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REVIEW

Emerging technologies in organ preservation, tissue engineering and regenerative medicine: a blessing or curse for transplantation?

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SUMMARY

Since the beginning of transplant medicine in the 1950s, advances in surgical technique and immunosuppressive therapy have created the success story of modern organ transplantation. However, today more than ever, we are facing a huge discrepancy between organ supply and demand, limiting the potential for transplantation to save and improve the lives of millions. To address the current limitations and shortcomings, a variety of emerging new technologies focusing on either maximizing the availability of organs or on generating new organs and organ sources hold great potential to eventully overcoming these hurdles. These advances are mainly in the field of regenerative medicine and tissue engineering. This review gives an overview of this emerging field and its multiple sub-disciplines and highlights recent advances and existing limitations for widespread clinical application and potential impact on the future of transplantation.

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Introduction

Despite the potential of organ transplantation to save and improve the lives of millions, there is a major imbalance between organ supply and demand. According to United Network for Organ Sharing (UNOS), 114 125 people are registered as wait-list candidates as of December 2018, with only 33 431 organ transplants having been performed since the first of January, 2018 [1]. The same trend is seen in the Eurotransplant (ET) region, where a total of 14 733 patients were active wait-list candidates in 2017 with only 6636 transplants performed in the same year [2]. According to UNOS, on average, 20 patients die each day while waiting for a

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life saving transplant – a number that dramatically underlines these shortcomings [1]. Some of the factors posing major barriers to meeting today's health care demands are inadequacies in organ storage and preservation as well as complex transplant logistics. Organ and tissue transplantations can only be carried out within a narrow timeframe, especially when standard preservation strategies are applied, limiting geographic distances over which organs and tissues can be transported and shared. In fact, a large proportion of donor kidneys get discarded simply because the maximum preservation time was exceeded before allocation could be completed [3,4]. In addition, time and geographic constraints complicate immunologic matching, a factor which increases the risk for transplant rejection and can result in an inferior outcome [5,6].

In order to truly meet the potential of organ transplantation, novel strategies to overcome these hurdles are desperately needed. In the last decade, the fields of regenerative medicine (RM) and tissue engineering – two terms commonly used synonymously – evolved, offering different approaches to tackle currently unsolved problems. Both fields cover a variety of subdisciplines combining engineering as well as biological principles [7] (Fig. 1). Transplant medicine benefits from various aspects of RM, as they offer potential strategies to increase organ utilization, expand the donor pool, generate new organ sources, reduce disparities in and enable fast access to transplantation ('off-the-shelf'), facilitate better matching and lower immunogenicity as well as allow more flexibility and better planning of transplantations [8]. The ultimate goal is the generation of immunosuppression-free, universally accessible organs-on-demand that lack damage from organ preservation and storage [9].

Thus far, research focuses on two distinct areas namely: strategies to improve organ preservation and the generation of new organs and organ sources [10]. The former is addressed by machine perfusion and cryopreservation, the latter by three-dimensional (3D) bioprinting, de- and recellularization, xenotransplantation, stem cell technology and interspecies organogenesis (Fig. 2). The following review will discuss these approaches and give an update on their current state.

Advances in organ and tissue preservation

For decades, organ cooling has been an essential part of organ and tissue transplantation. Low temperatures decrease metabolic rate by factor 1.5–3 per 10 °C, so cooling a donor organ to 4 °C reduces its metabolic rate to 10–12% of the baseline [11]. Slowing down metabolic processes reduce oxygen consumption and ATP depletion. After organ recovery, tissues and organs lack blood supply. This leads to oxygen deprivation, anaerobic respiration, metabolic waste accumulation and electrolyte imbalances, all contributing to *ischemic injury*. Upon reperfusion, the reintroduction of oxygen and subsequent production of reactive oxygen species (ROS) aggravates this damage (*reperfusion injury*). Despite the injurious potential, static cold storage (SCS) of donor organs is currently considered standard of care in organ transplantation. However, it only enables the preservation of organs and tissues for a few hours, depending on type of organ or tissue [12]. Using strategies to decrease tissue ischemic injury – through organ cooling or machine perfusion – is the basis of multiple preservation techniques aimed at increasing organ viability and thus availability.

Subzero organ preservation

One example of hypothermic preservation technique is that of subzero organ preservation. Nature has provided us with a series of examples of such strategies utilized by several animal species, suggesting that these are not a futuristic dream but actually pose a way to overcome the narrow timeframe set by SCS. This should, at least hypothetically, allow low enough temperatures for unlimited preservation time [13].

