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REVIEW ARTICLE

Prospects of 3D Bioprinting as a Possible Treatment for Cancer Cachexia

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ABSTRACT

Cancer cachexia is a multifactorial syndrome characterized by persistent muscle atrophy, functional impairment, anorexia, weakness, fatigue, anemia, and reduced antitumor treatment tolerance. As a result, the patients' quality of life suffers. Cachexia is responsible for approximately 22-25 percent of cancer deaths. This article discusses the signs and symptoms of cancer cachexia, as well as the mediators, treatment options, and future prospects for 3D bioprinting. Protein breakdown, inflammatory cytokines activation, and mitochondrial alteration are all factors that contribute to cachexia, according to research. Cachexia has eluded standard treatment despite the use of proper nutrition, physical activity, anti-inflammatory drugs, chemotherapy, and grafting attempts. By attempting to fabricate 3D constructs that mimic native muscle tissues, 3D bioprinting shows a lot of promise when compared to traditional methods. Some 3D bioprinting techniques have been discussed in this review, along with their benefits and drawbacks, as well as their achievements and challenges in in-vivo applications. Muscle atrophy can be repaired with neural integration or muscle-tendon units. However, properly bio-printing these complex muscles remains a challenge. Although new bio-inks or 3D printers can be used to fabricate high-resolution constructs, progress can be made. This review study uses secondary data to show why 3D bioprinting could be a viable alternative to treating cachexia.

Keywords: cancer cachexia, muscle atrophy, tissue regeneration, 3D bioprinting

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INTRODUCTION

In all cancer patients, weight loss has been identified as a common prognostic factor. However, when it happens for no apparent reason, even if the patient is eating properly, the patient is left wondering what went wrong. Cancer cachexia is a condition characterized by a number of abnormalities relating to weight loss [1]. Along with weight loss, other abnormalities such as muscle loss and insulin resistance are also observed. Cachexia kills nearly 2 million people every year [2,3]. Cachexia is said to be responsible for 22-25 percent of cancer-related deaths [4]. Cachexia is a multiorgan disorder that results in protein-tissue loss or muscle atrophy in the skeletal muscle. The loss of muscle mass can be as high as 75% and 85% of total body fat [5]. As a result, functional impairment occurs because the body's skeletal muscle can regenerate lost tissue up to a certain point after injury [6]. Furthermore, the patient begins to lose 30 percent of his or her body weight, which can be fatal if no therapeutics are used [7]. Because the abnormal metabolism of cancer cachexia affects fat tissues, which can target skeletal muscles, oncologists must estimate muscle loss rather than weight loss [8]. A nitrogen flux from the skeletal muscle may occur in the liver. This reduces the amount of branched-chain amino acids in the blood, which is needed to activate muscle protein synthesis [9]. Because of a decrease in mobility, fatigue, and physical activities, cancer cachexia has a negative impact on a patient's quality of life [5,10].

Cancer cachexia has been the subject of molecular mechanism studies for some time, and it is still unclear what causes it to develop. Due to increased exposure to surgical, radiotherapeutic, and chemotherapeutic treatment complications, patients experience asthenia, anemia,

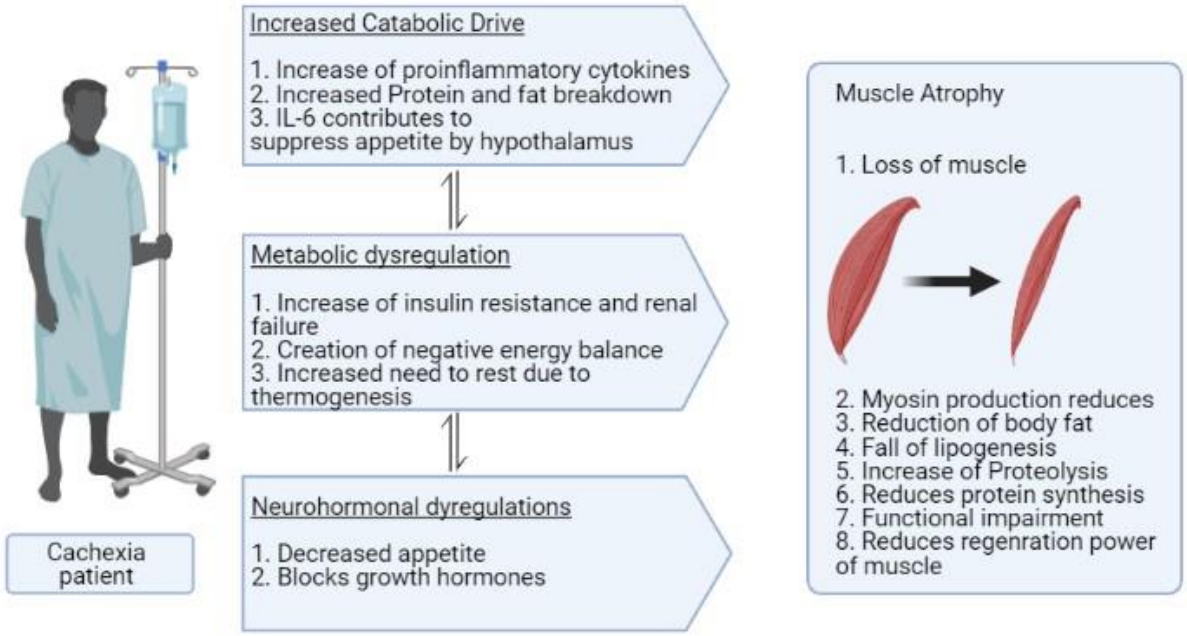

Figure 1. Factors that drive cancer cachexia – Increased catabolic drive, Metabolic dysregulations, and Neurohormonal dysregulations [8]

fatigue, and anorexia [8,11]. The timing of the advanced drugs and therapy administration was the cause of no beneficial clinical results in several cases [12]. According to experts, there are three stages of cancer cachexia: pre-cachexia, cachexia, and refractory cachexia. As a result, their treatments should be started as soon as possible in order to prevent or delay the progression of refractory cachexia [10,13].

