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Invited Review

Skeletal muscle tissue engineering

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Abstract

The reconstruction of skeletal muscle tissue either lost by traumatic injury or tumor ablation or functional damage due to myopathies is hampered by the lack of availability of functional substitution of this native tissue. Until now, only few alternatives exist to provide functional restoration of damaged muscle tissues. Loss of muscle mass and their function can surgically managed in part using a variety of muscle transplantation or transposition techniques. These techniques represent a limited degree of success in attempts to restore the normal functioning, however they are not perfect solutions. A new alternative approach to addresssing difficult tissue reconstruction is to engineer new tissues. Although those tissue engineering techniques attempting regeneration of human tissues and organs have recently entered into clinical practice, the engineering of skletal muscle tissue ist still a scientific challenge. This article reviews some of the recent findings resulting from tissue engineering science related to the attempt of creation and regeneration of functional skeletal muscle tissue.

Keywords: tissue engineering - skeletal muscle - cell culture - myoblasts - satellite cells - myopathies

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Introduction

Tissue engineering represents a scientific approach that attempts to mimic neoorganogenesis [1]. The creation of skeletal muscle tissue using tissue engineering methods holds promise for the treatment of a variety of muscle diseases, including skeletal myopathies such as muscular dystrophy or spinal muscular atrophy [2, 3]. In addition traumatic injury, aggressive tumor ablation and prolonged denervation are common clinical situations that often result in significant loss of muscle tissue requiring subsequent surgical reconstruction. Until now, only few alternatives exist to provide functional and aesthetic restoration of lost muscle tissues aside from transfer of muscle from local or distant sites. However, this technique of free tissue transfer, although a common practice, is associated with significant donor site morbidity causing functional loss and volume deficiency. Therefore plastic and reconstructive surgery also provides a wide field of indications, in which tissue engineered skeletal muscle could be a promising approach [4] [5]. Moreover regenerating or engineering new tissues may be a potential solution for the replacement of lost, damaged or failing tissues and organs in general [1, 6]. Even though these investigations have only recently been developed those techniques attempting regeneration of human tissues have recently entered into clinical practice in the case of tissues such as skin, bone or cartilage [7–12]. However the engineering of skletal muscle tissue remains still a challenge. This article reviews some of the recent findings resulting from „tissue engineering science“ related to the differentiation of muscle tissue cells within a three dimensional environment and discusses how tissue engineering techniques could be introduced to create and to regenerate skeletal muscle tissue.

Skeletal muscle tissue

Skeletal muscles are composed of bundles of highly oriented and dense muscle fibers, each a multinucleated cell derived from myoblasts. The muscle fibers in native skeletal muscle are closely packed together in an extracellular three-dimensional matrix to form an organized tissue with high cell density and cellular orientation to generate longitudinal contraction. After muscle injuries, myofibers become necrotic and are removed by macrophages [13]. A specialized myoblast sub-population called satellite cells scattered below the basal lamina of myofibers are capable of regeneration [14]. The incidence of satellite cells in skeletal muscle is very low (1%–5%) and depends on age and musclefiber composition [15]. These cells remain in a quiescent and undifferentiated state and can enter the mitotic circle in response to specific local factors [16]. This induces proliferation and fusion of myoblasts to form multinucleated and elongated myotubes, which self-assemble to form a more organized structure, namely muscle fiber [14]. Besides satellite cells migrate and proliferate in the injured area and can form a connective tissue network (muscle fibrosis). This process is called “scar tissue formation“ and leads to a loss of functionality [16, 17].

Engineering skeletal muscle tissue

The engineering of muscle tissue *in vitro* holds promise for the treatment of skeletal muscle defects as an alternative to host muscle transfer [1, 3, 4, 18, 19]. Skeletal muscle tissue engineering depends on the regenerative properties of the satellite cells and their potential for proliferation and differentiation, since these primary skeletal muscle cells can be harvested from adult muscle and successfully grown *in vitro* [15, 20]. Important requirements of engineering functional skeletal muscle are a parallel alignment of myofibrils with myosin/actin filaments, intracelluar calcium-strorage and acetylcholin receptors, which are needed for creating direct forces and functional use. Besides the neotissue must be biocompatible, needs to be vascularised and finally needs to be innervated [5, 21]. In order to obtain large volumes of tissue engineered skeletal muscle, myoblast cell cultures need to be expanded to a great extend. However, with extending passaging of primary cells, the differentiation process is difficult to induce. To overcome these problems in many studies focussing on *in vitro* generation of muscular tissue cell lines such as C2C12 which is an established cell line of satellite cells from skeletal muscle of C3H mouse, were used [22–25]. However this approach seems to have dis-

