Applications of In Vivo Bioprinting in Coordination with Robot Assisted Surgery for Musculoskeletal Tissue Repairs

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Bioprinting is the use of 3D printing technology, living cells, and bioinks to produce constructs. Bioprinting is a powerful tool that can help regenerate tissue in the body or print organs to be used in transplants. Although discoveries, such as the development of new printing methods and bioinks, have helped the scientific community learn more about the challenges of the bioprinting process, *in vivo* bioprinting, bioprinting that takes place directly in the body, is a common goal that many researchers are striving to achieve. This paper will review successful bioprinting processes and the different bioinks that have emerged. Also, the paper will offer new perspectives on how robot-assisted surgery could be combined with *in vivo* bioprinting to create new surgical technology, including injuries to the musculoskeletal system. The musculoskeletal system requires long and invasive procedures to fix problems that occur. Most musculoskeletal injuries are caused by strains on muscles that lead to tears in ligaments and the need for soft tissue replacement. Through *in vivo* bioprinting, the reconstruction and regeneration of skeletal tissue without long and invasive procedures is possible. Utilization of bioprinting could limit the need for complicated surgeries. Also, it would require the implementation of surgical robots.

I. Introduction

The musculoskeletal system (MSK) is heavily involved in movement, and it is made up of bones, cartilage, ligaments, tendons and muscle fibers. The MSK provides support throughout the body with skeleton and muscle connections [1]. As various parts of the MSK are used for menial and arduous tasks and stress is put on the system, injuries regularly happen. Injuries to the MSK are referenced as either blunt force trauma or penetrating trauma. Blunt force trauma occurs when the MSK is injured due to a person or object striking the body. Penetrating force trauma occurs when the skin and tissue are penetrated, resulting in a wound [2]. If a body part, such as the calf or shoulder, is over-extended or over used, a strain - an injury to the connection between the muscle and bone - can sometimes lead to a full tear of the tissue connecting the bones, resulting in a sprain [3]. Most injuries to the MSK are caused by stress put on the system stemming from accidents or physical contact activities, like sports. In 2012, 54% of musculoskeletal injuries were caused by traumatic events, such as car accidents, and 2.8 million sports-related musculoskeletal injuries were treated [4].

Serious injuries to the MSK require surgery. Surgery that targets the MSK may be invasive and carries risks of complications that can arise during and after surgery. Depending on what part of the MSK is injured, surgery could entail risk of bleeding if carried out close to highly vascularized tissue [5]. Hemorrhaging due to bleeding in the MSK could lead to the need for more surgery or complications [5]. These complications can range depending on the class of the hemorrhage and the location [5]. For example, hemorrhage in compact areas, such as the calf, can lead to compartment syndrome [5]. Although imaging is used before surgical procedures to plan out the course of action in order to repair the damages made to the MSK, the area that is repaired will never be exactly the same as it was before the injury occurred. The body also compensates for the surgical changes in the MSK, causing unwarranted stress to be put on other areas of the MSK. Additionally, the recovery process after musculoskeletal surgeries may be long and difficult. Physical therapy is usually utilized to help repaired musculoskeletal parts, such as ligaments or muscle fibers,in regaining strength [6]. If the repair is stressed too early, re-injury is very likely due to the lack of structure in the extracellular matrix during the reforming of scar tissue between healing fibers, causing pain and inflammation during the remodeling stage [6]. When musculoskeletal tissue is injured, the tissue heals in three different stages: inflammatory stage, fibroblastic stage, and remodeling stage [6]. For example, tendon and ligament tissue can take more than one year to fully regain strength [6].

Currently, there are multiple surgical processes that can be utilized for repairing the MSK. One of the most common procedures done is a soft tissue repair, in which tissue from various parts of the body, such as the hamstring tendon, is used to mend torn tendons and ligaments [7]. Although most musculoskeletal surgeries are completed with general infection rates of less than 1% [5], infections are still more likely to occur during complex surgeries where patients undergo operations for longer periods of time [8]. Furthermore, surgery to the MSK has the potential to damage other areas, and requires great precision to avoid this. Arthroscopic surgery is also a procedure used to seek out and repair problems with joints [9]. This technique is minimally invasive due to the use of a small incision to insert an arthroscope, a tube that has a camera inside of it, in order for the doctor to gain a better view at the area in question [9]. Although this procedure is routinely used, there is potential for the placement of the arthroscope to cause nerve damage and blood clots [10]. These complications that arise during musculoskeletal surgery and during recovery suggest that there may be a need for improvement in lowering the risk of infection [8] or nerve damage [10].

