Tissue engineering for skeletal muscle regeneration

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Summary

Stem cells and regenerative medicine have obtained a remarkable consent from the scientific community for their promising ability to recover aged, injured and diseased tissue. However, despite the noteworthy potential, hurdles currently hinder their use and clinical application: cell survival, immune response, tissue engraftment and efficient differentiation. Hence a new interdisciplinary scientific approach, such as tissue engineering, is going deep attempts to mimic neo-tissue-genesis as well as stem cell engraftment amelioration. Skeletal muscle tissue engineering represents a great potentiality in medicine for muscle regeneration exploiting new generation injectable hydrogel as scaffold supporting progenitor/stem cells for muscle differentiation reconstructing the natural skeletal muscle tissue architecture influenced by matrix mechanical and physical property and by a dynamic environment.

Key words: skeletal muscle, tissue engineering, hydrogel, mesoangioblast, stem cell.

Introduction

Muscles are primarily responsible for voluntary movement control and maintenance of the body posture. Skeletal muscle tissue can self-repair and regenerate activating resident (muscle satellite cells) or circulating (mesenchymal stem cells) stem cells in response to damage, but is unable to restore its integrity after a major loss of tissue or in long lasting chronic degeneration, such as large traumatic injuries or congenital diseases of skeletal muscle likewise muscular dystrophies[1]. Muscular diseases are difficult to treat, due to the nature of the skeletal muscle tissue that is composed of many large, multinucleated fibres whose nuclei cannot divide. The limited capacity of skeletal muscle to self-regenerate justifies the exogenous reconstruction necessity for structural and functional repair of large muscle damage[1]. Reconstructive strategies, such as autologous muscle transplantation and muscle progenitor cell injection, have so far achieved modest therapeutic effects due to a very poor tissue/cell engraftment, survival and integration into host regenerating muscle fibres. Sufficient experimental data are now available to recognize specific issues for myoblast transplantation in addition to difficulties associated with tissue transplantation, such as triggering immune responses and/or graft death[2]. One specific problem associated with myoblast transplantation is the amount of donor-derived tissue appearing to be dependent on the *in vitro* cultivation history of the implanted cells. Amongst novel therapeutic strategies for the treatment of muscular dystrophies, stem cell grafting and implantations of bioengineered muscle substitutes have been proposed recently as a promising alternative strategy[3,4], assuming that they can provide instant structural repair, prolonged implant survival and functional *in vivo* restoration (Fuoco et al., Skeletal Muscle under review). However, while these techniques may be of great importance in medicine in the near future, there is no widely available and reproducible method for the regeneration of living and functional muscle yet. A considerable body of evidence has recently emerged showing that recreating the proper environment in a tissue engineered muscle construct is a critical determinant for its survival, differentiation and proper functional maturation[5-11]. Is a wide accepted hypothesis that it is possible to promote functional muscle regeneration in a severely lost or damaged muscle ameliorating stem cell therapy enhancing cell engraftment, viability and myogenic differentiation employing biomaterial as scaffold to support implanted/injected cells[12,13].

Tissue engineering

The tissue engineering is an emerging multidisciplinary scientific approach aiming to replace lost, damaged or failing tissue and organs. Exploiting histology, stem cell biology and chemical engineering knowledge; tissue engineering aims to generate innovative biomaterials,

mimicking extracellular matrix (ECM) features (stiffness, elasticity and adhesion) in order to support cell survival, growth and differentiation, and to guarantee the scaffolding to reconstruct the complex tissue architecture. The regeneration of new muscle fibres through tissue engineering represents a powerful alternative for the replacement of skeletal muscle tissue after severe damage. Recently, implantation of engineered skeletal muscle tissues has been proposed as an alternative strategy with the potential for structural repair, prolonged implant survival, and accelerated functional recovery[13]. Tissue engineering utilizes two main components: one biological represented by stem cells with their regenerative potential, and one synthetic, natural or hybrid represented by biomaterial in which cells are embedded[12,14]. Biomaterials play central roles in modern regenerative medicine and tissue engineering representing a three-dimensional (3D) designable environment. The role of biophysical and biochemical cues guiding cellular behaviour and function is responsible for the complex processes involved in tissue formation and regeneration. Naturally derived polymers generally exhibit good biocompatibility but a low ductility whereas the synthetic polymers present a high tunability but may cause a negative immune response from the body. Biomaterials have been studied and used for many different applications, such as joint and bone replacement, artificial ligaments and tendons, dental implants for tooth fixation, blood vessel prostheses, heart valves, contact lenses and other several artificial tissues, each of this application forecast a different material characterized by specific physical mechanical properties likewise elasticity and stiffness. The hydrogel matrix demonstrated a noteworthy compatibility with muscular tissue, reproducing a suitable environment for myo-differentiation and muscle satellite cell (skeletal muscle resident stem cell) pool replenishment[13].

