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Transformative Materials to Create 3D Functional Human Tissue Models In Vitro in a Reproducible Manner

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Recreating human tissues and organs in the petri dish to establish models as tools in biomedical sciences has gained momentum. These models can provide insight into mechanisms of human physiology, disease onset, and progression, and improve drug target validation, as well as the development of new medical therapeutics. Transformative materials play an important role in this evolution, as they can be programmed to direct cell behavior and fate by controlling the activity of bioactive molecules and material properties. Using nature as an inspiration, scientists are creating materials that incorporate specific biological processes observed during human organogenesis and tissue regeneration. This article presents the reader with state-of-the-art developments in the field of in vitro tissue engineering and the challenges related to the design, production, and translation of these transformative materials. Advances regarding (stem) cell sources, expansion, and differentiation, and how novel responsive materials, automated and large-scale fabrication processes, culture conditions, in situ monitoring systems, and computer simulations are required to create functional human tissue models that are relevant and efficient for drug discovery, are described. This paper illustrates how these different technologies need to converge to generate in vitro life-like human tissue models that provide a platform to answer health-based scientific questions.

1. Introduction

Producing in vitro 3D models mimicking the anatomy and function of human tissues and organs in an automated, standardized, and reproducible manner remains a major challenge. This is particularly important since often rodent data cannot be translated into humans, which partly explains why many preclinical drug candidates fail in clinical testing. Therefore, the lack of efficient standardized human model systems limits progress in preclinical drug development, compound screening, adequate disease modeling, and outcome research (such as implant rejection). It hampers toxicity studies, testing of vaccines, and explains the scarceness of clinical success and high failure rates of preclinically well performing lead candidates. The high costs in drug development with high attrition rates during clinical phases are related to a lack of representativeness of current preclinical test procedures based on 2D cell culture or animal models.[1] In addition,

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for some human diseases, there are no adequate in vivo models available due to the absence of specific receptors or signaling pathways, such as for Shiga toxin-induced hemolytic uremic syndrome, which results in thrombotic microangiopathy leading to acute kidney injury.[2,3] Hence, there is a need for human-based 3D tissue models to better understand disease onset and progression, and to sequentially test newly designed drugs, drug delivery systems, and other therapies including cellular and immunotherapies. In addition, these models may identify effective drug candidates for humans despite low efficiency in animals. The recent changes in the US Food and Drug Administration rule lifting the requirement of animal testing for drug approval, and the fact that of the European Medicines Agency (EMA) encourages the 3R Principle (replacement, reduction, refinement) in their strategy until 2025, represent a paradigm shift. While the EMA does not yet allow for regulatory acceptance without animal testing, it strongly supports New Approach Methodologies (NAMs) to establish efficient and reliable alternatives. This reflects the confidence that the governments, medical professionals, and the public have in the biomedical community to deliver new methods for drug discovery and personalized medicine. Over the last 20 years, there have been numerous advances resulting from the merging of individual research areas, such as (bio)chemistry, material science, (stem) cell biology, and biofabrication into multidisciplinary fields. In **Figure 1**, we provide a timeline of the key evolutions made over the past decades regarding cell and material design and production, aiming to grow human functional tissue models. However, more convergence is needed between the different disciplines to achieve highly standardized human-based in vitro models that reinforce the 3R Principle in animal studies, support randomized controlled clinical trials as companion diagnostics, provide insights into disease mechanisms, and facilitate personalized medicine. With a clear aim for translation, automation, high-throughput systems, regulation, and ethics should be part of the development process.