The implementation of robot assisted surgery in the MSK is an option for improving treatment. As more surgical robots become available to use, trials have been conducted that conclude that robot assisted surgery leads to more accurate placement of implants and decreased chance of infection post-surgery [11]. Many robot assisted surgery systems have a separate console for surgeons to control the robot and view the operation from [12]. For example, Intuitive Surgical created the first FDA-approved robotic

surgery machine and instruments for their Da Vinci robot assisted surgery technology, allowing surgeons to make precise incisions to gain access to the surgical site and utilize specialized instruments to cut and seal vessels off in less than two seconds [12].

In addition to robot assisted surgery, *in vivo* bioprinting can be used to solve problems in repairs to the MSK. The down time after surgery, need for physical therapy in order to fully rehabilitate the repairs made, and the risk for further complications during and after surgery all point to the need for an improved approach to musculoskeletal surgery. *In vivo* bioprinting could be a viable alternative for other MSK surgical options. This paper will review bioprinting processes, different bioinks that have emerged, the current research that has been conducted for *in vivo* bioprinting and how *in vivo* bioprinting could combine with robot assisted surgery to improve the outcomes of musculoskeletal surgery.

II. 3D Printing and Bioprinting

The concept of additive manufacturing (AM) dates back to the mid-1900s. As one of the more common types of AM, 3D printing has made it possible to print freestanding structures through the use of a printhead that positions itself to print the structure layer by layer [13]. 3D printing has been introduced into many different lines of work, such as automobile manufacturing and consumer goods. It is a process of designing the product through a computer aided design (CAD) system and then choosing the specific materials that are used. As 3D printing technology has become more sophisticated, scientists have started to explore the use of biomaterials to print living cells in 3D structures [14]. This process is known as bioprinting. Bioprinting relies on the use of bioinks to print the 3D structures. The bioink is usually made up of a mixture of cells and biomaterials, such as hydrogel, ion-releasing biomolecules, nano-inorganic particles, and growth factors. This is involved in the cells' ability to survive, differentiate into the specific cell type needed, and promote regeneration of tissue during and after the printing process [15].

Extensive research has been conducted to explore the use of bioprinting in real life situations. The creation of *in vitro* tissue models has been a large step in the creation of full microenvironments where printed cells can fully function and be studied in the laboratory [16]. This makes the use of animals as a way to expand and study diseased cells [16]. The fabrication of tissue and organs outside of the body, also known as *in vitro* bioprinting, has allowed researchers to experiment with different printing techniques and bioinks [17]. These successfully printed organs have been used to test new drug formulations and dosage levels [16]. Some studies, including a project that shows the process of repairing osteochondral tissue with the use of bioprinting [2], shows that both *in vivo* and *in vitro* bioprinting have already taken steps in a promising direction.

III. In Vivo Bioprinting Techniques

Many techniques have emerged for bioprinting from past research. One of the most successful systems has been extrusion-based bioprinting. During extrusion-based printing, bioink is extruded through a nozzle that moves to create each layer of bioink that can build on top of eachother, creating 3D tissue [13]. Extrusion-based printing allows multiple printheads to be attached on the same machine, making it possible to use multiple materials if needed, and is able to operate at high cell densities, which is important in order to input a large amount of cells during the printing process [18][14]. There are some downsides to this process. For example, the stress that is put on cells during extrusion may cause cells to die due to the high pressure of the extrusion system and the low viscosity of the bioinks, hurting the viability of the printed tissue [14]. To help address this problem, nanoclay, a material that helps the printability and cytocompatibility of the bioink, is added [19].