Hydrogel

Hydrogels are a class of hydrophilic biomaterials made of water-soluble components exhibiting high porosity and high water uptake. Their structure is basically a network of macromolecules that can be stabilized either by chemical or physical cross-linking. The main properties of these materials are:

- High bio-compatibility;
- High tissue-like water content;
- Soft tissue mimicking mechanical and structural properties;
- Efficient diffusion of oxygen, nutrients and metabolites;
- Uniform encapsulation of cells;
- Injection as a liquid precursor and *in situ* polymerization;
- Re-absorption modulation.

Tissue engineering is one of the latest applications of hydrogels, which utilizes them as scaffold to generate new tissue[14]. Moreover the *in situ* liquid to solid transition (gelation) ability is particularly advantageous since it allows minimally invasive surgical procedures during the transplantation of the matrix embedded cells into the tissue. Hydrogels must address particular requirements, in particular hydrogel design has to consider physical parameters, such as mechanical and degradation properties, as well as cell adhesion and bio-compatibility[15]. The controlled degradation of the hydrogel is another important parameter for application in tissue engineering. Hydrogels degradation can occur due to hydrolysis and/or enzymatic degradation (proteolysis). The intend is typically to achieve a degradation rate that guarantees the time necessary for new tissue formation, which depends by the desired tissue type. Mechanical and degradation properties of hydrogels are generally tightly connected to each other: a more cross-linked and stiffer material will undergo a slower degradation process. The biological performance of the hydrogel is due to its interaction with cells, which influences cell adhesion, migration and differentiation. An effective cell-hydrogel interface is achieved thanks to the presence of biological domains inside the gels, such as peptides and growth factors, which stimulate cell adhesion and viability. These domains can be included in the network or adsorbed on the gel surface[15]. The gelling mechanism is also a critical feature of a hydrogel, since it might affect its biological performance one being photo-polymerization. Photo-polymerization is achieved using a radical photo-initiator, light sensitive compounds that exhibit high absorbance at a specific wavelength and initiates a radical polymerization when exposed to visible or UV light[16]. Photo-polymerization has several advantages over other cross-linking techniques, such as spatial and temporal control over polymerization and minimal heat production. For *in vivo* applications the polymerization requires acceptable conditions such as physiological pH and temperature, and, as previously mentioned, bio-compatible and non-cytotoxic photo-initiators. The bulk method is the most commonly used: the photo-initiator is dissolved in the liquid hydrogel precursor and the gelation is achieved by exposure to an appropriate wavelength. Injectable hydrogels can be subcutaneously injected as liquid solution and then polymerized *in situ* after exposure to visible or long wavelength UV light, which can be transmitted across skin[17]. A new class of polymeric temperature-responsive hydrogels that polymerize in presence of temperature changing has also been studied. Cohn and colleagues developed a poly(ethylene oxide)PEO-poly(propylene oxide)PPO-PEO triblock copolymer combining improved mechanical properties and injectability[18]. Shachaf et al. formulated a temperature-responsive biosynthetic hydrogel combining the tri-block copolymer Pluronic®F127 to fibrinogen, polymerizing at 37°C[19]. Lutolf et al. elaborated a PEG-based hydrogel, containing peptide sequences that are substrate domains of transglutaminase factor XIIIa and cross-link under the effect of this enzyme[20]. Poly(ethylene glycol) (PEG) (Fig. 1) is a widely studied and used polymer for biomedical applications, whose bio-compatibility and low toxicity have extensively been verified[15,21]. PEG has been used for different biomedical applications, such as localized drug delivery[22], cell encapsulation[23,24], biological adhesives for surgical procedures[25], wound dressings[26], and fillers for aesthetic procedures[27]. Furthermore, these materials are block copolymer made of bio-compat-

