Mena, 1:00
Team L12

THE USE OF BIOPEN AND BIOINK TO IMPROVE THE RECOVERY TRACK OF ARTHRITIS PATIENTS

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***Abstract*—*Arthritis is the leading cause of disability in the United States as it affects over 54 million adults and is caused by unsupported joints due to their inflammation. It is usually treated through using cartilage implants, but recently, researchers have turned to 3D-printing. At the University of Wollongong in Melbourne, Australia, scientists created the Biopen, a handheld device which outputs a fluid-like substance, bioink. This paper will examine the usage of Biopen as a means of treating arthritis patients, specifically by replicating the natural form and texture of the cartilage in a manner quicker than traditional methods. Biopen functions by adding the formulated Bioink directly onto the patient's bone layer-by-layer, with each layer being exposed to ultraviolet light to help replicate the rubber-like texture of cartilage. The bioink contains stem cells of hyaline cartilage extracted from the affected patients, a feature lacking in older cartilage implants. This treatment is beneficial because cartilage takes a long time to heal when damaged or inflamed and cannot regenerate. Through the eventual successful use of the Biopen on humans, it can more efficiently treat common joint problems specific to the patient's need with a shorter recovery rate and significantly reduce the number of arthritis surgeries. It is an important innovation for all engineers to recognize because it represents a convergence of engineering and medicine producing a comprehensive solution, as well as having the potential to revolutionize the recovery track of an arthritis patient.*

Key Words— Arthritis, Bioink, Biopen, cartilage, handheld, recovery, surgeries, 3D-printing

THE IMPACTS OF ARTHRITIS

Arthritis is a disease that affects millions of adults internationally and contributes twenty-seven billion dollars in health care expenditures annually [1]. Arthritis of the knee is inflammation and stiffness of the joint characterized by breakdown of the cartilage, bony changes of the joints, deterioration of tendons and ligaments, and various degrees of inflammation of the synovium surrounding the joint [2].

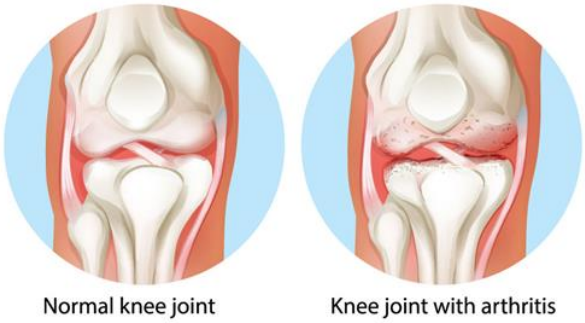

FIGURE 1 [3]
Impacts of Arthritis in the Knee

Figure 1 depicts this breakdown of cartilage, which shows the knee joint with arthritis breaking down due to the femur and tibia's direct impact on each other. Arthritis is often associated with pain due to swelling, decreased flexibility, cracking noise with joint movement, limit of weight-bearing activities, and in the long term, can impact one's quality of life.

Arthritis is currently being treated using pharmacotherapy, psychology, physical therapy, occupational therapy and arthroplasty [1]. Athroplasty is often the only way to completely eliminate knee pain for many arthritis patients, but there are often many complications associated with this invasive surgical procedure. Other traditional treatments, such as an Autologous Chondrocyte implantation, requires a double operation and often causes graft failure, tissue hypertrophy, and a loss of ability to produce reparative tissue [2]. This is not only time consuming for the patient due to extensive recovery and hospital trips, but also can be an extremely

painful process if other complications arise after the double operation. Yet the Biopen, an innovative, surgical device, allows patients to get the benefits of knee surgery, such as newly formed functional cartilage, without the complications caused by arthroplasty or an autologous chondrocyte implantation.

FIGURE 2 [4]
Creator Peter Choong using the Biopen on artificial knee

The Biopen improves the surgical procedure as it has the ability to "three-dimensionally print in 'real-time' multiple layers using different biomaterials and/or cells to reconstitute different tissues, simply by changing cartridges" [5]. This can be seen in Figure 2, which shows the Biopen being used to "treat" a replica knee. Additionally, the Biopen minimizes the complications post-application due to the simple way it functions. Along with the patients, surgeons also are at an advantage since it is an easy tool to manage and costs little to manufacture or to be used, hypothetically, in surgery. Although it is not yet being used on humans, it has the potential to revolutionize the recovery track of an arthritis patient.

THE ROLE OF THREE-DIMENSIONAL PRINTING IN MEDICINE

The Biopen utilizes three-dimensional printing in the realm of tissue engineering, a fairly recent innovation scientists have turned to, especially for creating surgical implants. Advances in both science and technology have allowed for engineers to construct complex and detailed biological scaffolds made out of living human tissue, to be used as implants that are effectively compatible with the human undergoing the surgery.

