Review of 3D-bioprinted Orthopaedic Soft Tissues

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Abstract:

Orthopaedic tissues control and protect our ability to move and interact with the world around us. Soft tissues such as cartilage, tendons, or ligaments provide some of the lubrication and absorb much of the forces to allow this interaction to occur and be sustained. As one of the most common injury types in the United States, scientists are working to develop new and innovative ways to repair these soft tissues after they are damaged or torn. The method of scaffold implantation is not new, but the traditional fabrication methods such as electrospinning or freeze drying are gradually being replaced by more controllable and precise methods such as E-jetting, fused deposition modeling, and 3D bioprinting. These methods allow for more biologically representative structures to be produced. Scientists are also currently studying the potential for cultured cells to have an impact on the ability to facilitate regrowth of orthopaedic tissues on scaffolds. Different types of cartilage, xenographic ECM, mesenchymal stem cells, differentiated somatic cells, along with many other materials are being studied in vitro and in vivo to develop new healthcare solutions for the future. Clinical trials have been undertaken to test the effects of newer scaffold manufacturing techniques, but most of the discussed methods are still in the stages of in vitro or in vivo development. There is much opportunity to develop the parameters of these methods for the future, combining the new manufacturing techniques with more knowledge of living cells to contribute to orthopaedic soft tissue healing.

1. Introduction

Orthopaedic Soft Tissues:

Orthopaedic soft tissues have key functions in the human body during movement. Soft tissue is a generic term for fat, muscle, fibrous tissue, blood vessels, or other supporting matrices. Specifically, orthopaedic soft tissues are a fibrous cord of connective tissue that offers support during movement. The most common soft tissues injured are menisci, ligaments, and tendons. The focus of this review is to document the latest techniques in 3D bioprinting the commonly injured orthopaedic soft tissues.

What are Ligaments?

Ligaments are found several places in the human body and are a frequently utilized tissue in the musculoskeletal system. Ligaments primary function is to serve as the tissue that connects bone to bone. One of the most common ligament injuries is a tear or sprain of the anterior cruciate ligament (ACL) in the knee that connects the Femur bone to the Tibia bone.

Ligaments have similar components to tendons such as the tough fibrous bands of connective tissue that serve to support the internal organs and hold bones together in proper articulation at the joints. There are many ligaments in the body, including ligaments in the knee, shoulder, elbow and ankle. In the knee alone there are four ligaments: ACL, MCL, PCL and LCL. There are two types of ligaments: visceral ligaments that serve as connective tissue that hold organs together and structures in place and skeletal ligaments that serves as connective tissue that bind bone to bone and must withstand great pressure. In this article we will focus on the skeletal ligaments.

Ligament injuries are only increasing. It was reported in 2016 that an “estimated 200,000 ACL-related injuries occur annually in the United States.” (Sherwin) Ligament injuries often occur in high impact sports like football and basketball where there is frequent brute forces exerted in the lateral directions. It is common to sprain or tear a ligament when the ligaments near a joint are stretched excessively. For example, this can occur in a basketball player when going up for a rebound then hyperextending their leg when hitting the ground.

Current Ligament Surgeries:

There are multiple surgeries that can be done to fix a ligament tear. One of the most common repairs for the tear is allograft reconstruction. A standard allograft arthroscopy reconstruction is performed by a doctor who replaces the patients torn ligament with a donated ligament from a cadaver. Another common method is an autograft. A ligament autograft procedure begins with a doctor harvesting a graft from a healthy part of the body of the patient prior to the surgery. The harvested ligament graft is then cut to fit the size of the ligament it will replace and is maintained in a sterile environment until another surgery is scheduled. This second surgery is performed by the doctor in which they implant the tissue in place of the ligament. The allograft procedure has the advantage of preventing donor site morbidity that would be caused by the autograft procedure. The allograft procedure also ensures the patient does not end up with too small of a graft and therefore provides a better graft size fit. However, the allograft procedure does have its disadvantages. The allograft procedure risks the rejection of the new ligament by the human body and it may not have the same mechanical properties the patient's previous ligament did.

There is an ongoing debate as to which reconstruction surgery is better, autograft or allograft but what is clear is that both methods are far from customized and can commonly cause pain and result in a long recovery time for patients. Therefore, Tissue Engineering and Regenerative Medicine (TERM) can become a better alternative than these methods to help provide a customized, less painful, and quicker recovery time.

