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REVIEW

Biomimetic Scaffolds and Extracellular Matrix-Based Strategies for Myofiber Regeneration in Volumetric Muscle Loss

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Abstract: Volumetric Muscle Loss presents a critical challenge involving the traumatic or surgical loss of over 20% of skeletal muscle mass by overwhelming the body's natural regenerative capacity. It causes functional decline of skeletal muscles leading to reduced quality of life. Current surgical interventions, such as autograft and allograft muscle transfers, often fall short of restoring full mobility frequently causing donor site morbidity and graft failure. The objective of this manuscript is to discuss the role of emerging regenerative strategies focusing on restoring muscle structure and regenerative microenvironment. Recent advances emphasize on extracellular matrix-based therapies that promote myogenesis and vascularization because of their ability to replicate the native structural as well as biochemical attributes leading to muscle fiber regeneration and innervation. Further, incorporation of growth factors like vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), or stem cells in the scaffolds help to recapitulate the complex structure and signaling of extracellular matrix promoting accelerated healing and recovery as observed in pre-clinical trials. However, despite of positive outcomes, there are challenges like immunogenicity, issues with batch to batch reproducibility, which hinder scalability and translation. Interdisciplinary collaboration between biomaterials science, tissue engineering, and clinical research can serve as solution to resolve this critical issue and will be helpful to advance these technologies potentially shifting the approach of VML therapeutic management from palliative to curative.

Plain Language Summary: Volumetric Muscle Loss leads to more than 20% loss of skeletal muscle fibers caused due to trauma or surgical removal. This leads to persistent weakness, impaired movement, and reduced quality of life. Traditional treatments such as muscle autografts or allografts often do not fully restore strength or coordinated function causing complications, including donor site damage, limited graft integration, or immune-related issues. Emerging regenerative approaches aim to rebuild both muscle tissue and its supportive environment. Biomaterial scaffolds designed to mimic the extracellular matrix can promote muscle fiber formation, blood vessel growth, and nerve integration. Incorporating growth factors like VEGF and IGF-1 or therapeutic cells into these scaffolds has shown encouraging results in preclinical studies. However, concerns regarding immune compatibility and manufacturing consistency must be addressed to enable reliable clinical translation.

Keywords: skeletal muscle, hyaluronic acid, fibrin, laminin, natural scaffold, synthetic scaffold

Introduction

Volumetric Muscle Loss (VML) is a severe musculoskeletal condition marked by the irreversible destruction of over 20% of muscle mass, surpassing the body's natural regenerative capacity.[1,2] Typically caused by traumatic injuries (eg, blast wounds, motor vehicle accidents), surgical resections (eg, tumor removal), or congenital defects, VML leads to chronic disability, impaired mobility, and long-term functional deficits.[3–6] Among trauma patients, it contributes to nearly 65% of long-term

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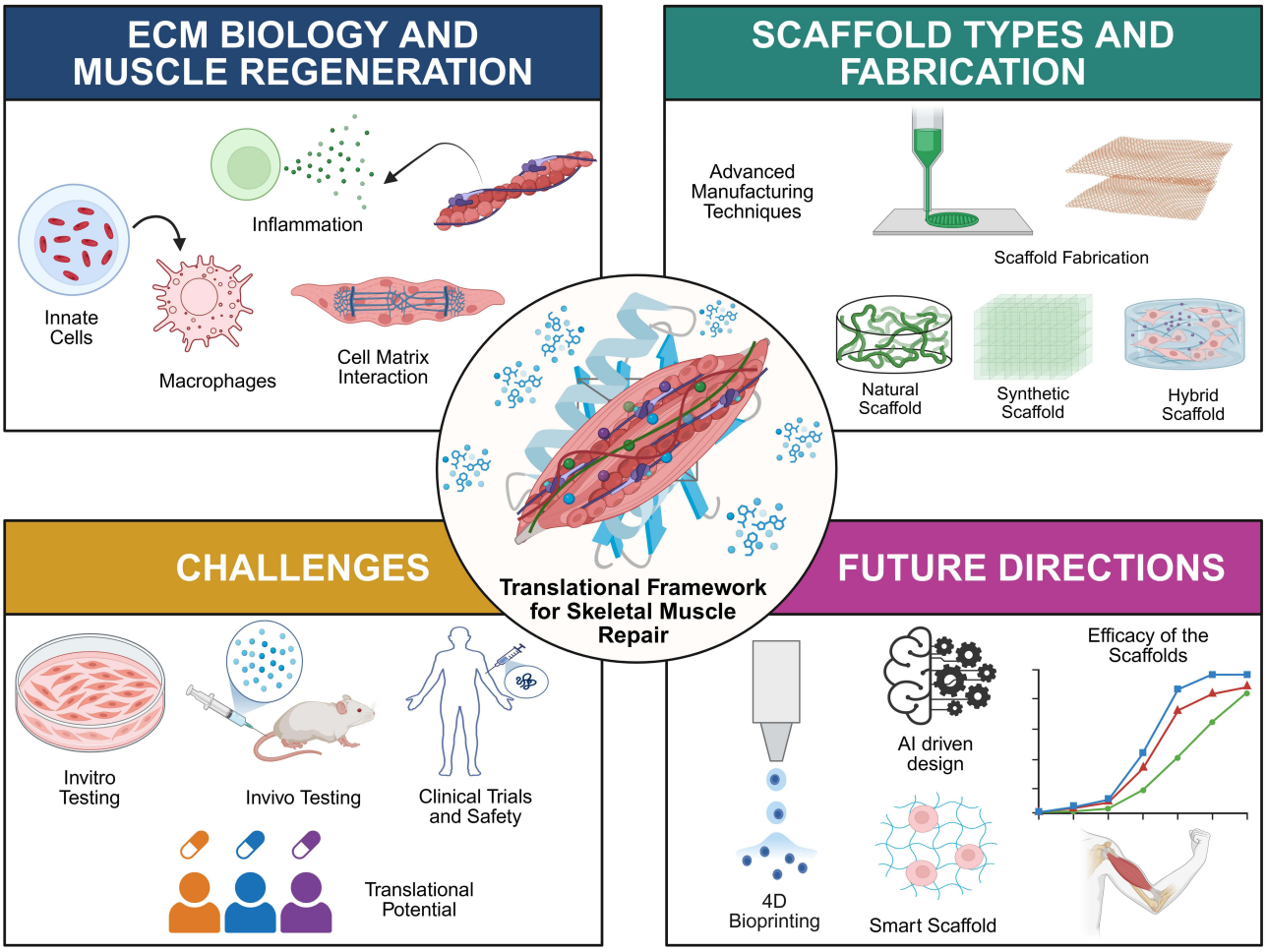
Graphical Abstract



disabilities, particularly in cases of severe open fractures.[7,8] In 2022, WHO reported that the global incidence of musculoskeletal injuries affects 1.71 billion individuals and more than three-quarters of these involve open wound injuries.[9] In the US, VML-associated injuries arise to approximately 150,000 open fractures, 30,000 gunshot wounds, and 13,000 soft tissue sarcoma excisions annually.[10,11] Similarly, in countries like India, trauma-related musculoskeletal injuries primarily from road accidents account for 61.9% of fatalities in adults aged 18–45 years, underscoring the global impact on productivity and healthcare systems.[12] For high-income countries, healthcare systems are well-funded to provide timely access to advanced diagnostic and treatment options, as well as specialist as well as multidisciplinary care, to ensure measurable outcomes.[13] Advanced palliative care and trauma care centers improve patient outcomes by reducing the country's mortality rate.[14] In contrast, healthcare infrastructure in low- and middle-income countries is limited, burdened with partially developed trauma centers and a shortage of trained specialists, limiting access to quality care.[15]

Existing therapies for VML, such as free functional muscle transfer, offer only partial functional recovery and are plagued by complications like donor site morbidity, graft failure, and inconsistent reinnervation.[16–18] Rehabilitation and physical therapy, though essential, often fail to restore lost muscle mass or strength, leaving many patients with permanent impairments. Compounding these challenges, VML injuries frequently involve concurrent fractures, vascular damage, and systemic inflammation, which further hinder recovery. Secondary injury mechanisms—ischemia-reperfusion injury, oxidative stress, and calcium overload—worsen muscle degeneration and impede regeneration.[19–21] Given these shortcomings, regenerative medicine has emerged as a transformative approach for VML. Strategies leveraging stem cells, growth factors, and

bioengineered scaffolds aim to restore functional muscle tissue rather than merely managing symptoms.[22,23] Among these, extracellular matrix (ECM)-based therapies stand out due to the ECM's critical role in muscle repair. The ECM not only provides structural support but also regulates satellite cell activation, angiogenesis, and fibrosis prevention—key processes involved in skeletal muscle regeneration. Recent advances in biomimetic scaffolds—engineered to replicate native ECM architecture—have shown remarkable preclinical success. These constructs, often combined with growth factors eg, VEGF, IGF-1, or stem cells, enhance muscle regrowth, vascularization, and histological muscle repair.[24–26]

This review examines the potential of ECM-based therapies for VML, focusing on their biological foundations and clinical applications. We first outline the mechanisms of skeletal muscle regeneration and the ECM's role in repair. Next, we discuss how VML disrupts these processes and evaluate current biomimetic scaffold designs. Finally, we analyze preclinical and clinical outcomes, address translational challenges, and highlight future directions for ECM-based solutions in VML treatment.

ECM Biology in VML

Structure and Function of the ECM in Skeletal Muscle

Skeletal muscle regeneration requires the activation and proliferation of muscle stem cells known as satellite cells essential to repair and rejuvenate the muscle function following an acute injury. Satellite cells were first identified in 1961 by Alexander Mauro as residing between the sarcolemma (plasma membrane) and the basal lamina, a form of external lamina that surrounds all muscle cells.[27] The injury influences the release of inflammatory markers particularly cytokines which contribute to muscle regeneration and their rapid expansion yields new myotubes and fuses with injured myofibers to regenerate muscle fibers.[28] The ECM forms a complex network of structural proteins (collagen, elastin), adhesive glycoproteins (fibronectin, laminin), and proteoglycans (hyaluronic acid) that provide both mechanical support and biochemical signaling in skeletal muscle. The ECM serves as a reservoir for growth factors and mediates critical functions including cell adhesion, migration, proliferation, and differentiation—all essential for muscle homeostasis and repair.[29]

Table 1 summarizes the primary ECM components involved in skeletal muscle regeneration and highlighting the respective structural and biological roles in tissue repair. Together, these ECM elements underscore the importance from maintaining structural integrity to regulating cellular signaling, inflammation, and growth factor availability.

Mechanism of Muscle Regeneration

The ECM, besides being a structural building block for tissues also have been recognized as one of the important factors in cell-to-cell communication during muscle regeneration acting as both a structural scaffold and a signaling hub.[39,40] Its receptors, such as integrin,[41] are abundant and it interacts with muscle cells to carry out the signaling pathways that

Table 1 Classification and Functional Attributes of Biomaterials for VML Repair

ECM Component	Structure/Function	Role in Regeneration	References
Collagen I/III	Forms tensile fibrillar networks	Guides myofiber alignment; provides a migratory scaffold	[30,31]
Laminin	Basement membrane glycoprotein	Maintains satellite cell quiescence via integrin binding	[32]
Fibronectin	Adhesive glycoprotein binding collagen/integrins	Mediates myoblast adhesion and migration	[33]
Hyaluronic Acid	Hydrated glycosaminoglycan polymer	Modulates inflammation; retains growth factors	[34,35]
Elastin	Elastic fibrillar protein	Supports tissue recoil after contraction	[36]
Decorin	Small leucine-rich proteoglycan	Regulates collagen fibrillogenesis; sequesters Transforming Growth Factor-β (TGF-β)	[37,38]

Note: This table summarizes the physical, chemical, and biological properties of natural, synthetic, and composite scaffolds, highlighting their specific mechanisms in promoting myofiber regeneration and structural support.

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control fundamental cellular processes like proliferation, differentiation, and migration.[42] These interactions are essential to orchestrate the integrated set of responses that contribute collectively towards effective tissue repair.