In recent decades, materials used in tissue engineering have focused on providing a scaffold for cells to grow, expecting that cellular processes may spontaneously trigger tissue formation. Now, we understand that these materials need to do much more than present a comfortable milieu for the cells to thrive, as their spatial organization and temporal functionality are crucial to form functional tissue. Therefore, transformative materials are required that adapt to the needs of growing tissue and actively direct cell behavior to transform biohybrid constructs into fully biofunctional tissues. Transformative materials directly interact with the cells and allow for the influence of external factors to recapitulate some aspects of native organogenesis, remodeling,

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1906	Hanging drop tissue culture as first example of 3D cell culture[4]
1960	First hydrogels for biological use (polyglycolmonomothacrylate)[5]
1980s	Matrigel produced from Engelbreth-Holm Swarm mouse sarcoma[6]
1988	First demonstration of bioprinting (fibrinogen as bioink)[7]
1993	Introduction of the concept of Tissue engineering[8]
1997	First tissue engineered ear (chondrocytes cultured on PLGA scaffold)[9]
1998	Human ESCs from blastocyst[10]
	FDA approves first allogenic TE product (Apligraf®)
2004	EU banned animal testing for finished cosmetics (testing of ingredients banned in 2009)
2007	iPSCs reprogrammed from human fibroblasts[11]
2008	First report of ESC self-organization into polarized cortical tissue[12]
2009	First report of organoids derived from mouse ASCs[13]
	Obama overturns ban on federal funding for stem cell research[14]
2010	First organ-on-a-chip (models alveolar-capillary interface of human lung)[15]
2011	First report of directed differentiation of human iPSCs into intestinal organoids[16]
2013	First report of iPSC-derived liver organoids transplanted *in vivo* to improve vascularization[17]
2015	First injectable MAP scaffold (based on PEG microgels)[18]
	First reports of human adult stem cell-derived liver[19], human pancreatic[20], human mammary gland[21], and fallopian tube[22] organoids
2016	First organoid cultivation in a synthetic matrix (intestinal stem cell organoid)[23]
2017	Injectable hydrogel for directed cell growth - Anisogel[24]
	EMA decision: Regulatory acceptance of 3R testing approaches
2018	Phenion full-thickness skin model for *in vitro* testing[25]
2019	Kidney organoids on a chip show flow-enchanced vasculature network[26]
2020	Mini-intestine - First scaffold-guided organoid self-organization[27]
2022	First clinical transplantation of an organoid into a patient with Ulcerative colitis (Tokyo medical and dental University)
	Implantation of allogeneic hiPSC-CM patches in a patient with ischemic cardiomyopathy[28]
	Rousselot Biomedical announces the world's first GMP grade GelMa (bioink)
2023	FDA decision: No animal tests required for human drug trial approval[29]

Figure 1. Timeline of groundbreaking developments in stem cell-based models and biomaterials.[4–29]

and regeneration to ensure proper tissue formation. In addition, transformative materials should allow for spatially and temporally controlled presentation and release of bioactive factors, and/or genetic modification of cells.[30] The challenge here is to present harmonious combinations and gradients of biochemical, mechanical, and physical signals in a perfectly timed sequence targeting specific cell populations. This can be achieved by programming the materials to respond to cellular (enzymes, transmembrane proteins, forces, etc.) and/or external triggers (e.g., light, force, temperature, magnetic fields, ultrasound, etc.) to align the appearance of these signals with the distinct developmental programs that guarantee organ-specific differentiation. Therefore, to create the next generation of native-like biohybrid constructs to grow life-like complex human tissues in vitro, we need to design and develop new platforms of transformative materials. These materials should be comprised of synthetic, biohybrid, biodegradable, and interactive molecules, as well as colloidal building blocks that respond to trigger for on-demand tuning of their mechanical and structural properties. Two main categories can be distinguished here: 1) biofabricated material platforms containing solid building blocks, such as fibers or hydrophobic polymer constructs, and 2) injectable hydrogels that can be pipetted or bioprinted. Hydrogels can be made from natural or synthetic building blocks, or a combination of both. Hydrogels obtained from natural sources, known to be present in the target tissue, aim to provide physiological cell–matrix interactions that drive tissue development,[31] while the mechanical, biochemical, and structural properties of synthetic materials can be better controlled and programmed to respond to specific triggers.

