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A review of manufacturing strategies to meet structural and functional requirements

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Tissue engineering of skeletal muscle, tendons and nerves: A review of manufacturing strategies to meet structural and functional requirements

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ABSTRACT

Additive manufacturing technologies have become at the forefront in tissue engineering, enabling the fabrication of complex tissues with intricate geometries that were not feasible using conventional manufacturing techniques. Due to the rapid progress in this field, it has become difficult not only to choose the most appropriate method, but also the optimal material, biological model (i.e., cells and bioactive compounds), and processing technique to fulfill the macro- and microstructural architecture and functions of biological tissues. The aim of this review is to describe recent advances in tissue engineering fabrication methods, from established electrospinning to emerging additive manufacturing technologies, with particular emphasis on tissues that exhibit hierarchically organized anisotropic architecture (skeletal muscle, tendons, and peripheral nerves). One of the current challenges is that the designs are usually dictated by the constraints imposed by the methods, rather than by criteria based on mechanical and biological requirements. Therefore, the review focuses on describing how the anatomical structure and function of muscles, tendons, and nerves should serve as the basis for an efficient three-dimensional design that considers both micro and macro aspects of the tissue. In addition, the individual factors that influence the fabrication strategy are discussed and related to the mechanical and biological properties of the three tissue types. The review highlights the advantages and limitations of each fabrication strategy and provides an overview of critical aspects relevant to future research strategies in this area.

List of abbreviations

2D	two-dimensional
3D	three-dimensional
AlgMA	methacrylated alginate
ASCs	adipose-derived stem/stromal cells
BCE	butylene cyclohexanedicarboxylate
BDNF	brain-derived neurotrophic factor
BM-MSCs	bone marrow-derived mesenchymal stem/stromal cells
BR	biological requirements
C2C12	mouse murine myoblast cell line
CAD	computer-aided design
CMCMA	methacrylated carboxymethyl cellulose
ColMA	methacrylated collagen
DLW	direct laser writing
DLP	digital light processing
ECM	extracellular matrix
EHD	electrohydrodynamic jetting
FDA	food and drug administration
FGF	fibroblast growth factor
GDF5	growth differentiation factor 5
GDNF	glial-derived neurotrophic factor
GelMA	methacrylated gelatin
GF	growth factor
GO-g-C3N4	graphite oxide-graphitic carbon nitride complex
HA	hyaluronic acid

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HAMA methacrylated hyaluronic acid
HDAC-3 histone deacetylase 3 enzyme
IGF insulin-like growth factor
iPSCs induced pluripotent stem cells
MWCN multiwalled carbon nanotubes
NB N-(2-aminoethyl)−4-(4-hydroxymethyl-2-methoxy-5-nitrosophenoxy) butanamide
NCSCs neural crest stem cells
NGC nerve guidance conduit
NGF nerve growth factor
NHDFs normal human dermal fibroblasts
NIH/3T3 mouse immortalized fibroblast cell line
NT-3 neurotrophin-3
MAI myelin-associated inhibitor
MEW melt electrowriting
MR mechanical requirements
MSC mesenchymal stem cell
MPCs muscle precursor/progenitor cells
PAA poly(acrylic acid)
PAN poly(acrylonitrile)
PANI polyaniline
PBCE poly(butylene 1,4-cyclohexanedicarboxylate)
PC12 rat pheochromocytoma cell line
PCL poly(ε-caprolactone)
PDGF platelet-derived growth factor
PEG poly(ethylene glycol)
PEG-DA poly(ethylene glycol) diacrylate
PEG-DMA poly(ethylene glycol)-dimethacrylate
PEGS-M poly(ethylene glycol)-co-poly(glycerol sebacate)
PEO poly(ethylene oxide)
PET polyethylene terephthalate
PGA poly(glycolic acid)
PHB poly(hydroxybutyrate)
PHBHHX poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)
PHBV poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
pHEMA poly(hydroxyethyl methacrylate)
PLA poly(lactic acid)
PLGA poly(lactic-co-glycolic acid)
PTFE polytetrafluoroethylene
PU polyurethane
PVA poly(vinyl alcohol)
SES solution electrospinning
SF silk fibroin
SIS small intestine submucosa
SkMMs primary skeletal muscle myoblasts
TECE triethylene cyclohexanedicarboxylate
TGFB transforming growth factor-beta
TE tissue engineering
VEGF vascular endothelial growth factor
VIT *in vitro* study
VIV *in vivo* study
VML volumetric muscle loss

1. Introduction

Disorders of muscles, tendons, and nerves result in dysfunction that significantly impairs the patient's ability to perform voluntary movements and carry out physical activities [1,2]. In particular, traumatic injuries to peripheral nerves and soft musculoskeletal tissues have a high prevalence worldwide and represent a significant socioeconomic burden [3,4]. Commonly, variable approaches using autografts are employed to treat traumatic soft tissue injuries [5,6]. However, one of the major limitations is donor site morbidity [7]. In addition, there is often not enough autologous donor tissue available [8]. Alternatively, allogeneic transplantation can be performed, although the drawback of limited donor supply should be considered as well. In an attempt to overcome these challenges, to improve success rates and to enhance overall clinical performance, research has focused on tissue engineering (TE) approaches. The main goal of TE is to develop methods to regenerate, repair, or replace damaged or diseased cells, tissues, or organs while maintaining and/or improving their performance [9,10]. This multidisciplinary research area combines expertise from developmental biology, materials science, cell biology, engineering and medicine.

In the development of tissue, knowledge should be gained about their macro- and microstructure and their functions. To better understand the complexity of their structure, Atala *et al.* [11] proposed to divide human tissues and organs into four groups: (i) flat tissue structures, (ii) tubular structures, which can be hollow or non-hollow, (iii) hollow viscous structures, and (iv) solid organs. While tendons and nerves belong to the non-hollow tubular structures category, skeletal muscles can also be included in this group because muscles are composed of tubular cells (the muscle fibres) arranged hierarchically in cylindrically shaped bundles (the muscle fascicles) [1,9].

Regardless of the complexity of the tissue being repaired, TE approaches typically involve various combinations of biomaterials, cells, and bioactive compounds that are processed into a construct in order to mimic the functional unit of the tissue as closely as possible [12–15]. This review addresses recent advances in the field of TE, which enable efficient repair of skeletal muscle, tendons, and nerves. This particular group of soft tissues is frequently exposed to traumatic injuries that result in impaired musculoskeletal function. Given recent advances in tissue engineering strategies, and more specifically in scaffold manufacturing and processing techniques, the focus of this review is to describe how anatomical structure and function should serve as the basis for efficient three-dimensional (3D) design considering both micro and macro aspects of these tissue types.

2. Definition of the design requirements based on the hierarchical tubular structure and function of muscles, tendons, and nerves

Before defining the design requirements for muscles, tendons, and nerves, the major anatomical and physiological features of these non-hollow tissue structures and the complex interrelationships amongst them should be considered, as each of them depends on the other to function appropriately. Skeletal muscle is the tissue responsible for contraction and is a key component within the soft musculoskeletal tissues. Peripheral nerves and tendons play a crucial role in ensuring proper muscle function: electrical pulses are transmitted through the motoneurons towards the neuromuscular junctions. After stimulation, neurotransmitters are released from the axon terminals of the motoneuron, and muscle contraction is initiated. The tendons serve as a bridge between the muscles and the bones, allowing the transmission of muscle movement into limb mobility [16].

2.1. Skeletal muscles

Skeletal muscle is one of the major components of the human body, accounting for about 40% of its mass [16]. It is known to generate mechanical force, using chemical energy, which can be applied, for example, for movement and posture, allowing the individual functional autonomy to participate in various daily activities in leisure and occupation. Skeletal muscles however also play an important role in regulating basal metabolism as they serve as storage for amino acids and carbohydrates and maintain body temperature. The smallest functional unit of skeletal muscle is the multinucleated tubular muscle fiber (cell), which is about 100 μm in size (Fig. 1). Every muscle fiber consists of myofibrils, which are the contractile muscle units. More specifically, the actin filaments and myosin bundles within the myofibril will initiate a contraction after stimulation when supplied with energy. Following stimulation, calcium ions are released from the sarcoplasmic reticulum and bind to troponin C which in turn ensures the temporarily

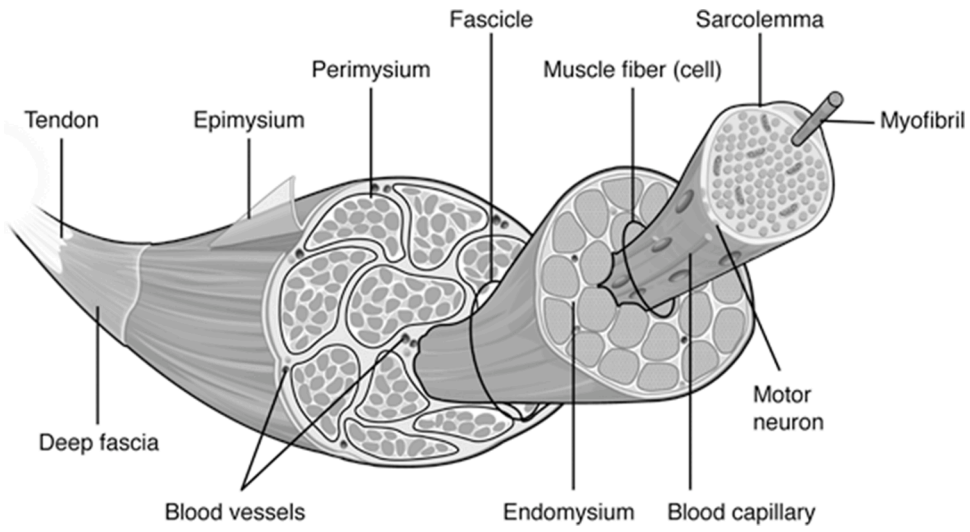

Fig. 1. Schematic representation of skeletal muscle and its subcomponents. Each skeletal muscle cell or muscle fiber is surrounded by a sarcolemma and consists of longitudinally oriented myofibrils, which are predominantly composed of myosin and actin. At the level of the neuromuscular junction, the motor neuron axon is in close contact with the sarcolemma, where electrical pulses initiate muscle contraction. Blood capillaries supply the muscle fibers with oxygen and nutrients. Different muscle fibers are separated from each other by a thin layer of collagen-rich connective tissue, the so-called endomysium. These collagen fibers are mainly longitudinally arranged in the muscle fibers. Different muscle fibers together form a fascicle which is surrounded by a layer of connective tissue, the perimysium. The latter also consists of different types of collagen fibers that are oriented in parallel to the underlying muscle fibers, with its thickness varying between muscles. The perimysium also contains larger blood vessels and nerve fibers. The epimysium is the outer connective tissue layer, which surrounds the entire muscle and has a similar composition as the connective tissue layers previously described, but with coarser collagen fibers [19–21]. Image reproduced from Ref. [21], ©2020, Oregon State University, Biga *et al.*

displacement of tropomyosin. As such, the actin binding sites on myosin are exposed which enables the binding of actin to myosin and, through a series of events, results in the contraction of the filament and, at the macro level, of the muscle itself. A more comprehensive description of skeletal muscle contraction and relaxation is found elsewhere [17]. The transition from muscle to tendon is also called the tendon-muscle interface, which consists of finger-like infiltrations of muscle into tendon, allowing transmission of its contractile force first into tendon and later into bone, through which movement is possible [16,18].

A range of disorders can seriously compromise normal muscle function. In particular, traumatic skeletal muscle injury, which is injury caused by bruising, extreme cold, or toxins, can cause severe cellular damage, drastically impairing muscle function [22]. Typical causes may include high-energy trauma in a traffic accident, combat injuries such as blast trauma, surgery (e.g., tumor excision), or during sport activities where a contusion can lead to acute muscle damage and loss. Up to 55% of all sports injuries result in damage at the myofiber level [23]. A loss of more than 20% of total muscle mass results in decreased function and impaired tissue regeneration [24]. In these severe cases, fibroblasts may proliferate disproportionately, and this scar tissue may partially or completely inhibit muscle fiber regeneration at the site of injury [25]. In addition, scarring may prevent the patient from returning to the pre-injury performance level by inhibiting axonal regrowth and, as such, muscle strength is reduced [26]. Another factor preventing optimal treatment outcome is lack of/delayed reinnervation of the muscle after injury, which can lead to extensive myofiber atrophy and consequently impaired future reinnervation [27]. Traumatic skeletal muscle injuries require surgery to reconstruct the damaged tissue, usually by autologous grafting of a muscle pedicle flap into the affected area, although in severe cases amputation of the affected limb may be required [15,24]. As already mentioned, it is not always possible to collect enough suitable grafts and post-transplant complications make this choice of treatment even more challenging [24]. Therefore, intense research efforts are currently focusing on alternative approaches to tackle these problems.

Tissue-engineered muscles represent an appropriate strategy to replace damaged or lost muscle tissue. A particular challenge in TE however is the production of constructs that accurately mimic the mechanical properties of muscles. Muscles are soft and elastic tissues that can withstand heavy uniaxial loads and stretch up to 60% before mechanical failure [28]. Gotti *et al.* have listed some important values regarding the optimal mechanical properties of skeletal muscle as a reference for TE approaches [28]. Nevertheless, mechanical properties alone are not sufficient for successful muscle regeneration. A high degree of fiber alignment, the presence of blood vessels, and neuronal stimulation are other crucial factors for successful muscle regeneration [28–30]. Moreover, skeletal muscle has a high metabolic activity, so an integrated vascular network is required for providing nutrients (e.g., glucose, oxygen) and removing metabolic waste away from the muscle cells. In addition to supplying oxygen and nutrients, the vascular network plays a fundamental role in the development, regeneration, and adaptation of skeletal muscle to physiological demands and is therefore a critical aspect of any tissue engineering approach. The vascularization process of skeletal muscle occurs by the following processes: vasculogenesis, angiogenesis, arteriogenesis and lymphogenesis [31]. Factors that stimulate vascularization in skeletal muscle include hypoxia, shear stress and muscle stretch, among other factors [31]. An overview of the muscles' anatomical key points, physiological functions, and the mechanical and biological requirements for the design of muscle constructs is shown in Table 1.

2.2. Tendons

Tendons are passive, relatively inelastic structures which have as main function to effectively transmit force from muscle to bone and enable body movement [32,33]. However, specific tendons, such as the human Achilles tendon or the equine superficial digital flexor tendon, have additional functional specializations that allow energy storage [34–36]. Tendons possess a highly organized structure consisting of parallel oriented collagen fibers (mainly collagen type I) embedded in an extracellular matrix (ECM). This ECM is composed of proteoglycans, glycoproteins, and elastin [32]. Fig. 2 shows the hierarchical structure of the tendon, in which collagen fibers assemble into subunits of increasing diameter. First, three collagen molecules form a triple helix (i.e., tropocollagen). Subsequently, five tropocollagen units form a microfibril, which are linked together to form a fibril. Depending on the functional role of the particular tendon, the diameter of these fibrils can range from 10 to 150 nm [33,37]. Different fibrils are grouped into fibers (i.e., primary bundle) and these are joined together in the fascicle (i.e., secondary bundle). Bundles of fibrils and fibers display an undulating pattern called crimp, which is established during embryonic development and acts as a shock absorber during loading. Between the primary and secondary bundles, the endotenon is present, a cell-rich layer that

Table 1
Anatomy, physiological functions and requirements per tissue type.

	Anatomical key points	Physiological functions	Construct design: Mechanical requirements (MR)	Construct design: Biological requirements (BR)	References
Skeletal muscles	Organized bundles of myofibers grouped together by connective tissue in bundles of increasing thickness relative to the direction of force generation. 1. Myofibrils: the smallest contractile unit primarily containing myosin and actin. 2. Myofibers (muscle cells): with innervation and capillary supply, surrounded by endomysium. 3. Fascicles: the largest bundles surrounded by perimysium with larger blood vessels in between. Different fascicles together are surrounded by epimysium.	- Allow movement/ force generation - Play a role in respiration and basal energy metabolism - Participate in maintaining core temperature - Storage for amino acids and carbohydrates - Production of endocrine/paracrine hormones (myokines) involved in various inflammatory processes.	1. Should allow shortening and shape recovery of the myofibers in response to a stimulus 2. Should allow for enough tensile strength [40–800] kPa and deformation [30–60] % 3. Should have appropriate amounts of resistance to deformation (elastic modulus in the longitudinal axis [30–8000] kPa)	1. High degree of muscle fiber alignment 2. Innervation, including the neuromuscular junction, necessary for activation, control and long-term muscle survival. 3. Presence of vasculature to provide oxygen and nutrients and to remove waste products. This is critical to allow the development of large size constructs	[16,28,30, 74,75]
Tendons	Highly organized connective tissues consisting of parallel orientated collagen fibers embedded within an extracellular matrix (containing cells, proteoglycans, glycoproteins and elastin). 1. Three collagen molecules form a triple helix (i.e., tropocollagen) 2. Five tropocollagen entities constitute a microfibril, that in turn form a fibril 3. Fibrils are grouped into fibers (primary bundle) and then assembled into a fascicle (secondary bundle), with an endotenon in between these two layers 4. Fascicles are bundled together to form the tertiary bundle, surrounded by the epitenon The tendon cells are tendon-specific stem cells and specialized fibroblasts or tenocytes	- Store elastic energy (through reversible stretching of collagen molecules) - Withstand large forces and assure effective load transmission between muscle and bone - Act as a buffer by absorbing external forces to limit muscle damage - Support the stability of the joints	Strongly dependent on the type of tendon and the exerted load. 1. Should have appropriate mechanical properties (general): max. strengths [13- 300] MPa, elastic modulus [4-8] GPa, strain and modulus at failure [6-50] % and [0.065 - 8] GPa Deep flexor tendons: Young's modulus [3.1 - 5.0] MPa, ultimate stress 4 MPa, ultimate strain [4-10-] % and tendon toughness [1000 - 4500] J/kg 2. Should allow the crimping mechanism to stretch and recoil: protects the collagen fibers and relevant stress-strain properties (up to 8 - 10% before macroscopic failure) 3. Should show appropriate levels of fiber sliding and degree of rotations (depending on the type of tendon). Should not show any gap formation in the repair zone.	1. Should mimic the hierarchical organization and fiber orientation. Remodeling of the tendon: tenocytes produce collagen and thus play a role in the crimping mechanism and collagen fiber deformation; use of bioactive components and mechanical stimulation to guide the remodeling 2. A limited blood supply is very important for metabolic activity; and innervation 3. Minimal scar formation (minimal adhesion formation and inflammation) during healing	[33,42, 75–82]
Nerves	The peripheral nervous system is responsible for the communication between the central nervous system and body parts, consisting of nerves branching out from the spinal cord and the brain. 1. Principally made of two cell types, (1) neuroglia (supporting cells) and (2) neurons. 2. All neurons have a cell body and extended arm-like process (-es) namely the dendrites and/or axons and other structures such as the glycocalyx, endoneurial fluid, endoneurium, perineurium and epineurium. 3. Axons bundled in groups called fascicles, which in turn are surrounded by the perineurium. 4. The neuronal cell body ranges from 5 - 140 μm in diameter with a single axon extending up to 1.2 meters.	- Send information through the entire body via electrochemical signals - Information is transmitted through sensory neurons (interpretation of sensory stimuli), motor neurons (signal relay to muscles or glands) and interneurons (signal transmission between neurons) - Sensory receptors of the sensory neurons respond to changes in the environment, converting chemical /mechanical energy in action potentials.	Diameter of the nerve varies throughout the body, depending on the location of the nerve. In general: 1. Appropriate mechanical properties: elastic modulus [15.87 ± 2.21] MPa, ultimate tensile strength [6.78 ± 0.57] MPa and elongation at break [0.61 ± 0.02] mm/mm. 2. When implanted *in vivo*, should undergo minimum tension at suture site 3. Ideal diameter of the conduit * (for reconstruction of nerves in the extremities): 3 - 4 mm.	1. Highly directional Schwann cell migration and axonal growth from the proximal to the distal end. 2. Micropores large enough to ensure nutrient exchange but small enough to obviate infiltration by myofibroblasts. 3. Adhesion of cells on the conduit wall.	[55,57,71, 83,84]

* a nerve conduit encloses the distal and proximal ends of a damaged nerve, aiming to guide the growth of the regenerating axons.

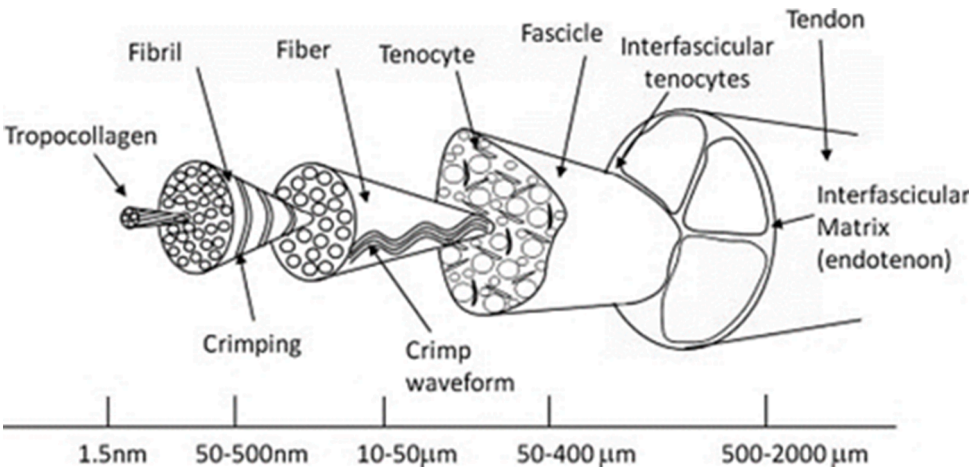

Fig. 2. Schematic hierarchical structure of a tendon with subunits of increasing diameter (from 1.5 nm to 500–2000 μm). From the smallest subunit onwards, the tendon consists of: (i) collagen molecules or tropocollagen, (ii) fibrils, (iii) fibers, (iv) fascicles composed of tenocytes, and (v) interfascicular matrix or endotenon. Reproduced from Ref. [33], ©2015 with permission from Elsevier.

facilitates sliding between fibers and/or fascicles. These fascicles are bundled together to form the tertiary bundles, which are surrounded by the epitenon (i.e., the fibrous sheath containing blood vessels and tracts for the nerves and lymphatics) and form the entire tendon [38]. Finally, the epitenon may be surrounded by a membrane or synovial sheath that facilitates smooth gliding of the tendon towards surrounding structures [37]. It ensures minimal friction and preserves the position of the tendon during muscle contraction.

The main component of tendons is water, which accounts for 55 to 70% of the tendon's wet mass [33]. Collagen molecules count for 60 to 85% of the dry mass of tendons [33]. While tendons are predominantly composed of type I collagen (more than 95%), the endotenon is primarily composed of type III collagen (less than 5%) [38,39]. Collagen is produced and secreted by specialized fibroblasts (i.e., tenocytes) located in the tendon. These cells are arranged in a layered composition and parallel orientation, which maximizes tensile strength [37]. When subjected to tensile stress, tenocytes stretch along the collagen fibrils in the form of longitudinal arrays [40]. In addition, they produce the ECM and assist with the orientation of the newly synthesized fibrils [41]. Furthermore, they control the degradation and remodeling processes of the ECM structure, which illustrates the continuous interaction between the tenocytes and the ECM [41]. Blood supply is also very important, although it is not as abundant present as in muscle and bone. Blood accounts for only 1 to 2% of the ECM [40]. Blood supply is directly related to metabolic activity, which means it is essential and even increased during the healing process after an injury. Furthermore, tendons have a very rich neural network and are often innervated by the muscles to which they are connected or by the local cuticular nerves [40]. The sensory innervation of tendons is of particular interest when considering tendinopathies and tendon repair [42].

Two partially oval/round tendons that rupture most often, are the Achilles tendon and the deep flexor tendons. Tubular tendons respond equally to tensile loads with parallel collagen patterns. In addition, the cross-sectional area is proportional to the maximum isometric force of the muscle [40]. The Achilles tendon provides a connective tissue link between the gastrocnemius and soleus muscles and the *os calcaneus*. The two main blood vessels supplying the Achiles tendon, are the posterior tibial artery that supplies both the proximal and distal sections, and the peroneal artery that supplies its middle section [36,43]. The Achilles tendon is innervated mainly by the sural nerve, with minor contributions from other smaller branches of the tibial nerve [36]. It is the strongest and thickest tendon in the human body. As such, force measurements have shown that the tendon is loaded with up to 9 kN during running, which is 2.5 times the body mass. The Achilles tendon is the best example of an energy storage tendon as it centralizes the force of different muscles [40]. The deep flexor tendons extend from the forearm through the wrist and across the palm, providing flexion to the fingers [39]. This is in contrast with the extensor tendons, which provide extension to the fingers. The flexor tendon structure of the hand consists of the tendinous extensions of the flexor muscles of the forearm which attach to the small bones of the thumb and fingers. In addition, the deep flexor tendons are subjected to greater flexion during movement and are therefore typically surrounded by a vascularized synovial sheath [44]. The presence of synovial fluid compensates for the limited vascular supply compared with other tendons (such as the Achilles tendon) [45]. In addition, synovial cells are present in this tendon sheath and provide lubrication to reduce friction during movement and loading, which supports the tendon to glide smoothly and efficiently [38,39,41].

Tendon injuries are painful, persistent, and can significantly affect the quality of life of patients who had inadequate healing or unsuccessful treatment [46]. More than 4 million cases of tendon injuries are reported annually worldwide [47]. Tendon injuries to the hand, in particular, are amongst the most common tendon disorders in the human body. Typically, flexor tendon injuries of the hand account for a significant proportion of trauma emergencies, affecting one in 2700 people each year [45]. The hand, as a performing unit of the human body, is essential in daily life, including sport activities and occupations [48]. For this reason, the tendons of the hand are often subjected to chronic overuse and ruptures. These injuries can have a significant impact on hand function. Early treatment with optimal recovery is critical to prevent permanent dysfunction [49].

Current surgical interventions include suture techniques (with needle and thread) or replacement tissue (i.e., biological and synthetic grafts) to repair tendon injuries. To date, none of these methods offer a

long-term solution because of the significant drawbacks limiting their success. Repaired tendons do not regain their full functionality and strength [50–52]. Ideally, a healing response should be induced at the injured tendon ends consisting of a repair site with low friction and minimal volume [39]. Adhesions are a frequently observed complication after tendon injury [53], as a result of the non-organized collagen production during the first phase of the healing process. Due to this scar tissue formation, injured tendons heal slowly, and a long recovery period is often required. In addition, inflammation plays a critical role in tendon injury and healing [54]. When surgical material is used, a strong inflammatory reaction might be triggered with inflammatory cells being attracted to the injured site. Following these inflammation and adhesion processes, the repaired tendon will be unable to regain its original mechanical properties, including ultimate tensile strength and elasticity. The latter illustrates how challenging it remains to realize regeneration instead of repair and restore the original mechanical properties of tendon by preventing the formation of adhesions and inflammation.

