

Tissue Engineering in Musculoskeletal Tissue: Review of Literature

Mary Bove, MD, Annalisa Carlucci, MD, Giovanni Natale, MD, Chiara Freda, MD, Antonio Noro, MD, Vincenzo Ferrara, MD, Giorgia Opromolla, MD, Mario Martone, MD, Roberto Cascone, MD, Gaetana Messina, MD, Anna Izzo, MD, Giovanni Vicidomini, MD, Mario Santini, MD, Alfonso Fiorelli, MD, PhD

Thoracic Surgery Unit, University of Campania Luigi Vanvitelli, Naples, Italy;

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Corresponding Author:

Alfonso Fiorelli, MD, PhD

Thoracic Surgery Unit,

Università della Campania “Luigi Vanvitelli”

Piazza Miraglia, 2

I-80138 Naples, Italy

Phone: +390815665228

Fax: +390815665230

E-mail: alfonso.fiorelli@unicampania.it

ABSTRACT

Tissue engineering, also called “regenerative medicine”, refers to attempt to create functional human tissue from cells in laboratory. This is a field that uses living cells, biocompatible materials, suitable biochemical and physical factors and their combinations, to create tissue-like structures.. To date, no tissue engineered skeletal muscle implants have been developed for clinical use, but it may represent a valid alternative to treat volumetric muscle loss in the near future. Herein, we reviewed the literature and showed different techniques to produce synthetic tissues with the same architectural, structural and functional properties of native tissues.

Key-words: tissue engineering; biocompatible materials; skeletal muscle

INTRODUCTION

In the XXI century life expectancy of aged population increased and the elderly expects good quality life with high levels of activity. However, as they grow, tissues fail due to illness or injury or simply the aging process and this can lead to a significant loss of quality life, particularly in individuals with chronic diseases. It is possible to observe this degeneration since the age of 30-35 and around the 10% of the world's population needs at least a tissue repair or replacement at some point during its lifetime. There are of course current options for tissue repair and replacement but all of these have limitations. None of these fully restore tissue function and the quality of life and the majority of them fail to grow and develop within the individual. Therefore it is imperative to ensure for the aging population that any new regenerative medical technology solution not only meets the clinical needs and increase patient expectation, but also that it is affordable in order to be clinically adopted.

Musculoskeletal disorders can be caused by trauma, genetic conditions, autoimmune and degenerative diseases. Whereas cartilage, tendons and ligaments have a limited self-repairing capacity, bones and skeletal muscles regenerate themselves after small injuries. When there is a severe volumetric loss, muscle regeneration fails leading to fibrosis, atrophy and ischemia. In this case the only operative technique in clinical practice is the use of muscle flaps to replace the damaged tissue. Tissue engineering, also called "regenerative medicine", refers to attempt to create functional human tissue from cells in laboratory. This is a field that uses living cells, biocompatible materials, suitable biochemical and physical factors and their combinations, to create tissue-like structures [1]. Its ultimate goal is to be a cure, not merely a treatment by repairing and replacing tissues or organs that fail due to diseases, genetic errors, congenital abnormalities or traumatic injuries [2]. To date, no tissue engineered skeletal muscle implants have been developed for clinical use, but it may represent a valid alternative to treat volumetric muscle loss in the near future.

TISSUE ENGINEERING STRATEGIES

The field of skeletal muscle tissue engineering has taken great strides since Vandenberg's first work in 1988, using cultured avian myotubes in collagen-coated tissue culture plates [3]. Tissue engineering strategies can be divided into two main categories: scaffold-based and scaffold-free approaches.

Scaffold –based approaches

Robert S. Langer and Joseph Vacanti [4] realized that, to build an organ, a framework that guides the cell's growth is needed: a "scaffold" to define the parts and hold them together. Scaffold's biochemical, topological and geometrical properties and fabrication methods affect behaviour in terms of differentiation, adhesion, and viability [5-6]. They have a structural framework similar to the EMC in order to provide support for tissue regrowth.

Synthetic scaffolds

Electrospinning

It uses an electric field to produce 3D scaffolds from biocompatible polymers. Electrospun scaffolds, fabricated using biodegradable polymers such as ECM proteins [7,8,9,10,11,12], have been used for muscle tissue engineering [13]. Elastin-like recombinant fibers are cytocompatible and allow for incorporating different functionalities, such as cell adhesion domains for fibroblasts and keratinocytes [14]. Collagen electrospun scaffolds seeded with myoblasts facilitate the regeneration of muscle fibers with low biocompatibility [9]. This method gives control over the scaffolds parameters important for cell adhesion and myotube formation, is quick and cost effective [15, 16].

