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Human pluripotent stem cell-derived engineered tissues: clinical considerations

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Abstract

The combined power of human pluripotent stem cells and tissue engineering promises to revolutionize medicine by building tissue patches and artificial replacement organs for patients battling diverse diseases. Here, we articulate some big questions that need to be addressed before such engineered tissues become mainstream in the clinic.

Introduction

Over the past several decades, the use of human pluripotent stem cells (hPSCs) for repair of failing organs has moved from a far-fetched concept to the forefront of regenerative medicine research. It is an exciting time – the combined power of hPSCs and tissue engineering has been harnessed to build human engineered tissues with increasingly complex structure and function from nearly every organ system. A major challenge for engineers, biologists and physicians alike will be translating this progress to successful treatment of diverse patient populations.

As new hPSC-derived engineered tissues continue to be developed, three clinical trials are already ongoing. First, Pfizer is implanting sheets of hPSC-derived retinal pigment epithelial cells immobilized on membranes in patients with age-related macular degeneration. Second, Viacyte is implanting multicellular hPSC-derived pancreatic beta cell progenitors encapsulated in an immunoisolating device in patients with type I/II diabetes. Finally, the Menasché group in Paris is implanting hPSC-derived cardiac progenitor cells embedded in a fibrin hydrogel onto the hearts of patients with severe cardiac failure. All of these trials are currently in Phases I or II. Furthermore, over a dozen clinical trials have been initiated to test direct injection of hPSC-derived cells in patients (Thies and Murry, 2015; Trounson and DeWitt, 2016).

Together, these ongoing clinical trials represent important scientific and logistical breakthroughs for the field. They have also provided valuable early lessons from which the entire field can benefit. Here, we reflect on these lessons and the critical remaining

challenges that must be overcome to broadly improve public health with safe and innovative hPSC-derived engineered tissues. Since human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are considered to be essentially equivalent in therapeutic potential, we broadly use the term hPSC encompass both cell types here. Furthermore, we focus on the use of hPSCs to engineer multicellular tissues (with or without biomaterials) for regenerative medicine, as therapies with dispersed cell preparations have been covered elsewhere (Thies and Murry, 2015; Trounson and DeWitt, 2016).

Hurdles for hPSC sources in product design

The first step towards clinical application of hPSCs is choosing a cell line. There is considerable line-to-line variation amongst hPSCs, even among clones from the same donor. This can be manifested as variations in growth rate, maintenance of pluripotency, genetic stability, and efficiency of differentiation into desired cell types. In practice, a clinically suitable cell line needs to be able to undergo enough population doublings to produce a master cell bank (e.g. 100 vials of 1 million frozen cells), each of which that can be subsequently expanded to create a series of comparably sized working cell banks of pluripotent cells. At the working bank stage, cells need to remain genetically stable and able to differentiate robustly into the desired cell type. It is worth mentioning that “genetic stability” does not have a strict regulatory definition. We use a combination of genome sequencing to identify mutations and undesirable variants, X-chromosome inactivation assays in female lines, and frequent karyotyping to ensure normal chromosomal complements. To select a final cell line for our program, we tested multiple lines head-to-head and chose the one that performed best in terms of expansion, genetic stability and ability to differentiate into cardiomyocytes.

Once a working cell bank is established, each vial is typically used for a single batch expansion, the size of which depends on the clinical demand. It is well known that hPSCs are immortal and amenable to large-scale expansion, but these insights came from small-scale cultures and arithmetic extrapolations rather than real-world practice. The field is just now learning the lessons of producing pharmaceutical-grade hPSCs at clinical scale and controlling their differentiation. Cell needs range from hundreds of thousands for retinal pigment epithelium to billions for cardiomyocytes, and expansion strategies will vary accordingly. Doses in the 10^5–10^6 range can be met through growth in monolayers and human handling. Higher doses will require adaptations like robotics or stirred tank bioreactors to produce billions of cells at a reasonable cost.

A major safety concern for hPSCs is tumorigenicity. This concern has two components: 1) teratomas, which are benign tumors that contain ectoderm, mesoderm and endoderm derivatives, and 2) malignant neoplasms that arise due to mutations in oncogenes or tumor suppressor genes, presumably acquired during cell culture. Teratomas should be avoidable through good control of stem cell differentiation, purification steps (if necessary) and quality control steps that demonstrate minimal residual undifferentiated cells in the final product. The risk of malignant neoplasms should be minimized by taking care to preserve genomic stability during cell expansion. Both types of tumors can be screened for by performing tumorigenicity assays with the final tissue product prior to clinical use. Fortunately,

numerous animal studies have demonstrated a good safety profile with pluripotent stem cell derivatives, but this is an area where researchers need to remain vigilant.

