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“Tissues in a Dish”: A Review of Organoids in Plastic Surgery

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Summary: Organoids are in vitro miniaturized organ models—or, colloquially, “organs in a dish.” These 3-dimensional, multicellular structures are classically derived from pluripotent or multipotent stem cells. When guided by tissue-specific molecular factors, these cells exhibit self-organizing abilities that allow them to accurately recapitulate the architecture and function of the organ of interest. Organoid technology is a rapidly expanding field that endows researchers with an unprecedented ability to recreate, study, and manipulate complex biologic processes in vitro. When compared with standard 2- and 3-dimensional culture systems, which rely on co-culturing pre-established cell types, organoids provide a more biomimetic model with which to study the intercellular interactions necessary for in vivo organ function and architecture. Organoids have the potential to impact all avenues of medicine, including those fields most relevant to plastic and reconstructive surgery such as wound healing, oncology, craniofacial reconstruction, and burn care. In addition to their ability to serve as a novel tool for studying human-specific disease, organoids may be used for tissue engineering with the goal of developing biomimetic soft-tissue substitutes, which would be especially valuable to the plastic surgeon. Although organoids hold great promise for the field of plastic surgery, technical challenges in creating vascularized, multilineage organoids must be overcome to allow for the integration of this technology in clinical practice. This review provides a brief history of the organoid, highlights its potential clinical applications, discusses certain limitations, and examines the impact that this technology may have on the field of plastic and reconstructive surgery. *(Plast Reconstr Surg Glob Open 2020;8:e2787; doi: 10.1097/GOX.0000000000002787; Published online 29 April 2020.)*

INTRODUCTION

In this review, we define organoids as 3-dimensional (3D) structures that resemble a parent organ in structure and function on a smaller scale and are derived from stem/progenitor cells through self-organization.[1,2] The impact of organoids since their development a decade ago has been substantial: these 3D “organs-in-a-dish” are named “Method of the Year” in 2017 by *Nature Methods*[3] and have been adapted to study nearly every organ in the body. Advancements in organoid technology have given researchers an unprecedented ability to investigate physiology and disease in a biomimetic in vitro environment. However, organoids have not yet been used to study the biologic processes most relevant to plastic surgery.[4–6]

Although in its infancy, the field of organoid science has demonstrated significant potential to transform the study of human disease and cell-based therapies.[7,8] Organoids can be used to investigate biologic processes such as tissue differentiation and disease pathophysiology, applications that are invaluable in determining the utility of potential diagnostic or therapeutic techniques. This article explores potential applications of this technology in the field of plastic and reconstructive surgery and anticipated challenges in translating organoids for clinical applications. We hope that this review will inspire readers involved in all aspects of plastic surgery—basic science research, clinical investigation, or surgical practice—to consider how this novel development may shape the field moving forward.

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A BRIEF HISTORY OF ORGANOID CULTURE

Traditional Cell Culture

Traditional cell culture provides an efficient platform for the expansion of many cell types. However, because biologic processes such as cell differentiation, signaling, and migration rely on intercellular communication within a 3D niche,[9–11] cells in 2D culture exhibit drastic changes in morphology, function, and gene expression.[12,13] These limitations have driven researchers to develop improved models for studying cells in vitro.