Ligament Cells:

In addition to scaffold fabrication techniques, cell source and cellular response are considered in ligament tissue engineering. In a study conducted by the Department of Orthopedic Surgery at the Allegheny University of the Health Services in Philadelphia, fibroblast cellular types ACL, PCL, MCL and LCL cell proliferation was compared. The study found that fibroblast MCL cells proliferated significantly more than did the other three ligament cell types. Mesenchymal stem cells (MSCs) are easy to isolate and culture and are thought to be able to build a strong ligament ECM. Therefore, it may be possible to use MSC to help build a durable ECM but there are still questions about how well these cells will proliferate on a polymer scaffold. Additionally, scientists still do not fully understand the precise molecular events involved in MSC differentiation.

The addition of growth factors and proteins can improve cellular proliferation in ligaments. Growth factors regulate synthesis of the ECM and the expression of type-1 collagen and type-3 collagen which helps improve the tensile strength of a ligament ECM.

What is the Meniscus?

The second orthopaedic soft tissue of interest is the meniscus. Widely present throughout animal kingdom, meniscus tissue is known for its versatile weight bearing functions. It not only helps disperse the forces driven on bones and joints, but also excels at lowering friction. There are five unique places meniscus tissue exists in humans, the knee, wrist, acromioclavicular (top of shoulder), sternoclavicular (between sternum

and clavicle), and temporomandibular joints (jaw). However, the meniscus that gets the most wear and tear is the knee meniscus. Not a surprise then it is one of the main focuses for regenerative medicine. Especially for orthopaedic soft tissue, as almost 200,000 people tear their meniscus every year in the US alone.

As a constant intake of friction, the knee meniscus is continually being worn down by daily motion and certainly enhanced by any rigorous physical activity. That is on top of the natural deterioration of the fibers and collagen that occur regardless of the amount of activity. As you age the meniscus begins to lose elasticity, lubrication, and becomes dramatically stiffer, which weakens its compression ability. On average once you hit age sixty-five the rate of tear increases by sixty percent. This is mainly because when the knee meniscus loses its cellular elements it creates a cystic area of fibrous tissue, which increases tear potential. The combination of these issues leads to roughly 850,000 meniscus surgeries a year. Needless to say, there is quite the market for the knee meniscus in regenerative tissue engineering.

Now unlike the other menisci, the knee meniscus has a unique crescent shaped geometry tissue specifically designed for strength, stability, and compression. There are two menisci in the knee. You have the lateral meniscus located on the outside of the knee and the medial meniscus located on the inside. Both act as the point of contact between the femur and tibia. The meniscus is mainly made of fibrous and cartilaginous tissue, giving it the technical term fibrocartilage. Specifically, white fibrocartilage when healthy and yellow when it's worn down. Overall the meniscus is relatively uniform in its transition from cartilage to bone.

Current Meniscus Surgeries:

Now being such a prominent injury there are a lot of current techniques to help resolve the issue. Such as grafting or arthroscopic repair, which is taking out a plug or biopsy from a less used part of the knee and plugging it to where the trauma is. However, the clear problem with this is that it is making an injury to resolve one. Plus, for those who are very active this won't allow for full motion of the knee. In addition, this can only help fix a small area of the meniscus and wouldn't be good for total meniscal repairs. Another procedure is a partial meniscectomy, which is the removal of all torn parts of the meniscus. Certainly, this isn't a solution, but a way to help the patient to cope with the pain. Although this generally leads to osteoarthritis over time. A third operation is a total meniscus repair. This is when the surgeon tries to repair the meniscus by stitching it back together. However, there are many issues with this, such as, it is highly dependent on the level of tear and if the cartilage is gone, there is nothing the surgeon can do to bring it back.

Overall these methods might be okay in the short term, but there is still a lot more that can be done in helping resolve menisci issues. This is exactly where tissue engineering, and regenerative medicine come in. Tissue engineering will help provide a more stable solution to the knee meniscus and in a way that can be highly scaled to reach the growing market.

Knee Meniscus Cells:

The knee meniscus is made up of fibroblast and chondrocyte cells. However, due to their interconnection links, for printing most consider it to be one cell type, fibrochondrocytes. This vastly expands the manufacturing possibilities as you don't have to worry about integrating two unique cells into your new bio fabricated meniscus, which is very difficult to do in a uniform way. Most complex organs, like kidneys, that have multiple cell types are unable to be produced in "subtractive" manufacturing for that reason. Stereolithography or fused deposition modeling are not used, since their vats of material can only contain one cell.