Figure 1[43] shows the ability of skeletal muscle fibers to repair and regulate their activity through a multistep process involving degeneration and subsequent repair due to necrosis. The underlying mechanism by which skeletal muscle-based scaffolds regulate the immune system involves macrophage polarization that is strongly influenced by the immunomodulatory effects of mesenchymal cells.[44,45] These cells contribute significantly to suppressing immune activity by secretion of several mediators and cell–scaffold interactions.[46] The acute inflammatory response initiated after injury is characterized by infiltration of neutrophils and macrophages.[46] M1 macrophages promote debris clearance by releasing pro-inflammatory cytokines such as Tumor Necrosis Factor—α (TNF-α), Interleukins (IL) like IL-1β and IL-6 and M2 macrophages enhance muscle tissue repair by transforming under the influence of mesenchymal cells, releasing anti-inflammatory cytokines such as IL-10 and IL-13 which facilitates regeneration.[29,47,48] The innate capacity for muscle repair and satellite cell migration along with proliferation controls inflammation while releasing growth factors like TGF-β1, Fibroblast Growth Factor (FGF), VEGF, and promoting structural organization and regeneration.[43] These factors guide satellite cell activation, proliferation, and differentiation into myoblasts that repair damaged matrix by enabling matrix breakdown and new matrix synthesis thus to make myofibers.[49,50]

ECM Dysregulation in VML

In VML, ECM functions are severely compromised. Traumatic injuries disrupt the ECM scaffold, causing an imbalance between degradation and synthesis that often leads to fibrosis.[51] Excessive collagen deposition creates a stiff matrix that impairs fiber alignment, cell migration, and neuromuscular junction reformation.[52] This aberrant

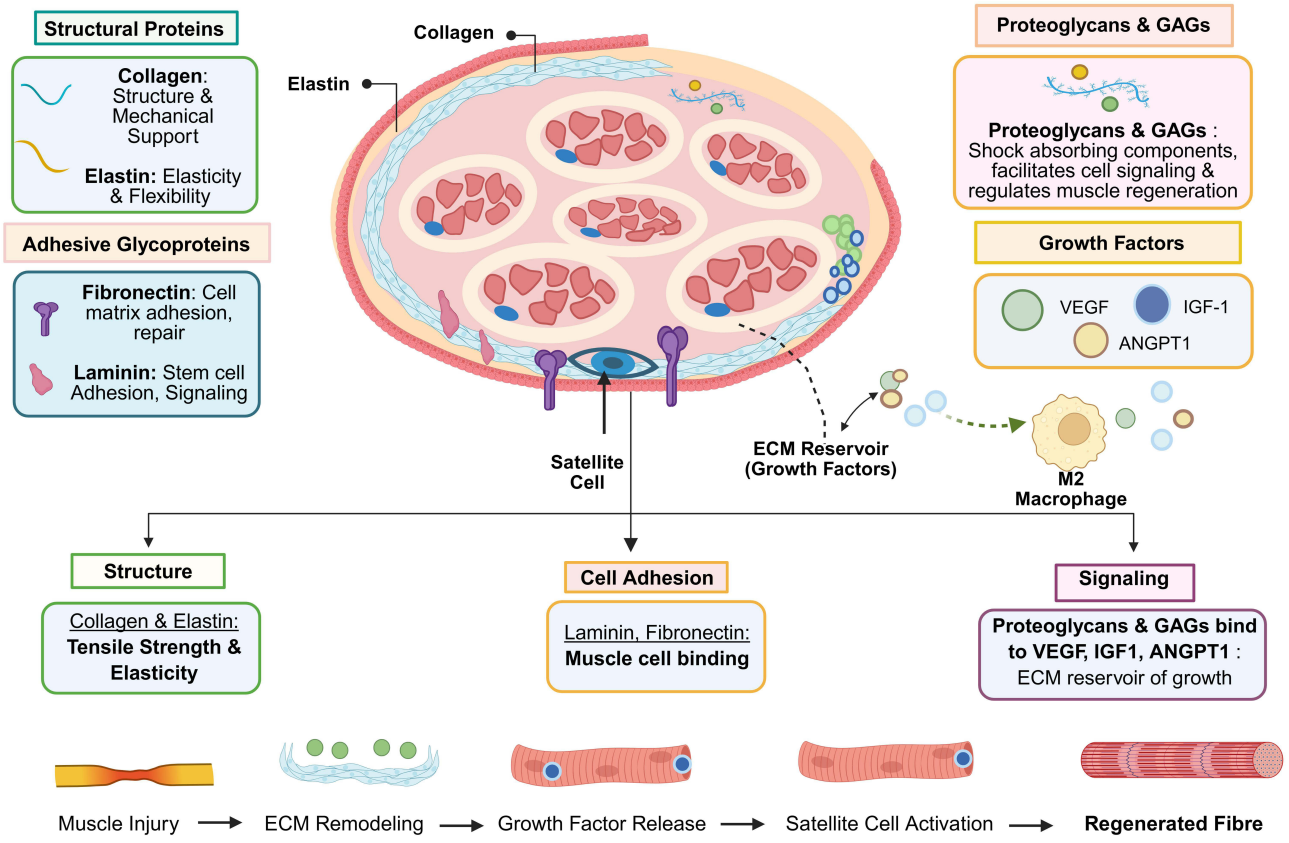

Figure 1 ECM composition: schematic representation of skeletal muscle ECM components and their functional roles (created with BioRender.com). The extracellular matrix (ECM) of skeletal muscle is a complex, hierarchical network comprising structural proteins (collagen, elastin), adhesive glycoproteins (laminin, fibronectin), and specialized molecules (proteoglycans and GAGs). This illustration highlights the dynamic interplay between the ECM and resident cells, including myoblasts, fibroblasts, and satellite cells. Key signaling pathways—such as VEGF for vascularization, IGF-1 for myogenesis, and ANGPT1 for immune modulation—are sequestered and presented by the ECM to orchestrate tissue repair and homeostasis.

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remodeling limits satellite cell function and regenerative capacity, presenting a major recovery barrier. ECM-based or biomimetic scaffolds offer promising solutions to restore the muscle microenvironment.[53] The muscle injury triggers dynamic reorganization of the stem cell niche. This specialized microenvironment contains multiple interacting components: resident muscle stem cells, recruited immune cells, and supportive stromal cells, all embedded within a carefully balanced ECM.[54] Together, these elements coordinate the complex process of tissue regeneration through precise cell-ECM signaling.

Table 2 outlines the sequential phases of skeletal muscle regeneration following an injury. The detailed coordinated events highlight the regulated immune responses that initiate debris clearance, activate myogenic progenitors guiding extracellular matrix deposition. The regenerative and remodeling phases enable myofiber formation, vascularization restoring impaired regeneration.

Any disruption in the equilibrium between ECM degradation and synthesis can result in fibrosis, a condition marked by an overabundance of collagen and other ECM elements, leading to stiff and non-functional tissue.[55] Fibrosis poses a significant obstacle to muscle function and presents a major challenge in scenarios where regeneration is hindered. The importance of the ECM in muscle regeneration is clear across various scenarios. In the context of aging and specific disorders like muscular dystrophy, the ECM tends to become stiffer and more fibrotic, which hinders the muscle's ability to regenerate effectively.[56] This decline in ECM quality is a contributing factor to the progressive muscle weakness seen in these conditions. Gaining insights into the ECM's role in these mechanisms have paved the way for innovative therapeutic strategies.[40]

Figure 2[57] describes that following injury, the muscle stem cell niche undergoes significant reorganization, involving multiple cell types like fibroblasts, macrophages, neutrophils, fibro-adipogenic progenitors (FAPs), and glial cells interacting within a remodeled ECM. Key ECM components including fibronectin, collagens (I, III, IV), laminins, elastins, tenascin-C, versican, proteoglycans, fibrillins, and thrombospondins (TSP-1, −2, −4) collectively mediate cell adhesion, migration, and signaling during repair. This schematic illustrates the complex interplay between cellular and extracellular components in the regenerating muscle microenvironment.[57]

Table 2 Engineering Strategies and Biological Outcomes of Biomimetic Scaffolds for VML

Phase	Key Events	Cellular Players	Molecular Signals	Outcome	Reference
Inflammatory Phase	• This phase begins immediately after injury marked by necrosis and hematoma formation • Rapid infiltration of neutrophils serving as first line of defense followed by polarization of macrophages • M1 macrophages dominate the early response and M2 macrophage emerge towards the repair	• Neutrophils are initial ones to respond releasing proteases and reactive oxygen species eliminating necrotic tissue • M1/M2 macrophages release pro inflammatory and promote tissue regeneration respectively.	IL-6, TNF-α, TGF-β, Damage-Associated Molecular Patterns (DAMPs)	Debris clearance; initiation of repair	[27–29,42]
Repair Phase	• The phase is initiated by satellite cell activation and proliferation. • The progenitor cells undergo expansion and differentiation into myoblast cells and fuse to form multinucleated myotubes. • Fibroblast cells deposition leads to ECM synthesis and angiogenesis restoring vascular network.	• Satellite cells are primary drivers of regeneration of new myofibers • Fibroblasts, endothelial cells promote neovascularization	HGF (Hepatocyte Growth Factor), FGF, IGF-1, VEGF, Matrix Metalloproteins (MMPs)	New myofiber formation; provisional ECM scaffold	[27–29,42]
Remodeling Phase	• ECM reorganization and scar resolution • Sarcomere maturation • Neuromuscular reinnervation	Fibroblasts, Schwann cells, myocytes	Tissue Inhibitor of Metalloproteinases (TIMPs), neurotrophins, collagen crosslinking enzymes	Functional restoration; optimized force transmission and tissue compliance	[27–29,42]

Note: This table delineates various scaffold fabrication techniques-including electrospinning, 3D bioprinting, and decellularization-and evaluates their specific impacts on myogenic differentiation, force production, and host-tissue integration.

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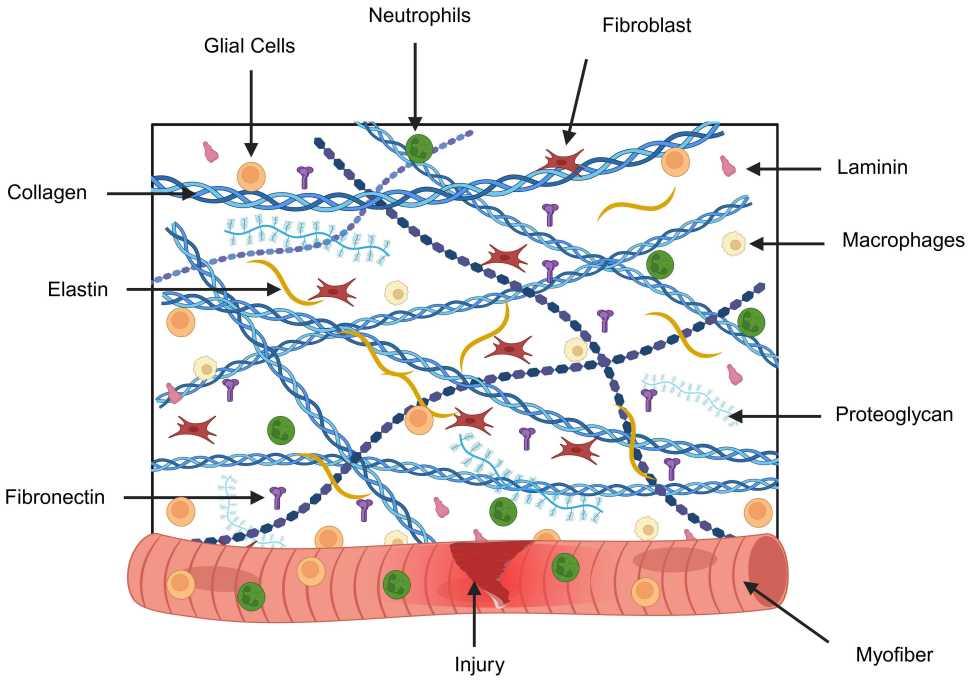

Figure 2 The skeletal muscle stem cell niche and ECM microenvironment (created with BioRender.com). This schematic illustrates the specialized microenvironment, or niche, that regulates muscle satellite cell behavior following injury. Structural and Adhesive Proteins: The niche is composed of a dense network of Collagen fibers and Fibrillins providing mechanical integrity, while Laminin and Fibronectin serve as critical anchors for MuSC adhesion and polarity. Specialized ECM Molecules: Proteoglycans and Elastin facilitate the sequestration of growth factors and provide the tissue with necessary recoil and elasticity. Cellular Constituents: The microenvironment is populated by a diverse array of resident and infiltrating cells. Fibroblasts actively remodel the matrix, while Macrophages, Neutrophils, and Glial cells provide biochemical cues that dictate the transition from the inflammatory phase to the regenerative phase. Regenerative Focus: The interaction between the cells and the damaged Myofiber at the site of Injury is highlighted, demonstrating how the biochemical and mechanical properties of the ECM scaffold are essential for directing stem cell activation, proliferation, and successful tissue integration.

Material Classes and Functionalization

Design Principles of Biomimetic Scaffolds

Biomimetic scaffolds for VML are designed to replicate the structural, mechanical, and biochemical properties of native skeletal muscle ECM, promoting functional tissue repair.[58,59] These scaffolds are to mimic the complexity of the ECM by adding necessary factors like collagen,[60] fibrin,[61] and glucosamine glycans[62] essential for muscle tissue generation and remodeling. These biomimetic scaffolds also feature mechanism-aligned topographies that direct the alignment of myoblasts to further boost muscle regenerative property during myotube formation.[63] An optimal scaffold must be biocompatible to avoid immune rejection, biodegradable to degrade in sync with tissue remodeling, and mechanically robust to withstand physiological stresses.[59] Porosity and microarchitecture should enable nutrient exchange, cellular infiltration, and aligned myofiber growth. Crucially, scaffolds must facilitate vascularization and innervation while supporting cell proliferation and differentiation.[64–66] These criteria ensure the scaffold not only fills the defect but also actively aids regeneration.

Figure 3 describes several scaffold fabrication approaches for VML repair and how they balance structural control, biological activity, and clinical practicality. Three-dimensional bioprinting allows precise, layer-by-layer placement of biomaterials and cells, enabling controlled pore size and spatial organization, although it can be limited by material properties and scalability.[67] Electrospinning produces fibrous meshes that resemble the alignment of native ECM, offering high surface area but often restricting deep cell infiltration due to small pore sizes. Freeze-drying creates highly porous, interconnected foam-like scaffolds that are relatively simple and scalable to produce, yet they may lack sufficient mechanical strength.[68] Decellular scaffolds, in contrast, retain the natural three-dimensional architecture and biochemical signals of donor tissue, enhancing bioactivity, but it is constrained by donor availability and potential immunogenicity.