Along with the printing technique, the material that is used in the printer is very important. Different materials are selected in order to ensure the cells stay intact during printing [21]. The material should have high print resolution, meaning that the material will stay in place and help the printer print accurately [20], and allow for fast crosslinking capabilities. This is done through techniques like photocrosslinking in order to join polymer chains and create a suitable microenvironment for the cells [21]. Bioinks are commonly used as the material, as they provide scaffolding for the cells during printing. In order for bioinks to properly print viable cells, the ink has to be set at a lower temperature than in the body and also provide a viable environment for cells to live while being printed [17]. Bioinks commonly use hydrogels to provide a high water content [22]. In the body, the extracellular matrix provides structure for tissues and cells to connect and reproduce through the network of proteins [23]. Through the use of polymers found in natural extracellular matrices, such as collagen, gelatin and hyaluronic acid combined with the use of hydrogels, bioinks are able to recreate the environment of a natural extracellular matrix and help the cells thrive while in the printing process [24]. Decellularized extracellular matrices are also used to perfectly replicate the environment [24]. For example, bioinks that utilize gelatin methacryloyl (GelMA) gels in their hydrogel have become popular in trials for extrusion-based printing due to GelMA's ability to help the bioink keep its printed structure and promote bioactivity [25].

The process of crosslinking, where gelation is induced on the hydrogel, is imperative for the hydrogel to keep its structure during and after printing [24]. Most bioinks have the capability of crosslinking through the addition of multivalent cations binding with the carboxylic groups of polymer chains that are in close proximity with each other [24]. This process promotes gelation of the polymer solution

[24]. For example, alginate, a naturally derived polymer, has instant gelation when Ca^{2+} ions are added [22]. The addition of different solutions that contain ions that start the crosslinking process is very applicable with extrusion-based printing due to the technique allowing the inclusion of multiple printheads and materials. For example, thrombin, an enzyme that is commonly found in blood plasma and causes blood clots [26], can be used as a crosslinking agent [21]. Also, the addition of ultraviolet (UV) light is used in crosslinking. This technique works by UV rays producing free radicals with the photoinitiator in the bioink, which react with hydrogel monomers to start the crosslinking process [22]. For example, Lui *et al.* tests how GelMA bioink would crosslink using UV light [25]. The results showed that the crosslinked GelMA had stabilized the structure [25].

In order for cells to take part in tissue regeneration after being printed, the correct cell type is needed to be used in the bioink. Stem cells are cells that have the properties to form differentiated cells to provide specialized functions [27]. Stem cells are commonly used in bioprinting due to their versatility as cells with self-renewing properties [28]. Mesenchymal stem cells are cells that can differentiate into osteoblast cells, myocytes, tenocytes, ligament cells, and smooth muscle cells, cell types in the MSK. Myocytes or myoblast cells can be differentiated from mesenchymal cells or inserted into bioinks directly [28]. These cell types have been printed to form cell types in actual tissue.

Table 1. Cell Types of MSK

Cell Types	**Cell Type Specifics**		
	Part of MSK	***Progenitor Cell***	***Potential Injury Implement ation***
Osteoblast cells	Osteoblast cells form bone tissue and are found on the surface of bones [29].	Mesenchymal stem cells [22].	Bone remodeling after fracture [29].
Myocyte	Myocytes are cells that make up muscle tissue and are found in cardiac, skeletal and smooth muscle [30].	Mesenchymal stem cells [22].	Muscle tears [31].
Tenocytes	Tenocytes are tendon cells that are found in tendons and help the synthesis of tendon fibers [32].	Mesenchymal stem cells [22].	Achilles repair [33].
Ligament cells	Ligament cells are found in ligaments [34].	Mesenchymal stem cells [22].	Anterior cruciate ligament (ACL) repair [33].
Smooth muscle cells	Smooth muscle cells are found in most parts of the body and are used for involuntary muscle contraction [35].	Mesenchymal stem cells [22].	Corporal veno-occlusive dysfunction [36]

