

BIOLOGIC SCAFFOLDS FOR MUSCULOTENDINOUS TISSUE REPAIR

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Abstract

Traumatic injuries to the musculoskeletal system are common events and volumetric muscle loss (VML) is no longer a rare occurrence. Surgical intervention is typically the only option for restoration of partial function. Surgical intervention for VML however does not regenerate the lost tissue and typically results in alterations of both the anatomy and biomechanics at the site of injury. Non-traditional approaches to the restoration of functional musculoskeletal tissue, including those provided by tissue engineering and regenerative medicine strategies, become viable alternative therapies when the expected outcome is bleak. One such strategy involves the delivery of constructive cues and modulation of the micro-environmental niche *via* biologic scaffold materials. These materials ideally retain the native structure and composition of the extracellular matrix of the tissue from which they are derived. Some of the recent advances in the use of biologic scaffolds to target key stages of the musculotendinous repair process and promote the restoration of functional tissue are described herein.

Keywords: Extracellular matrix; biologic scaffold; skeletal muscle; tendon; repair; tissue regeneration.

Introduction

Traumatic injuries to the musculoskeletal system are common events and volumetric muscle loss (VML) is no longer a rare occurrence. Survival following limb salvage for extremity tumors is contributing to the increased incidence of VML. More than 55 % of sport-related injuries involve skeletal muscle and/or tendon (Beiner and Jokl, 2001; Counsel and Breidahl, 2010; Garrett, 1996; Jarvinen *et al.*, 2005; Jarvinen *et al.*, 2000; Lehto and Jarvinen, 1991) and the most common injuries involve tears, lacerations and contusions, which have a robust capacity for restoration of full function. The presence of mononuclear myogenic satellite cells is largely responsible for the regenerative potential of skeletal muscle (Beiner and Jokl, 2001; Garrett, 1996; Jarvinen *et al.*, 2005; Jarvinen *et al.*, 2000; Lehto and Jarvinen, 1991). However, if more than 20 % of a given muscle is lost, the natural repair process will typically fail to repair the defect resulting in scar tissue formation and a loss of function (Aarimaa *et al.*, 2004; Crow *et al.*, 2007; Garrett *et al.*, 1984; Menetrey *et al.*, 1999; Terada *et al.*, 2001). In such cases, surgical intervention may be the only option for restoration of partial function. However, surgical intervention does not regenerate the lost tissue and typically results in alterations of the anatomy and biomechanics at the site of injury (Tesch *et al.*, 2008). It is not uncommon for extensive traumatic injury to result in amputation.

Non-traditional approaches to the restoration of functional musculotendinous tissue, including those provided by tissue engineering and regenerative medicine strategies, become viable alternative therapies when the expected outcome is bleak. As our understanding of developmental biology and the cellular and biochemical cues associated with tissue and organ formation, including musculotendinous tissue, has increased, an *in vivo* regenerative medicine strategy has emerged. This strategy involves the delivery of constructive cues and modulation of the microenvironmental niche *via* biologic scaffold materials. These materials ideally retain the native structure and composition of the extracellular matrix (ECM) of the tissue from which they are derived. The pathobiology of musculotendinous injury repair and some of the recent advances in the use of biologic scaffolds to target key stages of the repair process and promote the restoration of functional tissue are described herein.

Pathobiology of musculotendinous tissue repair

To discuss the concepts of tissue repair, tissue regeneration and constructive remodeling as they relate to

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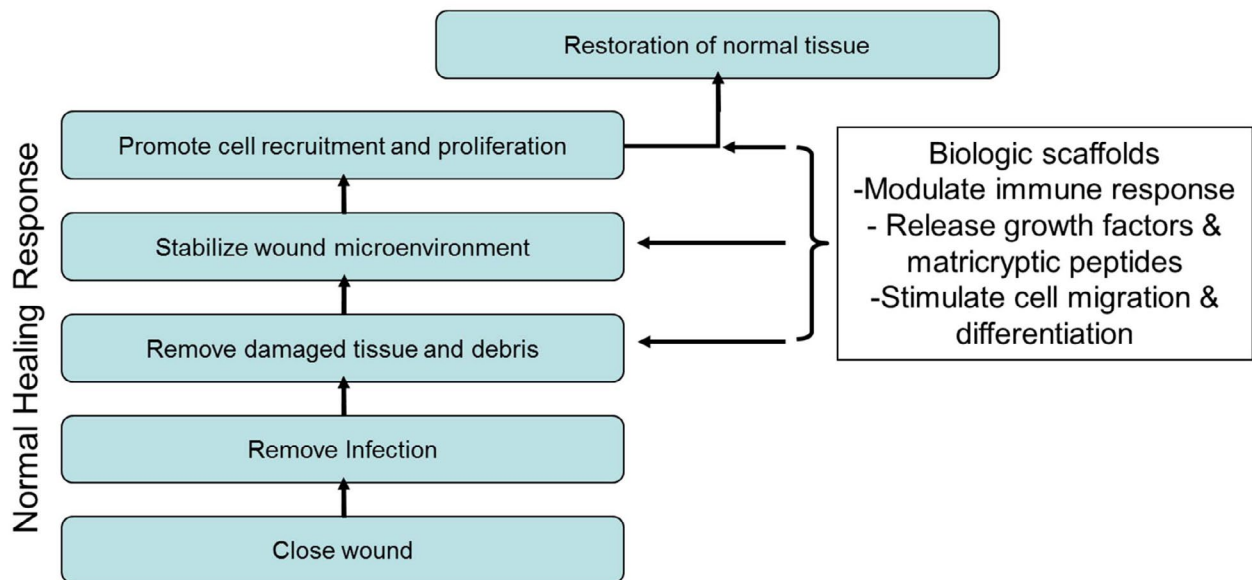