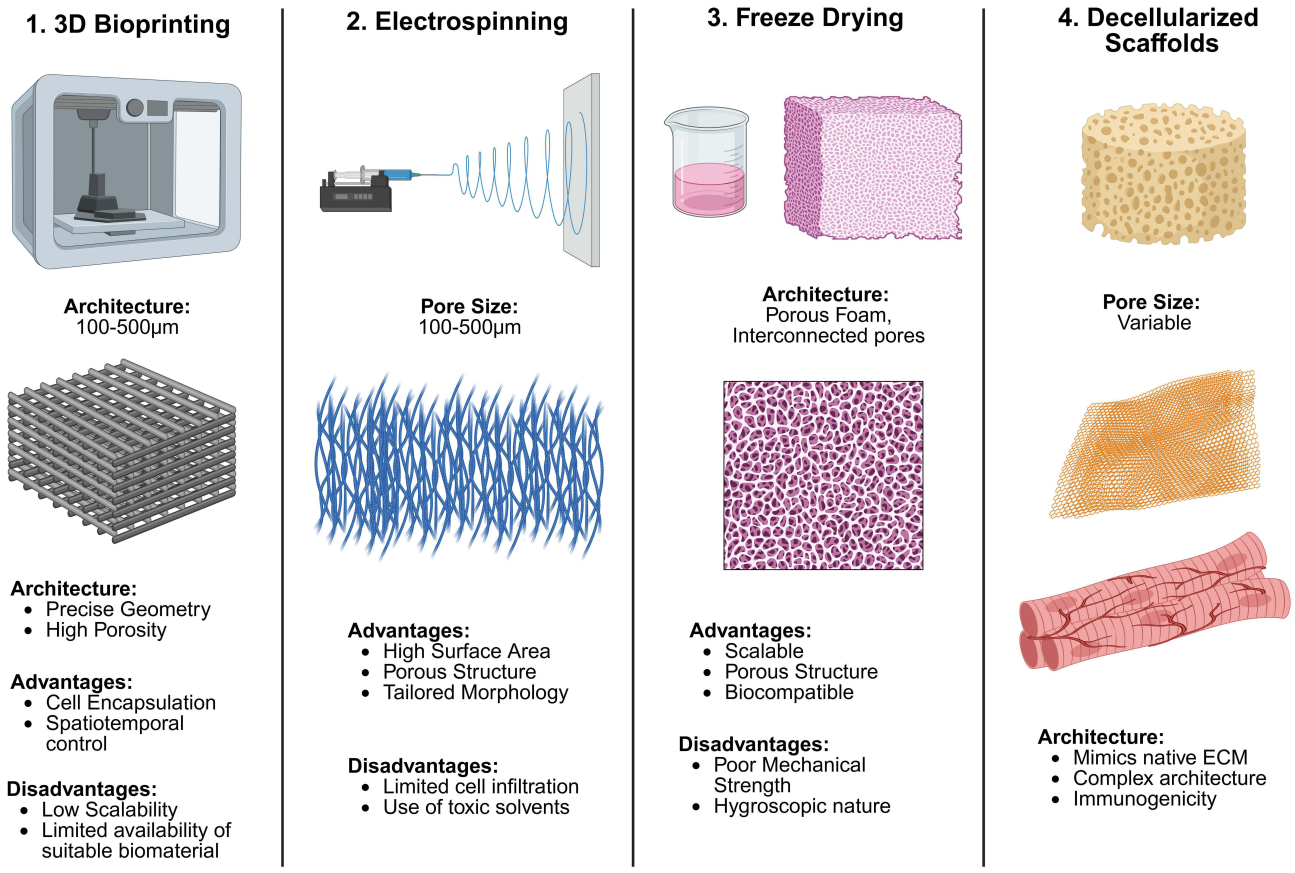

Figure 3 Scaffold fabrication techniques: comparative overview of scaffold fabrication techniques for VML repair (created with BioRender.com). A side-by-side comparison of four prominent fabrication methods: (1) 3D Bioprinting, providing high precision and cell encapsulation; (2) Electrospinning, producing fibrous meshes that mimic natural ECM alignment; (3) Freeze-drying, creating highly porous foam architectures; and (4) Decellularization, preserving the native complex architecture and bioactivity of donor tissue. The workflow, resulting architecture, and inherent advantages/limitations (e.g, scalability vs bioactivity) are detailed for each technique to guide scaffold selection.

Together, these techniques provide complementary strategies, and their selection depends on the required balance between structural precision, biological function, and translational feasibility.

Effective scaffold selection for VML treatment requires the use of robust materials that address specific defect characteristics and consider patient biology to maximize regenerative potential. The first step in defect analysis is to evaluate its size.[69] Depending on the area of muscles lost, scaffolds with mechanical integrity and stability can support the load required to support tissue growth.[70] Anatomical location is critical to understanding the type of scaffold required, its mechanical demands along with the specific biochemical environment.[71] To design a scaffold for the quadriceps muscle, one with high tensile strength and resilience is required to prevent the scaffold from failing and causing permanent functional impairment.

The study by Anderson S.E et al determined the regenerative, fibrotic, vascular, and neuromuscular responses by creating a complete muscle defect in quadriceps of female C57BL/6J mice with different defect sizes of 2 mm, 3 mm, and 4 mm in diameter. The relative quadriceps loss was 4.44 ± 1.85%, 15.49 ± 2.04%, and 32.16 ± 5.14% for a defect size of 2 mm, 3 mm, and 4 mm, respectively. Quantitative evaluation of wet muscle weight demonstrated the reduction in defect size for all groups on day 7, but the 4 mm defect size remained damaged and deteriorated on day 28. Among the three defect sizes, the 2 mm lesion largely dissolved fibrosis and inflammation on day 28, while the 3 mm lesion exhibited incomplete myofiber formation and deposition of collagen with unresolved CD68+ macrophage infiltration that was analyzed by histological examinations. Vascular analysis using micro CT angiography increased vascular volume confirming angiogenesis. The neuromuscular reinnervation at end of 28 days post injury was 44.3% with 2 mm defect and 55.7% in 3 mm defect due to the presence of fibrotic tissue. The overall study concludes 3 mm defect as the critical threshold for nonhealing VML in mouse quadriceps and 4mm injury as regenerative failure due to disorganized vascularization and hindered regeneration.[72]

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The study conducted by Panayi AC et al highlighted the improved functional performance of the quadriceps muscle by treatment with collagen-glucosamine glycan (CGAG)-based scaffold in a murine model of VML. Histological results are presented as the presence of fibrotic scar deposition with disorganized muscle architecture in the untreated group, whereas the scaffold-based intervention improved muscle hypertrophy after injury. Two weeks after injury, the scaffold group displayed lower fiber density (114 ± 7 fibers/HPF) compared to the uninjured control group (268 ± 13 fibers/HPF) and untreated VML (196 ± 18 fibers/HPF). Finally, by functional treadmill testing at 6 weeks, muscle repair was improved by 20–25% in the scaffold group compared to untreated VML. At the molecular level, upregulation of tissue inhibitor of metalloproteinase 1 (TIMP1) and colony stimulating factor 2 (CSG2) leads to ECM turnover by stimulating the differentiation of progenitor cells into macrophages and granulocytes, thereby increasing immune cell recruitment. Angiogenic factors such as VEGF, ANGPT1, HGF, and myogenic factors such as IGF1 and STAT3 ensured vascularization and gradual progression from injury to regeneration in scaffold treated animals, supported by histological evidence. In a broader context, the researchers also reinforce the fact that scaffolds not only support structural integrity but also regulate the host immune response, which decellularized ECM-based scaffolds lack, providing inconsistent and variable cell-scaffold integration and coherent regenerative gene expression.[73]

At the same time, patient attributes including age, coexisting medical conditions such as hypertension, diabetes, other vascular ailments, muscle regeneration capacity, and immune system response are also taken into account before selecting the scaffold. A person's aging significantly affects the body's response to regenerate muscle fibers efficiently and existing comorbidities can also hinder the results associated with the healing process. This eventually leads to a drastic change in the healing trajectory characterized by increased fibrosis and scar tissue deposition.[74] All of these factors are useful in governing the architecture of the scaffold, its stiffness, and resilience further shaping the biocompatibility profile essential to ensure fibrosis-free integration.

Researchers Kim J.T. et al, carried out a study to evaluate the modulating effect of age on VML muscle repair with a 3- and 18-month-old rat model with tibialis anterior defects. Decellularized skeletal muscle scaffold with minced muscle revealed a superior outcome in young 3-month-old rats by restoring torque to 79.9 ± 12.2% compared to the untreated 3-month-old rat that had a torque of 62.3 ± 10.1%. On the other hand, the 18-month-old rat model did not show significant muscle recovery compared to the untreated group, with an achieved torque of only 57.1 ± 8.7% and 58.6 ± 14%, respectively. The molecular mechanism implicated for superior regenerative outcomes in the 3-month-old rat model was the upregulation of myogenic genes such as MyoD, MyoG, and inflammatory biomarkers such as IL-β1 and TNF-α. Collagen deposition in young rats maintained normal collagen levels compared to older rats with markedly elevated fibrosis. The overall conclusion drawn from the study is the pronounced age-related response to the same treatment intervention.[75]

Types of Biomimetic Scaffolds

Natural Scaffolds

Elastin

In tissue engineering, elastin is a crucial ECM protein that provides elasticity and recoil to tissues-derived materials are increasingly used for their flexibility and biocompatibility. A key advancement involves methacryloyl-functionalized elastin (MeTro) combined with gelatin methacryloyl (GelMA) to create elastic hydrogels for 3D bioprinting. These scaffolds exhibit controlled degradation (12.7% by day 1, 17.9% by day 14) and maintain >90% cell viability, demonstrating strong cytocompatibility and structural support for muscle regeneration.[76–78]

Additionally, elastin-like recombinases (ELRs)—synthetic analogs mimicking elastin's properties—have been shown to modulate macrophage behavior in rat models, shifting from pro-inflammatory M1 to regenerative M2 phenotypes, reducing fibrosis, and enhancing myofiber formation.[79,80] Recent work with human elastin-like polypeptides integrated into collagen scaffolds has improved mechanical stability and vascularization, supporting aligned ECM remodeling for muscle regeneration.[81]

In recent years, elastin-like recombiners (ELRs) have been investigated as a biomaterial solution for enabling muscle regrowth in the context of volumetric muscle loss VML, which arises from chronic inflammation and subsequent fibrotic scar formation.[77] The study proposed that the biodegradable amphiphilic ELR-based hydrogels are able to provide a switching niche for converting of M1 macrophages into their pro-regenerative M2 counterpart. Rat tibialis anterior

muscle model was utilized to introduce chemically and physically crosslinked ELR hydrogels. These results show that the muscle treated with ELR hydrogels a higher percentage of M2 macrophages at 14 days and cell increases, which are responsible for reducing inflammation seconds to collagen production.[78,79]

More recently, a biomimetic human elastin-like polypeptide was proposed as an alternative to muscular volume loss assistance and enriched vascular tissue engineering. The objective of the current investigation was to develop human elastin-like polypeptide with improved mechanical properties, biodegradability, and cytocompatibility using several recombinant strategies. The results demonstrate that human elastin-like polypeptide addition to collagen scaffolds dramatically improves their tensile strength and stress resistance, approaching native tissue protein composition. These scaffolds can be useful in applications such as regenerative medicine, which require a certain mechanical integrity of the scaffold to ensure proper tissue regeneration. Human elastin-like polypeptide possesses thermosensitive characteristics which are beneficial for purification and scaffold integration.[82]

The enhancement of these biomaterials by combining them with the elements described here could further increase their regenerative properties towards vascular tissue damage and volumetric muscle atrophy, without which effective treatments are scarce. Through the developments using human elastin-like polypeptide, a versatile platform to design successful strategies for tissue engineering and regenerative medicine that can reconcile problems specific to native elastin in biomedical applications.[82,83]

An important leap in tissue engineering is achieved through the development of biomimetic elastin-like proteins that exhibit superior mechanical properties and biocompatibility for vascular as well as muscle regeneration applications. These modified elastin materials find wider scope to enhance clinical outcomes in regenerative therapies with the ongoing research work, particularly for reinstating function of lost or injured tissues.

Hyaluronic Acid

Hyaluronic acid, a naturally occurring biopolymer, is valued for its biocompatibility, hydration capacity, and low immunogenicity.[84]

In a study, a bioinspired semisynthetic acrylated hyaluronic acid (AcHyA) hydrogel was introduced as prospective therapy for VML of craniofacial muscles by researchers. In a novel rat injury model AnHyA was applied to a 5 mm × 5 mm lesion in superficial masseter. The results were shown to be an improvement relative to untreated controls following 16 weeks. From the images, muscle fibrosis was visibly reduced in hydrogel-treated muscles and there also appeared to be less damage (smaller defects) as well as higher myofiber cross-sectional area. AcHyA hydrogel promotes muscle sparing and reduces scarring in craniofacial VML injury, indicating a possible regenerative benefit to the damaged musculature. The findings highlight hydrogels as a therapeutic approach and provide hope for enhanced patient recovery from this disorder.[85]

Researchers in another study looked into the combination of 3D bioprinting and electrospinning with semisynthetic acrylated hyaluronic acid (AcHyA) hydrogels for VML repair. In rat masseter muscle injury models, AcHyA reduced fibrosis by 50% and defect size by 20%, while increasing fiber cross-sectional area (30%). These strategies allow creation of scaffolds that can more closely replicate the ECM, and as a result improve adhesion and alignment for muscle regeneration. There are also, investigations into whether using biological scaffolds with growth factors and cell therapies will increase muscle recovery even more. Yet, there are challenges to meet such that long-term clinical efficacy and immune responses toward the implanted materials can be more optimized on behalf of any effective VML treatment.[86]

Case studies have reported hyaluronic acid-based hydrogels are effective in treating volumetric muscle loss and enhancement of muscle lipogenesis by reducing fibrosis-associated features which can reduce the integration of better tissue.[34,87] In the future, advanced HA formulations and their combination with 3D bioprinting or even cell therapy could be promising to enhance tissue regeneration.

Collagen

Collagen, a vital component of the extracellular matrix, is crucial for tissue regeneration, especially in treating VML. Its types, particularly type I and III, is a fundamental ECM protein that provides structural support and facilitates cell adhesion.[30] The assembled cell-decorated collagen (AC-DC) bioprinting technique uses collagen microfibers to create aligned, high-strength

implants, which have improved muscle function in injury models.[88] However, collagen alone may lack sufficient mechanical mimicry of muscle, as seen in studies where collagen I hydrogels with minced muscle grafts yielded limited muscle repair.[89] A notable improvement is photo cross-linkable collagen methacrylate (CMA) hydrogels, which offer tunable stiffness and enhance vascularization. CMA scaffolds significantly boost angiogenesis and myogenesis compared to traditional collagen, making them promising for VML repair.[90]

Minced muscle grafts and collagen I hydrogels composite has recently been suggested for treatment of volumetric muscle loss (VML) by researchers. A more recent study showed that despite the gains in muscle recovery in grafts comprising 50% minced muscle, only about half as many new muscle fibers were created compared with whole grafts. These activity constraints were observed due to the limited ability of the collagen hydrogel in supporting muscle growth (though it had a strong angiogenic capacity). The defect of our hydrogels for mimicking the mechanical properties of native muscle led to limited migration and regeneration ability.[91]

In biomimetic VML treatments, collagen serves as a whorl to be remodeled in the same way that is seen with native tissue and integrates well into new vasculature development along with promulgating cell growth. This improves its effectiveness in muscle regeneration as it can be tailored for different forms such as hydrogels with adjustable properties.[31,92] The secondary studies should focus on improving collagen-derived materials involving better mechanical properties and integration with native tissue.[92] Breakthroughs in collagen tissue engineering may lead to improved VML therapies and other regenerative procedures. Given the vast unmet medical needs and tremendous potential benefits of fully developed organ bioengineering, such incremental innovation in this area can bring huge leaps forward to regenerative medicine or tissue engineering.