A particular challenge in tendon TE approaches is the fabrication of constructs with suitable mechanical properties. Tendons are stiff and resilient structures that have a high tensile strength: they can stretch up to 4% before damage occurs [36]. They have also high anisotropic mechanical properties: they ensure that the tendon is stiff along its long axis and can withstand its predominantly uniaxial loading environment by transmitting muscle forces along the length of the tendon to the skeleton. Actin and myosin are present in resident tenocytes, while the tendon itself may also have an inherent contraction-relaxation mechanism to regulate force transmission [36]. Besides the micro and macrostructure of the tendon, the biochemical and cellular structure should be taken into account as well in order to achieve its physiological functions. An overview of the anatomical key points, the physiological functions and the mechanical as well as biological requirements for the construct design is described in Table 1.

2.3. Nerves

Together with the endocrine system, the nervous system is responsible in the body for communication and control. Every thought, action, and emotion reflect its activity. Cells are able to communicate through rapid and specific electrical and chemical signals which provoke immediate responses. The nervous system has three main overlapping functions: (1) sensory input monitoring changes inside and outside the body, (2) motor output sent to effector organs such as muscles and glands that are activated depending on the sensory input, and (3) integration of the electrical signals at every step between the received sensory information and the effective motor output [55,56]. The structural units of the nervous system are the neurons, also called nerve cells. These highly specialized cells convey electrochemical pulses throughout the body. Neurons are extremely long-lived, amitotic and have an exceptionally high metabolic rate. They consist of a cell body or soma, dendrites and an axon. Dendrites are that part of the neuron that receive input from other cells. To this end, they often branch as they move towards their tips in order to increase their surface area significantly. The axon is the transmitting part of the neuron; electrochemical pulses are sent throughout the axon when a neuron wants to communicate with another neuron [53].

Fig. 3 illustrates the anatomy of a peripheral nerve with its main constituents, which include Schwann cells, fibroblasts, satellite cells, axons as well as the extracellular matrix. Schwann cells form myelin sheaths around peripheral neurons and provide both structural and metabolic support. Satellite cells surround the neuronal cell bodies and help to regulate the chemical environment. The endoneurium surrounds the nerve fibers, which in turn are surrounded by the perineurium to form fascicles. The epineurium is the outer layer of dense irregular connective tissue that surrounds the peripheral nerve and consists of multiple nerve fascicles and blood vessels. The number of fascicles and myelinated fibers of the nerves differs in different parts of the body [55,57].

Peripheral nerve injuries are caused by traumatic accidents, excessive drug abuse, tumor resection, iatrogenic side effects of surgery or repeated compression (tunnel syndromes). These injuries remain challenging and difficult to reconstruct surgically, despite the availability of important data on new, evolving neuroscience concepts over the past three decades [58]. With an annual incidence of more than 300,000 cases in Europe [59], peripheral nerve injuries can drastically limit the quality of life of patients suffering from partial or complete loss of motor function or secondary problems such as neuropathic pain [60–62]. Unlike tissues in the central nervous system, peripheral nerves are not protected by a thick or bony structure such as the spine or skull,

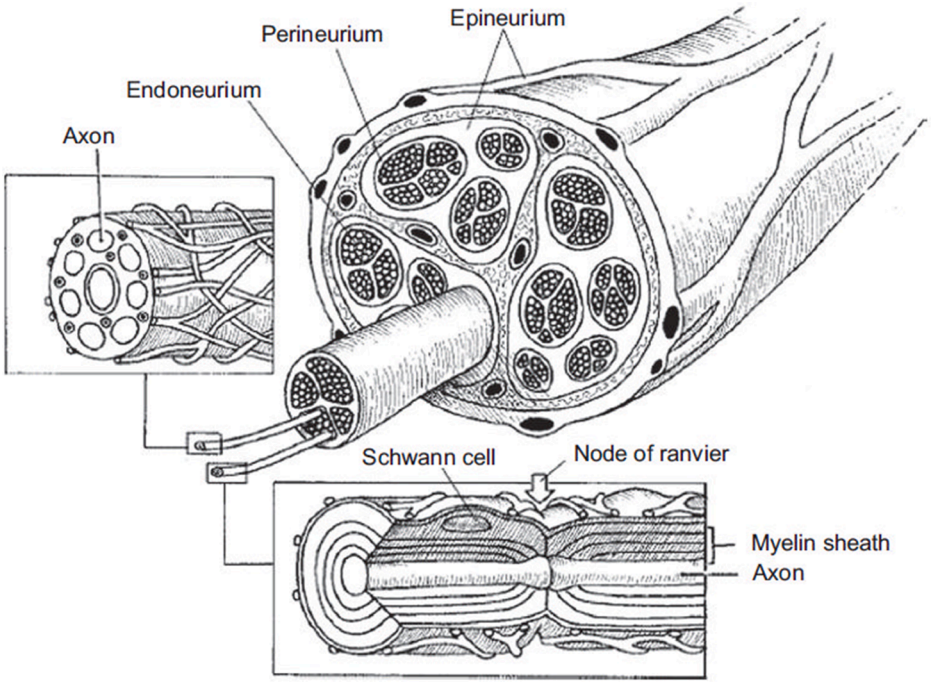

Fig. 3. Schematic representation of the anatomy of a peripheral nerve, consisting of an axon surrounded by the endoneurium. Groups of axons are bundled together and are surrounded by the perineurium. Subsequently, several fascicles bundle together to form the peripheral nerve, which is surrounded by the epineurium. Axons may be myelinated (inset) and range in length from 1 mm to approx. a meter, in case of the axon that spans from the brain to the spinal cord. Image reproduced from Ref. [70], ©2016 with permission from Elsevier.

rendering them delicate and susceptible to damage. But unlike axons of the central nervous system that do not regenerate when damaged, due to an adverse environment caused by myelin-associated inhibitors (MAIs) [63], peripheral nerves are characterized by their intrinsic ability to regenerate. However, depending on the severity of the injury, regeneration can be complex, making therapeutic intervention inevitable to support full recovery.

Two approaches of reconstructive surgical procedures are described to treat a peripheral nerve injury: manipulative and bridging peripheral nerve surgeries [64]. Manipulative nerve techniques are only performed when there is clean transection without tissue loss when two ends of the nerve are surgically attached without applying tension to the nerve. This technique allows for a quick recovery and reduces the likelihood of postoperative complications. When the bridging surgery technique is applied, a supportive structure is inserted between the proximal and distal end of the nerve in order to bridge the injured area. The current gold standard in bridging techniques is the autograft, which has been in use for over 50 years [6,60]. Autografts are obtained from the patient itself from functionally less vital nerves. They contain viable Schwann cells, neurotrophic factors, and provide the structural support required for axonal growth from the proximal to the distal end of the nerve. Disadvantages of this technique include donor site morbidity, axon mismatch, fibrosis, and scarring at both the resection and implantation sites, resulting in a success rate of only 50% [65]. Additionally, the length of an autograft is currently limited to maximum 5 cm in length, after which an allograft is used [12,66,67]. The use of allografts however is associated with an extensive immune suppression for up to 18 months, and consequently an increased risk of infection and tumor formation [68].

Given the drawbacks of autografts and allografts, the concept of nerve guidance conduits (NGCs) was introduced to provide a guiding channel for natural nerve regeneration [60]. NGCs are usually composed of a natural and/or synthetic polymer, similar to other scaffolds used for TE applications. These materials may be interspersed with other cells, growth factors, and bioactive compounds that further stimulate the growth of neurons in the underlying injury site [64]. NGCs offer a plethora of benefits, such as providing a vessel for the accumulation of neurotrophic factors, preventing the infiltration of myofibroblasts and thus preventing the formation of scar tissue and painful neuromas, to name a few [60,64,69], and, as such, represent a valuable alternative for the use of auto- and allografts [6].

When considering the mechanical and biological requirements for nerve TE, several features deserve special attention. Nerves are complex systems with a relatively high elasticity and ability to regenerate, with a Young's modulus of approximately 16 MPa, which is greatly reduced (to approximately 8 MPa) upon resection [71]. Because of this high modulus, care must be taken when implanting a potential NGC *in vivo* to avoid tension at the suture site, otherwise there is a high likelihood of painful neuromas, scarring, and inefficient regeneration to occur. Ma *et al.* have described in detail the mechanical properties of both intact and resected human median and ulnar nerves [72]. They tested the viscoelastic behavior of human ulnar and median nerves using tensile tester and also observed *in vivo* stress and deformation during a surgical procedure, followed by finite element models to determine the viscoelastic parameters of the nerves. Optimal cell growth from the proximal to the distal nerve end might require biological stimuli such as structural and chemical cues, depending on the gap that the sprouting axon must overcome. The outgrowth of neurites and the extension of cell bodies into a spindle shape are indicative for good cell adhesion and growth [73]. An overview of the anatomical key points, physiological functions, and mechanical as well as biological requirements for construct design is described in Table 1.

2.4. Summary of design requirements for each tissue type

Design requirements for TE applications are defined with consideration of the anatomy and physiology of the tissue or organ of interest. As outlined in the previous section, skeletal muscles, tendons and nerves have a typical hierarchical structure. In addition, they are highly organized, have anisotropic properties, are composed of different cell types, and a tissue-specific ECM. Each cell type and ECM component has a specific task to perform, both individually and as part of the overall structure, to enable the physiological function of the organ. These important anatomical points and physiological functions require very specific mechanical and biological requirements that should be considered in the design of the constructs (i.e., the tubular structure comprising biomaterials and/or cells). In addition, it is essential to evaluate the existing correlations between (i) the anatomy and hierarchical structure, (ii) the physiological function and (iii) the mechanical and (iv) biological properties of the tissue. Table 1 provides an overview of these four important factors for skeletal muscles, tendons and nerves, respectively, which serves as the basis for the discussion of current TE strategies to guide muscle, tendon, and nerve repair in the following sections.

3. Current tissue engineering strategies for skeletal muscle, tendon and nerve repair

The final properties of a TE construct depend largely on the specific design, the processing technique used, and its corresponding processing parameters. In addition, the specific design and the choice in processing technique are guided by, as previously described, the required mechanical and biological properties of the target tissue. A first correlation can be found between the different factors in the manufacturing process: (i) material selection, (ii) biological model (i.e., cells and bioactive compounds), and (iii) processing technique. The selection of these manufacturing process factors in turn influences the resulting mechanical and biological properties of the developed repair construct. Thus, there is a second correlation between (i) the requirements that the construct must meet (depending on the anatomy and physiology of the specific organ) (Table 1) and (ii) the achieved properties of the repair construct (depending on the manufacturing strategy). In the upcoming sections, each of the factors influencing the manufacturing strategy will be discussed and related to the mechanical and biological properties of skeletal muscles, tendons and nerves.

3.1. The biomaterial selection

Biomaterials have been used for over 20 years in TE and regenerative medicine to enhance tissue repair and to support transplantation of cells and/or growth factors [85]. While initially "inert" biomaterials were developed that elicit minimal immune response upon implantation, the emphasis has shifted in recent years to polymers, hydrogels, and other materials that can function as bioactive matrices [86]. Based on their chemical structure or nature, biomaterials for TE can be broadly classified into two different categories: synthetic and naturally derived biomaterials. Each category has advantages and disadvantages, and hybrid biomaterial combinations usually take advantage of both categories. The various types of materials used for skeletal muscle, tendon, and nerve repair and regeneration are listed in Tables 2, 3, and 4,

respectively, and are discussed below.

Synthetic polymers are widely used as biomaterials for scaffolds because of their ease of fabrication, high reproducibility, control of shape, architecture, and chemistry, versatility of processing techniques, and effective tunability of mechanical properties [87,88]. Synthetic materials used for TE include (i) polyesters such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly (lactic-co-glycolic acid) (PLGA), and poly(hydroxybutyrate) (PHB), (ii) poly(ethylene glycol) (PEG), (iii) polyurethane (PU), (iv) poly(vinyl alcohol) (PVA), and (v) poly(hydroxyethyl methacrylate) (pHEMA) [89–93]. Specific for muscle regeneration, the addition of electrically conductive nanoparticles to various synthetic polymers has been explored to create scaffolds capable of delivering electrical cues supportive of muscle regeneration while maintaining favorable mechanical properties [94]. Other studies have focused on changes in the surface wettability, mechanical properties, rate of degradation, and density of cell anchoring points of synthetic polymers in order to improve scaffold integration into skeletal muscle *in vivo* [95]. A comprehensive review of 3D *in vitro* skeletal muscle models can be found elsewhere [92]. For tendon repair, degradable polymers such as aliphatic polyesters including PLLA, PLGA, PCL, and PHB or polyurethane-based materials are most commonly used [14,33]. These degradable polymers generally have better long-term biocompatibility than non-degradable materials such as polytetrafluoroethylene (PTFE) and polyethylene terephthalate (PET), as they degrade over several months to physiological metabolites that are effectively excreted from the body [14,33]. For nerve repair, a variety of materials, including hydrogels, has been investigated as potential candidates in the form of films, mats, gels, sponges, nanoparticles, and so on. Hydrogels are a class of biomaterials that offer maximum flexibility and can be tailored to the requirements of neural TE. The most important factors to consider when selecting a hydrogel for neural TE include its mechanical properties, its ability to release growth factors or other bioactive molecules, its ability to promote neuronal cell attachment and growth, and its electrical conductivity. Some examples of hydrogels that have been used for nerve tissue engineering include alginate and hyaluronic acid hydrogels [96]. Boni *et al.* have published a detailed review of biodegradable and non-biodegradable synthetic and natural biomaterials for neural TE [90]. PLA and PGA were the first biopolymers to be tested for neural regeneration studies. Subsequently, the co-polymer PLGA provided new solutions for controlling permeability, swelling, deformation, and degradation rates by varying the ratio of PLA:PGA [90]. However, the use of synthetic materials also poses some limitations, including lack of biochemical cues, poor long-term patency and compliance, and possibly toxic degradation products [97–99].

Natural biomaterials, on the other hand, possess some advantages over synthetic materials, including their low immunoreactivity, intrinsic biochemical and mechanical cues for cell attachment and proliferation, and non-toxic degradation products [100,101]. However, extracted and processed natural materials often require a crosslinking step to become insoluble in physiological conditions. In addition, they have limited processability and mechanical strength and are subject to batch-to-batch variability [87]. Natural biomaterials can be subdivided into three groups: (i) polysaccharides and their derivatives: hyaluronic acid, chondroitin sulfate, cellulose, chitin, chitosan, (ii) proteins and their derivatives: collagen, gelatin, fibroin, elastin, silk, and (iii) materials derived from decellularized tissues: Alloderm™, small intestine submucosa (SIS), bladder acellular matrix (BAM) [101,102]. Further details on the types and properties of natural materials used for the fabrication of scaffolds for the regeneration of skeletal muscles [92,103,104], tendons [33,51,102], and nerves [105,106] have been described in literature and are not further discussed in this review.

Because of the intrinsic limitations of each group of biomaterials mentioned above, research has focused on hybrid biomaterials, which exploit the advantages of natural and synthetic materials. The combination of natural and synthetic biomaterials results in higher cell affinity, low immune response, and excellent biocompatibility of natural materials, together with the superior mechanical properties and control over shape, architecture, and structure of synthetic polymers [87]. Combining two or more polymers can be done simply by mixing, but also by advanced processing techniques. Research has been devoted to processing through coextrusion or coaxial pressure/electrospinning heads, where the core and sheath (and even more than one layer of the sheath) can be made of two or more different materials. Other possible combinations include post-processing steps for coating. Some of the approaches used for skeletal muscle repair include cell-loaded electrospinning of a 3D scaffold using an alginate/polyethylene oxide hybrid solution [107]. As an example, for tendon repair, Heidari *et al.* [108] described the fabrication of hybrid scaffolds (i.e., PCL and GelMA) by co-electrospinning or by a coating step after processing. In this way, the mechanical properties are improved compared to using a pure gelatin-based hydrogel, while excellent cell adhesion properties are maintained. For nerves, a combination of natural and synthetic polymers has been described by many research groups, with a synthetic polymer providing structural integrity and a coating of or a blend with a natural polymer [109,111]. This is mainly due to the need for a mechanically flexible yet biocompatible material. Yang *et al.* have explored the use of polypyrrole/alginate scaffolds that can be used as an electrically conductive biomaterial while maintaining a soft microenvironment [110] Abalymov *et al.* have listed hybrid materials for repair and cell growth in developing neural tissue [113].

3.2. The biological model

As highlighted in the previous sections, cells play an important role in the regeneration of damaged tissue. Therefore, cells are very often used as part of repair strategies, in combination with materials, or as a cell-based therapy. Depending on the type of tissue to be repaired or regenerated, specific cells are selected to achieve the appropriate physiological functions. For example, a well-established cell line known for its use in skeletal muscle regeneration studies is the murine myoblast line C2C12, typically in a 2D/3D monoculture [111–113]. For tendon repair, mainly tendon-specific stem and progenitor cells have been used in combination with autologous tenocytes and/or other co-cultures (e.g., adipose derived-stem cells, ASCs) [114], which have shown to increase collagen production and restore 3D collagen structure for both cell-based therapies and 3D scaffolds [14,115]. In many TE applications, transplantation of cells alone is not sufficient to repair and regenerate the tissue. Therefore, a viable alternative is the combination of biomaterials with a cell source [105], as these biomaterials provide the appropriate microenvironment for the cells and may also contain bioactive molecules that can stimulate cell growth . In nerve TE approaches, the preferred *in vitro* model has been the PC12 cell line, mostly because a large amount of data is already available on their ease of manipulation by pharmacological agents, proliferation, and differentiation profile [56,116–119]. They are also easy to culture and are an excellent model for neurotoxicity evaluation [120]. In addition, co-cultures of Schwann cells [121–123] and ASCs [73] have been

explored to gain insight in the behavior of nerve cells.

In addition to cells, a variety of bioactive compounds have also been evaluated for TE applications. Bioactive compounds, including growth factors (GF), cytokines and signaling molecules play an important role in the healing and repair of a damaged tissue. Growth factors have the capability to amplify the healing response by enhancing cell recruitment, proliferation, differentiation as well as ECM synthesis at the repair site [124] For example, fibroblast GFs (FGFs) are a family of cell signaling proteins that promote the growth of tenogenic progenitor cells, resulting in histological and biomechanical improvement of the repaired tendon [125]. Other GFs used in tendon repair include transforming GF-beta (TGFB), vascular endothelial GF (VEGF), platelet-derived GF (PDGF), and insulin-like GF (IGF) [33]. In addition, the correct GFs and their precise ratio should be upregulated at the correct time point during the various stages of the healing process, resulting in an increase in cell numbers and tissue volume [93]. For a more detailed discussion on the use of bioactive compounds in tendon repair, we refer to reviews from Gomes *et al.* [33] and Bianchi *et al.* [14]. The role of GF in tendon regeneration has been described by Randeli *et al.* [126], in which the effect of various GFs as well as platelet-rich plasma on different tendinopathies are discussed in detail. For example, growth differentiation factor 5 (GDF5) has been shown to stimulate stromal cells to produce a soft, collagenous musculoskeletal tissue that can be used in tendon regeneration [127]. Pharmacologically active compounds and drugs have also been applied in TE and regeneration approaches. In tendon repair, both anti-adhesive and anti-inflammatory drugs have been introduced into drug-eluting structures to efficiently enhance tendon regeneration [128–131]. Antibacterial drugs have also been studied in this regard [132]. In case of nerve damage, regeneration of the proximal end of the nerve and degeneration of the distal end begin almost immediately. In such cases, in addition to growth factors and bioactive molecules, there have been some experimental studies exploiting pharmaceutical agents to catalyze the nerve repair process [133]. Gold *et al.* [134] and Bota *et al.* [135] have investigated various agents such as tacrolimus, hyaluronic acid (HA), melatonin, methylprednisolone, calcium and potassium channel blockers. Neurotrophic factors and cells have been extensively studied in neural TE [105], focusing on neurotrophins such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3), all belonging to the same family [136,137]. Other neurotrophins explored are glial-derived neurotrophic factor (GDNF) and acidic or basic FGF [105,138,139]. In contrast with nerve regeneration, the use of bioactive compounds for skeletal muscle regeneration is limited in literature. There are a few examples where gold nanoparticles were used to impart electroactive properties to the material or to increase the thickness and orientation of myotubes [140,141]. Another example includes the use of scaffolds coated with poly-norepinephrine to increase the adhesion and proliferation of muscle cells [23].

3.3. *The manufacturing process*

Regeneration of non-hollow tubular tissues should focus on structural cues to promote cell adhesion, proliferation, and spatial alignment. On the other hand, cell-biomaterial interactions and, in particular, the mechanical and architectural impact (i.e., roughness, stiffness, porosity, orientation) of the material and/or scaffold on the cells are also important factors to consider. Thus, in addition to the selection of materials, cells and bioactive components, the processing technique plays an important role as well with regard to the required and resulting mechanical and biological properties of a construct.

Within the fabrication techniques for processing biomaterials to serve TE needs, extrusion-based 3D printing (3DP), solution

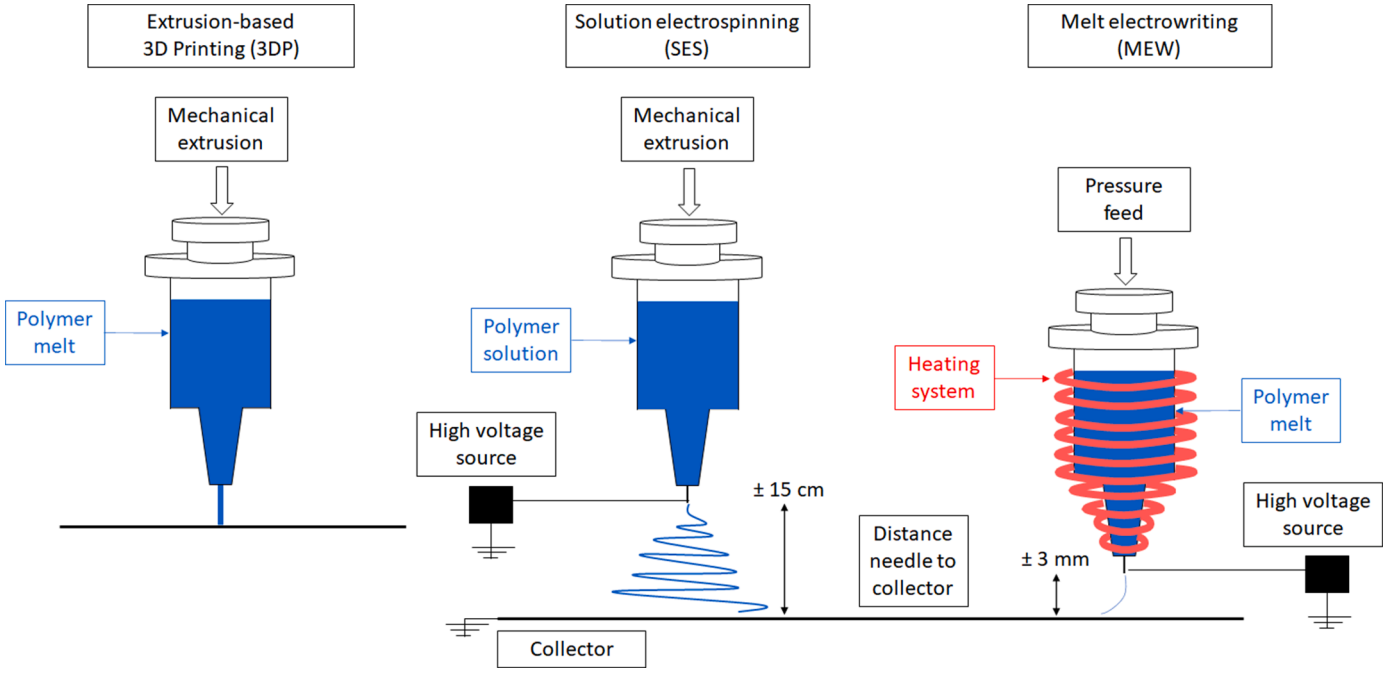

Fig. 4. Schematic overview of the set-up of three-dimensional printing (3DP), solution electrospinning (SES) and melt electrowriting (MEW) for biomaterial processing.

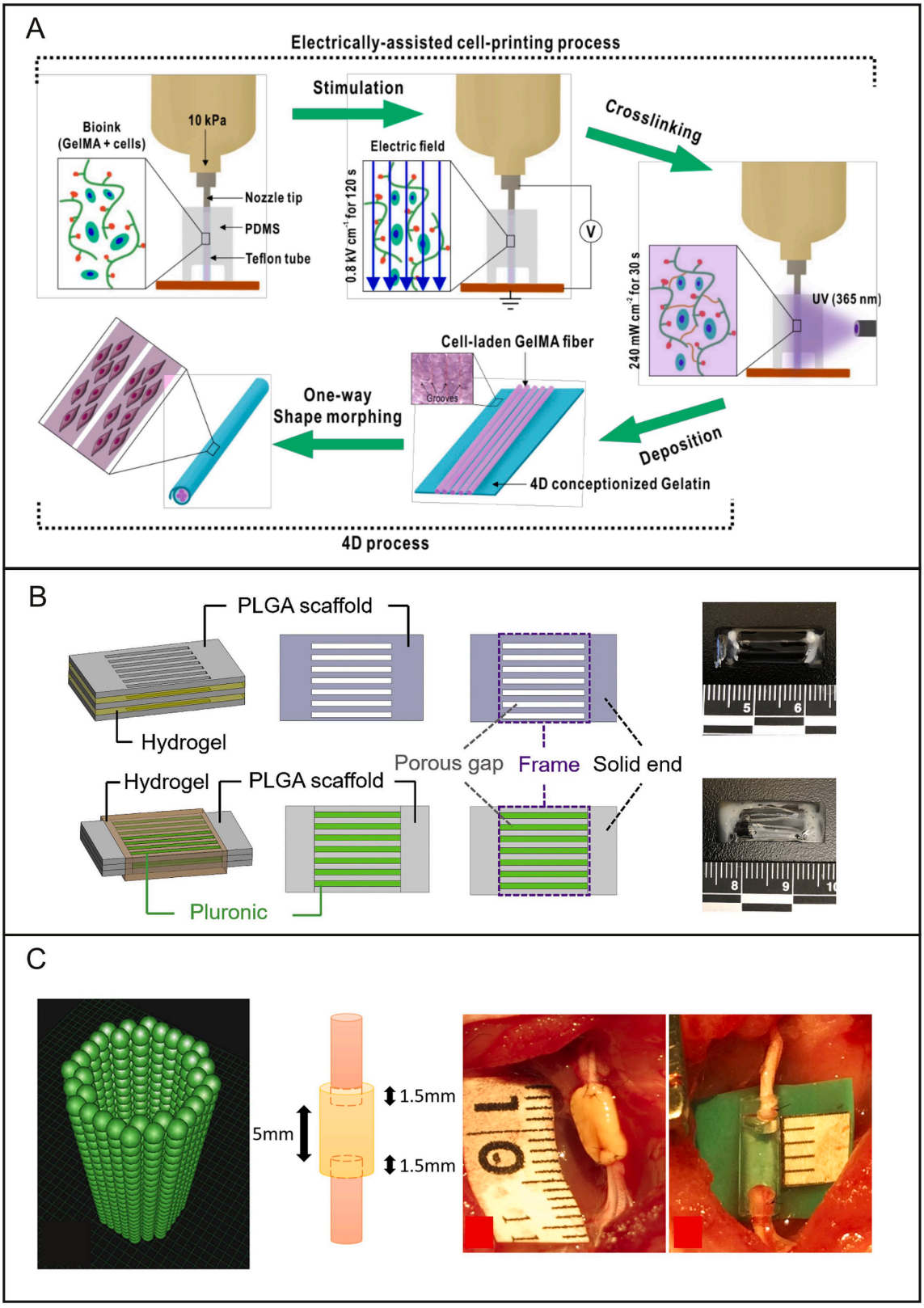

Fig. 5. *Examples of 3D printing approaches applied for TE of muscle, tendon, and nerve. (A) A cell-laden hydrogel (methacrylated gelatin, GelMA) was printed by means of an electrically assisted process. After UV crosslinking, the fibers were rolled to produce a 3D structure resembling the skeletal muscle fascicle. Adapted from Ref.* [181]*, ©2021 Yang et al. (B) Schematics of two poly(lactic-co-glycolic acid) (PLGA) scaffold models with collagen-fibrin hydrogels in a separate layer-by-layer structure (top) and in a tri-layered structure (bottom) for the preparation of hydrogel-loaded scaffolds for application in tendon regeneration. Adapted from Ref.* [163]*, ©2020 Jiang et al. (C) Design of a 3D tube-like structure based on homogeneous multicellular spheroids (left) and the bio 3D conduit* versus *silicone tube (right) interposed into the nerve defect. Adapted from Ref.* [178]*, ©2017 Yurie et al.*

electrospinning (SES), and melt electrospinning (MEW) have emerged as promising methods for fabricating tissue constructs suitable for the repair and regeneration of the three tissue types discussed in this review. Each of these fabrication techniques has specific advantages and limitations that should be considered when developing TE constructs to repair or regenerate damaged tissue (Fig. 4).