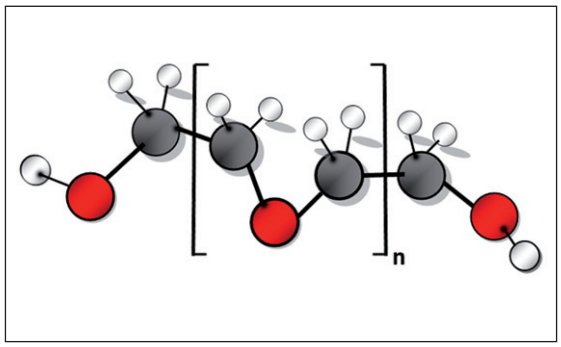

Figure 1. Poly(ethylene glycol) (PEG) molecular structure: Carbon atoms (grey), Hydrogen atoms (white) and Oxygen Atoms (red).

ible PEG conjugated with short oligopeptides, which are cleavage sequences for targeted enzymes. Reactive acrylate groups at each end allow cross-linking by exposure to UV light, with formation of hydrogel networks. Seliktar and colleagues developed a photo-polymerizable PEG-fibrinogen hydrogel as scaffold for tissue engineering, in order to exploit the properties of a hybrid hydrogel made of PEG and fibrinogen fragments, combining the advantages of both synthetic and natural scaffolds, assuring stiffness design of the matrix (PEG) and natural signal for survival, differentiation and adhesion (Fibrinogen)[28].

Mesenchymal stem cells

Several mesenchymal stem cells have been isolated and characterized revealing limited capacity to differentiate into skeletal muscle tissue[29]. In 2002 Minasi et al. reported the identification and initial characterization of a novel type of mesenchymal stem/progenitor cell associated to vessel, named "mesoangioblast" (Mabs)[30]. Their existence has been suggested by previous studies, which demonstrated unorthodox myogenesis occurring in cells from embryonic dorsal aorta[31]. Mabs has been isolated from embryonic and post natal muscles from different species (mouse, dog, human), showing the remarkable capacity to undergo robust *in vitro* myogenesis when reaching high confluence population (Fig. 2). Systemic injection of Mabs overcomes previous myoblasts transplantation issues, leading to long term survival of donor cells, restoring muscle structure and function of dystrophic mice[32] and dog[33]. Lately, the corresponding human cells have been isolated and well characterized, for the phase I/II clinical trial for muscle recovery of Duchene Muscular Dystrophy affected patients. A combination approach utilizing advanced state of the art tissue engineering could be useful in muscle dysfunction representing a new and attractive treatment option for skeletal muscle regeneration ameliorating Mabs based cell therapy.

Discussion

Skeletal muscle tissue engineering is still a hard task even if recent data showed encouraging results[13]. The main problem is linked to the peculiar organization of skeletal muscle tissue represented by parallel aligned myofibres with myosin/actin filaments sarcomere arranged, intracellular calcium storage and acetylcholine receptors, guaranteeing muscle functionality and than contraction. In 1991 Vandenburgh et al. demonstrated the pivotal role played by kinetic force to promote muscle organization reconstruction *in vitro* in a stretching bioreactor. The mechanical device pulling and realising rhythmically avian myogenic cell culture system promoted muscle tissue like generation reproducing the natural architecture of skeletal muscle tissue with parallel aligned myofibres[34]. Therefore, the structural support using biomaterial as scaffold in a static environment

