The Future of 3D Printing and Tissue Engineering

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The use of three-dimensional printing (3D Printing) and biotissue engineering appears to be a new and effective methodology to implement stem cell regeneration in repairing tendon injuries. Damage to the tendons has a great impact on an individual's active lifestyle ranging from daily ambulation to competing in sports. Current approach to repairing tendons usually only requires conservative measures. If surgery is needed, techniques involve either direct reconnection of the ruptured tendon or utilizing tendon donor grafts or autografts. Growth factors and stem cells have already demonstrated a positive effect in repairing ruptured tendons. However, limitations to applying these factors in vivo have been encountered. The progressive use of 3D bioprinting offers an exciting new way to approach tendon repair. Researchers have demonstrated potential use in treating conditions like tendon rupture, tendinopathy and tenosynovitis. Further studies need to be performed to establish efficacy, safety and clinical applications of 3D printing and tissue engineering, but the future of improved treatment options for tendon injuries looks promising.

Introduction

3D Printing and tissue engineering are the leading force in regenerative treatment in tendon tissues. Scaffolds that are made from three-dimensional printed biomaterial are imbued with growth factors that are then released in the area of injury. With traditional treatments like surgery and physical therapy, lasting problems still stand like loss of function, infection, and scar tissue. In this paper, numerous articles and studies were researched to figure out how effective and in what direction 3D printing and tissue engineering are going in the medical field. Since findings of tissue engineering and 3D printing have shown to reduce risk by cutting down surgery time, cut down costs, and optimize the benefit of the treatment, they are advancing their way into treatment options.

Tendon Overview

Part of the musculoskeletal tissue, the tendon is responsible for connecting muscle to bone. Collagen is one of the main proteins that is used in making skin, muscles, bones, tendons, and ligaments. In a healthy tendinous tissue, tightly packed collagen fiber bundles are aligned so that it is parallel to the direction they stretch (See Figure 1). Tendon, mostly formed with type I collagen, is made up of fibrils that hold a stiff structure. Therefore, these tendons allow for the body to exert a large amount of force under tension caused by contracting one's muscles, resulting in bone movement. Since the tendon is the connector of muscle to bone, it is critical in bone movement and proper joint functioning. Being such a vital part of our daily lives, injury to the tendons is incredibly detrimental and occurs frequently.

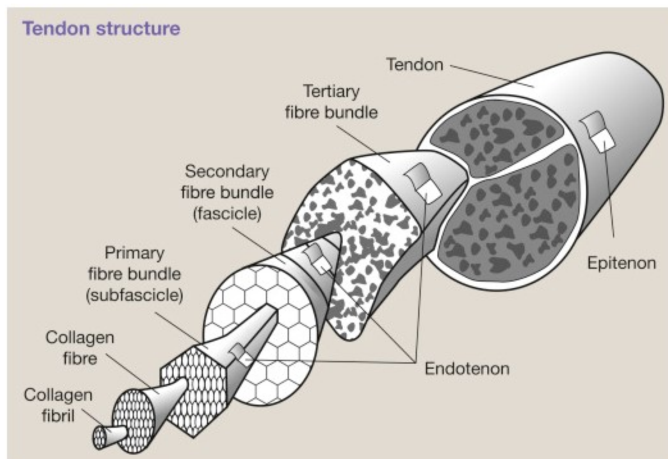

Fig. 1 Image provided by Jenna Zan, from the Tendon and Ligament: basic science, injury and repair - shows a zoomed image of a tendon's structure - from collagen fibril all the way to the epitenon that surrounds the entire tendon.