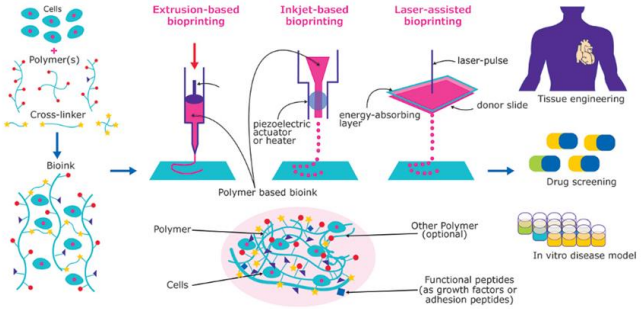

FIGURE 3 [6]
The different processes of converting cells to printable ink

There are several types of bioprinting that are commonly used, but the three main types are extrusion-based, inkjet-based, and laser-based, as shown in figure 3. The advantages of the extrusion-based model are its quick printing speeds and highly controllable structures, whereas the negatives are the low shear stress of the ink which can impact the cells' viability post-extrusion [6]. On the other hand, the inkjet-based model is very cost effective, has the fastest printing speeds, and is compatible with biological components, but it requires low viscosity materials. The laser-based model can print viscous materials with the highest accuracy, but it's laser also generates a lot of heat that can damage cells. As all three models have their advantages and disadvantages, choosing a superior model is dependent on what it will be used for. In the case of the Biopen, highly controllable structures and compatibility with living cells were the most important factors, so the extrusion-based model, which is also able to print structures rapidly and precisely [6].

Researchers at the University of Wollongong in Australia state that the use of bioprinting in surgery is superior to standard joint surgery repairs since it allows them to "tailor implants to the anatomy of the defect" [7]. Yet, most bioprinters are computer-controlled robotic devices that are inconvenient for time-dependent, exposed wound sites and for the surgeons, who consequently work only indirectly on their patient. Thus, the researchers and engineers came together to build a surgical device called the Biopen. Its main advantages are its small size, which facilitates the manufacturing process and cost, and its ability to be used directly on patients by surgeons. Additionally, since the Biopen uses an extrusion-based printing method, the surgeon is then able to implant the biomaterials more precisely while managing the handheld tool solely under his control.

The method of 3D printing, which is more commonly "referred to [as] additive manufacturing or solid free-form fabrication," is a fairly lengthy process that typically occurs layer-by-layer [8]. A necessary step in the process of bioprinting is allowing the cells to cultivate properly within a laboratory before their implantation and later becoming

functional, which lasts at least two days. With the Biopen used in surgery, though, the device can 3D print the cells directly onto the bone in a speedy process due to its engineering design and the make-up of the Bioink it extrudes.

THE CONSTRUCTION AND DESIGN OF BIOPEN

The Biopen engineering design allows surgeons to easily replicate the natural form and texture of the cartilage quicker than traditional methods. This is beneficial because cartilage takes a long time to heal when damaged or inflamed. The Biopen's design is also crucial in its development and in ensuring efficient printing and furthermore, proper layering of each Bioink. Engineers prioritized "design[ing] a philosophy which emphasizes ergonomics and sterilisability" [7]. The Biopen's sterility is easily maintained since it is smaller and more versatile than typical three-dimensional printers, as it can be taken apart for cleaning. The device, which is sterilized using similar methods done on other tools in surgery, has components directly exposed to cells (the ink cartridges, extrusion pistons and extrusion nozzle) that can be removed for separate sterilization by an autoclave [5]. This cleaning method minimizes the risk of complications without diverging from nominal surgical procedures, due to a lack of cross contamination.

The Biopen is efficiently made of only four main components: the body, a series of foot pedals, a titanium extruder, and a dim ultraviolet light [7]. The body, or more formally referred to as the chassis, is the 3D-printed component of the Biopen that holds the two ink chambers with separate but similar forms of Bioink. The right chamber contains Bioink composed of various hydrogels and infrapatellar fat pad adipose stem (IPFP) cells, while the left chamber contains Bioink with the same hydrogels but also with the photoinitiator VA-086 [7]. The IPFP Bioink acts as the "core" while the other acts as the surrounding "shell," which purposefully contains the photoinitiator so that it is successfully photocured by the ultraviolet light, providing protection for the cells and "mechanical support to the printed biomaterial" [5].

Although not shown in figure 4, the extrusion is controlled by two foot pedals connected to pressure regulators and the ultraviolet light source is connected to a third pedal [7]. These allow for the surgeon's optimal usability and precision by directly controlling how much Bioink is applied. The Biopen's extruder nozzle is three-dimensionally printed using a combination of medical grade Acrylonitrile Butadiene Styrene-like material and a sturdy titanium alloy. It has a small, circular tip so that the surgeon can directly print, in real-time, a stratified bioscaffold. It is intentionally designed as "a modular interchangeable component" that is effectively colinear, as seen in Figure 4 (D), accounting for the two types of ink separated in the right and left chambers above it [7]. The nozzle's structure can create two-dimensional gradients once ink is extruded and will also confine cells encapsulated in the "core" [7]. This colinear geometry also improves the printing resolution of the device as well as the proper shell-core layer orientation of inks once printed.