Because of the anisotropic fibrous structure of skeletal muscle tissue, the scaffold should contain physical cues for muscle cell orientation. The chemical cues are taken into account in the selection and modification of the biomaterial, e.g., by using collagen and gelatin derivatives. The three main strategies for incorporating directionality into skeletal muscle scaffolds include (i) cellularization (i.e., cell seeding) or bioprinting of aligned scaffolds, (ii) micropatterning of muscle progenitor cells in linear architectures using external fields during biofabrication of the construct, and (iii) alignment of cells by internal or external stimuli after fabrication [142]. For tendon repair, the effect of instructive cues originating from native tendon topography on guiding cell shape, phenotype, and function of tendon-related cells has also been studied. Dede Eren *et al.* [143] have demonstrated that native tendon topography contributes to the tenocyte phenotype. This was also observed in the work of Perikamana *et al.* [144], where favorable topographic cues (together with biochemical cues) showed their importance in the tenogenic differentiation of ADSCs. Schoenenberger *et al.* [145] have investigated how intrinsic topological cues from electrospun biomaterials and extrinsic mechanical loads cooperate to guide macrophage activation and macrophage-tendon fibroblast cross-talk. Their work gives insight into how a biological response might be therapeutically modulated by rational biomaterial designs that address the biomechanical niche of recruited cells [145]. In the case of nerve TE, it is important to design a construct that not only provides a favorable environment for nerve growth and regeneration, but also allows alignment of neurons. Some of these strategies were listed in the work of Daly *et al.* [6]. Extensive research has been done to explore different shapes and sizes of topography such as dots, pits, grooves, etc. at micro and nano-scales. The selected topography has a great impact on the behavior and growth of cells, which has been described in detail for different cell types by Yang *et al.* and Ma *et al.* [146,147].

The rise of additive manufacturing techniques has enabled the cost-effective development of patient-specific scaffolds in a high-throughput manner and is discussed here for the incorporation of topographic features into the scaffold. Incorporation of micropatterns into a scaffold to achieve directional organization can be achieved with patterns such as grooves, ridges, holes, channels, cantilevers [148], microchannels [149], microtopographic cues resulting from polymer fibrillation/leaching [150] or nanofiber structures by electrospinning techniques (see Table 3), among others [151].

This section provides an overview of the state-of-the-art of each processing technique used to engineer skeletal muscle, tendon or nerve. Within each subsection, Tables 2, 3, and 4, respectively, provide a summary of the materials, cells, and bioactive compounds used and the resulting mechanical and biological properties. The mechanical and biological pertinence indicated in these three tables (i.e., Table 2, 3, and 4) corresponds to the mechanical and biological requirements as listed in Table 1 (e.g., MR1 is the mechanical requirement number 1 from Table 1). These abbreviations are also used in the upcoming paragraphs.

3.3.1. *Three-dimensional printing (3DP)*

Three-dimensional printing is an additive manufacturing method based on computer-aided design (CAD) that creates a 3D construct in a layer-by-layer fashion. The operating principles of the various available 3D printers are described elsewhere [8,152–154]. Amongst the main advantages of this technique, is the precise control of biomaterial deposition at the micrometer scale, resulting in controlled porosity (both in terms of geometry and size) and an accurate mimicry of the macroscopic structure of native organs [155]. In addition, this technique allows the combination of multiple materials and multiple cell types in one process, enabling the fabrication of complex tissue architectures.

Most articles that applied 3D printing for skeletal muscle TE have reported the use of hybrid materials, followed by natural materials. All studies included *in vitro* assays, while only a few of them combined the *in vitro* studies with *in vivo* assessment [24,160]. In one third of the studies [157,158,160], GelMA was used, while in the vast majority of studies the most common cell type used was the C2C12 cell line [113,160,156–160]. Yang *et al.* have developed a C2C12-containing GelMA scaffold (Fig. 5.A) in which an effective myotube formation was demonstrated in response to stimulation with an electric field [181]. Regarding the requested biological and mechanical requirements (Table 1), muscle fiber alignment (biological requirement 1) was evaluated in almost all studies, while some of the studies included innervation or vasculature (biological requirements 2 and 3, respectively) [24,176]. One single study [159] evaluated the resistance to deformation (mechanical requirement 3).

In tendon repair, most research efforts have focused on synthetic materials or hybrid materials because tendon repair imposes high mechanical demands on the materials used. Hybrid materials often cover biological requirements as well [163,166,167]. In terms of cell type, mesenchymal stem cells [161,163,164] and tendon progenitor cells [162], known for their ability to differentiate into tenocytes, are most frequently used to study tendon tissue repair, besides tenocytes [165,167] or fibroblasts [161,169]. Especially the appropriate mechanical properties (i.e., max. strengths, elastic modulus, strain and modulus at failure – mechanical requirement 1) as well as the hierarchical organization and fiber orientation (biological requirement 1) were studied [51,162,177]. For the development of tubular constructs mimicking heterogeneous structures and mechanical properties, different approaches have been evaluated. The study of Jiang *et al.* compared the fabrication of a cell-laden scaffold in a separate layer-by-layer structure and a multilayered structure as a whole (Fig. 5.B), both demonstrating growth, proliferation and tenogenic differentiation of human ASCs [163]. It should be mentioned however that most research groups focused mainly either on the mechanical requirements or on the biological requirements. To the best of our knowledge, there are no scientific reports so far in which all the mechanical and biological requirements are studied.

Different research groups approach three-dimensional bioprinting applying potential NGCs from different perspectives. The group of Ramesh *et al.* [119], for instance, is active in reverse engineering the entire printing process, while other researchers mainly focus on the design of the conduit. Another example can be found in a scaffold-free approach, as shown by Yurie *et al.* who have developed a 3D conduit composed entirely out of cells to promote nerve generation (Fig. 5.C) [178]. However, when considering the development of nerve constructs, the mechanical properties are quite challenging, which explains the

Table 2
Summary of studies using 3D printing (3DP) as a fabrication technique for muscles, tendons and nerves. MR and BR indicate mechanical and biological requirements described in Table 1. VIT and VIV indicate *in vitro* or *in vivo* studies. Color coding was used to indicate whether the research study investigated/explored (green) or not (red) the mechanical and biological pertinence [24,56,116,122,124–126,153,159–180].

3D Bioprinting for regeneration of	References / study	Material choice	Cells & bioactive compounds	Scaffold architecture / morphology	Design idea and focus on anatomical key points / physiological functions in the study	Mechanical and biological pertinence							
						MR 1	MR 2	MR 3	BR 1	BR 2	BR 3	VIT	VIV
Skeletal muscles	Dixon *et al.* (2018) [159]	Silk fibroin / Collagen I / Matrigel	Human SkMMs Mouse glioma cells (NG108-15)	3D human myoblast-laden hydrogel; Shear-induced alignment; T-shaped cantilever (height: 5 mm, thickness: 2 mm, width (max): 6 mm)	Creation of 3D co-culture of muscle and neuron aiming to replicate alignment in gel cast approaches.								
	Kang *et al.* (2016) [160]	Gelatin / fibrinogen / HA / glycerol hydrogel, with PCL and Pluronic hydrogel scaffold elements	C2C12	Fiber-like (~400 μm diameter) patterned hydrogel with microchannels (650 x 450 μm^2) and supported by PCL pillars; Shear-induced alignment; 15 x 5 x 1 mm^3	Creation and evaluation of 3D muscle construct for in vivo maturation.								
	Mozetic *et al.* (2017) [161]	Pluronic / alginate	C2C12	Mesoporous hydrogel structure by Pluronic elution; Shear-induced alignment	Evaluation of direct wire bioprinting technology to create vastly organized constructs for muscle regeneration.								
	García-Lizarribar *et al.* (2018) [162]	GelMA and GelMA composite: CMCMA / PEG-DA / AlgMA	C2C12	Composite hydrogel scaffold; Shear-induced alignment; Young's modulus = 2-7 kPa; Cylinder: 16 mm diameter x 400 μm thickness; (200 μm nozzle inner diameter)	Characterization of various composite hydrogels' mechanical and geometrical properties and their effect on proliferation and alignment.								

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Table 2 (*continued*)

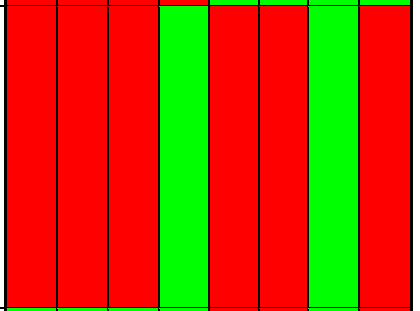
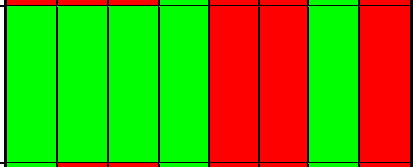
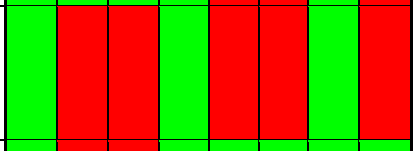
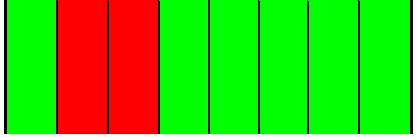
	J. H. Kim *et al.* (2020) [24]	PCL Fibrinogen based bioink Gelatin based bioink	Human MPCs Human NSCs	Fiber-like (~300 μm diameter) patterned hydrogel with microchannels (~300 μm diameter) and supported by PCL pillars; Shear-induced alignment; 10 x 7 x 3 mm^3 (300 μm nozzle inner diameter)	Evaluation of NMJ's effect on innervation, development and functionality.	
	W. J. Kim & Kim (2020) [163]	GelMA / ColMa	C2C12 Human ASCs Human MPCs	Mesh scaffold (~300 μm strut width); Shear-induced alignment; G'~400Pa; 12 x 5 x (<1mm) mm^3	Evaluation of pre-cultured bioinks and extrusion stress on myotube alignment and maturation.	
	Y.-J. Choi *et al.* (2016) [164]	Decellularized muscle ECM bioink	C2C12	Parallel, hexagonal or chain-patterned hydrogel (500, 1500, 5000 μm strut width) within a PCL framework; Shear-induced alignment; Young's modulus~12kPa	Creation of functional muscle constructs with mdECM bioink and evaluation of mechanical factors/ cell functionality.	
	Distler *et al.* (2020) [116]	Oxidized alginate-gelatin	C2C12	Hydrogel (600-900 μm strut width) with parallel grooves (600-900 μm strut spacing); Shear-induced alignment; 15 x 15 x 0.3 mm^3	Evaluation of the usability of oxidized alginate-gelatin as a bioink and influence of printing direction on cell alignment.	
	Lee *et al.* (2021)[153]	dECM / dECM-MA / GELMA / PVA	Primary human muscle progenitor cells	Hydrogel (~350 μm strut width) with parallel grooves and microtopographic cues; Shear-induced alignment; G'~2-8 kPa; 10 x 7 x 3 mm^3	Self-aligned myofibers in 3D bioprinted ECM-based construct accelerate skeletal muscle function restoration.	

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Table 2 (*continued*)

	Yang *et al.* (2021) [165]	GelMA	C2C12	Tubular structure by folding of gelatin film with grooves (150 μm spacing) coated with cell-laden Gel-MA hydrogel fibers (250-350 μm diameter); Shear-induced alignment; Young's modulus ~ 50-150 kPa; Folded tube (10 mm diameter x 25 mm length)	Combination of an electric field with 3d printing to aid cell alignment and differentiation.								
Tendons	Nakanishi *et al.* (2019) [166]	None	Human dermal fibroblasts MSCs	Scaffold-free ring-like structure assembled by MSCs (diameter 500-600 μm; 9x9 needle-array)	Evaluation of tendon formation using a scaffold-free 3D-bioprinted construct.								
	Zhang *et al.* (2021) [167]	GelMA NB and HA	Tendon stem / progenitor cells Hyaluronic acid	Cuboid GelMA/HA-NB cell-laden scaffolds (2x4 mm, with inner tunnels side length: 200 μm); Young's modulus ~ 40 MPa; Stiffness ~ 9 N/mm	Enhanced ability in promoting functional tendon repair and regeneration by combining small molecule-based culture system with 3D printing technology								
	Jiang *et al.* (2020) [168]	PLGA Collagen-fibrin	Human ASCs	Multi-layered PLGA scaffold with collagen-fibrin cell-laden hydrogel (20x15 mm rectangle, thickness 1.15 mm); Stiffness ~ 55-70 N/mm	New strategy for rotator cuff tendon repair by combining 3D printing of PLGA with cell-laden collagen-fibrin hydrogels.								
	Chae *et al.* (2021) [169]	Tendon-derived decellularized ECM / bone derived decellularized ECM	Human BM-MSCs	Spatially-graded tendon-to-bone patches (3x3x0.6 mm); Young's modulus ~ 30-40 MPa; stiffness ~ 45-80 N/mm	Novel therapeutic platform to achieve spatially-graded physiology for functional tendon-bone interface in rotator cuffs.								
	Wu *et al.* (2017) [170]	PCL	Human tenocytes	2 layers of thick printed fibers, four layers of thin fibers; leading to fibers and micro-ridge/grooves. Mesh was rolled to be ~2.5 mm diameter and 2.2 cm	Degradation behaviors of geometric cues and mechanical properties in a 3D scaffold for tendon repair.								

(*continued on next page*)

Table 2 (*continued*)

				length; pore size of ~110 μm × 2000 μm; Young's modulus ~ 227 MPa		
	Merceron et al. (2015) [171]	Poly(urethane) (PU) and PCL Bio-ink: HA / gelatin / fibrinogen	C2C12 NIH/3T3	Cell-laden muscle-tendon unit constructs (20x5x1 mm); Final construct was elastic on the PU-C2C12 muscle side (E = 0.39 ± 0.05 MPa), stiff on the PCL-NIH/3T3 tendon side (E = 46.67 ± 2.67 MPa) and intermediate in the interface region (E = 1.03 ± 0.14 MPa).	Integrated organ printing (IOP) technology to develop an integrated muscle-tendon unit construct.	
	Laternser *et al.* (2018) [172]	GelMA PEG-DMA	Human SkMMs Rat tenocytes	Dumbell-shaped lines bioprinted with tenocytes and myoblasts (container structure or two-channel structure)	3D bioprinting to develop a microplate platform for the engineering of muscle and tendon tissues.	
	Wu *et al.* (2021)[173]	Dopamine / PCL and TGF-β1	Primary tenocytes	Rectangular hydrogel scaffold of 10x3x1 mm	TGF-β1 loaded tendon scaffold to prevent tendon adhesion and to promote tendon repair.	
	Zhang *et al.* (2021) [174]	PCL / Pluronic	Mouse embryonic fibroblasts (C3H10T1/2)	3D printed scaffold on a wafer plate; 4 cm x 4 cm x 0.5mm; Fiber diameter 20-25 μm; pore size 375-390 μm; contact angle 20-70°C (depending on material formulation); Young's modulus 27-67 MPa (depending on material formulation)	PCL and Pluronic F127 scaffolds to study the repair of rat Achilles tendons.	
Nerves	Marino *et al.* (2013) [175]	OrmoComp®	PC12	N/A	Topographic effects of micromimetic patterned surfaces on neuronal cell behavior observed via Direct laser writing (DLW)	

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Table 2 (*continued*)

Zhu *et al.* (2018) [124]	PEG-DA GelMA	Sciatic nerve SCs	Hollow NGC with microchannels, branched conduit	Rapid continuous 3D printing (projection printing and continuous fabrication) with 4 microchannels to facilitate structural cues for optimal directional growth	
Vijayavenkataraman *et al.* (2018) [56]	PCL	PC12	Multilayered porous scaffold rolled into a tubular structure	3D printing using electrohydrodynamic jetting (EHD) system to form a multi-layer scaffold	
Singh *et al.* (2018) [176]	Modified PCL Chitosan / gelatin cryogel	NGF Neuroblastoma cells (Neuro2a)	Hollow cylindrical tube, 4-channel cylindrical tube	Four armed photocrosslinkable oligomer synthesised from PCL and filled with NGF containing cryogel	
Hsiao *et al.* (2020) [177]	PLA	Human dental pulp MSCs	Flat parallel alternating struts	3D printing using fused deposition modeling; the effect of gap width between parallel microstruts on the surface was analyzed	
Xu *et al.* (2019) [178]	GelMA Monomethoxy PEG - PCL nanoparticles	PC12 SCs HDAC-3 selective inhibitor (RGFP966)	Hollow cylindrical tubes with guidance filaments	3D printing with slow drug releasing nanoparticles-based resin	
Chen *et al.* (2019) [125]	PU / polydopamine / decellularized ECM	Human SCs	Hollow cylindrical tube	Assessment of physical properties, biodegradability, cytocompatibility, neural related GF, protein expression and cellular	

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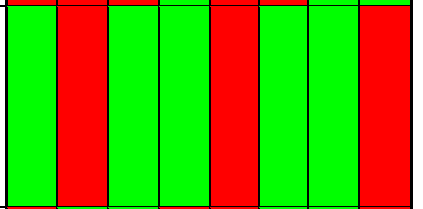
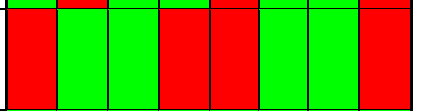
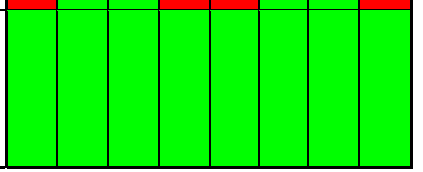
Table 2 (*continued*)

					adhesion and proliferation	
	Wu *et al.* (2020) [179]	Gelatin-sodium alginate hydrogel blend	Rat SCs	Multichannel tube	Gelatin and alginate used to encapsulate SCs for 3DP	
	Ye *et al.* (2020) [126]	GelMA	PC12 NCSCs	Multichannel hollow cylindrical tube	DLP printing of NGCs with (i) microchannels, (ii) NCSCs based bioink	
	Ramesh *et al.* (2021) [122]	PLA	PC12	Cylindrical tube filled with nanoflower design	Reverse engineered nerve guidance conduit	
	Zhang *et al.* (2022) [180]	GelMA-Vitamin B12 blend	PC12	Branched cylindrical conduit	Customized branch nerve conduit fabricated using DLP, resulting in the formation of a complex nerve network, confirmed with electrosphysiology	

Abbreviations: 3D, three-dimensional, AlgMa, methacrylated alginate; ASCs, human adipose stem/stromal cells; BM-MSCs, human bone marrow-derived mesenchymal stem cells; BR, biological requirements; C2C12, mouse murine myoblast cell line; CMCMA, methacrylated-carboxymethyl cellulose; ColMA, methacrylated collagen; DLW, direct laser writing; DLP, digital light processing; EHD, electrohydrodynamic jetting; GelMA, methacrylated gelatin; HA, hyaluronic acid; HDAC-3, histone deacetylase 3 enzyme; MPCs, muscle precursor cells; MR, mechanical requirements; MSCs, mesenchymal stem cells; NB, N-(2-aminoethyl)-4-(4-hydroxymethyl-2-methoxy-5-nitrosophenoxy) butanamide; NCSCs, neural crest stem cells; NGF, nerve growth factor; NHDFs, normal human dermal fibroblasts; NIH/3T3, mouse immortalized fibroblast cell line; PC12; rat pheochromocytoma cell line; PCL, poly(ε-caprolactone); PEG, poly(ethylene glycol); PEG-DA, poly(ethylene glycol) diacrylate; PEG-DMA, poly(ethylene glycol)-dimethacrylate; PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PU, polyurethane; SCs, Schwann cells; SkMMs, skeletal muscle myoblasts; TSPCs, tendon stem/progenitor cells; VIT, *in vitro* study; VIV, *in vivo* study.

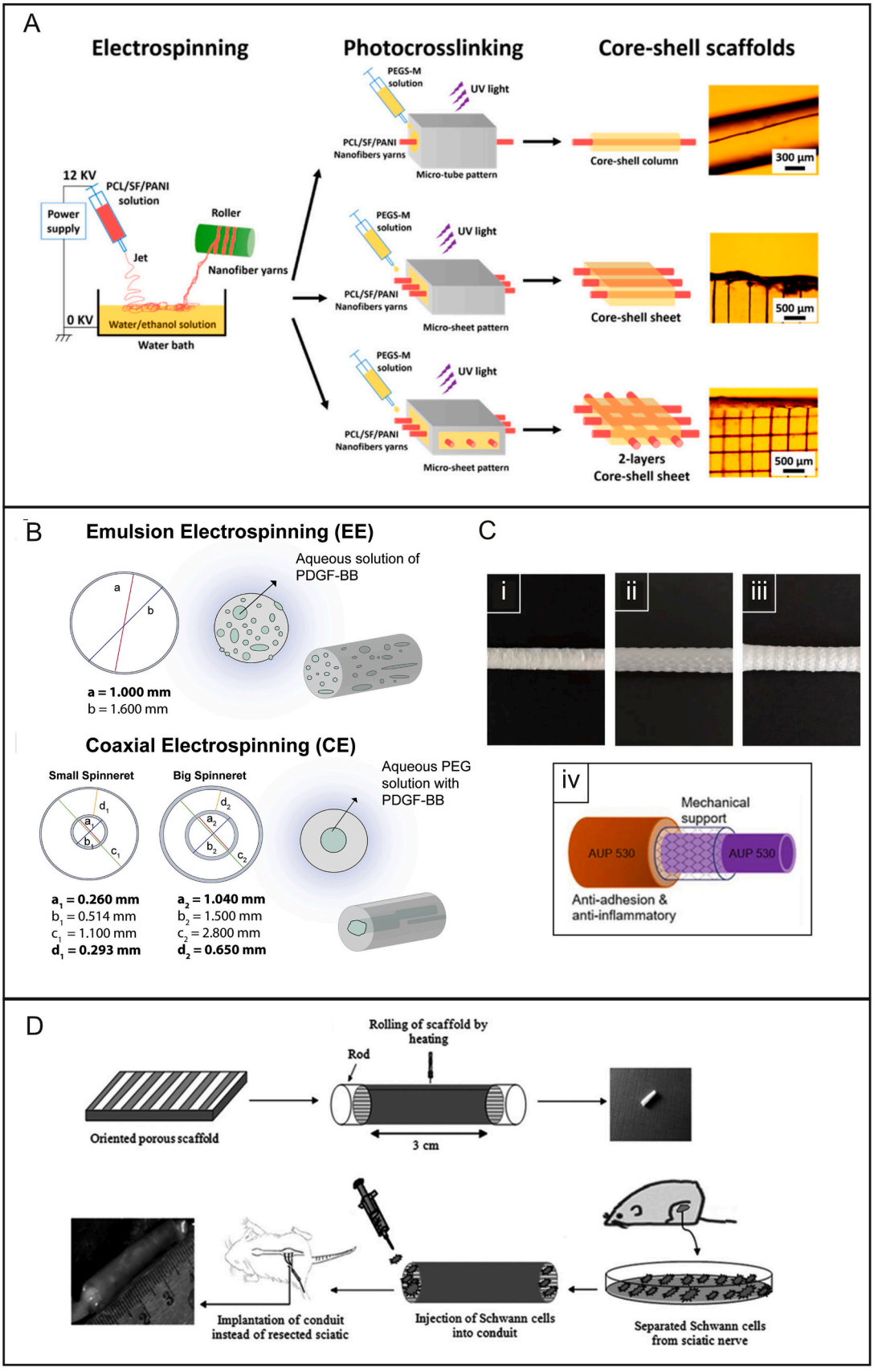


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Fig. 6. Examples of solution electrospinning approaches applied for TE of muscle, tendon, and nerve. (A) Preparation scheme of core-shell column and sheet scaffolds that mimic the native skeletal muscle tissue by combining aligned nanofiber yarns via electrospinning and hydrogel shell via photo-curable microfabrication. Adapted with permission from Ref. [194], ©2015 American Chemical Society. (B) Sketch of the spinnerets used for emulsion electrospinning and coaxial electrospinning for the fabrication of tubular constructs allowing a sustained delivery of growth factors. Adapted from Ref. [199], ©2019 with permission from Elsevier. (C) Schematic of a multi-layered electrospun tubular construct for tendon repair by combining a mechanical (i.e., mechanical support) and a biological (i.e., incorporation of anti-inflammatory and anti-adhesion drugs) approach. Adapted from Ref. [205], ©2022 by Pien *et al.* (D) The grafting process of an artificial nerve conduit in a resected sciatic nerve segment: oriented film fabricated by micropattern wafers, tube formation by rolling of the film by a heat sealing process, followed by injection of Schwann cells and implantation. Adapted from Ref. [213], ©2014 by Karimi *et al.* Abbreviations: PCL: poly(ε-caprolactone), SF: silk fibroin, PANI: polyaniline, PEGS-M: poly(ethylene glycol)-co-poly(glycerol sebacate), PDGF-BB: platelet-derived growth factor BB, PEG: poly(ethylene glycol, AUP: acrylate-endcapped urethane-based polymer.

common use of hybrid materials or coating a natural polymer with another physically tough, yet synthetic polymer. To provide an appropriate biocompatible environment for optimal cell adhesion and proliferation, a natural polymer should be used, which was confirmed by thorough *in vitro* testing with PC12 cells [56,173,179], Schwann cells, and neural stem cells [123]. Furthermore, additional bioactive compounds can be incorporated into the printed scaffold to enhance cell viability, such as NGF [122,171] and gradual delivery of drugs encapsulated in nanoparticles [173]. In summary, flexibility and cell adhesiveness have been extensively researched in almost all studies, whereas the effects of pore size in the scaffold wall on myofibroblast infiltration have not been evaluated. One study from Singh *et al.* and more recently, Zhang *et al.* covered all mechanical and biological requirements [171, 175].