However, transformative materials are just one piece of the puzzle to grow human functional tissue in vitro. They have to be combined with technologies, such as human (stem) cell culture, and automated platforms to manufacture, culture, employ and evaluate these complex tissue models. For example, high-throughput systems (HTS) will enable a systematic analysis of numerous combinations of cells, biomaterials, and culture conditions to screen a large parameter window aiming at successful creation of viable tissues. Combined with a smart Design of Experiments, this approach could resolve which combinations of parameters will aid in stem cell expansion and differentiation as well as tissue maturation in a standardized and reproducible manner. Hence, advances in creating applicable and on-demand tunable materials, accompanied by organ-on-a-chip and bioreactor platforms, are essential to create a reproducible environment for the cells to differentiate toward multiple developmental lineages and ultimately create structured, perfusable, and functional millimeter-scale tissues in high-throughput.

By combining transformative materials with patient derived induced pluripotent stem cells (iPSCs), personalizing medicinal products to the individual biology of the patient, a true personalized medicine approach can be achieved.[32–35] However, developing personalized treatment options based on autologous cells remains a logistical and financial challenge, which calls for allogeneic alternatives. To overcome these limitations, iPSC stocks based on cells derived from superdonors that are homozygous for the most relevant human leukocyte antigen variants in Japan are developed, which thereby cover around 32% of the Japanese population and is especially relevant for regenerative medicine to avoid graft rejection upon transplantation.[36] Regarding drug discovery and testing, relying on pooled iPSCs from multiple donors could allow for the formation of representative 3D functional tissue models in a more cost-efficient manner instead of utilizing patient-derived cells to still facilitate precision medicine and provide treatment options closer to the patient's needs.

Numerous differentiation protocols have already been established to successfully develop specific miniature organs on a dish by self-assembly and organization of stem cells into organoids. These microstructures replicate some of the structural and cellular complexity of tissues but are limited in size and lack complex tissue architecture.[37] Although organoids have been used in personalized medicine for cystic fibrosis[38] and in pharmacodynamics studies to test investigational new drugs[39] now in phase 2 trials, they have thus far not led to new drug discoveries. Therefore, the aim is to go beyond organoids and create more complex and physiological human tissues in order to better replicate human mechanisms that provide answers to those questions that still cannot be solved by current more simple models. However, the absence of physiological elements, such as blood flow and appropriate microenvironmental cues, still prevents these models from reaching the level of tissue maturity needed to recapitulate all aspects of their target tissue, including nutrient and oxygen supply and waste removal. Hence, the realization of functional human tissue on a millimeter scale under the control of dynamic environmental cues, including the immune system and hemodynamic properties, is still in its infancy.

In this article, we describe the current advances in the field for creating functional life-like human tissue in vitro. We discuss the different building blocks needed to create robust and functional, complex 3D tissues, spanning from engineered cells to transformative biomaterials, in a reproducible manner, and include essential tools, such as automated high-throughput production and screening, bioprinting, and dynamic bioreactor systems, including monitoring, in silico modeling and artificial intelligence-based analysis. To translate these tissue models to personalized medicine and clinical practice, we have to focus on creating the evidence needed for clinical development, ensure quality assured production, seek regulatory advice, and early plan the transfer to the pharmaceutical industry.

2. Stem Cell-Based Tissue Engineering

2.1. Stem Cell Types

Human cells are essential building blocks to create human in vitro tissue models. They come in the form of immortalized cell lines, primary cells, or stem cells. Immortalized cell lines can be easily expanded to generate a large supply without replicative senescence. They may provide more consistent results than primary cells due to the absence of donor variation.[40] However, their biological relevance is reduced, as immortalization and continuous acquisition of cellular changes during culture expansion can obscure cellular characteristics. Therefore, primary cells, directly isolated from tissues, are often used for tissue engineering. While they maintain the characteristics of the target tissue, their use is hampered by donor variation and these cells often undergo changes during cell culture, reaching a state of cellular senescence with ultimate growth arrest within a short time span.[41,42]

In addition, for certain tissues, primary cells such as neurons or cardiomyocytes are not easily accessible in humans.