Transitioning to 3D Culture

Three-dimensional cell culture more accurately recapitulates the in vivo cellular environment[14] and has long been used to study cell and tissue biology. For instance, spheroids (condensed 3D cell clusters) have proven useful in the study of cancer biology because they mimic tumors' highly proliferative exterior cell layer and oxygen-poor, necrotic center.[15] However, spheroids cannot be used for high-throughput screening due to the lack of standardization in spheroid formation.[15] Researchers have also used ex vivo cell culture models utilizing cell scaffolds (eg, hydrogels) composed of a variety of materials including agarose, collagen, and hyaluronic acid, to serve as extracellular matrix (ECM) substitutes in which cells can grow in 3D.[15] Although these fibrin matrices are convenient for culturing cells ex vivo, they may alter the phenotype and mechanical behavior of the cells cultured.[15] More recently, microfluidic technology has been used to culture mammalian tissue ex vivo to combat some of the shortcomings of traditional 3D culturing systems. Researchers have attempted to culture skin explants using this technique as they allow for continuous diffusion of nutrients through a tissue sample. Unfortunately, these explants undergo significant degradation in this culture system due to poor tissue diffusion and buildup of cellular waste.[16] Some have tried to improve nutrient exchange by agitating the culture media (through constant rotation, shaking, etc); however, this results in uncontrolled disruptions of the microenvironment.[17] As a result of these limitations, ex vivo systems are hampered in their ability to stably culture tissue for extended periods of time.

Stem Cells and the Genesis of the Organoid

Recent advancements in stem cell biology have further expanded possibilities in cell-based research. Complexities such as lineage restriction and lack of appropriate culture methods historically limited in vitro stem cell research. However, with evolving techniques, researchers recently observed that stem cells self-organize under certain conditions into 3D cell clusters; these cell clusters are now commonly referred to as "organoids."[6,18]

Organoids provide a unique advantage over traditional in vitro systems in their ability to more accurately recapitulate in vivo cellular interactions. Traditional 3D culture involves co-cultivating defined cell types (eg, keratinocytes and fibroblasts) to develop multi-layered structures. The organization of these distinct cell types in culture is generally established by the researcher (for instance, a layer of keratinocytes cultured over fibroblasts to mimic the native organization of skin). In organoids, multiple cell lineages differentiate from stem/progenitor cells and concurrently self-organize, following developmental patterning programs native to the tissue being modeled. Rather than being entirely dictated by the researcher, this process instead takes advantage of progenitor cells' intrinsic properties, thus allowing organoids to more accurately recapitulate the cellular interactions that govern organ development, architecture, and functionality in vivo.[1]

In 2009, Sato et al developed the first organoids from stem cells from the mouse small intestine.[7] By "re-creating" the intestinal epithelial niche in vitro, the researchers were able to expand single cells into 100-cell 3D structures comprising multiple cell types, with microarchitecture closely resembling that of the native intestine. These organoids could be dissociated and replated to form new organoids with no loss of efficiency, enabling them to be perpetuated for 8 months in culture.[7,19] The creation of organoids was a significant breakthrough in translational medicine and opened organoid science as a major avenue of research.

MODERN ORGANOIDS: AN OVERVIEW

The concept of an "organoid" is continually evolving, and precise definitions remain elusive. However, modern organoids are generally considered to share several key elements:

1. Organoids are 3D multicellular tissue constructs grown in vitro.
2. They contain multiple cell types specific to the corresponding gross organ.
3. They mimic key components of that organ—for instance, tissue architecture/microanatomy, cell populations, and organ-specific functions.
4. They are to some degree self-renewing, enabling modeling of organ development and function over time in culture.

Factors including conservation of native developmental and molecular signaling processes are also important determinants of an organoid model's utility. As the field advances, our ability to create biomimetic organoids and the concept of what defines an organoid will continue to evolve.

Organoids can be generated from several different cell/tissue sources, each with unique benefits and drawbacks. The organoids developed in the last decade span a wide breadth, from "mini-brains" to patient-specific cancer organoids to hair-growing "skin-in-a-dish." In this section, we overview existing organoid models, grouped broadly by derivation method (methods summarized in Fig. 1; organoid models summarized in Table 1).