Lastly, the ultraviolet light source, Omnicure LX400+, is attached at a slight angle directly next to the pen's nozzle, which ensures it will photocure all the Bioink applied. The Omnicure LX400+ light emits a relatively low wavelength of 365 nm, allowing the Bioink to only slightly harden the outer protective layer to a point where proper photocrosslinking of the hydrogels can successfully occur and it settles with a texture akin to cartilage.

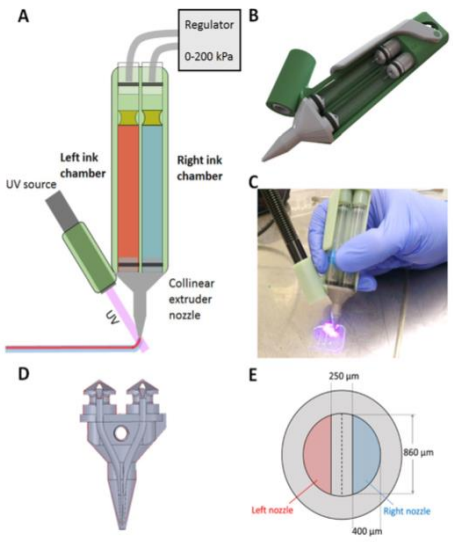

FIGURE 4 [9]
(A) Biopen Schematic (B) Three-dimensionally printed Portion of the Biopen (C) Image of Biopen being tested (D) CAD model of the colinear extruder nozzle (E) Annotated diagram of the tip of the nozzle

Figure 4 (A) shows a diagram of the Biopen including the ultraviolet light source, the extruder, and the two chambers containing the two different Bioink solutions which are controlled via a pneumatic system. The Biopen's main two components, shown in Figure (B), are, as mentioned earlier, three-dimensionally printed, so it can be produced cost effectively and uniformly compared to other building methods. Compared to traditional three-dimensional printers, Biopen has an increased surgical dexterity which allows for deposition within crevices or beneath overhangs in native tissue [5]. As seen in Figure 4 (C), the Biopen is not overly complex to use, making it easy for surgeons to learn in the future.

The researchers also sought to improve the structural integrity of the implants. "To control the size and shape of the scaffolds extruded by the Biopen, [shown in figure 5], [they] used polydimethylsiloxane (PDMS) cylindrical molds to create a desired shape with regulated cell numbers, providing reproducible structural organization with three-dimensional geometry for mechanical testing. Immediately after extrusion, samples were irradiated with ultraviolet light for photopolymerization" [12]. Using this method, it ensured the structure's stability.

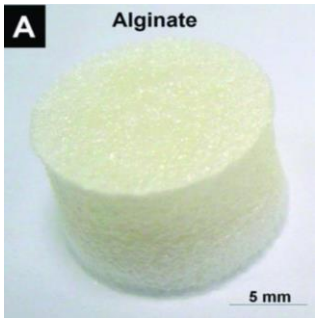

FIGURE 5 [11]
A 3D-printed alginate scaffold

BIOINK: A MIRACLE FORMULA

Scientists had to create a unique solution to mimic cartilage since there is no known natural substance that can fully replace it. The composition of the two different Bioinks are fairly complex but necessary for surgical compatibility, especially for, in the specific case of the Biopen, knee surgery. The first formula of the Bioink, acting as the "core", has three main components: infrapatellar fat pad adipose stem (IPFP) cells, alginate, and various hydrogels. In former surgical processes, the cells that were used were cloned stem cells that were more likely to die once implanted. Scientists behind the Biopen wanted to ensure that all of the components within the ink were biologically compatible with the same cells already in a patient's affected knee joint so that no infection or serious damage would occur. So ideally, the infrapatellar fat pad adipose stem cells would be derived directly from the knee joint of the patient undergoing surgery. These types of cells, in particular, were the most viable option due to their "ease of harvest and abundance" when implanted within the body in previous procedures and their relevant ability to transform into cartilage via the process of chondrogenesis [7]. Once in the body, the cells multiply, then differentiate into nerve cells, muscle cells or bone cells and eventually become functioning cartilage [13].

Both Bioinks contain alginate, a naturally-derived, commonly used biopolymer in 3D bioprinting, that stems from brown algae [6]. The alginate, shown in Figure 6, acts as a preservative for the adipose stem cells, allowing them to "retain viability and maintain sterility" [9]. The alginate would help eliminate the several-day process of cultivating the cells before surgical implantation. Thus, the cells can be fully functional and eventually transform into cartilage once implanted.

FIGURE 6 [11]
Alginate hydrogel in its natural form

Lastly, both Bioinks have several hydrogels, a class of highly hydrated polymer networks with cell-friendly aqueous environments, and suitable structural and mechanical properties, which allow for cell interactions and biochemical signaling [14]. It is important for these hydrogels to be in aqueous environments to begin with because the once the Bioink is in the patient, it will not break down when exposed to water. Additionally, it is essential to the cartilage that the cells can interact because the cells have to stay alive within the joing or the Bioink will not be effective. Overall, the hydrogels help maintain the ink's structural stability and semi-liquid state of matter.