A majority of the research has shown that the use of fibrochondrocytes works best for the

replications. Simply taking a biopsy from the knee and seeding the scaffold with these cells has worked well. However, when meniscal replacements are needed, most of the time it is because of tearing or a worn-down meniscus. Therefore, the trauma to the tissue makes it very difficult to get cells from that area, since not as many cells still exist. Some of the time cells from the inner parts will be available, but if not, other options need to be looked at. Those that have been shown to have the most potential in order are mesenchymal stem cells, non-differentiated progenitor cells, and cells found in the synovium of joints.

That being said if you are able to get some cells from the current meniscus you want to optimally culture them. Once the biopsy is taken it should be sliced then soaked in .05% hyaluronidase for five minutes then .2% trypsin for 30 min. Followed by a standard incubation over-night. Then the standard cell culturing process can resume with the help of growth factors to overcome the low cell count. Here are two of the most common growth stimulants currently in use

-TGF-b: stimulates the production of GAGs and biglycan, while enhancing ECM production. Very good for cells that are about to be added to the scaffold as ECM growth is key to their success.

-IGF-I: being that it is very similar in makeup to cartilage, IGF-I, has proven to stimulate the articular chondrocytes on the knee cells and help with the production of collagens.

What is the Tendon?

Tendons are widely present in the human body and are considered one of the most important components of the musculoskeletal system. Tendons are important components since they link muscle to bones so that force from the bones can be transmitted to the body for movement. A familiar tendon to most is the achilles tendon, but tendons are also present in shoulders, arms, hips, legs, hands, feet, head, neck and torso. Essentially, every time the body moves, a tendon is being used.

Tendons are made of tough, fibrous connective tissue that translates the produced force during any locomotion. The size and shape of tendon tissues are based on where they are located in the body. For example, sometimes when the muscle connected to the tendon is thin and wide, the tendon is only a thin sheet. The thin sheet for tendons connected to thin muscles is referred to as aponeurosis.

Tendon injuries are rampant. In fact, half of the musculoskeletal injuries in the US are comprised of tendon injuries. Tendon injuries occur near joints such as the elbow, knee and ankle. Tendon injuries seem to happen instantly, but actually they usually are the result of many tiny tears that happen overtime. For example, an achilles tendon injury usually occurs during an explosive jump, but any stretching or moving prior to the jump can strain the tendon and put it in a vulnerable spot for injury.

Current Tendon Surgeries:

Tendon repair is needed in order to bring back normal movement to a joint if it is lost. Tendon injuries can occur anywhere in the body, and the corrective procedure for the torn tendon is dependent on the severity of the tear.

Generally, during tendon repair, the surgeon will make small incisions in the damaged tendon and sew the torn ends back together. It is important to check the surrounding tissue to make sure no injuries occurred to the blood vessels or nerves during incision.

If the tendon tear is severe, or if there isn’t enough healthy tendon to reconnect, the surgeon will perform a tendon graft. The graft comes from another tendon area in the body. The risks associated with this tendon repair method is scar tissue, loss of joint use, or retear.

Sewing the tendon together or tendon grafting are two methods that work to aid in tendon repair, but there are improvements to tendon repair are currently being made. The latest tendon repair method is 3D-bioprinting the tendon for implantation to the affected patient.

Tendon Cells:

Techniques are being developed with hopes to standardize tendon repair with regenerative medicine permanently. Regenerative medicine requires cell sources for scaffold integration and proliferation, but the prime source of cells is not yet discovered. Many cell sources have been employed in tendon regeneration techniques. Somatic differentiated cells were the main source for proof of concept, but over the past few decades, mesenchymal stem cells (MSCs) became a popular cell source for applied research for tendon scaffolds.

Tenocytes are elongated fibroblast type cells, and are also referred to as tendon cells. In vitro expansion of tenocytes remains a challenge due to a phenotype drift and the direct loss of number of cells. While culturing tenocyte cells for tendon regeneration may seem like an obvious choice, it is not proven to be the best method.

Currently, bone marrow-derived mesenchymal stem cells are the most commonly used MSC type for tendon engineering. Significant progress has been achieved by identifying the MSC cell, since they are close to the tenogenic phenotype. The MSC cell is comparable to the tenogenic phenotype based on tensile strain and topographical structure with electrochemically aligned collagen threads.