Organoids from Adult Stem Cells

In 2009, Sato et al[7] generated the first self-organizing organoids from adult stem cells (intestinal stem cells). Similar progenitor cells have since been used to culture epithelial organoids for other gastrointestinal organs,

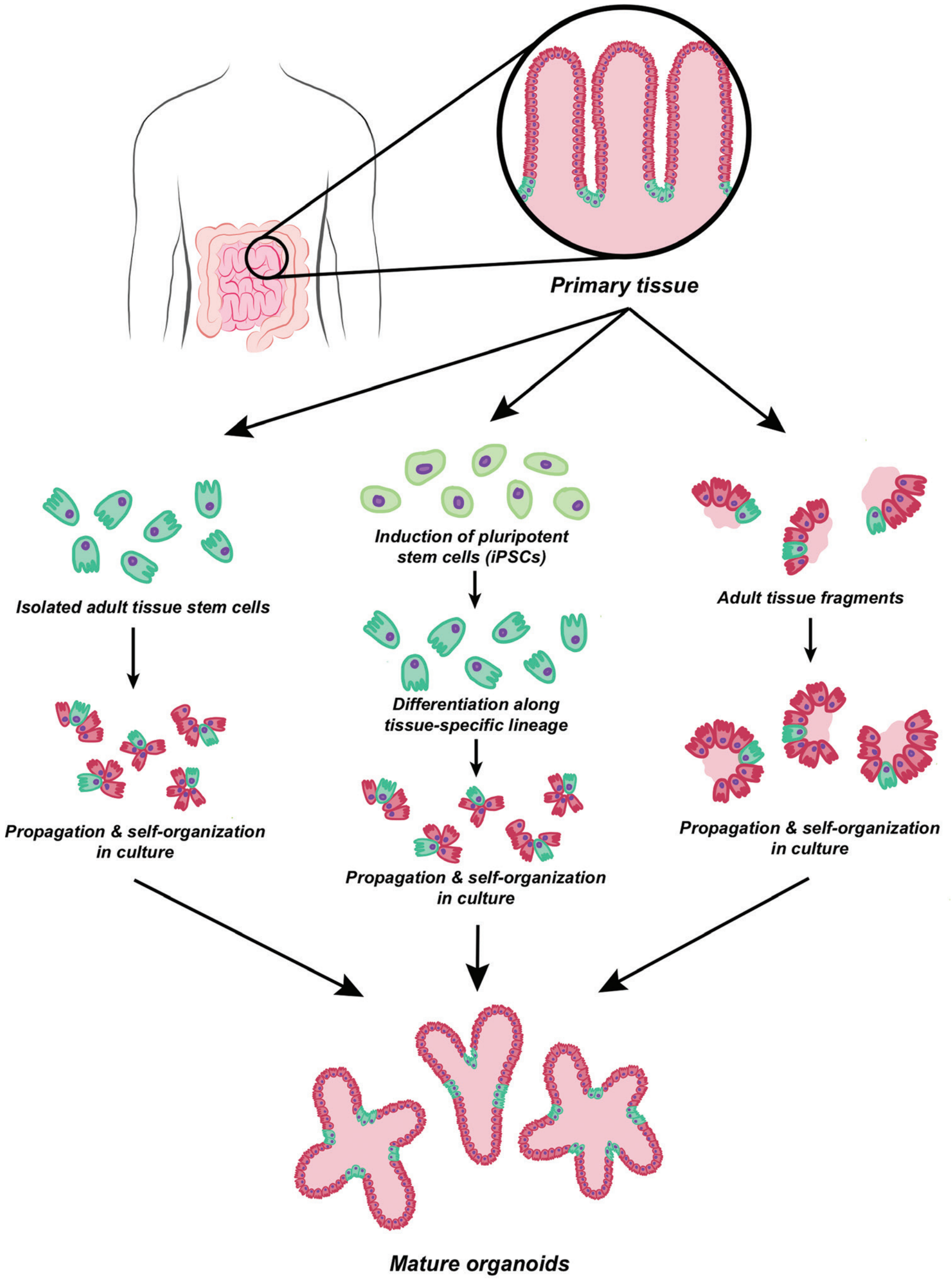

Fig. 1. Schematic depicting general methods of organoid derivation from primary tissue. Organoids can be generated from organ-specific stem/progenitor cells (left column); from pluripotent stem cells, either ESCs (not shown) or iPSCs (middle column); or from stem cell–containing intact tissue fragments (right column). ESC indicates embryonic stem cell.

and mammary glands and prostate[8,20–24] (Table 1). The self-assembly of organoids is a complex molecular process that relies heavily on the starting cell type, culturing environment, and endogenous/exogenous signaling cues (Fig. 1).