The main type of hydrogel in the Bioink is gelatin methacrylamide (GelMa). When it is in contact with chondrocytes post-implantation, it causes their higher production of extracellular matrix, which is the fluid within the cells that enhances their functionality by aiding in the differentiation and growth of more cells within the joint [7]. Scientists and tissue engineers had to strongly consider the Bioink's rheology, or the study of how materials deform in response to forces, to ensure that the material being printed from the Biopen's small nozzle would flow properly and settle within the patient's joint properly. Thus, they combined a miniscule 2% concentration of hyaluronic acid (HA) hydrogels with the 10% concentration of GelMa in order to increase its viscosity and the Bioink's ability to flow [7].

Additionally, HA is "a key component of articular cartilage" and have "binding sites for chondrocytes", thus furthering compatibility with the chondrocytes already within a patient's joint [7]. Therefore, this allows the Bioink to fuse onto the patient's cartilage easier than without the HA, so the

body does not detect it as a foreign substance and cause infection. Researchers developing Bioink found that HA shows high potential as a component of engineered tissue since it encouraged further differentiation of chondrocyte stem cells and further cartilage growth or even regeneration [5]. The Biopen has two immiscible aqueous phases of GelMA mixture and polyethylene oxide (PEO) which are photocrosslinked to fabricate predesigned cell-laden hydrogel models by extrusion bioprinting or digital micromirror device-based stereolithographic bioprinting [14].

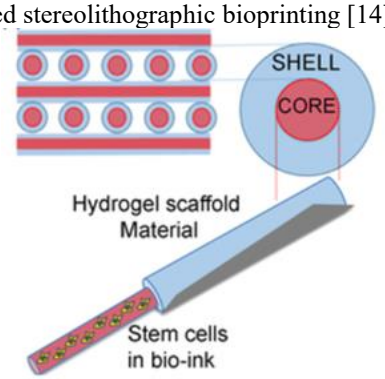

FIGURE 7 [5]
Shell-core layering of Bioink

In other words, the two phases (GelMA and PEO) form a covalent bond between the inner core and the shell to combine and produce a coherent Bioink. The porous structure of the three-dimensional bioprinted hydrogel construct is formed by subsequently removing the PEO phase from the photocrosslinked GelMA hydrogel. Porosity refers to the ratio of the fluid volume occupied by the fluid phase to the total volume of porous materials, therefore, the more porous it is, the less dense it is [15]. Porosity is a critical feature in the Bioink because large pores are shown to increase elastic moduli, improve nutrient mass transport, and provide interstitial space for extracellular matrix deposition [9].

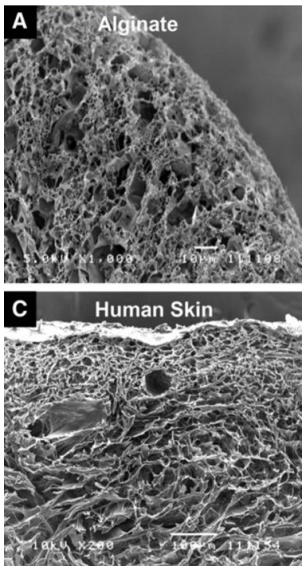

FIGURE 8 [11]
The comparison of the scanning electron microscopy images of alginate and human skin scaffolds

In figure 8, it shows the similarities between the scaffolds. The Alginate scaffold should ideally resemble the size of the pores, porosity, and function of the human skin scaffolds: “Three different cell types (human hepatocellular carcinoma cells, human umbilical vein endothelial cells, and NIH/3T3 mouse embryonic fibroblasts) within the 3D-bioprinted porous hydrogel patterns show enhanced cell viability, spreading, and proliferation compared to the standard, non-porous hydrogel constructs” [9].

Finally, the second Bioink, acting as the “shell”, lacks the IPFP stem cells but rather has a small 0.5% concentration of the photoinitiator VA-086 added to the hydrogels for the sole purpose of successful photocuring once outside of the Biopen and in contact with the UV light [7]. Altogether, the hydrogel formula present in both Bioinks provides an adequate viability for the chondrogenesis of the adipose stem cells. This material was found to be most compatible in its ability to maximize the viability of the cells it surrounds following extrusion from the nozzle. Thus, researchers observed that the Biopen device itself did not impede the functional abilities of the cells, which was a significant advantage [7]. Moreover, their formulas for the Bioink showed promise when tested for its rheology in withstanding various temperatures.

BIOPEN IN SHEEP TRIALS

As a step toward conducting human trials, researchers at the University of Wollongong in Australia, who developed

the Biopen, tested the surgical device on six young male sheep. They used the same composition of the two different Bioinks for each of the chambers within the pen, where the stem-cell Bioink contained "infra-patellar fat pad [cells] of recently euthanized sheep" which were cleansed and filtered before being added to the characteristic hydrogel combination, as previously discussed.

