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## Abstract

Congenital or acquired surgical conditions are associated to high morbidity and mortality, and most of the time functional replacement of the missing or damaged organ remains an unmet clinical need. Regenerative medicine has recently been established as an emerging interdisciplinary field focused on the repair, replacement, or regeneration of cells, tissues,

or organs. So far, there has been success in the production and surgical replacement of organs such as the urethra, bladder, and trachea.

While regenerative medicine encompasses a number of different disciplines, when it is aimed toward organogenesis, the important components are appropriate cells and an appropriate scaffold. The employment of induced pluripotent cells may overcome the use of immunosuppression associated with embryonic stem cells. Moreover, amniotic fluid stem cells may be specifically relevant to the cure of congenital malformation and reduce the hazards associated with pluripotent cells such

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as tumorigenesis, rejection, difficulty in isolation, and ethical issues. Research on scaffold-cell interaction has indicated that beyond the need for suitable biomechanical properties and micro-architecture, an optimal scaffold must support cell-matrix signaling; decellularized scaffolds may hold an advantage toward this.

The success obtained so far in transplanting tissue-engineered structures has paved the way for the regeneration of more complex and solid organs. Herein, regenerative medicine could represent a valid solution to the shortage of donors for organ transplantation.

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**Keywords**

Regenerative medicine · Stem cell · Congenital malformation · Tissue engineering · Transplantation · Organ decellularization

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**Introduction**

We are certainly going through the biggest revolution in medicine since the introduction of antibiotics. While medicine of the XVIII century was about discovering disease and, in the XIX, doctors devoted their career to develop new medicine and operations that could stop the evolution of diseases, the future generation of physician will have the instruments for regenerating tissues and organs when they are absent or damaged. This new paradigm in medicine will change forever the way we treat diseases. Regenerative medicine will dramatically change the way we look at both congenital and acquired disease (De Coppi 2013).

Regenerative medicine can offer the opportunity to functionally restore tissues and organs with the aim of giving patients a better and longer life. In 2014, we are still unable to restore the majority of congenital malformations with functional tissues. Patients born with a large diaphragmatic defect receive a patch of synthetic material that, in the best scenario will integrate with the native muscle but it will never function as the native muscle, giving the chance for the hernia to reoccur. And even when the diaphragmatic hernia can be effectively repaired, we are unable to replace or regenerate the underdeveloped lung

(De Coppi 2013). If children with severe congenital diaphragmatic hernia undergo respiratory failure, the only option is lung transplantation, which is associated with a poor outcome because of limited organ supply and immunosuppression.

The first clinical applications of regenerative medicine have recently become a reality, after years of basic science research and proof of principle experiments on animal models of disease. The rapid development of the field has been driven by the unmet clinical needs of patients requiring healthy tissues and organs, but for whom allotransplantation is not an option mainly due to the limited availability of appropriate grafts of human origin. So far, scientists around the world have been successful in tissue engineering structurally simple organs with the main functions of allowing passage (i.e., trachea, urethra) or storage (i.e., bladder) in the body, but it is likely that in the next few years, more complex structures will be prepared in bioreactors before being transplanted into patients. Alternatively, it is possible that regeneration may occur directly in patients using their own body as a bioreactor (Elliott et al. 2012). This would simplify the experimental design, avoid risks related to *in vitro* cell expansion, and reduce cost. Ultimately, tissue engineering may provide a long-term solution to the problem of shortage of organs available for transplantation (Table 1).

Congenital and acquired surgical conditions represent a major cause of morbidity and mortality during the first years of life and childhood. In those complex conditions, prosthetic materials are used because of the lack of biocompatible tissues able to replace or regenerate damaged organs. Besides the risk of infection, the major drawback of using a prosthetic patch closure is the risk of dislodgment and subsequent recurrence of the initial problem. Moreover, foreign body reactions and implant rejection occur when synthetic polymers are used. Regeneration of natural tissue from living cells to restore damaged tissues and organs is the main purpose of regenerative medicine. This relatively new field has emerged by the combination of tissue engineering and cell transplantation as a possible strategy for the replacement of damaged organs or tissues. So far, most of

**Table 1** Summary of clinical applications of tissue engineering

Organ	Scaffold		Cells		Patient number	Follow-up	Results	References
	Type	Size	Type	Number				
Bronchus	Decellularized trachea	7 cm	Epithelial cells	1*10 <sup>6</sup> /mL	1	4 months	Normal mechanical properties and appearance of graft, improved quality of life, no immunosuppression	Macchiarini et al. (2008)
			Chondrocytes	1*10 <sup>6</sup> /mL				
Trachea (whole)	Decellularized trachea	7 cm	Epithelial cells	2.5*10 <sup>8</sup>	1	2 years	Normal CT scan, appropriate growth, patient well	Elliott et al. (2012)
			Epithelial cells	Patches				
Vagina	Intestinal submucosa segments	7 × 10 cm	Epithelial cells	1*10 <sup>7</sup> cells per cm <sup>2</sup>	4	up to 8 years	Normal structural and functional variables	Raya-Rivera et al. (2014)
			Smooth muscle cells	1*10 <sup>7</sup> cells per cm <sup>2</sup>				
Bladder	Collagen matrix or collagen-PGA matrix	70–150 cm <sup>2</sup>	Urothelial cells	50*10 <sup>6</sup> /cm <sup>3</sup>	7	46 months	Volume and compliance increase, preservation of renal function, adequate structural architecture and phenotype	Atala et al. (2006)
			Smooth muscle cells	50*10 <sup>6</sup> /cm <sup>3</sup>				
Urethra	PGA-PLGA matrix	5 cm	Epithelial cells	1*10 <sup>7</sup> /mL	4	71 months	Maximum urinary flow rate 27.1 mL/s, no strictures, normal architecture (biopsy, 3 months)	Raya-Rivera et al. (2011)
			Smooth muscle cells	1*10 <sup>7</sup> /mL				

