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REVIEW ARTICLE

Mini-organs forum: how to advance organoid technology to organ transplant community

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SUMMARY

The generation of human mini-organs, the so-called organoids, is one of the biggest scientific advances in regenerative medicine. This technology exploits traditional three-dimensional culture techniques that support cell-autonomous self-organization responses of stem cells to derive micrometer to millimeter size versions of human organs. The convergence of the organoid technology with organ transplantation is still in its infancy but this alliance is expected to open new venues to change the way we conduct both transplant and organoid research. In this Forum we provide a summary on early achievements facilitating organoid derivation and culture. We further discuss on early advances of organoid transplantation also offering a comprehensive overview of current limitations and challenges to instruct organoid maturation. We expect that this Forum sets the ground for initial discussions between stem cell biologists, bioengineers, and the transplant community to better direct organoid basic research to advance the organ transplantation field.

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Introduction

Within the last decade, we have been living the emergence and explosion of a cutting-edge technology aimed to produce micrometer to millimeter versions of human organs, the so-called organoids. Though these developments seemed new, we like to recall that these powerful tools are the result of seminal works carried out from the beginning of the last century. Indeed, by that time the main question on how cells self-organize and subsequently arrange in space and time within the developing organ(s) imposed the design of apparently rudimentary culture systems to monitor and understand early steps of organogenesis (some of them still under use). Other studies by the 1900s pioneered the hanging drop culture system to study the origin of nerve cells by culturing fragments of frog embryonic nerves in a drop of lymph employing a simple coverslip [1]. Collectively, these and other approaches allowed for the direct observation of growing tissues of different embryonic origins culminating by the 1950s in the generation of methods sustaining the culture of tissue fragments (by the watch glass method—Strangeways and Fell, 1926) [2] and organ slides (through the lens paper method—Trowell 1954, 1955) [3,4]. All these methodological advances ran in parallel with pioneering experiments highlighting the intrinsic capacities of tissues to follow predetermined developmental and functional patterns during regeneration and homeostasis [reviewed in [5]].

Together with all these discoveries, other pioneer works defined proper conditions to isolate cells from tissues and sustain their growth and survival in vitro. These advances included the purification of cell tissue culture reagents such as collagen or collagenase, the later allowing for the generation of mammary organoids [6]. Furthermore, the research on the composition of the extracellular matrix (ECM) of chondrosarcomas [7,8] culminated in the generation of Matrigel [9] the most used ECM in the organoid field today. By 1980, technical skills and knowledge of ECM biology served as a major tool to continue exploring on the derivation of 3D cultures, a key part to set the basis for organoid culture. Illustrating the first examples of organoids, Mina Bissell and colleagues pioneered the development of 3D mammary gland cultures, starting from single mammary cells embedded in laminin-rich ECM [10]. Collectively, all this knowledge was applied by Hans Clevers and colleagues to culture single-cell suspensions of Lgr5+ intestinal stem cells embedded in Matrigel in the presence of defined culture conditions generating 3D intestinal organoids with a crypt-villus architecture [11]. Soon after the derivation of mini- or micro-organs has been also achieved from human pluripotent stem cells and human adult stem cells, and within the last five years, the organoid technology has allowed to start addressing complex questions related to organ-specific stem cells biology, homeostasis, and regeneration [reviewed in [5]]. Nevertheless, the increasing refinement in the procedures to generate these complex culture systems has imposed developments from the field of bioengineering with the aim to externally control the extent of differentiation and function of these cell culture platforms. In this regard, the Lutolf laboratory has pioneered on the design and fabrication of microfluidic devices capturing important aspects of the gut anatomy, namely, villi shape and fluid flow, leading to the generation of gut homeostatic organoid culture systems with an increased life span [12]. Other works from the same group also led to the definition of biomaterials phenocopying physical properties of the gut native tissue (namely rigidity) overall leading to novel developments to externally control gut organoid derivation [13,14]. Similar approaches have been used to derive kidney organoids from human pluripotent stem cells [15] Besides the development of these cutting-edge technologies, it is remarkable that the field nowadays makes use of culture systems developed almost one century ago to maintain the organoid culture in vitro as well as to test the differentiation potential of these culture systems ex vivo (Fig. 1).