Fig. 1. An overview of wound healing and the potential for intervention with biologic scaffolds.

musculoskeletal regenerative medicine, an understanding and definition of these terms as used in this manuscript is necessary. All tissues and organs within the body have a capacity to repair, but very few have a capacity to regenerate. Tissue repair represents a response to damage or loss of tissue that leads to restoration of tissue continuity by the formation of scar tissue without the complete replacement of normal functional tissue. In contrast, regeneration restores tissue structural and functional integrity through the synthesis of new tissue that is specific and appropriate to that anatomic location. Stated differently, regeneration restores normal tissue structure and function while repair does not. Adults typically respond to traumatic injury, in most tissues, by repairing the injured anatomic site. However, certain body sites, such as the bone marrow, selected epithelial tissues and liver, have the capacity for spontaneous regeneration following injury. The goal of a regenerative medicine approach is to modify the default response to injury toward a regenerative process.

While true regeneration would result in tissue that was indistinguishable from uninjured tissue, the response elicited by inductive biologic scaffolds may more appropriately be termed constructive remodeling; i.e. the restoration of at least partial, site-appropriate, structure-function relationships. Fig. 1 shows an overview of the wound healing process and the potential points for intervention to enhance the healing process.

The normal mammalian response to tissue injury occurs in a continuum of overlapping but distinct stages: hemostasis, inflammation, new tissue formation and tissue remodeling (Gurtner *et al.*, 2008). This repair process has been described in detail (Garrett *et al.*, 1984; Huard *et al.*, 2002; Jarvinen *et al.*, 2005; Lehto and Jarvinen, 1991) and a brief overview is described herein. The first stages of wound repair, hemostasis and inflammation, occur immediately following tissue injury.

Polymorphonuclear leukocytes migrate to the wound site in response to molecular signals from damaged cells. Mononuclear cells, including monocytes that differentiate into proinflammatory macrophages, subsequently engulf and/or attempt to destroy any pathogens and necrotic debris. Macrophages play a critical role in the wound healing process, particularly that which occurs following skeletal muscle injury. Macrophage populations with a preferred phenotype sequentially accumulate in the injured muscle tissue. First, pro-inflammatory, M1 phenotype macrophages phagocytose necrotic tissue and secrete a host of cytokines that stimulate the chemotaxis and proliferation of resident progenitor cells. The M1 macrophages are followed by tissue remodeling M2 phenotype macrophages, which facilitate progenitor cell differentiation (Tidball and Villalta, 2010).

The first week to 10 days following injury is characterized by the migration and proliferation of cells within the wound site. During the repair process, nerves and blood vessels advance into the wound area. In skeletal muscle, tissue satellite cells migrate and differentiate into myoblasts that fuse with other myoblasts or with existing myofibers to form new skeletal muscle. In tendon tissue, fibroblasts and tenocytes begin to replace the necrotic tissue of the wound with new ECM, including the formation of granulation tissue. The ECM produced by these fibroblasts is mostly collagen and will ultimately constitute the bulk of the ECM within the mature tissue. Furthermore, some fibroblasts differentiate into contractile myofibroblasts, which contribute to wound contraction. There is intense interest, and controversy, regarding the contribution of stem and progenitor cell populations, such as bone marrow derived cells, to tissue repair either through differentiation or by secretion of paracrine factors that affect surrounding cells (Quintero *et al.*, 2009; Sun *et al.*, 2009; Tedesco *et al.*, 2010; Ten Broek *et al.*, 2010).

The final stage of wound repair, remodeling, may take up to 12 months to complete and is characterized by a reduction in cell density at the wound site and maturation and organization of the ECM in response to focal microenvironmental factors, such as mechanical loading and blood supply. Macrophages, fibroblasts and myofibroblasts that are present within the wound site undergo apoptosis or migrate from the wound. In skeletal muscle this remodeling process can often be associated with marked reorganization of the muscle tissue, including the formation of forked fibers, satellite myofibers or orphan myofibers that form outside the basal lamina (Schmalbruch, 1976) and the ends of the damaged muscle fibers may not reunite, but instead form a new myotendinous junction with the interposed scar tissue. In tendinous injuries, the fibroblasts and tenocytes that remain within the wound secrete matrix metalloproteinases (MMPs), which facilitate the turnover of the ECM and the formation of a more mature tissue that consists predominantly of type I collagen, which strengthens the repaired tissue.

Macrophages in response to skeletal muscle injury

The type and intensity of the inflammatory response following injury defines the dynamic microenvironment that influences either a scarring or a constructive remodeling response. The role of macrophages in skeletal muscle injury and repair has been extensively reviewed by Tidball (Tidball, 2005; Tidball and Villalta, 2010; Tidball and Wehling-Henricks, 2007). The macrophage represents a bone marrow-derived cell that circulates as a monocyte, exits the peripheral circulation in response to chemotactic stimuli, and subsequently differentiates into a macrophage. Macrophage phenotype and the role of macrophages in normal and pathologic processes have been the subject of intense study during the past decade (Delavary *et al.*, 2011; Fleming and Mosser, 2011; Martinez *et al.*, 2008; Ploeger *et al.*, 2012; Tidball and Villalta, 2010). It is now recognized that a wide spectrum of macrophage phenotypes exists, ranging from the classically activated proinflammatory M1 macrophage to the regulatory, tissue rebuilding M2 macrophage phenotype. Phenotype is determined by the profile of secreted cytokines, chemokines, effector molecules, and cell surface markers. These secreted molecules contribute to the local tissue milieu, which, in turn, has marked effects on downstream events.