One of the most famous examples of subzero preservation is the wood frog *Rana sylvatica* [14], which survives winter with up to 65% of its total body water frozen solid. Yet, along with the *R. sylvatica*, various other insects [15] and fish species [16,17] take advantage of ice tolerance and ice avoidance strategies. Tolerance strategies are addressed by selective and highly orchestrated ice formation limited to the extracellular space [18], preferably absent in vital organs to avoid crystallization and dehydration and thus lethality. Ice avoidance – utilized by animals such as the brine shrimp *Artemia* – can be achieved by freezing point depression, a strategy facilitated by increased concentrations of cryoprotectants such as glucose, glycerol and blood colloids referred to as 'antifreeze peptides' [16,19] increasing molality of body fluids [20]. For application in human organ preservation, cryoprotective agents (CPA) for infusion have been discovered, mimicking of the naturally occurring ice avoidance strategies [21]. Despite the need for these agents, many currently have toxic properties, substantially contributing to post-transplant morbidity and mortality [4].

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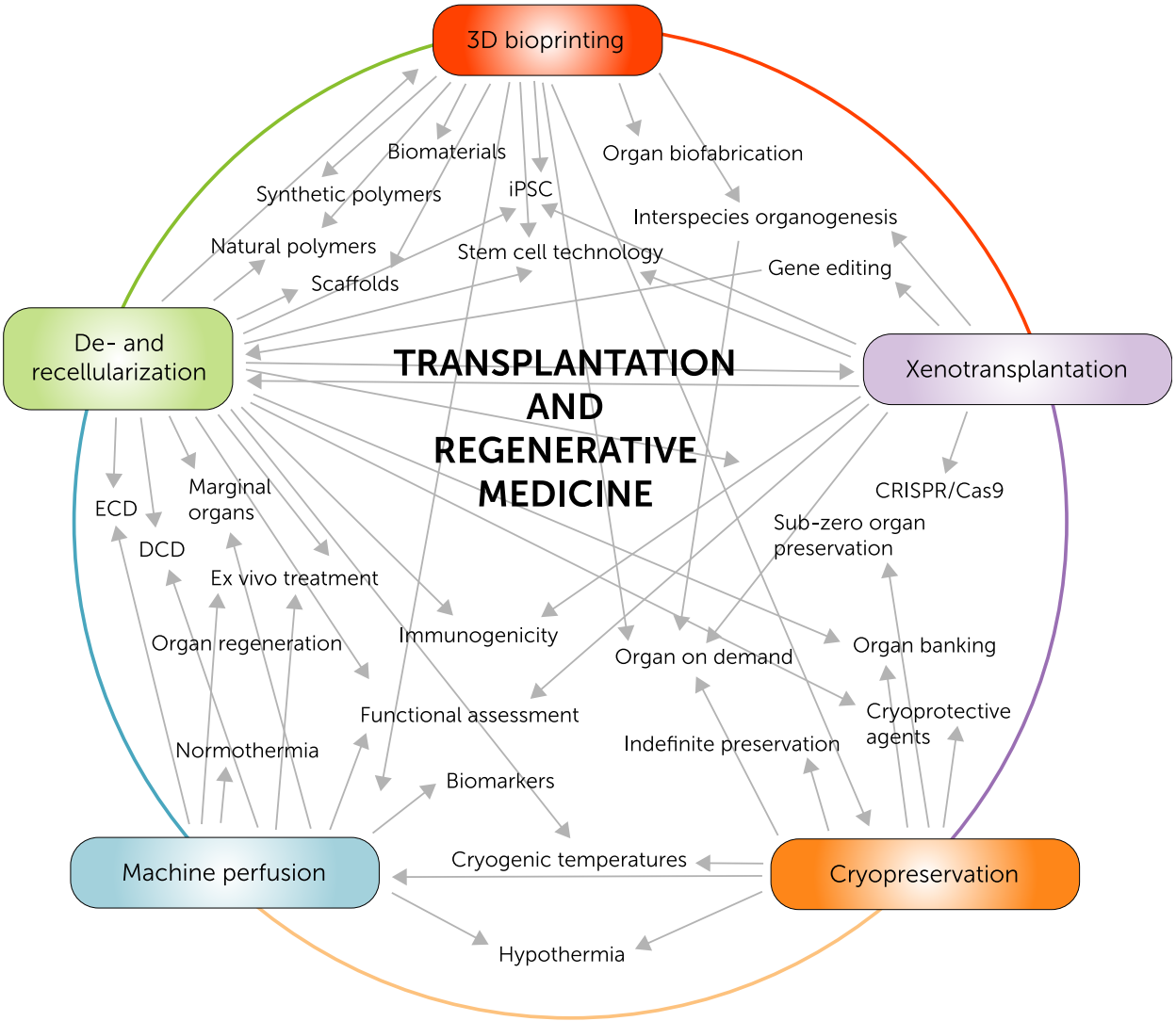

Figure 1 Interactions of different novel technologies in regenerative medicine and organ transplantation.