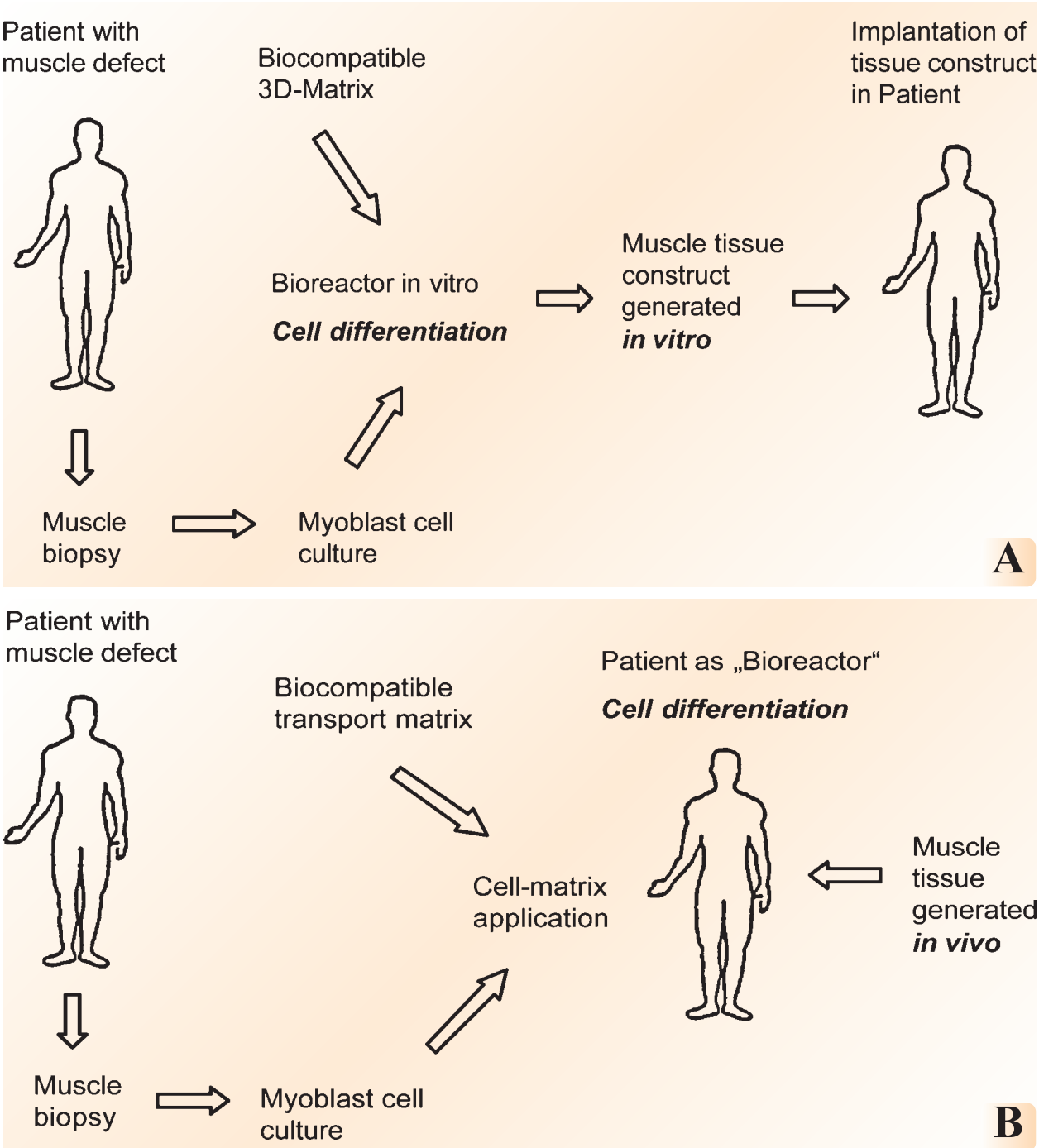

Fig. 1 **A**. The concept of *in vitro* - tissue engineering approach. **B**. The concept of *in vivo* - tissue engineering approach.

advantages, because established cell lines approximate myogenesis less closely than primary myoblasts. Therefore primary cultures derived from satellite cells from myofibers grown *in vitro* are the preferred source of myoblasts because they recapitulate muscle development more precisely than immortal myogenic cell lines [5, 26, 27]. Studies on the replacement of muscular tissues using tissue engineering methods have only recently started and many investigators have focussed on the creation of functional muscle tissues *in vitro* [23, 28, 29]. However, few studies on differentiation of myoblasts within a 3-D matrix have been reported and living tissue substitutes for functional skeletal

muscle replacement have not yet been developed successfully. To achieve this goal it is necessary to investigate novel approaches for culturing functional, differentiated skeletal muscle tissue *in vitro* using primary myoblasts for autologous transplantation. An understanding of the molecular control mechanisms of muscle development and differentiation is therefore an important prerequisite. It is becoming apparent that the circumstances related to the growth of cells in three-dimensional scaffolds *in vitro* are revealing aspects of the phenotypes of cells and insights into cell behaviors that would have otherwise escaped view. In this regard skeletal muscle tissue engineering has become a general model for understanding many fundamental principles of development, including mechanisms for cell differentiation, morphogenesis and the antagonism between growth and differentiation [30, 31]. Many of the steps involved in the development of myoblasts from mesodermal precursor cells and their subsequent differentiation into multinucleate muscle fibers correspond to the expression of specific transcription factors and signalling systems controlling each developmetal event. The factors which play a major role in controlling the events leading to skeletal muscle development are MyoD, myf-5, myogenin and myf-6/MRF4/herculin, a family of myogenic basic helix-loop-helix transcription factors [32-34]. The proper spatial and temporal expression of these transcription factors is critical for successful myogenesis [32].