At the same time and with regard to the recapitulation of complex features encountered in native organs such as vascularization, seminal work from the Lewis laboratory explored on the use of microfluidic devices to culture human pluripotent stem cells derived kidney organoids under constant fluid flow to achieve a higher degree of vascularization compared to static culture conditions [16]. Other approaches directed to provide vascularization into organoid models stand in the use of co-culture systems facilitating these processes. A recent example has relayed in the addition of amniotic epithelial cells into pancreatic organoids as a new mean to improve viability, function, and vascularization in these systems [17] (Fig. 2). Furthermore, in vivo vascularization (host-derived vascularization) has been partially achieved after organoid transplantation under the kidney capsule of immunodeficient mice profiting from the high vascularization capacity in different organoid model systems derived from human pluripotent stem cells, including brain organoids [18], nephron progenitor cells [19,20], or kidney organoids [21]. In a different approach, it has been shown that after transplantation in the chicken chorioallantoic membrane, kidney

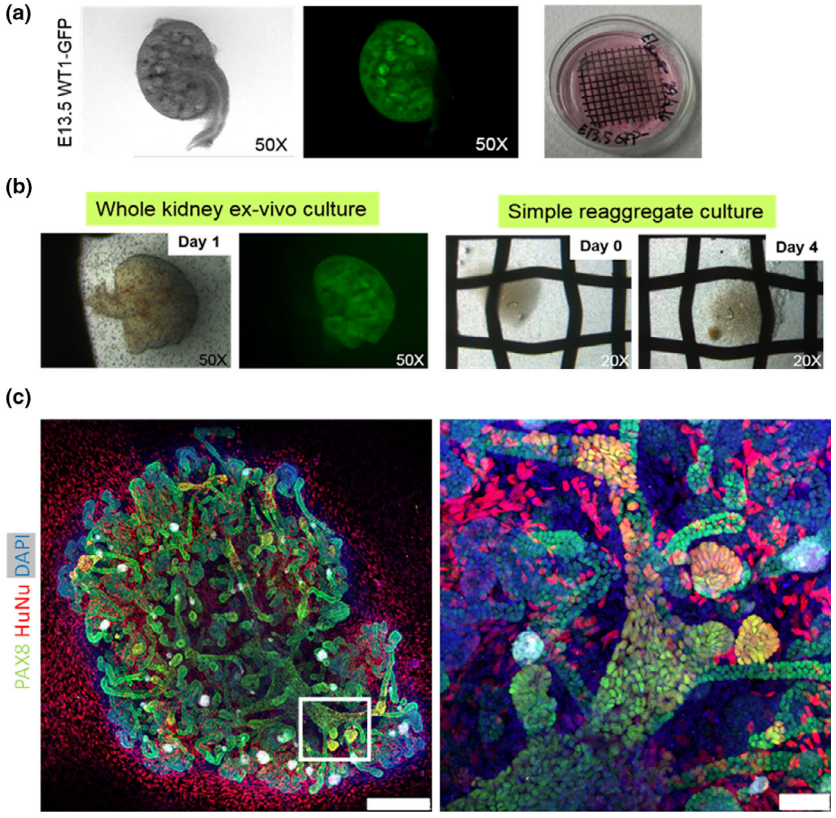

Figure 1 Techniques supporting the culture of mouse embryonic kidneys can help to dissect the early steps of kidney development and to develop chimeric mini-organs. (a, b) Mouse embryonic kidney at day 13.5 during embryogenesis isolated from WT1-GFP reporter mice can be kept in culture under organotypic culture conditions based on the Trowell method [3,4] for a period of 5 to 7 days. Kidney rudiments from WT1-GFP reporter mice show GFP (green) expression under the control of the endogenous WT1 promoter. Alternatively, mouse embryonic kidneys can be enzymatically digested leading to re-aggregated cultures, which self-assemble and develop into kidney rudiments ex vivo. (c) Generation of chimeric kidney organoids making use of human pluripotent stem cells derived renal progenitor cells re-aggregated with dissociated mouse embryonic kidney cells. Representative confocal image of a chimeric kidney organoid containing cells of human origin (labeled with human nuclear antibody—HuNu—in red) that engraft in nascent renal structures (labeled with Pax8, in green). A magnified view of the boxed region shows the detail of chimeric tubular structures in which human cells are detected by the co-expression of HuNu and Pax8. Scale bars, 250 and 50 μm.

organoids exhibit capillary loop stage glomeruli-like structures with endothelial cells of human origin in close contact to podocyte-like cells [15].

In sum, up to date, transplantation has been one of the major approaches to boost endogenous vascularization in organoids to further deliver oxygen and nutrition as a mean to expand organoid maturation and life span. In this regard, we believe that human organoids transplantation may help developing new interventions improving graft survival. As an example, it has been recently shown on the possibility to derive human islet-like organoids (HILOs) from induced pluripotent stem cells (iPSCs) supporting for robust ex vivo glucose-stimulated insulin secretion. Upon transplantation, HILOs rapidly re-established glucose homeostasis in diabetic NOD/SCID mice. Furthermore, by the overexpression of the immune checkpoint protein programmed death-ligand 1 (PD-L1) the authors demonstrated that HILO xenografts avoided the immune attack such that they were able to restore glucose homeostasis in immunocompetent diabetic mice for 50 days [22]. All in all, the generation of glucose-responsive islet-like organoids able to avoid immune detection provides a promising alternative to device-dependent therapies in the treatment of diabetes in the future. Furthermore, this study opens new venues on the exploitation of organoid technology to address similar questions in other organ systems.