Adult stem cell–derived organoids have several advantages. These cells may be easier to obtain than pluripotent stem cells (PSCs). Further, starting from lineage-specified progenitor cells may involve a simpler differentiation process, compared with fully PSCs. However, notably, growth factor supplementation is still required for differentiation, likely due to absent epithelial–mesenchymal paracrine signaling that governs cell behavior in vivo.[7] Further, adult stem cell–based approaches are currently limited to epithelial organoids and are thus unsuitable for studying organs without a significant epithelial component, or for evaluating stromal components. Finally, stem/progenitor cells have

Table 1. Select Examples of Existing Organoid Models Classified by Source of Cells/Tissue

Organoid Models by Source of Cells		
Tissue Stem Cells	**Tissue Fragments**	**Pluripotent Stem Cells**
Small intestine[7]	Small intestine[29]	Cerebral cortex[34–40]
Liver[25,26]	Stomach[30]	Kidney[41–43]
Stomach[27]	Pancreas[30]	Lung[44,45]
Pancreatic ducts[28]	Barrett's esophagus*[8]	Intestine[46]
Mammary gland[22]	Colon and colorectal adenocarcinoma*[8]	Pancreas and pancreatic adenocarcinoma[47,48]
Prostate[23,24]	Pancreas and pancreatic ductal adenocarcinoma*[31]	Placenta[49]
	Mammary gland*[32]	**Skin**[50]
	Lung*[33]	

Organoid models with the most potential relevance to plastic and reconstructive surgery applications are highlighted in bold font.
*Epithelial-only organoids derived from tissue fragments (in contrast to those incorporating mesenchymal elements).

not been elucidated for all organs and may be difficult to obtain from patients noninvasively.

Organoids from Intact Tissue Fragments

Shortly after the first published organoid model, Ootani et al[29] developed an organoid from “tissue fragments” by growing small pieces of mouse intestinal tissue in a partially air-exposed collagen matrix. These cells formed “intestinal spheres” containing underlying stromal cells (eg, myofibroblasts) and in vivo–like microstructures. Similar methods were subsequently validated for other gastrointestinal tissues,[30] and patient tissue fragments have been used to generate organoids for a range of cancers, fibroses, and other diseases[8,31,33] (Table 1).

Tissue fragments offer the potential advantage of incorporating both parenchymal and stromal elements, which may better recapitulate the complex in vivo microenvironment and is critical in evaluating drugs or pathologies targeting the stroma (eg, cancer therapeutics, scarring/skin fibrosis). However, due to their increased complexity, these stromal–epithelial organoids remain limited to gastrointestinal tissues. It remains to be seen whether similar methods can be expanded to other tissue types.

PSC-derived Organoids

“PSCs” can differentiate into any adult cell type. PSCs include “induced PSCs (iPSCs)”[51] generated through conversion from mature cells (eg, skin fibroblasts), enabling easily accessible adult cells to ultimately give rise to multiple organ-specific cell types. Figure 2 summarizes the process by which organoids are generated from iPSCs.

PSC-derived organoids have been heavily used to model brain development and disease, including for drug discovery.[34–40] Human PSC-derived organoids have also reconstituted architecture and pathology of numerous other organs[41,44,45,49] (Table 1). Excitingly, skin organoids recently developed from mouse PSCs reconstituted not only epidermal and dermal layers but also key appendages and cells including hair follicles, sebaceous glands, and adipocytes.[50]

PSCs benefit from their ability to reconstitute a virtually unlimited range of cell types. However, the derivation of a mature organoid is complex, often requiring a precisely coordinated sequence of factors for differentiation,[45,46] and thus may demand a priori knowledge of organ development and patterning. In fact, PSC-derived organoids often resemble fetal-like structures.[44,47] Despite the challenges of harnessing pluripotency for organoid