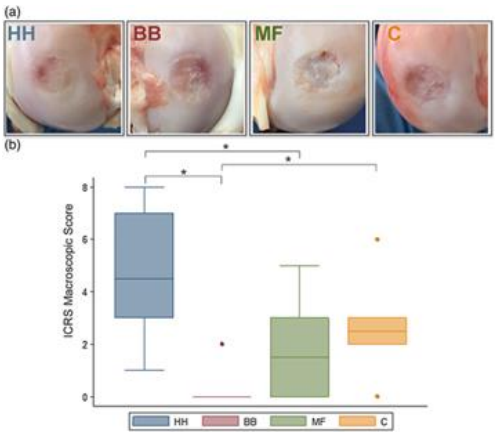

FIGURE 9 [5]
(a) Images of knee joints post-operation used for macroscopic evaluations of each group
(b) Box-whisker plots displaying data of ICRS assessments

The researchers fabricated joint defects on each knee of each sheep so that there would be four independent variables in terms of surgical repair procedures: treatment with the Biopen (HH group), with standard premade bioscaffolds (BB group), with microfracture surgery (MF group), and one without any treatment (negative control group).

The two bioinks were made the day of the procedure and printed "directly on the chondral defect" [5]. In contrast, the bioscaffolds made of the same Bioink containing the fat stem cells, hyaluronic acid, and GelMa, were cultured and incubated a whole week before direct implantation in surgery. For the MF group, researchers followed a standard simple, yet costly, microfracture surgical procedure by drilling five 1.0 mm microfractures into the bone surrounding the defective joint area in order to encourage natural regeneration and growth of cartilage [5].

Post-surgical procedure, the researchers interpreted the results in three main ways. In their macroscopic evaluation, they had scientists blindly rank the success of each treatment using the "International Cartilage Repair Society (ICRS) assessment protocol", which focuses on measuring the level of successful repair, surface texture of the defective area, and proper integration of the implant [5]. The HH group obtained the highest score, which was statistically significant, based on this assessment.

They also tested the biomechanics of the cartilage by analyzing the value of its Young's modulus. For context, Young's modulus is defined as the ratio of shear stress to shear strain and so is directly proportional to a material's rigidity.

Hooke's Law

$$\sigma = E\varepsilon$$

FIGURE 10 [16]
Young's modulus, E, in Hooke's Law

It can be calculated from Hooke's Law using the equation in figure 10, where σ is the stress, E is Young's modulus of elasticity and ε is the strain [16]. In the HH and BB group, which both used Bioink, the Young's modulus was found to be only slightly lower than those in the MF and control groups, indicating the cartilage formed in the first two groups was able to maintain a desired rubber-like texture that is not too rigid.

In the researchers' microscopic analysis, they used immunohistochemistry, a method for detecting antigens or haptens in cells of a tissue section by exploiting the principle of antibodies binding, to evaluate the makeup of the implanted cartilage and its protein expression, particularly collagen, as shown in Figure 11. They identified a significant amount of chondrocyte cell regeneration in the HH group with low variability compared to the other groups. The new hyaline cartilage formed in the HH group was abundant in collagen fibers and was the only one that displayed proper alignment of the chondrocyte cells, as seen in the figure. Also, the bone under the joint in HH group was not misshapen and had "minimal lateral integration", whereas other groups displayed "severe fibrillation" on the joint surface and formation of cysts on the bone, especially in the MF group [5].

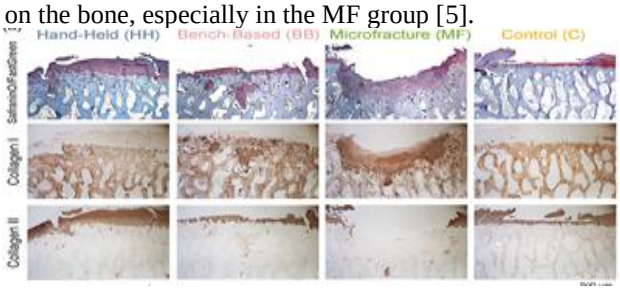

FIGURE 11 [5]
Microscope slides of cartilage samples, stained with or without Collagen I or Collagen II

As for the surgeons, it was a very practical and useful device that proved to be easy to use. They claimed that it had the familiar feel of other surgical tools and so did not require any additional training before using it. The surgeons stated that the bioscaffold from the pen "perfectly fitted the

defect['s] shape and depth" and thus found that the Biopen successfully overcame the main limitation of premade bioscaffolds in its lack of ability to "recapitulate the correct radius of curvature of the defect" [5]. Since it was a handheld device, the surgeons were able to effectively utilize their developed dexterity. Overall, the bioscaffold directly printed from the Biopen is much more compatible with both surgeons and the patients undergoing the knee surgery.