2 Commonalities of 3D orthopaedic Materials:

Much of the current research on tissue-engineered orthopaedic soft tissues has focused on the use of biological and synthetic polymers. The tested components that are critical to a successful material for orthopaedic soft tissues are compressive and tensile strength, structural stiffness and the ability to be anisotropy. In our research, we found three materials: Collagen, Polycaprolactone (PCL), and Poly(L-lactic) acid (PLLA), these stood out as common best materials for tissue-engineered orthopaedic soft tissues. Collagen, a biological polymer, accounts for more than 80% of the dry weight of a normal ligament making it an ideal fit. However, a drawback of collagen is its lack of mechanical strength, which is why collagen is often used in combination with other materials with stronger mechanical strengths.
Poly-caprolactone (PCL) is a biodegradable polymer that is easily manufactured and is very good at maintaining original strength.
Poly(L-lactic) acid (PLLA) is a biodegradable thermoplastic polymer. The advantage of thermoplastic as opposed to thermosetting is that the ladder sets at high temperature irreversibly rendering it unrecyclable.

	Advantage	Disadvantage
Collagen	Biocompatible	Weak mechanical strength
PCL	Easily manufactured	Very slow degradation rate
PLLA	Excellent cell adhesion	Acidic degradation byproduct

Figure 1: Highlights advantages and disadvantages of common material used in 3D orthopaedic soft tissues.

While thermoplastic can be taken to high temperatures, but it does not set making it recyclable. In figure 2, line “a” represents thermosetting where there is no plastic deformation which would not be a good material choice for an orthopaedic soft tissue. While line “b” represents thermoplastic where there is lots of deformation but can return to the original state making this a much more flexible material and a better choice for a tendon, ligament or meniscus replacement.

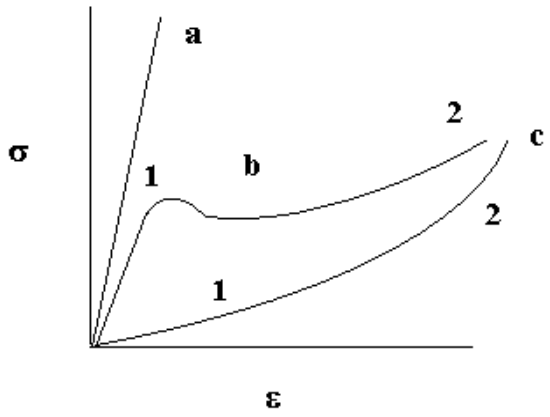

Figure 2: Stress vs. Strain graph. Lines A,B,C represent thermosetting, thermoplastic and elastomer respectively.

Mechanical Properties:

Much like with the materials, these three tissues have a lot of similar mechanical properties. First and foremost is the tensile strength. All three tissues have very high tensile strengths. This is crucial as each of the tissues is taking on an extremely high level of stress given its relatively small volume. Pound for pound they are some of the strongest tissues in the body. Plus, the stress that these tissues take is not only high, but continuous as the knee is constantly taking on force throughout our daily lives. That is why it is so important that the manufactured tissues can achieve such strengths. One key to their strength success is due to their fibers. All the tissues are made of thick intertwined fibers. Although it is important to note that the alignment is different. For ligaments and tendons, the fibers are aligned vertically and in the same direction as the stress. This is to avoid tearing when it is pulled. Whereas the meniscus fibers have a circumferential alignment to absorb more of a compression force or the “hoop” stress. Proper alignment of these fibers is crucial when manufacturing the new tissues. Without it the tensile strength with be nowhere near to optimal.

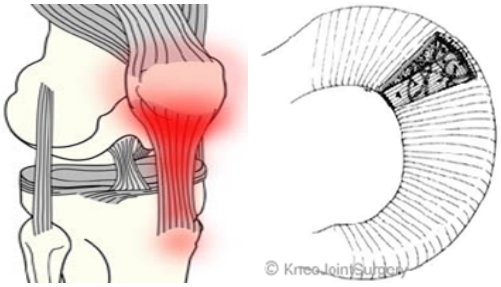

Figure 3: Vertical Fibers & Circumferential Fibers

The second mechanical property that is similar in each of the orthopaedic soft tissues is the elasticity. On top of the tensile and compressive forces, the knee also takes on a lot of unwanted horizontal or multidirectional forces. This is very common in a lot of activities or sports as your need goes through bending and twisting. Due to this, these tissues need to be elastic and flexible to absorb or adapt to these stresses. Clearly, if they were too stiff tearing would occur even more rapidly.

