Nutritional Requirements for Promoting Tendon Regeneration

By: Daniel Joseph Spicer

Submitted to:
Frank LaBanca, Ed.D.
Science Department Chair
Oxford High School
June 8, 2010

Abstract:

Tendons are the connective fascia linking muscles to bones and cartilage. They are composed of mostly tenocytes and collagen I. However, since they receive little blood and nutrition, they are likely to be strained and sometimes ruptured. Current research on regrowth of tendons is limited to tendon cell cultures, which allow for advancement of studies, however, on a smaller scale. Research done currently is on incubating tendon cell cultures to measure the amount of recovery, differences in material quality upon recovery, and factors that improve both of those studies. It may be possible to incubate tendons on a full scale to test factors in a more real life setting while avoiding the risk of performing on a person who might be injured by the experimentation. This project will test an environment and nutrition for chicken tendons, which are similar to those of humans, for sustainability of life in the tendons and growth in wounds. The tendons will be tested in a carbon dioxide rich atmosphere and poor oxygen to prevent growth of bacteria. The tendons are to be immersed in a PBS solution with copper, dextrose, amino acids, chondroitin, and vitamin C. A uniform incision of 1 cm will be made in all experiments, and growth over periods of one, two, and three days will be measured for success against tendons immersed in only the PBS.

Introduction:

Muscles have no ability to function without the use of tendons and other connective fascia. Tendons work as a connective tissue between skeletal muscles and bones, allowing the contractions of the muscles to move individual body parts as directed. Electric pulses sent through motor neurons to muscles from the brain cause them to contract, giving the motion one would see when he does a bicep curl or types on a key board.

However, by whatever measure causes it, some tendons rupture, tear, and weaken, prompting the need for recuperation. In the tendons, when a rupture or tear occurs, the tendons will form and deposit collagen into the area of the injury. As with all injuries to the body, the area will work to make the injured tissue stronger than it was before to avoid suffering the same injury in the future. Also, collagen III forms from the granulation of tissue, so it forms at a far greater rate than collagen I. when the body does this, in ligaments, tendons, ant the skin, it increases the amount of collagen III generated as the wound over the regular amount, causing a decrease in the principle collagen I.

Upon injury, mRNA for specific types of collagen are received in the tenocytes, collagen producing cells within the tendons, to begin the process (University of Michigan, 2009). Depending on the gene received, the tenocytes will synthesize procollagens. Procollagens are long protein strains with amino and carboxyl groups at the book ends and are soluble, allowing it to move effortlessly within the cell to be modified. The ends have peptides that are not found in collagen. The amino terminal on one of these ends forms disulfide bonds with two other procollagens to align them together. Following this, the procollagens will undergo many steps of modification and molding to form one of the most complex and organized structures found in the

body. Vitamin C deficiency will cause the production to fall, resulting in poor collagen production, referred to as scurvy (Lippiello, 2006).

Collagen I normally is found in bones, skin, tendons, organs, and ligaments lined up sequentially is the most abundant form of collagen found in the body. It is very resistive to tensile forces pulling and pushing on it because all of the individual fibrils align in a manner to accept forces, making it great for tendons that take immense pressure from cutting and running and falling in sports and daily life. However, collagen III, found in reticular fibers of connective tissue in a meshwork of crossing collagen proteins, is less likely to take the strains well due to its cross work of proteins.

Therefore, the areas where a tendon has been torn will be predisposed to become injured in future occurences. The reason that bones and skin can use this procedure well without injury reappearing is due to the fact that they do not have to stretch at odd angles; bones stay stiff, or they break and skin has no need to stay as rigid as tendons, so its looseness on the dermis allows it to take the pulls easily.

In research it is prevalent throughout that, in the biological field, a culture is made for the testing of procedures, chemicals, or conditions upon a subject to document its reaction. In order to create a culture, the cells that are wanted are isolated and suspended in a solution that enhances growth. The individual suspensions are then incubated to influence reproductionand allow for the process to occur more rapidly, since incubation creates the optimal conditions.