There is currently no treatment, medicine, or surgery for cancer cachexia that is both effective and free of side effects. As a result, it is strongly advised that people strive to live healthy lifestyles in order to avoid this condition. In our rapidly aging society, there is a huge medical need for therapies to treat degenerative muscle disease like cachexia. Furthermore, there is no disease-modifying medication available for cachexia [14].

Organ and tissue transplantation has had a 100% success rate in saving patients with incurable diseases since its discovery. However, the greatest disadvantage is that demand has outpaced the number of donors. Especially in the case of muscle tissue donors. However, in addition to availability, limitations in immune system response and organ rejection play a role. Tissue engineering with 3D bioprinting is a method of overcoming this limitation [15]. Because of its ability to control geometry, 3D bioprinting has emerged as the most promising method in tissue engineering. We can now bioengineer various functional skeletal muscle tissue constructs with complex geometry thanks to recent advances in 3D bioprinting technologies. It can fabricate a wide range of biomaterials, both with and without cells, in a precise and controlled manner [16,17]. A 3D-printed structure can also stimulate cellular activities, improving the activity of electrically stimulated muscle tissues. The 3D-printed constructs can assist in the repair or even replacement of muscle loss caused by cachexia [15]. Despite the fact that experiments were limited to rats or time constraints, 3D bioprinting appears to be a viable and impressive solution to cachexia and muscle loss.

CAUSES AND MEDIATORS

Dysregulation of metabolism, increasing catabolic drives for breaking down fat/protein, and dysregulation of neurohormones are the 3 main factors that drive this disease (**Figure 1**) [8].

Muscle loss usually occurs due to protein breakdown. Cancer cachexia makes the myofiber of the cell membrane weak, reduces dystrophin levels, and causes muscle dystrophy [18]. People with cancer cachexia mostly have a negative energy balance with an increasing need to rest. Their need to rest increases frequently due to constant thermogenesis, i.e., energy used is increased; energy intake is reduced. So, patients with a good diet and nutrition intake will still lose weight. This in turn makes them unable to do physical activities [19,20].

Blood in our body also plays an active role in cancer cachexia. They are means of transportation for tissue-wasting tumor mediators that include factors contributing to systemic inflammation (**Figure 2**) [21]. Additionally, suppressor cells derived from myeloid (MDSCs) that expand during cancer development were deemed to be a contributor to murine cancer cachexia. This inducted acute phase response (APR) and changed energy metabolic states [22].

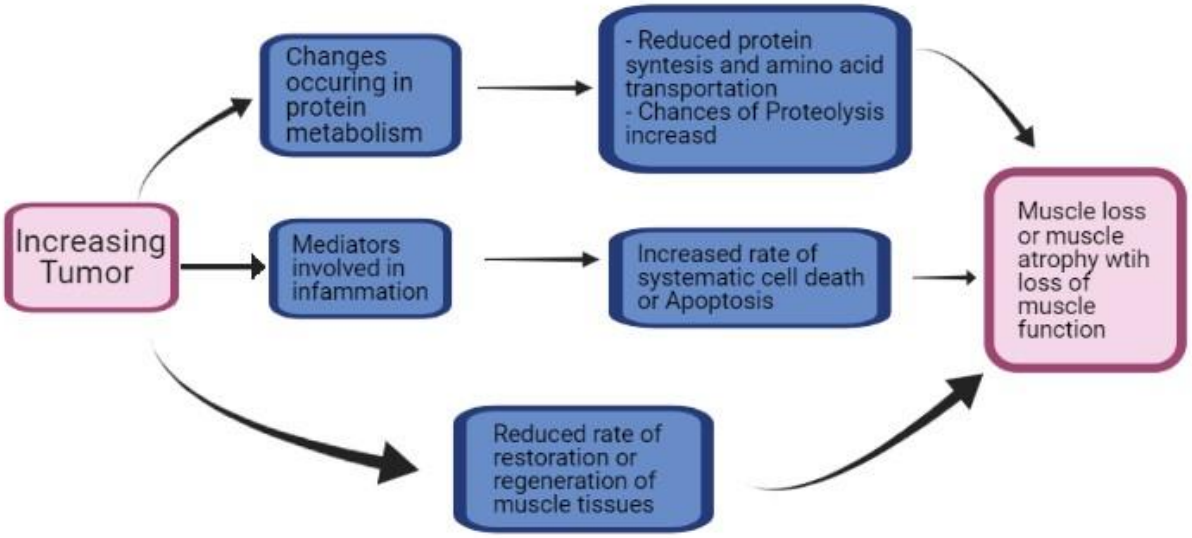

Figure 2. Cancer cachexia causing muscle-wasting, alteration in protein metabolism and reduces regeneration ability of muscles [19,21]

The presence of inflammatory cytokines like TNF-α, IL-6, and IL-1b are mediators that contribute to cancer cachexia [23,24]. The activation of TNF plays a role in suppressing appetite which leads to degradation of the proteasomal pathway [9]. This is a kind of alteration in mitochondria of skeletal muscle [9,19].