The initial response to skeletal muscle injury involves a proinflammatory M1 macrophage population. The secreted effector molecules of this phenotype stimulate proliferation and migration of muscle progenitor cells, including perivascular stem cells and satellite cells (Al-Shanti *et al.*, 2008; Chen *et al.*, 2005; Lolmede *et al.*, 2009; Torrente *et al.*, 2003; Wang *et al.*, 2008; Warren *et al.*, 2002). However, these accumulated cells fail to mature and differentiate into functional myocytes without an associated transition of the macrophage phenotype population from an M1 to the more constructive M2 phenotype (Szalay *et al.*, 1997; Tsujinaka *et al.*, 1996). The shift in phenotype from M1 to M2 macrophages coincides with, and is necessary for, myogenic differentiation (St

Pierre and Tidball, 1994), which is severely disrupted if M2 macrophages are depleted (Tidball and Wehling-Henricks, 2007). It is unknown whether there is plasticity among the tissue macrophages; that is, it is unknown whether the M1 phenotype transitions to an M2 phenotype *in situ* or if a separate population of infiltrating macrophages, the M2 phenotype, replaces the early arriving M1 cells, although there is evidence to suggest phenotype plasticity (Ploeger *et al.*, 2012). Regardless, this transition is necessary for the full process of skeletal muscle regeneration to occur. The spatial and temporal pattern of this process is an area of great interest and a more detailed understanding of the process may lead to novel strategies to promote and facilitate skeletal muscle regeneration following injury. In addition, the apparent requirement for macrophages in the restoration of skeletal muscle raises interesting questions regarding the use of anti-inflammatory medications and how these affect macrophage polarization.

Biologic scaffolds in skeletal muscle repair

As described above, the natural healing response of musculotendinous injuries follows a series of distinct phases. Biologic scaffolds have been shown to effectively reprogram key stages of the wound repair by altering the wound microenvironment from one that promotes fibrosis and scar tissue formation to one that stimulates constructive remodeling of the injured tissue (Badylak *et al.*, 2011). The ability of biologic scaffolds to alter the macrophage phenotype response, coupled with the release of latent growth factors and chemotactic degradation products, have made these materials attractive as scaffolds to promote skeletal muscle reconstruction following trauma and volumetric loss (Mase *et al.*, 2010; Merritt *et al.*, 2010; Turner *et al.*, 2010).

As shown in Tables 1 and 2 a wide variety of biologic scaffolds are now commercially available that differ in their tissue source, species of origin, and methods of preparation, and a large number of animal models exist by which these biologic scaffolds are evaluated. Typically, these materials are xenogeneic in origin and would, therefore, be anticipated to elicit a proinflammatory reaction when implanted. However, when processed in a manner that effectively removes resident cells and minimally distorts the native ultrastructure, biologic scaffolds induce a macrophage response that rapidly transitions from a proinflammatory M1 phenotype to one that is predominately M2 (Ariganello *et al.*, 2011; Badylak *et al.*, 2008; Brown *et al.*, 2009; Valentin *et al.*, 2009).

While the underlying mechanisms by which biologic scaffolds direct M1 vs. M2 polarization are still largely unknown, a number of important factors that influence this process have been identified. Processing methods play a critical role in determining the type of host response which occurs (Valentin *et al.*, 2009). Typically, ECM-based scaffolds that are not chemically cross-linked and which have minimal residual DNA content (at or below 50 ng/mg ECM dry weight) elicit an M2 macrophage polarization response with associated constructive tissue remodeling (Keane *et al.*, 2012; Valentin *et al.*, 2009). Chemically

Table 1: Examples of FDA approved biologic scaffolds.

PRODUCT	COMPANY	MATERIAL
<i>Porcine Tissue</i>		
Oasis® Wound Matrix	Cook® Biotech / Healthpoint	Non-crosslinked small intestinal submucosa ECM
Surgisis®	Cook® Medical	Non-crosslinked small intestinal submucosa ECM
Cuffpatch™	Biomet Sports Medicine	Crosslinked, hydrated small intestinal submucosa ECM
Restore®	DePuy	Non-crosslinked small intestinal submucosa ECM
Pelvicol®	C.R. Bard Inc.	Crosslinked, hydrated dermal ECM
Permacol™	Covidien	Crosslinked, hydrated dermal ECM
<i>Bovine Tissue</i>		
Xenform®	TEI Biosciences / Boston Scientific	Non-crosslinked fetal dermal ECM
Veritas®	Synovis® Surgical Innovations	Crosslinked pericardial ECM
Primatrix™	TEI Biosciences	Non-crosslinked fetal dermal ECM