3.3.2. Solution electrospinning (SES)

Solution electrospinning is a processing technique based on the application of a high-voltage electric field to enable the production of micro and nanoscale fibers from a polymer solution by depositing the fibers onto a suitable collector (i.e., plate, rotating mandrel, etc.). A detailed description of this technique and its fundamentals is beyond the scope of this review, but the interested reader is referred to a book from Bosworth *et al.* [180]. As mentioned above, the main advantage of SES compared to other material processing techniques is the possibility to produce a fibrous network that resembles the natural ECM in terms of hierarchical organization and properties. Therefore, this network generally provides an excellent microenvironment for cell adhesion, proliferation and differentiation [181,182].

Almost half of the studies applying solution electrospinning for skeletal muscle TE, used synthetic materials, followed by natural materials, and only a minority [107,184,194,195,211] used hybrid materials. The study of Wang *et al.* [194] used a dry-wet electrospinning method to develop hybrid scaffolds with a micro-sheet patterning that led to successful myoblast alignment and elongation (Fig. 6.A). Almost all studies were conducted *in vitro*, while less than half of the studies [23, 94,95,103,104,191] were conducted *in vivo* or included *in vivo* aspects. Considering material type, PCL was commonly used, while the C2C12 cell line was used in more than half of the studies, when considering cell type. The vast majority of papers evaluated muscle fiber alignment [95, 103,104,107,183–185,189–192,194–197,211]; almost half of them evaluated tensile strength and deformation [95,184,189,192–195,197], while the resistance to deformation was only assessed in some studies [183,189,192,193,195,197,211]. Only two studies [95,104] included innervation (biological requirement 2).

For tendon repair, it is clear that synthetic materials should be preferred in terms of mechanical requirements. They can be combined with natural materials. As with 3DP, the cell types of choice are tenocytes [198–200] and mesenchymal stem cells [201,212]. The effect of platelet-derived growth factor BB was evaluated in 2 of the listed studies [199,201]. When comparing the mechanical and biological requirements, none of the studies met all requirements from both the mechanical and biological perspective. One-third of the studies described the presence of adhesion and inflammation [131,199–202] (and hence scar formation) (BR3, Table 1), whereas fiber sliding, degree of rotation, and gap formation in the repair zone [131,199,200,202] (MR3) and blood circulation and innervation [131,201,202] (BR2) were only assessed in less than 50% of the studies. For the fabrication of tubular constructs for tendon repair (Fig. 6.B), either emulsion or coaxial [199] electrospinning have been proposed for the incorporation and delivery of growth factors. Another study used solution electrospinning as a drug delivery approach to incorporate and release anti-adhesion and anti-inflammatory components into the tubular construct (Fig. 6.C) [205].

Electrospinning is a popular fabrication method when considering nerve conduits, with an abundance of flexibility in terms of material selection and design strategies. PCL has been used in some studies due to its robust mechanical properties [207–210]. As in 3DP, PC12 cells are the preferred cell type for *in vitro* biological testing [116–118,208]. Most studies have shown that an additional stimulus can be provided to the growing cells, besides structural stimuli, to promote directed growth. In this regard, carbon/graphene-based materials have been used to provide electrical stimuli to increase the efficacy of neurite outgrowth [116,118, 206,208,210]. Due to the nanofibrous topology of the mats, more attention was paid to the diameter of these fibers, which is observed in all studies. Pore size (BR2) was only considered in the work of Ghobeira *et al.* [73] whereas only one *in vivo* study [118] was performed. Using a rat model, Karimi *et al.* [213] reported the successful repair of 30-mm-long sciatic nerve gaps. The authors applied electrospun PHBV scaffolds, which were rolled onto nerve guide conduits and loaded with Schwann cells (Fig. 6.D) [213].

3.3.3. Melt electrowriting (MEW)

MEW is a very recent and emerging technique being explored for various biomedical and TE applications. The process is similar to solution electrospinning, with that difference that the polymer solution is replaced by a polymer melt. The main advantage over SES is that the MEW process allows direct writing of a 3D scaffold with a predefined architecture at high reproducibility. Moreover, the scaffold design has a highly controlled pore size and interconnectivity. Like 3DP, this technique is also based on a computer-controlled layer-by-layer approach. An overview of the possible hybrid set-ups and applications, and of the opportunities in different areas of TE is described elsewhere [214–217]. Kade *et al.* [218] reviewed the various polymers that have been explored in MEW. By precisely controlling fiber deposition and alignment in specific 3D patterns, MEW can steer physiological cell organization and differentiation. As already described, cell alignment is important to recapitulate the physiological functions of the hierarchical structures of a given organ.

So far, the use of melt electrowriting for skeletal muscle TE has been described in only two studies: synthetic materials were evaluated in the first study [140] while a hybrid material was used in the second study [112]. Both studies assessed muscle fiber alignment (BR1) and tensile strength and deformation (MR2), while innervation (BR2) and vasculature (BR3) were not taken into account. Zhang *et al.* [223] have constructed a hierarchically organized, anisotropic and conductive scaffold (Fig. 7.A) and showed the formation and morphology of myotubes. Due to the small number of studies, no trend can be identified in terms of frequently used cell or material type.

Table 3
Summary of studies using solution electrospinning (SES) as a fabrication technique for muscles, tendons and nerves. MR and BR indicate mechanical and biological requirements described in Table 1. VIT and VIV indicate *in vitro* or *in vivo* studies. Color coding was used to indicate whether the research study investigated/explored (green) or not (red) the mechanical and biological pertinence [23,46,95,96,104,105,108,119–121,133,134,144,184,186,188–216].

Solution electrospinning for regeneration of	References / study	Material choice	Cells & bioactive compounds	Scaffold architecture / morphology	Design idea and focus on anatomical key points / physiological functions in the study	Mechanical and biological pertinence							
						MR 1	MR 2	MR 3	BR 1	BR 2	BR 3	VIT	VIV
Skeletal muscles	Aviss *et al.* (2010) [189]	PLGA	C2C12	Aligned fibers (0.6-0.9 μm diameter); Young's modulus ~ 800 MPa (parallel)/100 MPa (perpendicular); Thin fibrous sheet (~0.2 mm thickness)	Evaluation of the cell proliferation capacity and orientation within the material of interest.								
	Yeo & Kim (2018) [108]	Alginate / PEO	C2C12	Aligned hydrogel fibers (~300 nm diameter); Young's modulus ~5 MPa; Single strut (~100 μm diameter x 30 mm length)	Analysis of various degrees of electrospun fiber alignment effect on cell differentiation and organization.								
	McKeon-Fischer & Freeman (2011) [144]	Au–PLLA	Rat primary muscle cells Gold nanoparticles	Gold nanoparticles encapsulated in randomly aligned PLLA fibers (~1.5 μm diameter); Young's modulus = 50-100 MPa; Conductivity=0.05-0.1 S/cm; 1 x 1 cm^2	Assessment of different scaffold composition for biocompatibility and mechanical properties in order to lower the voltage used for electrical alignment								
	J. S. Choi *et al.* (2008) [190]	Poly(ε-caprolactone) (PCL) / collagen	Human SkMMs	Aligned fibers (200-400 nm diameter); Young's modulus ~ 4 MPa (parallel)/3 MPa (perpendicular); Tensile strength = 5 MPa (parallel)/3 MPa (perpendicular); Elongation at break = ~40% (parallel)/ ~100% (perpendicular); Nanofiber meshes (3.8 x 3.8 cm^2)	Study of the effect of fiber alignment on cell alignment, proliferation, differentiation and morphology of target cells.								
	Gilbert-Honick, Iyer *et al.* (2018) [104]	Fibrinogen / sodium alginate	C2C12	Hydrogel fibers (diameter N.A.); Young's modulus ~ 17 kPa; Microfiber yarn (~700 μm in diameter) on a	Development of aligned myofiber bundles of similar strength to native muscle, to assess their								

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			Human umbilical vein endothelial cells Human ASCs	supporting ABS frame (1.5 x 3.0 cm^2)	potential for muscle regeneration in vitro/vivo.	
	Smoak *et al.* (2021) [191]	Decellularized skeletal muscle ECM	C2C12	Aligned hydrogel fibers (~ 5 μm diameter); Young's modulus = 500-1000 kPa; 10 x 50 x 0.2 mm	Fabrication of different scaffold permutations with varying degree of fiber alignment, mechanical properties and its effect on cell alignment/differentiation	
	Narayanan *et al.* (2020, 2021) [192,193]	PLGA	PC12 C2C12	Aligned fibers (0.2-3 μm diameter); Young's modulus ~ 350 MPa	Characterization of the effect of P12 cells secretome on myoblast development and creation of a hydrogel mimicking ECM properties.	
	McKeon-Fischer *et al.* (2011, 2014) [95,194]	PCL - MWCN PAA / PVA	Acellular	Coaxially spun aligned fibers (1-4 μm diameter) with PCL/MWCN inner core and PAA/PVA outer hydrogel shell; Young's modulus ~ 12 MPa; Yield stress ~ 650 kPa; Conductivity ~ 0.4 S/cm; Aligned fiber mat (3 x 5 mm^2)	Determination of biocompatibility of scaffold material by in vivo evaluation/transplantation.	
	Y. Liu *et al.* (2017) [23]	PCL	Acellular Poly-norepinephrine coating	Randomly aligned fibers (400-900 nm); 3 x 2 cm^2 (2-10 μm diameter);	Implantation and evaluation of a new functionalized biomaterial to use for muscle regeneration in vivo	
	Bloise *et al.* (2018) [96]	PBCE / BCE / TECE	C2C12	Aligned fibers (400-1000 nm diameter); Young's modulus = 15 - 30 MPa; Elongation at break ~ 50%; Aligned fiber mat (0.2 mm thickness)	Study of a novel biomaterial, with focus on in vitro testing and in vivo transplantation in	

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Table 3 (*continued*)

				healthy and pathological models.	
Perez-Puyana *et al.* (2021) [195]	Fish gelatin type B / collagen protein / PCL	L6 cell line	Aligned hybrid protein-PCL fibers (~250 nm fiber diameter); Young's modulus ~ 2 MPa; Elongation at break ~ 50%	Assessment of a hybrid material of synthetic and biological components for mechanical properties, and cell alignment, to identify the most optimal scaffold alignment.	
Tang *et al.* (2019) [196]	PCL	C2C12 polydopamine and PEDOT: PSS coating	Aligned polydopamine and PEDOT: PSS (~400 nm particle size) coated fibers	Development of a novel, stimulus responsive scaffold to support muscle regeneration by achieving biocompatibility together with the ability to regulate the microenvironment.	
Gilbert-Honick, Ginn *et al.* (2018) [197]	Fibrinogen / sodium alginate	ASCs	Aligned hydrogel fibers; Young's modulus ~ 17 kPa; Microfiber yarn (~1 mm diameter) around a mylar frame (1.5 x 3.0 cm^2)	Development of a scaffold material with comparable mechanical properties to the endogenous muscle, stimulating cell differentiation in vitro and scaffold integration in vivo	
Chen *et al.* (2013) [198]	PANI / PCL	C2C12	Aligned fibers (~350 nm diameter); Young's modulus ~ 10-50 MPa; Conductivity ~ 15-60 mS/cm; Elongation at break ~ 40-60%; Tensile strength ~ 7-10 MPa; 60 x 20 mm^2	Optimization of scaffold composition and fabrication conditions and analyzing their effect on cell viability and alignment.	
Hosseinzadeh *et al.* (2016) [199]	PANI / PAN	Mouse satellite cells	Randomly aligned composite fibers (~100 nm diameter); Young's modulus ~ 150 MPa; Elongation at break ~ 50%; Peak stress ~ 1.5 MPa; 10 x 60 mm^2	Evaluation of composite scaffold material in terms of mechanical and cellular properties such as strength, cell proliferation and differentiation, showing that composite scaffolds are preferred to non-composite.	

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	Wang *et al.* (2015) [200]	PCL / SF / PANI / PEGS	C2C12	Aligned core-shell composite fibers (~ 500 μm); Core: PCL/SF/PANI (25 to 150 μm yarn diameter made up of 600-900 nm diameter electrospun nanofibers); Shell: PEGS hydrogel	Creation of a 3D scaffold/net of yarn-like composite nanofibers seeded with cells and incased in hydrogel. Focus on mimicking the structure of the nanofibers and the surrounding connective tissue.								
	HasmadHanis *et al.* (2018) [201]	PLGA fibers on human amniotic membrane	Human SkMMs	Aligned fibers (~ 1 μm diameter); Young's modulus ~ 250 MPa (hydrated); Ultimate tensile strength ~ 20-30 MPa (hydrated); 3 x 3 cm^2	Evaluation of amnion membrane to improve scaffold tensile strength shortcomings and provide alignment through electrospun fibers.								
	Gilbert-Honick *et al.* (2020) [105]	Fibrin	C2C12 Tethered heparan sulfate proteoglycan (agrin)	Randomly aligned hydrogel fibers; Young's modulus ~ 120 kPa; Microfiber yarn (~ 0.7 mm diameter) around an ABS frame (1.5 x 1.5 cm^2)	Incorporation Agrin into to the scaffold material to increase the re-innervation of the implant in vivo after VML								
	Guo *et al.* (2019) [202]	Fibrinogen / PEO	C2C12 (aggregates ~ 90 μm diameter)	Aligned hydrogel fibers; Microfiber yarn (~ 0.7 mm diameter) around an ABS frame (1.5 x 1.5 cm^2)	Direct incorporation of cells during electrospinning process and assessing their ability to conduct myogenesis								
	Ricotti *et al.* (2012) [203]	PHB	C2C12 and H9c2 myoblasts	Aligned fibers (200-400 nm diameter); Young's modulus ~ 270 MPa; Ultimate tensile strength ~ 10 Nmm^{-2}; Elongation at break ~ 13 %; 20 x 5 x 0.1 mm^3	Evaluation of properties of aligned and unaligned material of bacterial origin and their effect on cell differentiation and proliferation.								
	Yeo & Kim (2020) [184]	Alginate / PEO (mesh) PVA / PCL / collagen (supports)	Human umbilical vein endothelial cells and C2C12	Aligned cell-laden hydrogel fibers (~400 nm diameter) on micropatterned (PVA-leached) PCL or collagen 3D-printed strut; 30 mm length, ~ 300 μm diameter	Creation of composite constructs by cell electrospinning supplemented by 3D-bioprinting with mechanical properties similar to endogenous muscle.								

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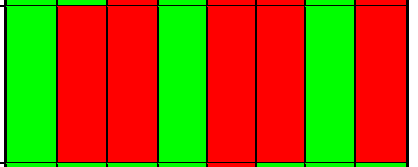
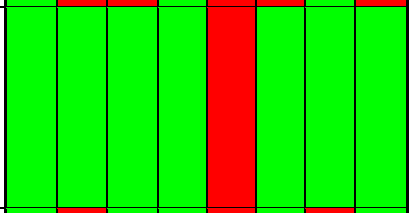
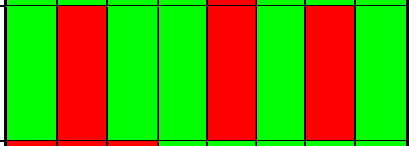
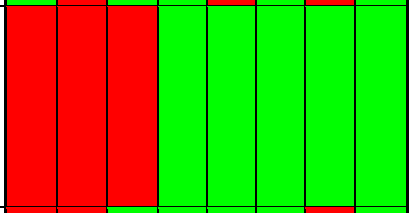
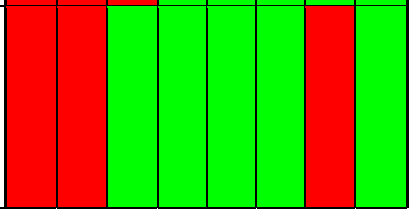
Tendons	Banik *et al.* (2020) [46]	PCL	Human MSCs	Tube-shaped scaffolds with bi-axially aligned fibers (diameter = 6.35 mm, length = 2.8 cm; thickness of tube wall = 0.0691 ± 0.0158, fiber diameter ~ 300 nm; n =20); Young's modulus = 35.8 MPa in toe region (0-2.7%)	Evaluation of a bioreactor as a possible therapeutic venture in tendon TE: effect on the scaffold's biomechanics and biofunctionality.	
	Deepthi *et al.* (2016) [204]	PLA / chitosan / alginate / collagen	Rabbit tenocytes	Tube-shaped scaffold with PLLA aligned nanofibers and chitosan / collagen coating; Tensile strength ~ 4-7 MPa; max. force ~ 6-7 N (3 layers)	Study on the effect of coating of the aligned electrospun membranes on protein adsorption and tenocyte proliferation and attachment.	
	Evrova *et al.* (2020) [205]	DegraPol®15	Rabbit tenocytes PDGF	Tube-shaped bioactive constructs by emulsion and coaxial ES; Young's modulus ~ 50 MPa; failure stress ~ 5-8 MPa	Delivery of growth factors at the injury site to improve the functional and biomechanical properties of repaired tendons.	
	Webb *et al.* (2013) [206]	Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) and collagen	Rat tenocytes	Porous PHBHHx tubular scaffold with varying number of fibers; Elastic modulus ~ 25 MPa; max. load ~ 10 N	Application of a novel material: PHBHHx in combination with/without collagen and/or tenocytes for tendon repair.	
	Manning *et al.* (2013) [207]	PLGA and a heparin/fibrin-based delivery system (HBDS)	ASCs PDGF	Aligned electrospun PLGA nanofiber mats and HBDS (11 alternating layers, ~ 100 μm, 3x7 mm mats)	Study on the ability of a layered electrospun scaffold to deliver both cells and growth factors.	
	Yousefi *et al.* (2018) [208]	Chitosan and ZnO particles	Acellular ZnO particles	Tube-shaped chitosan scaffolds (0.2 mm thick and 3.5 ± 0.5 inner diameter)	Fabrication of a chitosan/ZnO particles scaffold for reduced adhesion formation, improved gliding function and histopathological analysis.	

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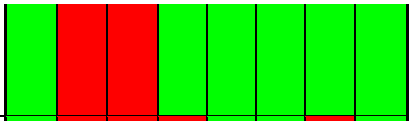
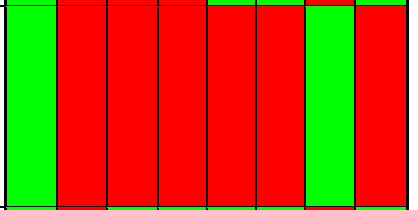
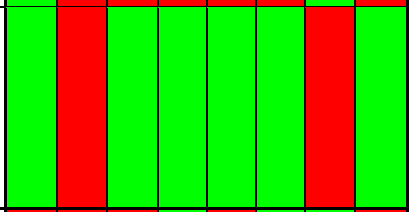
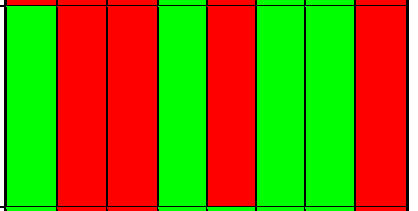
	Dong *et al.* (2021) [209]	PCL / SF	Human bone marrow MSCs	Composite fibrous scaffold (rectangular 30x10x0.3 mm)	Functionalized electrospun fibers for mitigating foreign-bode reaction and tendon adhesion	
	Uyanik *et al.* (2022)[210]	PLGA	None	Nanofibrous scaffold (rectangular 5x10x0.5 mm)	Bioabsorbable nanofiber to prevent peritendinous adhesions	
	Pien *et al.* (2021) [133] Pien *et al.* (2022) [211]	Acrylate-endcapped urethane-based polymer / PCL	Human foreskin fibroblasts; tenocytes and MSCs HA and naproxen	Tube-shaped electrospun scaffolds (35 mm length; 1 mm thickness; 5 mm diameter); Young's modulus ~ 9 MPa; Ultimate stress ~ 6 MPa	Reinforced drug-loaded electrospun construct using a mechanical and biological approach for flexor tendon repair	
	Peeters *et al.* (2021) [134]	Acrylate-endcapped urethane-based polymer / PCL	Acellular HA and naproxen	Tube-shaped electrospun scaffolds (15 mm length; 1 mm thickness; 2.5 mm diameter)	Comparison between i) modified Kessler sutures, ii) drug-loaded electrospun construct, iii) reinforcement construct, iv) reinforced drug-loaded electrospun construct in an *in vivo* rabbit model	
Nerves	Zhu *et al.* (2018) [212]	PAN-carbon nanofibers	Mouse NSCs	Nanofibrous mat	Evaluation of electrospun nanofibrous mats with biphasic electrical stimulation to promote stem cell proliferation	
	Wang *et al.* (2018) [213]	PCL-lignin copolymer PCL	Schwann cells Dorsal root ganglion cells	Dried nanofibrous mats	Evaluation of solvent-free ring opening polymerization, followed by engineering into nanofibrous scaffolds to assess cell adhesion and outgrowth	
	Shrestha *et al.* (2018) [119]	Chitosan grafted polyurethane	PC12	Aligned oriented inner nanofibrous layer	Development of self-electrical stimulated double layered NGC with aligned inner wall and	

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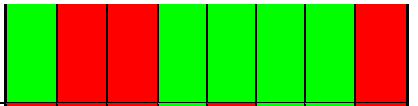
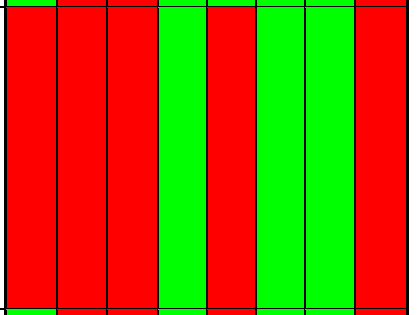
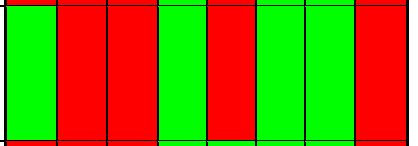
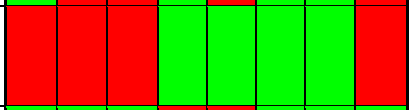
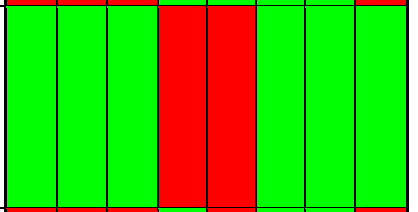
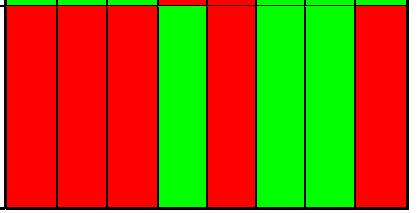
Table 3 (*continued*)

		Carbon nanotubes coated with polypyrrole	Schwann cells	Random oriented outer nanofibrous layer	random oriented outer wall	
	Wang *et al.* (2019) [214]	PCL Silk fibroin Carbon nanotubes	PC12 Dorsal root ganglion cells	3D core shell scaffold of hydrogel shell and aligned nanofiber yarns	Development of core-shell scaffold based wet-dry electrospinning to produce conductive nanofiber yarns that are encapsulated in hydrogel	
	Saudi *et al.* (2019) [120]	PVA / Poly(glycerol sebacate)	PC12 cells	Electrospun nanofibrous mat	Development of PGS based electrospun fibers with a quicker synthesis time: detailed analysis of mechanical properties and morphology	
	Ghobeira *et al.* (2019) [215]	PCL	ASCs	Aligned and random oriented fibers on a cylindrical collector	Evaluation of plasma treatment on electrospun aligned and randomly oriented fibers	
	Fang *et al.* (2020) [121]	Reduced graphene oxide-GelMA-PCL	PC12 cells Rat Schwann cell line (RSC96)	Electrospun on a rotating mandrel to obtain a hollow tube	Evaluation of electrospun fibers with enhanced electrical conductivity and biocompatibility	
	Xia *et al.* (2021) [216]	PCL Polyanion-polyglycerol sulfate modified graphene oxide	iPSCs	Electrospun nanofibrous sheet	Evaluation of electrospun fibers coated with a multivalent/bioadhesive 2D nanosheets	

Abbreviations: 2D, two dimensional; 3D, three-dimensional; ABS, poly(acrylonitrile-butadiene-styrene); ASCs, human adipose stem/stromal cells; BCE, butylene cyclohexanedicarboxylate; BR, biological requirements; C2C12, mouse murine myoblast cell line; iPSCs, induced pluripotent stem cells; MR, mechanical requirements; MWCN, multiwalled carbon nanotubes; PAA, poly(acrylic acid); PAN, poly(acrylonitrile); PANI, polyaniline; PBCE, poly(butylene 1,4-cyclohexanedicarboxylate); PC12; rat pheochromocytoma cell line; PCL, poly(ε-caprolactone); PDGF, platelet-derived growth factor; PEGS, poly(ethylene glycol)-co-poly(glycerol sebacate); PEO, poly(ethylene oxide); PHB, poly(hydroxybutyrate); PHBHHX, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); PLA, Poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PVA, poly(vinyl alcohol); SF, silk fibroin; TE, Tissue engineering; TECE, triethylene cyclohexanedicarboxylate; VIT, *in vitro* study; VIV, *in vivo* study, VML, volumetric muscle loss.

