



Review article

Regenerative medicine: Clinical applications and future perspectives



Federica Colombo^a, Gianluca Sampogna^a, Giovanni Coccozza^a,
Salman Yousuf Guraya^b, Antonello Forgione^{a,*}

^a *Advanced International Mini-invasive Surgery – AIMS Academy, Milan, Italy*

^b *Department of Surgery and Consultant Colorectal Surgeon, College of Medicine, Taibah University, Almadinah Almunawwarah, KSA*

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ABSTRACT

After many years of basic research, regenerative medicine (RM) is now beginning to represent a valuable tool to cure several clinical conditions in both acute injuries and chronic diseases. The aim of this study is to update readers on current clinical applications of some selected organs and pathologies which may benefit from RM. An extensive literature research was performed using PubMed, Google and specialized journals. RM has achieved great successes, but there are still several challenges to tackle before it could be used on a daily basis in clinical practice. The crucial point of this revolution is represented by the appropriate and valid translation from bench to bedside.

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1. Introduction

Regenerative medicine (RM), from tissue engineering (TE) to cell therapy, offers valuable treatment options, which are rarely considered in daily clinical settings [1]. Doctors, surgeons, clinicians and, in general, health-care policies are not prone to substitute conventional approaches with innovative therapies without extended and thorough experiments. A major concern that limits the spreading of RM is related to different challenges to solve definitively, e.g. live tissue handling and manufacturing [2].

In this article, the authors show how RM principles may be applied, presenting some examples of artificial tissues and organs, which could be considered as a valid purpose in daily clinical settings [3,4]. At present, RM is not the gold standard treatment for all the most common diseases. On

the one hand, clinicians are not well informed about the current applications; on the other hand, there are not many studies confirming safety, efficiency and effectiveness.

Therefore, we provide a brief reflection on the current pre-clinical and clinical applications with their advantages on traditional approaches and the issues that still need to be solved before a massive spread. Further research is mandatory to offer the definitive cure for both acute injuries and chronic diseases, but, first of all, clinicians should be informed of these possibilities and, later, form multidisciplinary team to adopt and deliver these new treatments.

2. Materials and methods

An exhaustive research of the latest clinical applications of RM was conducted using appropriate index terms (i.e. name of the organ AND tissue engineering or regenerative medicine AND clinical applications). These words were typed in scientific databases and search engine, like PubMed, Scopus, ScienceDirect and Google Scholar, to

* Corresponding author. Tel.: +39 0264447605.

E-mail address: antonello.forgione@aimsacademy.org (A. Forgione).

obtain articles from specialized journals (e.g. The Lancet, The Journal of Urology, Future Medicine Journals or the open access scientific journal PLoS ONE). We selected original articles written in English and published within the last two decades. Actually, most papers dated back to last five years; the older ones are related to key steps in the delivering of latest solutions.

Images were taken from PLoS ONE, as they could be freely used and inserted into the present review [5].

3. Specific organs and tissues

Scientists are developing all kinds of tissues and organs (from bladder to heart, from nerve to ovary etc); unfortunately, just some of them have been already implanted successfully in humans, while others are still in pre-clinical phase, or have been developed recently [6]. We selected and reported below some artificial tissues and organs, which have the potential to be implanted into humans on routine clinical settings.

3.1. Bladder

Urinary bladder is a hollow organ, made up of two functional layers: an inner epithelium and an outer smooth muscle layer. Therefore, its regeneration requires both these layers in order to be successful. The gold standard treatment for patients with pathologic bladders, which are not responsive to any drugs, is to substitute or augment the original bladder with bowel segments. However, the gastrointestinal (GI) tissue is not adequate to stay in contact with urine, as it is supposed to adsorb solutes, which are normally accumulated and excreted. Therefore, it leads to several complications: mucous production, urolithiasis, infection, perforation, metabolic disturbances and even cancer [7]. These issues have generated sufficient debate and novel research strategies are demanded to avoid the unwanted effects mentioned above. However, the storage and voiding functions associated to a good compliance and capacity of this organ are challenging to reproduce with TE.