*PLCA* poly-caprolactone, *PLA* polylactic acid, *PGA* polyglycolic acid, *PLGA* poly(lactic/glycolic acid)

the attention has been focused on degenerative diseases such as Parkinson or Alzheimer, while very little has been done for the treatment of congenital conditions. However, the knowledge acquired in the last years from stem cell biology and regenerative medicine strategies could lead to new ways of repairing or replacing injured organs and systems, even during fetal development, and therefore pediatric patients could largely benefit from the evolution of this new exciting field. In order to give rise to a new functional organ-like structure, several variables, such as local environment, nutrients, and metabolites, are pivotal. These variables, in the contest of tissue engineering, are mainly dependent on the provision of a three-dimensional growth structure termed "scaffold." Scaffolds are usually made by natural materials, which are essentially bioactive but lack mechanical strength, or synthetic materials, which lack inherent bioactivity but are mechanically strong and can be engineered with the desirable macrostructure and microstructure and might possess desired bioactive properties to make possible cellular growth and organogenesis. Despite scaffolds could ultimately represent the exclusive tool for tissue engineering and several attempts to generate whole organs, such as liver, have been done by developing structures with vascular channels to ensure an adequate network of vascular supply, major developments in regenerative medicine have been achieved after the discovery of cells capable to be isolated and expanded in number outside the body. Stem cells (SCs) are unspecialized cells with the capacity of undergoing to asymmetric division which offer them the unique power of self-renewal and to give rise to multiple different specialized cell types. Three are the main source SCs in human and animals: from embryonic, fetal, and adult tissues. Adult SCs have a limited cellular regeneration or turnover that could represent a limitation for tissue engineering application where a large number of cells is necessary. They can be identified in many adult mammalian tissues, such as bone marrow, skeletal muscle, skin, and adipose tissue, where they contribute to the replenishment of cells lost through normal cellular senescence or injury (Pittenger et al. 1999). In contrast, SCs derived from

embryonic sources have the ability to give rise to cells that not only proliferate and replace themselves indefinitely but also have the potential to form any cell type. Embryonic stem (ES) cells are derived from the inner cell mass of preimplantation embryos, are pluripotent, and demonstrate germ line transmission in experimentally produced chimeras (Thomson et al. 1998). More recently, cells with intermediate potential could be derived from the amniotic fluid (fetal SCs) (De Coppi et al. 2007) or reprogramed from adult SCs using various factors implicated with the maintenance of pluripotent potential of ES cells (Takahashi and Yamanaka 2006). This chapter would like to offer an insight on the latest evolution of SCs with a glance at their possible application for regenerative medicine and the recent clinical translations, particularly in the pediatric surgery field.

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## Stem Cells

### Embryonic Stem Cells

ES cells derive from the inner cell mass of a blastocyst stage embryo. They are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm; hence, they possess the potential to develop into most of the cell types within the body (Thomson et al. 1998). The field of ES cell research began with the study of teratocarcinoma cells in the 1950s and continued with the first mouse ES cell lines derived from the inner cell mass of blastocysts using culture conditions (fibroblast feeder layers and serum) in 1981 and expanded in 1998 when Thomson et al. (Thomson et al. 1998) first derived human ES (hES) cells. The maintenance of pluripotency in the hES is assured by the presence of different transcription factors like Oct-4, Nanog, and SOX2 that are essential to ensure the suppression of genes that lead to differentiation. The cell surface antigens most commonly used to identify hES cells are the glycolipids stem cells embryonic antigen-3 and -4 (SSEA3 and SSEA4) and the keratan sulfate antigens Tra-1-60 and Tra-1-81. ES cells could be

used not only to generate tissues but also could be employed as “cellular models” to study a range of human diseases and to test new drug candidates for efficacy and toxicity. ES cells, being pluripotent, require specific signals for correct differentiation, and if injected *in vivo* prior commitment, they will give rise to many different types of cells, causing teratomas. So far their potentials, together with the difficulties related to their allogenic origin, have limited their possible clinical applications. In particular, the political debate surrounding SCs began suddenly after hES creation because of the destruction of the derivative embryo. Recently, researchers opened the possibility of generating ES cell lines without destroying embryos by deriving cells from the early development of the embryo without impairing their further development. Moreover, in the last few years, ES cells have been used as a model for tissue differentiation, and rudimentary organs derived from ES cells have been engineered. Understanding the signaling necessary for terminal differentiation has been important to define lineage-specific transcription factors which can potentially be used to genetically engineer and terminally differentiate pluripotent stem cells increasing the chance of producing functionally differentiated tissues.