The third commonality is surface finish. Since there constant motion, the knee is prone to a lot of friction. This rapidly wears down the tissues and bones and can become extremely painful. To avoid this the tissues have a very fine surface finish to limit the amount of friction. Plus, they are well lubricated on the surface to further reduce friction. In fact, knee tissue fluid makes up 65% to 70% of its total volume. When it comes to manufacturing the optimal surface finish will play a key role as you will need very tight tolerances.

Porosity and Cell Metabolism of 3D orthopaedic soft tissues:

Porosity of the tissue scaffolds correlates to cell metabolism. The recommended pore size for scaffold design is similar for the ligaments, menisci, and tendons. Typically, conventional methods for building scaffolds of orthopaedic soft tissues encompass using a 50 μm interfiber distance for pore size. The recommended pore size is moving to a 100-150 μm interfiber

distance. This recommended size is based on how the cells align on the scaffold overall. If the cells metabolise within the pore but they do not align correctly along the scaffold, the scaffold is not completely biocompatible. Scaffolds with pore sizes ranging from 300 to 600 μm show significant cell growth, but cells do not align between the pores as well as they do on a 100 μm interfiber pore distance. This is why the 100-150 μm interfiber distance is the recommended pore size for all orthopaedic soft tissues.

3 Conventional Methods

Ligaments:

The traditional method of freeze drying for ligament scaffold fabrication has been one of the most popular. This was due to the positive results found in biological responses. Cellular proliferation, viable cell density, collagen-based matrix production are all positive benefits identified in the freeze-drying method. The freeze-drying method develops emulsification when a biopolymer is dissolved in a solvent mixed with water. The emulsification in the mold is then frozen and the pressure lowered. Next, the scaffold is heated, and the solvent evaporates and leaves behind pores in the polymer that are desired. However, freeze drying does not create a scaffold with and equivalent maximum tensile load or stiffness to a natural ligament. Additionally, one must mix the polymer with the solvent. This makes it difficult to get the solvent out depending on the shape of the object. Furthermore, this process is limited to one material at a time. Therefore, a lot of manufacturers have decided to look at other additive manufacturing techniques.

Knee Meniscus:

The conventional technique most common for meniscus scaffold replication was electrospinning. This is because electrospinning helps create the small pore size that is needed. It also can produce ultrathin fibers, micrometers to a few nanometers, which is optimal as it is very similar to the collagen fibers in the meniscus. However, there are clear limitation in this process. First is the geometry. The meniscus has a very unique crescent shape that is difficult to replicate. Unfortunately, electrospinning can't accurately or consistently reproduce the intricate geometry. In addition, you can't control the porosity with electrospinning so getting the right cell adhesion throughout is left to chance. This is why a lot of manufacturers have moved towards additive manufacturing.

Tendons:

The conventional method for tendons is the same conventional method for the knee meniscus. Electrospinning is a common technique for tendon repair because fibers for the tendon scaffold can be easily fabricated from the electrospinning process. An effective cue for inducing cell alignment is the production of highly oriented fibers, which electrospinning results in. However, electrospinning is not the best method for tendon scaffold development. The disadvantages with electrospinning tendon scaffolds is that the fibers have a small pore size and limited cell infiltration. The small pore size results in thin fibers that lack the capability of 3D tissue architecture generation. Another disadvantage of electrospinning is that it requires long setups for collecting the aligned fibers. Due to the lack of quality and efficiency for tendon scaffolds via electrospinning, manufacturers are moving to better techniques.

4 Current Techniques

Ligaments:

3-D Bioprinting, which is a rapid prototyping technique using layer-by-layer deposition of cells and matrixes may be the solution to creating a ligament scaffold. This process

improves the strength by creating a scaffold that can be tailored to a specific mechanical stiffness. Additionally, this process allowed for multiple materials to be used for the constructs. In a study from the Biofabrication Journal by W. Schuurman, found that scaffolds displayed a much higher Young's modulus when they combined hydrogel with a PCL support. The hydrogels served to help cells grow while the PCL laid the foundation for the scaffold and gave it strength. The process of scaffold fabrication for a 3D-bioprinter would ideally begin with a CT or MRI scan to provide a individual custom-design implant. The CT or MRI scan would automatically generate a design in a computer-aided manufacturing (CAM) software that would then generate an STL file that could be hooked up to the 3D bioplotter and printed.

