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REVIEW

Current approaches and future perspectives on strategies for the development of personalized tissue engineering therapies

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ABSTRACT

Personalized tissue engineering and regenerative medicine (TERM) therapies propose patient-oriented effective solutions, considering individual needs. Cell-based therapies, for example, may benefit from cell sources that enable easier autologous set-ups or from recent developments on IPS cells technologies towards effective personalized therapeutics. Furthermore, the customization of scaffold materials to perfectly fit a patient's tissue defect through rapid prototyping technologies, also known as 3D printing, is now a reality. Nevertheless, the timing to expand cells or to obtain functional *in vitro* tissue substitutes prior to implantation prevents advancements towards routine use upon patient´s needs. Thus, personalized therapies also anticipate the importance of creating off-the-shelf solutions to enable immediately available tissue engineered products. This paper reviews the main recent developments and future challenges to enable personalized TERM approaches and to bring these technologies closer to clinical applications.

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Regenerative medicine approaches, including stem cells therapies and tissue engineering, hold the potential to revolutionize the management of numerous diseases and trauma in the upcoming years. More recently, the importance of personalized medicine in tissue engineering and other cell-based therapies has been recognized, envisioning the development of customized approaches, where bioengineered products are tailored to meet patient requirements and to improve patient outcomes, including patient recovery time. This anticipates a significant decrease in the health care and social co-lateral costs associated to ineffective or inadequate approaches (Figure 1).

In this review, recent developments toward personalized tissue engineering and regenerative medicine (TERM) approaches will be discussed, highlighting those achieved through cell-based therapies using cell sources with autologous potential and induced pluripotent stem cell (iPSC) technologies and through custom-made systems exploring rapid prototyping technologies and injectable systems to tailor patient-oriented treatments. Moreover, emerging technologies and strategies for creating off-the-shelf solutions to enable immediately available customized therapies will be also addressed. Finally, the challenges and future perspectives toward the development of personalized strategies will be discussed in the light of the possible advances that these may enable for bringing TERM technologies closer to routine clinical applications.

Paving the way for personalized regenerative medicine

Cell-based therapies: stem cell sources

Several cell-based therapies, and tissue engineering approaches, rely on the use of stem cells that are isolated from human tissue samples, selected, expanded and/or manipulated (e.g. differentiated into a specific cell type) *in vitro* before being delivered via transplantation to stimulate regeneration and enhancement of functionality after damage, injury or degeneration of a tissue or organ. A common methodology, applicable to a certain extent as therapeutic agent, is the use of cells previously isolated and harvested from autologous or allogenic tissues.

Although mature resident cells from tissues seem to be an obvious choice, in recent years, considerable disadvantages that include local tissue morbidity, limited availability of tissues or organs for transplantation, or even rejection and disease transmission risks, limited the studies and progression toward clinical therapies. Moreover, the invasive procedures to access the tissues

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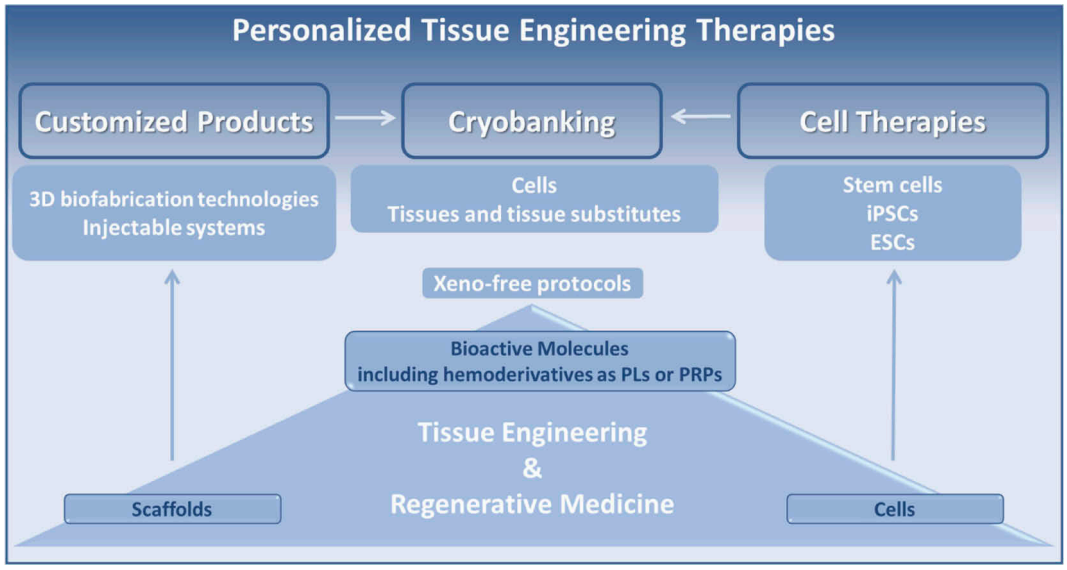

Figure 1. Schematic representation of the major areas of TERM strategies towards personalized medicine approaches, highlighting the relevance of these strategies in the development of innovative and more efficient therapies.

to harvest these cells together with the low self-renewal and proliferative capacities of tissue resident cells pursuit for alternative cell sources to overcome these limitations.

Advances in biology and cell sciences lead to the application of stem cells to stimulate regeneration mechanisms. Stem cells can be obtained from practically all tissues in the body, from embryonic [1] to adult tissue sources [2–5], allowing the development of several exploratory approaches that can be more easily employed in an autologous context aiming at personalized cell therapies.

Embryonic stem cells

Embryonic stem cells (ESCs) derive from the inner cell mass of the blastocysts, have a great capability to differentiate into innumerous cell types, being therefore designated pluripotent cells, and can be maintained and expanded *in vitro* for long periods of time [6]. However, the risk of tumor formation, the immunological compatibility of the transplanted cells, the ethical considerations related to the manipulation of human embryos and to the development of new research procedures also reflects their limitation in new research advancements [6]. Nevertheless, clinical applications in human patients refer the effectiveness of using ESCs in the treatment of blindness-associated diseases as Stargardt's macular dystrophy and macular degeneration [1].