Micropatterned substrates

They are synthetic polymers designed to control many aspects of cellular behavior, including spatial organization, migration, proliferation, and differentiation [17, 18, 19]. Skeletal muscle is made of multiple bundles of fiber formed by the fusion and alignment of myoblasts into myotubes, necessary to produce appropriate contraction. That is the reason why alignment and fusion of myotubes are crucial aspects for muscle tissue engineering. Micropatterned scaffolds have been used to guide these processes. Soft lithography uses an elastomeric master that is easy to mold or emboss that can be used directly as a substrate for biological applications or as a mold. It is widely used for the patterning of cells and proteins through various techniques such as microcontact printing, microfluidic patterning, and stencil micropatterning [20, 21, 22]. It can be used: patterning of ECM proteins such as collagen, fibronectin or laminin; printing of self-assembled monolayers with cell-repellant molecules; a combination of cell-repellant and cell-adhesive molecules [23, 24, 25]. Shimizu et al. [26] used a stencil membrane seeded with C2C12 myoblasts that proliferated and differentiated, forming a pattern of single myotubes. Direct inkjet printing technique has been used to obtain myoblast's patterning, improving cell alignment and tissue formation [27]. Contractile C2C12 myotube line patterns embedded in a fibrin gel have been developed to afford a physiologically relevant and stable bioassay system by Nagamine et al. [28]. Huang et al. [29] used micropatterned polydimethylsiloxane (PDMS) or poly2-hydroxyethyl methacrylate (pHEMA) as scaffold and transferred the aligned myotubes to biodegradable collagen gel. Functional nanomembranes, ultrathin polymeric films of fibronectin and fibril carbon nanotubes, can be micropatterned to promote myoblasts alignment, elongation and differentiation [30]. In a five layers 3D tissue, made of human umbilical vein endothelial cells sandwiched between myoblasts sheets, endothelial cell connections and capillary-like structures were found through the layers. After transplantation into the subcutaneous tissue of nude rats, the endothelial networks connected the host vessels, allowing the graft's survival [31]. Takahashi et al. [32] discovered that an anisotropic cell

sheet placed on top of other cell sheets induced myoblasts alignment. Guillaume-Gentil et al. [33] used an electrochemical strategy for the micropatterning and detachment of heterotypic cell sheets. These methods present the possible creation of co-cultured cell sheets and the creation of cellular constructs which mimic the cellular complexity of native tissue.

Protein based scaffolds

They provide a structural pattern like the fibrous protein components of the ECM. These are available as scaffolds in gel form or suspended into matrices with a determined pore size by cryogelation [34,35]. Synthetic polymers allow control over the scaffold structure and micro-architecture. Scaffold structural properties, such as diameter, alignment and porosity guide cell response, proliferation and differentiation [36], even after implantation [37]. Nigarawa et al. [38] used fibrin-based scaffolds seeded with mesenchymal stem cells to demonstrate their high potential for differentiation into skeletal muscles *in vitro*. Then, they implanted the construct *in vivo*, into mouse damaged tibialis anterior muscle, showing that transplanted cells can accelerate the functional recovery of injured muscles. Dynamic scaffolds can change their diameter, alignment and porosity through temperature change or magnetic fields, miming the complexity of environment changes *in vivo*. Fraley et al. [39] presented an integrated study of ECM proteins parameters in different ECM configurations using self-assembling 3D collagen and showed how each parameter relates to others and to cell motility: cellular motility was significantly predicted by fiber alignment; cellular protrusion rate, orientation, speed of migration and invasion distance showed biphasic responses to increasing collagen density. Also Wang et al. [40] demonstrated the relationship between fiber alignment and cells motility along the direction of fiber alignment and the change from unaligned to aligned morphology. Although fiber micro-architecture influences cellular behavior, a limitation of synthetic polymer scaffold is the lack of cell signaling provided by native ECM. In order to reply the native ECM environment, proteins and growth factor can be

incorporated into the scaffolds [41]. Hydrogel-based scaffold with integrin binding domains improves mesenchymal stem cells attachment and bone repair upon implantation [42, 43].