Preliminary attempts in subzero organ preservation have been undertaken by organ freezing, but limitations of this strategy still pose significant difficulties in application. In contrast to single-cell suspensions [22] such as blood products, bone marrow, or in the context of fertility treatment [13,23], freezing of whole organs is much more complex. Ice crystal formation itself generates mechanical stress and can cause cell rupture [24]. In addition, the freezing process leads to an increase in extracellular electrolyte concentration which further generates nonphysiological osmotic pressure gradients, leading to fluid efflux and subsequent cell dehydration and shrinking [21,25,26]. It is, therefore not surprising that early after the first successful liver transplantation in 1963, the first organ freezing attempts of canine livers with addition of 33% glycerol as cryoprotectant did not show the anticipated outcome; only limited survival for a few hours could be achieved after heterotopic transplantation [27]. Because of the destructive potential of ice, approaches of *ice avoidance* play a central role in subzero organ and tissue preservation.

Vitrification

Vitrification, a method where organs and tissues are cooled down to cryogenic temperatures (i.e. −150 °C) without ice crystal formation, is another promising strategy [21]. Ice avoidance is facilitated by the addition of a mixture of different cryoprotectants with a very rapid but highly controlled cooling rate [28].

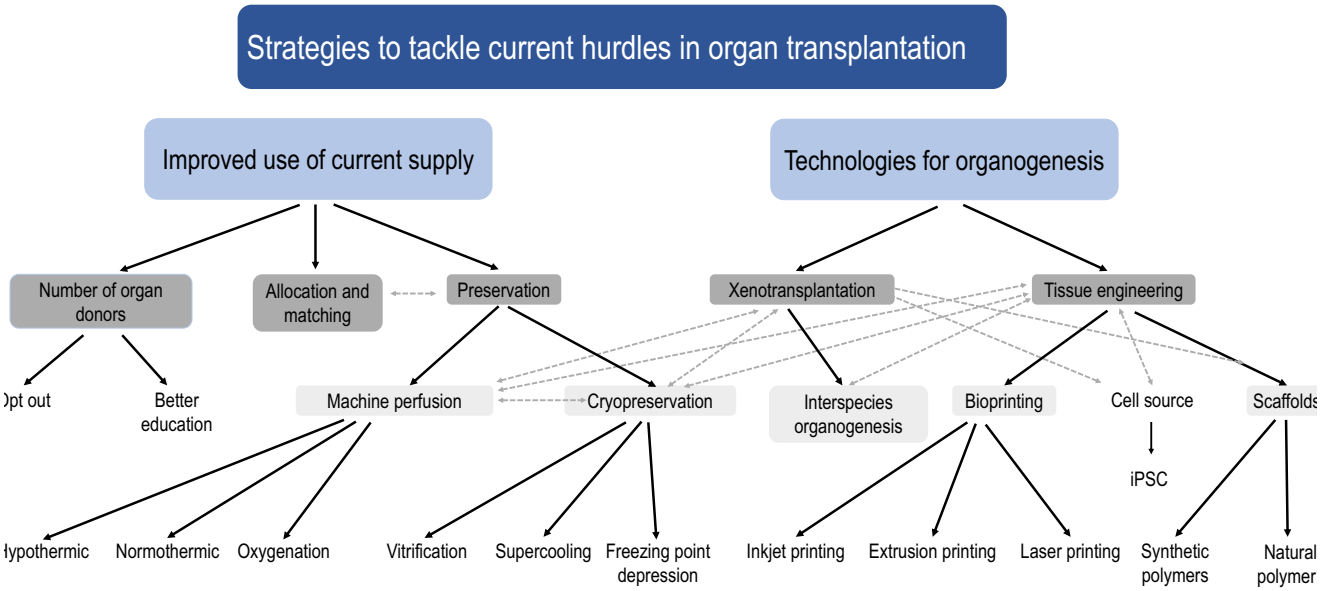

Figure 2 Strategies to tackle current hurdles in organ transplantation that might benefit from new advances in regenerative medicine.

Cryopreserved organs and tissues reach the glass transition temperature – about −137 °C for water – where preserved items enter an amorphous or glass-like conformation, allowing indefinite preservation [29]. Along with potential toxicity of CPAs, the process of rewarming following vitrification remains a major obstacle – as rewarming must occur at a certain rate to prevent ice crystal formation [30] – and new strategies to optimize this process are currently in development [31].