Induced pluripotent stem cells

iPSCs hold great promise for personalized therapies as iPSCs can be developed into any cell type in the body. The reprogramming of somatic cells into iPSCs through a small number of specific transcription factors like Oct3/4, Sox2 and c-Myc has great potential for tissue-specific regenerative therapies, avoiding ethical issues associated to the use of ESCs [7]. Moreover, iPSCs can recapitulate human disease with potential to repair or replace diseased, injured or aged cells within the human body, allowing the production of patient-specific cells and the development of cell replacement therapies without the need for immunosuppressive drugs. The generation of human iPSCs-derived retinal pigment epithelium meets the clinical use requirements with applicability in tissue replacement therapy of age-related macular degeneration [8]. The combination of human iPSC-derived cardiomyocytes, endothelial cells and smooth muscle cells in a 3D fibrin patch also demonstrated to improve heart function and metabolism in a porcine model, without inducing ventricular arrhythmias [9]. Furthermore, iPSC technology provides a unique platform for the development of disease models and for screening the efficacy of new therapeutics [10]. iPSCs-derived cardiac cells are currently being used to assess the cardiotoxicity of drugs [11,12] and to realize the pathogenesis of diseases such as myocardial infarction, [13] diabetic cardiomyopathy [10] and right ventricular dysplasia [13] envisioning primary therapeutic uses.

However, studies on iPSCs are still limited and some evidences suggest these cells may have low induction efficiency and share tumorigenicity with ESCs. The fact that iPSCs maintain an epigenetic memory of the cell type of origin may also limit their clinical application [14].

Adult stem cells

Adult stem cells are multipotent, and thus have a more limited differentiation potential in comparison to ESCs. Nevertheless, as endogenous mediators of regeneration mechanisms, adult stem cells are considered an important strategic tool for studies related to tissue and organ regeneration with minimal ethical concerns. Among stem cell sources, bone marrow, blood from the umbilical cord and adipose tissue are the ones mostly studied, stem cells from bone marrow, especially hematopoietic stem cells and the umbilical cord are more frequently referred in clinical therapies.

Bone marrow stem cells

Bone marrow-derived stem cells (BMSCs) have been clinically used for over 40 years. They are formed by heterogeneous cell populations of hematopoietic, endothelial and mesenchymal stem cells (MSCs), being considered one of the main stem cells sources for therapeutic purposes [3]. In fact, their high differentiation potential and low morbidity during the harvesting constitute very significant advantages. However, there are also some disadvantages such as the highly invasive procedure for cell harvesting or the limitation of cell availability that significantly decreases with aging [4,15].

In the last decades, several studies using BMSCs verified a great potential for cell therapy applications, including for the treatment of amyotrophic later sclerosis or ischemic cardiomyopathy, in which the transplantation of MSCs derived from bone marrow was shown to be a safe procedure and to improve the recovery and survival of patients suffering from these conditions [16–18].

Umbilical cord blood stem cells

Since the first umbilical cord blood stem cell transplantation in 1988, umbilical cord blood has become an important stem cell source not only due to its abundant supply, painless collection and faster self-renewal, but also due to its potential to differentiate into a variety of cells, including osteoblasts, chondrocytes and adipocytes, among others [19].

Besides the essential role of human cord blood stem cells (hCBSCs) in the regeneration of blood and immune system, treating blood diseases and inherited metabolic disorders, new and emerging uses of these cells in regenerative medicine have been investigated. For instance, cell therapies using umbilical cord blood-derived stem cells were shown to be clinically safe in patients with decompensated liver cirrhosis and spinal cord injury [20,21]. These studies suggest that hCBSC can improve the function of the damaged tissues and the quality of life in most patients [20,21].

Adipose-derived stem cells

Adipose tissue is an excellent source of stem cells, holding a big promise in regenerative therapies and for the creation of a wide range of autologous substitutes [3]. Adipose tissue is very abundant and stem cells are relatively easy to harvest [22] as compared to other sources and can be expanded *in vitro* rapidly [3,23]. Besides, adipose-derived stem cells (ASCs) exhibit a long-term genetic stability and an important differentiation potential for several musculoskeletal tissues including bone, cartilage and tendon [2,24]. Moreover, ASCs also exhibit immunomodulatory functions from their interaction with cells of both innate and adaptive immune systems.

Due to interest in the application of human adipose for regenerative medicine, human ASCs (hASCs) have been clinically explored. The autologous ASC transplantation was demonstrated to be safe and feasible in the treatment of ischemic cardiomyopathy and nonrevascularizable critical limb ischemia [25,26].

Although cell-based therapies have shown successful and promising outcomes in the management of a handful of diseases and pathologies, there are plenty of treatments and new strategies to be investigated, especially for tissue and organ regeneration that may not be completely fulfilled with current cell-based interventions and available medical procedures.

The versatile and successful clinical application of stem cells in regenerative medicine and cell-based therapies depends to some extent on the selection of the stem cell source. In recent years, the search for a universal cell source has highlighted the potential role of allogenic cells to mediate regenerative actions avoiding invasive harvesting procedures and time-demanding methodologies to expand autologous cells. Nevertheless, autologous cells from widely available sources as adipose tissue, whose harvesting procedures patient comply to renew the interest toward autologous clinical therapies. The use of autologous stem cells is highly desirable once it avoids the problem of biological incompatibilities and intraspecific variability, with minimal ethical constrains and simplified translational regulatory procedures. Indeed, a total of 5223 clinical trials with stem cell from different tissue sources are ongoing or were recently completed, as described in Figure 2. These studies roughly represent 17% of all clinical trials associated to cell therapies. Also, about 33% of the studies using stem cells correspond to studies with autologous stem cells.