Tendon Injuries

There are many tendinous injuries that one can endure, ranging from chronic (long term) to acute (abrupt) symptoms after a partial or complete tear of the tendon, but they can be generalized under 3 main categories: Tendonosis, tears that occur because of constant use; Tendinitis, inflammation of the tendon; and Tendinopathy, a combination of tears and degeneration. Patients with chronic tendon injuries like tendinopathy have a condition that prohibits any type of regeneration to the injured area. Acute injuries, on the other hand, usually occur because of an irregular movement of effort. Management of tendon injuries initially involves conservative measures such as rest and pain relievers. However, it usually takes months for tendon healing, especially for more extensive and significant injuries. Currently, medical and surgical treatment options are few due to the limited knowledge of tendon biology. Most common surgery techniques involve either direct suturing together of a ruptured tendon or transplanting a healthy tendon taken from elsewhere in the body. Operating on a tendon is challenging because the tendon may be too swollen or too damaged. Additionally, postoperative symptoms could cause the tendon to stop generating new cells. So, through research, scientists hope to advance in developing treatments that can effectively repair injuries like tendinopathy and abrupt ruptures. 3D printing and tissue engineering have been one of the outstanding potential treatment options that could accomplish this goal. This novel treatment plan could offer many advantages since 3D printing helps create organized and spatially controlled tissue. This tissue could help aid in cellular retention and organize and deliver growth factors to stimulate cellular growth and regeneration in a specific desired location and structural order.

Printing and Tissue Engineering

3D printing is the process of making or "printing" a physical object from a three-dimensional digital model. 3D printing and tissue engineering go hand in hand. Tissue engineering's purpose is to make a functioning tissue in regenerative medicine. 3D printed tissues provide a specific way to organize tendon cellular growth in order to better generate healthy tendons. After surgical tendon repair, there is a risk of rupturing it over and over again because the tendon-to-bone cells do not regenerate. 3D printing and tissue engineering could alleviate this risk by allowing improved growth and regeneration of the tendon.

Traditionally, tissue engineering uses prefabricated 3D scaffolds as the support system to load stem cells[1]. These 3D printed scaffolds required bioactive materials, porosity and compressive strength to support growth and proliferation of the desired cells. However, these previously conventionally made scaffolds are limited by their ability to accurately replace the complexity of the bio-tissue it is trying to mimic, as well as represent the spacing and distribution of cells as in the actual tissue[2]. 3D bioprinting appears to improve scaffold engineering for tissue regeneration by utilizing living pluripotent cells and biomaterials in a layer-by-layer technique. Current bioprinters' ability to place cells and materials with micrometer level accuracy compared to previous fabrication techniques carries a distinct advantage of creating a more representative scaffolding system to the tissue it is trying to replicate[3]. There are a few different methods of 3D printing of tissue such as inkjet, laser, and extrusion based bioprinting which mainly utilizes two types of 3D printing material, synthetic or natural.

The first step in 3D bioprinting is to create the desired 3D biological model using computer assisted design software[4]. An appropriate biomaterial known as "bioink" needs to be selected to be used for successful printing of cells and growth factors. Bioinks are typically either natural (e.g. gelatin, collagen, fibronectin, and hyaluronic acid) or synthetic (e.g. polyethylene glycol, polylactide). Some are a combination of natural and synthetic materials[5]. Beyond selecting the optimal bioink, specific stem cells need to be selected and cultured to achieve enough to achieve the desired tissue. Stem cells have the main advantage for tissue regeneration given their pluripotency and ability to self-renew. Several types of stem cells have been utilized in 3D bioprinting such as human mesenchymal cells (hMSC), adipose derived stem cells (ASC) and human amniotic fluid-derived stem cells (hAFSC). Aside from stem cells and tissue scaffolding systems to support cellular growth, appropriate cellular medium and growth factors are needed to provide the stimulus to guide and support the development of the desired tissue. Growth factors are water soluble signaling molecules that basically control how the stem cell develops. Thus, the correct growth factor will control the cells growth and proliferation and ultimately, its differentiation to a specific tissue[6].

Scaffolds that are placed to repair the tendon rupture are imbued with growth factors constructed with the 3D material and are released into the area that was injured. The growth factors that are released from the scaffold can then act as a stimulus that continues the healing process. If these growth factors prove to show promising results, the advantages of 3D printed tendon constructs and benefits over current methods of repair could revolutionize surgical therapy of tendon injuries. However, growth factors could act in negative ways; including cancer formation, cell degradation, and inflammation.