Among several studies assessing bladder reconstruction with exogenous biomaterials, the Atala's group achieved excellent results in seven patients, aged 4–19 years, suffering from myelomeningocele [8]. These patients needed augmentation cystoplasty, as they had severe urinary incontinence because of their neuropathic bladders (high-pressure or poorly compliant). Their bladders were reconstructed using a collagen scaffold seeded with patient's own bladder cells either with or without omental coverage, or a combined poly-glycolic acid (PGA)-collagen scaffold covered with omentum. The three patients with the composite collagen-PGA scaffold plus omental wrap showed better results, with several improvements in terms of preoperative values: bladder filling pressures decreased 56%, while the volume and the compliance increased 1.58-fold and 2.79-fold, respectively. After a mean follow-up of 46 months, no metabolic consequences and renal stones were noted and the mucous production and renal function remained normal. At last, the engineered bladders biopsies showed an adequate structural architecture and phenotype.

Despite this excellent outcome, during the phase II of a clinical study on eleven pediatric patients treated with the PGA matrix seeded with autologous urothelial cells, there was no clinical or statistical improvement in bladder capacity and compliance after a mean follow-up of 36 months. Adverse events, such as bladder rupture ($n=3$) and bowel obstruction ($n=3$), occurred in most of the patients and needed to be treated through other surgeries [9]. The failure of this clinical study could be explained considering the disease condition of the bladder which offered poor status cells to seed onto scaffolds. In this way, it was demonstrated that the use of artificial urinary bladder is still far from replacing the classic use of GI tissue for augmentation cystoplasty.

Another solution, which was first reported in rats after partial cystectomy, is the use of bladder acellular matrix (BAM), prepared by removing mechanically and chemically cellular components from bladder tissue [10]. Despite many years of intense research, BAM alone had never been able to achieve full bladder regeneration, as muscle layers developed incompletely [11]. Therefore, BAM is being associated with stem cells to improve tissue regeneration (Figure 1) [12].

Current available biomaterials for bladder TE are classified in biological and synthetic ones. In addition to acellular matrixes, other examples of biological scaffolds used for treating the bladder are primarily made of collagen and alginate; synthetic polymers include poly L-lactide (PLLA), PGA and silk derivatives instead [13]. The advantages of BAM and biological materials are related to good biocompatibility and biodegradability, while the processing of synthetic materials allows to obtain scaffolds with more reproducible and predictable physical behavior [14]. Although bladder TE is a promising alternative to traditional approaches, no strong benefits emerged comparing several biomaterials introduced in experimental studies [9,11,13]. Along with the definition of an adequate biomaterial and scaffold, bladder TE has still many challenges to solve, such as the appropriate choice of animal models; each animal model presents, indeed, its own specificity [15]. For example, the composition of rabbits urine conducted to the formation of bladder stones when the animal is subjected to exogenous bladder material implants [16]. Moreover, larger animals have a similar anatomy to human body than rats or rabbits and this help in being as close as possible to human situation in clinical evaluation [17]. Another issue of the studies reported in literature is the absence of a long follow-up characterized by the comparison with a control group treated with the gold standard treatment (GI tissues) [18]. All these parameters need to be taken into account and they are fundamental to find the optimal procedures for bladder regeneration before considering the clinical application.

Besides the importance of preclinical study standardization, greater interest is being reserved to obtain adequate stem cells for urological tissue-engineered organs. In this respect, Bharadwaj et al. [19] derived stem cells from excreted urine and differentiated them into bladder urothelial and smooth muscle phenotypes, demonstrating a potential future perspective for urological care.

