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Review

Challenges to acellular biological scaffold mediated skeletal muscle tissue regeneration

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ABSTRACT

Volumetric muscle loss (VML) injuries present a complex and heterogeneous clinical problem that results in a chronic loss of muscle tissue and strength. The primary limitation to muscle tissue regeneration after VML injury is the frank loss of all native muscle constituents in the defect, especially satellite cells and the basal lamina. Recent advancements in regenerative medicine have set forth encouraging and emerging translational and therapeutic options for these devastating injuries including the surgical implantation of acellular biological scaffolds. While these biomaterials can modulate the wound environment, the existing data do not support their capacity to promote appreciable muscle fiber regeneration that can contribute to skeletal muscle tissue functional improvements. An apparent restriction of endogenous satellite cell (i.e., pax7^{+}) migration to acellular biological scaffolds likely underlies this deficiency. This work critically evaluates the role of an acellular biological scaffold in orchestrating skeletal muscle tissue regeneration, specifically when used as a regenerative medicine approach for VML injury.

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1. Introduction

Skeletal muscle regeneration after traumatic injuries is often incomplete and can lead to permanent disability [1,2]. Particularly challenging are injuries in which a critical portion of a muscle or muscle unit is abruptly removed, either by trauma or surgery. This type of muscle injury, named volumetric muscle loss (VML) injury [3], is irrecoverable both in terms of contractile tissue and strength loss, and there is currently no standard regenerative therapy for this condition. Volumetric muscle loss injury within the abdominal, extremity, and facial muscles has been the context for which many tissue engineering and regenerative medicine therapies have been developed or tested, particularly over the past decade, during which VML was presented frequently among United States Servicemembers with battlefield injuries [1,4]. In general, these approaches implant surrogates for one or more of the missing components of native skeletal muscle that are influential, if not required, to regenerate muscle tissue (e.g., satellite cells and basal lamina). The primary purpose of these therapies is to promote *de novo* muscle tissue regeneration that integrates with the remaining host musculature and contributes to active force production.

A vital component of therapies for VML injury is a scaffold, upon which skeletal muscle regeneration may be initiated. In particular, acellular biological scaffolds, which are processed, decellularized extracellular matrices, have been investigated in numerous preclinical animal models [5–12] and VML patients [13,14]. Acellular biological scaffolds have characteristics that embody an ideal near-term therapy for VML repair because autologous donor muscle tissue is not required and there are existing FDA-approved acellular biological scaffolds with demonstrated safety for other soft tissue applications. That being said, there is significant concern within the field about the apparent sub-physiological levels of muscle tissue regeneration consistently observed following scaffold implantation. The low regenerative performance of biological scaffolds appears to be due to incongruity with the complex spatiotemporal events that are known to underlie mammalian skeletal muscle regeneration.

This work reviews the literature investigating acellular biological scaffolds specifically for repair of VML and VML-like injuries.

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The collective experimental successes and failures of these studies are examined under two primary tenants. First, that scaffold-mediated skeletal muscle regeneration follows canonical mechanisms of endogenous adult skeletal muscle regeneration. And second, that functional deficits after VML injury are not completely due to a loss of muscle tissue and therefore present multiple therapeutic targets.

2. What is volumetric muscle loss?

Mammalian skeletal muscle is highly plastic and possesses a remarkable capacity to self-repair and regenerate after non-destructive forms of injury. A defining characteristic of these recoverable injuries is that components of the damaged skeletal muscle, such as satellite cells [15] and the basal lamina [16,17], remain *in situ*. It is these remaining components that are essential for muscle fiber regeneration. In contrast, traumatic muscle injuries often abruptly remove a portion of muscle and therefore effectively ablate all essential regenerative components from the defect. The remaining musculature does not spontaneously orchestrate regeneration of the ablated muscle tissue [18–20]. The result is a chronic loss of contractile skeletal muscle tissue and extensive fibrotic tissue deposition [19]. These injuries are exceptionally complex and heterogeneous and there are no regenerative medicine therapies currently available to overcome the catastrophic loss of tissue [21]. This destructive form of injury has been defined as VML injury [3].

2.1. Epidemiology of VML injury

The incidence of VML injury is not currently known but is pervasive in musculoskeletal trauma. As an estimate in civilian trauma, approximately ~250,000 open fractures occur per year in the US, which commonly involve VML injury [22]. In military populations, epidemiological assessment of battlefield injuries among approximately 14,500 Servicemembers that were evacuated from war from 2001 to 2013 [23,24] suggests a high relative incidence of VML injury. Approximately 77% of all evacuated Servicemembers had musculoskeletal injuries [25] and commonly presented open soft tissue extremity wounds [2,25]. Because no regenerative standard of care exists for VML injuries in civilian or military medicine and this type of injury is by definition irrecoverable, the prevalence of unresolved VML injury among civilian and military trauma patients is high.