An important lesson from ongoing clinical trials of hPSC-derived tissues is that the simplest therapies, such as those with the fewest cell types, are quickest and easiest to translate. All three hPSC-derived tissues that have successfully reached patients contain just a single cell population. Despite clear advantages of simplicity, preclinical studies indicate that including several cell populations in iPSC-derived tissues may be superior to monotherapy (Ogle et al., 2016). For example, the addition of endothelial and stromal cells to hPSC-derived heart and liver tissues improves engraftment, vascularization, and/or function of these tissues upon implantation (Ogle et al., 2016; Takebe et al., 2013). As hPSC advances provide increasingly sophisticated cellular toolkits, a key challenge is to harness the power of multicellular interactions while simultaneously streamlining hPSC-derived tissues for clinical translation (Atala et al., 2012).

A final challenge on the cell front is determining the optimal maturation state of hPSC derivatives for transplantation (Gerbin and Murry, 2015). Although shortcomings in deriving cells with adult phenotype from hPSCs *in vitro* are commonly lamented, it remains unknown whether adult cells are even needed for transplantation. Indeed, some studies suggest that cells with an intermediate phenotype might be best. For instance, mature adult cardiomyocytes injected directly into the heart do not survive transplantation, whereas immature hPSC-cardiomyocytes do engraft (Gerbin and Murry, 2015). Whether these findings will also be the case when hPSCs are delivered in engineered tissues, which may improve cell survival, structure (e.g., cardiomyocyte alignment) and functionality, is an open question. Importantly, hPSC-derived tissues may mature progressively upon implantation (Takebe et al., 2013). Further experiments that carefully characterize the engraftment, maturation, and morphogenesis of iPSC-derived tissues with varying initial developmental states are needed.

Towards “off-the-shelf” products

When iPSCs were developed there was widespread enthusiasm for their autologous use, which should circumvent immune rejection. Ten years in, autologous use is looking less promising. The first issue is the enormous amount of work that is required to meet safety requirements and fine-tune expansion and differentiation (Hunsberger et al., 2015). At present, it costs millions of dollars to qualify a single cell line, and this would be cost-prohibitive for common diseases. In the Japanese efforts to treat macular degeneration with autologous iPSCs, identification of mutations in a line derived from one patient temporarily halted the trial (Trounson and DeWitt, 2016), and these investigators are now pursuing allogeneic cells. The second issue pertains to timing. The process of obtaining patient-specific somatic cells, reprogramming to iPSCs, differentiating to relevant cell type(s), constructing tissues, and performing proper quality control would currently take a year, and it is hard to imagine shortening this process to less than 6 months (Gerbin and Murry, 2015). This also precludes use of autologous tissues in clinical acute injury settings, such as acute myocardial infarction or spinal cord injury, which are likely to be some of the most critical indications.

A key challenge in the field thus is to develop an “off-the-shelf” allogeneic hPSC-derived tissue product, in which production can be scaled-up. Such a product requires fastidious and comprehensive quality control metrics for each tissue component (hPSCs, scaffold, bioactive factors) and for the final tissue product (Hunsberger et al., 2015). Furthermore, any product that comes into contact with each tissue component must be derived from Good Manufacturing Practice (GMP) sources. To do this, the field needs to overcome supply-chain challenges associated with consistent and reliable sourcing of all tissue components, highly reproducible and automated platforms for biofabrication, and rigorous and standardized quality control metrics (Hunsberger et al., 2015). Finally, allogenic products bring immunogenicity challenges. Pharmacological immunosuppression is one important toolset, although lifelong high level immunosuppression increases risks of renal injury, opportunistic infections and cancer. Another approach is creation of stem cell banks (including HLA homozygous “super-donor” lines), where major HLA haplotypes can be matched and reduce immunogenicity. A logistical challenge to this approach is gaining widespread coverage in populations with great HLA diversity such as the US. Finally, we are encouraged by exciting new strategies to engineer “universal donor” lines, akin to O-negative blood (Gerbin and Murry, 2015), e.g. via gene editing to create HLA class 1- and 2-negative cells, or by engineering cells to secrete local immunosuppressive signals. Challenges with this approach include possible clearance of the HLA-null cells by NK cells and the theoretical possibility of immune-stealthy cells to harbor viruses.

Hurdles in tissue assembly

Two of the three hPSC-derived tissues in clinical trials are simple flat (cornea) and small (pancreatic beta cell) structures that can be fed by nutrient diffusion. However, for most applications it will likely be desirable to build engineered tissues that recapitulate the native structure of organs such as liver, heart, and kidney. Although emerging biofabrication technologies (bioprinting, micromolding, photopatterning) offer increasingly refined positioning of cells, trade-offs between pattern complexity, resolution, and scaling still need to be overcome. Consider the emerging area of bioprinting. While considerable progress is being made, bioprinted tissues remain sparsely cellularized, simplistic in geometry (where “Lincoln Log” structures are state-of-the-art), and an order of magnitude coarser in resolution than native tissues. An elegant solution to fabrication of hPSC-derived tissues may include cellular “self-assembly” processes, which rely on intrinsic cell-cell and cell-matrix receptor-ligand interactions to drive morphogenesis and create architectural relationships found in native tissue (Hayashi et al., 2016; Huch et al., 2017). Engineering principles may be most useful when we discover and provide elements that are missing. Finally, a key challenge will be to address these assembly issues while also determining the structural complexity that is truly needed to treat human disease.