Induction of diffentiation

There are several attempts to induce fusion of myoblasts to myotubes *in vitro*, imitating the *in vivo* conditions during myogenesis. Critical issues include an understanding of the effects of mechanical and electrical stimulation on cultured myoblasts and the role of the extracellular matrix (ECM) in the migration, proliferation and differentiation of the cells [5, 21, 24]. Mechanical stimulation is one important factor during myogenesis which influences gene regulation, endogeneous protein expression, protein acculmulation and metabolic activity. [35-37]. Both passive and active mechanical forces play an important role in the transition of skeletal muscle from the embryonic to the mature state [36]. Directed mechanical tension is important to organize myoblasts into functional aligned myotubes and provides a stimulus for the expression of mature isoforms of myofibrillar proteins [38]. Besides it has been shown that mechanical forces also have important impact in mature skeletal muscle on myofiber diameter, cell number and myofiber composition [36, 39]. Based on this knowledge Powell et al reported the development of three dimensional human skeletal muscle tissue using a 3D-scaffold based on collagen and Matrigel® by mechanical stimulation [37]. This model allows to determine the cellular effects of mechanical stimulation, particulary those associated with cytoskeletal rearrangements. In order to improve the ratio of muscle fibers and extracelluar matrix Powell and coworkers created a mechanical cell stimulator that is able to stretch and relax the cell cultures *in vitro*. A force transducer was able to measure passive forces and viscoelastic properties. The mechanical stimulation improved the structure of the engineered skeletal muscle by increasing the mean myofiber diameter and the elasticity. However, the tissue that resulted on these studies is still not an appropriate substitute for functional implantation *in vivo*. Other studies focussing on the *in vitro* creation of skeletal muscle showed also a quite different morphologic and functional appearance without mechanical stimulation in comparison to native skeletal muscle. The extracellular matrix content was significantly higher, myofiber density was low and maturation was incomplete without stimulation [21, 28]. To summarize, the cellular effects of applied mechanical force seem to be an important aspect to the *in vitro* development of differentiated functional muscle tissue. Another approach of developing a higher differentiated and more functional skeletal muscle tissue is the application of electrical stimulation [28, 40–42]. This mimics the nerve stimulation during myogenensis and during regeneration of injured skeletal muscle. Induced contractile activity was shown to promote the differentiation of myotubes and results on directly stimulated, aneural myotubes indicated that neurally transmitted contractile activity may be an important factor in modulating phenotype expression of secondary myotubes. Moreover chronic electrical stimulation of primary myoblasts was shown to change and modulate myosin heavy chain expression depending on different impulse patterns

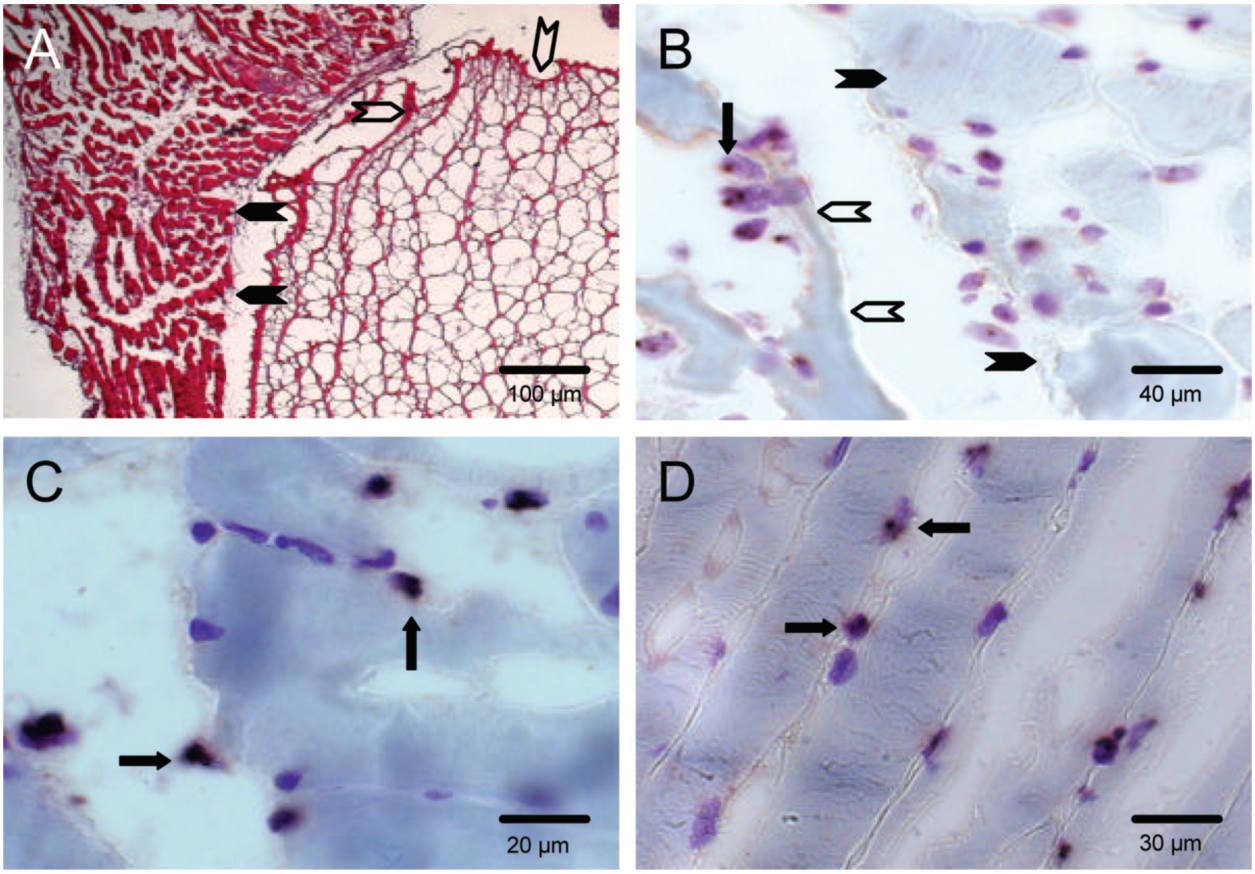

Fig. 2 Y chromosome in situ hybridization of transplanted myoblasts. Positive nuclei of syngeneic male donor rats indicated by arrows.
A + B) 2 days after transplantation: fibrin matrix still present (host muscle fibers of gracilis muscle indicated by arrow heads, fibrin matrix is indicated by hollow arrow heads);
C) 14 days after transplantation: male myoblasts fused with female host myofibers of the gracilis muscle;
D) 50 days after transplantation: male myoblasts fused with female host myofibers of the gracilis muscle, resulting in several mosaic multinuclear muscle fibers, consisting of cells of male and female origin.