Table 2: Examples of animal models of ECM-mediated skeletal muscle repair

Species	Muscle group	Mechanism of Injury	Reference
Mouse	Quadriceps	Partial resection of tensor fascia lata and rectus femoris	(Sicari <i>et al.</i> , 2012)
Mouse	Latissimus Dorsi	Resection of 18 mg tissue Resection of ~50 % of muscle	(Corona <i>et al.</i> , 2012) (Machingal <i>et al.</i> , 2011)
Mouse	Anterior Tibialis	Complete resection	(Perniconi <i>et al.</i> , 2011)
Rat	Abdominal wall obliques	Partial thickness partial resection	(Valentin <i>et al.</i> , 2010)
Rat	Lateral gastrocnemius	Partial resection	(Merritt <i>et al.</i> , 2010)
Rat	Rectus abdominus	Full thickness partial resection	(Vindigni <i>et al.</i> , 2004)
Rabbit	Abdominal wall	Partial resection of external oblique	(Gamba <i>et al.</i> , 2002)
Rabbit	Anterior tibialis	Compartment Syndrome	(Daly <i>et al.</i> , 2011)
Rabbit	Vastus Lateralis	Partial resection	(Kin <i>et al.</i> , 2007)
Dog	Quadriceps	Partial resection of vastus lateralis and vastus medialis	(Turner <i>et al.</i> , 2010)

cross-linked products, or poorly-decellularized tissues, elicit an M1, pro-inflammatory response with downstream fibrosis and scar tissue formation (Badylak *et al.*, 2008; Brown *et al.*, 2009; Keane *et al.*, 2012; Valentin *et al.*, 2009). These cross-linked products promote a foreign body response as the host macrophages cannot degrade the material and effectively isolate the scaffold from

the body in a fibrotic capsule. Conversely, degradable biologic scaffolds release matricryptic peptides and other bioactive molecules that signal a phenotypic change in the macrophages towards M2. The importance of macrophage phenotype in the repair of skeletal muscle has been clearly demonstrated, and the ability of an implanted material to influence this phenotypic switching may be critical in

promoting repair in severe traumatic injuries. Of note, acellular biologic scaffolds promote a more constructive remodeling M2 macrophage phenotype than even autologous tissue grafts when used in a model of muscle regeneration (Badylak *et al.*, 2008; Valentin *et al.*, 2009). Reider *et al.* (2005) showed that decellularization of tissues does not inhibit the activation of macrophages, while Ariganello *et al.* (2010) demonstrated that decellularization lowered esterase and phosphatase activity, suggesting that biologic scaffolds activate alternative (M2) signaling pathways. The ability of a biologic scaffold to promote an increase in M2 polarization may be a causative factor for improved myoblast differentiation and fusion and an increase in the amount of functional muscle restored following injury.

The manipulation of the macrophage response alone cannot account for the constructive remodeling responses that result from implantation of a biologic scaffold. Additional factors play an important role the ability of ECM scaffolds to manipulate the wound microenvironment, such as the release of latent growth factors and/or degradation products that can act as chemoattractant factors for myogenic precursor cells.

Growth factors are essential regulators of the muscle repair process, controlling the proliferation and differentiation of muscle progenitor cells (Charge and Rudnicki, 2004; Ten Broek *et al.*, 2010). The sequence of their release appears important to the control of muscle repair (Hayashi *et al.*, 2004). Many cells essential for skeletal muscle repair release latent growth factors that are sequestered by the ECM and which only become active following degradation of this ECM during muscle repair (Kresse and Schonherr, 2001; Miura *et al.*, 2006; Schultz and Wysocki, 2009). Biologic scaffolds are being used commonly to promote tissue repair in many body sites including muscle (Badylak, 2007; Borschel *et al.*, 2004; Jensen and Host, 1997; Owen and Shoichet, 2010; Robinson *et al.*, 2005; Silver and Pins, 1992; Turner *et al.*, 2010). Several studies have demonstrated that biologic scaffolds are rich in latent growth factors, particularly basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) which have been shown to be essential in the promotion of angiogenesis and constructive remodeling, particularly in the repair of skeletal muscle (Germani *et al.*, 2003; Menetrey *et al.*, 2000). The concentration of these growth factors within the biologic scaffolds can be altered by the methods used to prepare the material (Reing *et al.*, 2010). Equally, variations in growth factor content can occur as a consequence of source animal age (Tottey *et al.*, 2011b). Nonetheless, the significance of these growth factors in promoting constructive tissue remodeling is largely unknown as no studies exist where growth factors have been specifically depleted from these biologic materials. Potentially more important than overall growth factor content may be the distribution of these growth factors within the 3D architecture of the scaffold. Biologic scaffolds often possess anatomic site specificity; that is, their growth factor content and ultrastructural architecture are unique and specific to the source tissue from which they are derived. It may therefore be possible to tailor a biologic scaffold to maximize the constructive

remodeling response. For example, growth factors such as hepatocyte growth factor (HGF) and insulin like growth factor-1 (IGF-1) are known to be critical for muscle growth and myoblast proliferation (Chakravarthy *et al.*, 2001; Charge and Rudnicki, 2004; Hsu *et al.*, 1997; Menetrey *et al.*, 2000). HGF is a primary regulator of satellite cell proliferation (Allen *et al.*, 1995; Gal-Levi *et al.*, 1998; Li *et al.*, 2009; Sheehan *et al.*, 2000), with HGF expression increasing in proportion to the degree of injury (Suzuki *et al.*, 2002; Tatsumi, 2010; Tatsumi *et al.*, 2002). Thus, ECM derived from tissues rich in HGF, such as liver, may prove more beneficial to the skeletal muscle constructive remodeling response than ECM derived from tissues low in HGF.