Beginning in 1902, researchers have been creating tissue culture cells to study the process of reproduction in a controlled environment. Previously, scientists and researchers were unable to study the processes used by plants to reproduce cells, allowing for the continuation of life. The

main goal of the experiment was testing for totipotency of plant cells, the ability to perform all reproductive properties of a zygote without usual surrounding tissues of the organism present. (Multilab, 2007)

The 1902 experiment, performed by Haberlandt, submersed single cells in a salt solution heavily enriched with sucrose, and showed growth of cells for about a month. The cells did not perform the processes of a zygote in the ability to devide, but they performed the usual actions that plant cells do besides reproduction, like producing starches. From this, Hanning made cultures that followed similar guidelines in 1904 that grew dearly mature embryos to maturity

From the amazing and unexpected results from the Haberlandt experimentation, Gautheret and White in France and America, respectively, developed processes for cultivation in the twenties and thirties. It was further developed in the fifties and sixties by Skoog to near completion. The outlines Skoog developed are the basis of all guidelines for culture processes used in current scientific research. (Multilab, 2007)

The transfer from plant to animal tissue cultures was not needed or attempted until the 1940s and 1950s, at which point polio epidemics were running rampant across America and various other nations, inflicting terror into the eyes of many. The reason for the many bacterial cultures available but very few animal and, more importantly, human cultures was the time necessary for the reproduction of cells in animals versus bacteria. While animal cells require 18 to 24 hours to perform one cycle of reproduction, bacteria can do it in as little as 30 minutes, which is up to 48 times as fast as the cells of animals (Chaundry, 2004). Animal cultures that had bacteria in it would become overrun by the bacteria because of their ability to reproduce so

quickly, consuming all present fuel like sucrose, and possibly consuming the tissue from animals along the way.

The need was for cultures of human cells was the ability they allowed researchers of the time to perform tests on the virus of polio to make a cure or a treatment for the many civilians suffering from its symptoms. A 1949 experiment showed growth of the polio virus in human cultures. The cultures allowed the polio viruses to be grown in large quantities. Once the virus was grown, it was killed, leaving only a shell of the debilitating animal that affected so many before (Chaundry, 2004). The shell introduced the virus to one's body and allowed the body to form a defense for it without being weakened by the real thing.

Since then, cell cultures have been further developed to create more types of treatments for diseases. In the seventies, cultures were developed to create and replicate proteins commonly synthesized in animals. They previously hadn't been synthesized because bacterial cultures could not add sugar to the proteins to make them longer. Later on in the seventies, two different cells were able to be combined in a culture to make antibodies, the common treatment in diseases today (Chaundry, 2004).

In order for the culture to properly mature in a manner that allows for reproduction and survival without being consumed entirely by bacteria, incubation is utilized. Incubators are devices that heat an object to promote growth and influence maturation of the object. Since late ancient Egyptians employed fire-heated rooms to warm eggs, humans have been artificially heating objects for the purpose of growth in an unnatural environment (McGrath, & Travers, 1999).

Problem:

As previously stated, tendons that become injured form scar tissue that is callous and the tendons are not as pliable as they were before. The loss of elasticity and ability to take tensile forces as it was able to do before makes the tendons more apt to rupture or tear once again. Therefore, patients of these injuries have a predisposition to become reinjured, which is not too healthy to the patient.

Carbon nanotubes are quite possibly the most structurally stable compound known to man, so it has a strong ability to resist decomposition. They also are able to take strains and absorb forces that tendons may be incapable of taking. The nanotubes may stretch to accommodate the amount of strain given, yet they will not break.

Therefore, due to all the evidence and information available, it is possible to assume that one can use the technology of carbon nanotubes to make the process of healing tendons in the short term and the long term better for the patient. In certain situations where a tendon is taken from another person, cadaver, or part of the body, the tendon is out in the open. At this point, the tendon may be able to be applied to the tissue in order to make the healing process easier or faster. The nanotubes may be placed around the point of incision or the point where the tendon is connected to bone or muscle tissue.

Materials and Procedures:

Materials

Chemicals/Consumables	Supplies	Equipment
Store-bought chicken legs 8g NaCl 0.2g KCl 1.44g Na_2HPO_4 0.2g KH_2PO_4 Amino Acids Dextrose	Razor blades Solution Sterilization Filter Sterile Test Tubes	Microscope Incubator Rocking Machine Microscope Camera Computer

Procedure

For tendon cultures, the tenocytes are isolated by rinsing in trypsin and collagenase, which occur naturally in tendons throughout the production of collagen, in Dulbecco's modified Eagle's medium. This removes most of the collagen, but there is a small amount of collagen remaining and the tenocytes must be separated, so they are centrifuged and then suspended in DMEM to be incubated after. The process, including chemical costs, comes out to almost $3000, making it difficult for production with limited resources (Maffuli, 2000).