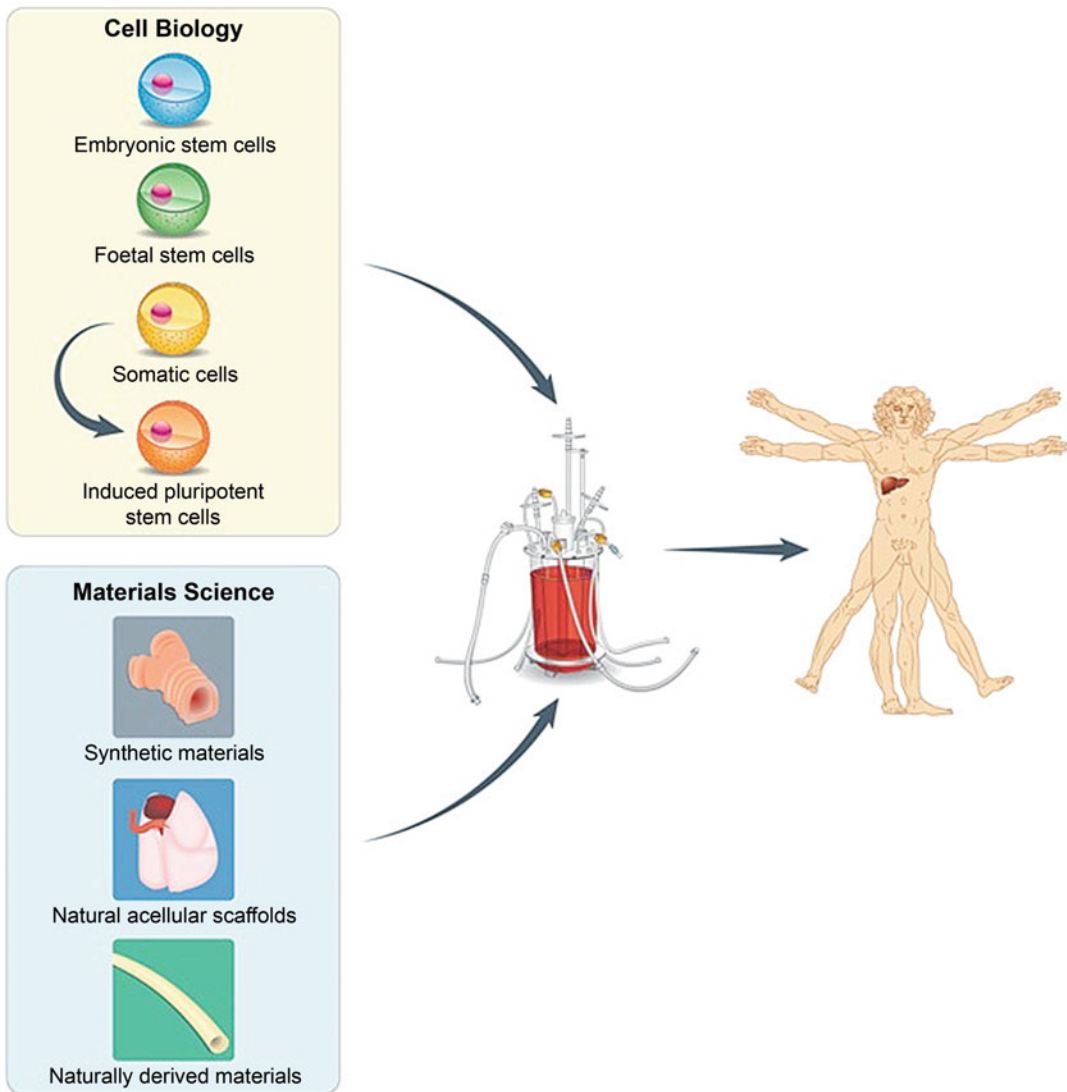
### Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT) has also been adopted to create patient-specific SCs and avoid problems related to the creation of allogenic tissue. This procedure entails specifically the removal of an oocyte nucleus in culture, followed by its replacement with a nucleus derived from a somatic cell obtained from a patient. Cells yielded by this induction would be genetically identical to the donor and would not be rejected by the patient. SCNT can potentially be used for three purposes: (a) reproduction, leading to generation of an embryo for continuation of life (a notable example in 1996 was the generation of the first mammal, a sheep named Dolly, derived from an adult somatic cell by the use of this technique (Campbell et al. 1996)); (b) therapy, generating blastocysts for SC

derivation; and (c) research and regenerative medicine. The first is scientifically and ethically condemned. The second has important implications for the future of ES therapies, allowing the production of non-immunogenic ES lines. Besides, these cells could be stored and used subsequently for the treatment of future medical conditions. As a consequence this could be relevant for the creation of autologous tissues also in children who are born with complex malformations in which tissue viability represents a problem. Patient-specific cells could be created *in vitro*. ES cells derived using SCNT would have the same genetic background of the patient who has donated the initial genetic material, and the tissue created would not be rejected after transplantation. ES cells have in fact the advantage of being extremely plastic facilitating the *in vitro* engineering of complex organ such as the heart, liver, and kidney. Nevertheless, in spite of the ethical considerations, the limitation of this technique is related both to the low efficiency, leading to a high loss in cell yield and the inadequate supply of human oocytes.

### Induced Pluripotent Stem Cells

Since the major objection to hES research is their immunogenicity, it would be advantageous to develop a method of creating SCs from autologous tissue. Generation of induced pluripotent stem (iPS) cells, described by Yamanaka et al. in 2006, avoids both the immunological and the ethical problems (Fig. 1). iPS cells can be developed from non-pluripotent cells, usually adult somatic cells, by causing a forced expression of several genetic sequences such as Oct4 (POU5F1), the transcription factor Sox2, c-Myc proto-oncogene protein, and Klf4 (Krueppel-like factor 4) as key genes, sufficient to reprogram fibroblasts which are able to produce viable chimeras if injected into developing embryos and teratomas in immunocompromised mice (Takahashi and Yamanaka 2006). In 2007, successful iPS cells were obtained from human fibroblasts both using Oct3/4, Sox2, Klf4, and c-Myc with a retroviral transfection and using OCT4,



**Fig. 1** Tissue engineering combines expertise on the fields of cell biology and material science aiming to generate tissues/organs that could be used in alternative to allotransplantation

