defect site. One of the molecules was a protein called bone morphogenetic protein-2. This protein is a growth factor that stimulates mesenchymal stem cells from the bone marrow to transform into bone tissue producing cells.

The biodegradable properties of the polymers we used in the film would help to release the protein into the surrounding tissue when the scaffold is implanted.

In addition to identifying molecules that would give us the function and properties that we desired, we had to choose an assembly pH that would ensure they had the desired charge.

To test our hypothesis that these materials would lead to bone growth, we implanted our coated scaffold into a rat quadriceps muscle.

In this model, the scaffold was placed in a location where bone is not normally found, so that we would know that any bone created on the scaffold was due to the coating.

Our experiments were successful in that we saw the deposition of bone minerals and collagen on our scaffolds within 4 weeks. Of course, there are more experiments to be done to see if this system will work in the same way in a large defect site.

The main lesson I learned in these experiments was that for bone tissue regeneration to occur, my multilayer-coated scaffolds needed to provide two key functions: to encourage bone producing cells to attach, migrate through, and deposit tissue in the scaffold; and to encourage mesenchymal stem cells to transform into bone producing cells.

In this video, we hope that you saw how general chemistry concepts such as pH and pKa continue to be

useful beyond the classroom and into research settings. Here, we saw how we need to consider a molecule's pKa when assembling polyelectrolyte multilayer films at a given pH.

We also saw how considering the desired function and properties of a material can help us rationally design its structure.

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