[40, 41]. Another study conducted by Dennis et al compared the excitability and contractility of three-dimensional skeletal muscle constructs, engineered from C2C12 myoblast and 10T1/2 fibroblast cell lines, primary muscle cultures from adult C3H mice, and neonatal and adult rats. Created myooids were 12 mm long, with diameters of 0.1-1 mm, and were excitable by transverse electrical stimulation, and contracted to produce force. After approximately 30 days in culture, the specific force generated by the myooids was 2-8% of that generated by skeletal muscles of control adult rodents [42]. Besides it has been shown that electrical stimulation of murine skeletal muscle cells enhances the expression of the angiogenetic factor VEGF and *in vivo* studies revealed that after 5 days of stimulation blood flow increased significantly [43]. This is an interesting finding for the development of a tissue engineering approach with regard to provide functional muscle tissue in a clinical scenario, since vascularisation of tissue constructs is therefore an important prerequisite. The composition of the extracellular matrix (ECM) plays also a key role in the alignment and differentiation of myoblasts. The ECM should provide a framework for cell adhesion and tissue growth, which includes cell proliferation and differentiation. The matrix must be biocompatible and should be bioresorbable [22, 24, 44, 45]. The matrices used in tissue engineering are divided into synthetic and biologically-derived biomaterials [46-48]. Saxena and collegues used polyglycolic acid (PGA) meshes seeded with myoblasts to transplant

cells *in vivo*. After 6 weeks a vascularized muscle like tissue could be noticed [49]. Other investigators established *in vitro* cell cultures cultivating muscle cells on Matrigel® [37, 50]. However Matrigel®, an extract from the Engelbreth-Holm-Swarm mouse sarcoma, contains various extracellular matrix proteins and growth factors in undefined concentrations. Besides it has the ability to change gene expression in cells [50]. This matrix has been used in combination with collagen as a 3 dimensional scaffold, but in our opinion due to its origin its utility is limited for experimental models and not appropriate for a clinical setting. Various other biomaterials including collagens and alginate hydrogels have been used to replace the ECM *in vitro*, either to enhance the attachment of myoblasts or to alter their growth [24, 29, 44, 46, 51]. However, these matrices are not biodegradable and some are potentially immunogenic [46, 47]. Dennis and Kosnik introduced a way of designing a three dimensional skeletal muscle without using a primary matrix to provide a structural growth scaffold [28, 42]. They developed skeletal muscle tissue constructs by coculture of myoblasts and fibroblasts. The fibroblasts formed an extracellular matrix which was surrounding the myotubes. Furthermore Borschel and co-workers recently produced engineered skeletal muscle using an acellularized mouse extensor digitorum longus muscle as a scaffold. C2C12 myoblasts were injected into the acellular muscle matrix and isometric contractile force testing of the constructs demonstrated production of longitudinal contractile force on electrical stimulation. Electron microscopy studies demonstrated recapitulation of some of the normal histologic features of developing skeletal muscle [52]. Since *in vitro* skeletal muscle tissue engineering involves culturing isolated primary myoblasts in an environment leading to the formation of a three-dimensional tissue construct, ideal matrices for such an approach should provide a high surface area for cell-matix interactions, sufficient space for extracellular matrix generation, and a minimal diffusion barrier during *in vitro* culture [53, 54]. Moreover, the matrix should be resorbable once it has served its purpose of providing a primary structure for the developing tissue [46]. In many studies fibrin has been shown to provide these basic conditions as an ideal cell culture matrix: it is biocompatible and biodegradable [31, 54, 55] and consists of key-proteins of the ECM. Since cellular growth and differentiation depend on a structured environment which the cells need to interact with, fibrin supports the migration capacity of cells, allows the diffusion of growth and nutrition factors and has a high affinity to bind to biological surfaces [56]. These properties are basic features of the hybrid skeletal muscle tissues which were developed by the authors: the incorporation of the myoblasts into a three-dimensional fibrin matrix [31]. In our studies a high proliferation rate of the primary myoblasts within the fibrin-matrix could be observed, indicating the feasibility of fibrin as 3-D matrix. In order to evaluate the designed skeletal muscle tissue, we focused on myogenic transcription factors like MyoD and myogenin, and development of the acetylcholin receptor. We could show that myoblasts can proliferate and fuse to myotubes in the three dimensional fibrin matrix. Thus in our culture model the fibrin 3-D matrix was obviously the structural basis and the promotor of cell survival, proliferation and cell differentiation. Moreover, we established a co-culture system with neuronal slices of the spinal cord and myoblast in a three dimensional fibrin matrix. The results of our study confirmed that obviously the presence of a three-dimensional environment and of neuronal tissue is required for the understanding of the control mechanisms which are essential for *in vitro* regenerating of highly differentiated skeletal muscle tissue [31]. However, aside from the induction of the differentiation of muscle cells, issues such as vascularization and innervation of *in vitro* generated muscle tissue constructs have to be more addressed, to provide functional muscle tissue in large volumes for clinical applications [5, 57].