TGF- β induced differentiation of MSC toward chondrogenic and osteogenic lineages depending on fiber alignment [44]. PDGF induced tenogenic differentiation of adipose-derived stem cells in an aligned collagen nanoparticle composite fiber [45]. Quarta et al. [46] engineered a biomimetic microenvironment that enabled maintenance of quiescent, potent mouse Muscle Stem Cells and human Muscle Stem Cells. Both mouse and human Muscle Stem Cells showed an enhanced engraftment and self-renewal potential. The possibility of culturing MuSCs for long time period without loss of potency gives the chance to correct genetic mutations, in order to transplant only corrected cells and replace pathological tissue in muscle disorders [47,48].

Acellularized tissue scaffolds.

They are natural scaffolds derived from tissues or organs, in which the cellular and nuclear contents are eliminated, but the tridimensional (3D) structure, composition and microenvironment of the extracellular matrix (ECM) are preserved. They have to be similar to the tissue that has to be repaired [49]. Recent works were performed with animal and human skeletal muscle models. Porzionato et al. [50] decellularized human skeletal muscle and used it as a scaffold in a rabbit model with an abdominal wall defect. It gave good results in terms of integration, but recellularization was not completely achieved. Wilson et al. [51] compared decellularized skeletal muscle taken from rectus femoris and supraspinatus, showing that muscle type influence material properties. They demonstrated that these scaffolds biodegrade at a rate that corresponds to the regeneration of the damaged muscle: biological scaffolds has not to be considered as permanent implants, but as a temporary support to ECM turnover by resident cells. Davari et al. [52] implanted cryopreserved and decellularized patches, derived from the hemidiaphragm of a deceased, in a canine model. They compared the two methods and demonstrated that the healing process was similar, with lower inflammatory cells and foreign body granulomas on decellularized patch.

However, ECM derived from decellularization of a tissue may be used for tissue engineering of another tissue type, as demonstrated by Wolf et al that compared quadriceps, hamstring and intestine derived scaffolds seeded with C2C12 cells implanted in the abdominal wall of a rat. After 35 days, muscle fiber structure had been restored using either scaffold [53]. A limitation of acellularized tissue scaffolds is the limited donor tissue and the potential for immune rejection. Trials of recellularization have been performed and demonstrated that the most promising cell types are mesenchymal stem cells and induced pluripotent stem cells, thanks to their capability of differentiation through different lineages, stimulated by the ECM [54].

Scaffold-free approaches.

Although classical tissue engineering is based on a combination of cells, scaffolds and signals, scaffold free techniques have emerged. They exploit cells' capability of synthesizing tissues and responding to signals, offering advantages over traditional scaffold-based techniques. Cell viability is increased because they do not use electric fields, elevated temperatures or toxic chemicals [55]. Scaffold less approaches provide a biomimetic microenvironment allowing cell communication and the maintenance of cell phenotype in order to increase ECM production [56-59]. Tissue regeneration can happen rapidly, because there is no need for scaffold degradation. They also do not determinate immune rejection in the host [60]. The advantages of scaffold less technology over classical scaffold based tissue engineering make them promising solutions for the use in clinical practice in the near future.

Self-organization process.

It produces organized tissues with the use of external forces, such as physical manipulation or thermal input [61-62]. Cell-sheet engineering, pellet culture aggregates or spheroids are examples of self-organization.

In Cell-sheet engineering, cells are cultured in monolayers either on functionalized substrates or on thermo responsive polymers. In the first case they are removed via mechanical or enzymatic cleavage of their cell-matrix attachments to the surface; in the second case through the change in conformation induced by variation in temperature [63]. The last method preserves the cell-matrix-binding interactions. After that, monolayers go through the process of tissue fusion. Through tissue fusion isolated cell populations make contact and adhere [64]. Fusion of multiple cell-sheet layers can be used to generate tissues of greater thickness to replicate the architecture of a target tissue [65].

Pellet culture consists in a centrifugation of cells inside a conical shaped tube and then cell pellets are cultured in medium in order to obtain cell differentiation and ECM deposition [66, 67]. This method fails in creating tissues with mechanical properties suitable for clinical use.