SOX2, NANOG, and a different gene LIN28 using a lentiviral system, improving transduction output (Yu et al. 2007; Takahashi et al. 2007). The viral transfection systems used to insert the genes at random locations in the host's genome created concern for potential therapeutic applications of these iPS cells, because it might increase the risk of tumor formation. To overcome these dangers, some studies have used adenovirus to transport the four sequences into the DNA of mouse somatic cells. Since the adenovirus does not

combine any of its own genes with the targeted host, the danger of creating tumors is eliminated (Meissner et al. 2007). Yamanaka et al. have since demonstrated that reprogramming can be accomplished via plasmids without any virus transfection system at all, although at very low efficiencies. IPS cells show morphological resemblance to hES cells, express typical human ES cell-specific cell surface antigens and genes, give rise to multiple lineages in vitro, and form teratomas when injected into immunocompromised

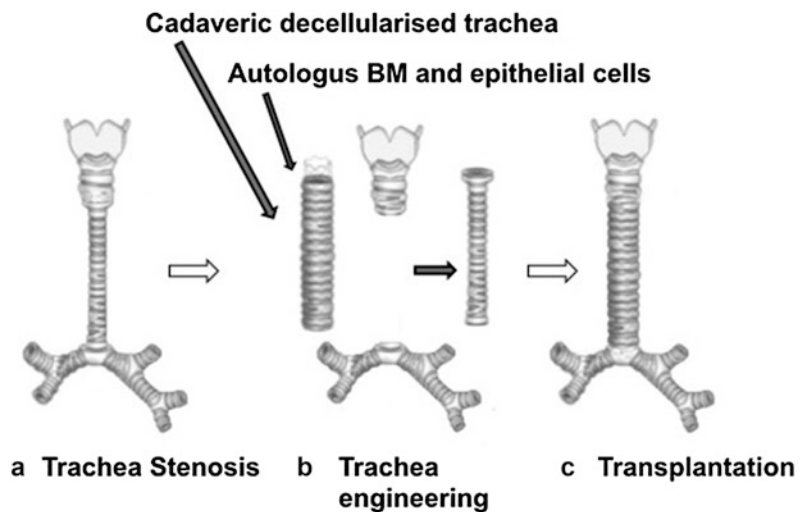
mice. The efficiency of reprogramming adult fibroblasts has been low (<0.1%). However, since reprogrammed clones could consistently be recovered and expanded with existing gene combinations, for practical applications, the low reprogramming efficiency itself was not really considered an issue, unless reprogramming selects abnormal genetic or epigenetic events that were stably propagated in the resulting iPS cell lines (Yu and Thomson 2008). Recently, Jaenisch's group found an elegant way to derive iPS from somatic cells of patients, free from reprogramming factors using Cre-recombinase excisable lentiviruses. The efficiency of reprogrammed iPS is very high with a low number of proviral vector integrations, and the cells maintain a gene expression profile more similar to hES than to hiPS and can be subsequently differentiated into dopaminergic neurons, such that Parkinson's disease patient-derived induced pluripotent stem cells can be free of viral reprogramming factors. Interestingly to the surgical community, it has been recently demonstrated the possibility to derive and isolate of enteric nervous system (ENS) progenitors from human pluripotent stem cells and their further differentiation into functional enteric neurons. ENS precursors derived in vitro are capable of targeted migration in the developing chick embryo and extensive colonization of the adult mouse colon.

Moreover, iPS cells can be generated from fetal cells without any genetic manipulation. It was recently described that iPS cells can be easily derived from first-trimester amniotic stem cells when grown on Matrigel in hES cell medium supplemented with the histone deacetylase inhibitor valproic acid (VPA) (Moschidou et al. 2012). It is possible that, because of their fetal origin, amniotic fluid stem (AFS) cells (discussed in detail later) could be the ideal for cell banking of patient-specific pluripotent cells and possible applications in pharmaceutical screening.

### Fetal Stem Cells

Fetal cells may represent the ideal source for therapy (Fig. 2a, b). Like ES cells, they are still plastic and easy to expand, and, in common with their adult counterparts, they are less controversial, not tumorigenic, and can be accomplished in an autologous setting. The latter is particularly important in the context of congenital malformation as surgical treatment is often complicated by insufficient available tissue at time of repair. Regularly, artificial materials have been the only option for reconstruction in such cases, with high rates of morbidity. Recently, fetal tissue engineering has emerged as a promising concept in surgical reconstruction of birth defects.

**Fig. 2** Schematic representation of the tissue-engineered trachea transplanted at Great Ormond Street Hospital in 2010. During surgery the airway was found to be severely stenotic with multiple stents including one entering the ascending aorta. (a and b) The old homograft trachea was removed and replaced by the engineered graft (c) Elliott et al. (2012)



Redundant or purposely obtained fetal cells could be harvested, cultured, and manipulated *in vitro* during the remainder of pregnancy and later used for tissue engineering of graft material that will be applied in postnatal reconstruction. Indeed, in the case of prenatal diagnosis of structural defects, there is the possibility of obtaining homologous cells at the time of invasive sampling such as chorionic villi biopsy, cordocentesis, or amniocentesis. Sampling of amniotic fluid (AF) is ideal for prenatal/neonatal applications with the advantages of being (i) relatively easy to perform; (ii) low risk for both the mother and fetus; and (iii) a widely accepted method of prenatal diagnosis. AFS cells represent about 1% of all whole cells in cultures of human amniocentesis specimens obtained for prenatal genetic diagnosis and can be immunoselected using c-Kit (CD117). AFS are described as broadly multipotent stem cells that can differentiate into adipogenic, osteogenic, myogenic, endothelial, neurogenic, and hepatogenic lineages, inclusive of all embryonic germ layers. In contrast with mesenchymal cells, AFS cells show hematopoietic engraftment (Ditadi et al. 2009) and a functional and stable integration into the skeletal muscle stem cell niche, highlighting their value as cell source for the treatment of muscular pathologies and skeletal muscle defects (Piccoli et al. 2012). This group of cells can be steadily expanded in cultures without a feeder layer and has a typical doubling time of 36 h. Sub-confluent cells show no evidence of spontaneous differentiation, but nevertheless, under specific inducing conditions, these cells are able to differentiate and, if injected *in vivo*, show no evidence of tumor growth in severe combined immunodeficient mice. AFS cells are positive for a number of surface markers commonly expressed by MSC, but not by ES cells, such as CD29, CD44 (hyaluronan receptor), CD73, CD90, and CD105 (endoglin) (Cananzi et al. 2009). Human AFS cells are also positive for stage-specific embryonic antigen (SSEA)-4, also expressed by ESC, and >90% of the cells express the transcription factor Oct4, which has been associated with the maintenance of the undifferentiated state and the pluripotency of ES and EG cells. First-trimester AFS cells can also be