Thus far, successful cryopreservation using vitrification has mostly been achieved in small structures that can be rapidly cooled and rewarmed such as cell suspensions, blood vessels and reproductive organs [23]. For vitrification of whole organs, studies are limited to the kidney. Fahy *et al.* [32] were able to successfully autotransplant a rabbit kidney after vitrification with concomitant contralateral nephrectomy. This is currently the only report of a successful vitrification in solid organ transplantation. In the setting of vascularized composite allotransplantation (VCA), successful vitrification and replantation of rodent epigastric flaps [33] as well as above-knee amputated hindlimb grafts have been reported; the latter, however, had a postoperative follow-up limited to 72 h [34].

Supercooling

The most promising results in the field of cryopreservation thus far have been seen in subzero preservation using supercooling techniques. In these regimens, temperatures range just below 0 °C, and ice formation is prevented by the addition of CPAs [35,36]. Even a slight decrease in temperature below 0 °C can result in markedly improved preservation, as demonstrated by increased ATP levels in liver grafts stored at −0.8 °C compared to 4 °C [37].

Using this technique, rat liver grafts were successfully transplanted by Yoshida *et al.* [37] after 72 and 96 h of supercooling at −6 °C. For loading of cryoprotectants [38] and post-supercooling recovery, a subnormothermic machine perfusion system was used [39]. With preservation using supercooling, animal survival was 100% after 72 h preservation and 58% after 96 h [40]. Inspired by the fact that the freezing point of water can be decreased down to −20 °C under high pressure, Takahashi *et al.* demonstrated that pressurized rat liver grafts could be cooled down to −2 and −3 °C for 5 h using a pressure of 5 MPa. All transplanted animals survived for 2 weeks; however, a pressure-dependent diffuse haemorrhage and vacuolar degeneration of liver grafts was observed [41]. Experiences in supercooling of lung and heart grafts are limited to *in vitro* experiments [24].

Machine perfusion

Machine perfusion (MP) poses another possible strategy to improve and prolong *ex vivo* organ and tissue preservation. The first clinical attempts in this field were made in the 1960s by Belzer *et al.* [42], but they failed to demonstrate superior outcomes compared to SCS [42–44]. Over the last two decades, however,

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because of progressive organ shortage and a higher proportion of extended criteria donors (ECD), machine perfusion has re-emerged. Now, systems for both hypo- and normothermic machine perfusion are commercially available for heart [45], lung [46,47], liver [48–50] and kidney grafts [51,52]. Most available systems are oxygenated and either preservation solutions or diluted blood serve as perfusate [53]. Whereas hypothermic approaches focus on slowing down metabolic rates and preserving ATP concentrations, normothermic ones try to generate an *ex vivo* environment mimicking physiologic *in vivo* conditions [54]. With this approach, organs are metabolically active and, in contrast to hypothermic models, functional markers of donor organs can be assessed (i.e. bile production, urine output, oxygenation) [55]. Both normothermic and hypothermic approaches allow measurement of biomarkers which could give additional information on organ quality. Several studies focus on predictive biomarkers, including, but not limited to, neutrophil gelatinase-associated lipocalin, kidney injury molecule-1, liver-type fatty acid binding protein, endothelin 1, micro ribonucleic acids (miRNAs) and antisense oligonucleotids [51,53,56–58]. Because of the large number of perfusion systems and preclinical and clinical trials, we exemplarily discuss the potential of machine perfusion in the setting of liver perfusion in the following section. Various reviews and reports on perfusion systems for the heart [45,59], lung [46,47,59–61], kidney [51–53,62–66], and initial studies in pancreas [67–69] and VCA [70–75] are available elsewhere.

Machine perfusion in liver transplantation

Currently, two devices are available allowing hypothermic MP in the context of liver transplantation. One, Liver Assist (Organ Assist, Groningen, the Netherlands) is used for 'Hypothermic Oxygenated Perfusion' (HOPE) [76]. Inital results indicate a significant reduction in peak alanine-aminotransferase (AST) levels, intrahepatic cholangiopathy and biliary complications along with significantly improved 1-year graft survival when compared to SCS with donation after cardiac death (DCD) donors. In addition, end-ischemic dual hypothermic oxygenated machine perfusion (DHOPE) – a technique that combines oxygenation and perfusion through canalizing of the portal vein and hepatic artery – has a significant influence on biliary ischemia/reperfusion injury (IRI) in DCD livers, leading to a decrease in non-anastomotic biliary strictures (10% vs. 35%; $P = 0.15$) [50].

Another hypothermic device, currently in the process of securing regulatory registration, is LifePort® Liver (Organ Recovery Systems) Transporter. Prototype studies confirmed lower early allograft dysfunction, fewer biliary complications and a shorter hospital stay when compared to SCS [77,78].