Laminin

Laminin is an essential building block of the extracellular matrix and participates in many cellular events like adhesion, differentiation, or migration. Its importance is most substantiated with respect to muscle repair and regeneration, especially in the setting of VML that results from severe forms of trauma or surgical excision. However, treatments for VML are unfortunately few and far between. More recent studies have begun to investigate the ability of laminin–111 in determining muscle regeneration and functional outcomes for these challenging cases.[32,93]

In a study, it was evaluated that a lower dose of minced muscle grafts with hyaluronic acid hydrogel augmented with laminin-111 (HA+LMN) in comparison to autologous muscle transfer, and interestingly found the gel was effective at increasing skeletal myogenesis/volume re-growth. The most striking result is the 42% relative improvement in peak tetanic torque with respect to untreated limbs. It was discovered that the combination treatment dramatically and synergistically improved functional outcome, though did not surpass minced muscle graft control. Histological analysis showed the HA+LMN compound was associated with reduced macrophage activity and, in particular, an increase satellite cell density primarily outside of rather than within the defected area. The results indicated a remarkable 42% improvement in peak tetanic torque compared to untreated limbs. Interestingly, while the combination treatment showed significant benefits, it did not outperform the control group that received only minced muscle grafts. Histological analyses revealed that the HA+LMN treatment led to decreased macrophage activity and increased satellite cell density, predominantly in the surrounding muscle tissue rather than the defect area itself. The results collectively demonstrate that HA+LMN could be a promising therapeutic option for VML, although further research is needed to fully understand its mechanisms and optimize its effectiveness.[94]

In another study, where the tibialis anterior muscle of rats was treated for VML using fibrin hydrogels enriched with laminin-111 at a concentration of 450 μg/mL. At 28 days post-injury, the treatment group exhibited augmentation in myogenic protein expression and an increase in contractile area. Supplementation of hydrogels with laminin-111 was most effective, enhancing muscle strength (measured as torque production) by up to 60%. Additional studies of laminin-111 enriched hydrogels for VML should be pursued given the results that are suggestive in nature but also promising (eg, functional gain, muscle regeneration).[34,83,87] These data have significant implications and suggest that laminin-111 may offer this population novel therapeutic approaches to address the consequences of VML.[93]

When introducing laminin-111 into hydrogels it creates a microenvironment that supports muscle regeneration. These findings imply that laminin-111 supplemented hydrogels might provide a new treatment strategy for the restoration of

VML-related functions by enhancing myogenic activity, contractile tissue formation, and muscular strength. The factors, such as concentration of laminin 111 to use for optimal effects, composition of the hydrogel in which they are loaded and long-term efficacy required detailed investigation.[95]

Synthetic Scaffolds

Synthetic polymers serve as controlled, reproducible platforms for muscle tissue engineering due to their tunable mechanical properties, degradation kinetics, and structural architecture. Their compatibility with fabrication techniques like 3D printing and electrospinning makes them valuable for VML repair.[60] However, their limited bioactivity requires surface modifications or incorporation of biological molecules to improve cellular interactions.

Poly ε Caprolactone (PCL)

Poly ε caprolactone (PCL), an aliphatic polyester, is valued for its mechanical strength, slow degradation, and compatibility with electrospinning and 3D printing. Its ability to form aligned nanofibers replicates the anisotropic structure of native skeletal muscle, promoting myoblast alignment and fusion into myotubes.[96,97] For example, electrospun PCL/gelatin scaffolds with aligned fibers significantly enhanced myotube formation by guiding cell orientation. Mechanical stimulation further increased myotube width (12.92 ± 3.29 μm) and nuclear fusion index (95.73 ± 1.05%), underscoring the importance of mechanical cues in regeneration. Despite these advantages, PCL's hydrophobic surface and lack of cell-adhesive motifs limit bioactivity. Strategies to address this include surface modification with ECM proteins (eg, collagen, laminin) or blending with natural polymers. PCL/gelatin composites, for instance, improved wettability, myotube contractility, and actin density compared to pure PCL, demonstrating the benefits of hybrid materials.[98]

The study carried out by Kim I et al, demonstrated the study carried out using myotubes induced myogenic progenitor cell embedded in PCL scaffold using electrospinning technique for skeletal muscle regeneration by incorporation of Matrigel. The scaffold rapidly induced myofiber formation enabling its alignment upto 12.8° ± 5.3° possibly due to cell signaling highlighting the role of biomimetic agents along with mechanical strength and biodegradation offered by PCL.[99]

Polylactic Acid (PLA)

Polylactic Acid (PLA), an FDA-approved biodegradable polymer, degrades faster than PCL, making it suitable for temporary mechanical support in muscle regeneration. Electrospun PLA scaffolds facilitate myoblast adhesion and alignment, while porous structures enhance vascular infiltration.[100] However, PLA's brittleness and acidic degradation byproducts can cause localized inflammation. Blending PLA with hydrophilic polymers like polyethylene glycol (PEG) improves elasticity and reduces acidic microenvironments.[101] For example, PLA/PEG/RosA/GO membranes exhibited a threefold increase in tensile strength (2.6 MPa) and improved hydrophilicity, reducing inflammation while enhancing tissue compatibility. Incorporating ceramics like β-TCP or hydroxyapatite further strengthens PLA scaffolds for musculoskeletal applications. However, replicating muscle-like elasticity remains challenging, prompting research into crimped fiber geometries or elastomeric additives.[100]

Polyethylene Glycol

Polyethylene glycol (PEG) PEG, a hydrophilic and non-toxic polymer, is widely used in hydrogel scaffolds due to its tunable crosslinking and high water content, which supports nutrient exchange—critical for VML repair. However, PEG's bioinert nature necessitates functionalization with adhesive peptides (eg, RGD) to promote cell adhesion. PEG-modified PLA scaffolds, for instance, enhanced endothelial cell attachment and collagen deposition, improving vascularization in vivo. Beyond structural support, PEG-based scaffolds serve as drug delivery platforms.[102] The PLA/PEG/RosA/GO system delivered rosmarinic acid and graphene oxide, achieving 99% antibacterial efficacy and accelerating wound healing in murine models. Despite its advantages, PEG's low mechanical strength requires reinforcement with stiffer polymers like PLA or PCL for load-bearing applications in large muscle defects.[100]

In the study carried out by Alarcon Y et al, the potential solution for skeletal muscle regeneration is PEG diacrylate. The authors fabricated copolymer of PEG diacrylate and acrylic acid with collagen methacrylate further promoting cell

adhesion and cell proliferation. The results positively showed C2C12 myoblast adhesion and differentiation with sustained metabolic activity over 14 day in vitro study.[103]

Hybrid Scaffolds

Hybrid scaffolds integrate the strengths of both natural and synthetic materials, creating composite platforms that balance mechanical performance with bioactivity. By combining the structural integrity and tunability of synthetic polymers with the cell-instructive properties of natural extracellular matrix (ECM) components, these scaffolds address the limitations of single-material systems.[104] This synergistic approach is particularly valuable for volumetric muscle loss (VML) repair, as it enhances both mechanical support and biological integration.

Collagen–PCL Hybrid Scaffolds

Collagen–PCL hybrid scaffolds illustrate this strategy effectively. Electrospun PCL fibers provide mechanical strength and structural alignment for myotube formation, while collagen enhances hydrophilicity and offers integrin-binding sites to improve cell adhesion and signaling. Studies show that collagen-coated or blended PCL scaffolds significantly improve myoblast proliferation, alignment, and differentiation compared to pure PCL.[104] In VML animal models, these scaffolds increase muscle fiber density, reduce fibrosis, suggesting strong clinical potential.[92] Advanced fabrication techniques, such as co-electrospinning or coaxial electrospinning, allow for composite fibers with gradient or layered architectures that better mimic native muscle tissue. For example, research comparing PCL/collagen and PCL/gelatin scaffolds found that collagen incorporation reduced fiber size, increased hydrophilicity, and softened mechanical properties, all of which promote cell growth and musculoskeletal tissue development.[105,106]

Fibrin-Based Hybrid Scaffolds

Fibrin-based hybrids, often combined with synthetic polymers like PLA, PLGA, or PEG, are widely explored due to fibrin's natural role in wound healing and its bioactive properties. These scaffolds enhance vascular infiltration, ECM remodeling, and satellite cell recruitment in VML defects.[107] Fibrin–PLGA constructs, for instance, act as transient matrices that degrade gradually while supporting early regeneration phases, eventually allowing native tissue deposition. Additionally, fibrin's ability to encapsulate and release growth factors (eg, VEGF, IGF-1) makes it useful for modulating the regenerative microenvironment. This controlled delivery can enhance muscle repair by promoting angiogenesis and myogenesis.[108]

Hyaluronic Acid Hybrids

HA-based hybrid scaffolds are gaining traction due to HA's anti-inflammatory, pro-angiogenic, and cell-adhesive properties. When incorporated into synthetic networks like PEG hydrogels or PLGA frameworks, HA improves scaffold integration and host–tissue interaction. These systems enhance myogenic differentiation, vascularization, and immune modulation in vivo. For example, HA-modified PEG hydrogels functionalized with RGD peptides and loaded with muscle progenitor cells have demonstrated superior tissue integration and myofiber organization in murine VML models. Similarly, HA-chondroitin sulfate hydrogels accelerate skeletal muscle healing by promoting cell migration and angiogenesis.[109,110]

Hybrid scaffolds offer significant advantages, including customizable architectures (gradient or multi-layered designs) and controlled release capabilities, better mimicking native muscle complexity. However, their fabrication can be technically challenging, and ensuring consistent material integration without compromising functionality remains a key hurdle. Future research should focus on optimizing fabrication techniques and improving scalability for clinical translation.[111,112]

A comparative assessment of scaffold classes for VML repair is described in Figure 4 underscoring clear material-dependent trade-offs across mechanical competence, biological performance, and translational readiness. Natural scaffolds demonstrate strong bioactivity due to inherent cell-binding ability and tissue-specific attributes, supporting adhesion, proliferation, and differentiation. However, they generally exhibit limited mechanical strength and present challenges related to large-scale manufacturing and immunological variability. In contrast, synthetic scaffolds provide tunable and reproducible mechanical properties with high structural precision and scalability, yet they lack intrinsic bio-

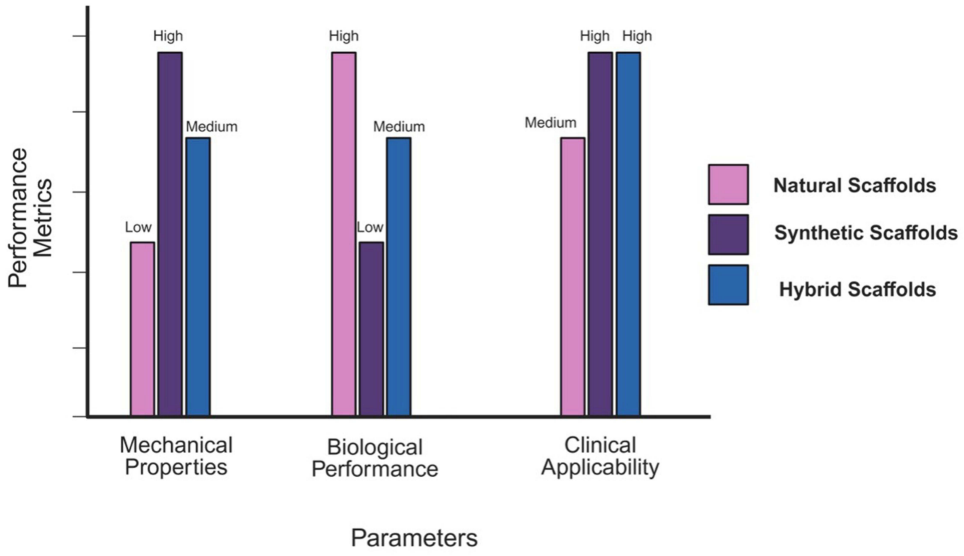

Figure 4 Performance metrics of natural, synthetic, and hybrid scaffolds for VML repair (created with BioRender.com).

instructive signals and often require functionalization to enhance cellular interactions. Hybrid scaffolds integrate components from both systems to balance structural robustness with biological functionality, thereby offering improved overall performance and enhanced clinical applicability. This comparative framework highlights the importance of aligning material selection with the specific mechanical and regenerative demands of VML repair.

Functionalization Strategies

Functionalization is a critical strategy in enhancing the therapeutic potential of biomimetic scaffolds, transforming them from passive structural supports into bioactive platforms capable of directing tissue regeneration. One common approach involves the incorporation of growth factors, such as VEGF, insulin-like growth factor-1, and FGF. These signaling molecules orchestrate key regenerative processes: VEGF stimulates angiogenesis, promoting the formation of blood vessels necessary for oxygen and nutrient delivery; IGF-1 activates satellite cells and enhances myogenic differentiation; and FGF supports the proliferation and migration of muscle progenitor cells. Controlled and localized delivery of these factors from scaffolds ensures sustained biological activity at the injury site.[113,114]

Moreover, electrical and mechanical stimulation has emerged as a powerful tool to mimic the native physiological environment of skeletal muscle. Electrical stimulation of scaffolds enhances myotube formation, contractile function, and alignment, mimicking neuromuscular signaling.[104] Similarly, mechanical loading promotes fiber alignment, cellular orientation, and ECM deposition, all of which are essential for functional muscle tissue. Incorporating conductive materials or designing dynamic bioreactor systems enables the delivery of these physical cues during in vitro maturation or post-implantation.[115]

The list of key biomimetic scaffolds used in VML are described in Table 3. It provides an overview of several scaffold types highlighting the material composition. Further the mechanism by which the scaffold supports modulation of myogenesis, vascularization, and innervation are discussed.