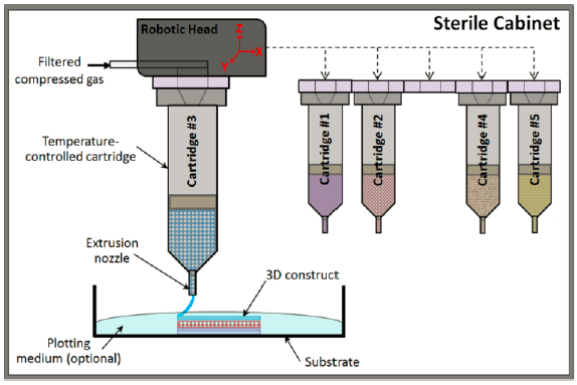

Figure 4: 3D bioprinter ability to print multiple materials

In the current process researchers have manually created designs in a CAM software generated an STL file and then printed. For clinical translation, the next step would be to implement CT or MRI scans that allow for a custom-designed implant. 3D bioprinting is preferred to traditional methods because it provides the ability to control the sterility of the environment that the constructs are printed in. Additionally, 3D bioprinting seems to be the best option available to create repeatable scaffold architecture. The ability to create a custom-designed implant, control the sterility and create a repeatable process will be critical aspects in clinical translation.

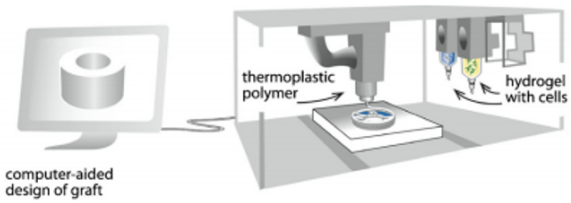

Figure 5: 3D bioprinter concept

Knee meniscus:

Fused deposition modeling, which is your standard 3D printer, was the first technique that seemed to be the solution to creating the meniscus scaffold. This process eliminated the geometry problem by consistently reproducing the crescent shape. Plus, this process allowed for controlling of the exact pore size and porosity. However, researchers are now finding this too has its limits. First, there is a long filament preparation time, which slows down the process. Second, researchers found a high level of buckling failures when heating and cooling the liquefier. Now although these failures are easily fixed it still requires operator intervention, which further slows down the process. For optimal clinical translation you want complete automation and that is why researchers began using precision extrusion deposition.

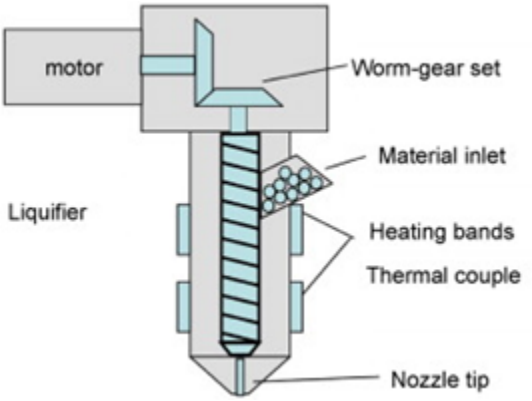

Figure 6: Precision Extrusion Deposition

As seen in the figure, the PED has a mini extruder attached to the top which allows for a wider range of materials to be used. Plus, it helps solve the set-up time issue as no filament preparation is needed. However, when extruding viscous materials, there are a lot of blockages in the nozzle, which means there is still a more optimal solution out there.

Finally, researches have landed on what seems to be the most optimal solution, at least for now, electrohydrodynamic-jetting. EHD jetting is much like a combination of electrospinning and ink jetting. There is an electric current passed through the nozzle which allows for the production of micron to nano fibers. Although different from electrospinning, it can control the porosity and structure. All while producing high resolution structures and patterns, with proprietary options for optimal path planning. Plus, it has be shown that it can consistently replicate the structures and make real-time adjustments from unpredicted disturbances. Such as material impurities or voltage fluctuations. Although more research still needs to be done on the optimal parameters for the meniscus scaffold such as the current, voltage, nozzle distance, feed rate, etc. we can rest assured knowing at least when those are found this machine is capable of performing them.

Tendons:

Currently, the most effective tendon tissue repair method is E-jetting. E-jetting is a novel technique that was employed specifically to fabricate scaffolds. The process for engineering the scaffold through E-jetting begins by loading a prepared PCL solution into a syringe, which is connected to a stainless-steel blunt needle. The solution is dispensed using a syringe pump and fibers are deposited onto a wafer at a consistent distance and at a high voltage. Fibers for the scaffold are oriented via the movement of the stage.