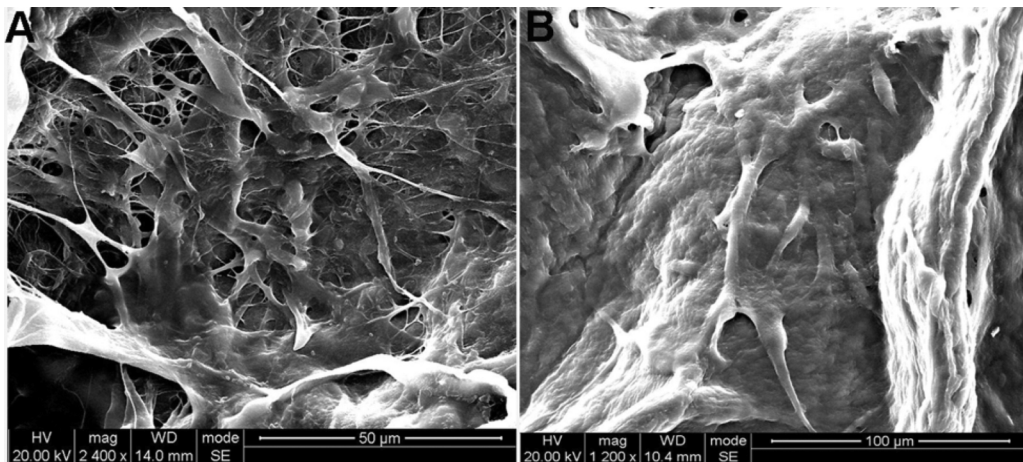


Fig. 1. Images of BAM seeded with MSCs from human umbilicus taken by Scanning Electron Microscope (SEM). **A** At 5 days the cells migrate and proliferate inside BAM (magnification $\times 2400$). **B** At 10 days MSCs from human umbilicus fill in the surface of BAM (magnification $\times 1200$).

Source: Yuan H, Zhuang Y, Xiong J, Zhi W, Liu L, Wei Q, Han P. Human umbilical mesenchymal stem cells-seeded bladder acellular matrix grafts for reconstruction of bladder defects in a canine model. *PLoS One*. 2013 Nov 20;8(11):e80959. doi: 10.1371/journal.pone.0080959. eCollection 2013.

3.2. Blood Vessels

Blood vessels are a striking example of application of RM strategies for engineering tubular organs. Since tissue-engineered vessels could be useful for common and life-threatening vascular lesions, research has been advanced greatly [20,21].

A traditional TE approach is the construction of functional living vascular grafts outside the body by using 3 elements: cells, scaffolds and bioreactors. Among all the organs, artificial blood vessels are the ones that are significantly developed by means of bioreactors: devices or systems able to reproduce biochemical/biophysical stimuli as in a physiological conditions. Computer-controlled pulsatile bioreactors seem to be superior then the old ones as they could permit the adjustment over a range of pressure and pulse rates, thus making great improvements in vascular TE [22].

As an alternative to autologous grafts, also xenogeneic or synthetic materials had largely been used, but, besides lacking of growth potential (key feature with pediatric patients), they were related to an increased risk of stenosis, infection and thromboembolization [23–25].

Therefore, the right strategy proved to be the use of biodegradable scaffolds seeded with patient's own cells. In this way, the autograft does not provoke immune responses and it degrades itself, leaving new tissue instead of a foreign body. This technology had been tried not only in laboratory or in animal models, but even in clinical setting. Indeed, a tubular biodegradable scaffold, created with polycaprolactone-poly(lactic acid) (PCL-PLA) copolymer, reinforced with woven PGA and seeded with endothelial cells, was implanted to substitute a stenosed pulmonary artery, repaired previously [26]. The cells were obtained from patient's stem cells through a blood sample. The tissue-engineered vascular autograft (TEVA) was later placed in a bioreactor system to acclimatize it to the

conditions of the body until implantation. Neither occlusion nor aneurism of TEVA was observed in the recipient 7 months after implantation. Subsequently to Shin'oka's successful approach, 25 extracardiac cavopulmonary connections with TEVA as a conduit were performed on pediatric patients with mean age of 5.5 years [27]. Long-term follow-up at 4 years after implantation demonstrated no instances of graft-related mortality, no cases of aneurysm formation, graft rupture nor calcification. Only two death were reported from late cardiac failure. This first human clinical trial confirmed the efficiency of TEVA and the absence of adverse events, such as graft rupture or aneurismal change.

Even for tissue-engineered small diameter vessels (< 6 mm), their development had been complicated by either occlusion or thrombosis of the current strategies for long time [28]. However, Kurobe et al. recently succeeded in the construction and implantation in mice of a tissue engineered vascular graft (TEVG) for small diameter arteries as shown in Figure 2 [29].

Despite the promising clinical results with large diameter engineered vessels, there are still some significant problems to solve, from the choice of material, geometry and scaffold composition, which should be standardized, to the definition of an accurate process of sterilization, preservation and transport of engineered grafts.