The same proteases that degrade the remnant ECM of native tissue following injury are also responsible for degrading a surgically placed ECM scaffold. This degradation process releases small peptides, termed matricryptic peptides, which activate multiple cell surface receptors and alter the cellular response to tissue injury (Davis, 2010; Davis *et al.*, 2000). Calve *et al.* (2010) demonstrated that ECM deposited during amphibian limb regeneration provided instructional cues for both myoblasts and satellite cells that are vital for muscle repair. Similarly, degradation products of ECM, generated by *in vitro* digestion, have been shown to have potent biologic effects including mitogenic and chemotactic effects on muscle progenitor cells (Crisan *et al.*, 2009; Tottey *et al.*, 2011a; Vorotnikova *et al.*, 2010).

Biologic scaffolds have been successfully used to repair or replace a variety of damaged or diseased tissues, including cardiac (Akhyari *et al.*, 2008; Badylak *et al.*, 2006; Kochupura *et al.*, 2005), esophageal (Nieponice *et al.*, 2006; Nieponice *et al.*, 2008), dermal (Brigido *et al.*, 2004), and musculotendinous (Aurora *et al.*, 2007; De Deyne and Kladakis, 2005; Dejardin *et al.*, 2001; Snyder *et al.*, 2009) tissues, among others.

In the context of skeletal muscle repair, Conconni *et al.* (2005) have shown benefits using decellularized abdominal muscle seeded with cultured myoblasts. This group showed that, when implanted in the abdominal wall location, these constructs generated a complex skeletal muscle tissue that was contractile and vascularized within 9 days. Similarly, Merritt *et al.* (2010) have shown that a biologic scaffold seeded with bone marrow-derived stem cells can successfully regenerate lost muscle tissue in a mouse model of volumetric muscle loss in the quadriceps. In many cases, the acellular ECM alone is capable of restoring functional tissue without an additional cellular component. Valentin *et al.* (2010) showed that acellular ECM derived from small intestinal submucosa (SIS) induced almost complete replacement of the defect site with islands and sheets of skeletal muscle after 6 months in a rodent abdominal wall model. This new muscle generated ~80 % of the contractile force of native muscle (Valentin *et al.*, 2010). Similarly, in a model of severe gastrocnemius muscle trauma with massive tissue loss, the implantation of SIS-ECM promoted the formation of vascularized skeletal muscle that was functionally innervated and almost indistinguishable to native muscle by 6 months (Turner *et al.*, 2010). These findings have translated into the successful restoration