FUTURE IMPROVEMENTS

Biopen Improvements

Like any product, there must be added innovations and altercations to improve the Biopen. From the sheep trials, they found it will be helpful in the future to more effectively employ the interchangeable cartridge feature already present in the Biopen. They plan to conduct studies in the future where the Biopen's chambers will be filled with biomaterial that would also generate bone tissue. This will help account for the subchondral bone, which was unaffected in the young sheep, but realistically, in human trials, patient's cartilage will be more developed, and so its associated bone must be "reconstructed or at least supported" [5].

Consequently, researchers must conduct more testing of the Biopen in order to enhance its efficiency and performance. With only the short experiments being conducted using the Biopen, it has limited power in detecting differences between control and experimental groups and it is hard to judge the quality of cartilage regeneration overall [5]. In the short term, the results can also be misleading and it is important to address this issue, since the Biopen will have to be an effective tool that, when used on human patients, will be able to last their lifespan. Another improvement that is being addressed by the researchers of the Biopen is the nozzle tip size, which is directly correlated to the accuracy of the Biopen: the smaller the tip the more intricate designs they can create. However, it increases the importance of creating successfully generated hydrogel constructs with large pore sizes using the 3D bioprinting techniques, since the solid particles with larger sizes tend to easily clog the nozzles, in particular in the case of extrusion bioprinting [14].

To this end, integration of droplet templating with 3D printing provides a promising strategy to produce porous scaffolds by simultaneous deposition of two immiscible phases. The benefit of this technology is that it could control the porosity and interconnectivity of the printed scaffolds by fine-tuning the composition, viscosity, and concentration of the emulsion inks. The current nozzle, separated into two half circles for each chamber, has a tip width of 400 μm and a length of 860 μm. If the nozzle tip could be slightly smaller, it could allow the device to be more precise. Also relevant to the nozzle, the creators are looking into creating a coaxial rather than colinear nozzle in order to enhance the protective role of the photoinitiator shell surrounding the adipose stem cells [7]. Present research though only has evidence based on the colinear model, which has still proved to be successful.

A third improvement is improving the Biopen's sensitivity to temperature. Due to the viscosity of the Bioink, there is a strong dependence on temperature and pressure, which can affect the extrusion stability rates of the solution. The body heat transferred from the user's hand while using the Biopen disturbs the thermal equilibrium, which can slightly increase the print-rate [7]. One proposed solution is adjusting the extrusion pressure to ensure a consistent volumetric flow by using a mechanical extrusion mechanism rather than a pneumatic mechanism and by also incorporating a thermally-controlled chassis [7].

Bioink Improvements

The first improvement to be made on the Bioink is the ability to maintain the scaffold's position after being extruded from the chambers of the Biopen. From the sheep trials, researchers found that "in some cases, the macroscopic and microscopic appearances of the HH and BB groups were similar to the control group, which indicates that the scaffold may have moved from its original position" [5]. It is crucial that the scaffold stays in its original position or else it will not cushion the knee joints, and there will continue to be bone on bone impact. One possible reason for this instability of the printed bioscaffold may be its lack of complete and "immediate adhesion to the host cartilage", which they believe can be accounted for by slightly modifying the chemical makeup of the Bioink [5].

There have been many proposed solutions to improve the Bioink's components; however, the development rate is being hindered by the capabilities of these materials, since they have to be able to withstand extrusion, maintain structural integrity for long periods of time, and permit adequate nutrient diffusion of cells under all conditions [9]. One solution that has been proposed is to use hybrid multicomponent gels, which incorporates the physical properties to improve the current single component gel Bioink. Hybrid multicomponent gels have a structure similar to the natural extracellular matrices, high water content, and high permeability for oxygen and essential nutrients. They are also able to preserve living cells and be manipulated easily in various environments [18]. One combination that is being tested currently is alginate, a current component in the original formula for Bioink, covalently tagged with arginylglycylaspartic acid, a tripeptide sequence present in the cellular adhesion protein fibronectin.

Through a time-course study conducted at the University of Bristol, this new Bioink can produce a "biomaterial that can be extruded at a high resolution and effectively crosslinked to produce cytocompatibility

constructs with long-term structural fidelity," and additionally, can exhibit an adequate amount of elasticity, since it displayed increased shear thinning, compressive modulus, and shear modulus [9]. This means that the newly printed cartilage will be able to withstand any twisting, compression, or stretching with more resistance and so further damage is less likely. This feature of the bioprinted cartilage is ideal since it is part of the knee, a very mobile joint. In the future, this improved technology for the Bioink can help to develop other, more complex biological structures that can extend Bioink's use from cartilage to three-dimensionally printing alternate internal tissues. The use of this matrix production can significantly improve the lives of those with arthritis as the Bioink improves also, which will ensure that the cartilage is reliable in all conditions.

In general, cartilage has proven to be a challenge to recreate using Bioink, or any other laboratory-made biomaterial, due to its low cell numbers, aliphatic environment, distinct collagenous structure, aggregated proteoglycans, and cell matrix organization [19]. Simply put, the cartilage is a unique product to be reproduced with many limitations due to its complex chemical makeup. In the current model of the Biopen, it utilizes a Bioink that is limited by its concentration of the different biomaterials and the temperatures it can be used in, which can lead to a degradation of the printed cartilage upon application [5]. This can be a major problem for future clients as failure of the Bioink at the time of application could cost patients valuable time and money.