Myostatin is a muscle differentiation and growth regulator that acts as a negative autocrine. Myostatin activates and signals through ActRII/SMAD2,3-related pathways [23,25]. Activin-A is expressed and secreted in skeletal muscle as a result of tumor burden [26]. GDF11 and MIC-1/GDF15 were discovered to act as cachexia mediators in recent studies, exerting effects on appetite control via the recently discovered receptor GFRAL. TGF- also played a role in cancer-related muscle weakness [27,28].

SYMPTOMS AND CONSEQUENCES

Cachexia is characterized by a loss of weight and muscle mass, as well as metabolic abnormalities. Fatigue and anemia are the most common symptoms, which cause the patient to be more tired than usual due to a gradual depletion of the body's energy and protein reserves [7,29]. Furthermore, it makes patients more susceptible to drug-related toxicity, resulting in a poor prognosis [30,31]. Cancer cachexia causes cardiac muscle wasting, remodeling, and dysfunction in addition to skeletal muscle loss. As a result, the risk of cardiac death rises [32,33]. By increasing energy loss in tumor glycolysis production and converting lactate to glucose, cancer cachexia affects the liver's functions (**Figure 3**) [34].

Patients experience chemosensory distress, hyper-catabolism, and systemic inflammation as a result of their reduced food intake [13]. Patients may experience side effects such as anorexia, anemia, asthenia, diarrhea, and nausea during chemotherapeutic sessions. They also deal with a lack of food intake, body pain, depression, and insomnia [7,30]. Another issue with cachexia is that it cannot be reversed with nutritional methods because the anabolic response is disrupted [11,13].

Cachexia also activates hepatic acute-phase protein, which encourages IL-1- and IL-6-producing macrophages to infiltrate the liver. This can have a significant impact on the escalation of systemic inflammation in cachexia [26,35,36]. Cachexia also causes bone loss (**Figure 3**) [37,38].

AVAILABLE REMEDIES AND TREATMENTS

The importance of a well-balanced nutritional diet cannot be overstated. It is impossible to gain or maintain mass and body weight without adequate energy and nutrient supply. As a result, patients are nutritionally monitored before they lose weight. This monitoring entails providing nutritional and metabolic support to patients as needed [10,13,39]. It was discovered that fish oil derived from fatty acids has the ability to regulate pro-inflammatory cytokines and improve insulin sensitivity [40]. Branched-chain amino acids help to prevent muscle loss and protein breakdown [41]. However, as previously stated, this disease cannot be reversed solely through proper nutrition.

Modulation of skeletal muscle metabolism can improve insulin sensitivity, regulate cellular homeostasis, and promote myogenesis with physical exercise [42-44]. Exercising is necessary for the metabolism of skeletal muscle [45]. Cachexia patients, on the other hand, face challenges due to their physical limitations. They are prone to fatigue, anemia, and cardiac problems, so physical activity takes a toll on them [46].

Many anti-inflammatory drugs aid in the reduction of inflammation caused by cachexia. Corticosteroids are a type of medication that temporarily reduces fatigue and increases appetite [47,48]. However, they are not advised for long-term use because they can cause muscle wasting [49,50].

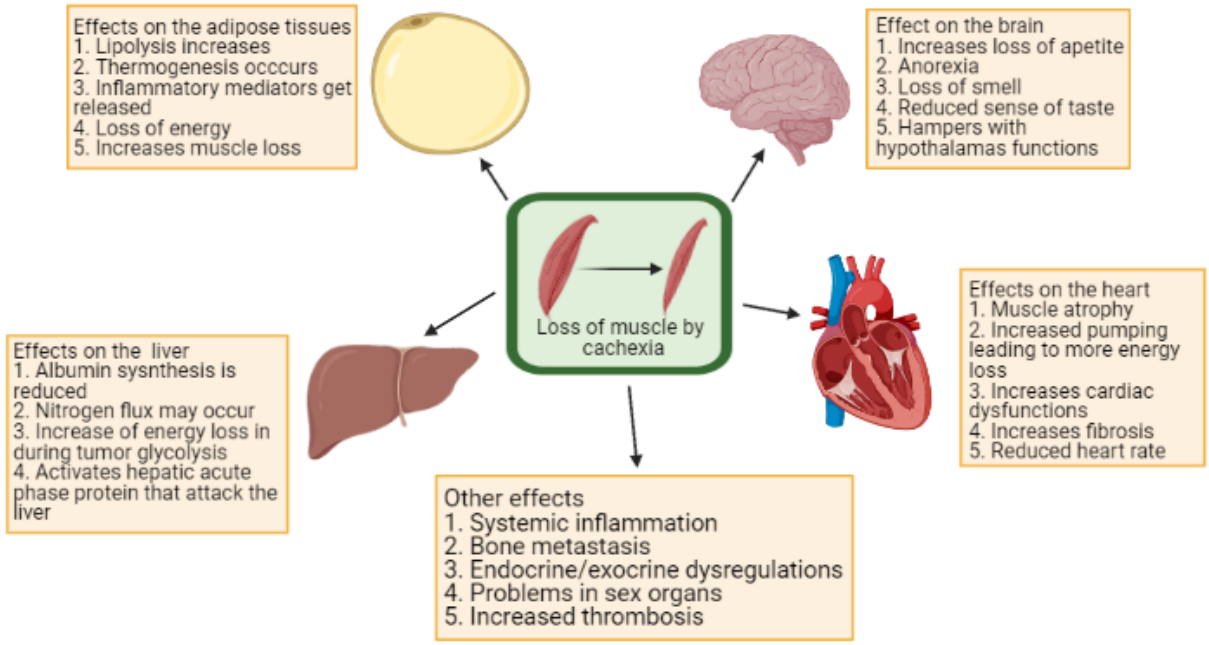

Figure 3. Cancer Cachexia is a multifactorial disease that causes skeletal loss, which contributes to several other problems [5,6]