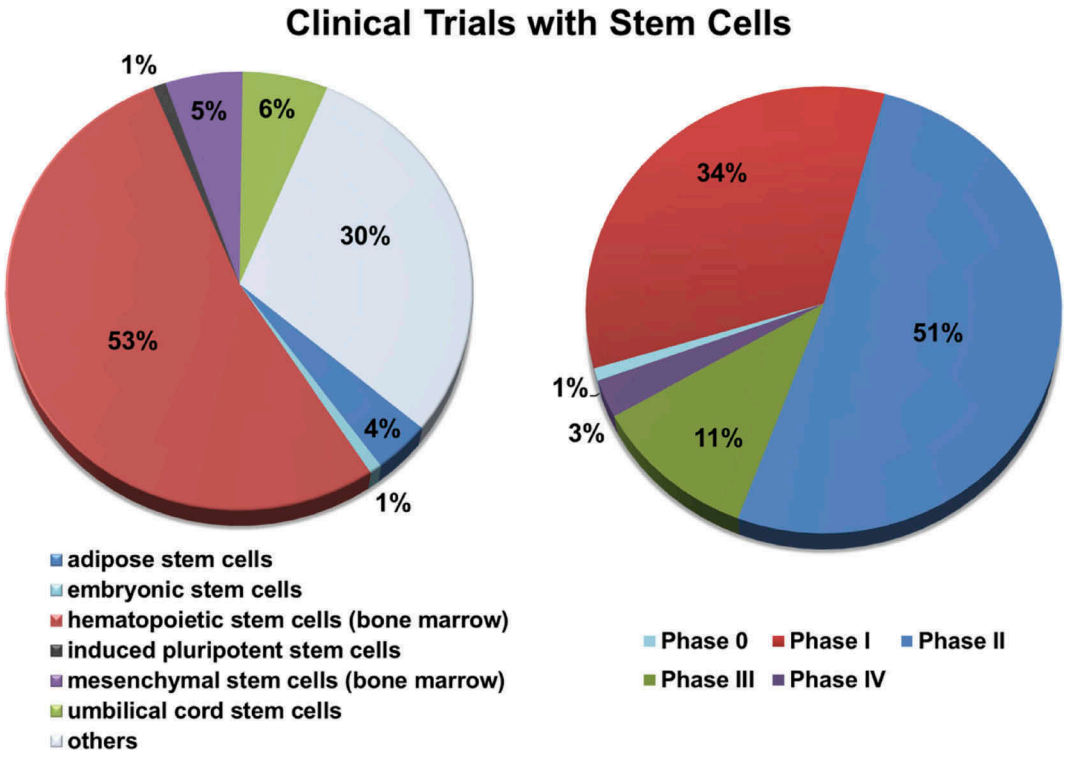

Figure 2. Graphic representation of clinical trials involving stem cells. Data obtained from the website "clinicaltrials.gov" (accessed date: 05 Nov 2015) using the keywords: "stem cells", "bone marrow hematopoetic stem cells", "adipose stem cells" "umbilical cord stem cells", "induced pluripotent stem cells", embryonic stem cells" and "bone marrow mesenchymal stem cells". These studies were also divided accordingly to the clinical phase. Phases 0 to 4 represent different categories of clinical studies, namely: Phase 0: Exploratory study involving very limited human exposure to the drug (in this case stem cells represent the therapeutic agent), with no therapeutic or diagnostic goals; Phase 1: studies that are usually conducted with healthy volunteers and that emphasize safety; Phase 2: studies that gather preliminary data on effectiveness; Phase 3: studies that gather more information about safety and effectiveness by studying different populations and different dosages and by using the drug in combination with other drugs, Phase 4: studies occurring after FDA has approved a drug for marketing.

The fundamental role of stem cells in tissue homeostasis and regeneration is not limited to stem cell properties as self-renewal and multilineage differentiation, but growing evidence suggests that paracrine mechanisms and associated release of cytokines, growth factors or microvesicles, often reported as stem cell secretome, may constitute stem cells' most biologically significant role toward tissue regeneration. Beyond the self-renewal capacity, proliferation and multilineage potential, stem cells secrete trophic paracrine factors that mediate growth, differentiation, angiogenic, anti-apoptotic or immunomodulatory actions, among others, deeply influencing cell niche dynamics and regulating the therapeutic effect of stem cells.

Hemoderivatives and their potential role in cell-based therapies

Another important issue concerning personalized cell-based approaches relates to the use of humanized media for preparing culture methodologies for clinical studies. Traditionally, culture media protocols involve the use of fetal bovine serum (FBS) as a growth supplement [27]. The significant batch-to-batch variation [27], a possible contamination with animal pathogens or even the risk of xeno-immunization, has generated numerous attempts to replace FBS by animal-free alternatives using commercial cocktails and hemoderivates, such as platelet lysates (PLs) [15,28] and platelet-rich plasma (PRP) [29,30]. In fact, human PLs have been proven as an extremely effective cell culture additive with potential for autologous approaches, minimizing the risk of immunological reactions or infections [31] and does not compromise the genomic stability or stem cell differentiation [32,33]. Moreover, PLs are a pool of growth factors available at physiological dosages with potential for regenerative strategies. The implementation of human PLs into standard culture media protocols represents a promising and safe tool for the development of personalized cellular therapies [26,30]. As a hemoderivative, there are already established protocols that can facilitate PLs' translation for clinical

application, including scale-up production with good manufacturing practice compliance.

The widespread use of human hemoderivatives as medium supplement may be also supported by studies in which both PLs and PRPs have been used as therapeutic agents [22,34–37]. Local application of PRP was shown to reduce the pain and accelerate the functional recovery of the repaired rotator cuff [34] and assist cartilage repair [36], and that intra-articular injections of PRP can also be useful for the treatment of early degenerative articular pathology of the knee [35,37].

Moreover, a search for PRP and PLs in clinicaltrials.gov (accessed 10 November 2015) resulted in 197 and 20 clinical studies, respectively, with particular incidence to treat knee joint-associated diseases such as osteoarthritis, tendinopathies and overall musculoskeletal diseases.

Customization of scaffold architectures

Tissue engineering strategies offer the possibility of regenerating injured or degenerated tissues and organs through the combination of cells within a 3D architecture for tissue replacement. Traditionally, TE strategies stand on three main pillars: cells, scaffolds and bioactive molecules, mostly growth factors, often combined into complex systems to recapitulate tissue requirements and assist tissue regeneration aiming at full restoration of tissue functionality. In these multidimensional systems, scaffolds act as cells and/or growth factor carrier/vehicle, but also as mechanical support and conduit until new tissue is formed. Therefore, the quest for advanced and sophisticated scaffolds able to mimic the complexity and functionality of the native tissues to guide local cells and stimulate tissue regeneration is increasingly important.

Scaffold fabrication

Mimicking tissues in organization, architecture and ultimately in complexity is challenging especially considering customization and personalized treatments to the precise anatomical shape and dimensions of the tissue defect or lesion of individual patients. Thus, scaffold fabrication requires high-precision tools to allocate cell and biomaterials in a precise manner in order to generate a fully functional 3D construct. Addictive manufacturing (AM) technologies, also known as solid free-form fabrication, rapid prototyping and 3D printing, employ a highly automated process that builds a 3D object through successive deposition of layers of a material under computer control and are especially advantageous for fabricating highly controlled multifunctional 3D templates using fast, high geometric precision with enhanced productivity and cost-efficient computer-controlled equipment [38,39]. The three most commonly used 3D printing technologies in medical applications are fused deposition modeling (FDM), thermal inkjet printing and selective laser sintering (SLS) [40]. The methodology chosen will depend on the biomaterials to form the 3D template as well as their intrinsic characteristics and final application.

SLS has been used, for example, to fabricate a composite scaffold of hydroxyapatite and polycaprolactone. Results in rabbit femur defects suggest that 3D scaffolds seeded with bone marrow MSCs induced new bone formation, showing large potential for orthopedic and reconstructive applications [41]. In another work, scaffolds of polyurethane (PU) inkjet printing produced demonstrated good hierarchical structures, allowing a good diffusion and ability to support fibroblast cell attachment and growth, envisioning a possible application in vascular tissue engineering [42]. Furthermore, the viability of human fetal MSC was improved and the extracellular matrix was homogeneously distributed in polycaprolactone and tricalcium phosphate scaffolds fabricated by FDM [43].