To overcome these limitations, stem cells have become an important source of cells for tissue engineering due to their capability of self-renewal and differentiation into multiple cell lineages (depending on the stem cell type), and self-organization into growing organoids.[34,43] Adult stem cells (ASCs) are stem cells derived from mature tissues, such as hematopoietic stem cells or mesenchymal stromal cells (MSCs) from human bone marrow. Other ASCs from the small intestine, stomach, liver, pancreas, lungs, and kidney biopsy tissue can even be used to generate spheroidal organoids that recapitulate some features of their tissue of origin. ASC-derived organoids are commonly multicellular structures and consist of a relatively simple architecture. A clear advantage of this model is that ASC cultures allow for long-term expansion of primary epithelium in 3D, which has been successfully used to model human disease and screen drug libraries.[44] However, this technology is restricted to (a subset of) epithelium, preventing the generation of life-like tissues in all their facets. ASCs are still bound to replicative senescence, can have high interdonor and interexperiment variability and do not develop critical interstitial compartments and vasculature.[44]

Some of the abovementioned limitations can be circumvented by iPSCs. Somatic cells can be reprogrammed into iPSCs using the Yamanaka factors, and in the pluripotent state, iPSCs can be expanded in culture without any signs of replicative senescence state (**Figure 2**a-i). In fact, virtually all signs of cellular aging are reversed by reprogramming and are gradually reacquired upon the subsequent differentiation of iPSC-derived cells.[45,46] Derivation of cells from iPSCs is possible, and reliable protocols have been established to differentiate iPSCs toward specific cell types in 2D and into multicellular 3D organoids.[43] The need for 3D organoid cultures rather than 2D cultures was recently shown in the kidney field. The cellular characteristics of podocytes in 3D kidney organoids closely mimicked their in vivo counterparts, while 2D iPSC-derived podocytes lost key markers, such as nephrin and podocin. Moreover, downstream signaling events following injury are absent in 2D iPSC-derived podocytes.[47] Such marker expression loss is also evident when podocytes are isolated from organoid glomeruli and recultured in 2D.[48] Despite the lack of full mature tissue and limited anatomical organization, some iPSC derivatives acquire similar, albeit not identical, epigenetic and transcriptomic characteristics as primary cells, e.g., as MSCs,[49] hematopoietic progenitor cells,[50] or neurons.[51] Furthermore, iPSCs allow for personalized medicine and can easily be subcloned to generate cell lines with patient-specific somatic mutations or for genetic engineering, e.g., with CRISPR-Cas9 technology. For example, iPSC-derived hematopoietic cells were established that mimic aspects of systemic mastocytosis,[52] of neurons with specific mutations present in erythromelalgia patients,[53] and 3D multicellular kidney organoids to study congenital renal disorders (Figure 2a-ii).[47] Next to disease modeling and their potential for regenerative medicine, iPSC derived human organoid models show potential for preclinical drug testing including pharmacodynamic studies.[39] Despite the potency of iPSC in biomedical sciences, critical challenges are still pending. iPSC cultures require delicate handling to avoid spontaneous differentiation and, importantly, differentiation efficiency into the target organ varies among protocols but also between batches.[54,55] The variations in cell proportion is largely driven by the presence and abundance of off-target cells during differentiation.[56] This off-target population can be reduced by pharmacological inhibition of pathways that drive off-target cells or by in vivo transplantation.[55,56] Regardless, it would still be favorable to establish robust iPSC-derived models without the need for inhibitors or transplantation into animals. In the future, differentiation conditions for iPSC toward specific cell types need to be further refined. To this end, self-organized differentiation in 3D culture, as well as in instructive biomaterials, will play an important role. The use of automated and standardized cell cultures will be essential in creating robust models and will improve the iPSC maintenance and differentiation pipeline.