Furthermore, despite its immunomodulatory and anti-inflammatory properties, thalidomide is not recommended due to its severe side effects [51-53]. The activin type-II-B receptor pathway can be blocked using ActRIIB decoy receptors, according to a study, resulting in resistance to muscle wasting. But it didn't work because it caused internal bleeding in the patients [54,55].

Weight loss is a common occurrence during chemotherapy or chemo-radiotherapy sessions, owing to muscle atrophy. The side effects of cytotoxic and targeted cancer therapies are extremely direct [30].

When muscle atrophy occurs in larger areas, autologous muscle transfer is used, but this can result in trauma or nerve injury, impairing motor functions [56,57]. In most cases, grafting healthy muscle from a donor site is used to restore the impaired function [58]. However, such grafting causes morbidity [59]. Furthermore, most grafting procedures can or will fail due to necrosis or infection caused by the donor [60]. Allografts and xenografts can trigger a severe immune response, resulting in rejection. The presence of antigens in donor tissue causes this [61-63].

Because cancer cachexia is a multidimensional syndrome, most unimodal approaches are unlikely to be effective. Overall, there are no agents, effective therapies, surgeries, or medicines that are 100% effective in the treatment of cancer cachexia.

3D BIOPRINTING

3D bioprinting is a relatively new strategy that, by creating tissue constructs, can yield positive results in regenerative medicine. This strategy closely resembles the natural structure of the tissue being targeted [64]. The 3D structure that is retrieved via CT scan, MRI, and ultrasound imaging is checked using a 3D scanner [15].

Bioprinting can be done in three ways: biomimicry, autonomous self-assembly, and mini-tissues [65]. By simulating the cellular microenvironment, biomimicry aids in the replication of specific cellular functional components of tissue [66]. For more complexity, autonomous self-assembly employs a guide. As 3D-biostructures, this guide has stem cell and embryonic organ properties [67]. Smaller functional building blocks can be printed on scaffolds and integrated into a larger macrostructure using mini-tissues [68,69].

For both non-biological and biological applications, inkjet printers are used [70]. Inkjet-bioprinters are used in the bioprinting of tissues and organs due to the availability of commercial products and ease of modification (Figure-4). The ease of access to a bioprinting platform and the high processing speed at a relatively low cost are two major advantages. However, one significant disadvantage is the limited selection of bio-ink materials available. To be shot out of the nozzle, the material must be liquid and viscous. Another issue is cell density, which can clog the nozzle and cause damaged cells [15,17].

Modified laser direct writing and laser-induced forward transfer techniques are used in laser-assisted bioprinting (LAB). It can print a wide range of cells while retaining viability (**Figure 4**) [65]. LAB can print high-cell densities and hydrogel precursors by positioning small drops of