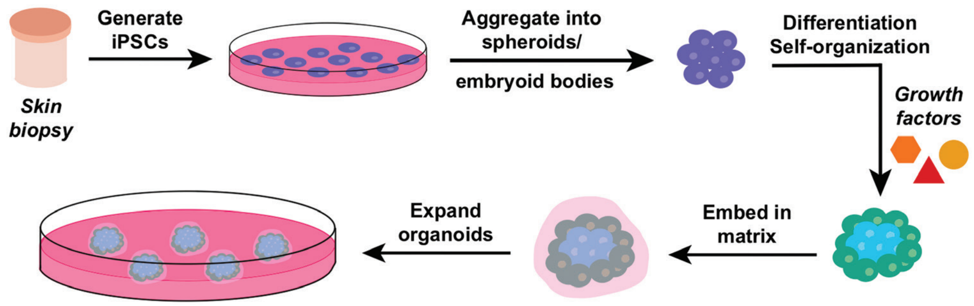

Fig. 2. Schematic depicting the steps by which organoids can be generated from patient tissue-derived iPSCs. In this example, iPSCs are derived from mature patient cells, such as dermal fibroblasts obtained from skin biopsies. These cells are then allowed to aggregate into small clumps/spheroids, known as embryoid bodies. Through the addition of exogenous molecular factors specific to the tissue of interest, these clusters of stem cells are coaxed to differentiate down tissue-specific lineages. Due to this extrinsic signaling and intrinsic patterning, as they differentiate, these cell clusters self-organize into multiple layers comprising different cell types (for instance, epidermis and dermis, for skin organoids). These clusters are then embedded into a 3D matrix and maintained in culture, where they continue to recapitulate tissue-specific microarchitecture and developmental patterning.

development, the synergy of organoid technology and iPSCs has unprecedented potential for studying development, physiology, and disease in vitro.

APPLICATIONS IN PLASTIC AND RECONSTRUCTIVE SURGERY

Given that plastic and reconstructive surgeons operate across a wide range of tissues, recreating complex biologic systems in vitro is of particular relevance to the field. Applications of organoid technology span the research and clinical pipeline, from creating personalized models of disease to potential use of organoids themselves as therapeutic agents (Fig. 3).

Organoids as Models of Disease and Development

Organoids have proven invaluable for studying cancer because traditional culture methods fail to incorporate elements such as stroma that play an important role in cancer progression.[52] Patient-derived organoids have been generated to model cancer in multiple organs[8,47,53]; to study complex phenomena such as the tumor immune microenvironment[54] and tumor single-cell diversity[55]; and to elucidate the effects of genetic manipulation using Clustered Regularly Interspaced Short Palindromic Repeats, CRISPR-associated protein 9 (CRISPR-Cas9) technology.[56–58] However, these studies have almost exclusively examined gastrointestinal tumors. The cancers most commonly encountered in plastic surgical practice, such as basal/squamous cell cancer, melanoma, and sarcoma, remain to be explored, and organoid modeling of these pathologies may offer similarly valuable insights.

Organoids have also been used to explore human development and organogenesis. For example, the integral role of bone morphogenic protein inhibition in posterior foregut formation[59] and fibroblast growth factor and Hedgehog signaling in pulmonary development[60] were discovered in organoids. Similar studies applied to craniofacial development may uncover aberrant signaling pathways that lead to deformities such as cleft lip and