Recently, the attention shifted towards scaffold-free blood vessels realized by the bioprinting technology. [30,31]. The latter uses a computer-controlled printing device to deposit cell aggregates, cells and biomaterials or cells encapsulated in hydrogels into defined 3D geometries. In this case, blood vessels with different diameters and cell patterns were printed using spherical and cylindrical multicellular systems as bioink. Since the fabrication method resulted accurate, reliable and scalable, further studies are now required to take advantage of bioprinting benefits to

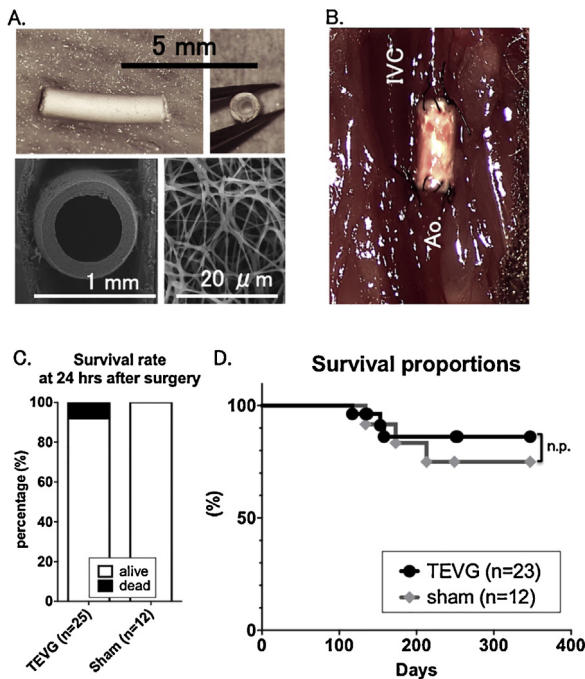


Fig. 2. Comparison between TEVG implantation to sham group. **A** TEVG was made of biodegradable electrospun polylactic acid (PLA) nanofibers (length=3mm; inner luminal diameter 500–600 μ m). **B** implantation of TEVG. The image shows inferior vena cava (IVC) and aorta (Ao). **C** Survival rate at 24 hours after surgery. < 10% of mice implanted with TEVG died within 24 hours after TEVG implantation because of bleeding or thrombosis. **D** Kaplan-Meier survival curve considering the 12 months of experiment. No significant statistical difference was highlighted between the two groups.

Source: Kurobe H, Maxfield MW, Tara S, Rocco KA, Bagi PS, Yi T, et al. (2015) Development of Small Diameter Nanofiber Tissue Engineered Arterial Grafts. *PLoS ONE* 10(4): e0120328. doi: 10.1371/journal.pone.0120328.

obtain reproducible implantable blood vessels in the near future.

3.3. Skin

The skin is probably the best well-known engineered organ and the daily clinical application of artificial skin is not far at all. The clinical gold standard in full-thickness injuries treatment is an autologous split-thickness graft, which consists in the surgical removal and subsequent stretching of epidermis and superficial dermis from an undamaged skin site of the patient; the autograft is thus applied on the full-thickness wound or burn. However, in case of extensive injuries, donor sites for autografts harvesting are not available. Allografts could be an additional option, but they still suffer the need of immunosuppressive therapies. To overcome these inconvenient, many research groups worldwide focused their attention on creating bioengineered skin substituted [32–34]. Some of the commercially available dermo-biopolymer composites for clinical applications are listed in Table 1 [35–41].

In addition to the current commercial skin substitutes, different approaches has been developed to meet the

demand of skin replacement. Among the most innovative solutions, bioprinting is emerging as a potential tool for skin TE applications. A printer designed to print skin cells onto burn wounds had recently been built and tested at the *Wake Forest Institute for Regenerative Medicine (WFIRM)* [42]. Before printing, a scanner is used to determine wound size and depth to calibrate the use of the ink, which, actually, includes different kinds of skin cells, collected from a skin patch one-tenth the size of the burn. The same research team has recently worked on the possibility to use the amniotic fluid-derived stem cells (AFSCs) to wound treatment in a mouse model of skin regeneration [43]. After two-week observation, the AFSCs deposited in a collagen/fibrin gels accelerated closure and re-epithelialization of full-thickness wounds in mice faster than gel-only controls and were as effective as bone marrow-derived mesenchymal stem cells (MSCs) treatments. Additionally, histological examinations showed that AFS cells induced stronger angiogenesis and blood vessel maturation compared with the other two groups. In conclusion, together with commercial skin substitutes, bioprinting approach will become an optimal treatment for burns and skin wounds without requiring a secondary surgical site (as in case of gold standard treatment) and without provoking the patient's immune response by using AFSCs.

3.4. Trachea

The success of a tissue-engineered trachea is determined only by its ability to conduct air lifelong, becoming a sustainable biological conduit. Many attempts had been tried to create an artificial trachea, but none showed superior.