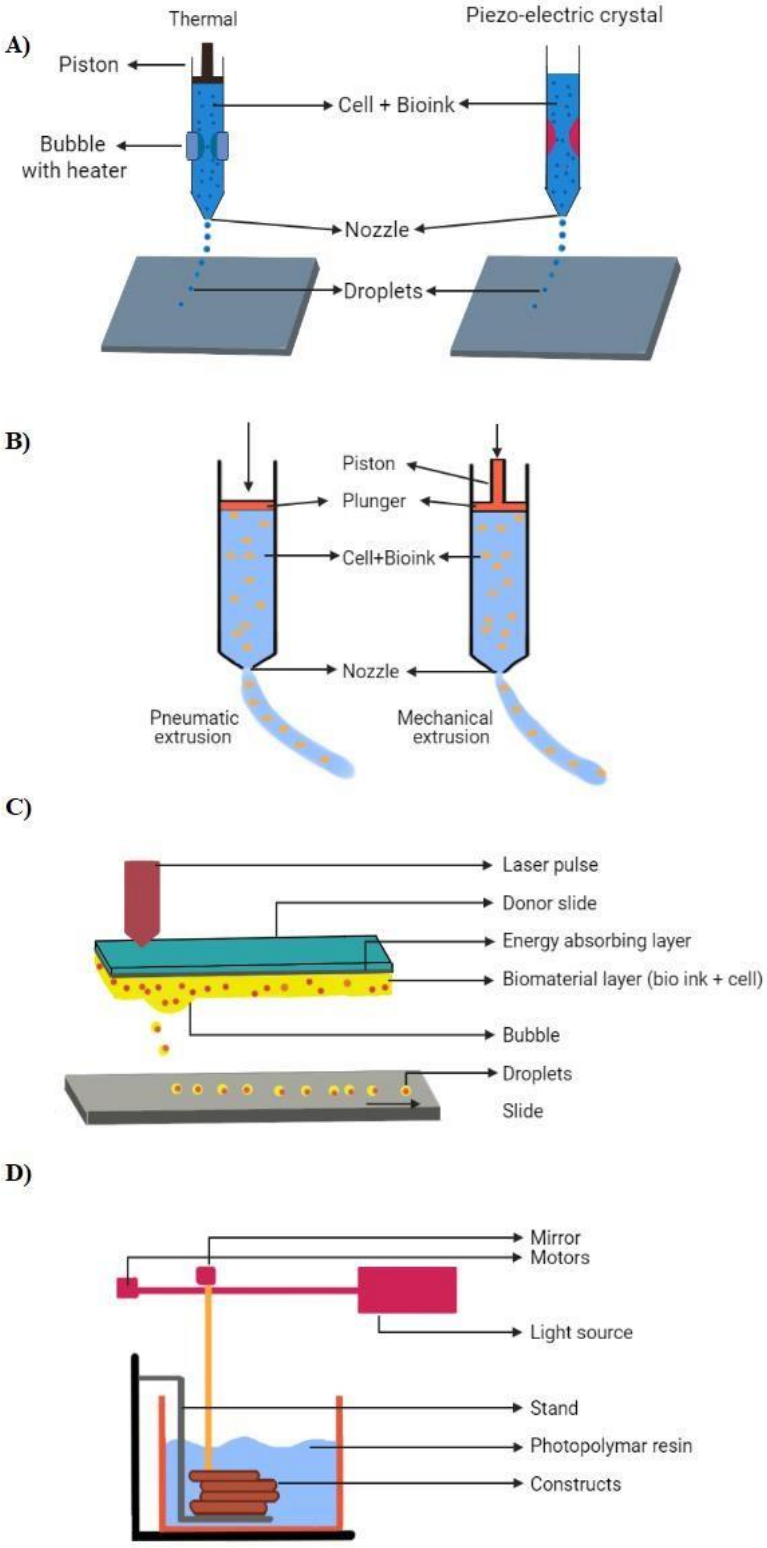

Figure 4. Different methods of 3D bioprinting- A) Inkjet Bioprinting. Cell droplets are printed using either thermal heaters or piezoelectric crystals. B) Extrusion bioprinting. It uses a piston to create air pressure or use mechanical force to get droplets. C) Laser-assisted-bioprinting. The light source helps create a laser that forms bubbles in the bio-material layer and the droplets are got. D) Stereolithography. Here 3D-constructs are created in a layer-by-layer step with the help of photochemical processes [15,61]

biomaterial at high resolution. This can be done with any viscosity you want [71,72]. However, the time required for rapid fabrication is lengthy and inconvenient [73]. Lasers that emit UV light can also have a negative impact on cells.

Biomaterials used in extrusion bioprinters are more diverse. Biocompatible copolymers, hydrogels, and cell spheroids are examples. They have enough viscosity to be printed (**Figure 4**). However, it has drawbacks, such as cell death caused by shear stress and rapid cell encapsulation [15,17].

A laser-assisted bioprinting system drives the stereolithography (SLA) process. This system produces realistic microstructures by photocuring photopolymerizable liquid polymers, resulting in 3D structures (**Figure 4**) [74].

BIO-INKS

Bio-inks are living cells and biomaterials that, after 3D printing, can mimic the extracellular matrix environment, cell adhesion, and proliferation. It's a type of biomaterial that's used to make living tissue. They usually have cells suspended in a liquid solution [75]. It is made up entirely of cells. Most have a 3D-molecular scaffold made of biopolymer gel as an additional carrier material. Cells can grow, spread, and proliferate when they attach to this. Natural or synthetic polymers with good biocompatibility are usually chosen. The bio-ink is what keeps the cells safe during the printing process [76].

3D bioprinting employs a variety of bio-inks to create cell-laden tissue constructs with sufficient strength and the ability to keep cells moist while printing without clogging the nozzle. Gelatin, Poly (ethylene glycol) alginate, hydrogels, collagen, and hyaluronic acid are among the materials used. Printability, biocompatibility, mechanical property, and ease of spatial arrangement are some of the most important characteristics of a bio-ink [70].

PRINTABLE BIOMATERIALS

A major obstacle for bioprinting is finding new biomaterials where cells can survive with their potency intact after being printed [77]. The biomaterials need to have an enhanced surrounding that helps host tissue formation. Strong and stiff mechanical strength is needed to provide sufficient support, handling, and implantation for cells [78,79]. The biomaterials need proper, so that, the internal structures do not break apart [80]. The biomaterials should also have maturation, proliferation, biocompatibility, biodegradability, differentiation, and be less immunogenic [78,81].

The biomaterials used for printing are categorized into synthetic and natural polymers. Synthetic polymers have the mechanical strength needed for printing and processing [82]. They help to precisely control molecular weight and functional groups but lack motifs that are cell-responsive. On the other hand, natural polymers are biodegradable and biocompatible. But they are mechanically weak [83].

Some examples of bio-ink can be alginate, gelatin, collagen, fibrin, hyaluronic acid (HA), agarose, chitosan, silk, decellularized extracellular matrix (dECM), poly(ethylene glycol) (PEG), etc. [84].