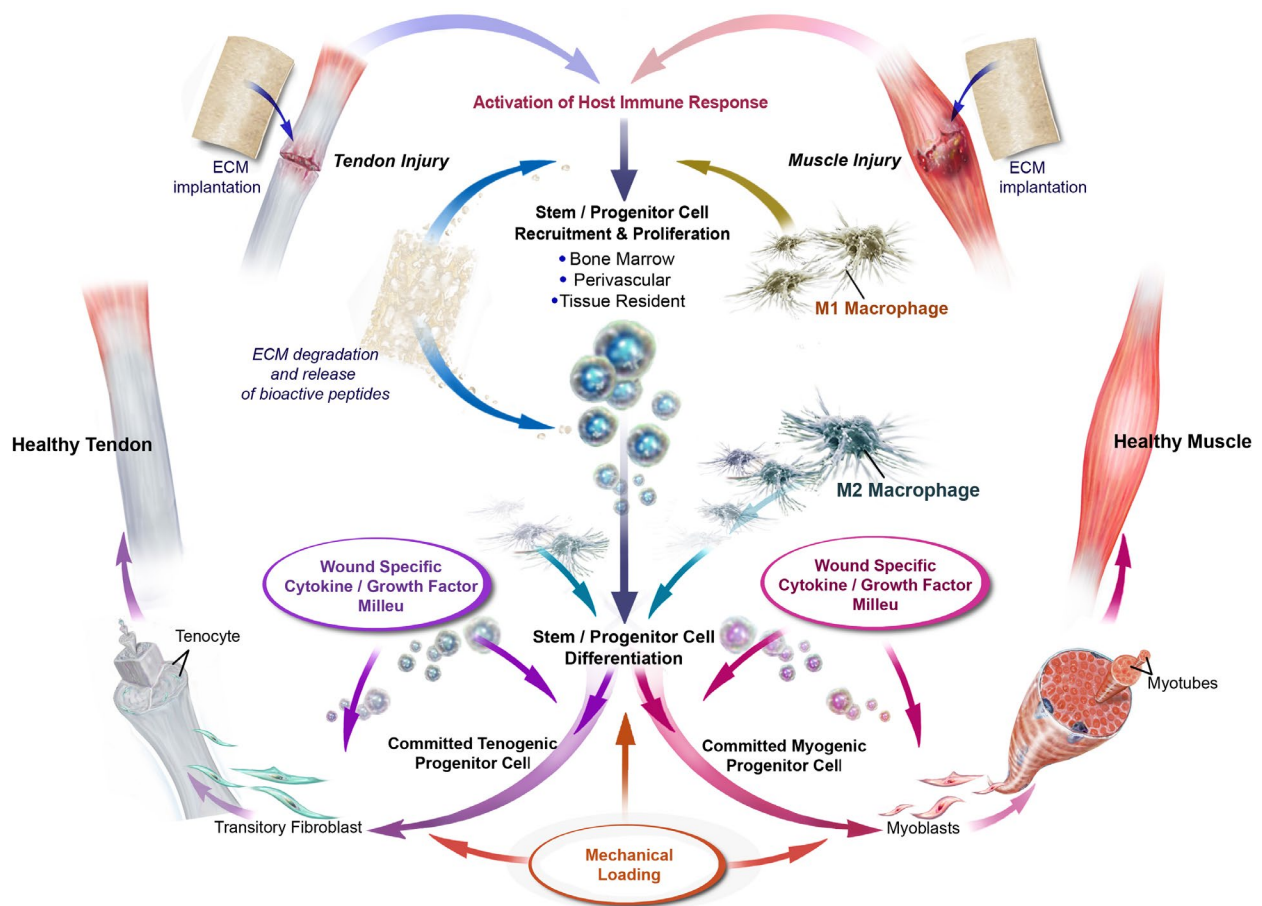


Fig. 2. Overview of ECM-mediated constructive remodeling of musculoskeletal injuries. Implantation of a biologic scaffold modifies the host immune response and stimulates progenitor cell recruitment and differentiation. Wound-specific factors determine cell fate resulting in site-specific tissue remodeling.

of skeletal muscle in human patients that have suffered volumetric muscle loss. Mase *et al.* (2010) have shown that placement of an ECM scaffold in a large quadriceps muscle injury, where all previous treatments had proven unsuccessful, resulted in the restoration of new functional skeletal muscle and a significant increase in isokinetic performance.

Stem and progenitor cells in biologic scaffold mediated musculoskeletal repair

As detailed in Fig. 2, the process of constructive tissue remodeling of either muscle or tendon tissue, which is induced by biologic scaffolds is complex and involves a number of factors including the unique wound microenvironment that defines the injury site. A key motivator of the constructive remodeling process is the recruitment, proliferation and differentiation of stem and progenitor cells elicited by the release of growth factors and degradation products from the biologic scaffolds. Following volumetric muscle loss, the proliferation of tissue resident cells and migration of circulating myogenic progenitor cells is particularly important and requires a cell population that is capable of sustained proliferation,

self-renewal, myogenesis and resistance to oxidative or hypoxic stress. Perhaps the most obvious cell population to target is the muscle satellite cell (Cossu and Biressi, 2005; Jarvinen *et al.*, 2005). However, these cells typically constitute only 1-3 % of total cells in skeletal muscle and in injuries with volumetric muscle-loss would be lost along with the excised tissue. A number of other myogenic stem cell populations have also been identified which are distinct from satellite cells, although the precise origin, identity and location of these cells remain speculative. These include myogenic progenitor cells characterized as CD56+, CD34-, CD144-, CD45- and CD146-; myo-endothelial cells characterized as CD56+, CD34+, CD144+, CD45- and CD146-; perivascular progenitor cells characterized as CD56-, CD34-, CD144-, CD45- and CD146+; and a muscle-derived side population (MDSP) that has similar properties to hematopoietic stem cells in the bone marrow (Crisan *et al.*, 2009; Deasy *et al.*, 2004; Huard, 2008; Jankowski *et al.*, 2002; Kallestad and McLoon, 2010; Lecourt *et al.*, 2010; Peault *et al.*, 2007; Qu-Petersen *et al.*, 2002; Quintero *et al.*, 2009; Ten Broek *et al.*, 2010; Usas and Huard, 2007; Wu *et al.*, 2010). The number of MDSCs increases rapidly following muscle trauma (Jackson *et al.*, 2011; Nesti *et al.*, 2008). Circulating CD133+ progenitor cells have also been identified with myogenic potential.

Following severe muscle trauma with massive tissue loss, replacement of the defect with an ECM-scaffold resulted in the recruitment of CD133+ cells to the site of injury, which was associated with the formation of new skeletal muscle and blood vessels in the first 4 months following injury (Turner *et al.*, 2010).

As an alternative strategy to *in situ* recruitment of cells, ECM has been commonly used as a delivery vehicle, where myogenic progenitor cells are seeded on a scaffold – which is then transferred to the *in vivo* site of interest (Beier *et al.*, 2009; Borschel *et al.*, 2004; Liao and Zhou, 2009; Merritt *et al.*, 2010; Moon du *et al.*, 2008; Vindigni *et al.*, 2004). However, these techniques generally produce muscle tissue with minimal contractile forces since the amount of scaffold material is high and can inhibit myoblast fusion. Many approaches use a gel-based construct of mainly collagen, laminin or fibrin that do not necessarily replicate the complex ultrastructure of native tissue (Beier *et al.*, 2009; Huang *et al.*, 2005; Matsumoto *et al.*, 2007; Rowlands *et al.*, 2007; Vandenburg *et al.*, 1988).

The use of acellular biologic scaffolds which maintain the complex 3D ultrastructure of the native tissue represents an alternative approach. For example, a biologic scaffold derived from skeletal muscle tissue may retain endomysial tubes and remnant neural and vascular pathways that can promote myoblast alignment and fusion and stimulate neurovascular growth (Borschel *et al.*, 2004; Di Benedetto *et al.*, 1994; Gillies *et al.*, 2011). In addition, the use of acellular scaffolds overcomes many of the hurdles associated with cell seeding, such as the necessity for prolonged *in vitro* cell culture, or the requirement for specific bioreactors that provide physiologic stimulation of the cells to promote differentiation.

