Collagen Hydrogels

Abstract

Collagen is another widely used biomaterial that has found popularity in commercial applications, such as photography. Collagen has more recently been used in tissue engineering applications, by creating hydrogels that provide structure to engineered tissue.

This video introduces collagen as a biomaterial, demonstrates how it is harvested from porcine skin, and shows how the material is used to create a hydrogel for tissue engineering applications. Finally, several applications of the material and these techniques are shown.

Transcript

Collagen Hydrogels act as a biologically compatible three dimensional structure, similar to that of native tissue, which facilitates the development of engineered tissue. Collagen, the main component of these gels, is an extracellular matrix protein found in connective tissue. And is used to fabricate hydrogels which, are 3D polymer networks comprised primarily of water. This video will introduce the physiological role of collagen, a procedure for the isolation and processing of collagen from porcine skin, the fabrication of hydrogels from the purified protein, and finally some applications for the use of these collagen hydrogels.

Within the extracellular matrix of connective tissues there are specific cells responsible for the majority of collagen manufacturing called Fibroblasts. These cells synthesis long, stiff, polypeptide chains. Which, are assembled into individual triple helical ordered polymers called Collagen Molecules. Collagen molecules are then further bundled into thicker and larger Fibris. Which, are then even further bundled into larger fibers to provide structural integrity to the extracellular matrix for the various mechanical loads it may experience. Different configurations of collagen are produced according to the mechanical needs of specific tissues. Bone tissue, for instance, utilizes a highly compact intense network of fibers in order to withstand mechanical loads. However, other tissues, like the intestinal wall, use a more dispersed structure. Once extracted from connective tissue the collagen polymer can be used to create various materials. Because collagen is a hydrophilic polymer it is highly absorbent. Thus, it can form a hydrogel, which is a polymer network that holds up to 90% water. These polymer networks are formed by crosslinking individual polymer chains using various methods. Such as, chemical crosslinkers, heat, or UV light. The degree of crosslinking affects the mechanical properties of the hydrogel. Thus, enabling these materials to be used in a wide range of applications. Now that the principles of collagen hydrogels have been explained, let's take a look at how collagen is extracted from porcine skin and used to create a hydrogel.

To begin the processing of collagen from a dermal sample, first rinse it in ice cold distilled water and then use depilatory cream for wool, fur, or hair removal. Using a single edged razor blade, scrape the sample clean of connective tissue and fat, and rinse once more in ice cold water. Then slice the skin sample into centimeter squared pieces. To remove the non collagenous solubilized material, first weigh out 5 grams of dermal squares per 50 milliliter conical tube, and then add 30 milliliters of ice cold 0.5 Molar sodium acetate to each sample. Then mix the tubes in a bench top homogenizer. After one minute discard the supernatant and mix the samples again in more ice cold sodium acetate for a total of seven wash cycles. After the last wash, rinse the sample in ice cold distilled water, and mix it one more time to remove the residual sodium acetate. Then compress the sample against the tube to remove excess liquid and transfer each processed 5 gram dermal sample to fresh 50 milliliter conical tubes. To extract the collagen wash the sample twice in 2 milliliters of .075 Molar sodium citrate buffer per gram of sample. Discard the supernatants and compress the samples after each wash has previously demonstrated. After the second wash, add a fresh aliquot of sodium citrate buffer, and then perform six sequential agitation cycles without removing the buffer between each cycle. Now, transfer the supernatants to individual collection tubes and add an additional one milliliter of .075 Molar sodium citrate buffer per gram of sample before performing a final agitation cycle. Next, centrifuge the supernatant again in a centrifugal filter device to purify the extracted collagen. Finally, store the purified collage at 4 degrees Celsius.

Now that the collagen has been purified, a collagen hydrogel can be fabricated and populated with cells to form an engineered tissue construct. To begin, thoroughly mix the purified dermal collagen in a conical tube. Then, add the cells immediate to the collagen solution and place it on ice. Pipette the ice cold mixture into a sterile, non tissue cultured treated, surface to minimize the attachment and growth of cells outside the collagen gel. Use a pipette tip to evenly spread out the solution. Allow the gel to polymerize at room temperature for approximately 10 to 15 minutes. Then, cover the gels and move them to a 37 degree Celsius incubator for an additional 60 minutes to finish polymerization. Upon polymerization, the gel will turn opaque. Once polymerized, add two to three milliliters of media. Then the populated hydrogel is ready to use in tissue culture studies.

Now that you have learned how to construct collagen hydrogels, let's look at some practical applications of these materials. Collagen hydrogels are often used to mimic native tissue, as shown here. However, changes in the collagen fiber organization can occur in natural tissue. Resulting in aligned structures. As a result, engineered collagen matrices can be constructed in either random or aligned configurations. Collagen hydrogels are often used as tissue scaffolds for the development of artificial tissues. By providing a customized three dimensional structure for cells to inhabit, the collagen matrix is subsequently reorganized to mimic the structure and function of real tissue. For example, scaffolds can be seeded with osteoblasts, the cells responsible for bone formation, to reorganize the matrix to more closely resemble the structure and function of native bone tissue.

You've just watched Jove's introduction to collagen hydrogels. You should now understand collagen structure, how it is isolated, and the fabrication of collagen hydrogels in addition to its various applications in the bioengineering field. Thanks for watching!