The use of 3D CT and other imaging techniques has provided an automated way to replicate the 3D shape of a target organ or tissue. For this, when the patient goes for a presurgery CT scan or magnetic resonance imaging, the scanning exam with the patient's 3D volumetric information obtained from medical imaging can be converted into a 3D digital model through a reverse engineering process that perfectly meets the defect dimensions and shape (Figure 3).

3D cell printing

3D printing has been investigated in medicine since the early 2000s, being firstly used to make dental implants and custom prosthetics. Since then, 3D printing systems have been fabricated with increased complexity and hierarchical organization in creative and spatial combinations of cells and biomaterials (often in the form of gels or fibers) for the development of biological substitutes providing enlightened possibilities of TERM.

Indeed, the capability of a 3D print with accurate distribution of different elements, including structural and cellular constituents, necessary to a functional tissue replacement, in an organized way, and be able to provide an optimized microenvironment to the growth of 3D structured tissue, is one of the most exciting prospects of bioprinting [44].

Despite the potential of this technology, printing of living entities as cells is limited to specific techniques and biomaterials. The printing process must be cytocompatible and operate in an aqueous environment

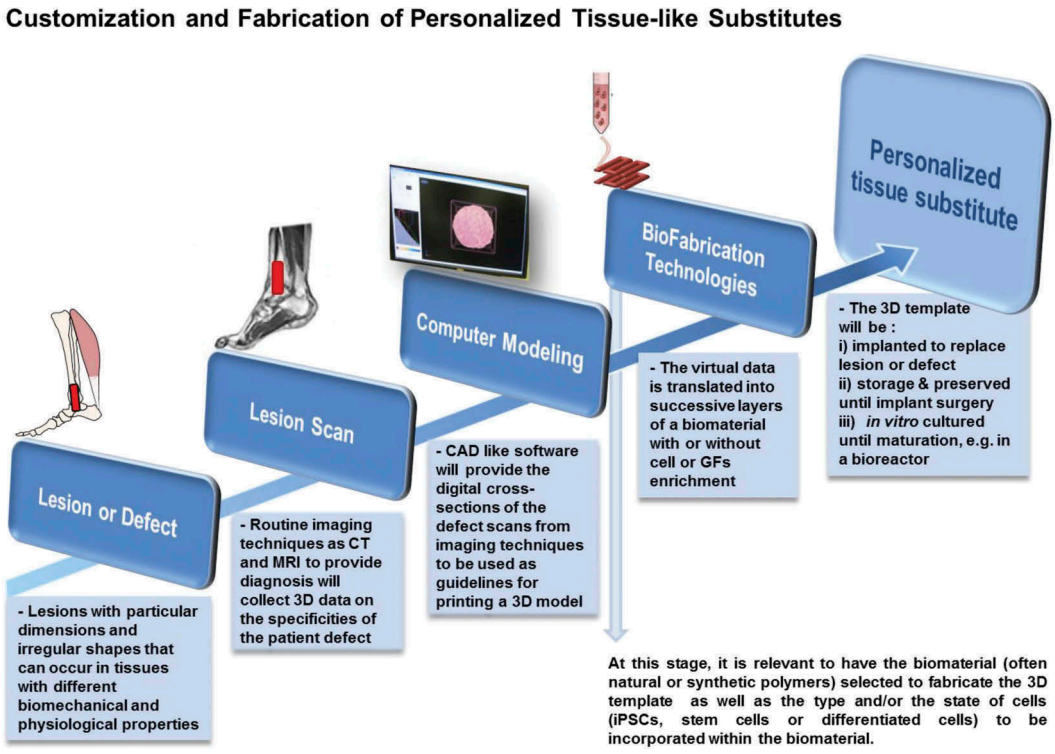

Figure 3. Schematic representation of the customization process to design and fabricate personalized tissue engineering substitutes oriented to patient individual requirements. The ability to accurately screen the lesion and recreate the defect in a 3D template with living cues is a significant achievement for successful medical treatments of a wide range of injured or diseased tissues and organs.

[44,45], so as to fabricate structures capable of providing an optimized condition to the growth of a tissue or organ [44]. Thus, natural-based hydrogel matrices are an interesting option for cell printing to a wide range of tissue substitute applications from liver to bone [46].

Bioinks for cell printing

Among the diverse technologies developed for TERM, the 3D printing systems were revealed to be one of the most attractive and powerful strategies for the development of personalized constructs that mimic a real 3D tissue or organ. However, the overall lack of suitable bioinks for the generation of larger 3D constructs is hampering both the progress in the field of 3D bioprinting technologies and its translation toward clinical application. From the polymeric materials point of view, it remains a challenge to develop unique bioinks, taking in account the required biological competence and the physical requirements dictated by the biofabrication process.

A 3D bioprintable and cell-compatible bioink should have tunable properties as stiffness, bioactive motifs and suitable degradation rate so that the printable solution could be self-supporting during layer-by-layer fabrication and gelate rapidly on the printing substrate [47]. Thus, they should exhibit viscous fluid behavior within the printing head but polymerize shortly after extrusion. This implies decreased shear rates that are present inside a nozzle or orifice during biofabrication, followed by a sharp increase in viscosity (resulting in a high-printing fidelity) upon deposition.

Hydrogels are interesting materials to be used as bioinks and to meet the bioprinting logistics as well as be biocompatible assisting cell viability within a highly hydrated environment [47,48]. Hyaluronic acid and gelatin have been developed into a bioink system to create liver constructs with high viability [47] while PU and poly(ε-caprolactone) were applied to bioprint a complex structure for engineering the muscle–tendon unit [49].

Clinical application of 3D templates

The creation on demand of personalized scaffolds with precise match to tissues or organs that require replacement through transplantation envisions newer opportunities to produce specific and individually customized scaffolds, with clinical applicability.

AM technologies allow the manipulation of biocompatible materials and the inclusion of cells and therapeutic agents within the 3D constructs in order to enhance the biological response and patient follow-

up. Moreover, it is widely accepted that the interface between the tissue substitute and the host tissue should be as intimate as possible.

Patient-specific medical implants have been digitally designed to remake a facial bone defect using SLS from polyamide 2200, which was shown to precisely fit into the defect [50]. Achieving the perfect fit has several advantages, including the reduction time of surgery, as the time spent in manual intraoperatory modeling of the graft/scaffold is practically eliminated and contributes to stable outcomes and to good healing [51]. In some cases, vascularization and biocompatibility of the scaffold post implantation can be also improved.

Other studies refer that computer-assisted reduction technique combined with 3D printing was used to fabricate a customized external fixator for treating tibial fractures, showing good results on all three tibial fractures under treatment [52].