AVAILABLE REMEDIES VIA 3D BIOPRINTING

Muscle Tissue Regeneration by Electrospinning

Injury to the musculoskeletal system is fairly common, and poor healing can result in long-term impairment [85]. Several studies and experiments using 3D bioprinting have yielded positive results and a number of benefits in muscle reconstruction [86]. Electrospinning is a technique for achieving a fibrous structure. This allows synthetic/natural polymers to be used to control arrangement, structural, and biochemical properties. Miji Yeo and GeunHyung Kim conducted research in which micro-fibrous bundles were stretched uniaxially to create a fully aligned 3D structure. With the help of the electrospinning process, the authors developed an electrohydrodynamic (EHD) printing process. They used micro-sized poly(-caprolactone) (PCL) to create a 3D-fibrous structure [87]. Collagen-coated surfaces were also extremely biocompatible. Although all of the scaffolds showed high cell viability and proliferation, differentiation differed between them. They stretched the randomly distributed fibers where the 3D-printed cells had a homogeneous distribution to achieve optimal stretching. As a result, it has been demonstrated that this can boost cellular activity. The native muscle structure was used to extract the final structures. It was thus possible to regenerate muscle tissue [88]. Patients with cachexia experience skeletal muscle loss. With more research and testing, muscle tissue regeneration for their muscle loss may be possible using electrospinning. When muscle transplants are performed on patients, the high vitality and proliferation with a homogeneous distribution that increases cell activities could play a big role.

Creating 3D-Functional Muscle Constructs Using Bio-ink and 3D Bioprinting

Despite having properties such as good proliferation and differentiation, natural hydrogels (collagen) are mechanically weak and unstable during the loading process [89]. In the long run, it might not be feasible. As a result, Choi et al developed a functional muscle construct using mdECM (extracellular matrix) bio-ink and 3D bioprinting technology in a study. They created a 3D-muscle construct by printing C2C12 myoblasts encapsulated in mdECM bio-ink. Decellularization was used to remove components while preserving extracellular molecules. To supply nutrients and oxygen to the tissue construct's cells, the shape and porosity of the construct were changed. This improved the viability and function of the cells [90]. The study's findings revealed that mdECM bio-ink could print various shapes of 3D-muscle constructs efficiently. This meant that the bio-ink could be used to design and create original muscle structures before they were implanted. It also had a high cell viability (>90%) and minimal cell death [91]. Unlike CPCs, cell proliferation was seen to increase in MPCs (mdECM bio ink-

printed constructs) (collagen bio ink-printed constructs). MPCs had higher myogenic gene expression, resulting in increased cell stimulation and myogenic maturation. There was evidence of the formation of structurally and functionally mature fundamental contractile apparatus [92]. Furthermore, the 3D-printed muscle constructs were capable of contracting in response to electrical stimulation. This research found that using 3D cell printing and mdECM bio-ink, researchers were able to create a biomimetic architecture and induce mature myogenic development [93]. This method of 3D bioprinting holds a lot of promise because it allows you to print different 3D-muscle constructs that are similar to the original structures but have more vitality. It has the potential to create functional engineered muscle that can fight cancer cachexia, for example. Cachexia patients lose different proportions of muscle tissue and cells from their bodies. It can be very useful to be able to replace lost tissues based on the original architectural structure that was lost.

Treating Skeletal Muscle Defects Using 3D-Bioprinted Muscle Constructs

Kim et al conducted a study to investigate the feasibility of using 3D-bioprinted muscle constructs to treat skeletal muscle defects, based on their initial success with the ITOP (Integrated tissue-organ printer) system. For functional muscle tissue reconstruction, they created skeletal muscle constructs with structural integrity and skeletal muscle tissue organization in this study. A skeletal muscle construct with structural organization was bioengineered using ITOP technology. Human muscle progenitor cell (hMPC)-laden hydrogel bio-ink, sacrificing acellular gelatin hydrogel bio-ink, and a supporting poly(-caprolactone) (PCL) polymer made up the muscle construct. Multiple myofiber bundles were highly organized in 3D-bioprinted muscle constructs in the live/dead analysis. When compared to non-printed muscle constructs, bio-printed muscle constructs had a higher cell viability. It was also discovered that 3D-printed organized muscle structure can speed up tissue maturation. The microchannel structure allowed nutrients and oxygen to diffuse into the bio-printed constructs, ensuring cell viability. These findings demonstrated that the ITOP system can produce skeletal muscle constructs with highly viable, differentiated, densely packed myofibers across a wide cell density range.

In mice, they created a muscle defect by removing 30–40% of the original TA (Tubagus anterior) muscles [94]. Without treatment, this defect resulted in irreversible functional deficits [95]. The bio-printed muscle constructs were inserted into the defect. In those who were not treated, the created defect resulted in severe muscular atrophy. The bio-printed group, on the other hand, maintained their original muscle volume. In addition, their tetanic muscle force and TA muscle weight increased significantly. When compared to non-printed groups, they had an 82 percent restoration of their TA muscle. The bio-printed group's TA muscle weight also increased. The bio-printed muscle group had superior muscle volume maintenance and myofiber formation with organized architecture when stained with H&E and Masson's trichrome. The development of the other groups was limited. For reconstructing the extensive muscle defect injury, the bio-printed muscle constructs were more mature and maintained their cellular organization. The 3D-ITOP system used in this study allows the bioengineered skeletal muscle to overcome its current size and spatial organization limitations. This study was able to create viable skeletal muscle constructs that could mimic the cellular function of native skeletal muscle by printing three components at the same time. Because large-scale cell-based constructs limit oxygen and nutrient supply, a microchannel structure was created in bio-printed muscle constructs [96,97]. The feasibility of using 3D-bioprinted muscle constructs containing human primary muscle cells was demonstrated in this study. It had a lot of positive qualities and outcomes. It's impressive to be able to print a high-viability muscle tissue construct from a wide range of cell densities. Patients with cachexia experience muscle and weight loss. However, if 3D bioprinting with PU and PCL is done correctly, there is a chance of 82 percent muscle mass restoration and good maintenance. However, more research is needed to see if constructs can completely replace native muscle tissues in humans, both functionally and structurally. Because of the use of rat cells in this method, drug screening in humans may be hampered [65,98,99].