fully reprogrammed without using any genetic manipulation (Moschidou et al. 2012). AFS cells can be used as carrier for congenital monogenic disease, which could be theoretically corrected by a combined approached gene-cell therapy (Fig. 2a). Following this approach, AFS cells could be isolated, the defect identified and corrected using the corrected sequence, and injected back to the donor avoiding both fetal and maternal immunorejection. Alternatively, in case of structural anomalies, AFS cells could be expanded and used to engineer the missing tissue/organ (Fig. 2b). Mesenchymal stem cells derived from amniotic fluid have already been used in large animal models for the repair of surgically created diaphragmatic defects (Fuchs et al. 2004), and engineered diaphragmatic tendon graft using mesenchymal amniocytes has obtained preclinical validation and may soon become a clinical reality (Turner et al. 2011).

## Adult Stem Cells

At the farthest end of the spectrum, we have stem cells, which are present in the postnatal life, are more committed, and classified as either multipotent or unipotent (Fig. 1). Classically, hematopoietic stem cells (HSC) have successfully been used for more than 25 years and transplantation of bone marrow (BM) and cord blood (CB) HSC is routinely performed for hemato-/oncological disorders. Following preliminary studies by Friedenstein et al. (Friedenstein et al. 1968), other cells, defined as mesenchymal stem cells (MSC), distinguishable from HSC because of the capacity to grow in adherent culture, were described. MSC are multipotent stem cells with fibroblast-like morphology, which can differentiate into osteogenic (bone), chondrogenic (cartilage), and adipogenic (bone marrow stroma) lineages “*in vitro*.” Their existence was first hypothesized when tissues biopsied (skeletal muscle, skin, heart, and even brain) from patients, who previously received a BM transplant, showed donor cell engraftment. To date, MSC have been isolated both in the fetus and postnatally from the blood, liver and bone marrow, amniotic fluid,



lung, pancreas, dental pulp, and periosteum. They have been isolated also from the umbilical cord blood, Wharton's jelly, and the placenta. These parts, previously considered a "biological waste" today are among the most interesting for MSC isolation. Three major criteria have been introduced to define MSC by the International Society for Cell Therapy (Horwitz et al. 2005):

- (i) The cells must be plastic adherent when maintained under standard culture conditions.
- (ii) >95% of the MSC population must express CD73 (ecto 5'-nucleotidase), CD90 (Thy-1) and CD105 (SH2 or MCAM or endoglin), LNGFR (low-affinity nerve growth factor receptor), CD166, ALCAM adhesion protein, CD146 (PIH12), CD29, and CD106 (vascular adhesion molecule-1, VCAM-1), and >98% MSC should be negative for hematopoietic cell surface antigens: CD45, a pan-leukocyte marker; CD34, a marker of primitive hematopoietic progenitors and endothelial cells; either CD11b or CD14, markers for monocytes; either CD19 or CD79a, B-cells markers; and human leukocyte antigen (HLA Class 2).
- (iii) MSC should be capable of differentiating into osteoblasts, chondroblasts, and adipocytes when placed into an appropriate induction/differentiation medium.

Adult stem cells or progenitors have already been used clinically to engineer urological constructs such as bladders and urethras, blood vessels, cornea, esophageal epithelium, bronchi, and tracheas. Moreover, MSC have been tested for cellular therapy in pediatric patients for several clinical indications, such as inborn errors of metabolism (metachromatic leukodystrophy, Hurler syndrome, infantile hypophosphatasemia), osteogenesis imperfecta, and GVHD (Horwitz et al. 1999; Le Blanc et al. 2005). However, in contrast with the aforementioned cells, MSC also have a limited life span and become senescent when cultured *in vitro*. This is a major problem for therapy since, regardless of the isolation procedure, the MSC quantity obtained from primary

tissues is not sufficient for any downstream MSC application in clinical settings. *In vitro* expansion can affect biological properties of the cells, and MSC go through very significant changes in phenotype and gene expression as a result of cell culture adaptation. Although considered a safer source compared with ES, the prospective clinical applications of MSC require meticulous examination.

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## Scaffolds

In order to develop a tissue-engineered construct to replace missing or damaged tissue, the combination of appropriate cellular engineering and material science is needed. Material science is concerned with the production of acellular scaffolds that can be seeded with cells, allowing and promoting their growth. General attributes that a scaffold must fulfill include:

- (i) Biocompatibility that does not lead to an immunogenic response from the host
- (ii) Biodegradation in a suitable time period that permits sufficient cellular growth while not producing harmful degradation products
- (iii) Mechanical properties that are in line with tissue growth; a significant amount of three-dimensional support in the early stages without obstructing cellular ECM production in the later stages

More specific scaffold attributes are related to aiming to recreate the nonmechanical attributes of the ECM. These properties include the mediation of cell adhesion via integrin receptors, as well as positive influence on cell survival and proliferation by means of growth factors and cytokines. What is more, the ECM has been argued to be involved in mechanochemical transduction, as shown by the differentiation MSC to neurons, muscle cells, and osteoblasts when seeded on substrate mimicking the elasticity of each of those host tissues respectively.