IV. Current Application of In Vivo Bioprinting in the Musculoskeletal System

In vivo bioprinting research has helped to produce new viable treatment options for repairing the MSK. For example, a study was conducted on a rabbit that was exhibiting volumetric muscle loss due to injury [37]. A handheld, extrusion-based bioprinter that accepted cartridges of bioink was used to print bioink inside the body, while a UV light was used as a crosslinking agent [37]. The bioink that was used contained vascular endothelial growth factor (VEGF) - a growth factor used to prompt vascularization and myofiber regeneration in the muscles-GelMA hydrogel, and Laponite® nanoclay [37]. Growth factors are used to direct cellular function in order to promote tissue growth [15]. The study concluded that the inclusion of this bioink to help volumetric muscle loss resulted in the decreased fibrosis and increased hypertrophy [36]. Due to less fibrosis, muscle tissue is able to regenerate at a faster rate due to the lack of scarring on the tissue [38]. This study shows how many different factors are used to

create a working and printable bioink that reduces the formation of fibrosis. Also, the printing mechanism and the method used to input bioink into the printer shows that extrusion-based printing can be translated into robotic technology, including the ability to implement different ink cartridges easily.

Wang *et al.* studied the viability of stem cells when implanted *in vivo* into the MSK [39]. Human adipose-derived stem cells (hASCs) are stem cells that are derived from adipose tissue, connective tissue that makes up body fat, and differentiate into specialized mesenchymal cells [40][41]. These cells were used to create bone matrices during skeletal remodeling called osteoblasts [41]. During this study, a scaffold made of alginate and gelatin that was crosslinked by $CaCl_2$ was produced. The scaffold was then cultured *in vitro* for seven days [39], and implanted into mice's dorsal subcutaneous area [39]. The results of this study concluded that bone matrix formation was found in the mice eight weeks after the scaffold was implanted. To see the formation of the collagen fibers in the tissue, researchers embedded sections of tissue in paraffin and used H&E and Masson trichrome staining, in which the sections of paraffin and new formation of bone matrix could take up the stain and be visualized under a light microscope [42][39]. This study is a prime example of how *in vivo* printing can advance the way injuries are looked at and treated. Bone fractures in the United States are predicted to increase by 50% and costs of treatment are estimated to increase by 37-175% by 2025 [43]. By changing the standard treatment process, cost of treatment could decrease with the implementation of *in vivo* bioprinting in addition to strong bone regrowth and formation without the downtime or cost of surgery.

Another study utilized bioprinting to create human cartilage *in vivo* [43]. The study used extrusion-based bioprinting to print scaffolds of bioink containing Nanofibrillated Cellulose (NFC) hydrogel, chondrocytes and mesenchymal stem cells that were crosslinked using a $CaCl_2$ solution [44]. Chondrocytes are cells that are used to sustain articular cartilage by secreting collagen and glycoproteins that make up the extracellular matrix. Mesenchymal stem cells promote chondrogenesis and allow for the formation of cartilage [45][44]. These scaffolds were then implanted into mice and were analyzed thirty and sixty days after implantation [44]. The study found that chondrogenesis was present after thirty days and increased at the sixty day mark through the use of alcian blue and van Gieson staining to highlight the extracellular matrix and a component of cartilage used for physical function [46][44]. Cartilage reconstruction surgeries are usually time consuming and can result in multiple complications such as infection, pain and tissue necrosis [44]. By implementing *in vivo* bioprinting to cartilage reconstruction surgical procedures, the process could speed up the healing process and decrease tissue necrosis.

V. Implementation of Robot-Assisted Surgery in In Vivo Bioprinting

The implementation of robot-assisted surgery combined with *in vivo* bioprinting could increase the success rate for current procedures. The most important aspect of robot-assisted surgery that could optimize *in vivo* bioprinting is the minimally invasive nature of the procedures. The Da Vinci Surgical System uses a First Access accessory to create an anchor point where multiple tools could enter into the surgical area [47]. *In vivo* bioprinting could be integrated by creating an attachment or a series of attachments that includes a print head, a crosslinking machine, and a camera. The creation and use of these series of attachments could help create a better patient experience.