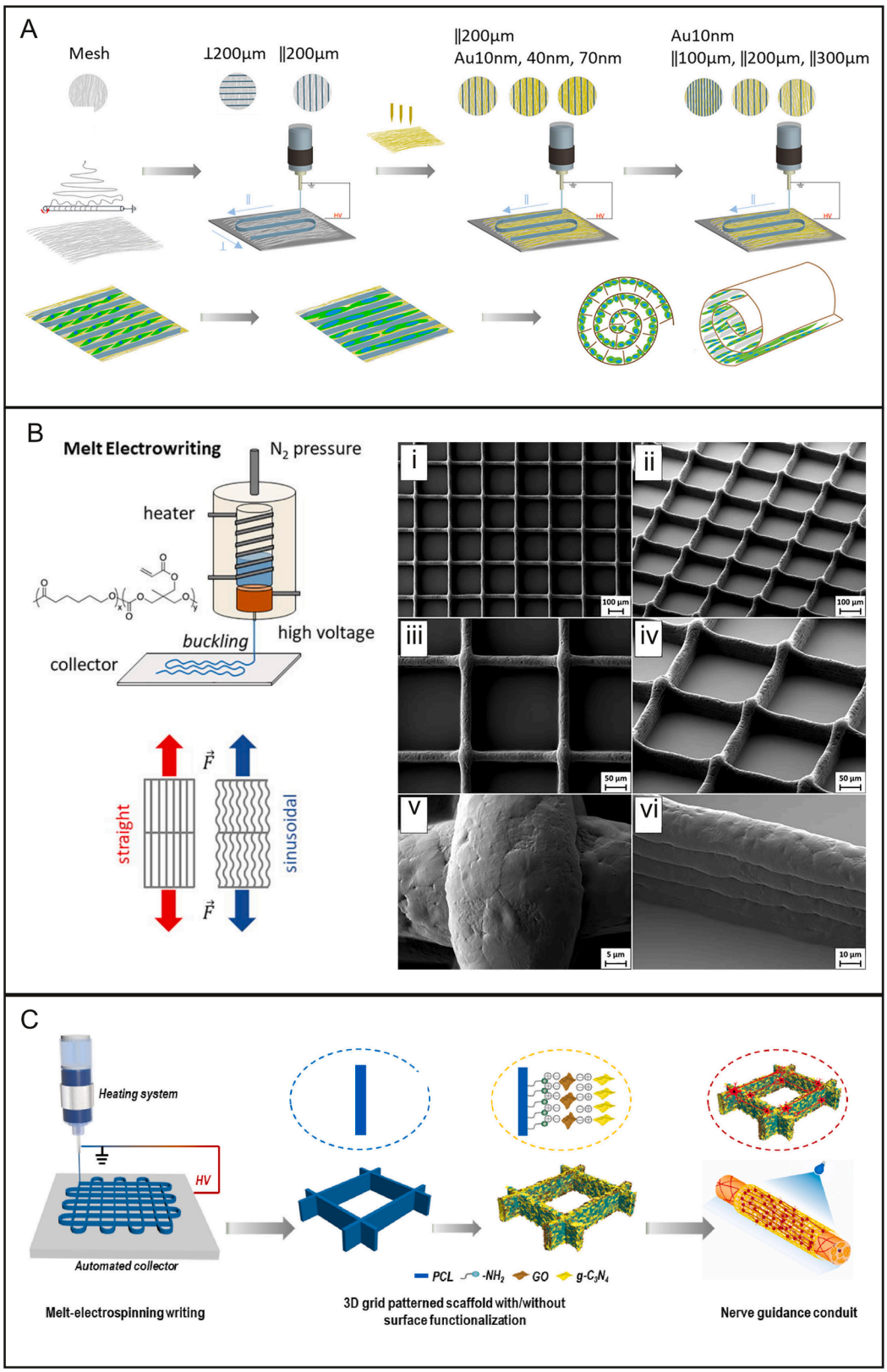


Fig. 7. Examples of melt electrowriting approaches applied for TE of muscle, tendon, and nerve. (A) Schematic outline for the preparation of 3D patterned fibrous scaffolds for skeletal muscle regeneration (⊥ means microgrooves perpendicular to aligned nanofibers, ∥ means microgrooves parallel to aligned nanofibers). Reproduced from Ref. [223], ©2020 with permission from Elsevier. (B) Development of fibrous scaffolds with sinusoidal patterns by melt electrowriting, mimicking the non-linear biomechanical behavior of tendon and ligament tissue. (i-vi) SEM images of a 10-layered scaffold indicating the accurate scaffold structure. Adapted from Ref. [219], ©2018 Hochleitner *et al.* (C) Scheme illustration of 3D polymeric grid patterned scaffolds produced by melt electrowriting, with or without surface modification, to serve as nerve guidance conduits. Reproduced from Ref. [222], ©2020 with permission from Elsevier. Abbreviations: Au: gold, PCL: poly(ε-caprolactone), GO: graphene oxide, g-C3N4: graphitic carbon nitride.

Table 4
Summary of studies using melt electrowriting (MEW) as a fabrication technique for muscles, tendons and nerves. MR and BR indicate mechanical and biological requirements described in Table 1. VIT and VIV indicate *in vitro* or *in vivo* studies. Color coding was used to indicate whether the research study investigated/explored (green) or not (red) the mechanical and biological pertinence [115,143,225–228].

Melt electrowriting for regeneration of	References / study	Material choice	Cells & bioactive compounds	Scaffold architecture / morphology	Design idea and focus on anatomical key points / physiological functions in the study	Mechanical and biological pertinence							
						MR 1	MR 2	MR 3	BR 1	BR 2	BR 3	VIT	VIV
Skeletal muscles	Uribe-Gomez *et al.* (2021) [115]	PCL / PU / HAMA	C2C12	Aligned MEW PCL-PU fibers (15-20 μm diameter) on 3D-printed HAMA hydrogel film (20 x 5 mm^2), folded into multilayer scroll tubes (~400 μm); Young's modulus ~ 2-15 MPa; Tensile strength ~ 5-20 MPa	Fabrication of a biodegradable elastic 3D scaffold with inlayed fibers to guide cell alignment.								
	Zhang *et al.* (2020) [143]	Gold-coated PCL	H9c2 myoblasts Gold nanoparticles	MEW PCL microgrooves (~100 μm height, 100-300 μm spacing, ~10 μm fiber diameter) on electrospun (~300 nm diameter) aligned PCL fibrous mesh and gold nanoparticle coating (10-70 nm thickness), folded into tubular structure; Young's modulus ~ 1.5 MPa; Resistivity = 0.1-10 kOhm/cm	Development of an organized 3D scaffold at a micro/nano level with topographic and electric stimuli in order to mimic the structure of muscle ECM and promote cell alignment and differentiation.								
Tendons	Hochleitner *et al.* (2018) [225]	Poly(L-lactide-co-e-caprolactone-co-acryloyl carbonate) and poly(e-caprolactone-co-acryloyl carbonate)	Murine fibroblast cell line (L929 CC1)	Box-structured scaffolds with straight fibres and a mesh width of 200 μm in x- and y-direction (10 layers); or sinusoidal-shaped fibres with a distance of 250 μm in between and a stacking of three fibres above each other (6 layers); Fiber diameter ~ 20-70 μm; Straight and sinusoidal fibres have similar maximum strength (~50 MPa) and elongation (~90%)	Fabrication of crimped elastomer scaffolds with non-linear extension behavior mimicking ligaments and tendons								
	Warren *et al.* (2019) [226]	PCL	Acellular	12 mm × 12 mm (square) or 35 mm × 5 mm (rectangular) scaffolds: alternating layers of PCL fibers oriented in the longitudinal (0°) or transverse (90°) directions; spaced 0.2 mm apart (2 or 10 layers total); Fiber diameter ~ 25-80 μm; Young's modulus ~ 7-22 MPa	Evaluation of MEW parameters: fiber morphology and tensile mechanics in scaffolds for musculoskeletal soft TE.								

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Table 4 (*continued*)

Nerves	Wang Y. *et al.* (2020) [227]	Surface modified PCL	PC12 cells	Grid-like framework	Evaluation of a construct fabricated using melt electrospinning writing to obtain straight fibers, printed into a 3D grid and surface modification using dip coating of graphite oxide-graphitic carbon nitride complex (GO-g-C3N4)	
	Zhang Z. *et al.* (2020) [228]	Surface modified PCL	PC12 cells	3D grid patterned scaffold	Evaluation of a construct using melt electrospinning writing followed by surface modification with different nanolayers of sputtered Au to increase electrical conductivity	

Abbreviations: 3D, three-dimensional; BR, biological requirements; C2C12, mouse murine myoblast cell line; HA, hyaluronic acid; HAMA, methacrylated hyaluronic acid; GO-g-C3N4, graphite oxide-graphitic carbon nitride complex; MEW, melt electrowriting; MR, mechanical requirements; PC12; rat pheochromocytoma cell line; PCL, poly(ε-caprolactone); PU, polyurethane; TE, tissue engineering; VIT, *in vitro* study VIV, *in vivo* study.

To date, only one group of researchers has published two studies on tendon repair using MEW [219,220] indicating that this technology is still young and is taking the first steps toward developing scaffolds for specific applications. The first study [219] fabricated a scaffold that mimics the nonlinear stretch behavior of ligaments and tendons using PCL-based materials (Fig. 7.B), followed by a second study [220] evaluating the tensile mechanics of the scaffold.

Similar to muscle and tendon TE, few articles reported on the use of MEW for neural TE. Again, PCL was explored as potential material of choice [221], as it remains the gold standard for MEW [218]. This is mainly due to its semicrystalline and biodegradable properties, low melting temperature (60 °C) and rapid solidification, and the U.S. Food and Drug Administration (FDA) approval for multiple clinical applications [218]. Moreover, PCL is fairly hydrophobic [218,224], as such enabling surface treatments such as dip coating with a graphite complex solution or sputtering with gold to increase biopotential conductivity [222]. However, it is not yet possible to adequately compare research papers using MEW for neural TE as this method is still relatively recent. MEW did show potential in the development of scaffolds with anisotropic, micro-fibrous architectures (Fig. 7.C), which were proposed to guide neurite extension [222].

4. Meeting the structural and functional requirements of skeletal muscle, tendon and nerve tissue

4.1. Current advances and opportunities by tissue type

For muscle TE, the ideal material in combination with cells, bioactive factors, and processing techniques has yet to be developed. With current TE approaches it is not possible to produce fully functional muscle tissue, especially because no material has shown to offer optimal biodegradability or is capable of supporting fully functional maturation [225]. Regarding the use of bioactive compounds, these were only explored in a small subset of the investigated studies which could potentially be limiting in biomaterial optimization. The widespread use of SES in the published studies could be explained by the relative ease to obtain a anisotropic 3D scaffold that mimics the structure of the *in vivo* ECM [226]. Fiber alignment at the microscopic level, which is a critical component for muscle regeneration, can be achieved at the microscopic level by adjusting basic parameters in the SES setup. Furthermore, SES is a more established technology than 3DP or MEW and is technically less challenging. Very few studies attempted to mimic parts of the tubular architecture of the muscle when developing their constructs (Tables 2, 3 and 4). Positive effects on muscle differentiation, alignment and mechanical properties were observed. Particularly in the study by Wang *et al.* [194], high cell survival, alignment and differentiation was reported when developing electrospun structures that simulated myofiber maturation and extracellular matrix deposition [194]. However, the mechanical properties of the entire construct were not analyzed which may limit its suitability *in vivo*. In the study of Kang *et al.* [176], myofiber-like structures were generated with a custom 3DP using PCL and a sacrificial hydrogel to obtain rows of aligned PCL tubes that were seeded with mouse myoblasts. Similarly, cell proliferation, alignment, and differentiation in culture were demonstrated, as well as evidence of innervation when implanted in mice. However, functional assessment of the implants showed significantly lower muscle function when compared to the controls [176]. Alignment is the most studied biological requirement, but alignment alone is not sufficient to create a fully functional muscle. Other important factors such as innervation and blood vessel ingrowth have rarely been studied. In terms of answering the mechanical requirements (Table 1), it has become evident that few of the studies included mechanical testing of the developed scaffolds. The lack of mechanical testing could result in the development of suboptimal scaffold materials which might hamper their clinical application, as mechanical parameters such as material stiffness are known to affect muscle development [227,228]. Therefore, mechanical testing

should be included in future studies to provide additional information on the suitability of the fabricated scaffolds to be applied in *in vivo* preclinical studies. As discussed in Section 3.3, most studies focus either on biological or on mechanical requirements, but rarely on both. Such studies, however, would not only provide important information about the suitability of a particular scaffold to support muscle regeneration, but knowledge would be gained about its potential performance *in vivo* as well. The study by Choi *et al.* [229], for example, combined the evaluation of all mechanical requirements (contractility, elastic modulus, tensile stress) and alignment, with biological requirements such as differentiation and cell survival in a muscle ECM-derived bioink. Mechanical properties were significantly improved when compared to collagen I, a commonly used bioink [229]. The use of external fields (acoustic, magnetic and electric) during biofabrication has been used successfully [160] but has the disadvantage of increasing complexity, which limits its use for large scaffold fabrication. There are numerous reports of *in vitro* stimulation techniques, where static or dynamic electrical or mechanical stimuli were shown to effectively induce alignment and maturation of muscle progenitor cells [230,231].

In tendon regeneration, research has focused on the delivery of cells into scaffolds and in a predefined pattern using 3DP. However, the incorporation of bioactive compounds such as growth factors has not been reported (Table 2), indicating that the latter should be explored in the near future. In contrast to 3DP, research using ES explored the fabrication of electrospun constructs containing bioactive compounds known to enhance tendon healing (i.e., growth factors, zinc oxide particles, hyaluronic acid, and naproxen) [130,131,199,201,202] (Table 3). The latter were provided to improve protein adsorption, cell attachment and proliferation, but also to reduce the presence of adhesions and improve tendon gliding function [130,131,199,201,202]. However, the physiological microstructure of the tendon was not taken into account, as ES does not allow for precise deposition of fibers nor for incorporation of cells during processing, resulting in either inferior mechanical or biological properties. Although research on MEW for tendon repair is scarce, the technique holds advantage over the other two techniques, especially regarding the potential to mimic the nonlinear stretching behavior of ligaments and tendons by depositing microscale fibers in a predefined architecture. Considering tendon repair, researchers only begin to acknowledge that the micro and macro-levels of anatomical structure, physiological functions, and appropriate requirements must be considered in order to develop functional tendon tissue constructs. In this direction, recent work by Chae *et al.* [164] has looked at spatially graded architectures using 3D printing. Another example is the work of Hochleitner *et al.* [219] who developed a scaffold that mimics the crimping behavior of tendon tissue using MEW.

A considerable amount of research is being conducted with regard to potential TE conduit models for nerve repair, although they are not currently superior to autografts. Various bioactive compounds have been explored along with novel materials for nerve repair, including degradable and non-degradable conduits. The major disadvantage of using non-degradable conduits is the need for a second conduit resection, whereas with degradable materials, the rate of degradation cannot always be controlled, leading to premature conduit collapse. Several requirements must be considered in the development of nerve conduits, both from a biological and mechanical point of view. Research studies have addressed some or most of the requirements listed in Table 1, but the development of a conduit that meets all of them is still pending. A few fabrication methods for NGCs have been explored, with most groups using high-resolution 3DP focusing on design considerations such as the incorporation of micropores and parallel channels (as listed in Table 2), while authors using SES as a processing method incorporated bioactive compounds and drugs to stimulate nerve growth. Some research groups also focused on incorporating nanoparticles to provide electrical stimulation to the regenerating nerve to further accelerate the process. The only work reporting on MEW used an electrically conductive coating to promote nerve growth [221]. It should be mentioned that there remains still much potential for further experimentation and trials exploiting this processing technique. Recent perspectives on NGCs were presented by Ramesh *et al.* [119], who reversely engineered a nerve conduit using microCT and subsequent 3DP, and Yang *et al.* [238], who investigated the potential of a graphene-based foam to improve the electrical conductivity of their scaffolds. Functional recovery in large nerve defects could potentially be improved with reverse engineering, by mimicking epineural and endoneural microstructures through a high-resolution fabrication method such as 3DP.

4.2. Strengths and limitations associated with processing techniques

The strength of 3DP is that a design-specific and complex construct can be developed and thus tailored towards each application. This technique allows for the encapsulation of cells throughout the construct (also known as 3D bioprinting). In addition, multiple materials and cell types can be combined in one process. Compared to 3DP, the main advantage of SES is the excellent physiologically relevant mimicry of native ECM due to the fabrication of micro and nanofibers with a high surface-to-volume ratio. By combining the direct writing aspect of 3DP with the micro and nanofibers of SES, MEW enables the fabrication of constructs with a predefined architecture and precise control over pore size and interconnectivity. One of the biggest challenges in 3DP, and in 3D bioprinting in particular, remains the identification of novel bioinks whose properties could be dynamically adjusted to modulate cell behavior and ultimately control cell phenotype and function over time. In addition, processing temperatures or light wavelengths and applied stresses should be monitored and fine-tuned during processing to maximize cell survival. Although material properties such as the stiffness of the gel/substrate itself may affect cell growth and potential, these properties are rarely investigated. 3DP has the great advantage that cells can be incorporated, which is beneficial for mimicking and meeting the biological requirements of the targeted tissue. In addition, fibers can be deposited in predefined patterns using 3DP, but compared to MEW, the fiber diameters are bigger, so the microscale features of tissues cannot exactly be mimicked yet.

SES is still the technique of choice to mimic the ECM structure of native tissue. Although more advanced devices have been developed and SES allows the fabrication of tubular scaffolds on tubular rotational mandrels, there is no precise control over fiber deposition and architecture, although fiber orientation can be controlled to some extent by varying tension and rotational speed. Aligned scaffolds produced by electrospinning contain aligned nanofibrous structures for guiding muscle cell proliferation and differentiation but are limited to small, 2D constructs leading to a poor clinical translation potential [142]. For applications requiring more precision, research is shifting toward MEW. Since SES alone does not result in sufficient mechanical strength of the obtained structures to support load-bearing applications, it should be combined with 3DP or MEW to provide mechanical stability.

MEW is a very recent technique. Research is currently focused on optimizing printing and processing parameters and in the development of new materials that can be processed by MEW. To be MEW processable, polymers require a low melting point, slow thermal and hydrolytic degradation, and rapid solidification. The incorporation of cells is not being explored due to the high processing temperatures required by the materials currently in use. There is considerable room for further development and optimization as the state of knowledge is still limited. However, the very precise deposition of microscale fibers in a predefined pattern and architecture highlights the potential of MEW in mimicking tissues at the micro level, enabling more physiologically relevant functions.

5. Future perspectives and concluding remarks

All the above limitations of each processing technique could be solved by combining two processing techniques. In this way, a

synergistic effect could be achieved since the advantages of both techniques together would create the appropriate conditions for the development of fully functional tissues. As shown in Tables 2, 3, and 4, research currently focuses on either biological or mechanical properties, but rarely on both simultaneously. However, this is one of the most important issues to be addressed in the development of functional tissues. In particular, for cylindrical tissues such as skeletal muscle, tendons, and nerves, the design and development of these tubular scaffolds are far from optimized. Advances in processing techniques and material design should open new opportunities in this area. In addition, the use of bioactive compounds, including growth factors and drugs, could greatly enhance both cell-material interactions and cell proliferation, allowing the development of more complex constructs. With regard to tissue maturation after scaffold processing (with encapsulated cells or after cell seeding, depending on the processing technique chosen), the use of a bioreactor to control ECM modeling and TE is essential [232]. The bioreactor enables nutrient transport and provides the physiological environment as well as biochemical, biomechanical, and biophysical stimuli that are necessary for the development of functional tissue [233,234]. However, the importance of bioreactors for TE only recently became clear and they will certainly be used more extensively in the coming years.

The regeneration of muscle, tendon and nerve is also challenged by the complex multicellular crosstalk occurring in the microenvironment. There exists a great need for advanced models able to mimic the hierarchical architecture, cellularity and physiological signaling, along with the recreation of the integrated gradients of the tissue interfaces [235]. Growing evidence suggests that surface topography, substrate stiffness, mechanical stimulation, oxygen tension and localized density influence cellular functions and longevity [236]. This enhances tissue-specific ECM deposition and directs stem cell differentiation [236]. The cellular crosstalk in the microenvironment includes key players that drive or resolve inflammation (*vide infra*), neovascularization, and complex interfaces. Vascularization is one of the essential aspects of the musculoskeletal system TE and an indispensable process in the regeneration of most tissues. Despite considerable efforts, it has not yet been possible to reproduce the hierarchical organization and function of native blood vessels [237].

In addition, micro and macro-level differences at the interface between ligaments/tendons and bone and between tendon and muscle have not been adequately explored. However, these interfaces are important because they ensure a smooth transition between these tissues. Therefore, research should expand the scope of interest of each tissue to include a connection between two tissues based on the interface [238,239]. However, progress in this field is difficult as nor the individual tissues, nor the interfaces between these tissues, are not fully understood yet. Within these tissues, differences not only with regard to mechanical properties, but also in cellular heterogeneity and ECM composition are present. It is important to highlight the anatomical micro and macro-level of each tissue and their corresponding physiological functions (based on the mechanical and biological properties). Some research groups have recently focused on the engineering of muscle-tendon units [5,166,187,240], tendon-bone units [241–243] or neuromuscular junctions [244–248], paving the way towards the future of engineering skeletal muscle, tendon and nerves. Research on these individual units as well as the obtained insights and understanding will eventually lead to advancing tissue engineering strategies for musculoskeletal repair and regeneration, going from one multi-tissue unit to the combination of multiple, i.e., bone-tendon-muscle unit.

In this context, research has recently begun to evaluate the combination of multiple processing techniques to address the challenges at these interfaces. For example, Jiang *et al.* have combined 3D bioprinting and melt electrospinning to regenerate a functional rotator cuff tendon-to-bone interface [249]. Apart from the tendon-to-bone unit, the tendon-to-muscle unit is essential for the proper functioning of the musculoskeletal system. Each of these multi-tissue units requires critical consideration and thoughtful integration of clinical, biological and engineering aspects (including material design, processing and construct maturation) to achieve efficient bench-to-bedside translation [240].

Suitable biomaterials are needed to successfully mimic the spatio-temporal signaling profile observed in tissue healing [250]. Biomaterials must serve as a delivery and/or support template for the sustained release of proteins, genes and cells, and to provide an architecture for cells moving into the site of damage. The biomaterial choice and design influence the biophysical cues (i.e., topography, rigidity, mechanical stimulation, macromolecular crowding) that replicate the native microenvironment, in addition to the required biochemical and biological cues [235,251–253]. An important point to consider in TE is the immunogenic response of the body towards TE implants. The cellular and molecular events that determine implant success occur at the interface between the material and the host and are governed by innate and adaptive immune responses [254]. Research has been focusing on expanding the understanding of these responses, and in the immunological profile of biomaterials [255]. As has been clearly observed over the years, the surface and bulk properties of scaffolds, together with their 3D architecture have a significant impact on their biological performance [256]. Current strategies for biomaterial immunomodulation include biomaterial design that starts from the surface properties that have been shown to be central to the immune response, either passively acting on physico-chemical properties or actively acting through the incorporation of molecules or coatings [254]. In tendon repair for example, researchers have explored strategies that balance inflammation and tenogenesis. In particular, the role of macrophage polarization has been studied as well as how biomaterials can modulate this polarization to promote tissue regeneration [257–260]. For nerve TE, researchers typically exploit animal models to investigate immune responses towards implanted biomaterials. However, translation of these *in vivo* models remains challenging mainly because of the differences in immune responses occurring between mice and humans. In addition, the evaluation of neural TE in humans may be influenced by patient-specific factors, which for many researchers pose another barrier towards the use of biomaterials in clinical applications [261,262]. The immune response following implantation of TE skeletal muscle constructs has also been explored in various studies. The correlation between muscle regeneration and inflammation following acute injury is well-established, where leukocytes (monocytes or macrophages) become activated upon acute injury and release pro-inflammatory cytokines to facilitate removal of cell debris [263]. Following the inflammatory stage, the immune cells switch to the anti-inflammatory response thereby suppressing the local inflammatory response and enhancing muscle growth [264]. A strategy to fully integrate skeletal muscle TE constructs has involved the incorporation of host cells and biomaterials that can regulate tissue mechanics, inflammation and integrin binding [265]. However, the animal models in which these strategies were tested, were mostly immunodeficient or immunocompromised, so the models are not fully transferable to humans [142]. Further studies are therefore needed to investigate the interaction between the host and the implanted scaffolds, to better understand the role of the immune and inflammatory response following implantation.

On their way from the laboratory bench to clinical use, tissue-engineered constructs are subject to strict regulatory guidelines and important standards (e.g., ASTM, FDA, EMA) [266–268]. Despite some promising results, the clinical application of advanced tissue engineered and regenerative medicine products is still hampered by insufficient functionality at both the mechanical and biological levels. This is mainly due to the lack of knowledge about reliable cell sources, effective immunosuppression, and strategies to induce effective neovascularization and integration into the host [8,266,269]. For tendon diseases, the current regulatory practice and guidelines, as well as preclinical advances [250,270] toward novel integrated therapies have been described in detail by Freedman *et al.* [267]. In the context of skeletal muscle injury, it remains unclear whether there is a single

clinically significant treatment option for volumetric muscle loss (VML), as there are no clear comparisons between studies and the few published clinical reports [271]. The meta-analysis by Greising *et al.* concluded that in VML animal models, acellular biomaterials in combination with cellular components have proven to be the most effective VML treatment to date [271]. However, more studies involving direct comparison of regenerative and/or physical therapy under identical experimental conditions following VML injury are needed to provide a clear overview of the efficacy of the currently investigated treatment options.

In summary, TE strategies serving repair and regeneration of the musculoskeletal system, including skeletal muscles, tendons, and nerves, are still at an early stage. One of the key findings of this review is that current research focuses on either biological or mechanical requirements and properties, but rarely on the combination of these two aspects. Advances and developments in the fields that support the principles of TE, such as biomaterial chemistry, engineering and processing as well as and molecular biology, are needed to provide a strategy to address the complex challenges involved in the repair and regeneration of these complex, hierarchically organized tissues.