In the normothermic machine perfusion (NMP) sector, the Oxford group has successfully demonstrated that their OrganOx Metra NMP device (OrganOx, Oxford, the UK) leads to a reduction in peak AST levels and early allograft dysfunction despite more than 50% longer mean preservation time and a 50% lower rate of organ discard in the NMP group compared to SCS in donation after both brain and cardiac death [49]. Liver assist, which can operate from 10 to 38 °C, has also been assessed in NMP. After perfusion of 47 livers that had been considered unsuitable for transplantation by all UK centers, 22 livers could eventually be transplanted upon good performance on NMP [79]. Machine perfusion, regardless if hypo- or normothermic, is rapidly evolving, and several studies are currently ongoing to further evaluate its effectiveness (NCT02522871, NCT03124641).

Not only is machine perfusion beneficial to allograft preservation, storage and transportation, this technology also offers the perfect platform for *ex vivo* treatment of organs in order to minimize injury or even facilitate regeneration and tissue repair [64,80,81]. This is especially beneficial in the context of ECD and DCD donation, as these grafts are most susceptible to ischemic injury and would profit the most from reparative attempts. This provides the unique potential to deliver treatment solely to grafts during *ex vivo* perfusion, bypassing complications that arise from systemic delivery in the recipient. Different approaches of *ex vivo* therapies have already been tested including cytokine absorption [82], addition of noble gas [83], as well as stem cell and gene therapies. Cytosorb absorber, a nonselective filter of cytokines and vasoactive substances, showed improvement in initial renal blood flow in the setting of porcine kidney preservation [82]. The application of 70% argon during MP displayed favorable results on renal blood flow; however, no measurable effects on other different outcome parameters were detected [83].

Stem cell delivery is an upcoming treatment strategy in MP. These cells possess potent regenerative properties mediated through the release of paracrine factors. Several preclinical studies are currently under way to investigate the effect of mesenchymal stem cells (MSC) [84–86] and stem cell-derived extracellular vesicles [85,87,88] in the setting of kidney and liver MP with

promising preliminary results [89]. Gregorini *et al.* [85] demonstrated that MSC/MSC-derived extracellular vesicles were able to protect DCD kidneys of rats during 4 hours of HMP. Treated-rat kidney grafts displayed significantly less global ischemic damage as well as an up-regulation of genes encoding enzymes for improved cell energy metabolism and membrane transport. Along with in the murine setting, scientists are currently investigating the potential of MSC therapy in the setting of normothermic *ex vivo* perfusion in pig kidneys [86].

In addition, early studies investigating the role of gene therapies have been conducted, offering the possibility to alter gene expression in isolated organ and tissue grafts. This attempts to further attenuate injurious processes during storage and can even provide a mechanism to repair nonoptimal donor organs. First steps in this setting have been made by Brasile *et al.* [90] who demonstrated that the addition of recombinant adenovirus encoding the green fluorescence protein could actually target the vascular endothelium of canine kidneys on NMP. The potential of small interfering RNA (siRNA) has further been shown by Cui *et al.* [80]. The group was able to mitigate major histocompatibility complex class II expression on endothelial cells using siRNA targeting class II transactivator in human vessels and murine systems.

Organ and tissue biofabrification

The ultimate dream of transplant medicine would be the ability to generate on-demand a new and personalized organ for every patient in need of organ replacement. Whereas previously discussed techniques aim to fully utilize the potential of organs we already have; attempts in biofabrification aim to generate organs *de novo* or by seeding cells and biomolecules within a scaffold, combining tissue engineering and stem cell technologies [91].

Three-dimensional (3D) bioprinting

Bioprinting has been defined as a layer-by-layer deposition of biological materials along with living cells using computer-aided transfer processes [92,93]. The basic concept was developed in the 1980s, but only in 1993 Sachs *et al.* [94] were the first group to design a 3D printer for printing nonviable materials. In general, 3D bioprinting comprises of three steps [95–97]. First, a blueprint of tissues or organs has to be generated using a medical imaging technique (i.e. computed tomography or magnetic resonance imaging) [98,99] which is then further converted into a model incorporating material and cell composition and distribution. Next, by reducing it to a series of two-dimensional (2D) layers, the 3D structure can be printed layer-by-layer. Finally, the printed tissue or organ needs to be incubated and matured in a bioreactor [100,101].