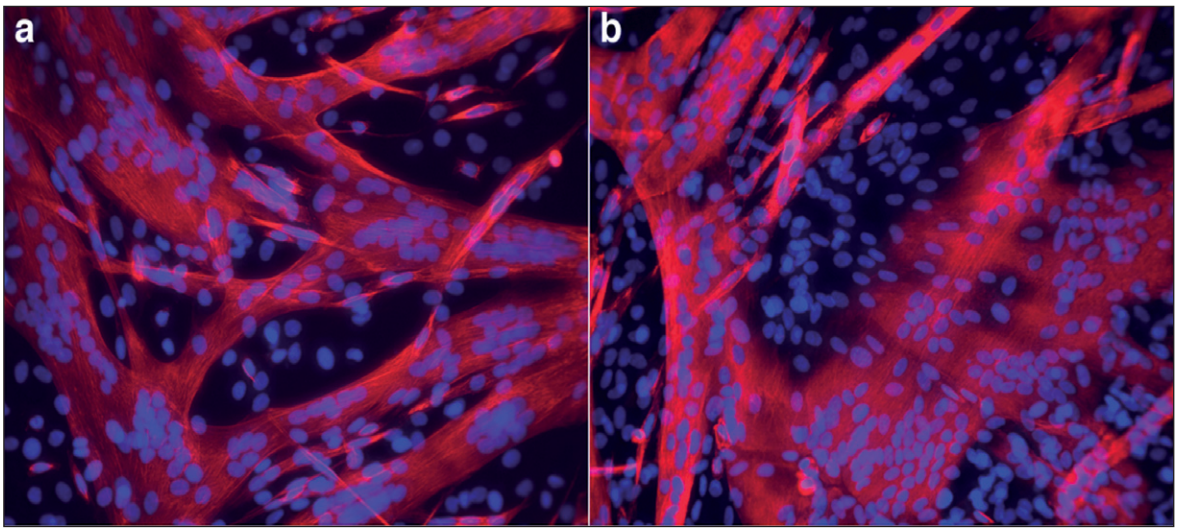

Figure 2. Human (a) and mouse (b) Mabs differentiating into skeletal muscle, revealed by immunofluorescence against Myosin Heavy Chain (red) and counterstained with 4, 6-diamidino-2-phenylindole (DAPI) (blue).

is not sufficient to reproduce the entire mechanical cues assuring artificial skeletal muscle tissue functionality. Recently a new strategy has been proposed to affect engineered myofibres alignment, developing an artificial muscle like tissue exploiting a bioreactor anatomical niche represented by the surface of a contracting muscle. Thus the dynamic environment offered by the mouse contracting muscle tibialis anterior (TA) as bioreactor anatomical niche, showed the possibility to obtain a supernumerary extra muscle engineered tissue presenting the characteristic parallel organization of the myofibres (Fig. 3) (Fuoco et al., Nature under review). Since displaying the remarkable capability to generate *in vivo* a healthy and functional artificial new muscle by skeletal muscle tissue engineering; representing a breakthrough in skeletal muscle regenerative medicine and than offering the possibility to replace or regenerate damaged, failing or missing muscle tissue portion. The development of an engineered muscular tissue should allow a noteworthy amelioration on muscle regeneration having a substrate to use in case of loss of tissue but above all to perform functional reconstruction in those cases were muscles are sacrificed because of robust damage or to permit a wide excision.

Conclusion

Tissue regeneration aims to create a biological substitute to damaged organs or tissues, in this context a fundamental role is played by the choice of the scaffold material. Ultimately the scaffolding system has the task of providing mechanical and bioactive signals to guarantee cell encapsulation. Bioactive signals are needed in order of facilitate cells to adhere to the matrix, survive, proliferate, form networks and eventually replace the dysfunctional tissue. Scaffold mechanical properties are fundamental in order to provide the right physical stimuli and mechanical support to the cell, which will thus be able to anchor and respond to the mechanical stimulation, eventually remodeling the matrix to accomplish tissue regeneration. On the other hand it is believed that matrix stiffness is the first hindrance leading the cells to elect their own fate, such as proliferation, differentiation and/or polarization. Since the scope to generate artificial skeletal muscle tissue, taking advantage of hydrogel and stem cell technology based on PEG-Fibrinogen new generation scaffold hydrogel and Mabs, open the possibility for quality life improvement in patients with several muscle affecting diseases, obtaining a complete and functional artificial muscle to pattern in different shape and size for different application