Tissue engineering *in vivo - in vitro*

Therapeutic treatments for acquired and inherited skeletal myopathies and loss of functional muscle tissue require the ability to either the implantion of differentiated muscle tissue constructs or the injection of muscle-precursor cells into sites of dysfunction or tissue deficiency for subsequent formation of new muscle tissue [4] [58]. The implantation of engineered myoblasts has been utilized as a poten-

tial therapy for genetic muscle diseases such as Duchenne´s muscular dystrophy or for the repair of damaged myocardial tissues (Myoblast Transfer Therapy) [59] [60] [58]. The rationale behind this strategy is that the implantation of large numbers of myoblasts result in the fusion of these cells to the affected tissue, thereby improving the functional status of the muscle [3]. Early results have demonstrated that exogenously introduced myoblasts are incorporated into local target sites and fuse with existing myofibers [61]. However, this techniques, although shown to improve the architecture and function of muscle as the myoblasts incorporate and differentiate, is limited by the large numbers of cells required and sites that must be injected [62] [53]. Nevertheless implanted and *in vitro* transfected myoblasts might serve as vehicles for the delivery of other recombinant proteins such as angiogenic factors and growth factors as insulin like growth factor 1, erythropoeitin and VEGF [21, 63–65]. This myoblast-targeted gene therapy with the potential for local production and release of needed therapeutic proteins holds promise for the treatment of several myopathies as well as other diseases [65–67], lacking important functional proteins [64, 68].

In contrast to these myoblast transfer strategies, other researchers in the field of muscle tissue engineering are more focussing on *in vitro* differentiation and maturation of satellite cells harvested from adult skeletal muscle. This approach of *in vitro* development of bioartificial muscle could be an alternative source for treating muscular disorders as described above [23] [4]. These attempts reflect the two general approaches to engineer skeletal muscle tissue. One approach uses *in vitro*-designed and pre-fabricated artificial muscle tissue equivalents to reimplant the neo-tissue after differentiation has taken place (*in vitro* tissue engineering) (Fig. 1A). The second approach uses the application of isolated satellite cells, after expansion of cells *in vitro* using an appropriate transport matrix, which allows differentiation into myotubes *in vivo* to occur (*in vivo* tissue engineering) (Fig.1B). Future developments and the decision regarding which approach is more promising depend on the elucidation of the relationships among cell growth and and differentiation, the cell integration capacity in the host in *in vivo* experiments and the capability to induce vascularisation of tissue equivalents *in vitro*.

Detection of transplanted cells

It has been shown that satellite cells can be successfully isolated and expanded *in vitro* from fetal and adult muscle biopsy [20]. In order to create a tissue *in vitro* that can be reimplanted *in vivo*, donor cells must be autologous or at least non-immonogenic [6]. Therefore, primary satellite cells or other stem cells, which can differentiate into skeletal muscle cells, are the ideal source for transplantation approaches in muscle tissue engineering. One major problem that cell transplantation studies imitating autologous transplantation are facing at the moment is the ability of detecting transplanted cells after integration in the host. Therefore we developed an approach to create functional skeletal muscle tissue *in vivo* using the transplantation of primary myoblasts precultivated within a three-dimensional (3D) fibrin matrix and to determine the fate of the transplanted cells using the Y chromosome detection technique in a syngeneic rat animal model [69]. 3D myoblast cultures were established derived from male donor rats and after 7 days of cultivation we performed an orthotopic transplantation of 3D-cell constructs into a created muscle defect within the gracilis muscle of syngeneic female rats (Fig 2A). These transplanted cells showed a poitive desmin immunostaining, supporting the assumption of the cells retaining their myogenic phenotype and Y chromosome in situ hybridization indicated the survival and integration of transplanted male myoblasts into the female recipient animal (Fig. 2B). After 50 days male donor cells still could be tracked, now as Y chromosome positive nuclei incorporated into host tissue, resulting in several mosaic multinuclear muscle fibers, consisting of cells of male and female origin (Fig 2C,D). These fibers were restricted to the area around the implantation site of the 3D-cell construct, indicating that transplanting myoblasts in a syngeneic rat model results in regeneration of skeletal muscle fibers by incorporation of myoblasts into host myofibers (Fig. 2C,D). Thus this approach to tissue engineering, transplantation of a small number of cells and growing the tissue *in vivo*, may bypass current difficulties in the *in vitro* engineering of large tissue masses for subsequent transplantation that are related to the lack of sufficient vascularization. Moreover this approach may be ideal for the utilization of small numbers of stem

cells in the regeneration of skeletal muscle tissue [70]. In summary, further progress in stem-cell technology [71], as well as discovery of conditions responsible for the control-mechanisms of proliferation and differentiation of adult satellite cells, combined with suitable techniques of vascularization might allow for the production of autologous artificial skeletal muscle-like tissue that is capable of correcting muscle injury and restoring impaired muscle function.

Conclusions

Tissue engineering and regenerative medicine is an exiting interdisciplinary field, which applies to the principles of engineering and biology to the development of viable substitutes that restore the function of damaged tissues and organs. Until now the efforts of tissue engineering science have provided new knowledge that have deepen our understanding of the phenotype and behavior of the skeletal muscle cells. This knowledge may in turn enable promising advances in musculoskeletal tissue engineering. Growing new tissues (neoorganogenesis) is a complex process that requires the teamwork of developmental and cellulare molecular biologists, engineers, material scientistes, and physicians. As the techniques of tissue engineering become more sophisticated, the usefulness of these methods for supporting the possibilities of reconstructive surgery will hopefully become reality.