New imaging technology could also play a large role in surgical planning. Currently, robot-assisted surgical systems use bone mapping technology to see the current state of a patient's surgical area [48]. This technology is able to manipulate different factors of the area and implant, such as the placement of the implant or the range of motion the patient would potentially have after the implant was placed in a certain orientation, in order to find the best surgical plan [48]. Another surgical system, the MAKO System, uses CT scans to create 3D structures of the surgical site [49]. By utilizing current imaging technology, *in vivo* bioprinting procedures could be used to prevent complications. Attachments that are already utilized in robot-assisted surgery could also be key contributors during *in vivo* bioprinting procedures. For example, the forceps attachment [12] could be used to maneuver around other organs during surgery, minimizing injuries to the area surrounding the surgical site. Using a combination of extrusion-based printing, digital imaging and robotic equipment, *in vivo* bioprinting could become a new alternative to musculoskeletal surgery.

In addition, there are currently many procedures that utilize grafts from other muscles in order to make repairs. One of the most common procedures that uses this method is an anterior cruciate ligament (ACL) reconstruction surgery. During this procedure, a graft is harvested from other muscles, usually the hamstring [50]. This graft acts as a tendon as it connects the bone to the muscle through two different holes made through the femur and tibia [50]. The graft also acts as a scaffold for a new ACL to develop on. By implementing *in vivo* bioprinting in this procedure, there would be no need for the hamstring graft. Although anchor points would still be necessary for the bioink through the implementation of holes in the femur and tibia, an extrusion-based bioprinting system could be used as a series of attachments on a robot-assisted surgery machine. These attachments could include one for extruding the bioink, one for crosslinking the bioink through UV light or the addition of solutions containing ions, and one that has a camera in

order for the surgeon to be able to see how the procedure is going and to make necessary adjustments in printing speed or placement of bioink.

VI. Conclusion

The creation of 3D bioprinting has already proven to be important in the medical field. 3D bioprinting has continued to develop to reach the end goal of *in vivo* bioprinting in humans. The combination of *in vivo* bioprinting and robot-assisted surgery technology could create the optimal treatment for many different injuries, specifically in the MSK, such as the use of 3D bioprinting to repair or replace cartilage in a knee joint. This review has provided an overview of the *in vivo* bioprinting process along with current robot-assisted surgery features, current research on this technology in the MSK and a perspective on how robot-assisted surgery could combine with *in vivo* bioprinting to create new procedures to treat MSK injuries. The future of *in vivo* bioprinting could change rapidly and become a revolutionary treatment for the musculoskeletal system.