To date, different bioprinting modalities, including inkjet-, extrusion- and laser- based printing technologies, have been developed and described in detail [91,102,103]. Not only is the complex cell component critical in bioprinting, but the development of printable biomaterials also poses a challenge, as they are required to exhibit distinct properties including specific viscosity and mechanical strength, biocompatibility, biodegradability and lack of immunogenicity [97]. Common biomaterials can be divided into synthetic and natural polymers [104]. Whereas synthetic polymers are processable and display favorable mechanical properties for printing, they typically lack cell-responsive motifs, negatively impacting cell proliferation and differentiation. Natural polymers, however, are highly biocompatible but often exhibit unfavorable mechanical characteristics [91].

Several clinical applications for 3D bioprinting exist, ranging from prosthetics and drug delivery systems and toxicity testing to tissue and organ regeneration [91,92,97,105]. For example, 3D printing can be used to fabricate individualized bone prosthetics displaying distinct mechanical properties for superior osteointegration [105,106] as well as cartilaginous structures. Zhou *et al.* [107] reported on the generation of a human ear-shaped cartilage using 3D printing for patients suffering from microtia, a congenital deformity of the outer ear. After seeding autologous chondrocytes and 3 months of *in vitro* culture, ear-shaped cartilage frameworks could be successfully implanted in a total of five patients.

Taniguchi *et al.* [108] generated a scaffold-free tubular trachea via 3D bioprinting using multicellular spheroids. Upon printing, the artificial trachea was matured in a bioreactor and transplanted into nine F334 rats. Average tensile strength of the artificial trachea was comparable to that of a naïve rat trachea, and histologic assessment of the artificial tracheal segment showed glycosaminoglycan deposits and some small capillary-like tubular formations consisting of $CD31^+$ cells, which increased in number after transplantation. Zhang *et al.* [109] were able to generate scaffold-free, 3D bioprinted nerve constructs using human gingiva-derived mesenchymal stem cell spheroids. In a rat model of segmental defects of the buccal branch of the facial nerve, nerve repair using this technology resulted in similarly

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organized nerve fascicles with similar immunofluorescence-measured expression levels of β-tubulin III and S-100β when compared to autografts.

Yet, bioprinting of whole vascularized organs for transplantation is still in an experimental stage due the complexity of biological tissues and its vascularization [91,92]. This and other discussed technologies' strategies further depend on a readily available, easily expandable and nonimmunogenic source of cells. Advances in stem cell generation, especially the discovery of induced pluripotent stem cells (iPSC), could potentially circumvent this obstacle [110].

Decellularization and recellularization

Another technique that provides promising strategies to generate tissue and organ scaffolds is organ de- and recellularization. These scaffolds, however, do not only serve as framework for cells; moreover, decelluarized extracellular matrix (dECM) has been shown to influence cell behavior relating to cell migration, proliferation and differentiation [111,112]. The final goal was to repopulate a size-matched extracellular-matrix (ECM) scaffold with patient-specific pluripotent cells and thus generate a tailored organ on-demand.

Decellularization of an organ or tissue is achieved through chemical, physical or enzymatic procedures which aim to remove all native cellular components while preserving the ECM with emphasis on maintaining its mechanical properties and ultrastructure. A large number of protocols and methods have been described in successful decellularization of various different tissue types and organs [113]. Most protocols are based on single or multiple freeze-thaw cycles, usually the first step of decellularization. During freezing and thawing, osmotic changes and ice crystal formation promotes cell lysis while only inducing minor changes in the mechanical properties of the ECM [114]. In a second step, different detergents are used to extract all natural cellular material from the organ, preferably without damaging ECM components either by immersion/agitation, vascular perfusion, via pressure gradients, or by using supercritical fluids [112,115–119]. Sufficient decellularization is essential to reduce immunogenicity [116] of scaffolds, but, in most instances, a small amount of cell components (i.e. DNA fragments, phospholipids) does remain [113]. Crapo *et al.* [111] describe standard metrics for effective decellularization after demonstrating that DNA residues over 50 ng were able to elicit immune response.

In order to rebuild and revive dECM scaffolds, seeding of terminally differentiated somatic cells or stem/progenitor cells is necessary [120]. This recellularization process is even more difficult to facilitate than the decellularization because of the huge diversity of the cell populations that need to be reconstituted. Recellularization is achieved via perfusion of cells through the vasculature [121,122] and direct injection into the dECM scaffold as well as into the ureter [123] or trachea [124] in kidney and lung grafts, repectively [125,126], with further maturation in special bioreactors [127,128]. Researchers recently were able to successfully recellularize the decellularized rat hearts [129] and human lungs [120,130] as well as rhesus monkey kidneys [131] with iPSCs.