References

1. **Mooney D.J., Mikos A.G.**, Growing new organs, *Sci. Am.,* **280**: 60-65, 1999
2. **Law P.K., Goodwin T.G., Fang Q., Deering M.B., Duggirala V., Larkin C., Florendo J.A., Kirby D.S., Li H.J., Chen M. et al.**, Cell transplantation as an experimental treatment for Duchenne muscular dystrophy, *Cell Transplant.,* **2**: 485-505, 1993
3. **Guettier-Sigrist S., Coupin G., Braun S., Warter J.M., Poindron P.**, Muscle could be the therapeutic target in SMA treatment, *J. Neurosci. Res.,* **53**: 663-669,1998
4. **DiEdwardo C.A., Petrosko P., Acarturk T.O., DiMilla P.A., LaFramboise W.A., Johnson P.C.**, Muscle tissue engineering, *Clin. Plast. Surg.,* **26**: 647-656, 1999
5. **Bach A.D., Stem-Straeter J., Beier J.P., Bannasch H., Stark G.B.**, Engineering of muscle tissue, *Clin. Plast. Surg.,* **30**: 589-599, 2003
6. **Bonassar L.J., Vacanti C.A.,** Tissue engineering: the first decade and beyond, *J. Cell Biochem. Suppl.,* **30-31**: 297-303, 1998
7. **Vangsness C.T. Jr., Kurzweil P.R., Lieberman J.R.**, Restoring articular cartilage in the knee, *Am. J. Orthop.,* **33**: 29-34, 2004
8. **Oakes B.W.**, Orthopaedic tissue engineering: from laboratory to the clinic, *Med. J. Aust.,* **180**: S35-S38, 2004
9. **Kopp J., Jeschke M.G., Bach A.D., Kneser U., Horch R. E.**, Applied tissue engineering in the closure of severe burns and chronic wounds using cultured human autologous keratinocytes in a natural fibrin matrix, *Cell Tissue Bank,* **5**: 81-87, 2004
10. **Kojima K., Bonassar L.J., Ignotz R.A., Syed K., Cortiella J., Vacanti C.A.**, Comparison of tracheal and nasal chondrocytes for tissue engineering of the trachea, *Ann. Thorac. Surg.,* **76**: 1884-1888, 2003
11. **Chang S. C., Tobias G., Roy A.K., Vacanti C.A. , Bonassar L.J.**, Tissue engineering of autologous cartilage for craniofacial reconstruction by injection molding, *Plast. Reconstr. Surg.,* **112**: 793-799, 2003
12. **Horch R.E., Debus M., Wagner G., Stark G. B.**, Cultured human keratinocytes on type I collagen membranes to reconstitute the epidermis, *Tissue Eng.,* **6**: 53-67, 2000
13. **Hurme T., Kalimo H., Lehto M., Jarvinen M.**, Healing of skeletal muscle injury: an ultrastructural and immunohistochemical study, *Med. Sci. Sports Exerc.,* **23**: 801-810, 1991
14. **Campion D.R.**, The muscle satellite cell: a review, *Int. Rev. Cytol.,* **87**: 225-251, 1984
15. **Allen R.E., Temm-Grove C.J., Sheehan S.M., Rice G.**, Skeletal muscle satellite cell cultures, *Methods Cell Biol.,* **52**: 155-176, 1997
16. **Hill M., Wernig A., Goldspink G.**, Muscle satellite (stem) cell activation during local tissue injury and repair, *J. Anat.,* **203**: 89-99, 2003
17. **Li Y., Huard J.**, Differentiation of muscle-derived cells into myofibroblasts in injured skeletal muscle, *Am. J. Pathol.,* **161**: 895-907, 2002
18. **Guettier-Sigrist S., Coupin G., Braun S., Rogovitz D., Courdier I., Warter J.M., Poindron P.**, On the possible role of muscle in the pathogenesis of spinal muscular atrophy, *Fundam. Clin. Pharmacol.,* **15**: 31-40, 2001
19. **Fauza D.O., Marler J.J., Koka R., Forse R.A., Mayer J. E., Vacanti J.P.**, Fetal tissue engineering: diaphragmatic replacement, *J. Pediatr. Surg.,* **36**: 146-151,2001
20. **Blau H.M., Webster C.**, Isolation and characterization of human muscle cells, *Proc. Natl. Acad. Sci. USA,* **78**: 5623-5627, 1981
21. **Vandenburgh H.H.**, Functional assessment and tissue design of skeletal muscle, *Ann. N.Y. Acad. Sci.,* **961**: 201-202, 2002
22. **Okano T., Satoh S., Oka T., Matsuda T.**, Tissue engineering of skeletal muscle. Highly dense, highly oriented hybrid muscular tissues biomimicking native tissues, *Asaio. J.,* **43**: M749-753, 1997