ETHICS

The Biopen and other bioprinting methods are revolutionary technologies as they can print human cartilage on demand using only the Bioink and living stem cells from the person's knee. While biomimicry aims to manufacture identical reproductions of cellular and extracellular components of cartilage through rational design, autonomous self-assembly takes embryonic organ development as a guide, replicating tissue by relying on the capacity of cells to generate functional and structural properties of tissue through self-organisation; this means that pluripotent stem cells, or cells that have a limited number of expansion cycles, will be used [17]. This process can be controversial because of the risks of using these cannot currently be calculated. However, unlike other bioprinting methods, the Biopen uses hydrogels which minimize these risks.

Additionally, all the studies in the short term had only promising outcomes. Like any new technology, the Biopen has done extensive research on preventing any risks and is overall a very ethically sound product. Furthermore, the Biopen addressed the issue of the controversial use of stem cells, by extruding them from the patient's knee rather than having them donated or using babies' stem cells.

FUTURE OF THE BIOPEN

Although there are some improvements the Biopen has to make before being used on arthritis patients, the University of Wollongong has constructed a pathway of what they believe are the necessary steps to be able to test on actual patients. As part of the BioMed Tech program, they are receiving millions of dollars from the Australian Government to be able to continuously improve the product [20]. "The grant will enable the partnership between Australian academia, healthcare and industry to lead and translate our research discoveries into the face of healthcare" [20]. Additionally, the investments in the Biopen will help test for future applications of the product as well as the feasibility of using it at general hospitals rather than only specialized facilities. This could improve the speed at which patients can be treated since there would be more places available where they can be treated.

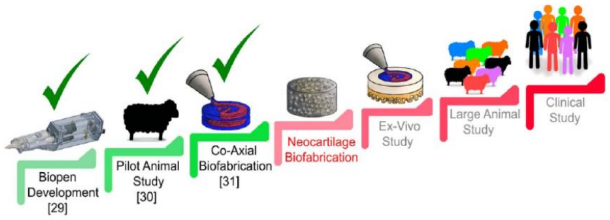

FIGURE 12 [19]
The Biopen's pathway towards a clinical study on humans

As shown in figure 12, the Biopen is in the third stage of their pathway towards a clinical study on humans. They already have a robust design of the actual product, completed their pilot animal study on sheep, and they finished the co-axial biofabrication of the Biopen. This biofabrication is an improvement from other treatments because the Biopen-treated joints were the only ones that had regenerated cartilage and properly aligned chromocytes [5]. The study demonstrated the capacity of the BioPen to produce human hyaline-like cartilage by coaxial extrusion of hADSC-laden in GelMa/HAMa hydrogel [12].

Overall, the Biopen is paving the way for more effective treatments for arthritis patients. Once the Biopen is able to be used on a global scale, it can significantly reduce the amount of people who suffer from the condition. Additionally, the Biopen is working towards using this technology not only for cartilage, but for other internal tissues. The Biopen is an important innovation for all engineers to recognize because it represents a convergence of engineering and medicine in producing a comprehensive solution to improve the lives of millions.

SOURCES

[1] "Arthritis By the Numbers." Arthritis Foundation. Accessed 01.16.2019. https://www.arthritis.org/Documents/Sections/About-Arthritis/arthritis-facts-stats-figures.pdf

[2] A. Alshami. "Knee osteoarthritis related pain: a narrative review of diagnosis and treatment". US National Library of Medicine. 01.08.2014. Accessed 02.05.19. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4039588/

[3] P. Edwin. "Arthritis of the Knee - Total Knee Replacement (Arthroplasty) and Other Treatments at HSS". Hospital for Special Surgery. 06.14.10. Accessed 02.05.19. https://www.hss.edu/conditions_arthritis-of-the-knee-total-knee-replacement.asp

[4] "Scope TV: Biopen." Scope TV. 12.04.2017. Accessed 02.28.2019. https://www.youtube.com/watch?time_continue=191&v=tVCCioe-yRU

[5] C. Augustine, S. Beirne, C. Di Bella, et al. "*In situ* handheld three-dimensional bioprinting for cartilage regeneration". Wiley Online Library. 05.17.2017. Accessed 01.27.2019. https://onlinelibrary.wiley.com/doi/full/10.1002/term.2476

[6] "3D Bioprinting: Bioink Selection Guide." Sigma-Aldrich. Accessed 01.29.2019. https://www.sigmaaldrich.com/technical-documents/articles/materials-science/3d-bioprinting-bioinks.html

[7] C. Augustine, S. Beirne, C. Bella, R. Cornock, C. O'Connell, F. Thompson. "Development of the Biopen: a Handheld Device for Surgical Printing of Adipose Stem Cells at a Chondral Wound Site." International Society for Biofabrication. Accessed 01.16.2019. https://iopscience.iop.org/article/10.1088/1758-5090/8/1/015019/pdf