Atala *et al.* [132] generated neo-bladders using donor-derived cells collected from bladder biopsies. As scaffold, either homologous decellularized bladder submucosa (first four patients) or biodegradable composite scaffold made of collagen and PGA (last three patients) was used. After *ex vivo* culture, muscle and urothelial cells were seeded on the scaffold and cultured for 5–6 days before implantation in the patients. In seven patients with myelomeningocele, an augmentation cystoplasty procedure was performed using the bioengineered bladder constructs. Except one urinary yeast infection, no postoperative surgical complications occurred, and the procedure increased volume and compliance as well as decreased mean bladder leak point pressure.

Despite these promising reports, recellularization of whole organ scaffolds remains challenging and further studies and refinements of the currently applied protocols are needed to make this field clinically applicable. Ongoing hurdles include the precise positioning of seeded cell types inside the organ or tissue scaffold, providing adequate oxygen and nutrient supply, enabling metabolic waste product removal and minimization of thromboembolic events during recellularization [112,115]. As well, there is currently a degree of difficulty in achieving adequate lymphatic drainage and vascularization of recellularized organs, something that needs to be addressed to optimize functional outcomes [9,133]. Nevertheless, other types of scaffolds and ECM generated via decellularization methods are already commercially available. Xenogeneic decellularized scaffolds, for instance, are regularly used for patients with wound-healing defects [115].

Xenotransplantation and interspecies organogenesis

The concept of interspecies transplantation, or the use of organ from other species than humans, is not a new

one. In 1905, Princeteau [134] inserted slices of rabbit kidney in a nephrotomy of a child with renal insufficiency. Despite a satisfying postoperative results with increased urine production, the child died on the 16th postoperative day as a result of pulmonary congestion. Initial, larger series of xenotransplantations using non-human primates as kidney donors were reported by Reemtsma *et al.* [135] in 1964 as the group was exploring alternative organ sources before human organs and dialysis were routinely available. He transplanted chimpanzee kidneys into a total of 13 patients with the longest patient and graft survival of 9 months. Starzl [136] was the first to perform chimpanzee-to-human liver transplantation in 1966, but only limited (<14 days) graft survival was achieved.

Xenotransplantation

Despite the initial experiences in xenotransplantation, where neither desirable patient nor allograft survival has been achieved, xenotransplantation has regained attention with the increasing organ shortage and the development of new techniques in the field of genetic engineering. As an organ source, pigs have proven to display the most favorable characteristics because of wide availability, good breeding potential, rapid growth, close anatomical similarity, low infectious transmission and social acceptance [137,138]. Still, a major obstacle to xenotransplantation is the immunologic barrier, which leads to hyperacute, acute and chronic allograft rejection [139] as well as dysregulated coagulation leading to consumptive coagulopathy [136,140,141].

To overcome these immunologic hurdles, genetic modifications have been made using various different approaches including somatic cell nuclear transfer, homologous recombination, zinc finger nucleases or transcription activator-like effector nucleases [142]. The recently discovered technique of clustered, regularly interspaced, short palindromic repeats-cas9 (CRISPR/Cas9) has significantly increased the speed and accuracy with which genetic modifications can be generated [143–145]. This novel technique has been used to knock out sugar epitopes specifically present in the porcine but not human system, such as α1,3-galactosyltransferase [146], N-glycolylneuraminic acid [147,148] and B1,4N-acetylgalactosaminyltransferase [149]. In addition, researchers also have targeted other epitopes that play distinct roles in xenograft rejection such as porcine von Willebrand factor [150] and complement component C3 [151], resulting in superior but still suboptimal graft survival.

In the pig-to-baboon setting, long-term allograft acceptance has been reported for heart xenografts. Mohiuddin*et al.* [152] demonstrated long-term survival (159–945 days in five consecutive recipient baboons) of cardiac xenografts from alpha 1–3 galactosyltransferase gene knockout pigs expressing human complement regulatory protein CD46 and human thrombomodulin transplanted into baboons using an αCD40 monoclonal antibody-based immune-modulatory regimen. Cessation of the αCD40 monoclonal antibodies after 100 days or 1 year caused recipient animals to developed anti-pig antibodies and resulted in xenograft failure.

In addition to immunologic challenges, the potential transmission risk of porcine endogenous retroviruses (PERVs), a provirus found in all pigs' DNA, raises concern [143]. With stress, this provirus can become activated and release viral particles into the bloodstream, though no case of human infection has ever been documented. Using CRISPR/Cas9 technology, Yang *et al.* [153] successfully inactivated 62 active PERV insertions, resulting in a substantial reduction in PERV infection in co-cultured human embryonic kidney cells.