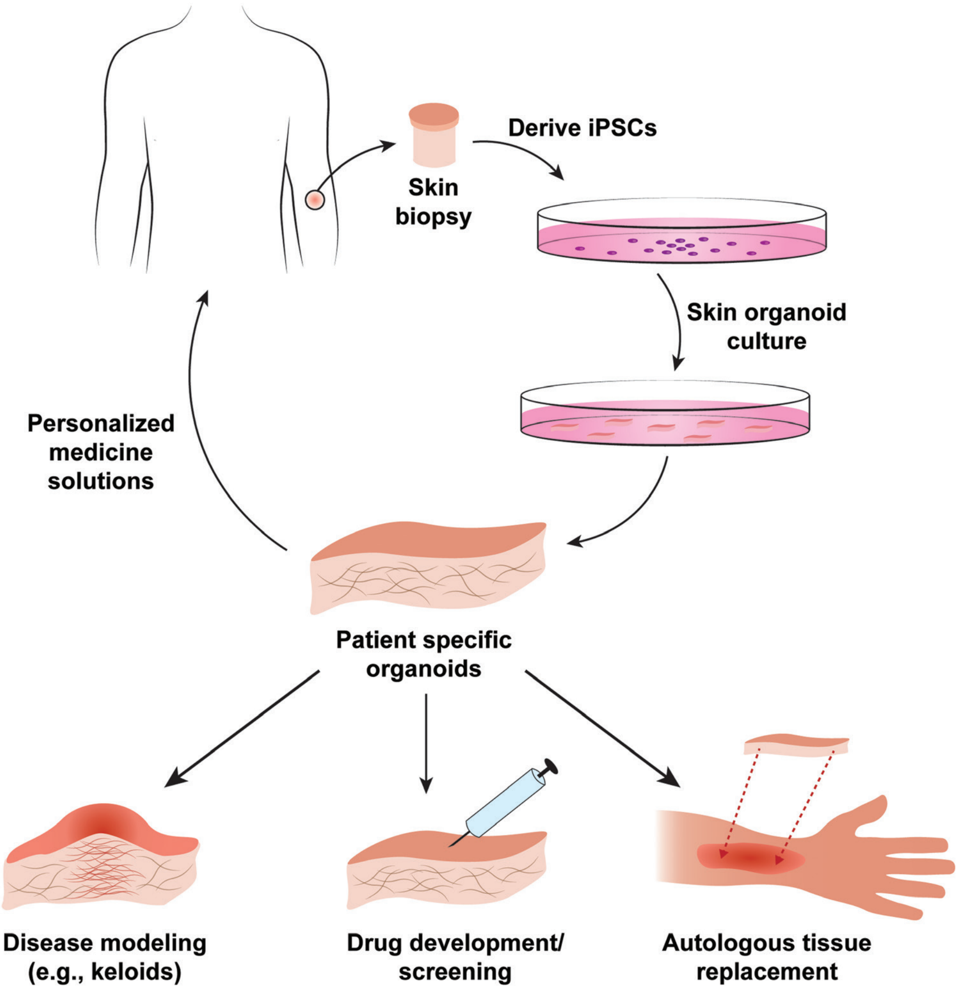

Fig. 3. Example of future applications for organoid technology in plastic surgery and reconstructive surgery. Personalized organoids can be generated from cells taken from a patient skin biopsy (top). These patient-specific/autologous organoids could then be used as a highly biomimetic model for disease modeling (bottom left), translational assessment of therapeutic agents (bottom middle), or even as a source of autologous tissue for transplantation (bottom right).

palate, 2 conditions commonly repaired by plastic reconstructive surgeons.

Although the field of wound healing and fibrosis is intimately connected to plastic surgery, in vitro study of these processes has historically been hampered by skin's resistance to 3D culturing techniques. Previous models relied on separately deriving and co-culturing different skin cell populations (eg, individually generating keratinocytes and fibroblasts, then combining into bilayered culture[61]); however, this method lacks the cell-layer communication that guides normal skin development and function in vivo.[62] Recently, mouse PSC-derived skin organoids are created that replicated both epidermal and dermal layers and exhibited spontaneous hair folliculogenesis[50] (whereas previous bioengineered skin models could only achieve hair folliculogenesis in the context of in vivo transplantation,[63–65] supporting the idea that organoids provide an increasingly "in vivo-like" cell niche). The generation of fully biomimetic skin organoids would significantly impact the study of plastic surgery–related pathologies such as skin infections, wound healing, and many others.