On March 2010, at Great Ormond Street Hospital, in London, a stem-cell based tissue-engineered trachea was implanted in a 12-year-old child, born with congenital long segment tracheal stenosis and pulmonary sling [44]. After a brief stimulation by granulocyte colony stimulating factor (G-CSF), bone marrow mesenchymal stem cells (BMSCs) were obtained preoperatively and seeded onto a scaffold with patches of autologous epithelium. Human recombinant erythropoietin (EPO) was applied topically to stimulate angiogenesis, along with Transforming growth factor beta (TGF- β) to support chondrogenesis. The graft revascularized within 1 week after implantation. Epithelium restoration became evident after 1 year, while the graft did not have biomechanical strength focally until 18 months, without requiring any medical interventions. Moreover, 18 months after surgery, he performed a chest CT scan and ventilation-perfusion scan, which resulted normal. At 2 years follow-up, he had a functional airway and returned to school, after growing 11 cm in height.

This study represents an evolution in the development of tissue-engineered tracheas, representing the foundation for future clinical applications.

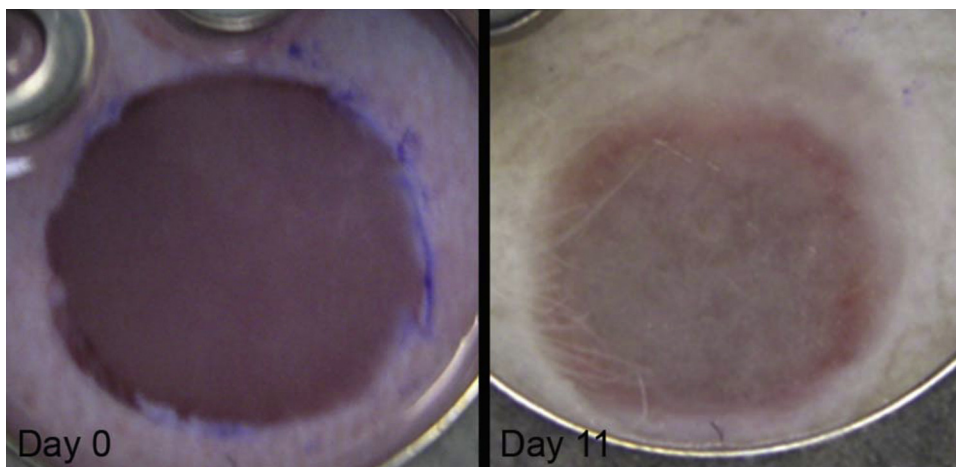
3.5. Vision

The eye presents some advantages that makes it as an optimal candidate for RM applications; in fact, it is easy to access in order to perform pre-treatment assessments and

Table 1

Some of the commercially available skin substitutes that have been successfully used in clinical practice.

Brand name/manufacturer	Scaffold material	Cells	References
Dermagraft® Advanced Biohealing, Westport, CT, USA	PGA and PLA fibers and silicon film	Fibroblasts	Hart et al. [35]
Apligraf® Organogenesis Inc., Canton, Massachusetts, CA, USA	Bovine collagen	Keratinocytes and fibroblasts	Falanga et al. [36]
OrCel® Ortec International, Inc., New York, NY, USA	Bovine collagen sponge	Keratinocytes and fibroblasts	Still et al. [37]
TissueTech Autograft System (Laserskin® and Hyalograft 3D®) Fidia Advanced Biopolymers, Abano Terme, Italy	Hyaluronic Acid	Keratinocytes and fibroblasts	Caravaggi et al.[38] Uccioli et al. [39]
Matriderm® Dr. Suwelack Skin & Health Care, Billerbeck, Germany	Acellular dermal substitute	Keratinocytes and fibroblasts	(Figure 3) -Micheal et al. [40] Min et al. [41]

**Fig. 3.** Tissue engineered skin construct inserted into the mouse wound after the implantation (left) and on the 11th day (right), when the wound fully healed.

Source: Michael S, Sorg H, Peck CT, Koch L, Deiwick A, Chichkov B, Vogt PM, Reimers K. Tissue engineered skin substitutes created by laser-assisted bioprinting form skin-like structures in the dorsal skin fold chamber in mice. PLoS One. 2013;8(3):e57741. doi: 10.1371/journal.pone.0057741. Epub 2013 Mar 4.

collect cells, it can be simply observed and evaluated during follow-up and it is an immune-privileged organ. However, the eye is composed by many different tissues, whose artificial reconstructions are at a different stage of research [45].