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Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

N. Pien: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **H. Krzyslak:** Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **S. Shastry Kallaje:** Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **J. Van Meerssche:** Visualization, Writing – original draft. **D. Mantovani:** Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **C. De Schauwer:** Funding acquisition, Supervision, Validation, Writing – review & editing. **P. Dubruel:** Funding acquisition, Supervision, Validation, Writing – review & editing. **S. Van Vlierberghe:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **C.P. Pennisi:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Reference

[1] E. Bayrak, P.Yilgor Huri, Engineering musculoskeletal tissue interfaces, Front. Mater. 5 (2018), https://doi.org/10.3389/fmats.2018.00024.
[2] J.B. Tang, D. Lalonde, L. Harhaus, A.F. Sadek, K. Moriya, Z.J. Pan, Flexor tendon repair: recent changes and current methods, J. Hand Surg. (2021), https://doi.org/10.1177/17531934211053757.
[3] A.M. Briggs, A.D. Woolf, K. Dreinhöfer, N. Homb, D.G. Hoy, D. Kopansky-Giles, K. Åkesson, L. March, Reducing the global burden of musculoskeletal conditions, Bull. World Health Organ. 96 (2018), https://doi.org/10.2471/BLT.17.204891.
[4] D.M. Wojtkiewicz, J. Saunders, L. Domeshek, C.B. Novak, V. Kaskutas, S. E. Mackinnon, Social impact of peripheral nerve injuries, Hand (2015) 10, https://doi.org/10.1007/s11552-014-9692-0.
[5] L. Baldino, S. Cardea, N. Maffulli, E. Reverchon, Regeneration techniques for bone-To-Tendon and muscle-To-Tendon interfaces reconstruction, Br. Med. Bull. 117 (2016), https://doi.org/10.1093/bmb/ldv056.
[6] W. Daly, L. Yao, D. Zeugolis, A. Windebank, A. Pandit, A biomaterials approach to peripheral nerve regeneration: bridging the peripheral nerve gap and enhancing functional recovery, J. R. Soc. Interface 9 (2012), https://doi.org/10.1098/rsif.2011.0438.
[7] B. Engebretson, Z. Mussett, C. Williams, A. Simmons, V. Sikavitsas, Tendon Tissue Engineering: Combined Tissue Engineering Approach for the Regeneration of Tendons, Elsevier Inc., 2015, https://doi.org/10.1016/B978-0-12-801590-2.00012-0.
[8] I. Holland, J. Logan, J. Shi, C. McCormick, D. Liu, W. Shu, 3D biofabrication for tubular tissue engineering, Biodes Manuf 1 (2018) 89–100, https://doi.org/10.1007/s42242-018-0013-2.
[9] A. Moriscot, E.H. Miyabara, B. Langeani, A. Belli, S. Egginton, T.S. Bowen, Firearms-related skeletal muscle trauma: pathophysiology and novel approaches for regeneration, NPJ Regen. Med. 6 (2021), https://doi.org/10.1038/s41536-021-00127-1.
[10] W. Balestri, R.H. Morris, J.A. Hunt, Y. Reinwald, Current advances on the regeneration of musculoskeletal interfaces, Tissue Eng. Part. B Rev. (2021), https://doi.org/10.1089/ten.teb.2020.0112.
[11] A. Atala, F.K. Kasper, A.G. Mikos, Engineering complex tissues, Sci. Transl. Med. 4 (2012) 1–11.
[12] R. López-Cebral, J. Silva-Correia, R.L. Reis, T.H. Silva, J.M. Oliveira, Peripheral nerve injury: current challenges, conventional treatment approaches, and new trends in biomaterials-based regenerative strategies, ACS Biomater. Sci. Eng 3 (2017), https://doi.org/10.1021/acsbiomaterials.7b00655.
[13] J.J. Paredes, N. Andarawis-Puri, Therapeutics for tendon regeneration: a multidisciplinary review of tendon research for improved healing, Ann. N Y Acad. Sci. 1383 (2016) 125–138, https://doi.org/10.1111/nyas.13228.
[14] E. Bianchi, M. Ruggeri, S. Rossi, B. Vigani, D. Miele, M.C. Bonferoni, G. Sandri, F. Ferrari, Innovative strategies in tendon tissue engineering, Pharmaceutics 13 (2021) 1–24, https://doi.org/10.3390/pharmaceutics13010089.
[15] H. Liao, G.Q. Zhou, Development and progress of engineering of skeletal muscle tissue, Tissue Eng. Part B Rev. (2009) 15, https://doi.org/10.1089/ten.teb.2009.0092.
[16] W.R. Frontera, J. Ochala, Skeletal Muscle: a Brief Review of Structure and Function, Behav. Genet. 45 (2015) 183–195, https://doi.org/10.1007/s00223-014-9915-y.
[17] Muscle fiber excitation contraction and relaxation, (n.d.). https://open.oregonstate.education/aandp/chapter/10-3-muscle-fiber-excitation-contraction-and-relaxation/(accessed December 16, 2022).
[18] B. Charvet, F. Ruggiero, D.Le Guellec, The development of the myotendinous junction. A review, Muscles Ligaments Tendons J. 2 (2) (2012) 53–63.
[19] C. Stecco, W. Hammer, A. Vleeming, R. De Caro, Deep fasciae, in: Functional Atlas of the Human Fascial System, 2015, https://doi.org/10.1016/b978-0-7020-4430-4.00003-8.
[20] P.P. Purslow, The structure and role of intramuscular connective tissue in muscle function, Front. Physiol. 11 (2020), https://doi.org/10.3389/fphys.2020.00495.
[21] L.M. Biga, S. Dawson, A. Harwell, R. Hopkins, J. Kaufmann, M. Lemaster, P. Matern, K. Morrison-Graham, D. Quick, J. Runyeon, Anatomy & Physiology, OpenStax, Oregon State University. (n.d.). https://open.oregonstate.education/aandp/chapter/10-2-skeletal-muscle/.
[22] G.L. Warren, T. Hulderman, N. Jensen, M. McKinstry, M. Mishra, M.I. Luster, P. P. Simeonova, Physiological role of tumor necrosis factor α in traumatic muscle injury, FASEB J. 16 (2002) 1630–1632, https://doi.org/10.1096/FJ.02-0187FJE.
[23] Y. Liu, G. Zhou, Z. Liu, M. Guo, X. Jiang, M.B. Taskin, Z. Zhang, J. Liu, J. Tang, R. Bai, F. Besenbacher, M. Chen, C. Chen, Mussel inspired polynorepinephrine functionalized electrospun Polycaprolactone microfibers for muscle regeneration, Sci. Rep. 7 (2017) 8197, https://doi.org/10.1038/s41598-017-08572-z.
[24] J.H. Kim, I. Kim, Y.-J. Seol, I.K. Ko, J.J. Yoo, A. Atala, S.J. Lee, Neural cell integration into 3D bioprinted skeletal muscle constructs accelerates restoration of muscle function, Nat. Commun. 11 (2020) 1–12, https://doi.org/10.1038/s41467-020-14930-9, 2020 11:1.
[25] T.A.H. Järvinen, T.L.N. Järvinen, M. Kääriäinen, H. Kalimo, M. Järvinen, Muscle injuries: biology and treatment, Am. J. Sports Med. 33 (2005) 745–764, https://doi.org/10.1177/0363546505274714.

[26] A.J. Quintero, V.J. Wright, F.H. Fu, J. Huard, Stem cells for the treatment of skeletal muscle injury, Clin. Sports Med. 28 (2009), https://doi.org/10.1016/j.csm.2008.08.009.

[27] A. Höke, T. Brushart, Introduction to special issue: challenges and opportunities for regeneration in the peripheral nervous system, Exp. Neurol. 223 (2010) 1–4, https://doi.org/10.1016/j.expneurol.2009.12.001.

[28] C. Gotti, A. Sensini, G. Fornaia, C. Gualandi, A. Zucchelli, M.L. Focarete, Biomimetic hierarchically arranged nanofibrous structures resembling the architecture and the passive mechanical properties of skeletal muscles: a step forward toward artificial muscle, Front. Bioeng. Biotechnol. 8 (2020) 767, https://doi.org/10.3389/fbioe.2020.00767.

[29] D. Gholobova, L. Terrie, M. Gerard, H. Declercq, L. Thorrez, Vascularization of tissue-engineered skeletal muscle constructs, Biomaterials 235 (2020), 119708, https://doi.org/10.1016/j.biomaterials.2019.119708.

[30] R.L. Lieber, J. Fridén, Clinical significance of skeletal muscle architecture, Clin. Orthop. Relat. Res. 383 (2001) 140–151, https://doi.org/10.1097/00003086-200102000-00016.

[31] K. Ranjbar, B. Fayazi, Vascularisation of Skeletal muscle. Muscle Cells - Recent Advances and Future Perspectives, 2020, https://doi.org/10.5772/intechopen.85903.

[32] Johanna Buschmann, Gabriella Meier Bürgisser, "1 - Structure and function of tendon and ligament tissues", Editor(s): Johanna Buschmann, Gabriella Meier B.. rgisser, Biomechanics of Tendons and Ligaments, Woodhead Publishing, 2017, Pages 3–29.

[33] M.E. Gomes, R.L. Reis, M.T. Rodrigues. Tendon regeneration: understanding tissue physiology and development to engineer functional substitutes, Elsevier Science & Technology, 2015.

[34] A. Shojaee, A. Parham, Strategies of tenogenic differentiation of equine stem cells for tendon repair: current status and challenges, Stem. Cell Res. Ther. 10 (2019), https://doi.org/10.1186/s13287-019-1291-0.

[35] M.L. Osborn, J.L. Cornille, U. Blas-Machado, E.W. Uhl, The Equine Navicular Apparatus as a Premier Enthesis Organ: Functional implications, Veterinary Surgery, 2021, p. 50, https://doi.org/10.1111/vsu.13620.

[36] M.N. Doral, M. Alam, M. Bozkurt, E. Turhan, O.A. Atay, G. Dönmez, N. Maffulli, Functional anatomy of the Achilles tendon, Knee Surg. Sports Traumatol. Arthrosc. 18 (2010) 638–643, https://doi.org/10.1007/s00167-010-1083-7.

[37] P.W. Ackermann, P. Salo, D.A. Hart, Tendon innervation, Adv. Exp. Med. Biol. 920 (2016), https://doi.org/10.1007/978-3-319-33943-6_4.

[38] C. Myer, J.R. Fowler, Flexor tendon repair. healing, biomechanics, and suture configurations, Orthoped. Clin. North Am. 47 (2016), https://doi.org/10.1016/j.ocl.2015.08.019.

[39] A.A. Salgado, Essentials of Hand Surgery, ed., InTech, 2018 https://doi.org/10.5772/intechopen.69751.

[40] M. Kaya, N. Karahan, B. Yılmaz, Tendon structure and classification, Tendons (2019) 1–9, https://doi.org/10.5772/intechopen.84622.

[41] J. Zabrzyński, Paczesny Łapaj, A. Zabrzyńska, D. Grzanka, Tendon — Function-related structure, simple healing process and mysterious ageing, Folia Morphologica (Poland) 77 (2018), https://doi.org/10.5603/FM.a2018.0006.

[42] M. Benjamin, E. Kaiser, S. Milz, Structure-function relationships in tendons: a review, J. Anat. 212 (2008) 211–228, https://doi.org/10.1111/j.1469-7580.2008.00864.x.

[43] P. Theobald, M. Benjamin, L. Nokes, N. Pugh, Review of the vascularisation of the human Achilles tendon, Injury 36 (2005) 1267–1272, https://doi.org/10.1016/j.injury.2005.02.012.

[44] G.L. Canata, P. D'Hooghe, K.J. Hunt, Muscle and tendon injuries: evaluation and management, 2017. https://doi.org/10.1007/978-3-662-54184-5.

[45] A.L. Titan, D.S. Foster, J. Chang, M.T. Longaker, Flexor tendon: development, healing, adhesion formation, and contributing growth factors, Plast. Reconstr. Surg. 144 (2019), https://doi.org/10.1097/PRS.0000000000006048.

[46] B.L. Banik, G.S. Lewis, J.L. Brown, Multiscale Poly-(ϵ-caprolactone) scaffold mimicking non-linearity in tendon tissue mechanics, Regen. Eng. Transl. Med. 2 (2016) 1–9, https://doi.org/10.1007/s40883-016-0008-5.

[47] W.L. Lim, L.L. Liau, M.H. Ng, S.R. Chowdhury, J.X. Law, Current progress in tendon and ligament tissue engineering, Tissue Eng. Regen. Med. 16 (2019), https://doi.org/10.1007/s13770-019-00196-w.

[48] V. Schöffl, A. Heid, T. Küpper, Tendon injuries of the hand, World J. Orthop. 3 (2012) 62–69, https://doi.org/10.5312/wjo.v3.i6.62.

[49] D.M. Avery, C.M. Rodner, C.M. Edgar, Sports-related wrist and hand injuries: a review, J. Orthop. Surg. Res. 11 (2016), https://doi.org/10.1186/s13018-016-0432-8.

[50] D. Docheva, S.A. Müller, M. Majewski, C.H. Evans, Biologics for tendon repair, Adv. Drug. Deliv. Rev. 84 (2015), https://doi.org/10.1016/j.addr.2014.11.015.

[51] F. Wu, M. Nerlich, D. Docheva, Tendon injuries: basic science and new repair proposals, EFORT Open Rev. 2 (2017) 332–342, https://doi.org/10.1302/2058-5241.2.160075.

[52] C. Lebled, L.M. Grover, J.Z. Paxton, Combined decellularisation and dehydration improves the mechanical properties of tissue-engineered sinews, J. Tissue. Eng 5 (2014), https://doi.org/10.1177/2041731414536720.

[53] C. Liu, S. Tian, J. Bai, K. Yu, L. Liu, G. Liu, R. Dong, D. Tian, Regulation of ERK1/2 and SMAD2/3 pathways by using multi-layered electrospun PCL–amnion nanofibrous membranes for the prevention of post-surgical tendon adhesion, Int. J. Nanomed. 15 (2020), https://doi.org/10.2147/IJN.S231538.

[54] S. Kokubu, R. Inaki, K. Hoshi, A. Hikita, Adipose-derived stem cells improve tendon repair and prevent ectopic ossification in tendinopathy by inhibiting inflammation and inducing neovascularization in the early stage of tendon healing, Regen. Ther 14 (2020) 103–110, https://doi.org/10.1016/j.reth.2019.12.003.

[55] E.N. Marieb, K. Hoehn, Human Anatomy & Physiology, 8th ed, Pearson, 2010, https://doi.org/10.1007/BF00845519.

[56] S. Vijayavenkataraman, S. Zhang, S. Thaharah, G. Sriram, W.F. Lu, J.Y.H. Fuh, Electrohydrodynamic Jet 3D printed nerve guide conduits (NGCs) for peripheral nerve injury repair, Polymers (Basel) 10 (2018), https://doi.org/10.3390/polym10070753.

[57] P. Rea, Introduction to the nervous system. Essential Clinical Anatomy of the Nervous System, 2015, https://doi.org/10.1016/b978-0-12-802030-2.00001-7.

[58] G. Lundborg, A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance, J. Hand Surg. 25 (2000), https://doi.org/10.1053/jhsu.2000.4165.

[59] H.S. Koh, T. Yong, W.E. Teo, C.K. Chan, M.E. Puhaindran, T.C. Tan, A. Lim, B. H. Lim, S. Ramakrishna, In vivo study of novel nanofibrous intra-luminal guidance channels to promote nerve regeneration, J. Neural Eng. 7 (2010), https://doi.org/10.1088/1741-2560/7/4/046003.

[60] D. Arslantunali, T. Dursun, D. Yucel, N. Hasirci, V. Hasirci, Peripheral nerve conduits: technology update, Med. Dev. 7 (2014) 405–424, https://doi.org/10.2147/MDER.S59124.

[61] Y. Huang, W. Wu, H. Liu, Y. Chen, B. Li, Z. Gou, X. Li, M. Gou, 3D printing of functional nerve guide conduits, Burns Trauma 9 (2021) 1–11, https://doi.org/10.1093/burnst/tkab011.

[62] S.S. Soman, S. Vijayavenkataraman, Perspectives on 3d bioprinting of peripheral nerve conduits, Int. J. Mol. Sci. 21 (2020) 1–16, https://doi.org/10.3390/ijms21165792.

[63] S.M.S. Eric, A. Huebner, Smelling, tasting, learning, Results Probl. Cell Differ. (2009) 339–351, https://doi.org/10.1007/400.

[64] X. Gu, F. Ding, Y. Yang, J. Liu, Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration, Prog. Neurobiol. 93 (2011), https://doi.org/10.1016/j.pneurobio.2010.11.002.

[65] A. Moradzadeh, G.H. Borschel, J.P. Luciano, E.L. Whitlock, A. Hayashi, D. A. Hunter, S.E. Mackinnon, The impact of motor and sensory nerve architecture on nerve regeneration, Exp. Neurol. 212 (2008), https://doi.org/10.1016/j.expneurol.2008.04.012.

[66] P.X. Zhang, N. Han, Y.H. Kou, Q.T. Zhu, X.L. Liu, D.P. Quan, J.G. Chen, B. G. Jiang, Tissue engineering for the repair of peripheral nerve injury, Neural Regen. Res 14 (2019) 51–58, https://doi.org/10.4103/1673-5374.243701.

[67] K. Dalamagkas, M. Tsintou, A. Seifalian, Advances in peripheral nervous system regenerative therapeutic strategies: a biomaterials approach, Mater. Sci. Eng. C 65 (2016), https://doi.org/10.1016/j.msec.2016.04.048.

[68] S. Kehoe, X.F. Zhang, D. Boyd, FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy, Injury 43 (2012) 553–572, https://doi.org/10.1016/j.injury.2010.12.030.

[69] G.N. Panagopoulos, P.D. Megaloikonomos, A.F. Mavrogenis, The present and future for peripheral nerve regeneration, Orthopedics 40 (2017) e141–e156, https://doi.org/10.3928/01477447-20161019-01.

[70] D. Petrov, K.S. Katiyar, L.A. Struzyna, J.P. Harris, D.K. Cullen, Extracellular matrix-derived tissues for neurological applications. Extracellular Matrix-Derived Implants in Clinical Medicine, 2016, https://doi.org/10.1016/B978-0-08-100166-0.00006-2.

[71] V. Chiono, C. Tonda-Turo, Trends in the design of nerve guidance channels in peripheral nerve tissue engineering, Prog. Neurobiol. (2015) 131, https://doi.org/10.1016/j.pneurobio.2015.06.001.

[72] Z. Ma, S. Hu, J.S. Tan, C. Myer, N.M. Njus, Z. Xia, In vitro and in vivo mechanical properties of human ulnar and median nerves, J. Biomed. Mater. Res. A. 101 A (2013) 2718–2725, https://doi.org/10.1002/jbm.a.34573.

[73] R. Ghobeira, C. Philips, L. Liefooghe, M. Verdonck, M. Asadian, P. Cools, H. Declercq, W.H. De Vos, N. De Geyter, R. Morent, Synergetic effect of electrospun PCL fiber size, orientation and plasma-modified surface chemistry on stem cell behavior, Appl. Surf. Sci. 485 (2019), https://doi.org/10.1016/j.apsusc.2019.04.109.

[74] A. Pratesi, F. Tarantini, M. Di Bari, Skeletal Muscle: An endocrine Organ, Clinical Cases in Mineral and Bone Metabolism, 2013, p. 10, https://doi.org/10.11138/ccmbm/2013.10.1.011.

[75] G. Bentley, Repair and regeneration of ligaments, tendons and joint capsule, Annal. R. College Surgeon. Engl. 89 (2007) 329–330, https://doi.org/10.1308/rcsann.2007.89.3.329b.

[76] J.B. Tang, Y.T. Gu, K. Rice, F. Chen, C.Z. Pan, Evaluation of four methods of flexor tendon repair for postoperative active mobilization, Plast. Reconstr. Surg. 107 (2001) 742–749, https://doi.org/10.1097/00006534-200103000-00014.

[77] A. Sensini, G. Massafra, C. Gotti, A. Zucchelli, L. Cristofolini, Tissue engineering for the insertions of tendons and ligaments: an overview of electrospun biomaterials and structures, Front. Bioeng. Biotechnol 9 (2021), https://doi.org/10.3389/fbioe.2021.645544.

[78] C.T. Thorpe, H.L. Birch, P.D. Clegg, H.R.C. Screen, Tendon Physiology and Mechanical Behavior: Structure-Function Relationships, Elsevier Inc., 2015, https://doi.org/10.1016/B978-0-12-801590-2.00001-6.

[79] C.T. Thorpe, H.L. Birch, P.D. Clegg, H.R.C. Screen, The role of the non-collagenous matrix in tendon function, Int. J. Exp. Pathol. 94 (2013), https://doi.org/10.1111/iep.12027.

[80] J. Bojsen-Møller, S.Peter Magnusson, Mechanical properties, physiological behavior, and function of aponeurosis and tendon, J. Appl. Physiol. 126 (2019) 1800–1807, https://doi.org/10.1152/japplphysiol.00671.2018.

[81] C.N. Maganaris, M.V. Narici, Mechanical properties of tendons. Tendon Injuries: Basic Science and Clinical Medicine, 2005, https://doi.org/10.1007/1-84628-050-8_2.

[82] C.N. Maganaris, P. Chatzistergos, N.D. Reeves, M.V. Narici, Quantification of internal stress-strain fields in human tendon: unraveling the mechanisms that underlie regional tendon adaptations and mal-adaptations to mechanical loading and the effectiveness of therapeutic eccentric exercise, Front. Physiol. 8 (2017), https://doi.org/10.3389/fphys.2017.00091.

[83] K. Ashley, F. Lui, Physiology, Nerve, 2020.

[84] Atlas of Anatomy of the Peripheral Nerves, 2017. https://doi.org/10.1007/978-3-319-43089-8.

[85] K.L. Christman, Regenerative medicine: biomaterials for tissue repair, Science 363 (1979) (2019) 340–341, https://doi.org/10.1126/science.aar2955.

[86] S.I. Stupp, Biomaterials for regenerative medicine, MRS Bull. 30 (2005) 546–553, https://doi.org/10.1557/mrs2005.148.

[87] N. Pien, S. Van Vlierberghe, P. Dubruel, D. Mantovani, Chapter 8. Hydrogel Processing Techniques and Vascular Tissue Engineering, 2021, https://doi.org/10.1039/9781839163975-00207.

[88] C. Archer, J. Ralphs, Regenerative medicine and biomaterials for the repair of connective tissues, 2010. https://doi.org/10.1533/9781845697792.

[89] H. Gregory, J.B. Phillips, Materials for peripheral nerve repair constructs: natural proteins or synthetic polymers? Neurochem. Int. 143 (2021) https://doi.org/10.1016/j.neuint.2020.104953.

[90] R. Boni, A. Ali, A. Shavandi, A.N. Clarkson, Current and novel polymeric biomaterials for neural tissue engineering, J. Biomed. Sci. 25 (2018) 1–21, https://doi.org/10.1186/s12929-018-0491-8.

[91] E. Alarcin, A. Bal-Öztürk, H. Avci, H. Ghorbanpoor, F. Dogan Guzel, A. Akpek, G. Yesiltas, T. Canak-Ipek, M. Avci-Adali, Current strategies for the regeneration of skeletal muscle tissue, Int. J. Mol. Sci. 22 (2021) 5929, https://doi.org/10.3390/ijms22115929.

[92] P. Zhuang, J. An, C.K. Chua, L.P. Tan, Bioprinting of 3D in vitro skeletal muscle models: a review, Mater. Des. 193 (2020), 108794, https://doi.org/10.1016/j.matdes.2020.108794.

[93] M. Beldjilali-Labro, A.G. Garcia, F. Farhat, F. Bedoui, J.F. Grosset, M. Dufresne, C. Legallais, Biomaterials in tendon and skeletal muscle tissue engineering: current trends and challenges, Materials (Basel) (2018) 11, https://doi.org/10.3390/ma11071116.

[94] K.D. McKeon-Fischer, J.H. Rossmeisl, A.R. Whittington, J.W. Freeman, Vivo skeletal muscle biocompatibility of composite, coaxial electrospun, and microfibrous scaffolds, Tissue Eng. Part A 20 (2014) 1961–1970, https://doi.org/10.1089/ten.tea.2013.0283.

[95] N. Bloise, E. Berardi, C. Gualandi, E. Zaghi, M. Gigli, R. Duelen, G. Ceccarelli, E. E. Cortesi, D. Costamagna, G. Bruni, N. Lotti, M.L. Focarete, L. Visai, M. Sampaolesi, Ether-oxygen containing electrospun microfibrous and sub-microfibrous scaffolds based on Poly(butylene 1,4-cyclohexanedicarboxylate) for skeletal muscle tissue engineering, Int. J. Mol. Sci. 19 (2018) 3212, https://doi.org/10.3390/IJMS19103212, 2018Page193212.

[96] P. Madhusudanan, G. Raju, S. Shankarappa, Hydrogel systems and their role in neural tissue engineering, J. R. Soc. Interface 17 (2020), https://doi.org/10.1098/rsif.2019.0505.

[97] M. Suzuki, S. Itoh, I. Yamaguchi, K. Takakuda, H. Kobayashi, K. Shinomiya, J. Tanaka, Tendon chitosan tubes covalently coupled with synthesized laminin peptides facilitate nerve regeneration in vivo, J. Neurosci. Res. 72 (2003), https://doi.org/10.1002/jnr.10589.

[98] M. Mir, N. Ahmed, A. ur Rehman, Recent applications of PLGA based nanostructures in drug delivery, Colloids Surf. B Biointerfaces (2017) 159, https://doi.org/10.1016/j.colsurfb.2017.07.038.

[99] H. Amani, H. Kazerooni, H. Hassanpoor, A. Akbarzadeh, H. Pazoki-Toroudi, Tailoring synthetic polymeric biomaterials towards nerve tissue engineering: a review, Artif. Cells Nanomed. Biotechnol 47 (2019), https://doi.org/10.1080/21691401.2019.1639723.

[100] G.S. Hussey, J.L. Dziki, S.F. Badylak, Extracellular matrix-based materials for regenerative medicine, Nat. Rev. Mater. 3 (2018) 159–173, https://doi.org/10.1038/s41578-018-0023-x.

[101] M.C. Catoira, L. Fusaro, D. Di Francesco, M. Ramella, F. Boccafoschi, Overview of natural hydrogels for regenerative medicine applications, J. Mater. Sci. Mater. Med. 30 (2019), https://doi.org/10.1007/s10856-019-6318-7.

[102] P.X. Ma, Biomimetic materials for tissue engineering, Adv. Drug. Deliv. Rev. 60 (2008) 184–198, https://doi.org/10.1016/j.addr.2007.08.041.

[103] J. Gilbert-Honick, S.R. Iyer, S.M. Somers, R.M. Lovering, K. Wagner, H.-.Q. Mao, W.L. Grayson, Engineering functional and histological regeneration of vascularized skeletal muscle, Biomaterials 164 (2018) 70–79, https://doi.org/10.1016/j.biomaterials.2018.02.006.

[104] J. Gilbert-Honick, S.R. Iyer, S.M. Somers, H. Takasuka, R.M. Lovering, K. R. Wagner, H.-.Q. Mao, W.L. Grayson, Engineering 3D skeletal muscle primed for neuromuscular regeneration following volumetric muscle loss, Biomaterials 255 (2020), 120154, https://doi.org/10.1016/j.biomaterials.2020.120154.

[105] L.R. Doblado, C. Martínez-Ramos, M.M. Pradas, Biomaterials for neural tissue engineering, Front. Nanotechnol. 3 (2021), https://doi.org/10.3389/fnano.2021.643507.

[106] B.E. Fornasari, G. Carta, G. Gambarotta, S. Raimondo, Natural-based biomaterials for peripheral nerve injury repair, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.554257.

[107] M. Yeo, G.H. Kim, Anisotropically aligned cell-laden nanofibrous bundle fabricated via cell electrospinning to regenerate skeletal muscle tissue, Small 14 (2018), 1803491, https://doi.org/10.1002/SMLL.201803491.

[108] B. Shiroud Heidari, R. Ruan, E.M. De-Juan-Pardo, M. Zheng, B. Doyle, Biofabrication and signaling strategies for tendon/ligament interfacial tissue engineering, ACS Biomater. Sci. Eng. 7 (2021) 383–399, https://doi.org/10.1021/acsbiomaterials.0c00731.