Organoids for Drug Development and Screening

Current techniques for disease modeling and drug screening rely heavily on manipulation of cell lines in 2D culture. Due to this model's inability to accurately recapitulate in vivo conditions, the attrition rate of drugs at this stage is high.[66] This has led to significant interest in developing physiologically representative culture methods, such as organoids, for drug screening purposes.[67]

A prime example of this application is the use of organoids in cystic fibrosis (CF) treatment. Using organoids derived from CF patient tissue, Ogawa et al[68] identified a therapeutic agent that stabilized and prevented improper folding of CFTR (cystic fibrosis transmembrane conductance regulator, the misfolded protein responsible for CF). In a subsequent blinded trial, this agent is tested in the patients from whom the organoids are derived, and their symptoms improved significantly.[69,70] This outcome powerfully demonstrates the utility of organoids for drug discovery.

Patient-derived organoids are important tools in the era of "personalized medicine." The ability to identify individual genetic signatures and phenotypic differences in patient-derived organoids may enable increasingly tailored and effective treatments. Living "organoid biobanks" have demonstrated utility for high-throughput drug and genetic screening.[69,71] For example, van de Wetering et al[71] performed genomic sequencing on a biobank of colon cancer patient-derived tumor organoids; therapeutic testing on this biobank identified targeted therapies for individual patients. Organoid disease models may be more likely to identify clinically actionable findings than simpler in vitro models, whose findings may be less translatable.

Similar techniques may aid in the development of novel treatment regimens for plastic and reconstructive surgery. For example, the spectrum of fibrotic skin pathologies (scleroderma, mixed connective tissue disease, hypertrophic scars, keloids, etc.) is wide. Although the burden of these diseases is significant, treatments are scarce due to the challenges of modeling disease and testing therapeutic outcomes in vitro. Organoids could provide a platform for studying disease mechanisms and assessing patient-specific phenotype and treatment response, in a setting that bridges in vitro and in vivo. For instance, organoids generated from cells of keloid-forming patients could facilitate the study of different cell types (eg, keratinocytes, fibroblasts, hair follicle stem cells, adipose cells) in a biomimetic setting to assess their distinct roles in lesion development and therapeutic response. Developing soft-tissue organoids to model diseases such as skin fibroses could open new doors in plastic surgical research and practice, both facilitating mechanistic analysis and allowing researchers to test potential therapies in vitro.

Tissue Engineering and Regenerative Medicine: Organoids as Treatment

The replacement of damaged, nonfunctional, or absent tissue is the raison d'être of reconstructive plastic surgery. Organoid technology may represent a novel source of patient-specific soft tissue for therapeutic use.

Autologous split-thickness skin grafts are the gold standard of tissue replacement. They enable replacement of "like with like," leading to favorable recipient site outcomes. However, significant obstacles include lack of suitable donor tissue (especially if the patient's skin is already compromised, eg, in large burns) and donor site morbidity including delayed healing, infection, and scarring.[72] Although allogeneic grafts are an active area of research, their utility is hampered by acute rejection not prevented with traditional immunosuppression.[73] Numerous synthetic and biologic skin substitutes have been developed, with varying resemblance to native skin. These materials, including both acellular matrices and cellular products [eg, Apligraf (Novartis, Basel, Switzerland)], facilitate healing by providing a scaffold for cell migration into the defect and a source of cytokines and growth factors.[74–76] Cultured epithelial autografts, which have been in clinical practice for decades, involve in vitro expansion of sheets of autologous keratinocytes used for tissue coverage. However, cultured epithelial autografts do not directly replace any components of the dermis, the most structurally and functionally critical skin layer.