3D printers have been also reported to materialize artificial customized templates for a wide range of human tissues, comprising heart tissue [53], spinal disk, alveolar ridge augmentation and musculoskeletal tissues, including bone defects [50,52,54–56], with clinical studies evidencing promising outcomes with 3D customized scaffolds as patient-tailored solutions.

Injectable hydrogels

While many applications benefit from innovative rapid prototyping technologies, particularly where the scaffold mechanical support is an essential feature, other applications may benefit with the use of injectable hydrogels (Figure 4).

Injectable hydrogels can be easily administrated via minimal invasive procedures, filling the irregular shape of the injury due to their pre-gelling fluidity [57,58], thus providing a better integration of the hydrogel with native tissue [59]. Indeed, this injectable methodology can also deliver a large number of therapeutic agents like drugs, growth factors and even cells, working like a promising injectable construct carrier (Figure 5). Moreover, injectable matrices are a potential system for cell delivery with impact in tissue regeneration. This is because, unlike cells that are directly injected at the injury site, cells within a hydrogel matrix are protected from local biochemical influence and from other cell-to-cell interaction immediately after delivery. Since the hydrogel also provides some physical resistance to cellular mobility, cells are maintained at the injection site for a longer period of time, which can be advantageous to exert cell therapeutic action at the desired location.

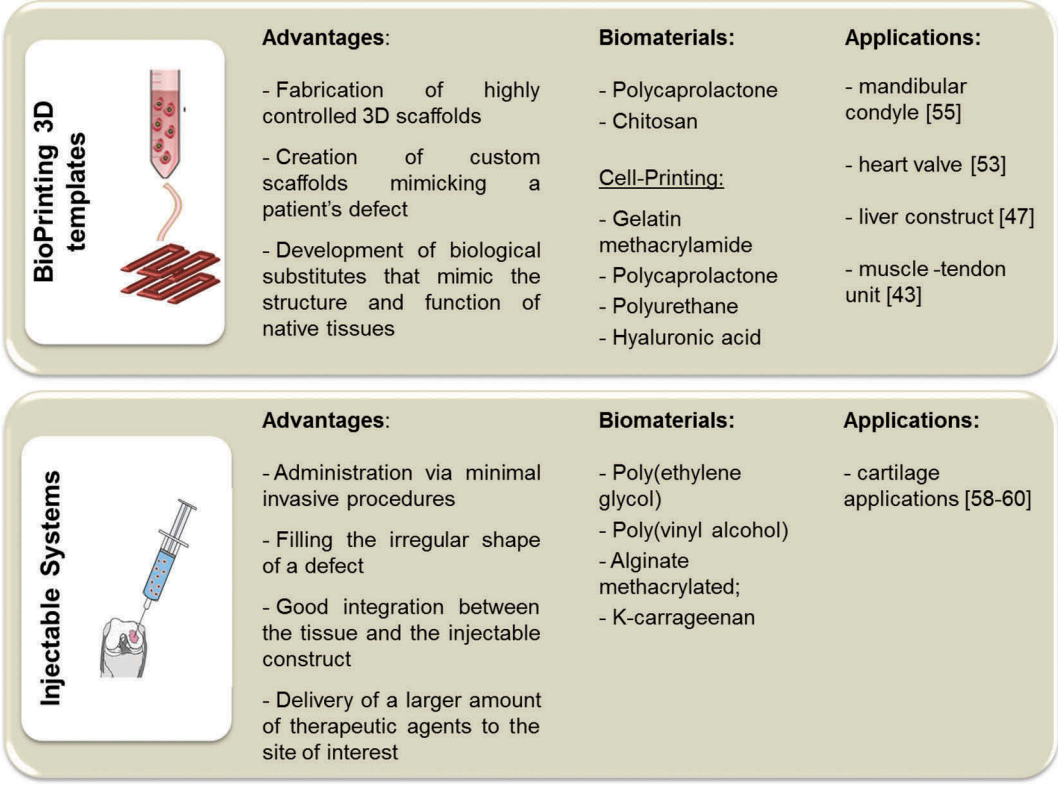

Figure 4. Graphical summary on the biomaterials and tools for 3D template fabrication aiming at personalized TERM approaches.

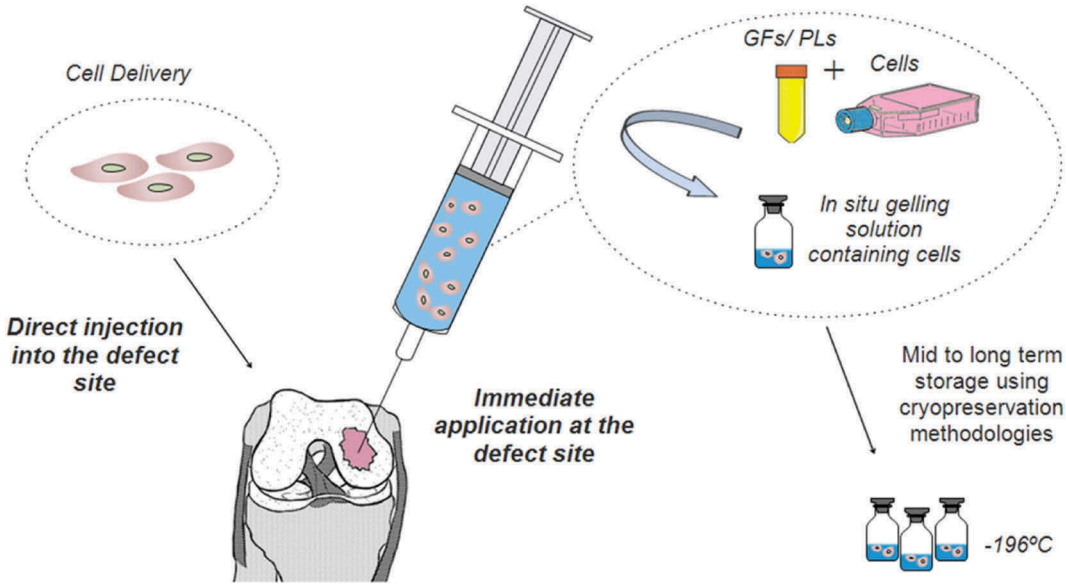

Figure 5. Schematic representation of methods using injectable systems with potential to be used as carriers of growth factors (GFs), platelet lysates (PLs) or cells for tissue regeneration and to be stored and preserved for ready to use approaches.

However, the use of these injectable systems in biomedical applications is very recent and mostly limited to *in vitro* studies. Investigations about cell-encapsulating hydrogels have given encouraging results, indicating that these systems can support the viability, proliferation and chondrogenic differentiation of hASCs [60], with significant potential as delivery systems for cell-based cartilage therapies [59]. Furthermore, injectable systems have also been used in the reconstruction of tissues lost or affected by tumor resection or congenital defects [61]. Thus, injectable systems can pave new ways for improved personalized therapeutics, oriented to fit the patient's defects and needs, using versatile matrices for the delivery of cells and therapeutic factors to the site of interest.

