Editorial

Clinical translation of tissue-engineered constructs for severe leg injuries

Nicolas L'Heureux[1], Didier Letourneur[2,3]

[1]Cytograft Tissue Engineering, Inc., 3 Hamilton Landing, Suite 220, Novato, CA 94949, USA; [2]Inserm, U1148, Cardiovascular Bio-Engineering, X. Bichat Hospital, 75018 Paris, France; [3]Université Paris 13, Sorbonne Paris Cité, F-93430 Villetaneuse, France

Correspondence to: Didier Letourneur. Inserm, U1148, Cardiovascular Bio-Engineering, X. Bichat Hospital, 46 rue H. Huchard, F-75018, Paris, France. Email: didier.letourneur@inserm.fr.

Submitted May 08, 2015. Accepted for publication May 13, 2015.
doi: 10.3978/j.issn.2305-5839.2015.05.03
View this article at: http://dx.doi.org/10.3978/j.issn.2305-5839.2015.05.03

An important component of critical traumatic injuries is severe limb damage. Bone repair, vascular supply integrity and wounds closure are immediate concerns but the prognosis for long-term functional recovery is largely conditioned by inadequate soft tissue regeneration, and in particular skeletal muscles (1). Skeletal muscles account for more than 40% of the body's mass and are central element of limbs. Among muscle damage, those accompanied by significant loss of tissue volume lead to important functional problems. Loss of muscle tissue can occur at the time of injury itself or be the result of diseases or surgical debridement of necrotic or damaged areas. Extensive muscle tissue destruction leads to a painful and disabling condition termed volumetric muscle loss (VML) that impairs normal muscular functions. Though muscles can often recover functionality through exercise-induced hyperplasia and satellite (stem) cells activation, regeneration does not occur in VML because the damage is too extensive.

Muscle tissue loss results in functional deficits that require costly and complex care from multiple specialties. Despite intense physiotherapy regimens, functional recovery can often be disappointing (1). Surgical options have been developed to correct these deficits. They rely on transplantation techniques of muscle flaps alone or combined with surrounding tissues (skin, fascia and muscle). These surgical approaches are dependent on the availability of a healthy donor site, compatible with the lesion, and are associated with a significant morbidity at the harvested site. As a result, there has been a growing interest in alternatives that could be delivered by regenerative medicine approaches, in particular the replacement of bulk tissue with biocompatible materials (1,2). If the use of biomaterials has already entered clinical practice for numerous orthopedic applications (bone, ligament, cartilage), there is currently no therapeutic solution for skeletal muscle regenerations.

In a study by a team led by Stephen Badylak from the University of Pittsburgh, Sicari and colleagues propose a technique of tissue engineering published in *Science Translational Medicine* (3) to induce muscle regeneration in VML patients using xenogenic extracellular matrix (ECM).

What is tissue engineering?

If you ask this question to scientists, engineers or clinicians, you might have a variety of answers. The term "tissue-engineering" was originally coined by YC Fung to refer to an engineering approach to the analysis of native tissue, but it was rapidly used to describe the on-going efforts to produce living tissues from cultured human cells. Pioneers like Howard Green and Eugene Bell developed cutaneous constructs that were rapidly translated to clinical use in the 80's and remain to this day arguably the most successful tissue-engineered products. In the 90's, the use of synthetic polymers as a delivery scaffolds for cells became an ubiquitous strategy as the field was enthusiastically adopted by chemical engineers and materials scientists. Since then, the terms "cell-based therapy" and "regenerative medicine" have also surfaced to describe rapidly expanding, and often overlapping fields. Regardless of the terminology, only a few non-cutaneous products have managed to reach commercialization in the US or EU despite the massive research efforts over the last 20 years. This is in part due to a heavy-handed regulatory process and a hefty quality control burden that hinders progress and challenges the

economical feasibility of such products. In Europe, such products are known as advanced therapy medicinal product (ATMP) and are composed of cells or tissues that may be viable or non-viable. It may also contain additional substances, such as cellular products, biomolecules, biomaterials, chemical substances, or scaffolds. For example, ChondroCelect® is a suspension of cultured autologous cartilage cells for repairing single symptomatic cartilage defects of the femoral condyle of the knee in adults (based on a technology developed in the 90's). MACI®, is next generation cartilage repair product composed of autologous cartilage cells cultivated on a collagen membrane. A fibrin sealant, made from blood clotting proteins, is used to hold MACI® in place on the cartilage (4). More ambitious products such as tissue-engineered blood vessels and bladders have shown great promise in clinical trials but have struggled with development costs and regulatory issues (5,6). On the other hand, decellularized tissues such as the one used by Sicari *et al*., either from human or animal origins, follow a less complex regulatory pathway and quality control burden.