[8] B. Duan. "State-of-the-Art Review of 3D Bioprinting for Cardiovascular Tissue Engineering" Springer Link. 04.11.2016. Accessed 02.13.2019. https://link.springer.com/article/10.1007%2Fs10439-016-1607-5

[9] J. Armstrong, M. Burke, B. Carter, S. Davis, A. Perriman. "3D Bioprinting Using a Templated Porous Bioink." Wiley Online Library. 06.22.2016. Accessed 01.16.2019. https://onlinelibrary.wiley.com/doi/full/10.1002/adhm.201600022

[10] B. Dume. "Biopen Speeds up Stem-cell Repair." Physics World. 03.20.2018. Accessed 01.15.2019. https://physicsworld.com/a/biopen-speeds-up-stem-cell-repair/

[11] C. Choong, Q. Loh. "Three-Dimensional Scaffolds for Tissue Engineering Applications: Role of Porosity and Pore Size." US National Library of Medicine. 06.25.2013. Accessed 01.29.2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3826579/

[12] T. Jeewandara. "3-D bioPen: A hydrogel injection to regenerate cartilage." Medical Xpress. 09.25,2018. Accessed 03.03.2019. https://medicalxpress.com/news/2018-09-d-biopen-hydrogel-regenerate-cartilage.html

[13] P. Choong. "BioPrinting: The Handheld BioPen." ARC Centre of Excellence for Electromaterials Science. Accessed 02.13.19. http://www.electromaterials.edu.au/case-studies/case-studies/case-study-1/

[14] X. Cao, R. Chai, S. Hassan, N. Jiang, S. Maharjan, A. Miri, J. Yang, Y. Yin, G. Ying, Y. Zhang. "Aqueous Two-Phase Emulsion Bioink-Enabled 3D Bioprinting of Porous Hydrogels." Wiley Online Library. 10.21.2018. Accessed 01.16.2019. https://onlinelibrary.wiley.com/doi/full/10.1002/adma.201805460

[15] M. Sadouki. (2018). "Experimental measurement of the porosity and the viscous tortuosity of rigid porous material in low frequency." 02.26.2018. Accessed 03.05.2019. https://doi.org/10.1177/1461348418756016

[16] "Mechanics of Materials." Boston University Mechanical Engineering Department. Accessed 02.23.2019. https://www.bu.edu/moss/mechanics-of-materials-strain/

[17] A. Jones, G. Haddow, N.Vermeulen, T. Seymour, W. Shu. "3D Bioprint Me: A Socioethical View of Bioprinting Human Organs and Tissues." Journal of Medical Ethics. 03.20.2017. Accessed 02.13.2019. https://jme.bmj.com/content/43/9/618

[18] X. Jia, K.Kiick. "Hybrid Multicomponent Hydrogels for Tissue Engineering." Wiley Online Library. 02.03.2009. Accessed 02.28.2019. https://onlinelibrary.wiley.com/doi/full/10.1002/mabi.200800284

[19] C. Onofrillo, S. Duchi, CD. O'Connell, R. Blanchard, AJ. O'Connor, M. Scott, GG. Wallace, PFM. Choong, C. Di Bella. "Biofabrication of Human Articular Cartilage: A Path Towards the Development of a Clinical Treatment." International Society for Biofabrication. 08.2018. Accessed 01.17.2019 https://www.researchgate.net/figure/Graphical-representation-of-the-experimental-procedure-and-the-corresponding-time-line_fig2_326911251

[20] S. Wright. "Biopen Receives Funding to Boost Health Innovations in Australia." University of Wollongong Australia. Accessed 01.17.2019. https://media.uow.edu.au/releases/UOW245623.html

ADDITIONAL SOURCES

T. Boland. "Cell and Organ Printing 1: Protein and Cell Printers." Clemson University. 2003. Accessed 01.16.2019. http://biocuriousmembers.pbworks.com/w/file/fetch/49775968/wilsonanatrecparta03.pdf

M. Dokmeci, P. Gungor-Ozkerim, I. Inci, A. Khademhosseini, Y. Zhang. “Bioinks for 3D Bioprinting: An Overview.” US National Library of Medicine. 05.01.2018. Accessed 01.16.2019. https://www.ncbi.nlm.nih.gov/pubmed/29492503
C. Frantz, K. Stewart, V. Weaver. The Extracellular Matrix at a Glace. Journal of Cell Science. 2010. Accessed 03.05.2019. jcs.biologists.org/content/123/24/4195
P. Janmey, M. Schlinwa. Rheology. US National Library of Medicine. 05.08.2008. Accessed 03.05.2019. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2895991/
C. Scott. “The BioPen: 3D Bioprinting Pen Lets Surgeons Draw New Cells Directly Onto Bone.” The Voice of 3D Printing. 04.04.2016. Accessed 01.16.2019. https://3dprint.com/127916/biopen-3d-bioprinting-pen/

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