Organoid technology carries the enticing possibility of in vitro generation of increasingly biomimetic, autologous tissue in quantities suitable for transplantation. Organoids have shown the capacity for substantial long-term expansion in vitro via repeated dissociation.[7] Such methods may be directly applicable in plastic surgery; for instance, we envision that many small, patient-derived skin organoids could be generated and distributed over a large defect. Precedent for such an application has been established by techniques including graft meshing, skin "micrografts,"[77] and automated devices such as the ART System (Medline Corius, Northfield, Ill.). Ultimately, skin organoids could represent a virtually unlimited source of autologous tissue that replicates key skin components and functions.[50]

ADDITIONAL CONSIDERATIONS FOR PLASTIC SURGERY

Organoid technology shows incredible promise for applications spanning from bench to bedside. We hope that this article will encourage our colleagues to consider how these novel methods might be applied to advance the field of plastic surgery. However, several unique challenges must be acknowledged when considering the application of organoid methods to our field.

One critical limitation of organoid technology is its inability to recapitulate the large-scale structural complexity found in organs in vivo. Recreating the in vivo ECM composition is challenging given the diverse cellular contribution to ECM,[66] but is important to consider given the importance of extracellular substrates in providing microenvironmental cues to cells. Further, existing organoids lack a component whose importance is intimately understood by plastic surgeons: a functional vascular system. The lack of blood vessels to circulate nutrients and oxygen limits the size of individual organoids. Incorporating vascular networks into organoid models has proven technically challenging, limiting researchers' abilities to develop organoids at true in vivo scale. However, Wimmer et al[78] have recently demonstrated the ability of human PSCs to self-organize into 3D blood vessels. Upon transplantation into mice, these organoids establish a fully perfused vascular tree. This advancement suggests the potential for overcoming organoids' size limitation, opening the door for ultimately generating at-scale soft-tissue replacements in vitro.

Although plastic surgeons work with tissues throughout the body, perhaps the most relevant tissue model is the skin. As addressed above, the derivation of skin organoids poses unique challenges. Compared with that of internal organs, the physiologic niche of the integumentary system is particularly difficult to recreate because it requires maintenance at an air–liquid interface.[79] Further, the skin is an extremely complex organ containing specialized appendages (eg, sweat glands, hair follicles, sebaceous glands) and a host of other cell types including immune and vascular cells, all of which are critical to skin's many roles (eg, wound healing, thermoregulation, barrier function). This remarkable diversity makes the skin inherently more challenging to faithfully replicate in vitro.

With the advent of organoids, many general challenges of these methods have also come to light. Due to their complexity, phenotypic variability between individual organoids is often high.[50] Furthermore, in many instances, it remains to be established how accurately organoids replicate in vivo development, physiology, and pathology. Organoid technology also presents unique ethical questions, including issues around patient privacy concerns, informed consent, and gene editing. As the field of organoid research is introduced into a broader range of biomedical fields, researchers, clinicians, and patients alike will be tasked with defining the precise role that organoids will play across the spectrum of medicine.

CONCLUSIONS AND FUTURE DIRECTIONS

This review describes the utility of the state-of-the-art technology in the rapidly maturing field of organoid science and highlights its potential applications within the field of plastic and reconstructive surgery. Progress is being made toward creating organoids that accurately model human skin; such a model will significantly improve our ability to accurately recapitulate epidermal–dermal biology in vitro. With technical optimization, organoids hold promise for use in testing and developing individualized therapeutic strategies in plastic and reconstructive surgery. We believe that with the development of consistently reproducible and cost-effective organoid models of skin and other tissues, and expansion of organoid technology toward ecto- and mesoderm-derived tissues, organoids could serve as a primary model for translational research that bridges in vitro and in vivo knowledge. Plastic and reconstructive surgery holds unique opportunities for applying organoids in clinical practice if they can be effectively adapted as autologous soft-tissue substitutes. Although key obstacles remain, it is our hope that this review will encourage our readers to consider how plastic and reconstructive surgeons can most effectively take advantage of this burgeoning technique, and what advancements may move this technology toward basic and clinical implementation in our field.

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