Standard of Care for Tendon Injuries

Achilles Tendon Rupture

The Achilles tendon is comprised of both the gastrocnemius and soleus tendons which connect the calf muscle to the posterior aspect of heel. The gastrocnemius muscle attaches to the medial femoral condyles, located on the inside part of the end of the knee. The soleus are the muscles that attach to the outer surface of the tibia and fibula. Over the past 30 years, an increased incidence of Achilles tendon ruptures have been noted, perhaps related to increased activity levels in the older population[7]. There are three degrees of severity to an injured Achilles tendon: Grade 1, a partial rupture that is less than 50%; Grade 2, almost a full rupture or if it is a full rupture (the gap between the tendon is less than 3 cm); and Grade 3, a full rupture that prohibits the ability to walk on it. This injury is fairly common amongst athletes due to excessive force applied or landing awkwardly. Rupture of this tendon occurs most commonly in men aged 20 to 39 years old. As of 2020, 1.8 people per 100,000 people have had an achilles tendon incident per the UT Southwestern Medical Center. Besides having a grade 3 rupture, patients are still able to ambulate on an injured achilles tendon; however, if not treated properly, the tendon may not return to it's preinjured strength and capability. There is no general consensus on whether surgery or non surgery provides the better outcome in terms of restoring functional strength, facilitating return to preinjury ability, and avoiding re-injury[8]. Surgical repair of the Achilles may provide quicker return to use, but there was no long term difference found in strength or rates of re-rupturing of the tendon. Surgery was associated with higher risk of wound complications and infection after surgery as opposed to non-invasive management. In fact, there may be a trend toward less surgery and more conservative treatment based on recent findings suggesting no measurable improvement in outcome with surgery versus no surgery[4].

Tendinosis

Tendinosis happens when tendons degenerate due to small tears or overuse. Tendinosis can occur in any tendon, but the most common locations are in the achilles, knee, and elbow. There are 3 tendinosis stages to consider: reactive, disrepair, and degenerative. The first stage usually happens after an overload in a short amount of time. This often leads to a tight and stiff feeling after workouts or long periods of rest. The second stage has a much greater magnitude of pain in the tendon as the tendon cells begin to stop healing and regenerating. Depending on how severe this stage is, the pain can make it difficult to carry out daily functions and exercise. Finally, the degenerative stage comes from overuse that actually results in cell death within the tendon. This type usually occurs in elderly people making it incredibly difficult to exercise. At this stage there is also a much higher risk of a tendon rupture. Tendinosis treatment typically consists of conservative measures like resting, stretching, massaging and wearing braces over the affected area helping about 80% of patients[9]. These methods are low cost and less invasive but can take up to 6 months for the tendon to recover and leave 20% of patients with tendinopathy symptoms[10]. Alternative treatments for cases, especially ones that are not responding well enough to more conservative modalities, include surgery and platelet-rich plasma (PRP) injections. Surgical methods typically either remove the damaged peritendinous tissue enabling the tendon to heal or make longitudinal incisions within the tendon[11]. PRP injections imbue the tendon with the person's plasma to promote the body's own tendon repair process and can be used alone or in conjunction with surgical treatment[12]. By releasing growth factors like PDGF, TGF-Beta, EGF, etc. through the PRP injections, there is a greater promotion of cell growth and synthesis of type I collagen that specifically help in the healing process of the tendon with very little risk. Synergistic activity of platelet rich plasma and high volume image guided injection for patellar tendinopathy. The goal of 3D printing would be to release similar growth factors to induce the synthesis of collagen needed to heal chronic tendon injuries like tendinosis. In cases of surgery, the success rate is about 95% and only takes 3 to 4 weeks to recover. Regardless of the treatment, those who make a full recovery are able to go back to daily life without any residual pain.