Cryobanking of cells, tissues, tissue-engineered substitutes as off-the-shelf products

The process of developing tissue substitutes is frequently labor intensive and requires long time spans once it involves not only the fabrication of the scaffold itself but often requires the isolation of cells, *in vitro* expansion, and, depending on the strategy, seeding and culture of cells in a construct before implantation [62,63]. During this process, the patient is hampered and waiting, which also implies social and health-care providing costs. In order to overcome this problem, the development of an effective preservation strategy would generate a reliable source of 'ready-to-use' bioengineered products that can be immediately available for patient demands [62,64–66]. Thus, mid- to long-term preservation and storage through cryobanking of cells, tissues, tissue substitutes or other tridimensional complex structures could be an attractive approach to translate bioengineered products with therapeutic value toward clinical applications.

The success of cell-based therapies requires not only the guarantee of a continuous production of cells, but also the identity and integrity maintenance as close as possible to their native origin that can be severely compromised in long-term *in vitro* cultures [67].

Traditional cryopreservation has been a routine practice and represents one of the oldest and the most common storage process for cell lines by cooling to low temperatures, typically to –196°C, using cryoprotectant agents (CPAs) [68]. Other methods such as vitrification [69] use ultra-fast cooling rates, and, more recently, magnetic cryopreservation [70–72] has also been explored to address the challenge of completely eliminating the cryoinjury, which affects to a certain extent survival and functionality of retrieved cells.

CPAs are used to avoid the damage caused by the ice formation during freezing and are classified as permeating or intracellular, such as dimethyl sulfoxide (DMSO), ethylene glycol (EG) and nonpermeating or intercellular compounds as polyvinylpyrrolidone (PVP), polyethylene glycol, trehalose or sucrose. The first ones penetrate the cell membrane, allowing the displacement of the inner water from cell, avoiding the intracellular ice formation, while intercellular CPA act from the cell outside promoting a quick dehydration of the cells, reducing the development of intracellular ice crystals [67,73–75].

Growing knowledge in cell biology and recent developments in stem cells technologies have motivated the creation of cryobanks of cells and stem cells making available a wide collection of cell sources, including bone marrow, cord blood and dental pulp, and, consequently, off-the-shelf cells for transplant, while allowing better timing for therapies. Cryopreserved stem cells from bone marrow and cord blood have shown a therapeutic effect on the treatment of multiple sclerosis [76], and graft versus host disease [30], highlighting the relevance of cryopreservation and banking for the implantation and effectiveness of cell-based therapies and as potential treatment for diseases that currently have no cure. Clinical trials employing cryopreserved stem cells are represented in Figure 6.

Although mammalian cell cryopreservation is considered a conventional and routine method worldwide, with significant benefits accomplished in cell-based therapies and in real clinical treatments, the challenge for preserving an integrated tissue or construct, while keeping cell features and the structural properties of the supportive matrix, remains a challenge.

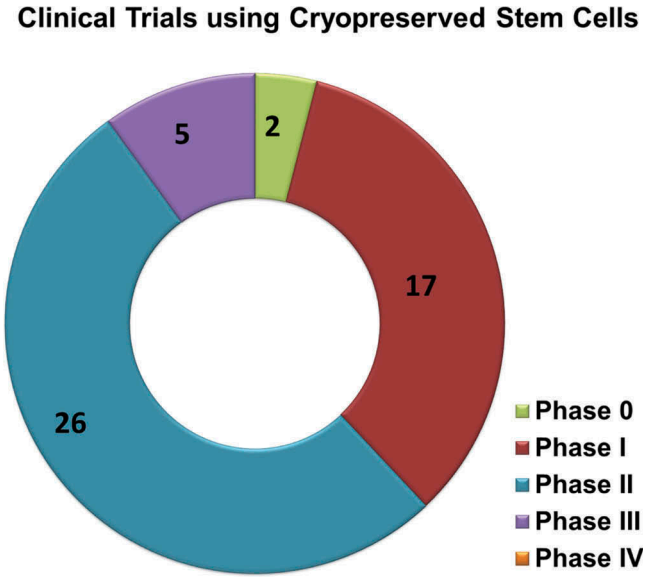

Figure 6. Graphic representation of clinical trials involving stem cells. Data obtained from the website 'clinicaltrials.gov' (accessed date: 05 Nov 2015) using the keywords: 'cryopreserved stem cells' The numbers within the graph represent the number of clinical trials divided accordingly to the clinical phase they were tested for a total of 55, corresponding to 1.05% of total clinical trials performed with stem cells. Phases 0 to 4 represent different categories of clinical studies, namely: Phase 0: Exploratory study involving very limited human exposure to the drug (in this case stem cells represent the therapeutic agent), with no therapeutic or diagnostic goals; Phase 1: studies that are usually conducted with healthy volunteers and that emphasize safety; Phase 2: studies that gather preliminary data on effectiveness; Phase 3: studies that gather more information about safety and effectiveness by studying different populations and different dosages and by using the drug in combination with other drugs, Phase 4: studies occurring after FDA has approved a drug for marketing.

Despite the favorable outcomes of cryopreservation, few studies have cryopreserved complex systems as tissue-engineered constructs. Altogether, these studies, which are compiled in Table 1, indicate that cryopreservation of tissue-engineered constructs is an effective approach, allowing the maintenance, and in some cases, the increment of the cellular recovery and viability, regardless of the type of the 3D support; scaffolds, microspheres, microcapsules, neurospheres, nanofibrous or even hydrogels.

Conclusion

This article reviewed the concepts and most recent strategies of TERM toward personalized medicine approaches with potential for developing innovative and more efficient clinical therapies.

The knowledge on stem cell biology and associated regenerative mechanisms has exponentially increased in the last few years, bringing new hopes and new options to the development of theranostic tools and to effective treatments.

Nevertheless, the mid- to long-term preservation platforms of cells as both disease and therapeutic agents, tissues or tissue-like substitutes to be ready on patient demand should be more deeply investigated to explore better solutions for human cell-based therapies that may also result in improved outcomes from pharmacological and biomedical sciences.

Although 3D fabrication technologies have been explored for decades, only in recent years have they been considered to be appealing for the development of 3D scaffold architectures for tissue engineering. Thus, the customization of tissue-like substitutes using these technologies is slowly arising but is expected to play a role in future regenerative strategies.

Altogether, the proposed TERM strategies may have an impact on the development of off-the-shelf tissue-engineered products as well as in innovative personalized tissue engineering therapies as alternative and effective treatments for a wide range of diseases and pathological conditions.