23. **Acarturk T.O., Peel M.M., Petrosko P., LaFramboise W., Johnson P. C., DiMilla P.A.,** Control of attachment, morphology, and proliferation of skeletal myoblasts on silanized glass, *J. Biomed. Mater. Res.,* **44**: 355-370, 1999
24. **Okano T., Matsuda T.**, Muscular tissue engineering: capillary-incorporated hybrid muscular tissues *in vivo* tissue culture, *Cell Transplant.,* **7**: 435-442, 1998
25. **Fuhrer C., Gautam M., Sugiyama J.E., Hall Z.W.**, Roles of rapsyn and agrin in interaction of postsynaptic proteins with acetylcholine receptors, *J. Neurosci.,* **19**: 6405-6416, 1999
26. **Marzaro M., Conconi M.T., Perin L., Giuliani S., Gamba P., De Coppi P., Perrino G.P., Parnigotto P.P., Nussdorfer G.G.**, Autologous satellite cell seeding improves *in vivo* biocompatibility of homologous muscle acellular matrix implants, *Int. J. Mol. Med.,* **10**: 177-182, 2002
27. **Blanco-Bose W.E., Yao C.C., Kramer R.H., Blau H.M.**, Purification of mouse primary myoblasts based on alpha 7 integrin expression, *Exp. Cell Res.,* **265**: 212-220, 2001
28. **Kosnik P.E., Faulkner J.A., Dennis R.G.**, Functional development of engineered skeletal muscle from adult and neonatal rats, *Tissue Eng.,* **7**: 573-584, 2001
29. **Neumann T., Hauschka S.D., Sanders J.E.**, Tissue engineering of skeletal muscle using polymer fiber arrays, *Tissue Eng.,* **9**: 995-1003, 2003
30. **Delfini M., Hirsinger E., Pourquie O., Duprez D.**, Delta 1-activated notch inhibits muscle differentiation without affecting Myf5 and Pax3 expression in chick limb myogenesis, *Development,* **127**: 5213-5224, 2000
31. **Bach A.D., Beier J.P., Stark G.B.**, Expression of Trisk 51, agrin and nicotinic-acetycholine receptor epsilon-subunit during muscle development in a novel three-dimensional muscle-neuronal co-culture system, *Cell Tissue Res.,* **314**: 263-274, 2003
32. **Weintraub H.**, The MyoD family and myogenesis: redundancy, networks, and thresholds, *Cell,* **75**: 1241-1244, 1993
33. **Molkentin J.D., Olson E.N.**, Combinatorial control of muscle development by basic helix-loop-helix and MADS-box transcription factors, *Proc. Natl. Acad. Sci. USA,* **93**: 9366-9373, 1996
34. **Molkentin J.D., Olson E.N.**, Defining the regulatory networks for muscle development, *Curr. Opin. Genet. Dev.,* **6**: 445-453, 1996
35. **Goldspink G.**, Gene expression in muscle in response to exercise, *J. Muscle Res. Cell. Motil.,* **24**: 121-126, 2003
36. **Goldspink G., Scutt A., Loughna P.T., Wells D.J., Jaenicke T., Gerlach G.F.**, Gene expression in skeletal muscle in response to stretch and force generation, *Am. J. Physiol.,* **262**: R356-R363, 1992
37. **Powell C.A., Smiley B.L., Mills J., Vandenburgh H.H.**, Mechanical stimulation improves tissue-engineered human skeletal muscle, *Am. J. Physiol. Cell Physiol.,* **283**: C1557-C1565, 2002
38. **Tatsumi R., Sheehan S.M., Iwasaki H., Hattori A., Allen R.E.**, Mechanical stretch induces activation of skeletal muscle satellite cells *in vitro*, *Exp. Cell Res.,* **267**: 107-114, 2001
39. **Noah E.M., Winkel R., Schramm U., Kuhnel W.**, Impact of innervation and exercise on muscle regeneration in neovascularized muscle grafts in rats, *Ann. Anat.,* **184**: 189-197, 2002
40. **Dusterhoft S., Pette D.**, Effects of electrically induced contractile activity on cultured embryonic chick breast muscle cells, *Differentiation,* **44**: 178-184, 1990
41. **Wehrle U., Dusterhoft S., Pette D.**, Effects of chronic electrical stimulation on myosin heavy chain expression in satellite cell cultures derived from rat muscles of different fiber-type composition, *Differentiation,* **58**: 37-46, 1994
42. **Dennis R.G., Kosnik P.E. 2nd, Gilbert M.E., Faulkner J.A.,** Excitability and contractility of skeletal muscle engineered from primary cultures and cell lines, *Am. J. Physiol. Cell. Physiol.,* **280**: C288-C295, 2001
43. **Kanno S., Oda N., Abe M., Saito S., Hori K., Handa Y., Tabayashi K., Sato Y.**, Establishment of a simple and practical procedure applicable to therapeutic angiogenesis, *Circulation,* **99**: 2682-2687, 1999
44. **Adams J.C., Watt F.M.**, Regulation of development and differentiation by the extracellular matrix, *Development,* **117**: 1183-1198, 1993
45. **Mulder M.M., Hitchcock R.W., Tresco P.A.,** Skeletal myogenesis on elastomeric substrates: implications for tissue engineering, *J. Biomater. Sci. Polym. Ed.,* **9**: 731-748, 1998
46. **Freed L.E., Vunjak-Novakovic G., Biron R.J., Eagles D. B., Lesnoy D.C., Barlow S.K., Langer R.**, Biodegradable polymer scaffolds for tissue engineering, *Biotechnology (N Y),* **12**: 689-93,1994
47. **Grande D.A., Halberstadt C., Naughton G., Schwartz R., Manji R.**, Evaluation of matrix scaffolds for tissue engineering of articular cartilage grafts, *J. Biomed. Mater. Res,* **34**: 211-220, 1997
48. **Cronin E.M., Thurmond F.A., Bassel-Duby R., Williams R.S., Wright W. E., Nelson K. D., Garner H. R.**, Protein-coated poly(L-lactic acid) fibers provide a substrate for differentiation of human skeletal muscle cells, *J. Biomed. Mater. Res.,* **69A**: 373-381, 2004
49. **Saxena A.K., Marler J., Benvenuto M., Willital G.H., Vacanti J.P.**, Skeletal muscle tissue engineering using isolated myoblasts on synthetic biodegradable polymers: preliminary studies, *Tissue Eng.,* **5**: 525-532, 1999
50. **Dusterhoft S., Pette D.**, Satellite cells from slow rat muscle express slow myosin under appropriate culture conditions, *Differentiation,* **53**: 25-33, 1993
51. **Rowley J.A., Madlambayan G., Mooney D.J.**, Alginate hydrogels as synthetic extracellular matrix materials, *Biomaterials,* **20**: 45-53, 1999
52. **Borschel G. H., Dennis R.G., Kuzon W.M. Jr.**, Contractile skeletal muscle tissue-engineered on an acellular scaffold, *Plast Reconstr Surg,* **113**: 595-602; discussion 603-604, 2004
53. **Rando T.A., Blau H.M.**, Methods for myoblast transplantation, *Methods Cell Biol,* **52**: 261-272,1997
54. **Ye Q., Zund G., Benedikt P., Jockenhoevel S., Hoerstrup S.P., Sakyama S., Hubbell J.A., Turina M.**, Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering, *Eur. J. Cardiothorac. Surg.,* **17**: 587-591, 2000
55. **Bach A.D., Bannasch H., Galla T.J., Bittner K.M., Stark G.B.**, Fibrin glue as matrix for cultured autologous