2.2. Automated and Standardized Cell Culture

Before embedding cells into 3D matrices and sequential assembly into tissue models, cells need to be expanded in culture. To produce the cells on a large scale in sufficient numbers for their use in tissue engineering and therapeutic applications, automated robot-based platforms are essential (Figure 2a-iii). These standardized production platforms avoid batch deviations and can enable reproducible and quality-assured cell cultures.[57–59] Stem cell factories, as they are known, have successfully been used to generate and expand iPSCs using defined and user-independent conditions with the economic advantage of a fully automated stem cell production and increased throughput.[59–62] As such, the integration of deep-learning-based algorithms into robotic pipelines has resulted in noninvasive routine assessment of iPSCs, concerted by the use of fluorescent reporter lines, to ascertain a standardized protocol for monitoring, e.g., the proliferation rate and cell confluency prior to splitting cells and further propagation.[60,63,64] Standardization is a key factor, as culture conditions can already prime cells toward specific functions. It has been shown that surface topography can direct the differentiation of MSCs and iPSCs,[65,66] whereas very soft fibrin-based hydrogels hardly affect the differentiation of MSCs.[67] It is also possible to seed cells as small cellular aggregates, rather than as individual cells, which may favor survival due to enhanced cell–cell interactions and, if needed, self-organized differentiation. Recently, a method has been described to generate such aggregates by a self-detaching mechanism from microcontact printed substrates,[68] which facilitates upscaling for automated production.[69,70] Such automated platforms enable quality assurance in production which is necessary for clinical application. For the development of an advanced therapy medicinal product (ATMP), reproducible quality has to be assured to meet the quality-related regulation requirements. For personalized treatment, it is mandatory to define optimal stem cell expansion and differentiation protocols, for example, to create iPSC-derived patient-specific therapies, such as allogeneic cardiomyocyte patches, joint implants, and CAR-T-cell therapy[28,58] To enable large-scale cell production and clinically GMP-compliant manufacturing, stirred bioreactor-based platforms have been established to expand and differentiate MSCs and iPSCs under controlled conditions,[57,71,72] for example, to produce iPSC-derived hematopoietic cells[73] and cardiomyocytes.[74] Bioreactor-derived