Interspecies organogenesis

Deeper understanding of organ development and advances in pluripotent stem cell technology allowed the recent development of interspecies organogenesis. Yamaguchi *et al.* [154] successfully generated rat-sized mouse-iPSC-derived pancreata after injection of mouse-iPSC into Pdx-1 deficient (apancreatic) rat blastocyst. Islets obtained from these pancreata could successfully normalize and maintain blood glucose levels when transplanted into mice with streptozotocin-induced diabetes with only brief (5 day) immunosuppression. This and other studies by the same group focusing on blastocyst complementation [155] demonstrate the requirement for a specific niche to facilitate donor-iPSC-derived organ development (i.e. the absence of pancreas, kidney, liver, or others in host species) and suggests that host species determines organ size irrespective of donor source [154,156]. Limitations arise from incompatibilities generated by xenogeneic barrier, which can hinder interspecies chimerism of different tissues at various developmental stages [157].

Author perspective

Taken together, there are many exciting new possibilities to further develop, expand, and eventually transform the field of transplantation that arise from

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combining favorable aspects of all of the technologies outlined above. Examples include the need for perfusion devices for loading and unloading of necessary cryoprotectants in prolonged cryopreservation using vitrification or supercooling as well as the need for alternative preservation strategies; both of these needs could be met with machine perfusion systems. Machine perfusion has already proven successful in prolonging graft viability in the kidney, liver, lung and heart. In addition, we have seen some indication of initial success of this method also in vascularized composite allografts [70–75]. However, there is still ample room for improvement and optimization before it can be widely applied clinically. Furthermore, success of prolonging graft viability and thus time and distance over which an organ can be donated will only increase organ prevalence within the confines of the current donor pool. Even with routine machine preservation of potential donor grafts, we still may not be able to meet demands unless strategies for *ex vivo* organ optimization are incorporated in such technologies. The same will be true for advances in cryopreservation, which could be utilized in a similar manner. Thus far, these technologies are still in their infancy, and we have seen only moderate success of cryopreservation in the experimental and preclinical setting. However, the proof of concept of this method in transplantation holds tremendous potential that we can preserve organs indefinitely for potential organ banking in the not-too-distant future.

The field of 3D bioprinting is ever-evolving, with constant improvement in printers and technology arising with increased speed and resolution, thus increasing in its allure and future potential in transplantation. Yet, the field is currently limited by the fact that fabrication of larger and more complex structures requires increased quantity and diversity of cell types, a process that is not yet feasible. Existing studies utilize only particular cell types or stem cells, but they continue to have limitations in expansion capacity and difficulty in differentiation to adequate lymphatics, innervation and neovascularization. In addition, while 3D printing of basic structures (like trachea) for transplantation was achieved in a few select cases, the majority of attempts was not successful. Overcoming these hurdles will be critical before this strategy can be implemented for organ restoration or as a clinical alternative to transplantation [158].

Additionally, the technique of blastocyst complementation has shown stunning preliminary results in generation of fully donor-derived organs using iPSC, but significant ethical and practical concerns remain [157].

Conceptually, interspecies organogenesis poses an ideal solution to an organ shortage, with a theoretical offer to grow an organ specifically for the individual recipient. Currently, we have seen the ability to grow a mouse pancreas in a rat, but we have been unable to generate kidneys in mice with renal agenesis [159]. Furthermore, all attempts with human stem cells or mouse blastocysts have had just limited success [155,160,161]. This implies that currently, while for some organs interspecies organogenesis is possible, it remains limited by species and organ type. Current research thus focuses on possible modifications of donor iPSCs, better engraftment strategies and enhanced the purity of cellular composition of generated organs [9].

Conclusion

Regenerative medicine and tissue engineering have emerged as approaches to tackle some of the currently most pressures limitations in transplant medicine. Both fields are widely overlapping and include approaches to maximize organ and tissue utilization of already available donor organs. This is achieved either via machine perfusion or advanced cryopreservation techniques as well as methods focusing on the fabrication of new organs either by optimization of discarded donor organs as source of organ scaffolds for repopulation, by 3D bioprinting, or via interspecies generation of organ sources using xenotransplantation or blastocyst complementation techniques. Major discoveries in gene editing, stem cell technology and biofabrication over the last decade have enabled significant advances and the advent of many truly disruptive approaches and technologies – some of which have been considered fantasies or science fiction for centuries – that could forever change the field of transplantation as we know it. However, it will need a very thoughtful and cautious approach to incorporate these technologies into the current practice of transplantation in order to ensure the best utilization of the potential they might hold to reduce the gap between the ever-growing discrepancy between organ supply and demand.

Authorship

M.F. performed literature search, wrote and revised the manuscript and provided figures, G.Y. wrote and critically revised the manuscript, E.J. wrote and critically revised the manuscript, B.G. conceptualized, critically revised, and finalized the manuscript.

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