Restoration of Muscle Function by Neural Cell Integrated 3D-muscle Constructs

Without nerve supply, skeletal muscles lose their contractility and face muscles atrophy [100,101]. Denervated bioengineered skeletal muscle constructs with cultured muscle cells are required to integrate quickly with the host nervous system [101,102]. Muscle atrophy will occur if it fails, and functional recovery will be hampered. Most studies did not go into great detail about this. As a result, Kim et al created a neural cell-integrated human skeletal muscle construct. Human muscle progenitor cells (hMPCs) and human neural stem cells were 3D bioprinted (hNSCs). Long-term survivability and maturation of the bio-engineered skeletal muscle construct were improved by neural integration within the construct. To test the feasibility of using this method, the bio-printed constructs were implanted in a rat model of tibialis-anterior (TA) muscle defect injury. The cell survival and maturation of the 3D-bioprinted skeletal muscle constructs were increased, and they were implanted in the defective sites for regeneration. The non-treated group showed no improvement and suffered from severe muscular atrophy. The 3D-printed group, on the other hand, had their TA muscle volume and weight restored. The restoration rate was 71.42 percent. This study found that incorporating neural cell components into 3D-bioprinted skeletal muscle constructs can speed up

muscle restoration and function in people who have lost a lot of muscle mass. In-vivo, the intervention could take up to 12 weeks. Rapid innervation of the host nerve is essential for constructs to restore muscle function in vivo. Intriguingly, muscle weight was quickly recovered in the 3D-bioprinted group. The 3D-bioprinted group showed complete restoration based on muscle force measurements.

As a result, the findings suggest that incorporating neural cell components into bio-printed skeletal muscle constructs could speed up muscle recovery. The surgically excised regions of the TA in the non-treated group showed no sign of muscle regeneration, but fibrotic tissue formed in the defect region, leading to muscular atrophy. Rapid innervation with the host nerve is critical for the success of bioengineered skeletal muscle constructs in restoring the function of injured muscle in vivo. Finally, in a rat TA muscle defect model, neural cell components can support bio-printed skeletal muscle constructs in vitro, resulting in rapid muscle function restoration [103]. Similar to the previous study, this method had the limitation of being tested on rats. This requires more research because it has the potential to help people with cachexia. Even though they are getting enough nutrition, the patients are losing muscle tissue. If a 3D bioprinting technique is used, this problem can be solved. This method demonstrated that muscle tissue can survive and mature for a long time. A restoration rate of 71.42 percent, and that too with speed, is something to consider in clinical trials for cachexia patients.

Engineering Integrated Muscle-Tendon Unit via 3D-Bio-fabricating Complex Structures

Typically, cells can be manually seeded into tissue-engineered constructs with a porous structure [104,105]. This method has drawbacks such as difficulty seeding a scaffold uniformly, inability to distribute multiple cell types, and poor scaffold micro-architecture control. These limitations could be overcome with 3D bioprinting [70,106]. Tyler and colleagues used 3D-bio-fabrication of complex structures to achieve this. They created an integrated muscle–tendon unit using a variety of synthetic biomaterials and cell types (MTU). The MTU construct was made up of two synthetic polymeric materials for the scaffolding and two cell-laden hydrogel-based bio-inks for the cellular component. The scaffolding component provided a biomechanical and functional structure, while the cellular component provided the biological basis for tissue development. The muscle side of the MTU was made with thermoplastic polyurethane (PU) and C2C12 myoblasts, while the tendon side was made with poly(-caprolactone) (PCL) and NIH/3T3 fibroblasts. These two were chosen because PU and PCL, respectively, can mimic muscle elasticity and tendon stiffness. Although the tensile strength did not differ, the PU side was more elastic than the PCL side. To re-create the MTU, a construct was created with three distinct regions: printed PU on the muscle side, printed PCL on the tendon side, and overlapped PU-PCL on the MTJ (muscle-tendon junction) region. The cells were discovered to have survived the printing process and had begun to develop into linearized tissue. It was designed to look like natural muscle and tendon in terms of biological architecture. Furthermore, it was discovered that the NIH/3T3 cells had formed dense collagen deposition. This was the start of the tendon's development. With C2C12 (92.72.5%) and NIH/3T3 (89.13.33%), this resulted in high cell viability [107]. Cells remained in their original positions and organized themselves into a predictable pattern. They were able to demonstrate that transcription of focal adhesion markers increased. The ability to expose constructs made from synthetic polymers and cell-laden bio-inks to biomechanical stimulation is a benefit of having them made from synthetic polymers and cell-laden bio-inks. As a result, they were able to print cells that were viable. These cells have increased MTJ-associated gene expression and were aligned into highly aligned morphology of muscle and tendon.

The time required to culture constructs was identified as a limitation in this study. A long period of time was required to create a fully integrated muscle-tendon tissue unit. It's because focal adhesions between the muscle and tendon can't form until collagen is deposited in the MTJ [108]. This study demonstrated that 3D-bioprinting can be used to print muscle cells. The end products would be structurally and biomechanically functional, with normal biological tissue development, and would be implanted in cachexia patients. After being printed, the 3D construct becomes a linearized tissue that can mimic natural muscle tissue. This may pave the way to regaining muscle mass lost to cachexia. The time constraint appears to be a minor setback in the context of a brighter future.