Tenosynovitis

Synovium is a thin membrane that lines the inside of joints. Tenosynovitis is an inflammation of a tendon and its synovial sheath. The synovial sheath is located where the tendon passes under the ligaments and reduces friction between the tendon and the rest of the body. Tenosynovitis most commonly occurs in the wrists, ankles, and feet and typically is seen in middle aged adults between the 30 and 50 years old. Symptoms of an inflamed synovium include local pain, redness and swelling on movement of the affected area. Like tendinosis, synovitis is often associated with joint overuse. Therefore, athletes and people who perform repetitive exertive movements are at higher risk. Treatments for synovitis are similar to that of tendinosis as well. Rest, time and ice, along with anti-inflammatory drugs are some of the best treatments because they are inexpensive and non-invasive. Mild forms of synovitis usually heal within a month, but surgery may be required for recalcitrant cases. Synovitis surgery or synovectomy where a lot of the synovium is removed is typically used for chronic cases. When the tendon is inflamed, there is an excess growth of synovium so taking out large amounts of it aids with the healing process of the inflammationn[13]. This surgery is usually performed with a minimally invasive approach and recovery takes about 3 to 4 months before allowing resumption of daily activities.

The Future of Tissue Engineering

Three-dimensional printing (3D printing) has made its way forward in numerous fields such as aerospace, food industry, consumer markets, and arts. Now, 3D technology is making advances in the biomedical field as well. Although not completely incorporated into the medical field, tissue engineering has seen recent progression in creating biological substitutes for degenerative tissues within the body. This curiosity stems from the fact that 3D biofabrication allows scientists to mimic cell architecture and organization which can be used to tailor cell function and regenerate useful biomaterials[13]. 3D printing is currently used in producing prototypes, and companies like Helisys, Ultimateker, and Organovo have already used 3D printing to make living human tissues. Even with this foreseeable benefit, only about 1.6% of the entire 3D printing economy is invested in medical applications. Tissue engineering aims to utilize scaffolds or constructs, cells and growth stimulating signals to create functional tissue in order to repair or restore damaged tissues or organs[14]. Recent advances in nanotechnology, cell biology and biomaterials has made 3D bioprinting more applicable to tissue engineering. There are three main bioprinting techniques: inkjet, laser assisted and extrusion. Inkjet printing was the first method created and has the advantage of faster speed, lower cost and easier accessibility. However, inkjet can be limited by droplet problems and nozzle clogging. Laser avoids using a nozzle and is non contact but is relatively complex to set up and perform relative to inkjet printing. Extrusion is related to inkjet printing but sends out a continuous line of bioink ("extrudes") rather than single droplets as done with inkjet[15].

Samples of 3D Printing in Tissue Engineering

Achilles Tendon Rupture

Tendinous tissue is the source of about 50% of musculoskeletal injuries in the U.S and has a poor ability to heal. Complete recovery after injury is something that doctors have yet to accomplish. Looking at specific cases, one potential treatment involves the use of a three dimensional printed scaffold with induced pluripotent stem cell-derived mesenchymal stem cells. Through extrusion 3D printing, the polycaprolactone (PCL) scaffolds were imbued with iMSC (induced mesenchymal stem cells) and were observed in rat Achilles tendon defects. These rats were tested in two groups: microgrooved scaffolds and sutured only. The different results that were looked at were gene expression, gait, histology and immunofluorescence. The 3D printed scaffolds were engineered by melt extrusion and printed into a dog bone shape in order to settle onto tensile grips. The histology and immunofluorescence results showed that compared to the achilles tendons that were only sutured, there was an increase in tissue formation in the scaffold and iMSC group. Overall, the testing revealed that the scaffold and iMSC group showed that in topographical and biomechanical characterization the tensile areas had a significantly lower stiffness than the microgrooved scaffold. Additionally, the gene expression of scaffold-seeded iMSC had a statistically significant upregulation of tenogenic markers compared to the non-patterned scaffolds. Overall, better functionality and histological growth was found in the 3D printed scaffold compared to traditional treatment. Although these results are promising, limitations arise in that rats have different loading characteristics than humans. Perhaps, improved understanding of integration of 3D printed stem cell laden scaffold delivery with current surgical techniques will improve surgical outcomes versus nonsurgical approach.