Implantation of a xenogenic non-muscle ECM for VML

The approach tested by Sicari *et al*. in five male patients, relies on the administration of a biological scaffold made of xenogeneic decellularized ECM from porcine urinary bladder, to stimulate and support regeneration. Native ECM is present in every tissue and is a mixture of proteins, glycosaminoglycans (GAGs), and growth factors that constitute the optimal microenvironment, or niche, for the differentiation, function and proliferation of specific cell types. The team of S. Badylak has been working extensively on the use of xenogeneic ECM for tissue repair for decades (7). The clinical use of these matrices is already widely established in human for applications such as rotator cuff repair, hernia repair, and the treatment of skin ulcers. Their success relies on both their role as physical support and their ability to create a microenvironment for the recruitment of cells capable of repopulating injured tissue. This trophic effect may be due to the presence of growth factors or angiogenic factors released during the degradation of the matrix by the host (8). The GAGs present in the ECM appear to play an important role in their ability to bind growth factors and induce cell signaling via receptors on the surface of recruited cells. This group has previously published the use of acellular ECM for treating VML in animal models (9,10).

The most widely used ECM material is prepared from the submucosa of small intestine (SIS) of the pig, a connective tissue layer rich in collagen and with low cell density. However, in VLM models, these matrices are not sufficiently resorbed by the host, give rise to a fibro-inflammatory response, and lead to a poorly organized tissue and a lack of muscle regeneration (9). On the contrary, non-crosslinked, but decellularized ECM matrices induce a macrophage polarization towards an anti-inflammatory phenotype (so-called "M2 phenotype") and the matrix is gradually resorbed to allow the development of muscle tissue (11). A preclinical study in a model of muscle damage in dogs has shown that this technique is transposable to large animals, however histology indicated modest results 6 months after transplantation, and islets of bone or cartilage tissue within the matrix near bones were observed (12).

In their most recent publication, the researchers from Badylak's team first conducted with decellularized porcine ECM, preclinical trials in mice that had sections of their quadriceps removed (3). Two weeks after implantation, they noticed perivascular stem cells near blood vessels in the region of the implant as well as in the progressively resorbed ECM. After 6 months, the regenerated muscle in treated mice was innervated and vascularized whereas there was no evidence of muscle repair in untreated mice. This approach was then translated to humans in 5 VML patients (including 3 military personnel) with a severely injured leg muscle (quadriceps or tibial), whose volumetric muscle tissue loss ranged from 58% to 90%, and who had undergone multiple surgeries and extensive physical therapy with limited improvement. Patients received a scaffold consisting of the ECM of porcine urinary bladder that was placed in the injured muscle close to the remaining living tissue. All patients followed a physical therapy program for post-surgical rehabilitation. At 6-8 months post-implantation, biopsies revealed the presence of muscle fibers of variable diameters, suggesting signs of regeneration. Blood vessels were found in the vicinity of the muscle cells. From a functional aspect, most patients (3 out of 5) showed a significant increase in force production (up to 220%) and improvements in tasks performance and balance.

This study represents an important step in the clinical translation for the field of tissue regeneration based on decellularized bioscaffolds. This clinical trial combined bioengineering with an intense physical therapy regiment to spur endogenous circulating stem cells that can target the site of injury and orchestrate a better tissue regeneration. The technique probably would work better after a recent

injury but researchers chose to start with old injuries where physical therapy alone could not trigger significant muscle regrowth. Though the number of patients examined in this study is very small, the results are very encouraging and call for further studies.

One key limitation of the repairs observed in animals or humans is that, if differentiated muscle tissue is demonstrated histologically, it is composed of isolated muscle fibers, or small islands of fibers, separated by connective tissue and without functional organization. While some functional improvements have been reported in various rodent models, they have been variable and, because of the variety of quantification methods used, difficult to interpret (13). Moreover, the simple bridging effect of the injured area for transmitting power independently of any contractile activity may contribute significantly to the reported functional improvements. In the clinical study, 2 patients out of 5 showed no functional improvement despite the presence of differentiated muscle cells. Although, these studies clearly show the potential of acellular ECM in the treatment of muscle damage with VML, these inconsistent outcomes highlight that further research is needed to understand the mechanism of the regeneration process.