Among all tissues, cornea is at the highest research domain. Corneal scarring from trauma and inflammation disrupts vision for millions worldwide, but corneal transplantation, the primary therapy for corneal blindness, is unavailable to many affected individuals.

As an alternative to cadaveric corneas transplantation, transparent thin gelatin gel (TGG) scaffolds, functionalized with heparin, were fabricated to support transfer of cultured human corneal endothelial cells (HCECs). Through a small incision in the cornea, the scaffold was implanted in rabbits' eye and gradually integrated with the surrounding tissue [46].

Cellular therapy seems to offer a valid alternative as well. Stem cells from different sources had been isolated, expended *in vitro*, differentiated and proved to function as

corneal cells *in vivo*. In Figure 4, the results associated to the treatment of corneas burned by alkali and later seeded with MSCs in rats are shown [47]. Moreover, Du et al. collected human stem cells and transplanted them into mice with genetically cloudy corneas, which, eventually, became transparent [48].

In addition to these pre-clinical treatments, Holoclar®, a stem cell-based medical product for the repair of the outer layer of cornea damaged by chemical or physical injuries, has recently been approved by European Commission. The therapy is based on the biopsy of a tiny portion (min 1–2 mm²) of the undamaged limbus and its growth in laboratory using specific techniques. The limbus is located between the sclera, the white part of the eye, and the cornea, the front part of the eye, and it is rich in stem cells able to heal the damaged cornea. Through this system, a novel sheet of cornea can be developed and transplanted back onto the eye. The combination of Holoclar® and corneal transplants can restore a normal cornea for previously incurable, deep corneal burns.