Materials used thus far for tissue engineering have been traditionally divided in three

categories, namely: (2A) naturally derived materials (e.g., collagen and elastin), (2B) synthetic materials (e.g., poly(L)-lactic acid [PLA], polyglycolic acid [PGA], and polylactic-co-glycolic acid [PLGA]), and (2C) natural acellular scaffolds.

### Naturally Derived Materials

Materials composed of naturally occurring macromolecules, in particular those that formulate the ECM, have been tested for tissue engineering purposes. They have the advantage of having a specific structure with which cellular integrins bind well to, promoting cellular adhesion. A classical example is collagen, the most abundant protein found in the ECM, making up a quarter of the total protein content in many animals. There have so far been 29 different types of collagen that have identified, though 90% of the collagen in the body is types I, II, III, and IV. In vertebrates approximately 22% of the total protein content is of collagen type I, and in vascular tissue, such as the aorta, 80–90% of the collagen content is of type I or III. Collagen extracted from animal and human tissues has been used due to its influence in cell adhesion and proliferation as well as low inflammatory and immunogenic responses. Mechanical properties of the collagen-based scaffolds, including resorption rate, may be altered according to porosity, fiber orientation, and cross-linking conditions. Elastin is another ECM protein and it is mainly found in blood vessels, making up a large part of the ECM content of arteries. It provides the arteries with their elastic properties and also has antithrombogenic properties. For example, in vascular tissue engineering production of a tubular scaffold containing both collagen and elastin allowed smooth muscle cells to orientate themselves in line with the fibers.

### Synthetic Materials

Synthetic materials were considered as a possibility for tissue engineering following their use

as biomaterials in other areas of medicine. They are advantageous because they are (i) easily and (ii) cheaply manufactured, (iii) can be formed in many different structures with the required dimensions, the microstructure and nanostructure can be well controlled, and (iv) they can be produced with a range of different mechanical properties and done so reproducibly. For all these reasons synthetic polymers should be the best choice for clinical translation; however, it has been clearly very difficult to replicate in the laboratory the millions of years of evolutions that has taken to design structure and composition of our tissues and organs. Some of those synthetic polymers, such as the polyester polymers, have been firstly used already and employed as sutures and orthopedic fixatives such as pins, rods, and screws. The advantage of those scaffolds is related to scaffold degradation that occurs through hydrolytic attack of the ester bond, and its rate is affected by adjusting various properties of the scaffold including crystallinity, molecular weight, and porosity. The existing wide clinical application of polyesters supports their biocompatibility, although some studies have suggested that there can be problems due to their propensity to disintegrate into small particles or toxicity associated with acidic degradation (Taylor et al. 1994). A commonly used biodegradable synthetic polymer is polyglycolic acid (PGA). Its degradation product, glycolic acid, is a natural metabolite and can therefore be readily metabolized without toxic effect and is already in use as a material for resorbable sutures. PGA is polyester with high crystallinity and as such is a stiff and brittle material. It also has a fast degradation rate of 6 to 8 weeks when fabricated as a mesh. When smooth muscle cells (SMCs) are cultured on PGA mesh in a bioreactor, it was observed a complete loss of PGA after 8 weeks and production of ECM products like collagen. Additionally, polylactic acid (PLA) is a semi-crystalline polymer with a similar rate of degradation to that of PGA and along with PGA is one of the few biodegradable polymers with Food and Drug Administration (FDA) approval for clinical use.

## Natural Acellular Matrix

Natural acellular matrices are organs from humans or animals that have been treated to remove cells and immunogenic material, producing natural scaffolds that maintain their architecture of origin (Fishman et al. 2013). Following decellularization these scaffolds may be implanted with or without prior cell seeding, aimed at regenerating a complete organ or improving tissue repair respectively. The benefits of this technique include the preservation of the ECM that favorably influences cell fate as well as of the macro- and micro-architecture. The macro-architecture is important in recreating the three-dimensional structure of the organ to be tissue engineered as well as maintaining a vascular tree that will allow for cell and nutrient delivery and waste removal. The micro-architecture is of value toward maintaining the mechanical properties and porosity of the tissue that will be optimal for cell seeding and growth. Since ECM components are preserved across species, immunogenic responses are removed (Totonelli et al. 2013). The decellularization process can be performed in a variety of manners including mechanical, chemical, and enzymatic methods. The aim is to remove cellular material while preserving the integrity and constituents of the ECM. Mechanical methods such as snap freezing, direct pressure, and sonication have been reported to disrupt ECM. The major chemical methods used for decellularization include ionic detergents such as sodium dodecyl sulfate and sodium deoxycholate, nonionic detergents such as Triton X-100, and zwitterionic detergents such as CHAPS. Our experience with intestinal and bladder decellularization have suggested that a combination of Milli-Q water, sodium deoxycholate, and DNase is superior to the use of Triton X-100 in preserving ECM structure and collagen/GAG content while removing cellular material (Totonelli et al. 2013). Comparing the benefits of each method is beyond the scope of this review, and when they are looked at, they should be examined in a tissue-specific manner. They have the added hypothetical advantages over synthetic scaffolds of not producing potentially toxic degradation products or inducing inflammation, characteristics that may be important