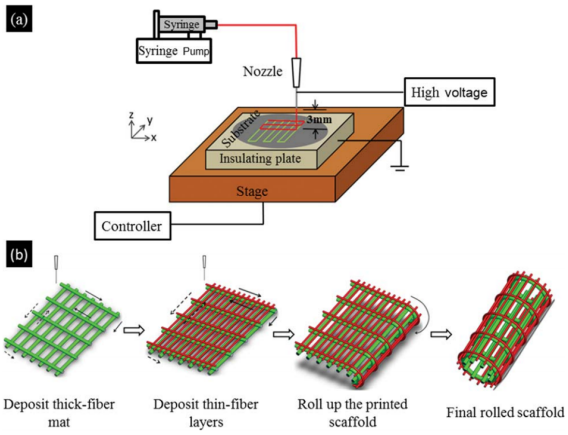

Figure 7: (a) E-jetting Process (b) Finish Scaffold

The efficacy of E-jetting requires culturing, harvesting, and binding of human tenocyte cells. The process to discover the best scaffold design including testing different pore sizes. Different pore sizes were tested and printed by E-jetting, and the most effective pore size for E-jetting the scaffold was 100-150 micrometers. The effectiveness is due to how the cells align on the E-jetted scaffold and how the scaffold translates to the human body.

Another important design component for the E-jetting process, is that it produces micrometer-size thick knitted fibers. Most conventional techniques do not produce knitted fibers. Knitted fibers correlate to better cell alignment and growth, and also increase the mechanical strength of the scaffold. The overall structural support of an E-jetted scaffold is very comparable to the Young's Modulus of a regular tendon.

Overall, E-jetting is the latest novel technique for engineering tendon scaffolds. E-jetting comprises of micrometer-size fiber bundles that are interconnected. E-jetting introduces the concept of a supporting layer made out of thick fibers. Due to the thick fibers, the scaffold has a stable stacking pattern that enhances the mechanical strength of the scaffold. Currently, E-jetting is the best method for producing a quality biocompatible tendon scaffold.

5 Clinical Trials

While biofabrication of tissues is a relatively new field, there have been some Universities and private companies to carry out clinical trials involving the implantation of biologic scaffolds.

Clinical Trials in Ligaments:

There have been numerous studies conducted regarding the viability of implanted scaffolds in aiding ligament regrowth. Unfortunately, none were found that included either the seeding or printing of cells within these scaffolds before implantation. Mowbray, Jadeja, and Petrou each worked with their respective teams and over the last twenty-five years released studies examining the efficacy of the ABC scaffold; a carbon fibre polyester product released in 1985 to serve as resorbable scaffold to aid in ACL regrowth. These studies tested the effect of the scaffold for years after surgery, and yielded mixed results. Mowbray noted the fact that the ABC scaffold showed tissue growth, and clearly aided in the healing of the ACL. However, the rate of failure was high, especially in the early years after surgery.

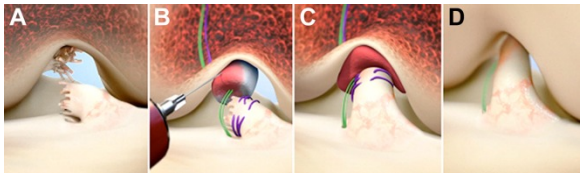

Figure 8: Rendered depiction of the BEAR ligament repair procedure

With regards to newer research, a study carried out at the Boston Children's hospital has proven the concept of a new type of scaffold through clinical trials. The study carried out by Murray tested a Bridge-Enhanced Anterior Cruciate Ligament Repair procedure, or BEAR. The procedure involves a combination of traditional suturing and a hollow scaffold surrounding the torn part of the ACL. While traditional suturing alone has been proven less effective than ACL reconstruction surgery, the combination of suturing with a scaffold is promising. It is unclear how the scaffold was manufactured, but it consists of extracellular matrix and proteins derived from bovine tissue. The hydrophilic scaffold became soft when put in contact with the blood around the injury, and was able to conform to the shape of the tear. This was a very preliminary study that was intended only to prove the potential for this treatment and to identify any potential adverse reactions to the scaffold's use in humans.

Clinical Trials in Menisci:

The development of meniscal products has faced the hurdle of having to prove its superiority over a partial or full meniscectomy. Unlike a tendon or ligament, a meniscus is not absolutely necessary to maintain safe mild use of the knee, making the removal of all or some of the meniscus a safe and viable treatment. Despite this, there are at least two synthetic scaffolds currently on the market that are intended to biodegrade in the knee and aid in the regrowth of meniscal tissue.