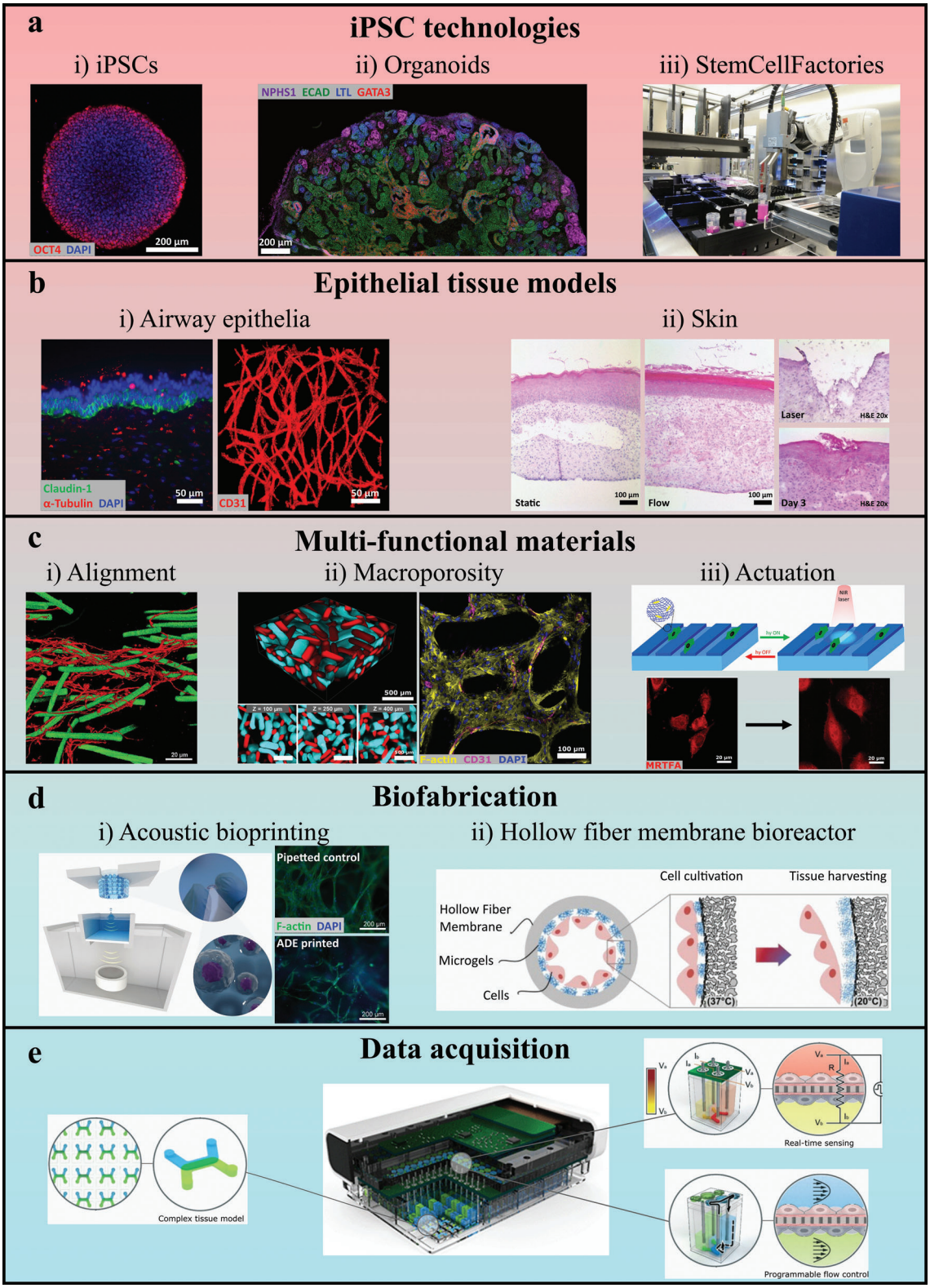

Figure 2. State of the art building blocks as first steps toward functional mm-scale tissue formation and analysis. a) (i) The development of iPSC technology has propelled the generation of tissue like structures using (ii) organoid formations. (iii) Upscaling requires technological advances in automated tissue culture techniques to help in the arduous process of iPSCs expansion. b) Initial epithelial tissue models such as those from (i) the airway epithelia and (ii) skin, have shown that these models require complex environments that include interfaces and other forces such as flow. c) Transformative materials are those that go beyond providing a safe environment for cells to grow but actively direct tissue formation in its native

iPSC macrophages closely resemble human macrophages isolated from peripheral blood mononuclear cells and have been successfully used as immunotherapy to stimulate pulmonary immunity and treat bacterial infections in a preclinical in vivo mouse model.[73] Such a yield of high-quality differentiated cells underlines the importance of translating 2D, low-yield, cultures toward 3D bioreactors that can facilitate the quantity and quality of iPSC-differentiated cells needed for clinical implementation in the future. In addition, the toolbox for a more holistic understanding of cell behavior and characterization has greatly expanded in recent years. The integration of omics technologies, including (spatial) transcriptomics, epigenomics, and proteomics, as well as spatial image-based platforms, allows for in-depth characterization of cellular differentiation processes. As such, an integrated analysis using an image-based framework of iPSCs has been developed, in which intracellular spatial patterns among distinct cell states can be identified.[64] Moreover, there is a growing perception that epigenetic parameters, which ultimately govern the process of cellular differentiation, are powerful to define cell populations.[75–77] Applying robotic pipelines accompanied by high-end cellular characterization will facilitate the development and realization of complex millimeter-scale human tissues for a reproducible provision of highly specific analysis systems for therapy diagnostics, where quality is ensured by design and production.