Another possible choice of ECM from muscle for VML

In theory, acellular ECM produced from muscle could provide a biomaterial whose composition would be more favorable to the development of muscle tissue by providing the optimal three-dimensional niche for the development of myocytes. Interestingly, Badylak's team recently provided data demonstrating that ECM from different tissues can have different regenerative potentials (14). In this study, ECM from the brain and urinary bladder where show to have different molecular compositions and to differentially influence macrophage phenotype. Since significant numbers of macrophages and granulocytes are found at the implantation site, from the first weeks up to several months after implantation (15), it is not surprising to learn that these differences in phenotype resulted in different regenerative responses The approach of using ECM from muscle has been tested in murine models with lesions of the muscles of the abdominal wall, back or limbs (15-17). In these studies, the injury and the ECM implantation were performed during the same surgery. It is unlikely that the complex interplay between myogenesis and angiogenesis in this setting is representative of the regeneration process that occurs when the ECM is introduced after the acute injury phase. A better understanding of the mechanistic aspect of the regeneration in a more clinically relevant temporal setting will be needed to facilitate the clinical translation of this technology.

A more complex solution with the cellularization of ECM

Recently, the group of G Christ reported the effect of cell seeding on the regenerative potential of urinary bladder ECM in a murine VML model. The addition of a heterogeneous population of muscle-derived cells resulted in better functional recovery and a more organized histology, with abundant muscle fibers, when compared to ECM alone (18). It is tempting to hypothesize that combining cell therapy and muscle-derived ECM could lead to even better outcomes for VML but this approach has yet to be tested clinically. The general decellularization and recellularization-based approaches are also currently under development for generating several tissue-engineered organs such as lungs (19).

Conclusions

The use of a xenogeneic bioscaffold lacking cells, such as the one presented by Sicari and colleagues (3), obviates a series of technical, biological and regulatory problems typically associated with living tissue-engineered products: extraction and delivery of living cells to patients, cell survival and differentiation in the patient, reproducible cell production, high costs, etc. This scaffold, with its natural biological composition and tridimensional organization, appears to be sufficient to stimulate a significant regenerative response. These results support a growing trend toward exploring the transition of proven tissue-engineered constructs from cellularized towards non-living or from autologous towards allogeneic (20,21).

In contrast, numerous approaches under development rely on the convenience of synthetic scaffolds combined or not with cells. In light of the results from Badylak's group, an hybrid approach that combines a cheap and versatile polymer with a natural ECM coating could provide an economical product. Indeed, medical devices integrating the mechanical properties of synthetic materials with the tissue remodeling properties of naturally occurring materials such as ECM or other biological materials (collagen, glycosaminoglycan, elastin, fibrin, silk, alginate…) can

have significant clinical applications. It is interesting to note that S. Badylak himself recently published a study to assess the effects of an ECM coating on the long-term host tissue response to polypropylene mesh in a rodent model of abdominal muscle injury (22). They found that an ECM hydrogel coating mitigated the chronic inflammatory response to a polypropylene mesh. We have also observed better integration of a polypropylene mesh when embedded in a polysaccharide hydrogel (23). However, the presence of a permanent synthetic scaffold may not be desirable in applications such as in muscle reconstruction in VML.