When cultivating tendons, chicken legs were acquired from a local supermarket as a recently harvested whole chicken. First, the legs were cut from the chicken, because they hold the greatest amount and largest tendons. The skin was then removed so the muscle was showing.

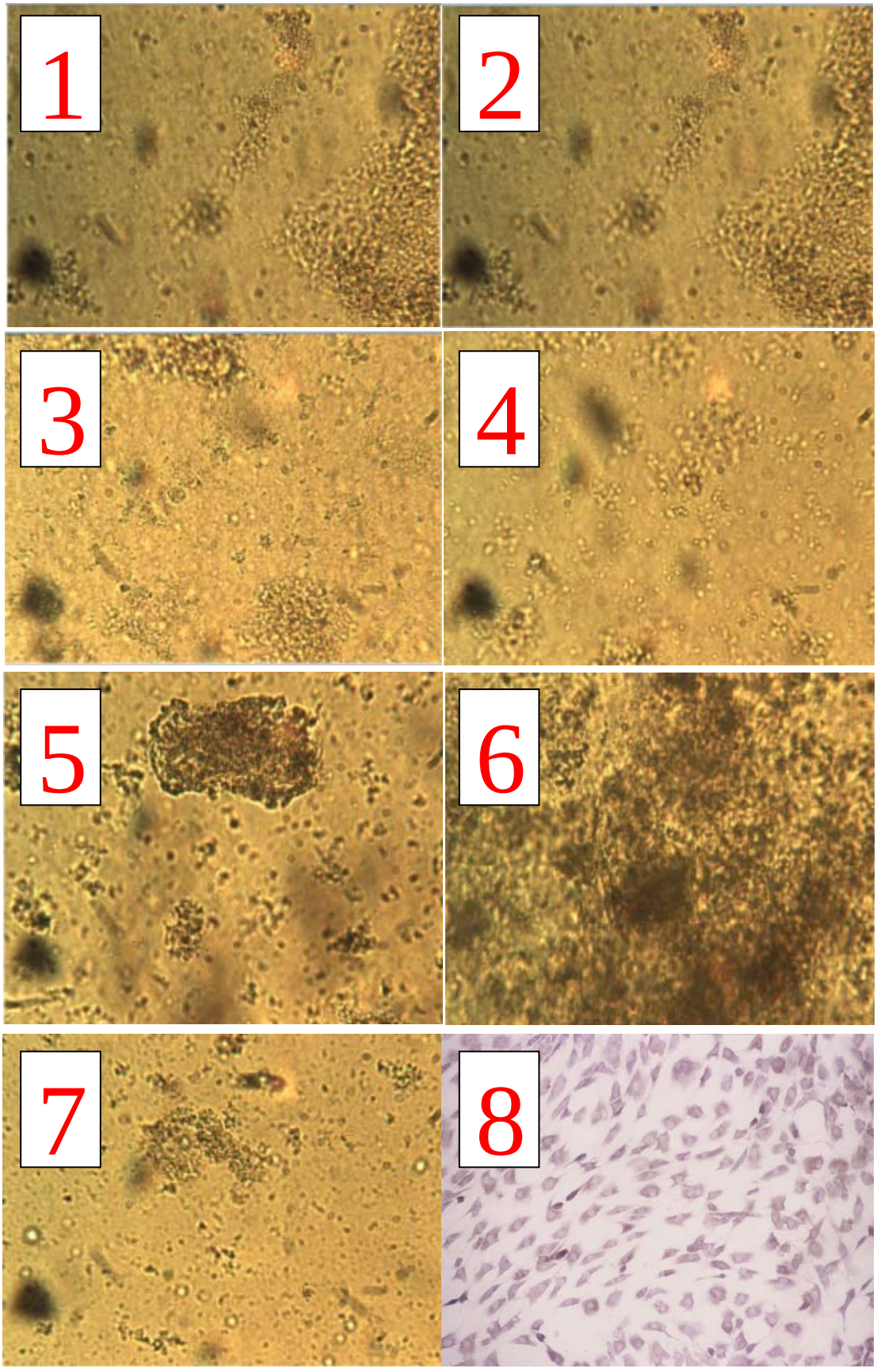
When finding the tendons one would look for white tough areas across the muscle. The tendons at the bode or cartilage end come to a tight rope-like structure while the areas attached to the muscle fan out in a thin sheet. A razor was used to free the tendon from the leg and where it was attached to the bone it was cut off as close to the bone as possible. The tendons were then

removed of all visible flesh and fat by scraping the edge of the razor along the tendon in a sawing motion.