Expert commentary

Since the first concept of personalized medicine, remarkable effort and progress toward the development of technological outcomes have been accomplished in order to become a real and successful model in the clinical field.

Table 1. Cryopreservation of tissue-engineered constructs.

Scaffolding material	3D template	Cell type	Cryopreservation protocol	Major achievements	Reference
Natural-based polymers					
Alginate	Gels constructs	Human dental pulp stem cells	Optimization of CPAs: (1) DMEM + 10% EG; (2) DMEM + 10% EG + 1.0 M sucrose (3) DMEM + 10% EG + 1.0 M sucrose + 0.00025 M PVP (4) DMEM + 10% EG + 1.0 M sucrose + 0.0005 M PVP (5) DMEM + 10% EG + 1.0 M sucrose + 0.00075 M PVP (6) DMEM + 40% EG +0.6 M sucrose (7) DMEM + 10% FBS + 12% DMSO The samples were stored at −80°C for 1, 7, 14, 21 and 28 days	DMEM supplemented with 10% EG, 1.0 M sucrose and 0.00075 M PVP was the most optimal CPA No visual differences between the cell viability of control (gel constructs without cryopreservation-thawing process) and cryopreserved gel was found The cell characteristics were maintained during encapsulation and cryopreservation	[63]
	Neurospheres	Brain cells isolated Wistar rat embryos	The cryopreservation media consisted of serum-supplemented medium or serum-free CryosStor-CS10 solution, both containing 10% DMSO The samples were stored in the liquid N_2 for 1–2 weeks	Cryostor-CS10 solution enhances cryopreservation and post-thawing recovery for both nonencapsulated and encapsulated neurospheres compared with standard culture medium	[77]
	Gel layer	Neuroblastoma N2a and colon adenocarcinoma Caco-2	Cryopreservation media: (i) Standard medium: DMEM supplemented with 10% heat-inactivated FBS and 4.5 g/L glucose (ii) Differentiation medium: DMEM+ 2% FBS + 15 µM retinoic acid) supplemented with 10% DMSO or CryoStor™ (BioLife Solutions, Bothell, WA, USA) Stored of the samples at −80°C for 1–2 weeks	Optimization of cryopreservation of adherent cells in a fully differentiated state The cryopreservation protocols facilitated the reduction in batch-to-batch variability and normalizing passage age	[78]
	Microspheres	Human MSCs	Cryopreservation medium: 10% FCS and DMSO at 5% and 10% Cooling was manipulated accordingly to three protocols: *Protocol 1*: 2 steps slow cooling *Protocol 2*: 3 steps slow cooling with induced ice formation *Protocol 3*: rapid 1 step freezing Cryopreserved samples were kept at −196° C for a month	The viability and metabolism of MSCs in alginate microspheres was higher with 10% DMSO The highest viabilities and metabolic rates were obtained following the protocol 2. After cryopreservation by protocol 2, alginate microspheres with encapsulated MSCs were capable of achieving multilineage differentiation	[79]
Alginate-gelatin	Cryogel scaffolds	Human MSCs	Cryopreservation with cryo-medium containing 10% DMSO The samples were stored at least 24 h in liquid N_2	Short culture times before cryopreservation (0.5 and 2 h) are preferable for adherent hMSCs than longer times (24 h), showing higher viability and recovery	[80]
APA	Microcapsules	Murine C_2C_{12} myoblasts	DMSO concentrations (1%, 5%, 10%, 20% and 30%) The cells were stored in liquid N_2 for 45 days	Freeze/thawed microencapsulated cells using 10% DMSO showed the most suitable features in terms of *Epo* release	[73]
	Microcapsules	MSCs genetically modified (D1-MSC)	CPA solutions combining DMSO, glycerol and trehalose at different concentrations (10% DMSO, 5% DMSO, 10% glycerol, 10% trehalose, 5% trehalose + 5% DMSO, 5% trehalose + 2.5% DMSO, 2.5% trehalose + 2.5% DMSO, 2.5% trehalose + 5% DMSO) Cryovials were stored in liquid N_2 for 2 weeks	10% DMSO represents the most suitable solution for encapsulated MSCs Nonpenetrating cryoprotectants such as trehalose do not provide an appropriate recovery for microencapsulated cells	[68]
Collagen	Scaffold	Mouse fibroblasts	10% DMSO, 15% FBS and 0.4% antibiotics The cells were stored in liquid N_2 for 7–12 days	The cryopreservation of fibroblasts immobilized within a PVF scaffold reveals an efficient process. Besides, no negative effects on cell recovery were observed	[81]
k-Carrageenan	Hydrogels	Human ASCs	10% DMSO in FBS cells were stored in liquid N_2 for 1 month	The hydrogels withstand the cryopreservation maintaining their structural integrity, while assisting cells proliferation and chondrogenic potential	[64]

(*Continued*)

Table 1. (Continued).

Scaffolding material	3D template	Cell type	Cryopreservation protocol	Major achievements	Reference
Silk	Nanofibers	Human MSCs derived from umbilical cord	Cryopreservation solutions: (1) 10% DMSO, 50% FBS (2) 40 mM trehalose (3) 40 mM ectoin (4) 40 mM trehalose and 40 mM ectoin (5) 40 mM trehalose, 40 mM ectoin in 100 µg catalase (6) 40 mM trehalose, 40 mM ectoin and 2.5% DMSO (7) 40 mM trehalose, 40 mM ectoin, 2.5% DMSO and 100 µg catalase as antioxidant The samples were stored in liquid N_2 for 7 days	Post-thaw cell proliferation rate was higher in solutions containing trehalose/ectoin with 2.5% DMSO than other freezing solution The addition of catalase used as antioxidant has marginally increased cell viability	[82]
Gelatin and collagen/elastin	Scaffolds	Human fibroblasts and keratinocytes	Cryopreservation medium: 70% FAD medium (F12: DMEM = 1:3), 10% FCS, 0.1 nM choleratoxin, 0.4 µg/mL hydrocortisone, 50 µg/mL ascorbic acid 20% serum and 10% DMSO The samples were stored 24 h in liquid N_2	Cryopreservation has no negative influence on vitality and differentiation capacity of cultured constructs	[83]
Natural and synthetic polymeric blends					
Polycaprolactone-gelatin	Nanofibrous scaffolds	Bone marrow-derived porcine MSCs	Vitrification strategy at low concentrations of CPA (10% EG; 25% EG) and VS (40% EG and 0.6 M sucrose) The samples were stored in liquid N_2 less than 24 h	Vitrification approach is effective in cryopreserving these TECs with high cell viability while maintaining their integrity	[69]
Blend of starch and poly (caprolactone)	Fiber mesh Scaffolds	Goat bone marrow stem cells	Cryopreservation medium of 10% DMSO and FBS The samples were stored in liquid N_2 during 7 days	Maintenance of cell-viability and scaffolds properties upon cryopreservation	[62]

ASC: adipose stem cells; APA: alginate-poly-L-lysine-alginate; CPA: cryoprotective agent; DMEM: Dulbecco Modified Eagle Medium; DMSO: dimethyl sulfoxide; EG: ethylene glycol; FBS: fetal bovine serum; FCS: fetal calf serum; MSC: mesenchymal stem cells; N_2: nitrogen; PVF: polyvinyl formal; VS: vitrification solution; PVP: polyvinylpyrrolidone; TECs: tissue engineering constructs.

