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Guidelines for quality assessment of intestinal cell lineages utilised for transplantation

WP 1 - Autologous cell derivation

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Contributors: Sarah Chan, Hans Clevers, Ryan Conder, Paolo De Coppi, Tracy C. Grikscheit, , Vivian S.W. Li, Mathias P. Lutolf, Ludovic Vallier

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1. Introduction

Treatment of short bowel syndrome (SBS) requires the development of innovative interdisciplinary engineering solutions. Here we discuss experimental considerations and challenges for tissue engineering via combination of cells derived from primary tissues or via directed differentiation of pluripotent stem cells, with either naturally derived or synthetic scaffolds to generate artificial organs.

2. Summary of activities and research findings

Restoration of organ function
Many disorders significantly affect organ function leading to severe complications and, in a worst-case scenario, death. One strategy for treating such patients is organ transplantation. Yet, the current demand for organs greatly exceeds the availability of donors. An alternative strategy, is to engineer functional organs *in vitro*. This, however, comes with significant biological, technical and translational challenges that need to be carefully considered in the context of current state-of-the-art.

Replacement therapies for patients with severe burns and devastating diseases such as junctional epidermolysis bullosa with normal and genetically corrected human cells have paved the way to engineer entire organ (Gallico et al., 1984; Hirsch et al., 2017). Indeed, these proof-of-principle epidermal transplantation studies have established the requirements for engineering tissue in vitro and also demonstrated that risk for adverse complications such as cancer can be tightly controlled. However, this therapy has also underlined the challenges for total replacement of complex organs. Procurement of multiple cell type in large quantity, with adequate functional level and scaffolds used to seed these in 3D environment mimicking organ architecture represent major challenges. Furthermore, it will be key to develop standardized procedures to eliminate adverse side effects such as graft rejection or tumor development and sustain long-term functionality. Here we discuss these different aspects taking the small intestine as a prime example for organ replacement.

The small intestine
The small intestine represents a complex organ consisting of concentric rings of muscle fibers (longitudinal and circular), and a submucosa that provides a mesenchymal framework for the epithelium that forms the barrier to the inside of the gut. Within these layers, the enteric nervous system regulates peristalsis and the controlled secretion of hormones and enzymes, blood vessels as well as lymphatics capillaries ensure that nutrients taken up are distributed to the rest of the organism, and an intricate balance between enteric immune cells and luminal microflora sustain gut homeostasis. The organ consequently relies on a

complex interplay between tissues, different cell populations and their environment in order to sustain gut function.

Short Bowel Syndrome (SBS) is a condition that occurs when part or the entire small intestine is missing or has been removed during surgery. This condition renders the bowel incapable of fulfilling its nutritional function (intestinal failure). There is no cure for SBS as parenteral nutrition is associated with low survival rates while small intestinal transplants are limited by shortage of organ donors. Thus, functional bowel reconstruction for patients with SBS through a tissue engineering strategy will provide therapeutic options currently not available.

Cell sources

A starting point to engineer an artificial small intestine is the identification and characterization of potential sources for the individual components required to make an engineered organ. Each of these options will be associated with pros and cons. These considerations will be similar irrespective of the organ in question, as combination of cells and scaffold are invariably required to support functionality and longevity.

Protocols now exist for the expansion of most primary cell types from the intestine including the epithelium, mesenchyme and nervous system. The obvious limitations are of course that if a patient suffers from organ failure, there might not be sufficient tissue left, or cellular functions might be irreversibly compromised. Even if a suitable donor can be identified as a source for derivation of primary cell cultures, this would still mean that the recipient will require extensive and life-long immune modulation. Moreover, some of the primary cells may have a limited lifespan and thus cannot be expanded quantities necessary to engineer of a entire intestine. As an alternative to cells derived from somatic tissues, protocols are now emerging, which enable the directed differentiation of human pluripotent stem cells (hPSCs) into essentially all specialized cell types of the intestine. Moreover, exciting studies demonstrate that enteric neurons, muscle and intestinal organoids all derived using such protocols interact in a coordinated manner (Schlieve et al., 2017). This means that we may now for the first time be able to create a functional intestine. Comparative studies between engineered and native intestine will subsequently reveal how closely the *in vitro*-derived organ resembles the real thing.

The scaffold

Most organs in our body supported by a 3D scaffold consisting of extra cellular matrix proteins which provide the spatial organisation essential for their function. In the small intestine, the finger-like protrusions, termed villi, projecting into the lumen provide an extended surface area for uptake of dietary nutrients, and pockets (crypts) in between villi form protective niches for intestinal stem cells. Importantly, insufficient surface area causes intestinal failure, and essential nutrients have to be supplied intravenously. The 3D architecture of the gut is therefore of essential for functionality, and any scaffold-based approach must be able to recapitulate these aspects. Interestingly, transplantation studies in mice have shown that intestinal cell populations self-organize into functional

domains suggesting that this can be achieved in a guided manner based on intrinsic features of distinct cell populations (Watson et al., 2014). Yet, it will be essential to define how gut function can be maintained long-term in a manner that can support nutrient demands.

