

Tissue-Engineered Articular Cartilage

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A star football player watches his knee buckle backward, hears an excruciating “pop,” and realizes his worst fear has come true – a meniscal tear. A 65-year-old woman rises from her chair to sense a stiffness in her ankles that forces her to limp, reminding her that her osteoarthritis is growing worse day by day. Despite their broad age gap, these two are victims of similar afflictions that are caused, at least in part, by damage to cartilage.

Cartilage is a compact connective tissue whose chemical and physical properties allow it to offer support and facilitate smooth movement in skeletal joints. It is comprised of cells known as chondrocytes and a surrounding extracellular matrix (ECM) which contains various proteins and other structural components. Together, these extracellular components form a gel-like mesh that gives cartilage its mechanical strength. However, if cartilage is damaged, chondrocytes cannot regenerate it, not only because they are too few, but also because they lack access to blood and nutrients [1]. This calls for a novel tissue engineering approach that allows us to create cartilage to insert into people’s joints that have suffered this kind of damage.

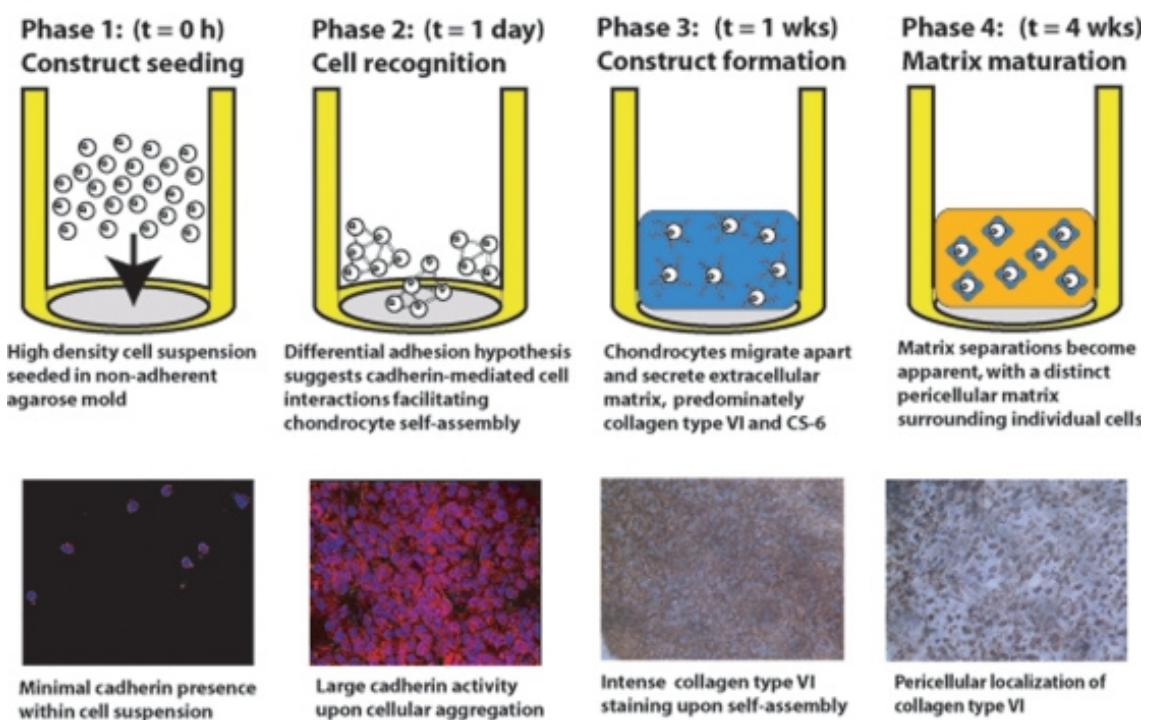
Many labs have worked towards developing such an approach, but in 2006, Rice Professor of Bioengineering Dr. Kyriacos Athanasiou and his group, most notably Dr. Jerry Hu, pioneered an unique self-assembly method of growing cartilage. This method involved seeding a high density of chondrocytes in agarose wells, which served as three-dimensional “molds” for the tissue constructs to form. Unlike most previous attempts, this new method did not rely on the construction of “templates” known as scaffolds to direct tissue formation [2]. The Athanasiou lab has continued to use the self-assembly method in creating articular cartilage (the smooth tissue lining the ends of bones at moveable joints) and in analyzing biochemical

and mechanical characteristics during tissue development. They have been able to modify this technique to closely mirror the developmental stages of native articular cartilage and even to identify key points for effective manipulation of the tissue growth.