USE OF 3D BIOPRINTING OTHER FIELDS AND THEIR LIMITATIONS

For a long time, 3D bioprinting has been studied and experimented with. It can also be used to treat cardiovascular diseases (CVD). Experiments with printing 3D constructs and putting them on mice, as well as several other trials, are underway. Tissue implants via grafting have been used in the past, but problems with tissue rejection and a lack of donors have arisen [109-111]. CVD causes the heart's cell structures to deteriorate, necessitating replacement. These replacements are made using 3D bioprinting technology. To restore the functions of damaged myocardium, cardiac patches made of biomaterials and bio-inks were created. Atmanli et al. developed 3D-functional cardiac patches that could maintain myocardial tissue structure [112]. Another study led by Ong et al was able to create 3D-biomaterial cardiac patches that were able to beat spontaneously [113]. Using ink-jet printing, Xu et al. created functional cardiac pseudo-tissues with structural support. It showed contractile behavior when exposed to mild electrical stimuli [114]. Inkjet printers, on the other hand, are only compatible with

low viscosity inks. As a result, ink-jet printer-made structures have weaker mechanical properties [70,115,116]. Furthermore, a discretized flow causes restriction to thin structures, as well as excessive thermal stress and the risk of cell lysis [115]. Cell viability and functionality may suffer as a result of such circumstances. High cell density, cell viability, and the selection of a single cell for transfer are all possible with the LAB system [110,117]. The resolution of LAB is dependent on a number of factors, and it is also expensive, so this system is not commercially available [110,118]. The SLA technique for bioprinting 3D-cardiac patches and heart valves has shown a lot of promise, including reduced printing time, improved fabrication accuracy, and increased cell viability [115]. However, because lasers are used, they have drawbacks, and the optics required are costly [74]. It is still difficult to create tissue with a high oxygen consumption rate. It's difficult to print capillaries at the submicron scale when bioprinting vascularized thick tissues [17,119].

To test the effectiveness of 3D-bioprinted vasculature, researchers used immunodeficient mice. Endothelium has been generated by colonizing endothelial cells in studies, but the native structure is so complex that proper replication is difficult [120,121]. After numerous trials, it was discovered that a solution of greater than 15wt percent is best to use for GelMA/C to achieve rapid gelation for 3D-bioprinting. Although it became difficult to handle when the bio-ink solution concentration exceeded 30% by weight. The reason for this was primarily due to the high viscosity of the liquid. The 3D-bioprinted vasculature, on the other hand, replicates biomimetic vessel structures with smooth muscle and endothelium. As a result, researchers are now looking into 3D bioprinting of tissue constructs, though some tweaking is still needed to improve the methods [122].

Similarly, 3D bioprinting has come a long way in skeletal muscle regeneration. Several studies have also been carried out over the years. Muscle tissue can be regenerated, for example, using electrospinning, according to future research and experiments. When muscle transplants are performed on patients, the high vitality and proliferation of constructs with a homogeneous distribution that increases cell activities could play a big role. Muscle atrophy can be reduced by different shapes of 3D-muscle constructs that are physically printed out according to their native structure. Cachexia patients lose muscle tissue despite consuming nutrition on a daily basis. Because cachexia patients lose muscle tissue in a deplorable manner, the ability to create replicants of lost tissues based on the original architectural structure can provide hope and motivation to continue fighting.

The use of 3D bioprinting techniques on mouse specimens yielded positive results. It's impressive to be able to print from a variety of cell densities. These 3D bioprinters, which will be implanted in the host subject, are expected to develop normally and resemble natural muscle tissue. They can work and function in the same way that the original muscle did before it was lost. However, because the experiments were carried out on mice, it is still unknown how well they will work for human grafting and implanting. Studies have already shown that muscle restoration and maturation can be achieved using a 3D-bioprinted muscle construct.

CONCLUSION

Several technologies and methods have been used to create 3D-muscle constructs, but none of them has been able to accurately mimic the morphology of muscle tissues in their natural state [123,124]. However, 3D bioprinting has emerged as a powerful tool for creating bioengineered skeletal muscle constructs. Because these methods can produce structurally complex cell-based constructs by precisely positioning multiple cell types, bioactive factors, and biomaterials within a single architecture to mimic native tissues, they are becoming increasingly popular [65,93,125,126]. 3D bioprinting has allowed for much more precise construction of dense constructs with rapid maturation [70,125,127]. However, more research and development in 3D bioprinting for human skeletal muscle is required. There are many cell sources for skeletal muscle tissue, but most of them can only be expanded in vitro to a limited extent. So, despite its progress to date, 3D bioprinting still faces significant challenges. Problems such as a lack of a biocompatible bio-ink with supportive mechanical properties for 3D-cell culture can cause cell accuracy and structural organization to suffer [93,125]. It does, however, provide hope and a chance of survival. Because 3D bioprinting allows for more flexibility in the development of engineering skeletal muscle tissues than traditional models [128]. Available 3D bioprinting methods may have drawbacks such as time constraints, tests limited to mice, and so on. However, these are minor setbacks that can be overcome with more research and experiments in the future.

Because cells and tissues can be constructed to create 3D-bioprinted muscle constructs and tendon units, it is expected that applications for 3D bioprinting will improve in the coming years as methods for 3D bioprinting technology become more widely used. These are sufficient reasons to incorporate these techniques into a cachexia application. The method can be improved by developing new bio-inks and printers capable of projecting high-resolution constructs. Future experiments may benefit from more in-depth research into muscle tissues and how they function. Finally, it is very likely that 3D bioprinting will be able to combat the muscle loss problem caused by cachexia.

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