[109] A. Abalymov, B. Parakhonskiy, A.G. Skirtach, Polymer-and hybrid-based biomaterials for interstitial, connective, vascular, nerve, visceral and musculoskeletal tissue engineering, Polymers (Basel) 12 (2020), https://doi.org/10.3390/polym12030620.

[110] S. Yang, L. Jang, S. Kim, J. Yang, K. Yang, S.W. Cho, J.Y. Lee, Polypyrrole/Alginate hybrid HYDROGELS: electrically conductive and soft biomaterials for human mesenchymal stem cell culture and potential neural tissue engineering applications, Macromol. Biosci. 16 (2016) 1653–1661, https://doi.org/10.1002/mabi.201600148.

[111] S.K. Hsieh, H.Y. Lin, C.J. Chen, C.F. Jhuo, K.Y. Liao, W.Y. Chen, J.T.C. Tzen, Promotion of myotube differentiation and attenuation of muscle atrophy in murine C2C12 myoblast cells treated with teaghrelin, Chem. Biol. Interact. 315 (2020), 108893, https://doi.org/10.1016/J.CBI.2019.108893.

[112] J. Uribe-Gomez, A. Posada-Murcia, A. Shukla, M. Ergin, G. Constante, I. Apsite, D. Martin, M. Schwarzer, A. Caspari, A. Synytska, S. Salehi, L. Ionov, Shape-morphing fibrous hydrogel/elastomer bilayers fabricated by a combination of 3d printing and melt electrowriting for muscle tissue regeneration, ACS Appl. Bio Mater 4 (2021) 1720–1730, https://doi.org/10.1021/ACSABM.0C01495.

[113] T. Distler, A.A. Solisito, D. Schneidereit, O. Friedrich, R. Detsch, A.R. Boccaccini, 3D printed oxidized alginate-gelatin bioink provides guidance for C2C12 muscle precursor cell orientation and differentiation via shear stress during bioprinting, Biofabrication 12 (2020), 045005, https://doi.org/10.1088/1758-5090/AB98E4.

[114] C. Long, Z. Wang, A. Legrand, A. Chattopadhyay, J. Chang, P.M. Fox, Tendon tissue engineering: mechanism and effects of human tenocyte coculture with adipose-derived stem cells, J. Hand Surg. 43 (2018), https://doi.org/10.1016/j.jhsa.2017.07.031.

[115] A.H. Huang, J.L. Balestrini, B.V. Udelsman, K.C. Zhou, L. Zhao, J. Ferruzzi, B. C. Starcher, M.J. Levene, J.D. Humphrey, L.E. Niklason, Biaxial stretch improves elastic fiber maturation, collagen arrangement, and mechanical properties in engineered arteries, Tissue Eng. Part C Methods 22 (2016) 524–533, https://doi.org/10.1089/ten.tec.2015.0309.

[116] S. Shrestha, B.K. Shrestha, J.I. Kim, S. Won Ko, C.H. Park, C.S. Kim, Electrodeless coating polypyrrole on chitosan grafted polyurethane with functionalized multiwall carbon nanotubes electrospun scaffold for nerve tissue engineering, Carbon N Y 136 (2018) 430–443, https://doi.org/10.1016/j.carbon.2018.04.064.

[117] A. Saudi, M. Rafienia, A. Zargar Kharazi, H. Salehi, A. Zarrabi, M. Karevan, Design and fabrication of poly (glycerol sebacate)-based fibers for neural tissue engineering: synthesis, electrospinning, and characterization, Polym. Adv. Technol. 30 (2019) 1427–1440, https://doi.org/10.1002/pat.4575.

[118] X. Fang, H. Guo, W. Zhang, H. Fang, Q. Li, S. Bai, P. Zhang, Reduced graphene oxide-GelMA-PCL hybrid nanofibers for peripheral nerve regeneration, J. Mater. Chem. B 8 (2020) 10593–10601, https://doi.org/10.1039/d0tb00779j.

[119] P.A. Ramesh, R. Dhandapani, S. Bagewadi, A. Zennifer, J. Radhakrishnan, S. Sethuraman, A. Subramanian, Reverse engineering of an anatomically equivalent nerve conduit, J. Tissue Eng. Regen. Med. (2021), https://doi.org/10.1002/term.3245.

[120] B. Wiatrak, A. Kubis-Kubiak, A. Piwowar, E. Barg, PC12 cell Line: cell types, coating of culture vessels, differentiation and other culture conditions, Cells (2020) 9, https://doi.org/10.3390/cells9040958.

[121] W. Zhu, K.R. Tringale, S.A. Woller, S. You, S. Johnson, H. Shen, J. Schimelman, M. Whitney, J. Steinauer, W. Xu, T.L. Yaksh, Q.T. Nguyen, S. Chen, Rapid continuous 3D printing of customizable peripheral nerve guidance conduits, Mater. Today 21 (2018) 951–959, https://doi.org/10.1016/j.mattod.2018.04.001.

[122] Y.W. Chen, C.C. Chen, H.Y. Ng, C.W. Lou, Y.S. Chen, M.Y. Shie, Additive manufacturing of nerve decellularized extracellular matrix-contained polyurethane conduits for peripheral nerve regeneration, Polymers (Basel) 11 (2019) 1–16, https://doi.org/10.3390/polym11101612.

[123] W. Ye, H. Li, K. Yu, C. Xie, P. Wang, Y. Zheng, P. Zhang, J. Xiu, Y. Yang, F. Zhang, Y. He, Q. Gao, 3D printing of gelatin methacrylate-based nerve guidance conduits with multiple channels, Mater. Des. 192 (2020), 108757, https://doi.org/10.1016/j.matdes.2020.108757.

[124] A. Prabhath, V.N. Vernekar, E. Sanchez, C.T. Laurencin, Growth factor delivery strategies for rotator cuff repair and regeneration, Int. J. Pharm. 544 (2018), https://doi.org/10.1016/j.ijpharm.2018.01.006.

[125] D. Tsekes, G. Konstantopoulos, W.S. Khan, D. Rossouw, M. Elvey, J. Singh, Use of stem cells and growth factors in rotator cuff tendon repair, Eur. J. Orthopaed. Surg. Traumatol. 29 (2019), https://doi.org/10.1007/s00590-019-02366-x.

[126] P. Randelli, A. Menon, V. Ragone, R. D'Ambrosi, F. Randelli, P. Cabitza, G. Banfi, The Role of Growth Factors in Tendon Stimulation, Elsevier Inc., 2015, https://doi.org/10.1016/B978-0-12-801590-2.00007-7.

[127] R. James, G. Kesturu, G. Balian, A.B. Chhabra, Tendon: biology, biomechanics, repair, growth factors, and evolving treatment options, J. Hand Surg. 33 (2008) 102–112, https://doi.org/10.1016/j.jhsa.2007.09.007.

[128] C. Akduman, I. Özgüney, E.P.A. Kumbasar, Preparation and characterization of naproxen-loaded electrospun thermoplastic polyurethane nanofibers as a drug delivery system, Mater. Sci. Eng.: C 64 (2016) 383–390, https://doi.org/10.1016/j.msec.2016.04.005.

[129] C.-.H. Chen, S.-.H. Chen, K.T.T. Shalumon, J.-.P. Chen, Dual functional core–sheath electrospun hyaluronic acid/polycaprolactone nanofibrous membranes embedded with silver nanoparticles for prevention of peritendinous

adhesion, Acta Biomater. 26 (2015) 225–235, https://doi.org/10.1016/j.actbio.2015.07.041.
[130] N. Pien, I. Peeters, L. Deconinck, L. Van Damme, L. De Wilde, A. Martens, S. Van Vlierberghe, P. Dubruel, A. Mignon, Design and development of a reinforced tubular electrospun construct for the repair of ruptures of deep flexor tendons, Mater. Sci. Eng.: C 119 (2021), 111504, https://doi.org/10.1016/j.msec.2020.111504.
[131] I. Peeters, N. Pien, A. Mignon, L. Van Damme, P. Dubruel, S. Van Vlierberghe, D. Mantovani, V. Vermeulen, D. Creytens, A. Van Tongel, S. Schauvliege, K. Hermans, L. De Wilde, A. Martens, Flexor tendon repair using a reinforced tubular, medicated electrospun construct, J. Orthopaed. Res. (2021), https://doi.org/10.1002/jor.25103.
[132] K.T.T. Shalumon, C. Sheu, C.-.H. Chen, S.-.H. Chen, G. Jose, C.-.Y. Kuo, J.-.P. Chen, Multi-functional electrospun antibacterial core-shell nanofibrous membranes for prolonged prevention of post-surgical tendon adhesion and inflammation, Acta Biomater. 72 (2018) 121–136, https://doi.org/10.1016/j.actbio.2018.03.044.
[133] A. Mekaj, Y. Mekaj, The role of pharmacological agents in nerve regeneration after peripheral nerve repair. Peripheral Nerve Regeneration - From Surgery to New Therapeutic Approaches Including Biomaterials and Cell-Based Therapies Development, 2017, https://doi.org/10.5772/intechopen.68378.
[134] B.G. Gold, FK506 and the role of immunophilins in nerve regeneration, Mol. Neurobiol. 15 (1997) 285–306, https://doi.org/10.1007/BF02740664.
[135] O. Bota, L. Fodor, The influence of drugs on peripheral nerve regeneration, Drug Metab. Rev. 51 (2019) 266–292, https://doi.org/10.1080/03602532.2019.1632885.
[136] G. Terenghi, Peripheral nerve regeneration and neurotrophic factors, J. Anat. 194 (1999) 1–14, https://doi.org/10.1017/S0021878298004312.
[137] S.P. Frostick, Q. Yin, G.J. Kemp, Schwann cells, neurotrophic factors, and peripheral nerve regeneration, Microsurgery 18 (1998) 397–405, https://doi.org/10.1002/(SICI)1098-2752(1998)18:7<397::AID-MICR2>3.0.CO;2-F.
[138] J. Bloch, E.G. Fine, N. Bouche, A.D. Zurn, P. Aebischer, Nerve growth factor- and neurotrophin-3-releasing guidance channels promote regeneration of the transected rat dorsal root, Exp. Neurol. 172 (2001), https://doi.org/10.1006/exnr.2001.7778.
[139] E.G. Fine, I. Decosterd, M. Papaloïzos, A.D. Zurn, P. Aebischer, GDNF and NGF released by synthetic guidance channels support sciatic nerve regeneration across a long gap, Eur. J. Neurosci. 15 (2002), https://doi.org/10.1046/j.1460-9568.2002.01892.x.
[140] Y. Zhang, Z. Zhang, Y. Wang, Y. Su, M. Chen, 3D myotube guidance on hierarchically organized anisotropic and conductive fibers for skeletal muscle tissue engineering, Mater. Sci. Eng.: C 116 (2020), 111070, https://doi.org/10.1016/J.MSEC.2020.111070.
[141] K.D. McKeon-Fischer, J.W. Freeman, Characterization of electrospun poly(L-lactide) and gold nanoparticle composite scaffolds for skeletal muscle tissue engineering, J. Tissue Eng. Regen. Med 5 (2011) 560–568, https://doi.org/10.1002/term.348.
[142] M. Samandari, J. Quint, A. Rodríguez-delaRosa, I. Sinha, O. Pourquié, A. Tamayol, Bioinks and bioprinting strategies for skeletal muscle tissue engineering, Adv. Mater. 34 (2022), https://doi.org/10.1002/adma.202105883.
[143] A. Dede Eren, A. Vasilevich, E.D. Eren, P. Sudarsanam, U. Tuvshindorj, J. de Boer, J. Foolen, Tendon-derived biomimetic surface topographies induce phenotypic maintenance of tenocytes *In Vitro*, Tissue Eng. Part A 27 (2021) 1023–1036, https://doi.org/10.1089/ten.tea.2020.0249.
[144] S.K. Madhurakkat Perikamana, J. Lee, T. Ahmad, E.M. Kim, H. Byun, S. Lee, H. Shin, Harnessing biochemical and structural cues for tenogenic differentiation of adipose derived stem cells (ADSCs) and development of an in vitro tissue interface mimicking tendon-bone insertion graft, Biomaterials 165 (2018) 79–93, https://doi.org/10.1016/j.biomaterials.2018.02.046.
[145] A.D. Schoenenberger, H. Tempfer, C. Lehner, J. Egloff, M. Mauracher, A. Bird, J. Widmer, K. Maniura-Weber, S.F. Fucentese, A. Traweger, U. Silvan, J. G. Snedeker, Macromechanics and polycaprolactone fiber organization drive macrophage polarization and regulate inflammatory activation of tendon in vitro and in vivo, Biomaterials 249 (2020), 120034, https://doi.org/10.1016/j.biomaterials.2020.120034.
[146] Y. Ma, H. Gao, H. Wang, X. Cao, Engineering topography: effects on nerve cell behaviors and applications in peripheral nerve repair, J. Mater. Chem. B 9 (2021) 6310–6325, https://doi.org/10.1039/d1tb00782c.
[147] C.Y. Yang, W.Y. Huang, L.H. Chen, N.W. Liang, H.C. Wang, J. Lu, X. Wang, T. W. Wang, Neural tissue engineering: the influence of scaffold surface topography and extracellular matrix microenvironment, J. Mater. Chem. B 9 (2021) 567–584, https://doi.org/10.1039/d0tb01605e.
[148] T.A. Dixon, E. Cohen, D.M. Cairns, M. Rodriguez, J. Mathews, R.R. Jose, D. L. Kaplan, Bioinspired three-dimensional human neuromuscular junction development in suspended hydrogel arrays, Tissue Eng. Part C Methods 24 (2018) 346–359, https://doi.org/10.1089/ten.tec.2018.0062.
[149] H.-.W. Kang, S.J. Lee, I.K. Ko, C. Kengla, J.J. Yoo, A. Atala, A 3D bioprinting system to produce human-scale tissue constructs with structural integrity, Nat. Biotechnol. 34 (2016) 312–319, https://doi.org/10.1038/nbt.3413.
[150] H. Lee, W. Kim, J. Lee, K.S. Park, J.J. Yoo, A. Atala, G.H. Kim, S.J. Lee, Self-aligned myofibers in 3D bioprinted extracellular matrix-based construct accelerate skeletal muscle function restoration, Appl. Phys. Rev. 8 (2021), https://doi.org/10.1063/5.0039639.
[151] P. Zhuang, J. An, C.K. Chua, L.P. Tan, Bioprinting of 3D in vitro skeletal muscle models: a review, Mater. Des. 193 (2020), https://doi.org/10.1016/j.matdes.2020.108794.
[152] H.J. Jeong, H. Nam, J. Jang, S.J. Lee, 3D bioprinting strategies for the regeneration of functional tubular tissues and organs, Bioengineering 7 (2020) 1–24, https://doi.org/10.3390/bioengineering7020032.
[153] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nature Publishing Group 32 (2014) 773–785, https://doi.org/10.1038/nbt.2958.
[154] J. Van Hoorick, L. Tytgat, A. Dobos, H. Ottevaere, J. Van Erps, H. Thienpont, A. Ovsianikov, P. Dubruel, S. Van Vlierberghe, Photo-)crosslinkable Gelatin Derivatives for Biofabrication applications, Acta Biomater. 97 (2019) 46–73, https://doi.org/10.1016/j.actbio.2019.07.035.
[155] J. Li, M. Chen, X. Fan, H. Zhou, Recent advances in bioprinting techniques: approaches, applications and future prospects, J. Transl. Med. 14 (2016) 271, https://doi.org/10.1186/s12967-016-1028-0.
[156] P. Mozetic, S.M. Giannitelli, M. Gori, M. Trombetta, A. Rainer, Engineering muscle cell alignment through 3D bioprinting, J. Biomed. Mater. Res. A 105 (2017) 2582–2588, https://doi.org/10.1002/JBM.A.36117.
[157] A. García-Lizarribar, X. Fernández-Garibay, F. Velasco-Mallorquí, A.G. Castaño, J. Samitier, J. Ramon-Azcon, Composite biomaterials as long-lasting scaffolds for 3d bioprinting of highly aligned muscle tissue, Macromol. Biosci. 18 (2018), 1800167, https://doi.org/10.1002/MABI.201800167.
[158] W.J. Kim, G.H. Kim, 3D bioprinting of functional cell-laden bioinks and its application for cell-alignment and maturation, Appl. Mater. Today 19 (2020), 100588, https://doi.org/10.1016/J.APMT.2020.100588.
[159] Y.-.J. Choi, T.G. Kim, J. Jeong, H.-.G. Yi, J.W. Park, W. Hwang, D.-.W. Cho, 3D cell printing of functional skeletal muscle constructs using skeletal muscle-derived bioink, Adv. Healthc. Mater. 5 (2016) 2636–2645, https://doi.org/10.1002/ADHM.201600483.
[160] G.H. Yang, W. Kim, J. Kim, G. Kim, A skeleton muscle model using GelMA-based cell-aligned bioink processed with an electric-field assisted 3D/4D bioprinting, Theranostics 11 (2021) 48–63, https://doi.org/10.7150/thno.50794.
[161] Y. Nakanishi, T. Okada, N. Takeuchi, N. Kozono, T. Senju, K. Nakayama, Y. Nakashima, Histological evaluation of tendon formation using a scaffold-free three-dimensional-bioprinted construct of human dermal fibroblasts under in vitro static tensile culture, Regen. Ther 11 (2019) 47–55, https://doi.org/10.1016/j.reth.2019.02.002.
[162] Y. Zhang, T. Lei, C. Tang, Y. Chen, Y. Liao, W. Ju, H. Zhang, B. Zhou, R. Liang, T. Zhang, C. Fan, X. Chen, Y. Zhao, Y. Xie, J. Ye, B.C. Heng, X. Chen, Y. Hong, W. Shen, Z. Yin, 3D printing of chemical-empowered tendon stem/progenitor cells for functional tissue repair, Biomaterials 271 (2021), 120722, https://doi.org/10.1016/j.biomaterials.2021.120722.
[163] X. Jiang, S. Wu, M. Kuss, Y. Kong, W. Shi, P.N. Streubel, T. Li, B. Duan, 3D printing of multilayered scaffolds for rotator cuff tendon regeneration, Bioact. Mater 5 (2020) 636–643, https://doi.org/10.1016/j.bioactmat.2020.04.017.
[164] S. Chae, Y. Sun, Y.J. Choi, D.H. Ha, I. Jeon, D.W. Cho, 3D cell-printing of tendon-bone interface using tissue-derived extracellular matrix bioinks for chronic rotator cuff repair, Biofabrication (2021) 13, https://doi.org/10.1088/1758-5090/abd159.
[165] Y. Wu, Y.S. Wong, J.Y.H. Fuh, Degradation behaviors of geometric cues and mechanical properties in a 3D scaffold for tendon repair, J. Biomed. Mater. Res. A 105 (2017) 1138–1149, https://doi.org/10.1002/jbm.a.35966.
[166] T.K. Merceron, M. Burt, Y.J. Seol, H.W. Kang, S.J. Lee, J.J. Yoo, A. Atala, A 3D bioprinted complex structure for engineering the muscle-tendon unit, Biofabrication 7 (2015), https://doi.org/10.1088/1758-5090/7/3/035003.
[167] S. Laternser, H. Keller, O. Leupin, M. Rausch, U. Graf-Hausner, M. Rimann, A novel microplate 3D bioprinting platform for the engineering of muscle and tendon tissues, SLAS Technol 23 (2018) 599–613, https://doi.org/10.1177/2472630318776594.
[168] G. Wu, B. Sun, C. Zhao, Z. Wang, S. Teng, M. Yang, Z. Cui, G. Zhu, Y. Yu, Three-dimensional tendon scaffold loaded with TGF-β1 gene silencing plasmid prevents tendon adhesion and promotes tendon repair, ACS Biomater. Sci. Eng. 7 (2021) 5739–5748, https://doi.org/10.1021/acsbiomaterials.1c00747.
[169] H. Zhang, Z. Pei, C. Wang, M. Li, H. Zhang, J. Qu, Electrohydrodynamic 3D printing scaffolds for repair of achilles tendon defect in rats, Tissue Eng. Part A 27 (2021) 1343–1354, https://doi.org/10.1089/ten.tea.2020.0290.
[170] A. Marino, G. Ciofani, C. Filippeschi, M. Pellegrino, M. Pellegrini, P. Orsini, M. Pasqualetti, V. Mattoli, B. Mazzolai, Two-photon polymerization of sub-micrometric patterned surfaces : investigation of Cell-Substrate Interactions and Improved Di ff erentiation of Neuron-like Cells, (2013).
[171] A. Singh, S. Asikainen, A.K. Teotia, P.A. Shiekh, E. Huotilainen, I. Qayoom, J. Partanen, J. Seppälä, A. Kumar, Biomimetic Photocurable three-dimensional printed nerve guidance channels with aligned cryomatrix lumen for peripheral nerve regeneration, ACS Appl. Mater. Interfaces 10 (2018) 43327–43342, https://doi.org/10.1021/acsami.8b11677.
[172] D. Hsiao, S.H. Hsu, R.S. Chen, M.H. Chen, Characterization of designed directional polylactic acid 3D scaffolds for neural differentiation of human dental pulp stem cells, J. Formosan Med.Assoc. 119 (2020) 268–275, https://doi.org/10.1016/j.jfma.2019.05.011.
[173] X. Xu, J. Tao, S. Wang, L. Yang, J. Zhang, J. Zhang, H. Liu, H. Cheng, J. Xu, M. Gou, Y. Wei, 3D printing of nerve conduits with nanoparticle-encapsulated RGFP966, Appl. Mater. Today 16 (2019) 247–256, https://doi.org/10.1016/j.apmt.2019.05.014.
[174] Z. Wu, Q. Li, S. Xie, X. Shan, Z. Cai, In vitro and in vivo biocompatibility evaluation of a 3D bioprinted gelatin-sodium alginate/rat Schwann-cell scaffold, Mater. Sci. Eng. C 109 (2020), https://doi.org/10.1016/j.msec.2019.110530.
[175] J. Zhang, J. Tao, H. Cheng, H. Liu, W. Wu, Y. Dong, X. Liu, M. Gou, S. Yang, J. Xu, Nerve transfer with 3D-printed branch nerve conduits, Burns Trauma 10 (2022), https://doi.org/10.1093/burnst/tkac010.