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References

1. *Musculoskeletal system: Arthritis, lower back pain, bones, Muscles*. Cleveland Clinic. (n.d.). Retrieved October 7, 2022, from https://my.clevelandclinic.org/health/articles/12254-musculoskeletal-system-normal-structure--function#:~:text=What%20is%20the%20musculoskeletal%20system,posture%20and%20help%20you%20move.
2. Keriquel, V., Guillemot, F., Arnault, I., Guillotin, B., Miraux, S., Amédée, J., Fricain, J.-C., & Catros, S. (2010). *in vivo* bioprinting for computer- and robotic-assisted medical intervention: Preliminary study in mice. *Biofabrication*, *2*(1), 014101. https://doi.org/10.1088/1758-5082/2/1/014101
3. Mayo Foundation for Medical Education and Research. (2020, September 1). *Muscle strains*. Mayo Clinic. Retrieved October 7, 2022, from https://www.mayoclinic.org/diseases-conditions/muscle-strains/symptoms-causes/syc-20450507#:~:text=A%20muscle%20strain%20is%20an,complete%20tears%20in%20these%20tissues
4. *BMUS: The burden of musculoskeletal diseases in the United States*. BMUS: The Burden of Musculoskeletal Diseases in the United States. (n.d.). Retrieved October 7, 2022, from https://www.boneandjointburden.org/
5. Wang, D., Dubois, M., & Tutton, S. (2015). Complications in musculoskeletal intervention: Important considerations. *Seminars in Interventional Radiology*, *32*(02), 163–173. https://doi.org/10.1055/s-0035-1549447
6. Kirkby Shaw, K., Alvarez, L., Foster, S. A., Tomlinson, J. E., Shaw, A. J., & Pozzi, A. (2019). Fundamental principles of rehabilitation and musculoskeletal tissue healing. *Veterinary Surgery*, *49*(1), 22–32. https://doi.org/10.1111/vsu.13270
7. Orthosportsmed. (2022, February 1). *Musculoskeletal surgeries explained*. Orthopedic & Sports Medicine. Retrieved October 7, 2022, from https://orthosportsmed.com/musculoskeletal-surgeries-explained/
8. Cheng, H., Chen, B. P.-H., Soleas, I. M., Ferko, N. C., Cameron, C. G., & Hinoul, P. (2017). Prolonged operative duration increases risk of surgical site infections: A systematic review. *Surgical Infections*, *18*(6), 722–735. https://doi.org/10.1089/sur.2017.089
9. Jonathan Cluett, M. D. (n.d.). *What you need to know about arthroscopic surgery*. Verywell Health. Retrieved October 7, 2022, from https://www.verywellhealth.com/what-is-arthroscopic-surgery-2548501
10. Mayo Foundation for Medical Education and Research. (2022, August 19). *Arthroscopy*. Mayo Clinic. Retrieved October 7, 2022, from https://www.mayoclinic.org/tests-procedures/arthroscopy/about/pac-20392974#:~:text=Overview,a%20high%2Ddefinition%20video%20monitor
11. Li, C., Wang, L., Perka, C., & Trampuz, A. (2021). Clinical application of robotic orthopedic surgery: A bibliometric study. *BMC Musculoskeletal Disorders*, *22*(1). https://doi.org/10.1186/s12891-021-04714-7
12. *Da Vinci Surgical Systems*. Intuitive.com. (n.d.). Retrieved October 7, 2022, from https://www.intuitive.com/en-us/products-and-services/da-vinci/systems
13. Ramya, A., & Vanapalli, S. L. (2016). 3D printing technologies in various applications. *International Journal of Mechanical Engineering and Technology*, *7*(3), 396-409.
14. Vanaei, S., Parizi, M. S., Vanaei, S., Salemizadehparizi, F., & Vanaei, H. R. (2021). An overview on materials and techniques in 3D bioprinting toward biomedical application. *Engineered Regeneration*, *2*, 1–18. https://doi.org/10.1016/j.engreg.2020.12.001
15. Brokesh, A. M., & Gaharwar, A. K. (2020). Inorganic biomaterials for regenerative medicine. *ACS applied materials & interfaces*, *12*(5), 5319-5344. https://doi.org/10.1021/acsami.9b17801
16. Liaw, C.-Y., & Guvendiren, M. (2017). Current and emerging applications of 3D printing in Medicine. *Biofabrication*, *9*(2), 024102. https://doi.org/10.1088/1758-5090/aa7279
17. Mota, C., Camarero-Espinosa, S., Baker, M. B., Wieringa, P., & Moroni, L. (2020). Bioprinting: From tissue and organ development to *in vitro* models. *Chemical Reviews*, *120*(19), 10547–10607. https://doi.org/10.1021/acs.chemrev.9b00789
18. Gillispie, G., Prim, P., Copus, J., Fisher, J., Mikos, A. G., Yoo, J. J., Atala, A., & Lee, S. J. (2020). Assessment methodologies for extrusion-based bioink printability. *Biofabrication*, *12*(2), 022003. https://doi.org/10.1088/1758-5090/ab6f0d
19. Zhang, Y., Wang, Z., Hu, Q., Luo, H., Lu, B., Gao, Y., Qiao, Z., Zhou, Y., Fang, Y., Gu, J., Zhang, T., & Xiong, Z. (2022). 3D bioprinted gelma‐nanoclay hydrogels induce colorectal cancer stem cells through activating wnt/β‐catenin signaling. *Small*, *18*(18), 2200364. https://doi.org/10.1002/smll.202200364
20. Chen, A. (n.d.). 3 crucial things to know about 3D printing resolution. Retrieved October 7, 2022, from https://www.cmac.com.au/blog/3-crucial-things-know-about-3d-printing-resolution#:~:text=Simply%20put%2C%203d%20printing%20resolution,is%20also%20called%20planar%20dimension.
21. MacAdam, A., Chaudry, E., McTiernan, C. D., Cortes, D., Suuronen, E. J., & Alarcon, E. I. (2022). Development of in situ bioprinting: A mini review. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.940896
22. Irvine, S., & Venkatraman, S. (2016). Bioprinting and differentiation of Stem Cells. *Molecules*, *21*(9), 1188. https://doi.org/10.3390/molecules21091188
23. *NCI Dictionary of Cancer terms*. National Cancer Institute. (n.d.). Retrieved October 7, 2022, from https://www.cancer.gov/publications/dictionaries/cancer-terms/def/extracellular-matrix