in the prevention of stenosis. A successful example of natural acellular tissue currently used in the clinic is small intestinal submucosa (SIS) harvested from mammalian small intestine and treated with peracetic acid, followed by antibiotic or radioactive sterilization. Beyond preservation of the ECM structure, GAG, and collagen content, SIS has been demonstrated to contain growth factors such as vascular endothelial growth factor (VEGF), FGF-2, and tumor growth factor- $\beta$  (TGF- $\beta$ ) (Voytik-Harbin et al. 1997). VEGF stimulates angiogenesis of the tissue, whereas TGF- $\beta$  has immunomodulatory properties, suppressing T-helper response and reducing inflammatory or immune reactions. Collectively, these properties provide an environment for tissue growth and vascularization while maintaining sufficient mechanical strength until endogenous ECM production occurs. Clinical uses of SIS include various types of hernia repair (Franklin et al. 2008), anal fistula closure (Champagne et al. 2006), and dural repair (Bejjani et al. 2007). Similar materials that are being examined for clinical use in tissue repair include amniotic membrane tissue, cadaveric fascia, and acellular dermis (which has been used for full-thickness burns since 1995). Decellularized esophageal scaffolds have been used with good reported results in both preclinical and clinical studies (Badylak et al. 2011). Significant heterogeneity exists among studies, both with respect to the type of scaffold, extent of surgery, and species used, which partly explains the range of results reported. Thus, regeneration of the muscularis propria layer is seen to take place in some studies. Badylak et al. laid sheets of SIS onto the raw internal surface of esophagus following endoscopic submucosal resection in five patients with superficial cancers (Badylak et al. 2011). With a follow-up of 4 to 24 months, the scaffold promoted physiological remodeling as evident by endoscopy and histological characterization following biopsy. Strictures still formed but only at areas outside those lined by SIS, suggesting possible technical improvements in scaffold delivery. In fact, when SIS was used to completely cover a  $3 \times 5$ -cm mucosal defect in the cervical esophagus, there was no stenosis or other complications, and endoscopy at 4 weeks demonstrated good integration of

the scaffold (Clough et al. 2011). Hypothetically, decellularized esophageal tissue should retain the signals, both chemical and structural, that will direct the appropriate migration and differentiation of host cells, in a way unlikely to occur with scaffolds originating outside the esophagus, such as SIS. Ozeki et al. compared two methods of decellularization of adult rat esophagus based on deoxycholate and Triton X-100, respectively (Ozeki et al. 2006), and assessed the resultant tissue using routine histology and biocompatibility. Those treated with deoxycholate showed superior mechanical properties, maintenance of the ECM, and a lower DNA content than those treated with Triton X-100. Bhrany et al. found a combination of 0.5% sodium dodecyl sulfate (SDS) and Triton X-100 to be effective in decellularization, albeit with a loss of tensile strength as measured by burst pressure studies (Bhrany et al. 2006) (Fig. 3).

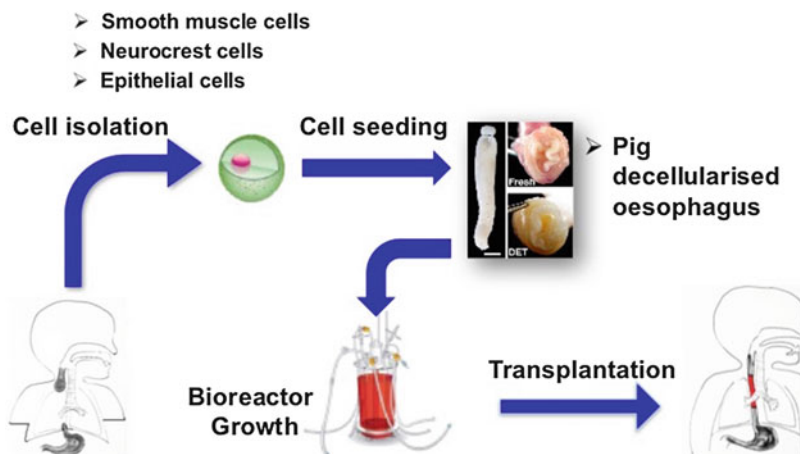
### Cell-Derived ECM Scaffolds

Instead of decellularizing blood vessels to obtain vascular ECM for cell seeding, some groups have taken the approach of culturing vascular cells to produce vascular ECM and then decellularizing. The cells can be allo- or xenogeneic and a much

greater degree of control can be had over the manufacture, increasing reproducibility. This is an important factor in good manufacture practices (GMP), necessary if one wishes to take a tissue-engineered device from lab to clinic. Dermal of saphenous vein fibroblasts were cultured for 21 days and then washed with deionized water and dried at room temperature for 8 h. The fibroblasts can produce a large amount of ECM, which autologous SMCs seeded on to it might not be able to do, depending on patient age, history, and site of extraction. The Niklason group at Yale have seeded SMCs from pigs, canines, or humans (Dahl et al. 2011) on to PGA scaffolds and cultured them in a biomimetic bioreactor for 10 weeks, before decellularizing the scaffold with a detergent treatment. The scaffolds can then be seeded with cells or implanted directly.