Aggregate culture are based on various methods that induce the aggregate formation. The aggregate cells lay in an environment that can induce cell differentiation and ECM synthesis and formation of neotissue. These methods, like pellet culture, are not suitable alone for in vitro biomimetic tissue engineering, because produce small cell aggregates. Anyway, these approaches can be used in tissue engineering with other technologies for the maintenance of many cell types [68-69].

Self-organizing musculoskeletal tissues show structural features and mechanical functions similar to those of the native tissues [70-71]. Donnelly et al. [72] showed that physiology and function of muscle can be improved in vitro using an electrical stimulation bioreactor. Electrical stimulation applied to monolayers in culture determined an increase in protein synthesis, while the stimulation of three-dimensional engineered muscle improved force production and excitability.

Self-assembling process

It produces tissues with spontaneous organization without the influence of external energy. These tissues are characterized by: the use of a non-adherent substrate to minimize tissue free energy; sequential phases that reassume native tissue formation; tissue constructs with clinically relevant size and morphology; functional properties like those of native tissue [73].

The process has been used especially in articular cartilage tissue engineering [74]. Differential adhesion of surface-bound molecules and differential interfacial tension are mechanisms that contribute to explain self-assembly. Williams et al. [75] produced scaffold free constructs and implanted them into the hind limb of a rat along the sciatic nerve. After one week in vivo the engineered constructs showed phenotypical modification: a developing capillary system, an epimysium-like outer layer of connective tissue, and an increase in myosin heavy chain content. In addition they increased contractile strength and no sign of immune rejection were observed. Carosio et al. [76] engineered a three-dimensional vascularized skeletal muscle tissue and implanted it in place of extensor *digitorum longus* muscle in mice, restoring the functionality of the damaged muscle.

Advantages of scaffold less techniques consist in the native tissue integration, enhanced ECM deposition and direct mechanic transduction, avoiding the release of harmful by-products. They also avoid issues of cytotoxicity caused by processing conditions required for the production of some biomaterials. Avoiding the use of synthetic materials, biocompatibility issues are mitigated. These technologies present some limitations. The engineered tissues have to present the same mechanical characteristics of the tissue that needs to be repaired. Dennis and Kosnik [76] produced a self-assembling skeletal muscle tissue in which the myotubes remained arrested in an early developmental state due to the absence of signals to promote expression of adult myosin isoforms. This limitation can be resolved by the development of a vascular network in vitro [77]. Cell source needs is also an important issue. Stem cells represent an attractive cell source, since they can

differentiate into different cells and tissues, so co-cultures of primary cells and stem cells should be explored [78]. The problem is that in many cases they don't totally differentiate into the target tissue, compromising the properties of the neo tissue. Another limitation for the creation of functional muscle tissue is the time required for myoblasts and fibroblasts to assemble a robust tissue through ECM. Even if the process is faster than the scaffold-based approaches, it takes almost a month to assemble an implantable muscle-tendon construct in vitro. For this reason scaffold-free constructs could be more appropriate for chronic reconstruction rather than acute repair of traumatic injuries.

Conclusion and future directions

Tissue engineering studies different techniques to produce synthetic tissues with the same architectural, structural and functional properties of native tissues. In the last decades, it made significant progress in creating functional tissues able to replace the ones damaged by age, disease or trauma. Scaffold-based and scaffold less approaches have been developed and studied during the years, with promising results, even if at the current state engineered tissues have not been used in clinical practice, especially for skeletal muscle injuries. Engineered muscle tissue has to be biocompatible, in order to permit muscle regrowth without immune reactions. It also has to be scaled up to replace clinically relevant volumes of tissue. Studies in vivo could be more effective than in vitro to obtain a biocompatible and large enough tissue. Development and standardization of appropriate animal models are needed to value long-term results and allow clinical application.

Then, when used in clinical practice, physician could develop rehabilitation techniques in order to not overload musculoskeletal tissue during the phase of maturation. Simultaneous use of scaffold and scaffold-free approaches is a promising method, depending on the characteristics of the tissue that needs to be repaired. The choice of the method should be based on the knowledge of the development of the tissue in vivo, in order to use an approach as close as possible to the biological

process. For example, the phases of self-assembling process are similar to the development of articular cartilage in vivo.

Further research into the biological mechanisms at the basis of muscular endogenous regeneration could guide new approaches that can be used in skeletal muscle tissue engineering. The ultimate aim of tissue engineering is to synthesize neo tissues with a high level of complexity that present the exact features of native tissue, in order to regenerate it.

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