Volumetric muscle loss contributes to disability in civilian and military trauma patients. In civilian trauma, soft tissue injuries have been shown to largely impact decision making regarding limb salvage versus amputation [26], with only approximately half of trauma patients with severe lower extremity injuries returning to work [27]. A prospective study of patients with severe soft tissue injuries that threaten limb salvage also indicated that already poor functional outcomes continue to decline from 2 to 7 years post-injury [27,28]. The impact of VML injury has been more definitively identified in military trauma. In a cohort of wounded Servicemembers with type III open tibia fracture [1], retrospective analysis revealed that VML injuries contributed to the majority (65%) of a permanent disability rating by a military medical review board. Also, within a cohort of all medical evacuees with battlefield trauma (i.e., various types of injuries), 8% of patients received a disability rating specifically for their VML injury, which also may worsen as post-injury time increases [29]. Among each of these cohorts, the estimated life-time disability cost per patient is $340,000–440,000, which does not account for medical costs, loss of lifetime wages, or Veteran's Affairs disability-related costs.

2.2. Chronic loss of function in VML patients

Volumetric muscle loss injuries cause a chronic loss of muscle strength, range of motion, and limb function, and can directly lead to disability [1,3,13,19,29]. The course of care and common clinical outcomes are characterized in case studies of Servicemembers with VML injury. A 19 year old Servicemember underwent two phases of physical therapy each of ~1.75 years without achieving significant restoration of function [13]. In comparison to the unaffected contralateral limb there was a ~72% deficit in isokinetic strength of the injured limb ~3.5 years post injury. A second case study described a 23 year old Servicemember with VML in the anterior and posterior compartment of the lower limb [19]. This patient had noteworthy limitations of active and passive range of motion about the ankle, in addition to significant limitations in the performance of standardized functional tasks (e.g., stair climbing) ~1.5 years post-injury. At this time, dorsi- and plantar-flexor isometric and isokinetic torque were reduced by 34–100% compared to the contralateral uninjured limb. Lastly, a 28 year old Servicemember with lower extremity VML injury presented full passive range of motion, but reduced active range of motion [30]. The patient presented an ~43% deficit in lower extremity function as examined by a battery of tests. All patients presented VML concomitant to fractures at the time of their initial trauma and in each case their fracture eventually healed. Also, all patients underwent prolonged physical therapy without achieving acceptable functional improvements. Collectively, these patients therefore highlight a common outcome following musculoskeletal trauma, successful fracture healing followed by persistent functional deficits due to unrepaired VML injury.

2.3. Animal models of VML present persistent strength deficits

Persistent functional deficits are effectively modeled in animals by the surgical creation of VML injury [21]. Isolated and composite tissue injury models of VML have been created in the mouse [7,12,14,31–33], rat [6,10,11,20,34–43], rabbit [44], dog [9,45], and pig (Figs. 1 and 2). Models that permit orthodox neuromuscular strength assessments have demonstrated persistent maximal isometric torque (i.e., strength) deficits at prolonged times post-injury [21]. The prolonged functional deficits observed, even with a relatively small VML injury (e.g., 20% loss of estimated muscle mass), are in stark contrast to the recovery of strength observed following canonical muscle fiber repair and regeneration after recoverable muscle injuries [19,46–49]. Histological assessment of the VML injured tissue indicates gross fibrotic tissue deposition within the VML defect and a chronic reduction in the number of muscle fibers within defect area of the traumatized tissue [6,18–20,36]. Interestingly, recent analyses [21] also indicated that persistent strength deficits are disproportionately large compared to the magnitude of VML (e.g., 10% initial loss of estimated muscle mass resulted in an ~80% strength loss [40]). Together, these findings highlight that VML-related strength deficits are caused by the immediate loss of muscle fibers and sub-optimal performance of the remaining musculature. Thus, VML injuries present multiple therapeutic targets, to include *de novo* muscle fiber regeneration, that may be treated to improve muscle function.

3. What are acellular biological scaffolds and how do they work?

Acellular biological scaffolds are the extracellular matrix remnants of decellularized tissues [for review see Ref. [50]]. Biological scaffolds are thus primarily comprised of organized collagenous structures enriched with various other extracellular matrix