Clinical translation and commercialization are obviously less challenging for simpler products. While they may have a more limited potential for delivering therapeutic breakthroughs, they may, paradoxically, have a greater potential to generate a commercial product and reach a larger patient population. However, despite the initial financial investment, it may make more sense in the long run, to first find a complex solution that works, and to subsequently simplify it, than to try many simple solutions that are likely to fail. This may be particularly true for the most complex applications that will likey require complex solutions. We are still at the early stages of clinical translation in the relatively recent field of tissue engineering.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Mertens JP, Sugg KB, Lee JD, et al. Engineering muscle constructs for the creation of functional engineered musculoskeletal tissue. Regen Med 2014;9:89-100.
2. Juhas M, Bursac N. Engineering skeletal muscle repair. Curr Opin Biotechnol 2013;24:880-6.
3. Sicari BM, Rubin JP, Dearth CL, et al. An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. Sci Transl Med 2014;6:234ra58.
4. Ram-Liebig G, Bednarz J, Stuerzebecher B, et al. Regulatory challenges for autologous tissue engineered products on their way from bench to bedside in Europe. Adv Drug Deliv Rev 2015;82-83C:181-191.
5. Atala A, Bauer SB, Soker S, et al. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 2006;367:1241-6.
6. McAllister TN, Maruszewski M, Garrido SA, et al. Effectiveness of haemodialysis access with an autologous tissue-engineered vascular graft: a multicentre cohort study. Lancet 2009;373:1440-6.
7. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: Structure and function. Acta Biomater 2009;5:1-13.
8. Teodori L, Costa A, Marzio R, et al. Native extracellular matrix: a new scaffolding platform for repair of damaged muscle. Front Physiol 2014;5:218.
9. Sicari BM, Agrawal V, Siu BF, et al. A murine model of volumetric muscle loss and a regenerative medicine approach for tissue replacement. Tissue Eng Part A 2012;18:1941-8.
10. Valentin JE, Stewart-Akers AM, Gilbert TW, et al. Macrophage participation in the degradation and remodeling of extracellular matrix scaffolds. Tissue Eng Part A 2009;15:1687-94.
11. Brown BN, Sicari BM, Badylak SF. Rethinking regenerative medicine: a macrophage-centered approach. Front Immunol 2014;5:510.
12. Turner NJ, Badylak JS, Weber DJ, et al. Biologic scaffold remodeling in a dog model of complex musculoskeletal injury. J Surg Res 2012;176:490-502.
13. Valentin JE, Turner NJ, Gilbert TW, et al. Functional skeletal muscle formation with a biologic scaffold. Biomaterials 2010;31:7475-84.
14. Meng FW, Slivka PF, Dearth CL, et al. Solubilized extracellular matrix from brain and urinary bladder elicits distinct functional and phenotypic responses in macrophages. Biomaterials 2015;46:131-40.
15. Garg K, Ward CL, Rathbone CR, et al. Transplantation of devitalized muscle scaffolds is insufficient for appreciable de novo muscle fiber regeneration after volumetric muscle loss injury. Cell Tissue Res 2014;358:857-73.
16. Chen XK, Walters TJ. Muscle-derived decellularised extracellular matrix improves functional recovery in a rat latissimus dorsi muscle defect model. J Plast Reconstr Aesthet Surg 2013;66:1750-8.
17. Corona BT, Wu X, Ward CL, et al. The promotion of a functional fibrosis in skeletal muscle with volumetric muscle loss injury following the transplantation of muscle-ECM. Biomaterials 2013;34:3324-35.
18. Corona BT, Ward CL, Baker HB, et al. Implantation of in vitro tissue engineered muscle repair constructs and bladder acellular matrices partially restore in vivo skeletal muscle function in a rat model of volumetric muscle loss injury. Tissue Eng Part A 2014;20:705-15.
19. Tsuchiya T, Sivarapatna A, Rocco K, et al. Future

prospects for tissue engineered lung transplantation: decellularization and recellularization-based whole lung regeneration. Organogenesis 2014;10:196-207.
20. Patterson JT, Gilliland T, Maxfield MW, et al. Tissue-engineered vascular grafts for use in the treatment of congenital heart disease: from the bench to the clinic and back again. Regen Med 2012;7:409-19.
21. Wystrychowski W, McAllister TN, Zagalski K, et al. First human use of an allogeneic tissue-engineered vascular graft for hemodialysis access. J Vasc Surg 2014;60:1353-7.
22. Faulk DM, Londono R, Wolf MT, et al. ECM hydrogel coating mitigates the chronic inflammatory response to polypropylene mesh. Biomaterials 2014;35:8585-95.
23. Abed A, Deval B, Assoul N, et al. A biocompatible polysaccharide hydrogel-embedded polypropylene mesh for enhanced tissue integration in rats. Tissue Eng Part A 2008;14:519-27.

Cite this article as: L'Heureux N, Letourneur D. Clinical translation of tissue-engineered constructs for severe leg injuries. Ann Transl Med 2015;3(10):134. doi: 10.3978/j.issn.2305-5839.2015.05.03