urothelial cells in urethral reconstruction, *Tissue Eng,* **7**: 45-53, 2001
56. **Albelda S.M., Buck C.A.,** Integrins and other cell adhesion molecules, *FASEB J.,* **4**: 2868-2880, 1990
57. **Saxena A.K., Willital G.H., Vacanti J.P.**, Vascularized three-dimensional skeletal muscle tissue-engineering, *Biomed. Mater. Eng.,* **11**: 275-281, 2001
58. **Miller R.G., Sharma K.R., Pavlath G.K., Gussoni E., Mynhier M., Lanctot A.M., Greco C.M., Steinman L., Blau H.M.**, Myoblast implantation in Duchenne muscular dystrophy: the San Francisco study, *Muscle Nerve,* **20**: 469-478, 1997
59. **Menasche P.**, Myoblast transfer in heart failure, *Surg. Clin. North Am.,* **84**: 125-139, 2004
60. **Atkins B.Z., Lewis C.W., Kraus W.E., Hutcheson K.A., Glower D.D., Taylor D.A.,** Intracardiac transplantation of skeletal myoblasts yields two populations of striated cells *in situ*, *Ann. Thorac. Surg.,* **67**: 124-129, 1999
61. **Gussoni E., Pavlath G.K., Lanctot A.M., Sharma K.R., Miller R.G., Steinman L., Blau H.M.**, Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation, *Nature,* **356**: 435-438, 1992
62. **Rando T.A., Pavlath G.K., Blau H.M.**, The fate of myoblasts following transplantation into mature muscle, *Exp. Cell Res.,* **220**: 383-389, 1995
63. **Prelle K., Wobus A.M., Krebs O., Blum W.F., Wolf E.**, Overexpression of insulin-like growth factor-II in mouse embryonic stem cells promotes myogenic differentiation, *Biochem. Biophys. Res. Commun.,* **277**: 631-638, 2000
64. **Powell C., Shansky J., Del Tatto M., Forman D.E., Hennessey J., Sullivan K., Zielinski B.A., Vandenburgh H.H.**, Tissue-engineered human bioartificial muscles expressing a foreign recombinant protein for gene therapy, *Hum. Gene Ther.,* **10**: 565-577, 1999
65. **Barr E., Leiden J.M.**, Systemic delivery of recombinant proteins by genetically modified myoblasts, *Science,* **254**: 1507-1509, 1991
66. **el Oakley R.M., Brand N.J., Burton P.B., McMullen M.C., Adams G.B., Poznansky M.C., Barton P.J., Yacoub M.H.**, Efficiency of a high-titer retroviral vector for gene transfer into skeletal myoblasts, *J. Thorac. Cardiovasc. Surg.,* **115**: 1-8, 1998
67. **Law P.K., Goodwin T.G., Fang Q., Quinley T., Vastagh G., Hall T., Jackson T., Deering M. B., Duggirala V., Larkin C., Florendo J. A., Li L.M., Yoo T.J., Chase N., Neel M., Krahn T., Holcomb R.L.**, Human gene therapy with myoblast transfer, *Transplant. Proc.,* **29**: 2234-2237, 1997
68. **Deasy B.M., Huard J.**, Gene therapy and tissue engineering based on muscle-derived stem cells, *Curr. Opin. Mol. Ther.,* **4**: 382-389, 2002
69. **Beier J.P., Kneser U., Stern-Strater J., Stark G.B., Bach A.D.**, Y chromosome detection of three-dimensional tissue-engineered skeletal muscle constructs in a syngeneic rat animal model, *Cell Transplant,* **13**: 45-53, 2004
70. **Young H.E., Duplaa C., Romero-Ramos M., Chesselet M.F., Vourc'h P., Yost M.J., Ericson K., Terracio L., Asahara T., Masuda H., Tamura-Ninomiya S., Detmer K., Bray R.A., Steele T.A., Hixson D., el-Kalay M., Tobin B.W., Russ R.D., Horst M.N., Floyd J.A., Henson N.L., Hawkins K.C., Groom J., Parikh A., Blake L., Bland L.J., Thompson A.J., Kirincich A., Moreau C., Hudson J., Bowyer F.P. 3rd, Lin T.J., Black A.C. Jr.**, Adult reserve stem cells and their potential for tissue engineering, *Cell Biochem. Biophys.,* **40**: 1-80, 2004
71. **Korbling M., Estrov Z., Champlin R.**, Adult stem cells and tissue repair, *Bone Marrow Transplant,* **32 Suppl 1**: S23-S24, 2003