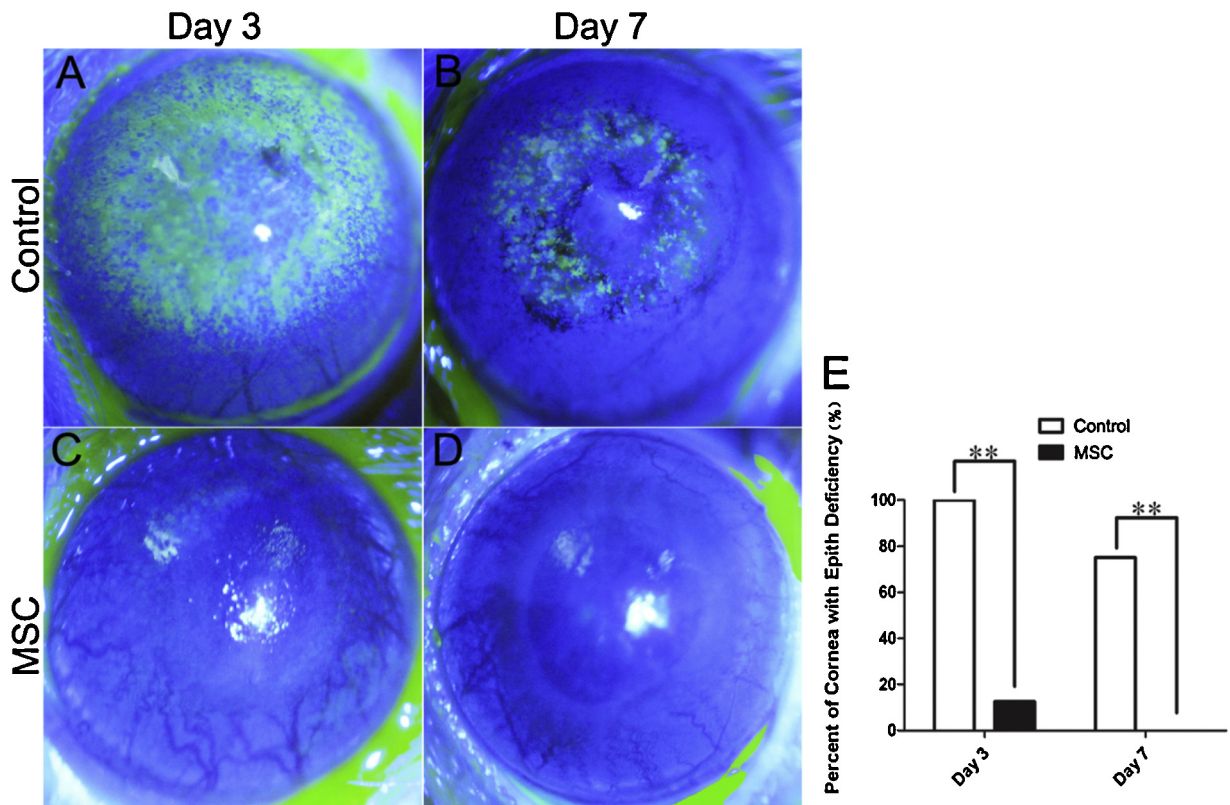


Fig. 4. MSCs improve corneal wound healing. **A–D** Fluorescein-stained corneas of the control group show reduced recovery in reference to group treated with MSCs. **E** Percentage analysis of fluorescein-stained corneas highlights a significant statistical difference between the two groups: MSC group stained in a very lower manner than the control group, confirming the acceleration of corneal wound healing.

Source: Yao L, Li ZR, Su WR, Li YP, Lin ML, Zhang WX, Liu Y, Wan Q, Liang D. Role of mesenchymal stem cells on cornea wound healing induced by acute alkali burn. *PLoS One*. 2012;7(2):e30842. doi: 10.1371/journal.pone.0030842. Epub 2012 Feb 17.

Together with cornea, also retina is involved in different eyes disease such as retinitis pigmentosa, diabetic retinopathy and age-related macular degeneration (AMD). The latter is considered as the leading cause of vision loss among older people. AMD is multifactorial disease which interests retinal pigment epithelium degeneration and it ends with the damage of the retina central portion that, eventually, conducts to the patient's vision loss. The traditional therapy consists of drugs able to stop neovascularization, but not able to repair the precedent damage. For this reason, recent progress have been made in the field of cell therapies for RPE treatment, including the use of induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) [51].

In particular, at the end of 2014 in Japan, at the RIKEN Center for Developmental Biology, a 70-year-old woman, suffering from AMD, was treated by using iPSCs, taken by hers and transplanted as a 1.3–3 mm RPE mono-layer without the use of synthetic scaffolds or matrices [52]. No complications appeared with the transplant surgery and a one-year monitoring period was set to evaluate the initial results of the research. The technology described above represents a real breakthrough and is able to give faith to all patients suffering from AMD.

Recently, an American stem cell-based company reported successful completion of two phase I/II clinical

trials for human ES-derived RPE cell suspension transplanted in 18 patients suffering from Stargardt's disease or with AMD. No major adverse events and immune responses were noted in any patient over a mean 22 months follow-up [53].

In conclusion, stem cell-based therapy could represent an effective solution for the cure of retinal degenerative disease.

4. Conclusions

Every 30 seconds a patient dies from diseases which could be treated with tissue replacement [54]. A tissue engineering and regenerative medicine (TERM) approach could probably offer the definitive solution for children with congenital malformations, young soldiers disfigured in war and old people suffering from chronic invalidating diseases, which are burdening more and more heavily on world's national economies [55]. As described before in detail, current researches are supporting valuable directions to improve clinical applications of TE [12,26,28,40,41,49,50].

Researchers are asked to direct their efforts towards the translation of the many different laboratory findings into modern and viable therapies: a close collaboration between clinicians and other scientists (bioengineers,

materials engineers, biologists) is mandatory to translate basic scientific discoveries in TERM therapies to help their spread. However, efficacy and efficiency of these new solutions must be carefully evaluated before clinical trials. Specifically, pre-clinical studies ought to be constructed according to the following principles:[18]

- Enrollment of large samples;
- Use of tissue/organ constructs which present similar dimensions as human ones;
- Appropriate choice of animals models which should not report over- or under- estimations;
- Selection of pertinent bioreactors (specially for vessels) to improve the construction of the artificial organ by means of adequate stimuli;
- Use of diseased tissues and/or dysfunctional animal organs in view of clinical applications;
- Long follow-up;
- Comparisons with the gold standard treatment.

In order to stimulate reader's research interests, the authors would like to highlight major challenges which need to be solved in future:

- Vascularization and innervation of artificial constructs;
- Engineering organs with different cell types;
- Construction of validated bioreactors to avoid animal testing;
- Conducting appropriate pre-clinical and clinical trials following the principles indicated above in order to make TERM approach the gold standard treatment for every diseases.

When all these issues are completely overcome, TERM approach will become superior to existing clinical therapies.

5. Conflict of interest

None declared.

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