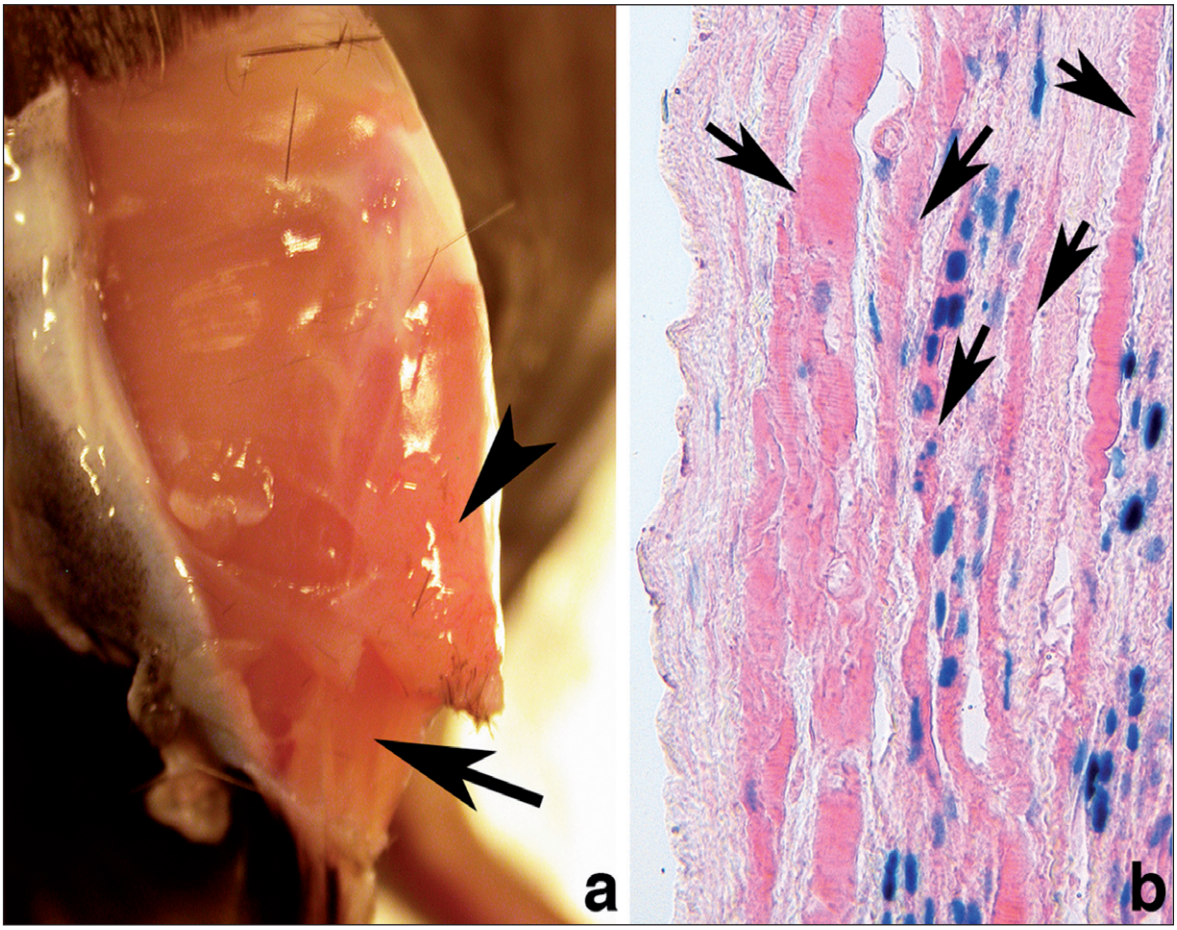

Figure 3. (a) Engineered skeletal muscle tissue (arrowhead) generated subcutaneously on the surface of tibialis anterior (arrow) from Mabs expressing LacZ embedded into 8mg/ml PEG-Fibrinogen. (b) X-Gal staining on histological section from (a) revealing artificial tissue muscular organization, highlighting implanted LacZ positive cells (blue) origin; section counterstained with Eosin.

likewise: groin hernia, sphincter incontinence and muscle ablation after tumor removal or traumatic iniury.

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