While engineers and biologists alike have developed diverse methods to fabricate hPSC-derived tissues, the vast majority of tissues created to date are simply not large enough to support clinically meaningful functions. Building tissues of scaled size requires incorporation of functional blood vessel networks. This brings substantial challenges in recreating the hierarchical cellular, structural, and mechanical complexities of mammalian vasculature. Even methods in which the architecture of the donor vascular tree is largely

intact, such as organ decellularization, need to address issues related to vascularization, including incomplete endothelialization, blood leakage, and thrombosis. As the field matures, hybrid engineering-biology strategies are emerging, such as constructing tissue "seeds" that grow coordinately with vascular ingrowth (Stevens et al., 2017). New methods to build and image perfused vasculature across length scales are also needed to assess the degree to which vascularization is a necessary complexity (Ogle et al., 2016).

Establishing a clear vision and therapeutic end-goal

Developing a successful tissue therapeutic requires knowledge of its mechanism of action. In general, there are two mechanisms through which tissue grafts may improve host function: direct replacement of the lost tissue, and indirect paracrine benefits to viable host tissue. The direct mechanism is conceptually straightforward, where engineered tissues form long-lived grafts in the host to repopulate or replace tissues lost to disease. Examples here include new myocardium that generates systolic force, new beta-cells that produce insulin, and new neurons that transmit impulses. The paracrine mechanisms are less straightforward and may include secretion of soluble factors, membranous vesicles and so on that promote viability of parenchymal cells, increase angiogenesis or reduce inflammation and fibrosis. In some circumstances, it may not be necessary for grafts to persist long term for paracrine benefits to be realized.

Most engineered tissues will likely require proper in situ delivery and almost seamless integration with the patient's tissue. All highly metabolic tissues need to be integrated quickly and efficiently with host vascular supply upon implantation. Furthermore, specialized tissues have additional integration constraints. For instance, iPSC-derived tissue for myocardial repair should integrate electrically with host myocardium to beat in synchrony and avoid arrhythmias, and tissue for hepatic applications should integrate not only with the vasculature but also likely with a ductal network that enables bile excretion. Host integration is also complicated by intrinsic variability between patients, such as patient-specific differences in wound healing response. Prior to clinical translation, how safely and effectively iPSC-derived tissues integrate with the host needs to be better understood.

With decades of technical infrastructure in place, the field now has the opportunity to develop hPSC-derived tissue products that are tailored to treat patients with specific diseases (Atala et al., 2012). A key task is to establish the most relevant product design, manufacturing, and regulatory considerations early in product development (Hunsberger et al., 2015). Even seemingly simple design criteria, such as the size of the tissue construct, are likely to vary considerably for different patient subsets. For example, liver tissue to treat cirrhosis or hemophilia need to replace markedly different levels of hepatic function (~30% or 1%, respectfully), and thus bring different tissue scaling needs. A one-tissue-fits-all approach (simplistic "heart", "liver", or "bone" tissue) is likely not enough in the clinic.

The quest to design iPSC-derived tissues to meet the needs of specific populations then raises the question: What patient population(s) should be targeted for first-in-human studies? Should patients be chosen based on greatest need or greatest likelihood to derive benefit (efficacy signal)? These two populations are likely to be very different. For instance, chronic

disease constitutes the majority of patients with heart, neurological and liver disease, and these are also the sickest patients. However, preclinical studies suggest that there is a temporal window for hPSC derivatives in treating heart or spinal cord injury, and efficacy may be lost in chronic conditions. While an argument can be made for initial safety testing patients with advanced chronic disease, in the end, tissue engineering will only succeed if it is developed in conditions where preclinical studies show a strong efficacy signal. Testing in high-risk patients (e.g. with no other options, or where the disease cannot be modeled) can only be justified when there are sufficient resources to continue product development if the trial fails to show efficacy.

Looking forward

The convergence of hPSC biology and tissue engineering has ushered in remarkable advances. Looking forward, continued cross-pollination with gene therapy, genome engineering, systems/synthetic biology, nanotechnology, and surgical automation will enable scientists and engineers to develop new technologies that address the pressing clinical challenges facing this field. For example, advances in biomarkers and molecular imaging may enable tracking of engraftment, integration, and remodeling of tissues in varying host settings in real-time (Atala et al., 2012). Gene circuits from synthetic biology may allow control of engrafted cells, such as using synthetic ligands to regulate their proliferation, secretion activity or apoptosis. Advances in tissue delivery may improve market viability by creating reliable products that can be administered by less invasive routes. For example, tissues may be constructed with “shape memory” that allows them to be delivered in compact form and expand into a large-area patch at the target site. Advances in robotic surgery and catheter delivery may reduce the invasiveness of the implant procedure. Together, approaches that harness the combined power of biology and engineering will likely improve the safety and efficacy of hPSC therapies and enable these cells to broadly advance human health.

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