In relation to the full application of organoid technology as an alternative source to cadaveric organs, the field needs to start collectively working toward the development of technological advances to overcome major challenges related to their generation, characterization, and bioprocessing. While true that up to date

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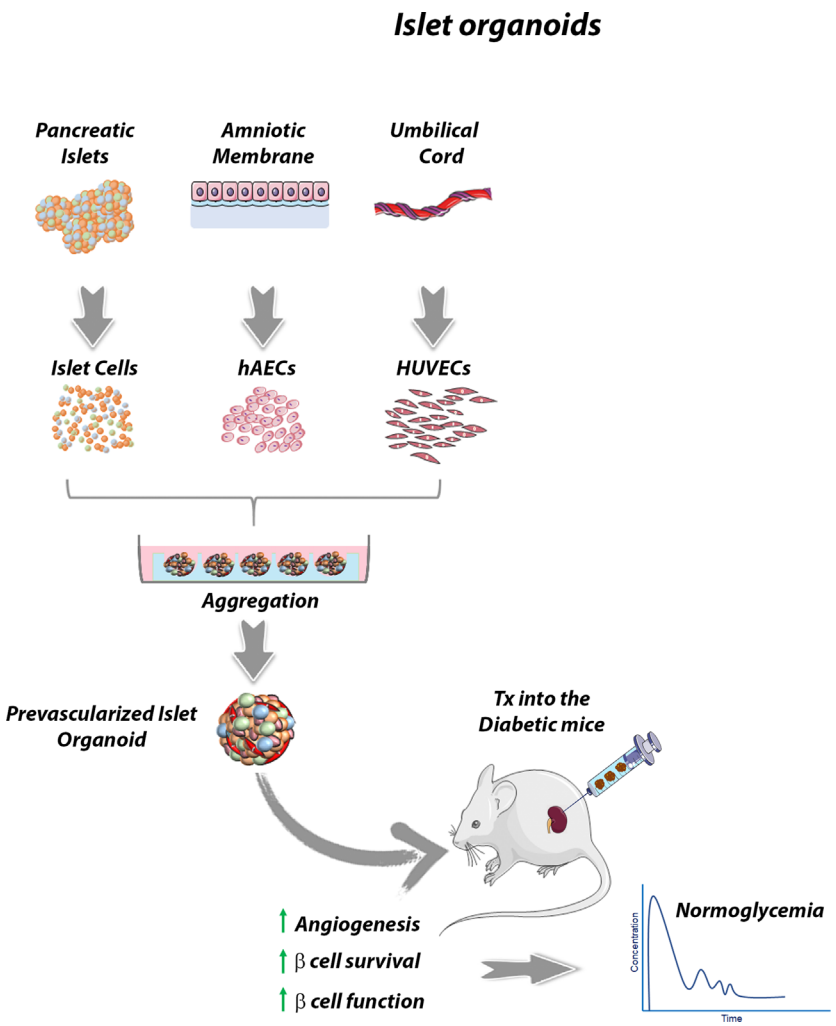

Figure 2 Beta-cell replacement strategy using vascularized islet organoids. *Organoid generation:* Dissociated adult islet cells mixed with endothelial (HUVECs) and human amniotic epithelial cells (hAECs) are seeded on microwell plates and cultured for several days to form organoids. *Transplantation:* Generated vascularized organoids are collected, packed, and transplanted into the diabetic mice. Diabetic mice rapidly return to normoglycemic state due to enhanced survival, vascularization, and engraftment of transplanted organoids.

both adult stem cells and human pluripotent stem cells have led to the successful derivation of micrometer size versions for different human organs, there are still important differences in terms of their differentiation extent and functional capacities when compared to native counterparts. Moreover, the observed organoid to organoid variability when organoids are generated in different laboratories [23] or even when comparisons are done between organoids of the same batch [24,25] remains yet an unsolved issue in the field. Importantly, there is an urgent need for the development of methodologies to faithfully measure the functional capacity of organoids. This would be especially important for disease modeling applications in where the definition of standardized functional assays and readouts for quality control of organoids would facilitate the establishment of reproducible organoid models. Furthermore, the development of novel transplantation approaches for validating functional organoids engraftment and safety would pave the way toward translating organoid technology for tissue replacement and regeneration therapies. For example, a recent work has demonstrated the possibility to use ex vivo-perfused human livers for showing the therapeutic engraftment of cholangiocyte organoids [26].