The scaffolds act as engineered biomimetic ECM-based guides for muscle regeneration by promoting muscle repair and functional restoration. Regeneration capacity depends on the type of material, its composition, presence of any growth factors, cellular incorporation, and distribution.[128] Since the architecture of the scaffold is highly influenced by the type of biomaterial used, it directly regulates cell signaling, adhesion, migration, proliferation, and differentiation.[129] The decision matrix for deciding the type of scaffold to be used is described in Table 4. The decision framework for selecting biomimetic scaffolds for VML was based on defect size, injury complexity, and patient-specific factors. It integrates material choice, scaffold architecture, and bioactive incorporation to align mechanical support with

Table 3 Critical Analysis of Preclinical Success vs Clinical Translation Barriers in VML

Scaffold Type	Composition	Mechanism of Muscle Regulation	Outcomes	References
Decellularized ECM	Laminin, Collagen, Fibronectin, growth factors	• This scaffold serves the purpose of muscle regeneration by recruiting the host cells like satellite cells and progenitor cells facilitating cell adhesion and migration. • The immune response is modulated by M2 macrophage polarization thereby promoting angiogenesis and reducing chronic inflammation.	• The trials on murine model demonstrated partial muscle restoration post treatment with the scaffold • After 6 months of implantation, increase in isomeric torque by 20–37% with increased limb strength and neovascularization. • Overall improved the limb motion by 27.1%	[116]
	dECM with autologous minced muscle grafts	• The scaffolds deliver satellite cells contributing to de novo muscle regeneration • The scaffold enables improved host – scaffold integration thereby offering mechanical support, vascularization and achieving functional restoration compared with only decellularized ECM. • This also reduces the fibrotic deposition	• The pre-clinical studies showed the recovery upto 28.2% 8 weeks post-surgery when compared with the untreated ones. • The increase in muscle mass and contractility was observed during histological examinations	
Laminin 111	Minced muscle along with Laminin and Hyaluronic Acid	• This acts as a conductive scaffold that supports the muscle fiber regeneration by modulating the inflammatory response like T-lymphocyte activation and increase in the satellite cells post 14 days of injury. • It was noticed that elevation of Pax7 cells was essential in normalizing the satellite cell count.	• The pre-clinical studies on rat tibialis anterior muscle showed the recovery upto 42% in scaffold group with respect to the untreated control group. • However, the strength was not significantly improved when compared with minced muscle group. • It overall highlights the use of cell-based scaffold approach for VML.	[94]
Adipose derived ECM	Decellularized adipose ECM scaffold embedded with adipose derived stem cells and L6 myoblast	• The adipose stem cells have the proliferative capacity to differentiate into myogenic lineage; however, it is low when delivered alone. • L6 myoblast function as auxiliary cells that push adipose-derived stem cells towards differentiation. • The molecular mechanism involved is expression of Mki67 proliferation marker, CD34, a marker for stem progenitor cells and Cyclin-dependent kinase 1 that regulates cell cycle progression. • Together all these expressions generate a regenerative cell pool capable of rapid expansion and muscle cell differentiation.	• The model used for preclinical studies was anterior tibialis of rat. • The outcome of this study was improved muscle regeneration and recellularization resulting in increasing muscle contraction strength. • The adipose tissue is low on myogenic differentiation which is addressed by the addition of L6 myocytes. • Further single nucleus RNA sequencing also proved the transformation of adipose stem cells leading to oriented myogenic differentiation.	[117]
Silk Fibroin	Silk Fibroin loaded with murine myoblast cells	• The scaffold when placed in the site of action, releases fibronectin due to cell-scaffold signaling and it leads to adhesion of muscle fibers due to endogenous ECM deposition ensuring efficient cell–cell attachment. • During the proliferation and differentiation phase, myogenic factors like Myf5 and MyoD1 are released. • Upregulation of the structural and contractile genes like myosin heavy chains (MYH1 and MYH 7) and actin which was marked by advanced myotube maturation.	• The study using silk fibroin with the murine myoblast cells showed better adhesion and biocompatibility to the human skeletal muscle myoblast. • Mechanical testing revealed that silk from Bombyx mori was able to maintain mature myogenic marker expressions with young's modulus closely resembling the native skeletal muscle which is (10–16 kPa).	[118]

(*Continued*)

Table 3 (Continued).

Scaffold Type	Composition	Mechanism of Muscle Regulation	Outcomes	References
3D printed muscle construct	Gelatin and PCL 3D muscle constructs with decellularized ECM	• The mechanism by which the 3D printed scaffolds help in de novo muscle regeneration was by the presence of the human nuclei cells adjacent to muscle fibers. • These help in aligning the myofibers further contributing to superior regeneration. • The myoblast activation was initiated by the biochemical signals from the 3D decellularized ECM scaffold leading to proliferation and migration of cells forming multinucleated myotubes.	• The scaffold demonstrated high cell viability upto 91.5% and cell alignment by significantly reducing the hypoxia owing to scaffold's microporous architecture. • On the other hand, the hydrogel-based scaffolds showed just 34.5% cell viability, decellularized ECM showed 87.1% cell viability. • The area of myotube regeneration in 3D printed scaffolds was higher by 3.6 folds in comparison to decellularized ECM sponge and by 2 folds with hydrogels. • The *invivo* results on the rats with 40% VML injury achieved restoration of contractile function of skeletal muscles using 3D printed scaffolds upto 71% in comparison to untreated ones and upto 78.6% muscle mass recovery when fabricated the scaffolds with co-axial printing. • The function of the regenerated muscle was evaluated by conducting a contractile force measurement where twitch force and tetanic force were measured to be highest for 3D printed scaffold (coaxial) as 0.44 ± 0.017 and 0.85 ± 0.005 mN respectively.	[119]
PLGA	Silk scaffold coated with PLGA nanofibers embedded with bone marrow derived mesenchymal cells	• The scaffold acts by attaching to the silk microfibers and increases area for cell adhesion leading to improved integrin binding and efficient cytoskeletal organization. • The cells further promote cell viability by reducing the apoptosis allowing continuous proliferation.	• The scaffold resulted in 11% more tensile strength (68.2 ± 6.72 N) in comparison to the unseeded counterparts (61.5 ± 3.43 N). • The cells when seeded on both the surfaces of scaffold, showed better proliferation upto 70% more at day 21 in comparison to the single surface seeding. • The combination of these scaffolds ensured mechanical resilience along with improved cellular growth.	[120]
	PLGA with Polydopamine (PDA) loaded with islet cells	• The mechanism was studied by staining scaffolds with phalloidin and DAPI which showed reorganization of actin filaments. • It was observed that Rho GTPase orchestrated the actin remodelling and cytoskeletal orientation by lamellipodia and filopodia formation allowing cellular adhesions. • The cellular adhesions promoted proliferation and differentiations of the endothelial and myoblast cells supporting angiogenesis. • The Islet cells further released insulin enabled due to the presence of glucose in the skeletal muscles.	• The scaffolds adhere to the cells and enhance cell proliferation due to increase in hydrophilicity of PLGA due to coating of PDA. • Morphologically, the cells had grown in slender shape along the nanofibrous scaffold. • The *invivo* studies on diabetic rat model showed cell infiltration at transplanted site with insulin secretion leading to significant decrease in glucose levels which lasted for 3 weeks • The presence of newly synthesized collagen increased with time with no pathological changes observed and the scaffold implantation had no adverse effects on the body.	[121]

(*Continued*)

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Table 3 (Continued).

Scaffold Type	Composition	Mechanism of Muscle Regulation	Outcomes	References
PCL	PCL, gelatin, hyaluronic acid	• The mechanism by which scaffolds act is by increasing the expression of proliferation and differentiation (Myf5, MyoD, MyoG). • The presence of Pax7+ satellite cells suggested activation of regenerative cells. • The release of pro-inflammatory macrophage M1 and its polarization to M2 due to F4/80+, CD68+ and CD163+ leads to phenotypic transformation regulating the immune system. • The phenotype transformation of macrophage proves to be favourable for skeletal muscle regeneration.	• The tri-layered scaffold showed a mixture of staggered and aligned arrangement of the fibers with abundant sites for cell adhesion. • The tensile strength of fibers was found to be 11.50 ± 0.89 MPa with elongation of 72.21 ± 11.81% indicating the strength to be greater than human muscle tissue. • Early cell adhesion influenced proliferation and differentiation of C2C12 myocytes with high MHC expression. • The *invivo* studies show that least inflammatory response was found in the tri-layered scaffold with additional neovascularization as observed by histological analysis. • The walking pattern analysis was carried out for rats 4 weeks after treatment that showed improvement in the tri-layered scaffold to 94.51 ± 6.12% when compared with remaining groups.	[122]
Collagen	Collagen, Chondroitin sulfate, polypyrrole nanoparticles, EDC/NHS	• The phenotype transformation of macrophage proves to be favourable for skeletal muscle regeneration. • Scaffold anisotropy mimics aligned muscle extracellular matrix (ECM), aiding myoblast alignment and maturation. • Inclusion of conductive polypyrrole particles aims to recapitulate skeletal muscle electrical excitability, enhancing myoblast proliferation and differentiation. • Open, interconnected porous structure (~150 μm pores) facilitates cellular infiltration, nutrient/waste transport. Scaffolds provide bioelectrical and mechanical cues supporting endogenous repair processes. • Persistent macrophage infiltration indicates ongoing remodeling and wound healing.	• Both Collagen and Collagen–polypyrrole scaffolds significantly improved functional muscle recovery in a rat tibialis anterior VML model at 12 weeks post-implantation, shown by increased isometric torque compared to non-treated muscles. • Scaffold-treated muscles displayed regenerating muscle fibers with centrally located nuclei, indicating myogenesis. • The scaffolds showed significantly enhanced neovascularization (higher CD31 and smooth muscle actin vessel markers). • Non-conductive CG scaffolds showed muscle innervation levels similar to native tissue, unlike conductive CG-PPy and untreated groups. • Remaining polypyrrole particles observed 12 weeks post-injury; collagen scaffold largely degraded by this time. • Scaffold-treated muscles had increased fiber cross-sectional area comparable to native muscle, indicating reduced muscle atrophy. • Persistent inflammatory macrophage presence (M1 and M2) suggesting ongoing wound healing response. • Gross morphology showed reduced fibrosis and better tissue integration in scaffold-treated muscles compared to no repair groups. • Despite improvements, muscle volume was still reduced compared to native tissue, highlighting need for further optimization.	[123]

(*Continued*)

Table 3 (Continued).

Scaffold Type	Composition	Mechanism of Muscle Regulation	Outcomes	References
Elastin	Poly caprolactone, Elastin	• Nanofibrous structure provides an aligned, high surface-area scaffold, encouraging skeletal myoblast adhesion and orientation. • Elastin inclusion increases hydrophilicity (lower contact angle), enhancing cell adhesion and proliferation. • Improved mechanical properties, with higher Young's modulus and maximum stress, better mimicking native muscle tissue. • Supports dynamic mechanical stimulation in bioreactors, which potentially enhances neovascularization.	• In vitro studies with rat skeletal myoblasts demonstrated better cell viability on PCL-elastin scaffolds than pure PCL. • Dynamic mechanical stimulation in a bioreactor was tested but did not show significant improvement in cell maturation compared to static culture due to low overall maturation levels. • Cell viability in static PCL-elastin scaffolds increased by day 14, while viability in dynamic scaffolds decreased, possibly due to cell detachment from mechanical forces. • In Vivo Outcomes: After 30 days implantation in Wistar rats, scaffolds showed slight acute inflammation and moderate chronic inflammation, comparable or somewhat improved relative to controls. • Fibroblast activity and collagen deposition were moderate, indicating constructive tissue remodeling without excessive fibrosis. • Neovascularization was greater in dynamically cultured scaffolds, showing enhanced blood vessel formation likely due to mechanical stimulation before implantation. • Histology indicated viable but immature muscle cells with incomplete differentiation in both static and dynamic scaffold groups. • Immunohistochemistry confirmed the presence of differentiating myogenic cells though further maturation is needed.	[124]
Electrospun nanofibrous composite scaffold	Polycaprolactone (PCL) doped with L-arginine	• L-arginine acts as a precursor for nitric oxide (NO) synthesis in cells. NO plays a key role in: • Regulating vascular tone, muscle contraction, and cell proliferation. • Enhancing angiogenesis (formation of new blood vessels) and myoblast differentiation. • Modulating cellular signaling pathways related to muscle regeneration and wound healing.	• Smooth, uniform nanofibers with L-arginine well distributed. • Increased hydrophilicity and surface energy, leading to improved cell attachment. • Enhanced cell adhesion and proliferation (notably fibroblasts and myoblasts). • Increased bioactivity due to NO-related cellular signaling. • Maintained biodegradability and biocompatibility. • Improved potential for muscle tissue engineering and vascular applications.	[125]
Decellularized bovine skeletal muscle scaffold	Collagen- and GAG-rich extracellular matrix	• Decellularised scaffold provides a native muscle ECM microenvironment, enabling muscle-derived (bovine fetal myoblast) cells to adhere, and thus potentially differentiate/regenerate muscle fibers. • By preserving ECM, the scaffold supports muscle cell interaction and regeneration.	• Successful decellularisation confirmed (DAPI staining for nuclei removal, DNA quantification) and structural integrity of ECM maintained • SEM confirmed 3D architecture preserved. • Water absorption ability demonstrated (indicative of scaffold porosity and hydration). • Cytocompatibility: bovine fetal myoblasts seeded on scaffold adhered and proliferated forming more dense cellular networks by day 10.	[126]

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Table 3 (Continued).