Biologic scaffolds in tendon repair

Perhaps one of the most widespread clinical uses of biologic scaffolds is in the surgical repair of tendinous injuries (Aurora *et al.*, 2007; Cheung *et al.*, 2010; Derwin *et al.*, 2010; Derwin *et al.*, 2006). As detailed in Fig. 2, many processes in biologic scaffold-mediated skeletal muscle repair are also involved in biologic scaffold-mediated tendon injury repair. The complex cytokine and growth factor milieu, as well as the unique mechanical forces that define the two different injury sites, ultimately determine the differentiation pathway the infiltrating stem and progenitor cells will follow. Thus, the same biologic scaffold material can have the potential to promote both tendon and skeletal muscle repair, possibly within the same injury site (Turner *et al.*, 2010). However, despite the growing clinical use of biologic scaffolds, there are still many questions related to their mechanism of action, surgical application and efficacy that remain unanswered. Repair of the rotator cuff with biologic scaffolds is well documented (Aurora *et al.*, 2007; Cheung *et al.*, 2010; Derwin *et al.*, 2010; Iannotti *et al.*, 2008; Tsiridis *et al.*, 2008), but the use of biologic scaffolds in the repair of flexor tendons is rarely investigated (Derwin *et al.*, 2004). In many cases, biologic scaffolds are primarily used as surgical meshes promoting strength and support

rather than constructive tissue remodeling. As a result, biologic scaffolds for tendon repair tend to be either thick multilaminar devices or chemically cross-linked scaffolds manufactured to increase structural strength. As previously noted, these modifications can have significant negative effects on the host constructive remodeling response. There is a paucity of data on biologic scaffold-mediated constructive tissue remodeling in tendon.

Recent research has shown that just as degradation products and growth factors released by biologic scaffolds can promote stem and progenitor cells migration and proliferation in skeletal muscle, these bioactive molecules also enhance tendon healing in a similar way. Bi *et al.* (2007) have shown tendon contains a specific population of tendon progenitor cells that exist within an ECM niche. In addition, this group showed that this ECM niche controls tendon progenitor cell fate and speculated that alteration of the ECM composition signaled tenogenesis versus osteogenesis of the progenitor cells. Zantop *et al.* (2006) showed that the use of a biologic scaffold to repair an Achilles tendon defect promoted the migration of bone marrow-derived progenitor cells to the site of injury that adopted a tenocyte-like morphology. Mienaltowski *et al.* (2012) further characterized the progenitor cell niche in tendon, identifying a series of regional differences in progenitor cell distribution. Cells within the tendon tended to express Sca-1, CD90 and CD44, while progenitor cells in the peritendon region were more similar to perivascular progenitor cells expressing vascular and pericyte markers. Interestingly, many of these progenitor cell populations express similar surface markers to progenitor cell populations identified to be involved in skeletal muscle repair suggesting there may be a common progenitor cell pool within adult tissues.

The importance of macrophage phenotype in the promotion of tendon repair has also recently been highlighted. Dakin *et al.* (2012) investigated the contribution of different macrophage phenotypes to tendon healing showing that while the M1 phenotype predominated in sub-acute tendon injuries, there was a switch towards an M2 phenotype with chronic injuries. However, while further work is required to characterize the macrophage response and its contribution to tendon healing, it is possible that, as in skeletal muscle injuries, biologic scaffolds may modulate phenotypic switching of macrophages.

Tendon injuries are among the most common musculoskeletal problems affecting the adult population. More than 50 % of individuals over the age of 80 experience rotator cuff injury requiring surgical repair (Longo *et al.*, 2012; Tempelhof *et al.*, 1999). In the USA every year, 11 % of runners will suffer from Achilles tendon injuries (Kaz *et al.*, 2007; Rees *et al.*, 2006). Incomplete healing of tendon injuries can lead to compromised function and pain requiring surgical intervention. Surgical intervention, particularly of large tendon pathology, can be problematic and frequently involves the repair of degenerating tendon tissue that may be unable to sustain the forces associated with activities of daily living and will never regain the strength of the uninjured tendon, which increases the risk of repeated injury. Tendon augmentation

with a biologic scaffold material provides an alternative approach to autografts, xenografts and tendon prostheses and overcomes the problem of limited donor tissue.

Rotator cuff tears are a common source of shoulder pain with an incidence in the general population of up to 27 % and 37 % for full and partial tears, respectively (Itoi *et al.*, 1995; Yamanaka and Matsumoto, 1994). Despite surgical intervention there is a high rate of recurrent injury (Aurora *et al.*, 2007; Conti *et al.*, 2009; Iannotti *et al.*, 2006; Longo *et al.*, 2012; Soler *et al.*, 2007; Walton *et al.*, 2007), particularly in multi tendon rotator cuff tears. Consequently, the use of biologic scaffold materials for rotator cuff repair has increased with the hope that ECM scaffolds can provide temporary mechanical support while also promoting host remodeling and improved tissue remodeling.

The most commonly used biologic scaffolds for rotator cuff repair include Graftjacket[®], Restore[™], Cuffpatch[™], TissueMend[®] and OrthADAPT[™]. These devices represent a range of source tissues including dermis, small intestinal submucosa and pericardium. In addition, processing/manufacturing of these biologic scaffolds often includes chemical cross-linking, lamination or lyophilization (or some combination thereof). As a result, remodeling responses for these biologic scaffolds vary considerably (Valentin *et al.*, 2006) and clinical results range from success to abject failure (Fini *et al.*, 2007; Walton *et al.*, 2007).