Indeed, advancements in the complex and multidisciplinary strategies of TERM as cell-based therapies, drug and cell delivery, as well as diagnosis and therapeutic tools, and the development of 3D tailored tissue like substitutes, have proved to be particularly interesting and promising for the progress and exploitation of specialized therapies.

The significance of stem cell technologies in cell therapies has been supported with clinical evidence. The selection of stem cell sources within an autologous context would allow customized exploratory approaches with potential to manage and treat several pathological conditions. Although universal cells can also impact regenerative medicine procedures, autologous sources will always be privileged especially if there are easily accessible and whose procedures patients comply with. Moreover, autologous cells are fully compatible and liberated from potential intraspecific restrictions.

Furthermore, the ability to generate multifunctional systems and the customization of scaffold architectures that perfectly fit to individual tissue defects using diagnostic scans or *in situ* delivery approaches, as injectable hydrogels, enables revealing and powerful technological tools that could revolutionize tissue regeneration and tailoring health care to the individual patient.

Furthermore, personalized TERM strategies are envisioned to evolve into more effective and successful 3D templates with regenerative action. Advances in 3D printing technologies will allow moving from time-and labor-intensive fabrication technologies into mass production of patient-oriented tissue and organ substitutes, which will lead to a cost reduction and an easier access to these technologies in medical institutions. It is also expected that 3D bioprinting advances will also influence the progress of imaging technologies. This will result in improved equipment with improved resolution and software to translate tissue scans with a higher level of detail and information into a virtual 3D model. Different properties of complex biological systems could be accurately combined, for instance, in the case of bone, the reconstruction of structural and biomechanical properties of cortical and trabecular bone, vascular vessels and marrow within the same 3D template using multiple biomaterials with different properties and cells or growth factors. These outcomes will also benefit interface tissues that require specific properties and structures that fuse with the nearby tissue with complementary functionality.

The combination of all these developments with the possibility to cryobank cells and more complex systems as tissue-engineered constructs assists in the translation of effective off-the-shelf strategies involving custom-made products available upon request.

Thus, the advances described are paving the way for enhanced personalized treatments searching for innovative and effective solutions to promote real tissue regeneration meeting individual patient requirements and needs.

Five-year view

Traditional medical practices often involve randomized strategies to treat the masses, but this approach has been revealed not to be always the best nor the most cost effective. Individual needs should be considered so that individual requirements can be successfully fulfilled.

Within the next five years, it is expected that medical practice will continue to evolve toward a better and customized health care, with new strategies to be developed so as to understand and improve current therapeutic limitations, envisioning an increased effort to tailor medical treatments to the individual characteristics of each patient. Ongoing technological advances in medicine will exponentially increase the level of detail and information, bringing new knowledge and the need to provide more precise diagnosis and pathology management.

Scientific developments and clinical trials will help to understand and guide personalized strategies toward a successful clinical scenario. This pathway will be assisted by the progress on stem cell technologies, upscaling and mid- to long-term storage strategies so that cell-based therapies can be widely available and routinely applied in medical procedures. Since intraspecific variations can interfere with follow-up and treatment outcomes, autologous setups would be an ideal option. With the iPSC technologies and stem cells from selective sources, these variations could be minimized or even eliminated and upscaling would become a more tangible reality.

In order to facilitate translation and to provide off-the-shelf products for patient on demand, preservation methodologies are a critical step of the process. Cryopreservation is a very promising methodology, and new protocols that require no cryo-additives or preservatives that may interfere with cell metabolism and function should be investigated. Magnetic cryopreservation may be a promising methodology to be more deeply explored in future.

Recently, cell banks have been established worldwide. Nevertheless, cryobanks of tissues, tissue-like substitutes as scaffolds or constructs, and other 3D complex structures are still at their infancy, but are expected to grow and facilitate tissue-like substitute storage for clinical translation.

Tissue-engineered scaffolds and constructs have been evolving with increased complexity, including improved functionalities and hierarchical structural motifs to recapitulate native tissue and to be recognized by the cells. Thus, it is likely that not only the scaffold will evolve but 3D biofabrication technologies will also progress into more sophisticated tools to accurately reproduce the detailed information from medical scans into a 3D defect model to be built upon request, through a simple and fast process with state-of-the-art software. It is envisioned that, in a relatively short period of time, this kind of technology may become available in clean rooms close to the surgical theater of some leading-edge hospitals to assist grafting or replacement surgeries with customized 3D scaffolds.

Undoubtedly in five years time several hurdles will remain, but surely several of these approaches and technologies will be one step closer to meet the enthusiastic challenges of personalized medicine and revolutionize the therapeutic field with custom-made therapies and effective tailored treatments for a wide range of pathologies.

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Key issues

- Personalized medicine brings a new hope for the development of patient-oriented effective therapeutics.
- Tissue engineering and regenerative medicine (TERM) strategies contribute to the development and exploitation of technologies with translational potential toward personalized medicine therapies.
- TERM strategies potentiate the generation of innovative off-the-shelf tissue-engineered products to be available on patient request.
- Cell sources play an important role, especially the ones explored in an autologous context that eliminate intraspecific variations and incompatibilities, and reduce ethical and regulatory procedures.
- Hemoderivatives such as platelet lysates and platelet-rich plasma have proved their versatile therapeutic action in several medical conditions and can also assist in the establishment of humanized (xeno-free) cell culture methodologies for clinical therapies.
- 3D biofabrication technologies are appealing for the development of patient-customized 3D scaffolds to replace damaged tissue or organs with precise architectures that tend to replicate native structures in complexity and at a cellular scale.
- Other strategies such as injectable hydrogels are also promising for personalized therapies that do not require a mechanical support while regeneration occurs.
- Cryobanking of cells or tissue-engineered constructs, as the ones developed by 3D technologies, facilitates the translation of custom-made products, available upon request, minimizing waiting time, and health-care and social associated costs.
- The customization of therapies and treatments will meet patient needs and improve patient recovery and follow-up with potential to revolutionize medical routine procedures.

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