Scaffold Type	Composition	Mechanism of Muscle Regulation	Outcomes	References
Electrospun nanofibrous scaffold	poly-caprolactone (PCL) with nano-hydroxyapatite (n-HA) particles, further surface-modified by ink-jet printed conductive polyaniline (PANI) patterns.	• The mechanism is via the conductive PANI pathways enabling electro-chemical signaling to cells: the conductive scaffold can transmit electrical stimulation to cells, thereby influencing cell adhesion, proliferation, migration and differentiation.	• Uniform bead-free electrospun fibers • Electrical conductivity of modified scaffold: bulk resistivity ~0.2–0.3 mS cm^{-1}. • Mineralisation: In simulated body fluid, apatite formation observed; PANI patterns did not hinder mineralisation. • Cell response: human osteoblast-type cells spread well, elongated morphology, good viability; alkaline phosphatase activity and mineral deposition were observed • The scaffold shows potential as a biomimetic conductive substrate for tissue engineering that could allow planned electrical stimulus to accelerate regeneration	[127]

Note: This table highlights the discrepancy between high functional recovery observed in small animal models and the limited efficacy in human clinical trials, identifying key physiological, immunological, and structural bottlenecks.

Table 4 Regulatory Framework and Quality Control Standards for Scaffold-Based Muscle Therapies

Defect Characteristics	Scaffold Material	Design Rationale	Expected Outcomes	References
Small defect or acute injury (< $5cm^3$, superficial)	Injectable hydrogels based scaffolds made up of natural polymers	• Minimally invasive approach promoting angiogenesis by bridging the gap between the injured myocytes. • Mimics ECM and has high water content • Provides pro-regenerative environment • Efficient delivery of growth factors.	• Promotes vascularization and innervation by increasing cell signaling. • Satellite cells migrate, proliferate, and differentiate • Rapid revascularization due to M2 macrophage polarization supporting myogenesis in host. • Tissue bridging achieved with improved muscle force.	[116]
Medium defect (5 to 20 cm^3, moderate depth)	Structurally aligned decellular ECM scaffolds with natural and synthetic polymers	• The scaffold has high porosity with anisotropic structure to facilitate cell infiltration. • Biodegradable and biocompatible polymers ensure tissue-specific matrix • Minimized immunogenicity • Mechanical strength due to presence of synthetic polymers like PCL, PLA etc.	• Partial muscle restoration due to the presence of scaffold and due to body's inherent capacity to regenerate • Treatment essential to ensure muscle restoration by satellite cell migration else leads to the formation of fibrotic scar tissue.	[130]
Large defect (> $20cm^3$, deep)	Scaffolds with decellularized ECM and synthetic polymers of high tensile strength to support the deeper injuries along with growth factors	• 3D printed scaffolds made of high tensile strength materials like PCL, PLGA, polyurethane • Synergistic balance between scaffolds and ECM layers	• With high mechanical demand, the scaffold provides both support as well as bioactivity. • The growth factors enable cell signalling and adhesion	[128]

(*Continued*)

Table 4 (Continued).

Defect Characteristics	Scaffold Material	Design Rationale	Expected Outcomes	References
Immuno-compromised patients	Inert decellularized scaffold materials with minimal immunogenicity	• The scaffolds provide biological framework with minimal immunogenicity reducing risk of immune rejection. • Bioactive like silver, antimicrobial agent further limit risk of infection • Decellularized components avoids immune overstimulation.	• Promote gradual host and tissue integration with reduced inflammatory response • Encourage cell infiltration, adhesion, proliferation, and differentiation. • Stable structural repair	[131]
Elderly patients		• Age-related decline in stem cell function and vasculature. • Scaffolds required that actively support regeneration without initiating any antigenic response. • The scaffold material with high tensile strength and biocompatibility preferred	• Neovascularization with cell immigration but at slower rate due to age. • Slow and gradual muscle restoration.	[132]
Patients with existing co-morbidities (eg Diabetes, vascular disorders etc)	Structurally aligned decellular ECM scaffolds with natural and synthetic polymers seeded with satellite cells or mesenchymal stem cells loaded with antioxidant and anti-inflammatory actives	• Controlled release of actives to overcome oxidative stress and inflammation. • Conductive polymers improve cellular communication and metabolic activity.	• Promotes regeneration attenuating chronic inflammation. • Decellularized scaffolds limit the antigenic response avoiding potential metabolic stress.	[133]
Complex defect with irregular shape	Customized 3D printed patient specific constructs loaded with cells and growth factors	• Complex defects require structural anisotropy and mechanical gradients allowing patient-specific geometry. • It allows controlled cell distribution and growth factors required to support regeneration process.	• Facilitates muscle growth but critical factor involved is uniform cell distribution. • Restoration of muscle volume gradually. • Caters to specific patient needs with respect to complexity, depth of injury and location of injury.	[134]
Chronic Injury	Scaffolds with anti-fibrotic drugs like nintedanib, losartan	• The presence of dense scar tissue is replaced by ECM-based scaffolds. • The growth factors and anti-fibrotic agents eventually reset the regenerative microenvironment. • Further seeding of satellite cells or mesenchymal stem cells are driving force for de novo muscle regeneration.	• The angiogenesis and vascular integration observed but partial recovery. • Increase in twitch and tetanic force yet limited functional restoration of the limbs with inflammatory challenges to be addressed • Reduction in fibrotic tissue due to the presence of drugs.	[135]
Injury with bone or tendon involvement	Hybrid Scaffolds with decellularized ECM using synthetic polymers of high tensile strength and natural polymers along with growth factors	• The synthetic polymers mimic the load bearing regions like tendon or bone like properties withstanding high tensile strength. • The area of muscles acting as supporting regions are fixed using natural polymers that mimic the extracellular matrix and the presence of growth factors enhance the muscle growth.	• Improved mechanical load and cell adhesion minimizing risk of graft detachment. • Satellite cell infiltration and myofiber alignment with vascularization. • Restoration of force in muscles post implantation followed by sufficient physiotherapy.	[136]
Young trauma or sports injury (acute injury)	Injectable fibrin and gelatin hydrogel loaded with FGF-2	• Rapid closure of wound • Minimal invasive • Boost angiogenesis	• Promotes macrophage polarization M2 • Endothelial migration and myoblast differentiation	[137]

(*Continued*)

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Table 4 (Continued).

Defect Characteristics	Scaffold Material	Design Rationale	Expected Outcomes	References
Post surgical defect	3D printed hybrid ECM- PCL scaffolds with neutrophic factor gradients and mechanical anisotropy	• Provides mechanical continuity for stump load bearing and nerve reinnervation	• Guided alignment of myofiber • Nerve sprouting across ECM interface	[138]
Elderly patients (age-related degeneration)	Exosome-functionalized decellularized ECM scaffold with pro-angiogenic miRNAs or NAD^+ precursors	• Counteracts reduced stem cell potency and vascularization • Promotes rejuvenation	• Exosomes activate aged satellite cells • ECM guides remodeling	[139]
High -load defect	PCL–collagen–hydroxyapatite mimicking myotendinous junction	• Transfer load efficiently between muscle-tendon interface	• Promotes myoblast differentiation and osteo conductive reinforcement	[140]

Note: This table outlines the critical ICH and FDA-aligned guidelines governing the characterization, stability, and sterility of tissue-engineered constructs. It highlights the essential parameters required for Good Manufacturing Practice (GMP) compliance and commercial standardization.

regenerative demands. This stratified approach emphasizes the necessity of personalized scaffold strategies to achieve functional muscle restoration.

While advances in scaffold design have been significantly observed and shown promise in preclinical studies, clinical translation for VML treatment faces persistent knowledge gaps in understanding the host's immune response to implanted material, inflammation, and fibrosis due to chronic immune activation, thereby ensuring biocompatibility to avoid immunogenic reactions.[141] While biomimetic scaffolds mimic the ECM, cell adhesion, and differentiation with adequate vasculature and long-term functional integration still remains a challenge. Approaches such as customizable 3D printed scaffolds show promising properties but have not yet been clinically established.[142,143] A major unresolved challenge in VML repair is the restoration of functional neuromuscular units rather than mere tissue filling. While scaffold-based systems often promote myofiber formation, they frequently fail to achieve synchronized vascularization and reinnervation, which are essential for sustained contractile function. Additionally, fibrotic signaling pathways remain insufficiently controlled in large defect models, particularly in chronic injury settings where immune dysregulation persists.[43] To overcome these limitations, future scaffold designs should incorporate dynamic, responsive materials capable of modulating the inflammatory milieu and adapting to the evolving regenerative microenvironment. The inclusion of mechanoresponsive elements may further enhance alignment and maturation of regenerating myofibers.[144] However, translating such multifunctional constructs to clinical settings demands careful balancing between biological complexity and manufacturability. There is also a need for standardized protocols for comprehensive biomolecular characterization to better understand host scaffold interactions in clinical trials. Multidisciplinary research focusing on precise biomimicry at the molecular and structural levels is needed to address these knowledge gaps, improved manufacturing technologies for clinical translation of biomimetic scaffolds to evaluate the long-term performance and safety of these scaffolds in muscle regeneration.[145] Strategies like integrating angiogenic factors or pre-vascularizing scaffolds are essential to ensure sufficient blood supply.

Preclinical to Clinical Translation

Critical Evaluation of Scaffold Performance

Biomimetic scaffolds for treating VML are extensively tested in preclinical animal models to assess how well they integrate with tissue, promote muscle regrowth, and support blood vessels and nerve formation.[146] Rodents, such as mice and rats with surgically created defects in muscles like the quadriceps or tibialis anterior, are popular choices due to their cost-effectiveness and genetic uniformity.[147] For example, studies using scaffolds made from small intestinal submucosa extracellular matrix (SIS-ECM) in these models have shown new muscle tissue forming with functional blood vessels and nerves. However, rodents differ significantly from humans in immune responses and tissue structure, limiting how well findings translate. Larger animals like pigs, dogs, and sheep better mimic human biology, particularly in processes

like scar tissue formation and blood vessel remodeling.[148] A study in mini-pigs, for instance, revealed that muscle injuries healed with extensive scarring and minimal regeneration, mirroring what happens in humans. Sheep models are especially useful for testing large scaffolds, as they highlight challenges like ensuring nutrients reach the core of implanted materials to prevent cell death.[149–151] Table 5 summarizes key animal models used in VML research. This table highlights how each model contributes unique insights into muscle degeneration, fibrosis, muscle repair, and biomaterial performance. By comparing reproducibility, anatomical relevance, and regenerative outcomes, it supports the discussion on preclinical validation of biomimetic scaffolds. These models collectively bridge mechanistic understanding and translational assessment of VML therapies.

Despite encouraging preclinical outcomes, several biomimetic scaffolds have underperformed due to inadequate immunomodulation, insufficient vascularization, and poor neuromuscular integration. Many constructs successfully replicate the structural aspects of native extracellular matrix but fail to actively direct macrophage polarization toward a pro-regenerative phenotype, resulting in prolonged inflammation and fibrotic encapsulation. Animal studies remain critical for evaluating the safety and efficacy of biomimetic scaffolds but raise ethical concerns, prompting adherence to the 3Rs principle—Replacement (using non-animal alternatives where possible), Reduction (minimizing animal use), and Refinement (enhancing welfare) to address welfare issues.[154] However, interspecies differences, such as faster healing in rodents compared to humans, complicate translation, leading researchers to prioritize larger models like pigs and dogs that better replicate human fibrosis and regeneration.[155] Additionally, human variables like age, comorbidities, and individual healing capacity further challenge the replication of clinical conditions in animals. Emerging alternatives, such as patient-derived organoids and humanized mice, offer promising avenues to bridge this gap, though.[156] Solutions include adaptive clinical trial designs, collaborative efforts between engineers and regulators, and post-market surveillance to ensure therapies meet safety and efficacy standards.

The clinical application of biomimetic scaffolds for VML remains experimental, with ongoing and completed trials yielding mixed outcomes.[157]

Regulatory and Manufacturing Considerations

Regenerative approaches for VML that include cell-based therapies, biomaterial scaffolds, and combination products occupy a complex regulatory space. This leads to inconsistency in product quality and results due to lack of standardized protocols related to safety, efficacy, and sustainability.[158] The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) is recognized for accelerating clinical translation for global

Table 5 Industrial Scalability and Clinical Adoption Matrix for VML Scaffolds

Sr. No.	Study Title	Animal Model	Key Findings	Reference
1	Long-term Longitudinal Study on Swine VML Model	Mini-pig (Sus scrofa domesticus)	Swine muscle showed poor regeneration and significant fibrosis, paralleling human scar tissue development.	[149]
2	Treatment of Volumetric Muscle Loss in Female Rats with Biomimetic Sponges	Female rats	Biomimetic sponges increased large myofiber area and reduced necrosis and inflammation post-VML.	[150]
3	Retrospective characterization of a rat model of volumetric muscle loss	Rat (6 mm biopsy of tibialis anterior muscle)	Demonstrated reproducibility and muscle weight dynamics, supporting its robustness as a translational platform.	[151]
4	Biomimetic Sponges Improve Functional Muscle Recovery Following VML and Fracture	Rat (VML + fracture model)	FK-506-loaded sponges enhanced myofiber regeneration and strength, but VML impaired concurrent bone healing.	[152]
5	Rodent Model of Masseter VML for Craniofacial Studies	Rat	Developed a model for craniofacial VML and created a standardized protocol for biomaterial evaluation.	[153]

Notes: This table evaluates diverse fabrication platforms based on their manufacturing throughput, cost-efficiency, and readiness for clinical translation. It provides a strategic comparison of traditional processing versus advanced automated systems like 3D bioprinting for large-scale production.