The medium that the tendons are to be incubated in is a solution of Potassium Buffer Saline (PBS) with a mix of assorted nutrients that have been determined to enhance be vital to the growth of tendons. PBS was made by adding 8g of NaCl, 0.2g KCl, 1.44g Na2HPO4, and 0.2g KH2PO4 to one liter of distilled water. At these conditions the pH was 7.4, which is not harmful to the tendons. Each of the following nutrients was picked to be added to the solution for varying reasons: Amino Acids and Dextrose contain building blocks for growth, which are proteins and carbon. Copper Chloride contains Copper which, along with other elements like Manganese and Silicon organizes the collagen in tendons into the order to accept tensile forces best. Ascorbic Acid, or Vitamin C, is integral in the breaking down of amino acids and building of procollagens in the tenocytes. In order to influence growth in the tendons, amino acids, along with Dextrose, Ascorbic Acid, and Copper Chloride were mixed into the PBS. After the solution was made it was sterile filtered and ten sterile test tubes each received 5mL of the solution.

When incubating the tendons to test for bacterial growth, the tendons that were previously cultivated were immersed in the PBS solution described before. The tendons were placed in the test tubes. In the incubator, the test tubes were placed on a rocking device in the upright and horizontal positions. The test tubes in the upright position were placed in a small beaker to allow for movement and sloshing about, which spread the solution throughout the tendons and gave nutrition to all areas. In the upright test tubes split half and half, with two cracked slightly open to allow for air flow and two sealed air tight. The test tubes laying down were sealed air tight to avoid spills. The incubator was set to 37^0 as a normal temperature for incubating tendon cultures. Samples were incubated for 2 days.

Results:



In the pictures shown above for the results, images 1-7 show pictures taken from a microscope of the tests performed on the tendons in the incubator. In the images, one can see how bacteria infected the cultures of tendons and overran the tendons. Image 8 shows a picture of a tendon after it had been stained. One can see how the images of the tested tendons cannot be stained because of the overwhelming presence of infection throughout the cultures.

Conclusions

Through testing of growth of tendons in a full sized scale it was determined that incubation of a tissue sample without it first being prepared as a culture is not a successful way to promote growth and perform tests. The results showed that after only two days of full sized tendon incubation in a culture-type setting, there was noticeable infection throughout the samples. Infection was noticeable in yellow discoloration to the normally white tendon tissue and foul odor of rot emanating from the samples.

In a normal tendon tissue sample incubated for that period of time, there would not be entire bacterial seizure of the samples as noted in the tendons tested here. Therefore, it has been noted that this is an unsuccessful procedure for testing on tissues. Bacteria normally around the tissue flourishes in the environment that culturing is incubated in. Preparing a culture for testing involves steps that remove harmful substances, such as immersion in slightly acidic solutions.

For this reason, one should prepare cultures in the procedures described literature of scientific journals.

Limitations

Because of the overindulgence of the bacteria that infected the experimented tendons consumed the tendons and all of the parts that they are comprised of, including the collagen I and III fibrils and the tenocytes.

Through staining of the tendons, it would be possible for one to determine growth of the tendons in the experimentation. However, when the tendons were examined after incubation, it was possible for one to see that the tendons had become consumed by the bacteria, and it would be impossible to perform the staining.

For staining to be performed, the tendon samples would need submersion in acetone and covered with antibodies that allow for staining. From there, the samples would require incubation for another 60 minutes to allow for the antibody to spread throughout the sample and allow for fluorescence of the fibrils and tenocytes within the tendons. Further incubation of the tendon samples would only further enhance the growth of bacteria and eventual death of the tendons. (Maffuli, 2000)

Future Studies

Because of the results of the testing, it is impossible to perform future studies in the field of this study. My goal was to see the ability of tendons to be incubated in a culture incubation environment. Through the testing of this project, it was found that tendons could not be incubated in such a way to promote growth of the tendons without infection in the systems.

Future studies of similar types might include testing cultures for their limitations of successful growth in multiple strenuous environments. Cultures are made and tested in such a way as to make sure as possible that they do not become infected with bacterial growth. However, doing this requires much time and precision with chemicals and gases. If tests could be performed on cultures of tendons with the least amount of chemicals and in the least stressful procedure, it would allow for more tests in a quicker manner, meaning that answers could be found more quickly.

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