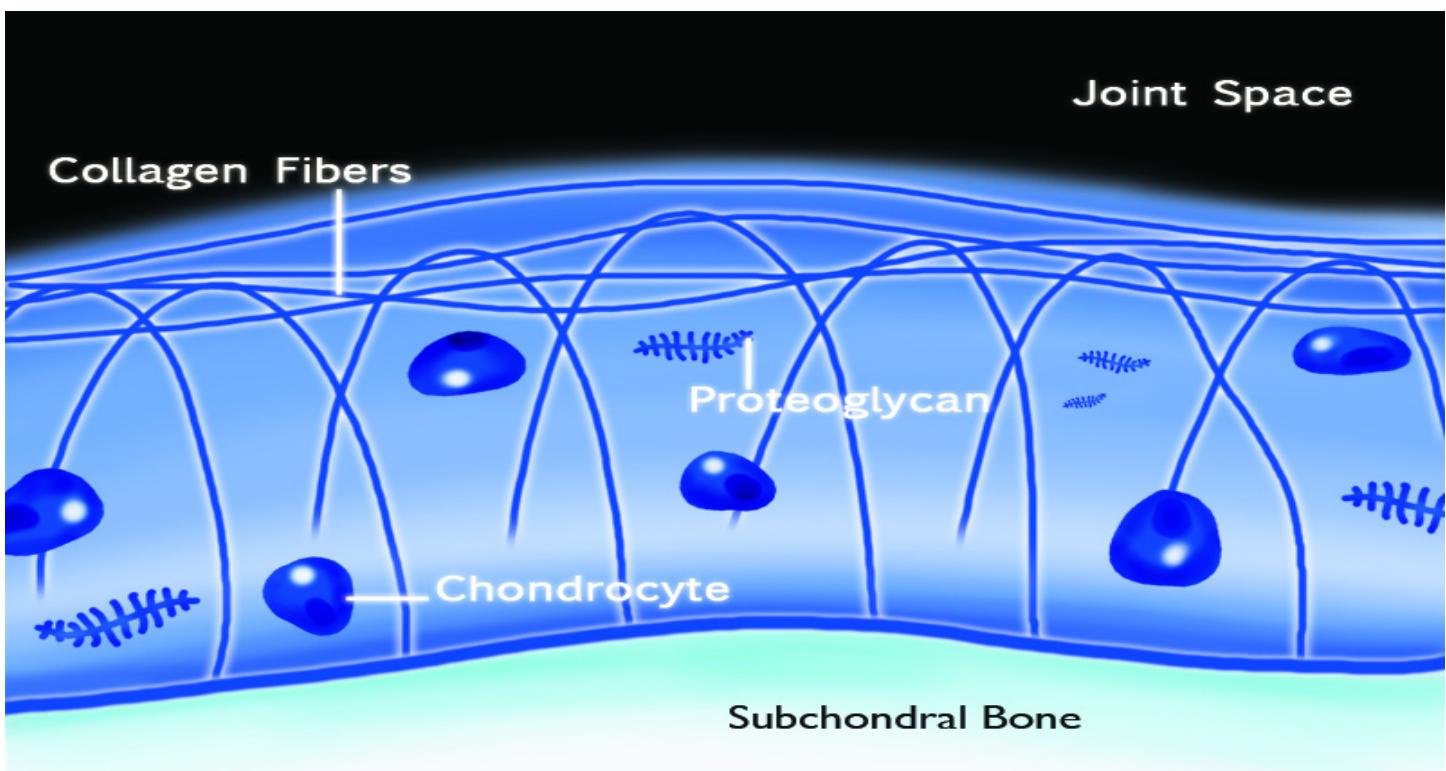
One of their research projects has focused on the ECM development during cartilage self-assembly and the specific trends in matrix components such as various collagen types, glucose and glycosaminoglycan (GAG) types, and N-cadherin. By relating these observations with the results of mechanical testing, Dr. Athanasiou’s team has identified structure-function relationships in the developmental process that make articular cartilage the strong material it is [3].

To create the cartilage tissue constructs, the lab members first prepared a mixture of bovine articular chondrocytes in a culture medium and inserted it into agarose wells. From this point onward, they conducted the experiment in two phases over a total of 8 weeks, collecting both qualitative and quantitative data on the ECM components. For example, immunofluorescent staining, which involves labeling specific molecules and structures using antibodies, was used to map the general occurrence of N-cadherin and collagen types I, II, and VI. Safranin-O staining was used to label GAG. Shifting to a more specific analysis, the

Tissue Development within the Self-Assembling Process



Cartilage development begins with seeding of chondrocytes and ends with formation and modification of the ECM. These changes correspond to trends in localization of collagen and GAGs. (Image courtesy of Dr. Kyriacos A. Athanasiou)



Articular cartilage consists of chondrocytes and the extracellular matrix (ECM) they secrete. The ECM contains a mesh of collagen fibers and various proteoglycans. (Graphic by Rodrigo Flores)

team collected biochemical data to find the net amounts of GAG, glucose, collagen, and individual collagen types contained in each construct. They found that overall GAG concentration increases during the growth of the constructs, but a certain type of GAG known as chondroitin 4-sulfate (CS-4) increases at a faster rate than the related chondroitin 6-sulfate (CS-6). Collagen type II increases and spreads throughout the tissue whereas collagen type VI decreases and ultimately remains in the region immediately outside the cells. N-cadherin, the protein responsible for intercellular adhesion of chondrocytes (and therefore crucial to the initial steps of the self-assembly process), has an increased presence only for a short time during the early stages of development.

The tissue samples from the second phase were exposed to mechanical stresses, such as tension and compression. These tests were then correlated with the biochemical data to reveal some interesting relationships between structural composition and function. For example, the growing trend in GAG levels corresponded with increasing compressive stiffness. The net amount of collagen as well as the collagen type present in the construct determined its tensile strength.

Another important finding in this experiment involved identifying the point at which significant changes in localization of collagen and GAGs occurred, which was around 4 weeks. The lab members believe this may be an effective time to introduce stimuli that will influence the overall development of the constructs, allowing them to enhance the self-assembly process and achieve one of the main goals of this research [3]. This project was aimed mainly at gaining a greater understanding of the self-assembly process in articular cartilage, but future projects will go

even further by using this knowledge to modify that process—to really engineer it.

According to one of the lab members, MD/PhD student Sriram Eleswarapu, one of the major problems facing tissue engineering is “making a three-dimensional tissue that is mechanically stable—this is an engineering feat in itself.” However, once the self-assembly process is improved, this feat will come closer to real clinical application. At the same time, the discovery of alternative cell sources such as embryonic, mesenchymal, and induced pluripotent stem cells, each of which are studied by the Athanasiou group, will eliminate the need to extract cells from individuals, making it more feasible to transplant them into patients with damaged cartilage.

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References

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