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access by ensuring the safety of biomaterials for VML-based therapies.[159] Current ICH initiatives related to quality and manufacturing of biologics and cell-based products include ICH Q5A (R2) which addresses genetically engineered viral vectors and other biotechnology products associated with cell-based therapies, ICH Q5D related to the derivation and characterization of cell substrate with detailed documentation, ICH Q5E when changes are made to the scaffold material and ICH Q6B to establish important quality characteristics of organic products.[160]

It highlights the importance of understanding regulatory pathways, manufacturing standardization, and implementation timelines. Regulatory authorities such as the FDA and EMA require a clear understanding of the frameworks typically under investigation of new drugs (IND). In the United States, the FDA oversees these constructs as combination products through the Office of Combination Products, classifying them based on their mode of action.[161] Developers must comply with current Good Manufacturing Practices (cGMP; 21 CFR Part 4) and submit appropriate regulatory submissions, either an Investigational Device Exemption (IDE) or an IND application, depending on whether the scaffold functions primarily as a device or as a biologic.[162]

In the European Union, the European Medicines Agency (EMA) governs these scaffolds under the Advanced Therapy Medicinal Products (ATMP) framework, which requires simultaneous compliance with GMP for biologics and ISO 13485 quality standards for device components.[163] Both regulatory bodies emphasize manufacturing standardization, including validated sourcing of biomaterials, process reproducibility, and rigorous sterility and performance testing, to ensure safety and effectiveness.[164] Early collaboration with regulators helps clarify classification, expedite documentation, and avoid delays during clinical progression. At the preclinical stage, researchers face the difficult balance of adhering to ethical animal research principles while generating meaningful data designed to protect animals often forces studies in unrealistically healthy specimens that poorly predict human outcomes.[149] Combination products that pair scaffolds with cells or biological factors fall into regulatory grey areas between device and drug classifications, requiring reviews from multiple FDA divisions. Cellular therapies bring additional layers of complexity—from ensuring stem cells will not form tumors to verifying genetic modifications will not cause unintended effects.[165] Manufacturing these complex products under strict quality standards drives costs into prohibitive ranges, with each component from biological materials to specialized equipment requiring rigorous validation.[166] Despite these substantial barriers, the field continues advancing through innovations in smart materials and precision manufacturing. However, realizing the full potential of these technologies will require more than scientific breakthroughs—it demands new frameworks for regulatory evaluation, standardized testing methods, and international cooperation to streamline the path from lab to patient while maintaining rigorous safety and ethical standards.[167] The coming years will test whether our current systems can adapt quickly enough to deliver these promising therapies to those who need them.

Realistically, the full clinical translation journey spans 8 to 12 years, encompassing approximately 2 years of preclinical optimization, 3 to 5 years of Phase I to III clinical evaluation, and 1 to 2 years of regulatory review and marketing authorization, followed by long-term post-market surveillance to ensure continued safety and performance.[168,169] Accelerating this process requires proactive regulatory engagement, automated and scalable manufacturing systems, and coordinated multidisciplinary collaboration between engineers, biologists, and clinicians to refine design, improve functionality, and validate efficacy through adaptive clinical trial frameworks.[170]

Challenges and Translational Potential

The clinical application of biomimetic scaffolds for VML faces several persistent biological and technical challenges. A primary concern is immune rejection, as scaffolds derived from animal or donor tissues frequently trigger inflammatory responses that lead to fibrosis and impaired integration with host tissue.[40,171,172] This hostile immune environment not only compromises scaffold stability but also disrupts the delicate process of muscle regeneration, highlighting the urgent need for advanced biocompatible materials with immunomodulatory properties. Equally problematic is the difficulty in establishing functional vascular and neural networks within implanted constructs.[29,173] The absence of rapid blood vessel formation results in oxygen deprivation and cell death, while inadequate nerve connections prevent proper muscle reinnervation, ultimately leading to tissue atrophy and loss of contractile function. Current strategies have yet to reliably replicate the body's natural ability to restore these critical networks in large-scale defects.[174] Another major hurdle lies in recreating the native muscle ECM, which provides essential mechanical and biochemical guidance

for regenerating tissue. Scaffolds must precisely mimic the ECM's unique combination of structural anisotropy, elasticity, and dynamic signaling cues.[175,176] Even minor deviations in stiffness or architecture can misdirect cell alignment and impair muscle fiber maturation, significantly reducing regenerative outcomes. These challenges are further complicated by the stark differences between preclinical models and human patients.[168,177] Although, preclinical studies in VML consistently demonstrate substantial histological improvements following scaffold-based interventions, including increased myofiber cross-sectional area, enhanced vascularization, and improved innervation density. However, these structural and cellular gains frequently fail to translate into proportional recovery of functional outputs such as twitch and tetanic force. This divergence highlights a persistent translational gap between tissue-level regeneration and meaningful restoration of contractile performance as shown in Figure 5. This gap calls for better human-relevant models and precision medicine approaches to turn research breakthroughs into successful clinical outcomes.

Addressing these multifaceted biological barriers will require innovations in material science, vascularization techniques, neural integration strategies, and ECM fabrication, along with improved translational models that better reflect human pathophysiology. Only through such comprehensive advances can biomimetic scaffolds fulfill their potential for treating VML in clinical practice.[59,63,178]

Scalability remains a critical bottleneck while advanced fabrication techniques like electrospinning and 3D bioprinting enable precise control over microarchitecture, they struggle to produce clinically relevant volumes without compromising structural integrity, particularly when attempting to replicate the anisotropic organization of native muscle tissue.[179] Material variability further complicates production, as batch-to-batch inconsistencies in natural polymers lead to unpredictable mechanical properties and cellular responses, undermining therapeutic reliability.[180] Controlled delivery of bioactive factors adds another layer of complexity, as current encapsulation strategies for growth factors (VEGF, FGF, PDGF) lack the spatiotemporal precision required for optimal tissue guidance.[181] The technical challenges are related to manufacturing and commercialization barriers, including exorbitant GMP production costs (>$50,000 per batch for decellularized ECM scaffolds and supply chain limitations for clinical-grade reagents.[182] While emerging innovations like stimuli-responsive smart scaffolds, spatially patterned bioinks, and hybrid natural-synthetic systems

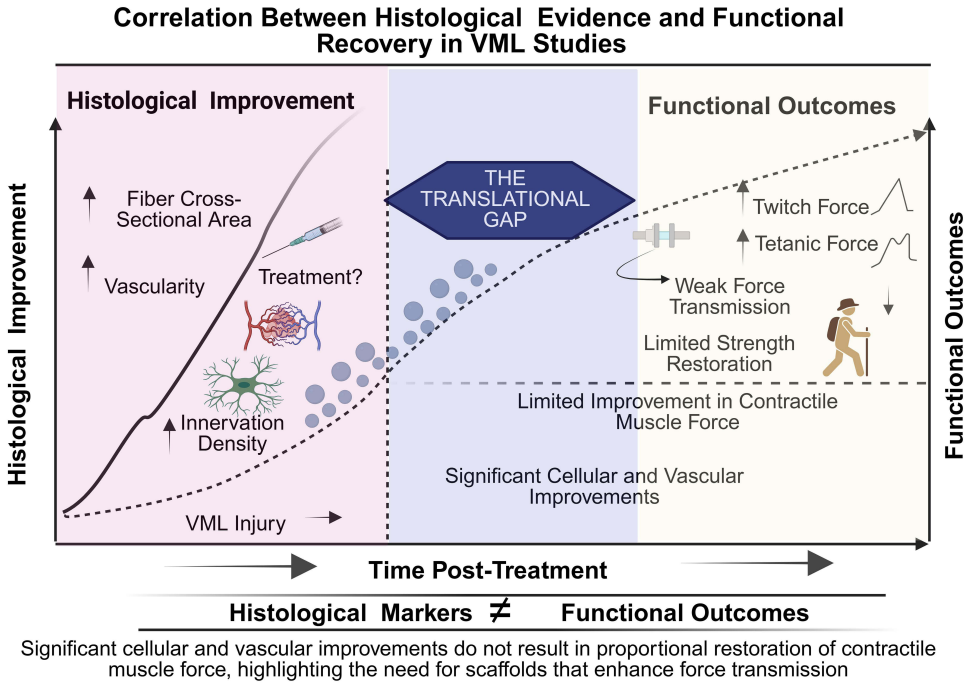

Figure 5 Functional recovery vs histological evidence (created with BioRender.com). Correlation between histological evidence and functional recovery in VML Studies. A dual-axis representation mapping histological markers (e.g, fiber cross-sectional area, innervation density) against functional outcomes (twitch/tetanic force). The overlay illustrates the "translational gap" often observed in preclinical studies, where significant cellular and vascular improvements do not always result in a proportional restoration of contractile muscle force, emphasizing the need for scaffolds that improve force transmission.

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offer promising solutions, significant development is still required to address the interrelated challenges of scalable production, material consistency, degradation control, bioactive delivery, and perfusion— all of which must be overcome to advance these technologies from promising laboratory prototypes to clinically viable therapies.[67,183]

Future Directions

The treatment of VML remains one of the most complex and unmet challenges in regenerative medicine. Despite significant progress in developing biomimetic scaffolds that recapitulate the native ECM and support muscle regeneration, substantial hurdles remain before these therapies can be widely implemented clinically.[184] Future research will depend on the integration of emerging technologies, advanced regenerative therapies, and personalized medicine approaches—underpinned by strong interdisciplinary collaboration and continued investment in translational research. To speed up the process, 1–3 years are necessary for refining scaffold composition, mechanical characteristics, and thorough pre-clinical testing. Followed by which another 3–5 years focus to be shifted towards advanced fabrication technologies which includes AI guided design and 3D bioprinting for production of patient-specific scaffolds with controlled cellular and biological cues. In long term (5–10 years), the scaffold should be clinically translated with adaptive biomaterials that is able to restore full muscle function.[185,186]

Technological advancements are revolutionizing how scaffolds for VML can be designed to match the geometry of the injury site and incorporate spatial gradients of cells, growth factors, and ECM proteins. This level of customization enhances tissue integration and may improve functional outcomes.[187] Another key innovation is the development of smart scaffolds engineered to possess responsive or adaptive properties. These include pH-sensitive,[188,189] thermosensitive,[190,191] and enzyme-responsive[192] materials that can alter their structure, stiffness, or release profiles in response to the local microenvironment. New emerging technologies like artificial intelligence and machine learning are helpful for transforming the scaffold design.[193] These advanced tools help in predicting the optimal scaffold architecture along with the materials to be used for catering various patient needs by integrating therapeutic elements like myoblast cells, growth factors, stem cells.

Recent advances in regenerative medicine demonstrate that combining biomimetic scaffolds with other innovative therapeutic approaches can significantly improve outcomes for VML treatment. A particularly effective strategy involves incorporating mesenchymal stem cells into scaffold designs. These cells show remarkable myogenic potential and secrete bioactive factors that enhance tissue repair. Research indicates that mesenchymal stem cells -seeded scaffolds promote muscle regeneration through multiple mechanisms: stimulating blood vessel formation, regulating immune responses, and facilitating the recruitment and differentiation of host cells.[46,194] Multifunctional scaffold integrating several cells, gene-editing technologies that provide a microenvironment to restore complex muscle architecture has shown potential in breakthrough applications. Gene therapy approaches, including CRISPR-Cas9 gene editing systems, represent another promising avenue for enhancing scaffold-based treatments.[195] These technologies enable precise modification of genetic pathways controlling muscle regeneration, such as activating pro-regenerative genes or correcting genetic defects that hinder repair processes. When delivered via biomimetic scaffolds, genetically modified cells can provide targeted therapeutic effects at the injury site.[196]

Emerging research highlights the potential of extracellular vesicle (EVs) and exosomes as cell-free therapeutic alternatives. These naturally occurring nanoparticles, derived from stem cells or immune cells, contain bioactive molecules (proteins, lipids, and RNAs) that can reduce inflammation, promote blood vessel growth, and stimulate muscle formation.[197,198] Incorporating EVs into scaffold systems offers advantages including localized, controlled release of therapeutic factors while potentially avoiding some of the immunological and regulatory challenges associated with cell-based therapies.[198]

The effective treatment of VML requires a multidisciplinary approach, integrating expertise from biomedical engineering, regenerative biology, materials science, rehabilitation medicine, and clinical practice to develop solutions that restore not only tissue structure but also functional strength and endurance.[11,148,199] Biomimetic scaffolds, when combined with tailored rehabilitation protocols, have demonstrated potential in enhancing neuromuscular integration and recovery, highlighting the need for close collaboration between tissue engineers and rehabilitation specialists to optimize both biological regeneration and mechanical performance.[200] Additionally, translational efforts must prioritize scalability,

regulatory compliance, and cost-effectiveness by standardizing fabrication processes, establishing reproducible preclinical models, and conducting rigorous long-term safety and efficacy assessments. Ensuring scaffold systems meet Good Manufacturing Practice (GMP) standards and align with clinical workflows will be critical for successful real-world implementation.[158]

Despite significant progress in the field, critical challenges remain in developing optimal ECM-based scaffolds, including determining ideal composition, mechanical properties, and degradation kinetics, as well as refining the dosage, timing, and delivery of adjunctive therapies like stem cells and growth factors to ensure long-term functional integration with native muscle and vasculature. Addressing these challenges requires increased investment in interdisciplinary research, with collaboration among funding agencies, academic institutions, and industry partners to accelerate next-generation regenerative therapies for VML.[111] Advancing clinical trials, streamlining regulatory pathways, and fostering public–private partnerships will be essential to translate these innovations into clinical practice. Biomimetic strategies that recreate native-like ECM environments, combined with stem cell therapy, gene editing, and personalized scaffold design within a collaborative translational framework, offer a transformative approach to restoring muscle function and improving the quality of life for patients with VML.[201]

Emerging Technologies

To further align biomimetic scaffold strategies for VML with emerging frontiers in regenerative engineering, it is essential to incorporate recent technological breakthroughs that reshape skeletal muscle regeneration. 3D bioprinting has emerged as a reliable approach for fabricating scaffolds with precise regulation of fiber alignment, pore geometry, and cell distribution. These features are crucial for recreating the anisotropic structure and contractile function of native muscle tissue. The three principal bioprinting modalities used for skeletal muscle engineering differ substantially in their printing mechanics, bioink requirements, and performance outcomes. Extrusion-based systems dispense continuous filaments of highly viscous bioinks through pneumatic or mechanical pressure, enabling fabrication of large, cell-dense constructs but exposing encapsulated cells to elevated shear stress and relatively coarse resolution.[202] Inkjet-based platforms generate discrete droplets using thermal or piezoelectric actuation, offering rapid and cost-effective patterning with reduced shear forces, although they are restricted to low-viscosity formulations and moderate structural resolution.[203] In contrast, laser-assisted bioprinting employs pulsed laser energy to transfer bioink droplets in a nozzle-free manner, minimizing mechanical stress on cells and achieving superior spatial precision, albeit with increased technical complexity and cost.[204] These distinctions highlight the need to align bioprinting modality with the specific architectural, mechanical, and cellular requirements of skeletal muscle regeneration which are described in Figure 6.