In the meantime, as basic research progresses, the possibility to generate autologous organoids from patient adult stem cells or from iPSCs opens new scenarios in which the primary cell source from which to generate organoids is not the limiting factor. However, the initial assumption that autologous iPSCs and their derivatives would evade immune surveillance has been

called into question by the evidence that ectopic expression of even a few developmental antigens causes immune rejection of iPSC-derived tissue [27]. Taking advantage of humanized mouse models (humanized mouse model reconstituted with a functional human immune system), Zhao and colleagues showed that whereas iPSCs-derived retinal pigment epithelial cells were immune tolerated, autologous iPSCs-derived smooth muscle cells were highly immunogenic even in an autologous setting [27]. In this regard, it would be important to investigate, side-by-side, the cellular components and the mechanism of the immune response toward patient-derived iPSCs versus iPSCs-organoid counterparts, and endogenous isolated cells, making use of humanized animal models. These findings overall highlight the necessity to define the initial variations between undifferentiated stem cells lines that upon organoid generation may trigger different degrees of immune activation upon transplantation. Such alterations may arise from disparities already found in the initial cell sources to be used as sources for iPSCs generation, leading to incomplete reprogramming. Similarly, epigenetic variations between same patient iPSC clones may also predispose them to differences in differentiation outcomes. In this regard, further comparative analyses from undifferentiated iPSC clones versus their derived organoid counterparts at different stages during the differentiation process would help to detect the amount of gene expression differences that can be tolerated by the host immune system after transplantation or that conversely can activate the immune response therefore requiring the adoption of a minimal and specific host-conditioning.

On the other hand, there are important aspects that the organoid community is also addressing when envisioning the manufacture of organoid-based therapeutic products for allogeneic and autologous replacement therapies according to good manufacturing practice (GMP) quality requirements. In this regard, there have been a series of technological advances in different aspects of organoid-based therapeutic product manufacturing including clinical-grade cell line development, large-scale banking, upstream and downstream processing, and quality assessment of the final cell therapeutic products. In this regard, the Hubrecht Organoid Technology (HUB) is one of the best examples of bringing organoids closer to clinical application. To date, the HUB accounts with a living organoid biobank including more than 1000 organoid models representing a variety of organs and disease types. In this regard, the HUB holds patient-derived intestine, liver, pancreatic, lung, and mammary gland organoids as well as healthy organoids model diseases such as cancer, cystic fibrosis, and inflammatory bowel disease. Other initiatives in Europe include the creation of the Spanish Network of Biobanks and Biomodels from the Institute of Health Carlos III, which facilitates services in organoid derivation and characterization from national units. The possibility to have access to such centralized systems is of great benefit for researchers, clinicians, and pharmaceutical companies. Soon, it is expected that the interaction between unique structures like these together with researchers from the field of organ transplantation will pave the way toward the generation of bona fide organoids for applications in transplant research.

Other examples of organoid clinical translation relate to recently approved phase I/II clinical trials for subcutaneous implantation of human embryonic stem cells derived pancreatic progenitors into type 1 diabetic patients [28,29]. These trials have generated strong preliminary evidence that stem cell-derived islet products have the capacity to survive and function in patients with type 1 diabetes. Furthermore, the Food and Drug Administration (FDA) has recently granted Fast Track Designation for VX-880—the first investigational stem cell-derived therapy utilizing fully differentiated, insulin-producing pancreatic islet cells for the treatment of type 1 diabetes through their infusion in the liver.

With recent technological advances in organoid research, patient's organoids are becoming the "center stage" of laboratories around the world. It is possible now to rapidly assess fundamental questions related to human developmental biology as to discover new therapies for human disease. Bioengineering strategies are playing an important role to control the inherent properties of stem cells to generate *bona fide* organoids resembling the native tissue. In this regard, it is expected that the definition of new allogenic biomaterials and fluidic (micro and macro) platforms will play a key role in catalyzing the realization of organoids in a controllable and scalable manner. Beyond the initial focus on developmental biology and precision medicine, organoids should advance transplant research by supporting long-lasting questions in the transplantation field, which have largely relayed in the use of animal models. This overarching goal can be achieved by creating an active and inclusive forum in Transplant International that facilitates the alliance bridging both organoid and organ transplant communities. Toward this aim, we will continue offering opinion papers and viewpoints in organoid research that may be of interest for transplant research community and *vice versa*.

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Conflicts of Interest

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