The collagen meniscus implant detailed in the report by Rodkey is currently on the market by the name CMI. According to this study, the implant has no seeded cells, and acts only as a scaffold to facilitate growth from cells within the knee. The patients enlisted only had partial meniscal tears, and were split into two groups: one receiving the collagen implant, and the other receiving a partial meniscectomy. Each of these treatment types were applied over two types of patients: those experiencing acute pain, and those with chronic issues persisting after previous surgeries. The results in the chronic groups showed a much higher rate of regrown meniscal tissue in the group receiving the implant, leading to better function and less pain. However, the acute groups showed no statistically significant differences.

The Verdonk study also tests the implantation of a meniscal scaffold, but this theirs was made of polyurethane instead of collagen. This study did not include a control group not receiving the implanted scaffold, but the researchers still

deemed that their results showed that the polyurethane scaffold can be a viable treatment to regrow meniscal tissue.

The common threads between these two products are that it is unclear exactly how they are manufactured, and that they were not seeded with cells or biologically living material before implantation. The first point is due to the proprietary nature of the products. The second point identifies an opening for potential future research, adding living cells to already proven methods and aiming for even better efficacy.

Clinical Trials in Tendons:

The use of biological scaffolds in clinical trials in tendons has been even more lacking than in the previously mentioned tissues. Most of the clinical trials involving biological scaffolds attempting to heal tendons have been the reapplication of a product designed for another purpose. For example, the GraftJacket and Zimmer Collagen Repair patch are both intended to aid the regrowth of skin. The Zimmer patch is a xenograft originating from pigs, while the GraftJacket is an allograft made of collagen derived from human skin. Both products (in separate studies, the Zimmer patch by Badhe, and the GraftJacket by Barber) were used as aids in patients undergoing rotator cuff surgery in their shoulders. While both studies exhibited mildly positive results with regards to pain and inflammation, this research was not enough to declare a definitive positive effect caused by the additional product.

Another biological scaffold has been tested in rotator cuff surgery, derived from pig intestines. The study was carried out by Iannotti, but yielded negative results. The control group had a much higher rate of successful surgery compared to the group that actually received the scaffold in addition to their surgery.

It should be noted that these studies did not attempt to test the efficacy of living cells in aiding the healing of tendons. The shoulder is a more vascular area of the body than say the knee, but it could still potentially benefit from the addition of cultured stem cells or host cells being seeded into the scaffold before implantation. More research is required.

Future Work in Clinical Translation:

All of the above mentioned clinical trials had many things in common. They were all built on the backs of years of material testing, in vitro testing, and in vivo testing. For any trial to be undertaken in the future, it would be difficult to bypass any of these steps without identically mirroring another previously performed study. This would take the element of originality and potential for a new discovery somewhat out of the equation. However, these studies can act as guides to develop the framework for a plan of action in the future.

While all potential solutions were first tested in vivo, they were not all performed in pigs. However, the one study we found that used a canine tendon repair as evidence for the potential benefit in humans (Iannotti) was ultimately unsuccessful in ineffective when tested in clinical trials.

The most definitive studies mentioned also compared a group of patients receiving a new method of treatment against a group of patients receiving either no additional treatment or a traditional treatment. Studies such as Rodkey's were able to have a much more well-defended conclusion than Badhe or Barber because Rodkey used a control group. Because of the high costs of developing a new product and bringing it to market, any new method must be shown to be not only safe and effective, but more effective than methods already on the market.

The largest opportunity for future clinical work is the combination of living cells (either stem or host) with a biologically compatible scaffold. While the cell proliferation and viability are being tested in vitro and in vivo, our research did not show any published results with this combination of treatments being tested.

6 Conclusion

In conclusion, this review is highlighting the most recent and relevant 3D-bioprinting techniques for orthopaedic soft tissues. Tissue injuries are very common, so it is important that we continue to engineer the most effective and reliable techniques to help heal orthopaedic tissue injuries. The tissues of interest for this paper include ligaments, menisci, and tendons. The tissues reviewed in this paper are very similar in regards to materials used for scaffold development. The mechanical properties for the three tissues are all enhanced by incorporating thick intertwined fibers into the design of the scaffold. Also, each scaffold design includes a similar porosity micro-meter size to allow for accurate cell alignment and binding. Conventional techniques for scaffold development of each tissue are compared to the latest and most novel technique for ligaments (3D-bioprinting), menisci (Fused Deposition Modeling & Precision Extrusion Deposition), and tendons (E-jetting). The review concludes with clinical translation of each tissue scaffold and the required steps before clinical translation for each of the current scaffold building techniques.

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