Apart from this, one of the most transformative advancements is 4D bioprinting, an evolution of conventional 3D bioprinting in which printed constructs are engineered to undergo controlled, time-dependent structural or functional transformations in response to external stimuli. In the context of VML, 4D bioprinted muscle constructs can be designed using shape-memory polymers or stimuli-responsive hydrogels that are engineered to respond to biochemical (pH, enzymes, reactive oxygen species), mechanical, or electrical signals present within injured muscle tissue.[205–207] This dynamic adaptability more closely mimics the native muscle microenvironment, where mechanical loading and electrical activity continuously influence fiber maturation and alignment. By enabling implanted scaffold to reorganize, 4D bioprinting addresses the limitation of conventional scaffolds, i.e. their inability to adapt to the evolving regenerative environment.[208]

For example, redox-responsive polymers can modulate growth factor release in oxidative inflammatory environments characteristic of acute VML, while mechanoresponsive matrices can alter stiffness in response to contractile forces, thereby promoting myogenic differentiation. Electrically conductive biomaterials incorporating graphene, polypyrrole, or gold nanostructures further enable synchronized myotube formation and neuromuscular integration by facilitating electrical signal propagation.[209]

Another transformative development is the application of organ-on-a-chip technologies to model VML pathophysiology and therapeutic screening. Microfluidic muscle-on-chip systems recreate the architectural alignment, vascular perfusion, and mechanical loading conditions of skeletal muscle in vitro, allowing real-time assessment of contractility, calcium flux, and metabolic activity.[210] These platforms enable high-resolution evaluation of scaffold–cell interactions

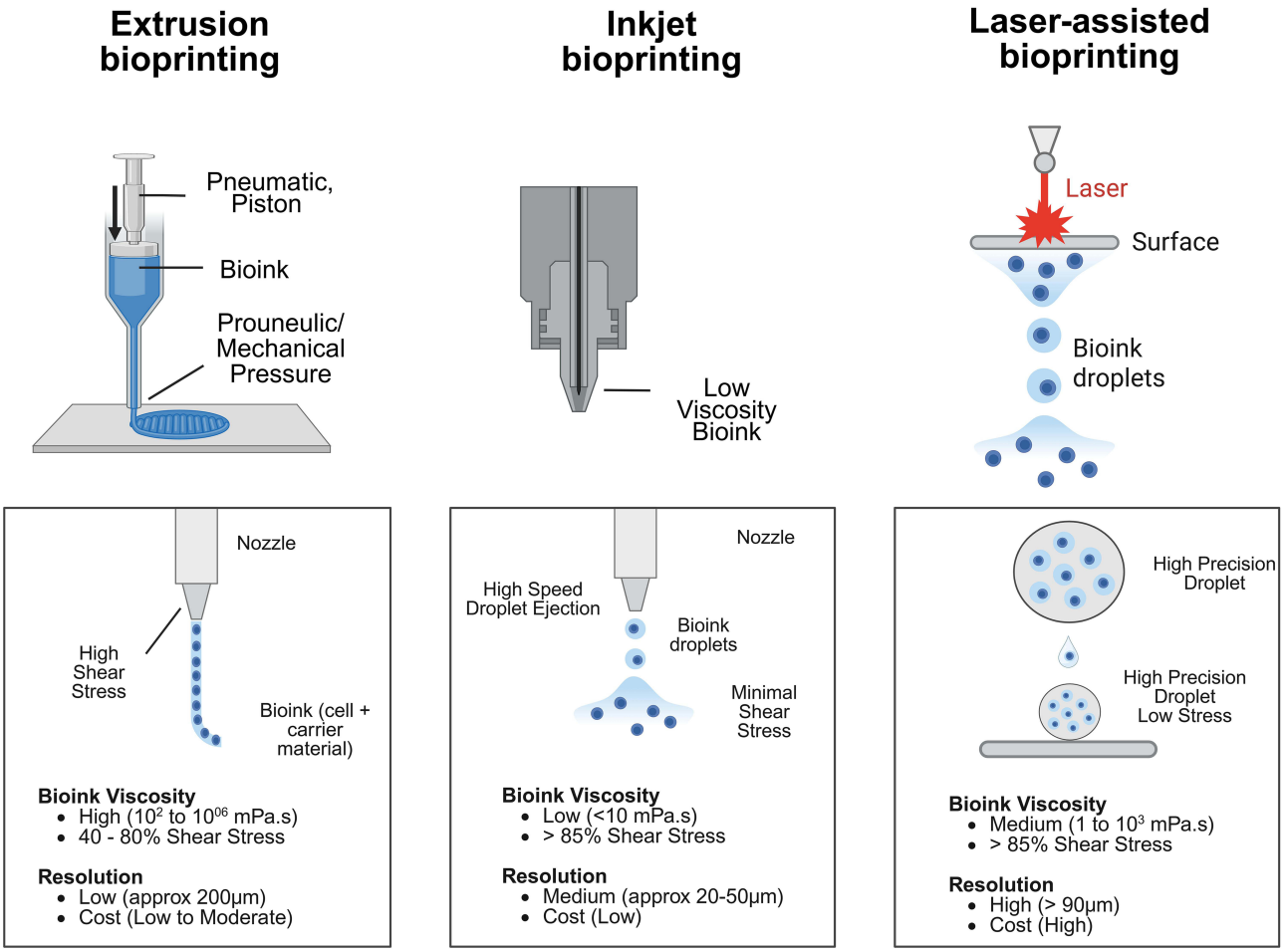

Figure 6 Technical comparison of the three primary 3D Bioprinting modalities for skeletal muscle engineering (created with BioRender.com). This illustration summarizes the mechanisms and comparative technical parameters of the three dominant bioprinting modalities used in the fabrication of skeletal muscle scaffolds: **Extrusion-Based Bioprinting**: Utilizes a piston or pneumatic-driven syringe to deposit continuous filaments of high-viscosity bioinks. It is highly effective for creating large-scale structural scaffolds but subject cells to high shear stress at the nozzle tip, which can lower viability (40–80%). It supports very high cell densities but offers lower resolution (~ 200um). **Inkjet-Based Bioprinting**: Employs thermal or piezoelectric actuators to generate discrete droplets of low-viscosity bioinks. While it provides faster printing speeds and lower costs, the low viscosity of the bioink often results in poor mechanical integrity of the final construct and limits resolution to a medium range (~ 20–50um). **Laser-Assisted Bioprinting**: Uses a laser pulse to create a high-pressure vapor bubble on a donor "ribbon", propelling precise droplets onto a substrate. This nozzle-free approach eliminates shear stress, maintaining high cell viability (> 95%) and achieving the highest resolution (< 10um), though it is characterized by higher operational costs and technical complexity. The summary metrics below the schematics provide a quick reference for Bioink Viscosity, Resolution, Cell Viability, and Manufacturing Cost to assist in selecting the optimal modality based on specific muscle tissue engineering requirements.

under dynamic conditions that better approximate in vivo physiology than traditional static culture models.[211] Moreover, integrating immune or endothelial compartments into multi-organ chips can elucidate the complex inflammatory and angiogenic cascades following volumetric muscle injury. Such systems not only accelerate preclinical optimization of biomimetic scaffolds but also reduce reliance on large-animal models by providing predictive, human-relevant data on functional restoration.[212]

Artificial intelligence (AI) and machine learning–driven scaffold design further represent a paradigm shift in regenerative biomaterials.[168] AI algorithms can analyze multidimensional datasets encompassing material composition, pore architecture, mechanical properties, and biological outcomes to identify optimal design parameters for muscle regeneration.[213] Generative modeling approaches enable rapid virtual prototyping of scaffold architectures tailored to patient-specific defect geometries derived from imaging data. Additionally, predictive modeling of growth factor kinetics, degradation profiles, and cell matrix interactions can streamline translational development by minimizing empirical trial-and-error experimentation. By integrating computational modeling with advanced manufacturing platforms such as

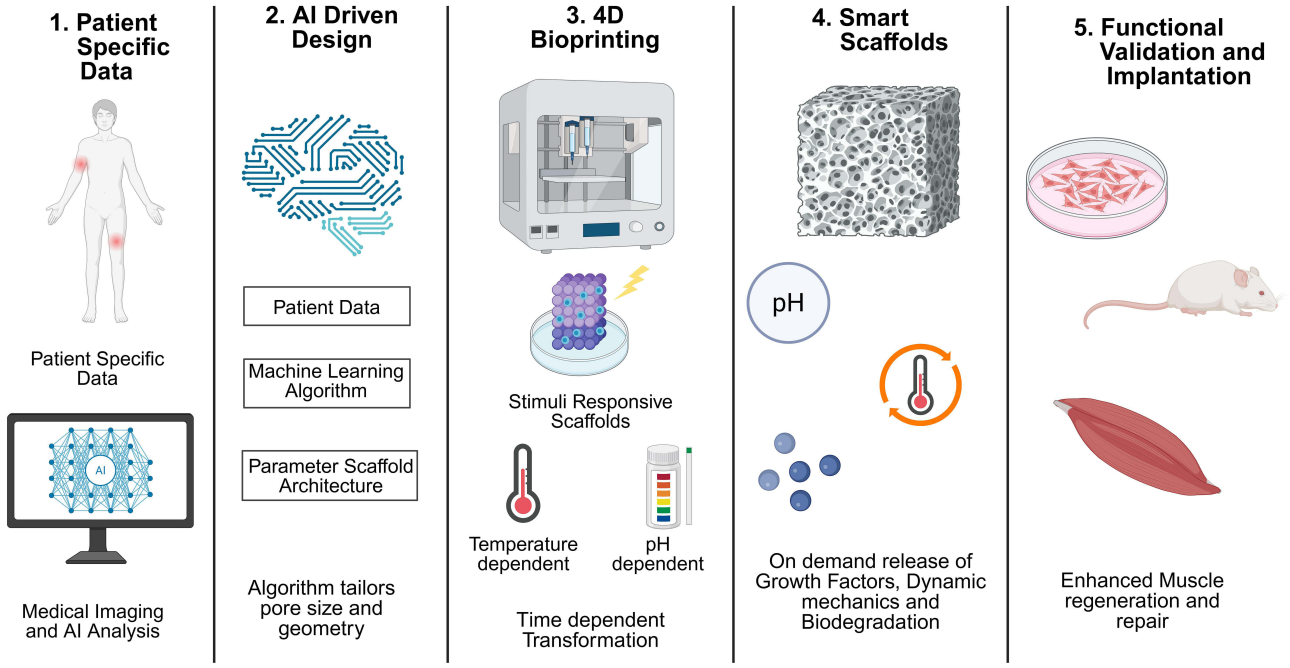

Figure 7 Emerging technologies revolutionizing scaffold design for VML treatment (created with BioRender.com). Integration of advanced technologies into the scaffold design workflow. (1) Patient Specific data is generated using medical imaging techniques (2) AI-Driven Design utilizes machine learning to optimize pore geometry based on patient-specific imaging. (3) 4D Bioprinting enables time-dependent structural changes in response to external stimuli. (4) Smart Scaffolds incorporate stimuli-responsive materials (pH, temperature, enzymes) for on-demand growth factor release, (5) validating it further for personalized and functional tissue engineering solutions.

bioprinting, AI-guided design fosters precision-engineered scaffolds capable of delivering reproducible and scalable therapeutic outcomes.[214]

Collectively, these emerging technologies signal a transition from static, structure-focused constructs to adaptive, data-driven, and physiologically integrated regenerative systems. Incorporating 4D bioprinting, smart scaffolds and AI-enabled design into VML research frameworks not only enhances mechanistic understanding but also establishes a forward-looking roadmap for clinically translatable muscle regeneration strategies which is described in Figure 7.

Conclusion and Outlook

VML remains a significant clinical challenge, characterized by irreversible tissue damage and functional impairment that profoundly impacts patients' lives. Current surgical treatments, including autologous muscle transfers, often provide incomplete recovery and introduce additional complications.

This review underscores the critical role of the ECM in muscle regeneration, serving as both a structural framework and a bioactive signaling platform that guides satellite cell behavior, angiogenesis, and neuromuscular reinnervation. Advances in biomimetic scaffold design, incorporating key ECM components such as laminin, elastin, fibrin, have demonstrated improved muscle fiber alignment, vascular network formation, and functional outcomes in preclinical studies.

This review emphasizes ECM-based biomimetic scaffolds as a transformative strategy in volumetric muscle loss (VML) management, shifting the paradigm from passive tissue replacement to active, instructive regeneration. The combination of the structural architecture, mechanical properties, and the signaling pathway of native ECM, substantiate the scaffolds to promote myogenesis, immune regulation, and muscle remodeling. The major outcome is the successful VML repair depends not only on replacing lost tissue volume but also by coordinating cellular interactions that restore muscle integrity.

However, replicating the native ECM's intricate architecture, controlling immune responses, ensuring consistent graft integration, scalability and compliance of regulatory requirements still remain key challenges. Overcoming these barriers demands a multidisciplinary approach, uniting experts in bioengineering, materials science, immunology, and clinical

rehabilitation to optimize scaffold design, manufacturing processes, and therapeutic protocols. Future progress will depend on integrating innovative techniques such as 3D bioprinting, dynamic biomaterials, and cell-based therapies to create patient-specific solutions. By aligning technological advancements with clinical needs, researchers can develop more effective and scalable treatments. In summary, ECM-inspired regenerative strategies represent a transformative opportunity in VML treatment. These approaches not only address structural repair but also aim to restore full muscle function, offering patients a meaningful improvement in quality of life. Equally important is developing the scalable and regulatory compliant materials of clinical relevance. The clinical trial should ensure robust outcomes with respect to strength and muscle recovery along with functional restoration thereby transforming the standard of care for patients suffering from VML.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflicts of interest in this work.

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