[176] H.W. Kang, S.J. Lee, I.K. Ko, C. Kengla, J.J. Yoo, A. Atala, A 3D bioprinting system to produce human-scale tissue constructs with structural integrity, Nat. Biotechnol. 34 (2016), https://doi.org/10.1038/nbt.3413.
[177] R. Costa-Almeida, I. Calejo, M.E. Gomes, Mesenchymal stem cells empowering tendon regenerative therapies, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20123002.
[178] H. Yurie, R. Ikeguchi, T. Aoyama, Y. Kaizawa, J. Tajino, A. Ito, S. Ohta, H. Oda, H. Takeuchi, S. Akieda, M. Tsuji, K. Nakayama, S. Matsuda, The efficacy of a scaffold-free Bio 3D conduit developed from human fibroblasts on peripheral nerve regeneration in a rat sciatic nerve model, PLoS ONE 12 (2017), e0171448, https://doi.org/10.1371/journal.pone.0171448.
[179] A. Marino, G. Ciofani, C. Filippeschi, M. Pellegrino, M. Pellegrini, P. Orsini, M. Pasqualetti, V. Mattoli, B. Mazzolai, Two-photon polymerization of sub-micrometric patterned surfaces: investigation of cell-substrate interactions and improved differentiation of Neuron-like Cells, ACS Appl. Mater. Interfaces 5 (2013) 13012–13021, https://doi.org/10.1021/am403895k.
[180] L.A. Bosworth, S. Downes (Eds.), Electrospinning for Tissue Regeneration, eds.1st ed, Woodhead Publishing, 2011.
[181] X. Mo, B. Sun, T. Wu, D. Li, Chapter 24 - Electrospun Nanofibers for Tissue Engineering, Elsevier Inc., 2019, https://doi.org/10.1016/B978-0-323-51270-1.00024-8.
[182] A.E. Oprea, A. Ficai, E. Andronescu, Chapter 4. Electrospun Nanofibers for Tissue Engineering Applications, Elsevier Inc., 2019, https://doi.org/10.1016/B978-0-12-816909-4.00004-X.
[183] K.J. Aviss, J.E. Gough, S. Downes, Aligned electrospun polymer fibres for skeletal muscle regeneration, Eur. Cell Mater. 19 (2010) 193–204, https://doi.org/10.22203/eCM.v019a19.
[184] J.S. Choi, S.J. Lee, G.J. Christ, A. Atala, J.J. Yoo, The influence of electrospun aligned poly(ε-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes, Biomaterials 29 (2008) 2899–2906, https://doi.org/10.1016/j.biomaterials.2008.03.031.
[185] M.M. Smoak, K.J. Hogan, K.J. Grande-Allen, A.G. Mikos, Bioinspired electrospun dECM scaffolds guide cell growth and control the formation of myotubes, Sci. Adv. 7 (2021) 4123–4137.
[186] N. Narayanan, C. Jiang, C. Wang, G. Uzunalli, N. Whittern, D. Chen, O.G. Jones, S. Kuang, M. Deng, Harnessing fiber diameter-dependent effects of myoblasts toward biomimetic scaffold-based skeletal muscle regeneration, Front. Bioeng. Biotechnol 8 (2020), https://doi.org/10.3389/fbioe.2020.00203.
[187] N. Narayanan, S. Calve, Extracellular matrix at the muscle–tendon interface: functional roles, techniques to explore and implications for regenerative medicine, Connect. Tissue Res. 62 (2021), https://doi.org/10.1080/03008207.2020.1814263.
[188] K.D. McKeon-Fischer, D.H. Flagg, J.W. Freeman, Coaxial electrospun poly (ε-caprolactone), multiwalled carbon nanotubes, and polyacrylic acid/polyvinyl alcohol scaffold for skeletal muscle tissue engineering, J. Biomed. Mater. Res. A. 99 A (2011) 493–499, https://doi.org/10.1002/jbm.a.33116.
[189] V. Perez-Puyana, P. Wieringa, Y. Yuste, F. de la Portilla, A. Guererro, A. Romero, L. Moroni, Fabrication of hybrid scaffolds obtained from combinations of PCL with gelatin or collagen via electrospinning for skeletal muscle tissue engineering, J. Biomed. Mater. Res. A 109 (2021) 1600–1612, https://doi.org/10.1002/JBM.A.37156.
[190] X. Tang, N. Saveh-Shemshaki, H.-.M. Kan, Y. Khan, C.T. Laurencin, Biomimetic Electroconductive Nanofibrous Matrices for Skeletal Muscle Regenerative Engineering, Regenerat. Eng. Transl. Med. 6 (2019) 228–237, https://doi.org/10.1007/S40883-019-00136-Z, 2019 6:2.
[191] J. Gilbert-Honick, B. Ginn, Y. Zhang, S. Salehi, K.R. Wagner, H.Q. Mao, W. L. Grayson, Adipose-derived stem/stromal cells on electrospun fibrin microfiber bundles enable moderate muscle reconstruction in a volumetric muscle loss model, Cell Transplant. (2018) 27, https://doi.org/10.1177/0963689718805370.
[192] M.C. Chen, Y.C. Sun, Y.H. Chen, Electrically conductive nanofibers with highly oriented structures and their potential application in skeletal muscle tissue engineering, Acta Biomater. 9 (2013) 5562–5572, https://doi.org/10.1016/J.ACTBIO.2012.10.024.
[193] S. Hosseinzadeh, M. Mahmoudifard, F. Mohamadyar-Toupkanlou, M. Dodel, A. Hajarizadeh, M. Adabi, M. Soleimani, The nanofibrous PAN-PANi scaffold as an efficient substrate for skeletal muscle differentiation using satellite cells, Bioprocess. Biosyst. Eng. 39 (2016), https://doi.org/10.1007/s00449-016-1592-y.
[194] L. Wang, Y. Wu, B. Guo, P.X. Ma, Nanofiber Yarn/Hydrogel core–shell scaffolds mimicking native skeletal muscle tissue for guiding 3d myoblast alignment, elongation, and differentiation, ACS Nano 9 (2015) 9167–9179, https://doi.org/10.1021/ACSNANO.5B03644.
[195] Y.Reusmaazran HasmadHanis, M.R. Rashid, H.I. Bt, C. Roy, Human amniotic membrane with aligned electrospun fiber as scaffold for aligned tissue regeneration, Home.Liebertpub.Com/Tec. 24 (2018) 368–378, https://doi.org/10.1089/TEN.TEC.2017.0447.
[196] Y. Guo, J. Gilbert-Honick, S.M. Somers, H.-.Q. Mao, W.L. Grayson, Modified cell-electrospinning for 3D myogenesis of C2C12s in aligned fibrin microfiber bundles, Biochem. Biophys. Res. Commun. 516 (2019) 558–564, https://doi.org/10.1016/j.bbrc.2019.06.082.
[197] L. Ricotti, A. Polini, G.G. Genchi, G. Ciofani, D. Iandolo, H. Vazão, V. Mattoli, L. Ferreira, A. Menciassi, D. Pisignano, Proliferation and skeletal myotube formation capability of C2C12 and H9c2 cells on isotropic and anisotropic electrospun nanofibrous PHB scaffolds, Biomed. Mater. 7 (2012), 035010, https://doi.org/10.1088/1748-6041/7/3/035010.
[198] S. Deepthi, M. Nivedhitha Sundaram, J. Deepti Kadavan, R. Jayakumar, Layered chitosan-collagen hydrogel/aligned PLLA nanofiber construct for flexor tendon regeneration, Carbohydr. Polym. 153 (2016) 492–500, https://doi.org/10.1016/j.carbpol.2016.07.124.
[199] O. Evrova, G.M. Bürgisser, C. Ebnöther, A. Adathala, M. Calcagni, E. Bachmann, J. G. Snedeker, C. Scalera, P. Giovanoli, V. Vogel, J. Buschmann, Elastic and surgeon friendly electrospun tubes delivering PDGF-BB positively impact tendon rupture healing in a rabbit Achilles tendon model, Biomaterials 232 (2020), 119722, https://doi.org/10.1016/j.biomaterials.2019.119722.
[200] W.R. Webb, T.P. Dale, A.J. Lomas, G. Zeng, I. Wimpenny, A.J. El Haj, N. R. Forsyth, G.Q. Chen, The application of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds for tendon repair in the rat model, Biomaterials 34 (2013) 6683–6694, https://doi.org/10.1016/j.biomaterials.2013.05.041.
[201] C.N. Manning, A.G. Schwartz, W. Liu, J. Xie, N. Havlioglu, S.E. Sakiyama-Elbert, M.J. Silva, Y. Xia, R.H. Gelberman, S. Thomopoulos, Controlled delivery of mesenchymal stem cells and growth factors using a nanofiber scaffold for tendon repair, Acta Biomater. 9 (2013) 6905–6914, https://doi.org/10.1016/j.actbio.2013.02.008.
[202] A. Yousefi, F. Sarrafzadeh-Rezaei, S. Asri-Rezaei, A.-.A. Farshid, M. Behfar, Fabrication of novel tubular scaffold for tendon repair from chitosan in combination with zinc oxide nanoparticles, Veterinary Res. Forum 9 (2018) 105–111, https://doi.org/10.30466/VRF.2018.29979.
[203] L. Dong, L. Li, Y. Song, Y. Fang, J. Liu, P. Chen, S. Wang, C. Wang, T. Xia, W. Liu, L. Yang, MSC-derived immunomodulatory extracellular matrix functionalized electrospun fibers for mitigating foreign-body reaction and tendon adhesion, Acta Biomater. 133 (2021) 280–296, https://doi.org/10.1016/j.actbio.2021.04.035.
[204] O. Uyanik, K.C. Pekkoc-Uyanik, S. Findik, A. Avci, Z. Altuntas, Prevention of peritendinous adhesions with electrospun poly (lactic acid-co-glycolic acid) (PLGA) bioabsorbable nanofiber: an experimental study, Colloids Surf. B Biointerfaces (2022) 209, https://doi.org/10.1016/j.colsurfb.2021.112181.
[205] N. Pien, Y. Van de Maele, L. Parmentier, M. Meeremans, A. Mignon, C. De Schauwer, I. Peeters, L. De Wilde, A. Martens, D. Mantovani, S. Van Vlierberghe, P. Dubruel, Design of an electrospun tubular construct combining a mechanical and biological approach to improve tendon repair, J. Mater. Sci. Mater. Med. 33 (2022) 51, https://doi.org/10.1007/s10856-022-06673-4.
[206] W. Zhu, T. Ye, S.J. Lee, H. Cui, S. Miao, X. Zhou, D. Shuai, L.G. Zhang, Enhanced neural stem cell functions in conductive annealed carbon nanofibrous scaffolds with electrical stimulation, Nanomedicine 14 (2018) 2485–2494, https://doi.org/10.1016/j.nano.2017.03.018.
[207] J. Wang, L. Tian, B. Luo, S. Ramakrishna, D. Kai, X.J. Loh, I.H. Yang, G.R. Deen, X. Mo, Engineering PCL/lignin nanofibers as an antioxidant scaffold for the growth of neuron and Schwann cell, Colloids Surf. B Biointerfaces 169 (2018) 356–365, https://doi.org/10.1016/j.colsurfb.2018.05.021.
[208] L. Wang, Y. Wu, T. Hu, P.X. Ma, B. Guo, Aligned conductive core-shell biomimetic scaffolds based on nanofiber yarns/hydrogel for enhanced 3D neurite outgrowth alignment and elongation, Acta Biomater. 96 (2019) 175–187, https://doi.org/10.1016/j.actbio.2019.06.035.
[209] R. Ghobeira, C. Philips, L. Liefooghe, M. Verdonck, M. Asadian, P. Cools, H. Declercq, W.H. De Vos, N. De Geyter, R. Morent, Synergetic effect of electrospun PCL fiber size, orientation and plasma-modified surface chemistry on stem cell behavior, Appl. Surf. Sci. 485 (2019) 204–221, https://doi.org/10.1016/j.apsusc.2019.04.109.
[210] Y. Xia, H. Yang, S. Li, S. Zhou, L. Wang, Y. Tang, C. Cheng, R. Haag, Multivalent Polyanionic 2D nanosheets functionalized nanofibrous stem cell-based neural scaffolds, Adv. Funct. Mater. (2021) 31, https://doi.org/10.1002/adfm.202010145.
[211] M. Yeo, G.H. Kim, Micro/nano-hierarchical scaffold fabricated using a cell electrospinning/3D printing process for co-culturing myoblasts and HUVECs to induce myoblast alignment and differentiation, Acta Biomater. 107 (2020) 102–114, https://doi.org/10.1016/J.ACTBIO.2020.02.042.
[212] B.L. Banik, J.L. Brown, 3D-printed bioreactor enhances potential for tendon tissue engineering, Regen. Eng. Transl. Med. 6 (2020) 419–428, https://doi.org/10.1007/s40883-019-00145-y.
[213] M. Karimi, E. Biazar, S.H. Keshel, A. Ronaghi, J. Doostmohamadpour, A. Janfada, A. Montazeri, Rat sciatic nerve reconstruction across a 30mm defect bridged by an oriented porous PHBV tube with schwann cell as artificial nerve Graft, ASAIO J. 60 (2014) 224–233, https://doi.org/10.1097/MAT.0000000000000044.
[214] P.D. Dalton, C. Vaquette, B.L. Farrugia, T.R. Dargaville, T.D. Brown, D. W. Hutmacher, Electrospinning and additive manufacturing: converging technologies, Biomater. Sci 1 (2013) 171–185, https://doi.org/10.1039/c2bm00039c.
[215] T.M. Robinson, D.W. Hutmacher, P.D. Dalton, The next frontier in melt electrospinning: taming the jet, Adv. Funct. Mater. 29 (2019), https://doi.org/10.1002/adfm.201904664.
[216] T. Jungst, I. Pennings, M. Schmitz, A.J.W.P.W.P. Rosenberg, J. Groll, D. Gawlitta, Heterotypic scaffold design orchestrates primary cell organization and phenotypes in cocultured small diameter vascular grafts, Adv. Funct. Mater. 29 (2019), https://doi.org/10.1002/adfm.201905987.
[217] T. Jungst, M.L. Muerza-Cascante, T.D. Brown, M. Standfest, D.W. Hutmacher, J. Groll, P.D. Dalton, Melt electrospinning onto cylinders: effects of rotational velocity and collector diameter on morphology of tubular structures, Polym. Int. 64 (2015) 1086–1095, https://doi.org/10.1002/pi.4948.
[218] J.C. Kade, P.D. Dalton, Polymers for Melt electrowriting, Adv. Healthc. Mater 10 (2021), 2001232, https://doi.org/10.1002/adhm.202001232.
[219] G. Hochleitner, F. Chen, C. Blum, P.D. Dalton, B. Amsden, J. Groll, Melt electrowriting below the critical translation speed to fabricate crimped elastomer

scaffolds with non-linear extension behaviour mimicking that of ligaments and tendons, Acta Biomater. 72 (2018), https://doi.org/10.1016/j.actbio.2018.03.023.
[220] P.B. Warren, Z.G. Davis, M.B. Fisher, Parametric control of fiber morphology and tensile mechanics in scaffolds with high aspect ratio geometry produced via melt electrowriting for musculoskeletal soft tissue engineering, J. Mech. Behav. Biomed. Mater. 99 (2019), https://doi.org/10.1016/j.jmbbm.2019.07.013.
[221] Y. Wang, Y. Zhang, Z. Zhang, Y. Su, Z. Wang, M. Dong, M. Chen, An injectable high-conductive bimaterial scaffold for neural stimulation, Colloids Surf. B Biointerfaces 195 (2020), 111210, https://doi.org/10.1016/j.colsurfb.2020.111210.
[222] Z. Zhang, M.L. Jørgensen, Z. Wang, J. Amagat, Y. Wang, Q. Li, M. Dong, M. Chen, 3D anisotropic photocatalytic architectures as bioactive nerve guidance conduits for peripheral neural regeneration, Biomaterials 253 (2020), 120108, https://doi.org/10.1016/j.biomaterials.2020.120108.
[223] Y. Zhang, Z. Zhang, Y. Wang, Y. Su, M. Chen, 3D myotube guidance on hierarchically organized anisotropic and conductive fibers for skeletal muscle tissue engineering, Mater. Sci. Eng.: C 116 (2020), 111070, https://doi.org/10.1016/j.msec.2020.111070.
[224] H. Jahani, F.A. Jalilian, C.Y. Wu, S. Kaviani, M. Soleimani, N. Abbasi, K.L. Ou, H. Hosseinkhani, Controlled surface morphology and hydrophilicity of polycaprolactone toward selective differentiation of mesenchymal stem cells to neural like cells, J. Biomed. Mater. Res. A 103 (2015), https://doi.org/10.1002/jbm.a.35328.
[225] B. Langridge, M. Griffin, P.E. Butler, Regenerative medicine for skeletal muscle loss: a review of current tissue engineering approaches, J. Mater. Sci. Mater. Med. 32 (2021) 15, https://doi.org/10.1007/s10856-020-06476-5.
[226] S. Agarwal, J.H. Wendorff, A. Greiner, Use of electrospinning technique for biomedical applications, Polymer (Guildf) 49 (2008) 5603–5621, https://doi.org/10.1016/j.polymer.2008.09.014.
[227] N.W. Pensa, A.S. Curry, P.P. Bonvallet, N.F. Bellis, K.M. Rettig, M.S. Reddy, A. W. Eberhardt, S.L. Bellis, 3D printed mesh reinforcements enhance the mechanical properties of electrospun scaffolds, Biomater Res 23 (2019), https://doi.org/10.1186/s40824-019-0171-0.
[228] M.T. Wolf, C.L. Dearth, S.B. Sonnenberg, E.G. Loboa, S.F. Badylak, Naturally derived and synthetic scaffolds for skeletal muscle reconstruction, Adv. Drug. Deliv. Rev. 84 (2015), https://doi.org/10.1016/j.addr.2014.08.011.
[229] Y.J. Choi, T.G. Kim, J. Jeong, H.G. Yi, J.W. Park, W. Hwang, D.W. Cho, 3D cell printing of functional skeletal muscle constructs using skeletal muscle-derived Bioink, Adv. Healthc. Mater 5 (2016), https://doi.org/10.1002/adhm.201600483.
[230] K.J.M. Boonen, M.L.P. Langelaan, R.B. Polak, D.W.J. van der Schaft, F.P. T. Baaijens, M.J. Post, Effects of a combined mechanical stimulation protocol: value for skeletal muscle tissue engineering, J. Biomech. 43 (2010) 1514–1521, https://doi.org/10.1016/j.jbiomech.2010.01.039.
[231] C.N.M. Ryan, M.N. Doulgkeroglou, D.I. Zeugolis, Electric field stimulation for tissue engineering applications, BMC Biomed. Eng. 3 (2021), https://doi.org/10.1186/s42490-020-00046-0.
[232] J. Kim, K. Kennedy, G. Vunjak-Novakovic, A.O. Oluseun Odeleye, C.-Y. Chui, Bioreactors in Regenerative Medicine, Elsevier, 2019, pp. 2–17.
[233] S. Pigeot, T. Klein, F. Gullotta, S.J. Dupard, A.Garcia Garcia, A. García-García, S. Prithiviraj, P. Lorenzo, M. Filippi, C. Jaquiery, L. Kouba, M.A. Asnaghi, D. B. Raina, B. Dasen, H. Isaksson, P. Önnerfjord, M. Tägil, A. Bondanza, I. Martin, P. E. Bourgine, Manufacturing of human tissues as off-the-shelf grafts programmed to induce regeneration, Adv. Mater. 33 (2021), https://doi.org/10.1002/adma.202103737.
[234] D. Lim, E.S. Renteria, D.S. Sime, Y.M. Ju, J.H. Kim, T. Criswell, T.D. Shupe, A. Atala, F.C. Marini, M.N. Gurcan, S. Soker, J. Hunsberger, J.J. Yoo, Bioreactor design and validation for manufacturing strategies in tissue engineering, Biodes Manuf (2021), https://doi.org/10.1007/s42242-021-00154-3.
[235] M. Gomez-Florit, C.J. Labrador-Rached, R.M.A. Domingues, M.E. Gomes, The tendon microenvironment: engineered in vitro models to study cellular crosstalk, Adv. Drug. Deliv. Rev. 185 (2022), 114299, https://doi.org/10.1016/j.addr.2022.114299.
[236] D. Cigognini, A. Lomas, P. Kumar, A. Satyam, A. English, A. Azeem, A. Pandit, D. Zeugolis, Engineering in vitro microenvironments for cell based therapies and drug discovery, Drug. Discov. Today 18 (2013) 1099–1108, https://doi.org/10.1016/j.drudis.2013.06.007.
[237] S. Chae, D.-.W. Cho, Biomaterial-based 3D bioprinting strategy for orthopedic tissue engineering, Acta Biomater. (2022), https://doi.org/10.1016/j.actbio.2022.08.004.
[238] J. Apostolakos, T.J.S. Durant, C.R. Dwyer, R.P. Russell, J.H. Weinreb, F. Alaee, K. Beitzel, M.B. McCarthy, M.P. Cote, A.D. Mazzocca, The enthesis: a review of the tendon-to-bone insertion, Muscl. Ligament. Tendons J. 4 (2014), https://doi.org/10.11138/mltj/2014.4.3.333.
[239] T. Lei, T. Zhang, W. Ju, X. Chen, B.C. Heng, W. Shen, Z. Yin, Biomimetic strategies for tendon/ligament-to-bone interface regeneration, Bioact. Mater. 6 (2021) 2491–2510, https://doi.org/10.1016/j.bioactmat.2021.01.022.
[240] D. Wang, X. Zhang, S. Huang, Y. Liu, B.S. chuen Fu, K.K. lun Mak, A.M. Blocki, P. S. hang Yung, R.S. Tuan, D.F.E. Ker, Engineering multi-tissue units for regenerative Medicine: bone-tendon-muscle units of the rotator cuff, Biomaterials (2021) 272, https://doi.org/10.1016/j.biomaterials.2021.120789.
[241] Y. Xu, W.X. Zhang, L.N. Wang, Y.Q. Ming, Y.L. Li, G.X. Ni, Stem cell therapies in tendon-bone healing, World J. Stem. Cells 13 (2021), https://doi.org/10.4252/wjsc.v13.i7.753.
[242] R.A. Sun Han Chang, J.F. Shanley, M.E. Kersh, B.A.C. Harley, Tough and tunable scaffold-hydrogel composite biomaterial for soft-to-hard musculoskeletal tissue interfaces, Sci. Adv. 6 (2020), https://doi.org/10.1126/sciadv.abb6763.
[243] A. Tits, D. Ruffoni, Joining soft tissues to bone: insights from modeling and simulations, Bone Rep 14 (2021), https://doi.org/10.1016/j.bonr.2020.100742.
[244] S. Barbeau, J. Tahraoui-Bories, C. Legay, C. Martinat, Building Neuromuscular Junctions in Vitro, Development (Cambridge), 2020, p. 147, https://doi.org/10.1242/dev.193920.
[245] J. Saini, A. Faroni, M.A. Al Samid, A.J. Reid, A.P. Lightfoot, K. Mamchaoui, V. Mouly, G. Butler-Browne, J.S. McPhee, H. Degens, N. Al-Shanti, Simplified in vitro engineering of neuromuscular junctions between rat embryonic motoneurons and immortalized human skeletal muscle cells, Stem. Cells Cloning 12 (2019), https://doi.org/10.2147/SCCAA.S187655.
[246] J.K. Ichida, C.P. Ko, Organoids develop motor skills: 3D human neuromuscular junctions, Cell Stem. Cell 26 (2020), https://doi.org/10.1016/j.stem.2020.01.003.
[247] K. Yoshioka, A. Ito, Y. Kawabe, M. Kamihira, Novel neuromuscular junction model in 2D and 3D myotubes co-cultured with induced pluripotent stem cell-derived motor neurons, J. Biosci. Bioeng. 129 (2020), https://doi.org/10.1016/j.jbiosc.2019.10.004.
[248] S. Das, K.D. Browne, F.A. Laimo, J.C. Maggiore, M.C. Hilman, H. Kaisaier, C. A. Aguilar, Z.S. Ali, F. Mourkioti, D.K. Cullen, Pre-innervated tissue-engineered muscle promotes a pro-regenerative microenvironment following volumetric muscle loss, Commun. Biol 3 (2020), https://doi.org/10.1038/s42003-020-1056-4.
[249] X. Jiang, Y. Kong, M. Kuss, J. Weisenburger, H. Haider, R. Harms, W. Shi, B. Liu, W. Xue, J. Dong, J. Xie, P. Streubel, B. Duan, 3D bioprinting of multilayered scaffolds with spatially differentiated ADMSCs for rotator cuff tendon-to-bone interface regeneration, Appl. Mater. Today 27 (2022), 101510, https://doi.org/10.1016/j.apmt.2022.101510.
[250] G. Walden, X. Liao, S. Donell, M.J. Raxworthy, G.P. Riley, A. Saeed, A. Clinical, Biological, and biomaterials perspective into tendon injuries and regeneration, Tissue Eng. Part B Rev. (2017) 23, https://doi.org/10.1089/ten.teb.2016.0181.
[251] C.N.M. Ryan, D.I. Zeugolis, Engineering the tenogenic niche in vitro with microenvironmental tools, Adv Ther (Weinh) 3 (2020), 1900072, https://doi.org/10.1002/adtp.201900072.
[252] F. Trensz, F. Lucien, V. Couture, T. Söllrald, G. Drouin, A.-.J. Rouleau, M. Grandbois, G. Lacraz, G. Grenier, Increased microenvironment stiffness in damaged myofibers promotes myogenic progenitor cell proliferation, Skelet Muscle 5 (2016) 5, https://doi.org/10.1186/s13395-015-0030-1.
[253] P.A. Walczak, P. Perez-Esteban, D.C. Bassett, E.J. Hill, Modelling the central nervous system: tissue engineering of the cellular microenvironment, Emerg. Top. Life Sci 5 (2021) 507–517, https://doi.org/10.1042/ETLS20210245.
[254] E. Mariani, G. Lisignoli, R.M. Borzì, L. Pulsatelli, Biomaterials: foreign bodies or tuners for the immune response? Int. J. Mol. Sci. 20 (2019) https://doi.org/10.3390/ijms20030636.
[255] A. Angeletti, C. Cantarelli, P. Cravedi, Immune responses towards bioengineered tissues and strategies to control them, Curr. Opin. Organ. Transpl. 24 (2019), https://doi.org/10.1097/MOT.0000000000000688.
[256] E. Antmen, N.E. Vrana, V. Hasirci, The role of biomaterials and scaffolds in immune responses in regenerative medicine: macrophage phenotype modulation by biomaterial properties and scaffold architectures, Biomater. Sci 9 (2021), https://doi.org/10.1039/d1bm00840d.
[257] J. Ye, C. Xie, C. Wang, J. Huang, Z. Yin, B.C. Heng, X. Chen, W. Shen, Promoting musculoskeletal system soft tissue regeneration by biomaterial-mediated modulation of macrophage polarization, Bioact. Mater 6 (2021), https://doi.org/10.1016/j.bioactmat.2021.04.017.
[258] V. Russo, M. El Khatib, G. Prencipe, A. Cerveró-Varona, M.R. Citeroni, A. Mauro, P. Berardinelli, M. Faydaver, A.A. Haidar-Montes, M. Turriani, O. Di Giacinto, M. Raspa, F. Scavizzi, F. Bonaventura, L. Liverani, A.R. Boccaccini, B. Barboni, Scaffold-mediated immunoengineering as innovative strategy for tendon regeneration, Cells (2022) 11, https://doi.org/10.3390/cells11020266.
[259] J. Lin, W. Zhou, S. Han, V. Bunpetch, K. Zhao, C. Liu, Z. Yin, H. Ouyang, Cell-material interactions in tendon tissue engineering, Acta Biomater. 70 (2018) 1–11, https://doi.org/10.1016/j.actbio.2018.01.012.
[260] J. Hou, R. Yang, I. Vuong, F. Li, J. Kong, H.Q. Mao, Biomaterials strategies to balance inflammation and tenogenesis for tendon repair, Acta Biomater. 130 (2021), https://doi.org/10.1016/j.actbio.2021.05.043.
[261] K.M. Adusei, T.B. Ngo, K. Sadtler, T lymphocytes as critical mediators in tissue regeneration, fibrosis, and the foreign body response, Acta Biomater. 133 (2021), https://doi.org/10.1016/j.actbio.2021.04.023.
[262] D. Xia, J. Chen, Z. Zhang, M. Dong, Emerging polymeric biomaterials and manufacturing techniques in regenerative medicine, Aggregate (2022), https://doi.org/10.1002/agt2.176.
[263] S. Kiran, P. Dwivedi, V. Kumar, R.L. Price, U.P. Singh, Immunomodulation and biomaterials: key players to repair volumetric muscle loss, Cells (2021) 10, https://doi.org/10.3390/cells10082016.
[264] J.G. Tidball, S.A. Villalta, Regulatory interactions between muscle and the immune system during muscle regeneration, Am. J. Physiol. Regul. Integr. Comp. Physiol. 298 (2010), https://doi.org/10.1152/ajpregu.00735.2009.
[265] B.J. Kwee, D.J. Mooney, Biomaterials for skeletal muscle tissue engineering, Curr. Opin. Biotechnol. (2017) 47, https://doi.org/10.1016/j.copbio.2017.05.003.
[266] N. Pien, S. Palladino, F. Copes, G. Candiani, P. Dubruel, S. Van Vlierberghe, D. Mantovani, Tubular bioartificial organs: from physiological requirements to fabrication processes and resulting properties. A critical review, Cells Tissues Organs (2021), https://doi.org/10.1159/000519207.

[267] B.R. Freedman, D.J. Mooney, Biomaterials to mimic and heal connective tissues, Advanced Materials (2019) 31, https://doi.org/10.1002/adma.201806695.
[268] I. Gherghescu, M.B. Delgado-Charro, The biosimilar landscape: an overview of regulatory approvals by the EMA and FDA, Pharmaceutics 13 (2020) 48, https://doi.org/10.3390/pharmaceutics13010048.
[269] J. Hunsberger, J. Neubert, J.A. Wertheim, J. Allickson, A. Atala, Bioengineering priorities on a path to ending organ shortage, Curr. Stem. Cell Rep 2 (2016) 118–127, https://doi.org/10.1007/s40778-016-0038-4.
[270] C. Hotz, T.R. Wagenaar, F. Gieseke, D.S. Bangari, M. Callahan, H. Cao, J. Diekmann, M. Diken, C. Grunwitz, A. Hebert, K. Hsu, M. Bernardo, K. Karikó, S. Kreiter, A.N. Kuhn, M. Levit, N. Malkova, S. Masciari, J. Pollard, H. Qu, S. Ryan, A. Selmi, J. Schlereth, K. Singh, F. Sun, B. Tillmann, T. Tolstykh, W. Weber, L. Wicke, S. Witzel, Q. Yu, Y.A. Zhang, G. Zheng, J. Lager, G.J. Nabel, U. Sahin, D. Wiederschain, Local delivery of mRNA-encoding cytokines promotes antitumor immunity and tumor eradication across multiple preclinical tumor models, Sci. Transl. Med. 13 (2021), https://doi.org/10.1126/scitranslmed.abc7804.
[271] S.M. Greising, B.T. Corona, C. McGann, J.K. Frankum, G.L. Warren, Therapeutic approaches for volumetric muscle loss injury: a systematic review and meta-analysis, Tissue Eng. Part B Rev. (2019) 25, https://doi.org/10.1089/ten.teb.2019.0207.