Tendinopathy

Research is also being done with chronic injuries like that of tendinopathy. Platelet-rich plasma (PRP) injections have become an increasingly popular and positive treatment on tendon repair. In addition, some studies have found PRP combined with stem cell therapy increases tendon functionality as well as relieving tendon pain. Currently, applying stem cell therapy clinically is mainly limited by the inability to retain enough stem cells in the desired location[16]. Also, PRP is loaded with growth factors to support tendon growth, but too much PRP can be inhibitory. In order to address these issues, Li's research study group used 3D printing to create an effective delivery system containing PRP laden with tendon-derived stem cells (TDSCs) in a rat model with tendinopathy[17]. In their project, methacrylic acid gelatine (GelMA) was used to create the scaffold. Using a projection-based 3D bioprinting system, a PRP-TDSC-GM (gel matrix) preparation was created to possibly promote tendon differentiation and rescue inflammatory response. The healing process was observed in a cellular assay by monitoring the migration of the TDSCs. A wound healing assay was used to determine optimal concentration of PRP in the gel matrix for TDSC proliferation. TDSCs were cultured in 10%, 20% and 40% PRP-GM groups. There was no significant difference between the 10% and 40% group, but the 20% group showed significantly better TDSC migration and consequently the 20% PRP concentration was used further in the study. Rat tendons were exposed to 3 test groups: a sham (no treatment), TDSC-GM, and PRP-TDSC-GM. The PRP-TDSC-GM group showed better tendon morphology, cellular characteristics, collagen arrangement and tensile strength. Overall, there have been much greater benefits to treatment of tendinopathy; however, there are concerns about how the overstimulation of the regenerative pathway may result in negative effects as well. This study did show a potential compatibility for tendon repair by facilitating the proliferation and differentiation of TDSCs with a 3D printed delivery system and by downregulating the P13K-AKT pathway (promotes metabolism, proliferation, cell survival, etc.) in the inflammatory areas.

Tenosynovitis

Aside from efforts to improve tendon repair and regeneration, focus has also been placed on the tendon sheath structure. The sheath surrounds many areas of tendons, providing separation and synovial fluid to allow the tendon to glide smoothly within the sheath. Damage to tendons through inflammation, injury or surgery can involve damage to the surrounding sheath as well which can lead to tendon adhesions and lead to limited joint range of motion. Reducing adhesions is key to trying to restore fully normal function of the tendon. Hyaluronic acid (HA) is found in high concentrations in synovial fluid and plays a major role in preventing tendon adhesion. Current attempts to repair sheath damage involve using bioengineered constructs to deliver chosen factors to the injured tendon. In chicken models, Xu et al. used polyglycolic acid constructs seeded with autologous tendon sheath cells and provided the necessary HA to prevent adhesions. Results showed a well developed sheath structure had formed around the damaged tendon with a clear space between the two indicating no adhesions had formed. Jiang et al. used Chitosan, a natural polysaccharide, as a scaffold seeded with synovial cells and growth factor TGF-B3 to study synovial sheath growth in vitro. To scientists' current knowledge, 3D bioprinted constructs have not yet been studied with regards to synovial sheath repair and regeneration or to prevent peritendinous adhesions. Existing studies have already laid the groundwork and offer encouragement for the possible incorporation of 3D printing into already established growth factors for synovial cells like HA or TGF-B3.

Discussion

3D printing and tissue engineering proves to be an advancing way of treatment. Studies have clearly shown that these 3D printed scaffolds and growth factors have had a tremendous impact on the cell promotion in areas of degeneration. Specifically, to the tendon, scientists were able to attach it to the parts of the most tension. They found that the micro grooved scaffolds showed much better results than a traditional treatment. However, as stated above, there are some limitations to the conclusions of the studies. The rats or other animal models in the studies do pose somewhat as a reliable model, but the strength of tendinous tissue in humans are very different. Traditional existing conservative or surgical treatments have been the most common because of the experience with each that they bring. 3D printing offers an improved way of delivering stem cells or growth factors necessary for repairing and regenerating tissue, which is a major obstacle in current tendon repair techniques. Since 3D printing is still being developed, there have not been enough results in human cases to move forward with certainty and switch over to this new treatment option completely. Moving forward, more research will need to be performed on human patients to demonstrate effectiveness and superiority over existing treatments of 3D printing and tissue engineering in repairing tendon injuries in all different age groups and different levels of physical activity. Additionally, there are concerns that arise regarding the ethicality of such treatment methods that may halt the progression of advancement in effectiveness and superiority.