The Restore[™] device, composed of small intestinal submucosa, was the first commercially available biologic scaffold. Several preclinical studies demonstrated the efficacy of Restore[™] in rotator cuff tendon repair. DeJardin *et al.* (2001) showed that SIS-ECM used in repairing a canine infraspinatus injury was completely replaced within 6 months by organized connective tissue similar to native tendon. Similar studies in the rabbit and rat also demonstrated the replacement of the SIS-ECM with organized collagenous tissue with superior biomechanical properties to unrepaired control groups (Chen *et al.*, 2007; Perry *et al.*, 2007; Zalavras *et al.*, 2006; Zheng *et al.*, 2005). Early clinical studies also suggested that Restore[™] showed successful rotator cuff reconstruction with post-operative follow up over a two-year period showing thickening of the cuff tendon and incorporation of the scaffolds in 11 out of 12 patients and improved functionality (Metcalf *et al.*, 2002). However, subsequent studies suggested that Restore[™] does not improve the rate of tendon healing and may be associated with a high rate of recurrent rupture. Sclamberg *et al.* (2004) showed that 6 months postoperatively 10 or 11 patients had failed repairs. Other investigators have shown that unaugmented groups perform equally or better than those treated with Restore (Walton *et al.*, 2007; Iannotti *et al.*, 2006). In the majority of these reports the major reported side effect was a non-infectious, sterile effusion. Given the success of SIS-ECM scaffolds in pre-clinical studies and in other body sites, the reasons for this high failure rate are not entirely clear. It is known that site-specific changes in the wound microenvironment, including extent of injury, post operative NSAID use and post-operative rehabilitation

(Galatz *et al.*, 2009; Galatz *et al.*, 2001; Iannotti, 1994; Longo *et al.*, 2012; Maffulli *et al.*, 2011; Tanaka *et al.*, 2010) have dramatic effects on constructive remodeling. In addition, the recruitment of progenitor cells, modulation of the innate immune response and generation of bioactive cryptic peptides over a period of weeks to months have known effects on the clinical symptoms of patients.

Other biologic scaffolds have shown greater success. Graftjacket[®], derived from human dermis, has shown efficacy in a number of studies. Adams *et al.* (2006) showed that use of Graftjacket[®] as an interpositional graft in the canine infraspinatus injury model results in a robust tendon-like tissue formed at the site of implantation within 6 months. A number of groups have shown positive outcomes using Graftjacket[®] for interpositional repair of large rotator cuff tears in human patients (Bond *et al.*, 2008; Rotini *et al.*, 2011; Snyder *et al.*, 2009). Significant improvement in mobility and a reported decrease in pain were reported (Bond *et al.*, 2008).

The paucity of controlled comparison studies using biologic scaffolds for rotator cuff repair makes it difficult to define indications for their respective use. However, it is clear that biologic scaffolds are best suited to situations where a poor healing outcome is likely, such as chronic and/or large rotator cuff tears. Data on the repair of other tendon injuries is limited, particularly in flexor tendon repair. However, a number of studies have shown biologic scaffolds to have efficacy in Achilles tendon repair.

In a canine model of Achilles tendon repair, it was shown that SIS-ECM scaffolds remodeled into neotendon that had organized collagenous architecture similar to normal healthy tendon, which was stronger than either the musculotendinous origin or bony insertion (Badylak *et al.*, 1995). Later studies showed the contribution of bone marrow-derived cells to long term remodeling of SIS-ECM in Achilles tendon repair (Zantop *et al.*, 2006). More recently, the ability of SIS-ECM scaffolds to promote the formation of a new, functional musculotendinous origin between the Achilles tendon and gastrocnemius muscle has been shown (Turner *et al.*, 2010). A lack of clinical data makes it difficult to determine whether these pre-clinical successes translate to successful tendon repair in humans. A number of clinical studies do exist which have evaluated Graftjacket[®] in the repair of Achilles tendon rupture (Branch, 2011; Lee, 2008; Lee, 2004; Liden and Simmons, 2009). Graftjacket[®] is reported to have the strongest mechanical properties among five other commercially available scaffolds for tendon repair. In a human cadaver model, Barber *et al.* (2006) showed that Graftjacket[®] significantly increased Achilles tendon strength and stiffness. In a series of clinical studies Lee *et al.* (2004; 2008) showed that repair of Achilles tendon rupture with Graftjacket[®] resulted in an early return to activity and good plantar flexion strength with no ruptures of chronic pain up to 30 months post-operatively.

It is important to note that all xenogeneic biologic scaffolds are approved by the FDA only for the reinforcement of soft tissues and not as interpositional grafts intended to replace normal body structure or function. As our understanding of biologic scaffolds

increases and the mechanism(s) with which they interact with the wound microenvironment becomes known, their constructive use will undoubtedly expand.

Conclusion

The use of biologic scaffolds for musculotendinous tissue reconstruction in the context of regenerative medicine represents a fundamentally different strategy from traditional surgical approaches. While their clinical use continues to increase, it is clear that a greater understanding of the biochemical, cellular and mechanical cues that stimulate the constructive remodeling response is required. Biologic scaffolds are known to promote the migration and proliferation of progenitor cells when implanted in an injury site. However, many questions remain as to the cues that cause these cells to then differentiate into site appropriate tissue. Future directions in developing the use of biologic scaffolds and/or the promotion of constructive tissue remodeling may focus on the importance of mechanical loading and physiologic stimulation on cell differentiation. In addition, further characterization of the composition of biologic scaffolds derived from different tissues may identify biologic scaffolds that provide superior constructive remodeling in certain injury sites. Similarly, an improved understanding of the methods of biologic scaffold preparation may provide further insight into the retention and release of growth factors and the generation of matricryptic peptides from these materials leading to improved clinical outcomes.

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