A scaffold that provides the 3D context for organ engineering has to be *i)* responsive to the environment, *ii)* sufficiently malleable to permit cellular self-organization into appropriate tissue domains, and *iii)* provide durability. It has to be a structure where cells can be seeded and stimulated to adopt their appropriate spatial localization and tissue-like pattern, and where flow of food within the organ and blood across the organ occur in the most appropriate manner. This can be achieved using either a completely artificial system such as those based on biodegradable polymeric scaffolds combined with bioprinting (Schlieve et al., 2017). Decellularized matrices constitute an alternative that has shown in vivo efficacy long-term (Elliott et al., 2012). A completely artificial scaffold will eliminate the dependence on cadaveric organs and offers the obvious advantage that it can be engineered in the preferred format, dimensions and shape, and to contain, in principle, any desired combination of growth factors and cytokines. A natural scaffold will on the other hand have the appropriate dimensions and spatial complexity that reflects distinct environments appropriate for e.g. muscle cells, vascularization, stem cell niches and villi.

Considerations for Proof of principle studies

Before being able to take any device into clinical trials in patients, important questions need to be addressed in preclinical animal studies. This ranges from concerns with regard to safety such as does the engineered organ can cause any adverse side-effects, to more practical concerns such as how stable is the scaffold, and how big should the engineered organ be to provide functionality. If the device needs to be at near human organ size, this requires extensive upscaling of all the methodologies for establishing cell cultures. Here one key challenge will be to translate the methods into large scale clinically compliant GMP protocols that will in case of the small intestine require billions of intestinal cells. Efforts worldwide spearheaded by consortia at Tokyo Medical and Dental Hospital, Yokohama City University, Cincinnati Children's Hospital Medical Center and the INTENS framework are developing GMP conditions for growth of intestinal cultures (Takebe et al., 2018). In addition, it might be difficult to coordinate the production of each cell type and it will accordingly be necessary to develop optimized methods of cryopreservation. In parallel, functional studies are required to fully demonstrate that multiple cells produced in vitro can functionally interact not only between themselves but also with endogenous cells such as endothelial and immune cells. With regard to all other cell types present within any particular organ, this also brings obvious scaling challenges.

Once these decisions have been made and the challenging requirements met, the cellularised scaffold needs to be tested in preclinical trials to assess safety, functionality and durability. The choice of recipient host animal will influence the ability to test these criteria. Immunocompromised mouse models have been used widely as a recipient for human cells, but it will be difficult to accommodate

larger scaffolds and thereby reliably test functionality. On the other hand, larger animals such as pigs are valuable for testing functionality, owing to their anatomical similarities to humans. However, here the challenge lies in avoiding immune rejection of foreign material. While patient safety should be considered of paramount importance to avoid false expectations and patient harm, the lack of appropriate large animal models is something to carefully consider when designing appropriate translation pathways (Cossu et al., 2018). Overall, the combination of diverse animal model might ultimately be necessary to assess safety and efficacy.

Most importantly and as illustrated by the diversity of challenges, the development of engineered organ is an interdisciplinary effort that has to involve bioengineers, biologists, clinicians, industry and ethics advisors. Moreover, early interaction with patient representatives is critical to design meaningful therapeutic strategies and lobby for dedicated funding, which can help ameliorating devastating conditions such as SBS.

3. Conclusions and future steps

A major hurdle for the subsequent translation to patients is to provide clear quality measures for the actual 'engineered graft' in preclinical trials. Such guidelines should be translatable into specific requirements for each of the individual components that are used for the engineered organ, as well as of the assembled organ. This should ascertain that once the graft is transplanted into a recipient, it is ready to perform the required task. For the small intestine, it is essential to provide barrier function, secretion of enzymes involved in nutrient degradation and uptake of amino acids, lipids, sugars, vitamins and minerals. It is consequently important that the epithelial cells introduced into an engineered organ can exert these functions, and that the functions are retained. Similarly, interactions between muscle and enteric neurons should provide the basis for the contractile forces that promote peristalsis and thereby movement of ingested material along the longitudinal axis of the intestine. It is not directly obvious how to connect the nervous system and the vasculature of the graft to the intestinal tract. In an ideal situation, it will be possible to test for all of these functions prior to assembly of the organ; yet this is unlikely to be feasible. Alternatively, correlation of e.g. expression analysis from every single culture with function, will provide empirical standards for assessing the likelihood of successful implementation of all functions into the engineered organ. Such standardization will however require repeated measurements of many cultures to provide a robust correlation matrix. Overall, it will be important to develop a set of common guidelines for scaffold monitoring and stem cell-based material utilized in patients to avoid adverse effects that will jeopardize the future use of stem cells in the clinic.