24. Ghavami Nejad, A., Ashammakhi, N., Wu, X. Y., & Khademhosseini, A. (2020). Crosslinking strategies for 3D bioprinting of polymeric hydrogels. *Small*, *16*(35), 2002931. https://doi.org/10.1002/smll.202002931
25. Liu, W., Heinrich, M. A., Zhou, Y., Akpek, A., Hu, N., Liu, X., Guan, X., Zhong, Z., Jin, X., Khademhosseini, A., & Zhang, Y. S. (2017). Extrusion bioprinting of shear-thinning gelatin methacryloyl bioinks. *Advanced Healthcare Materials*, *6*(12), 1601451. https://doi.org/10.1002/adhm.201601451
26. *Thrombin*. Thrombin - an overview | ScienceDirect Topics. (n.d.). Retrieved October 7, 2022, from https://www.sciencedirect.com/topics/medicine-and-dentistry/thrombin
27. Mayo Foundation for Medical Education and Research. (2022, March 19). *Answers to your questions about Stem Cell Research*. Mayo Clinic. Retrieved October 7, 2022, from https://www.mayoclinic.org/tests-procedures/bone-marrow-transplant/in-depth/stem-cells/art-20048117#:~:text=What%20are%20stem%20cells%3F,more%20cells%20called%20daughter%20cells.
28. Ong, C. S., Yesantharao, P., Huang, C. Y., Mattson, G., Boktor, J., Fukunishi, T., Zhang, H., & Hibino, N. (2017). 3D bioprinting using Stem Cells. *Pediatric Research*, *83*(1-2), 223–231. https://doi.org/10.1038/pr.2017.252
29. *Osteoblast*. Osteoblast - an overview | ScienceDirect Topics. (n.d.). Retrieved October 7, 2022, from https://www.sciencedirect.com/topics/materials-science/osteoblast
30. Rachel Baxter BSc, M. S. (2022, June 30). *Types of muscle cells*. Kenhub. Retrieved October 7, 2022, from https://www.kenhub.com/en/library/anatomy/types-of-muscle-cells
31. Ramos, L. A., de Carvalho, R. T., Abdalla, R. J., & Ingham, S. J. (2015). Surgical treatment for muscle injuries. *Current Reviews in Musculoskeletal Medicine*, *8*(2), 188–192. https://doi.org/10.1007/s12178-015-9272-0
32. *Tenocyte*. Tenocyte - an overview | ScienceDirect Topics. (n.d.). Retrieved October 7, 2022, from https://www.sciencedirect.com/topics/immunology-and-microbiology/tenocyte
33. *Ligament Tears*. Orthopedics | Ligament Tears Causes & Symptoms | Beaumont Health. (n.d.). Retrieved October 7, 2022, from https://www.beaumont.org/conditions/ligament-tears
34. Asahara, H., Inui, M., & Lotz, M. K. (2017). Tendons and ligaments: Connecting developmental biology to musculoskeletal disease pathogenesis. *Journal of Bone and Mineral Research*, *32*(9), 1773–1782. https://doi.org/10.1002/jbmr.3199
35. Jaslove, J. M., & Nelson, C. M. (2018). Smooth muscle: a stiff sculptor of epithelial shapes. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *373*(1759), 20170318. https://doi.org/10.1098/rstb.2017.0318 Ferrini, Monica G et al.
36. Ferrini, M. G., Kovanecz, I., Sanchez, S., Umeh, C., Rajfer, J., & Gonzalez-Cadavid, N. F. (2009). Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *The Journal of Sexual Medicine*, *6*(2), 415–428. https://doi.org/10.1111/j.1743-6109.2008.01105.x
37. Quint, J. P., Mostafavi, A., Endo, Y., Panayi, A., Russell, C. S., Nourmahnad, A., Wiseman, C., Abbasi, L., Samandari, M., Sheikhi, A., Nuutila, K., Sinha, I., & Tamayol, A. (2021). In vivo printing of nanoenabled scaffolds for the treatment of skeletal muscle injuries. *Advanced Healthcare Materials*, *10*(10), 2002152. https://doi.org/10.1002/adhm.202002152