Great strides have been made with experimentation in the laboratory. The successful use of 3D bio-printing for damaged tendinous repair demonstrated to date will eventually lead to applications in human subjects. Safety protocols and regulatory rules will need to be adhered to for clinical trials to take place. In the United States, as with any other form of medical treatment, the Food and Drug Administration (FDA), has established rules for any 3D printed bio-engineered tissue. Tissue engineered with 3D printing and intended for implantation, transplantation, infusion or transfer to a human recipient is regulated by The Center for Biologics Evaluation and Research (CBER). Under Title 21 Code of Federal Regulations (CFR) parts 1270 and 1271, in order to maximize public health safety, rules have been applied to these bio-engineered tissues. Institutions using human cell, tissue, and cellular and tissue-based products (HCT/P) will need to screen and test donors, prepare and follow written instructions on preventing the spread of any communicable diseases, and maintain good records of this. Additionally, under21 CFR 1271, any HCT/Ps will need to be listed with the FDA. Utilizing 3D bio-printing in the treatment of tendon related injuries appears to be progressing in America, but will always be monitored under the watchful eye of the FDA. To conclude, even with the FDA watching over future advancement, 3D printing and tissue engineering has shown great strides in being the best option for tendon repair.

Conclusion

The use of 3D scaffold printing and bio-tissue engineering appears to be a new and effective methodology to implement stem cell regeneration in repairing tendon injuries. Damage to the tendons has a great impact on an individual's active lifestyle ranging from daily ambulation to competitive sports. Current approach to repairing tendons usually only requires conservative measures. If surgery is needed, techniques involve either direct re-connection of the ruptured tendon or utilizing tendon donor grafts or auto-grafts. Growth factors and stem cells have already demonstrated a positive effect in repairing ruptured tendons. However, limitations to applying these factors in vivo have been encountered. The progressive use of 3D bio-printing offers an exciting new way to approach tendon repair because of the ability to produce more material at a quicker rate. Researchers have demonstrated potential use in treating conditions like tendon rupture, tendinopathy and tenosynovitis. Constraints with current printer technology, materials and cellular aspects challenge the progress of utilizing this method of tissue regeneration. Generally, the process of bio-printing with tissue engineering is a time-consuming process and only gets longer as the tissue construct desired enlarges. Along with time, bio-printing can be expensive, particularly with laser assisted and extrusion-based printers. Isolating and culturing cells needed for the tissue regeneration requires proper selection of bio-ink as well as other growth factors to ensure development and longevity of the cells. Optimal nutrition and oxygen need to be delivered to the developing cells, and waste products removed. Clinical applications of 3D bio tissue printing have obstacles related to activity of the tissue construct inside the human body. 3D bio-printing has enabled creation of a precisely configured construct but getting it to behave similarly to an actual donor graft can be challenging.

Despite the improvement in tissue scaffolding with bio-printing, replicating a tendon with similar physical attributes and mechanical properties has proven difficult so far[18]. Further studies need to be performed to establish efficacy, safety and clinical applications of 3D printing and tissue engineering. Minimizing cost of production, improving resolution of printing, enhancing or creating improved bio-materials, whether natural or synthetic, will hopefully improve the prospect of using 3D printing for bio tissue engineering and specifically for repair of tendon injuries. Further studies need to be performed to establish efficacy, safety and clinical applications of 3D printing and tissue engineering to ensure that the 3D printed scaffolds are not contaminated pre-operation and do not harm the body post-operation. Furthermore, it is more expensive to mass produce and acquire the material needed for this new development. If scientists and doctors are able to figure out a way to minimize costs and clear health and safety protocols, the future of improved treatment options for tendon injuries looks promising.

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