### Regenerative Medicine

In order to give rise to a new functional organ-like structure, several variables, such as local environment, nutrients, and metabolites, are pivotal. These variables, in the contest of tissue engineering, are mainly dependent on the provision of a three-dimensional growth structure termed a



**Fig. 3** Engineering of esophageal tissue for the repair of infants born with esophageal atresia. Decellularized pig esophagus is prepared using detergent enzymatic treatment (DET). Autologous cells are derived from affected infants,

and the neo-esophagus is conditioned in specific bioreactors before transplantation. The use of xenogeneic decellularized tissue has the advantage of not requiring immunosuppression Fishman et al. (2013)

“scaffold.” Scaffolds are usually made of natural materials, which are essentially bioactive but lack mechanical strength, or synthetic materials, which lack inherent bioactivity but are mechanically strong and can be engineered with the desirable macro- and microstructure and might possess desired bioactive properties to make possible cellular growth and organogenesis. Although scaffolds could ultimately represent the exclusive tool of tissue engineering and several attempts have been made generating whole organs, such as the liver, by developing structures with vascular channels to ensure an adequate network of blood supply, major developments in the clinic have been achieved in the last few years using relative simple scaffolds. Atala et al. presented in 2006 a series of seven children with myelomeningocele, aged 4–19 years, with high-pressure or poorly compliant bladders, who successfully received an engineered bladder tissues (Atala et al. 2006). Briefly, after undergoing a bladder biopsy, urothelial and muscle cells were grown and then seeded onto a biodegradable bladder-shaped scaffold made of collagen or a composite of collagen and polyglycolic acid. About 7 weeks after the biopsy, the autologous engineered bladder constructs were implanted and wrapped in omentum with good long-term (mean 46 months) results (Atala et al. 2006). The same group described in 2011 their experience with five boys who, following trauma, between 2004 and 2007 underwent urethral reconstruction using tubularized urethras seeded with autologous cells which remained functional for up to 6 years (Raya-Rivera et al. 2011). In 2001 a 4-year-old girl with a single right ventricle and pulmonary atresia, who underwent the Fontan procedure at the age of 3 years, was transplanted with a polycaprolactone-poly(lactic acid) copolymer (weight ratio, 1:1) reinforced with woven polyglycolic acid seeded with autologous cells to bypass the total occlusion of the right intermediate pulmonary artery. Ten days after seeding, the graft was transplanted with no postoperative complications, and at seven months the patient was doing well, with no evidence of graft occlusion or aneurysmal changes on chest radiography (Shin’oka et al. 2001). More recently, a 36-year-old man, with recurrent primary cancer

of the distal trachea and main bronchi, who underwent excisional surgery followed by a reconstruction of his airway with a tailored bioartificial nanocomposite previously seeded with autologous bone marrow mononuclear cells via a bioreactor for 36 h (Jungebluth et al. 2011). More complex organs, such as the liver or the kidney, may require more time before being applied for therapy. Atala’s group has described a perfusion decellularization technique for the liver and kidney generating decellularized organ scaffolds for organ bioengineering (Baptista et al. 2011). In an analogous fashion, Uygun et al. have reported a successful approach of decellularizing rat livers and recellularizing them with rat primary hepatocytes, showing promising hepatic function and the ability to transplant heterotopically these bioengineered livers into animals for up to 8 h (Uygun et al. 2010; Uygun et al. 2011). The decellularization approach was pioneered in the heart by Ott et al. who showed that it was possible not only to generate whole organ scaffolds using a perfusion decellularization system but also that neonatal rat cardiomyocytes (delivered through transmural injection) and endothelial cells (injected through the aorta) could generate a contractile construct (Ott et al. 2008). Using a similar approach, lungs have also been regenerated through the seeding of pulmonary epithelium and vascular endothelium on rat lung ECM (Ott et al. 2010). Among unmet clinical needs, intestinal engineering is particularly relevant, particularly because of the relatively poor outcome of intestinal transplantation. However, although pioneering investigations in the field of intestinal bioengineering date back to the 1980s, initial excitement has been blunted by the considerable limitations and roadblocks encountered in the course of experimental investigations. The main culprit of such stagnation is the complexity of intestinal anatomy and the various functions of the intestine. Vacanti’s group showed that rodent organoid units seeded on a nonwoven polyglycolic acid (PGA) fiber and implanted in rats having undergone the resection of 85% of their native intestine were able to partially replace gut function (Choi and Vacanti 1997; Kim et al. 1999; Grikscheit et al. 2004). Bioengineered intestinal

constructs were also successfully transplanted in pigs (Sala et al. 2009) but this technology is inefficient because several centimeters of bowel are needed to obtain a sufficient number of organoid units able to repopulate just a few centimeters of engineered intestine (Dunn 2008). Intestinal stem cells, which reside in the base of Lieberkuhn crypts and express Lgr5, have been shown to be capable of building crypt-villus structures in vitro without any mesenchymal niche (Barker et al. 2007). As these cells can be reliably expanded in culture, they could represent the ideal source of progenitor cells for intestinal bioengineering. Although representing only an initial step toward the ultimate goal of generating fully functional organs, decellularized matrices may help understand the functional structure of complex organ and may represent in the mid-term the bridge for transplantation since some of these structures may be difficult to mimic artificially (Totonelli et al. 2012). Those barriers could be overcome by producing three-dimensional vascularized cellular constructs of clinically relevant size, shape, and structural integrity. Very recently we have witnessed the first report of an integrated tissue/organ printer that could fabricate stable, human-scale tissue constructs of any shape. Biodegradable polymers and hydrogels could be printed to correct shape by representing clinical imaging data as a computer model of the anatomical defect and translating the model into a program that controls the motions of the printer nozzles, which dispense cells to discrete locations (Kang et al. 2016). Using this technology, the authors could demonstrate capabilities of the ITOP by fabricating mandible and calvarial bone, cartilage, and skeletal muscle. Future development of the ITOP may lead to the production of tissues for human applications and to the building of more complex tissues and solid organs.

## Conclusion

We are living in a very exciting time in medicine. Tissue and organ regeneration will change the treatment of diseases, as we know it now. It will be particularly interesting to observe the evolution

of surgical approaches to malformations and organ transplantation and the totally new possibility of correcting them using autologous tailor-made functional tissue.

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