38. Mahdy, M. A. (2018). Skeletal muscle fibrosis: An overview. *Cell and Tissue Research*, *375*(3), 575–588. https://doi.org/10.1007/s00441-018-2955-2
39. Wang, X.-F., Song, Y., Liu, Y.-S., Sun, Y.-chun, Wang, Y.-guang, Wang, Y., & Lyu, P.-J. (2016). Osteogenic differentiation of three-dimensional bioprinted constructs consisting of human adipose-derived stem cells in vitro and in vivo. *PLOS ONE*, *11*(6). https://doi.org/10.1371/journal.pone.0157214
40. Wang, X. F., Song, Y., Liu, Y. S., Sun, Y. C., Wang, Y. G., Wang, Y., & Lyu, P. J. (2016). Osteogenic differentiation of three-dimensional bioprinted constructs consisting of human adipose-derived stem cells in vitro and in vivo. *PLoS One*, *11*(6), e0157214. https://doi.org/10.1371/journal.pone.0157214
41. Dirckx, N., Moorer, M. C., Clemens, T. L., & Riddle, R. C. (2019). The role of osteoblasts in energy homeostasis. *Nature Reviews Endocrinology*, *15*(11), 651–665. https://doi.org/10.1038/s41574-019-0246-y
42. Zhou, X., & Moore, B. (2017). Lung section staining and microscopy. *BIO-PROTOCOL*, *7*(10). https://doi.org/10.21769/bioprotoc.2286
43. Tharakan, S., Khondkar, S., & Ilyas, A. (2021). Bioprinting of stem cells in multimaterial scaffolds and their applications in Bone Tissue Engineering. *Sensors*, *21*(22), 7477. https://doi.org/10.3390/s21227477
44. Apelgren, P., Amoroso, M., Lindahl, A., Brantsing, C., Rotter, N., Gatenholm, P., & Kölby, L. (2017). Chondrocytes and stem cells in 3D-bioprinted structures create human cartilage in vivo. *PLOS ONE*, *12*(12). https://doi.org/10.1371/journal.pone.0189428
45. Akkiraju, H., & Nohe, A. (2015). Role of chondrocytes in cartilage formation, progression of osteoarthritis and cartilage regeneration. *Journal of Developmental Biology*, *3*(4), 177–192. https://doi.org/10.3390/jdb3040177
46. Sharma, A., Wood, L. D., Richardson, J. B., Roberts, S., & Kuiper, N. J. (2007). Glycosaminoglycan profiles of repair tissue formed following autologous chondrocyte implantation differ from control cartilage. *Arthritis Research & Therapy*, *9*(4). https://doi.org/10.1186/ar2278
47. *Da vinci XI single-site technology - intuitive surgical*. (n.d.). Retrieved October 8, 2022, from https://www.intuitive.com/en-us/-/media/ISI/Intuitive/Pdf/1025290ra-isi-brochure-single-site-digital-low-res-394110.pdf
48. *Tka Gap Planning - Smith+nephew*. (n.d.). Retrieved October 8, 2022, from https://www.smith-nephew.com/global/assets/pdf/products/surgical/navio_tka_gap_planning_brochure_08776v1.pdf
49. *Mako smartrobotics overview*. Stryker. (n.d.). Retrieved October 7, 2022, from https://www.stryker.com/us/en/joint-replacement/systems/Mako_SmartRobotics_Overview.html#know-more
50. Mayo Foundation for Medical Education and Research. (2021, March 6). *ACL reconstruction*. Mayo Clinic. Retrieved October 7, 2022, from https://www.mayoclinic.org/tests-procedures/acl-reconstruction/about/pac-20384598#:~:text=During%20ACL%20reconstruction%2C%20the%20torn,or%20from%20a%20deceased%20donor