2.3. Materials that Support and Direct the Formation of Organoids

Organoids have taken center stage as a source of microtissues that replicate certain aspects of the native tissue architecture, function and, in some cases, pathologies in vitro.[78] For example, kidney organoids, often cultured at the air–liquid interface on transwell filters, have been developed to recapitulate podocytopathies, such as the nephrotic syndrome,[47,79] model the most commonly inherited polycystic kidney disease[80,81] and nephronophthisis ciliopathy,[82] and decipher SARS-CoV-2-induced kidney fibrosis.[47,83] Alternatively, ASC-derived organoids are often formed in Matrigel, which is a mouse sarcoma-derived matrix with a poorly defined composition. Matrigel does not reflect physiological conditions and differs from batch-to-batch, thereby limiting the reproducibility and validity of the respective organoids, as well as tuneability. To overcome the limitations of Matrigel and other animal-derived materials to culture organoids inside a 3D microenvironment, alternatives based on synthetic materials with a tailored polymeric network and variable mechanical, physical, and biochemical cues have been developed. For example, designer matrices with tunable degradability, stiffness, relaxation behavior,[23] and/or viscoelastic properties[84] are investigated to tailor the material properties according to the cells' needs. Recently, a synthetic lymphoid tumor microenvironment was developed by combining polyethylene glycol (PEG) hydrogels and lymphoma organoids that were engineered based on molecular information from more than 1100 patient samples. By tuning the hydrogel's mechanical properties to match the increased stiffness of lymphomas compared to noncancerous lymph nodes, and integration of bioadhesive peptides that mimic integrin binding in vivo, characteristic features of lymphomas were recapitulated in vitro. In this way, it was possible to identify advanced treatment options that are effective in vitro and in vivo. This platform may represent an accurate approach to identify effective lead compounds that can move to the next stage in drug development.[85]

In addition to matrix-related limitations, organoids are still unable to match the full architecture, complexity, maturity, and thereby function of organs.[86,87] To address these challenges, controlling and steering the process of organoid formation in a reproducible manner is crucial. Novel approaches complement the intrinsic self-organization of cells by providing external geometrical cues to guide cell morphogenesis, resulting in organoids with anatomical and functional features closer to their target tissues compared to conventional organoids. In this context, pioneering work has been done with perfusable "organoids-on-a-chip" that resemble the topology of the gut surface, allowing for the development and long-term culture of mini-intestines for more than two weeks.[27] Moreover, various methods, such as photopatterning of synthetic PEG-based stem cell niches, are being further developed to steer organoid development.[88] However, the complexity of these tissue models is still limited by the organoid size as larger tissues are necessary to reproduce some important functional features that organoid systems cannot reproduce. This is primarily due to limitations in nutrient and waste exchange, which often leads to a necrotic core region for organoid sizes as small as 200 µm.[89] Therefore, growing millimeter-scale functional tissues requires the incorporation of an internal vascular network capable of fulfilling this task, which, to date, remains one of the major challenges of tissue engineering.[90] One solution to this problem has been human organoid xenotransplantation in rodents. In vivo transplantation of organoids results in host engraftment and enhances the vascularization and maturation of organoids, as shown for, e.g., kidney, liver, and lung organoids.[17,91–93] Transplanted kidney organoids have been used for preclinical assessment of drug candidates where the localization of the drug is measured in the human kidney organoids and inside the rat host kidneys. This model allows for pharmacodynamic and pharmacokinetic modeling of new drugs and emphasizes the power of mature-like organoids for developing new therapies.[39]

form. Examples of these are (i) anisotropy, (ii) porosity, and (iii) mechanical forces amongst others. d) Novel, cell-friendly biofabrication techniques and bioreactors techniques are necessary to create tissues in the microscale such as (i) acoustic bioprinting based on the principle of acoustic droplet ejection and (ii) thermoresponsive hollow fiber membrane bioreactors. (e) Data acquisition systems in the form of microchip-embedded-microfluidic array systems combined with AI, need to be included in the tissue model design to achieve high-throughput screening. Reproduced with permission.[119,117] Copyright 2021, 2022, Elsevier. Reproduced with permission.[60] Copyright 2021, MDPI. Reproduced with permission.[115] Copyright 2022, Frontiers. Reproduced with permission.[24] Copyright 2017, American Chemical Society (ACS). Further permissions related to excerpts from these materials should be directed to ACS. Reproduced with permission.[120–122] Copyright 2020, 2021, 2022, Wiley–VCH GmbH. Reproduced with permission.[123] Copyright 2019, Springer Nature. Reproduced with permission.[124] Copyright 2021, Royal Society of Chemistry (RSC).

However, the still ongoing use of animals for the maturation of tissues presents further problems in standardization and production quality, calling for new ways to recreate functional vascularized and perfused tissues in vitro. Therefore, culture systems, including organs-on-a-chip,[26,94–96] capable of growing a vascular network with controlled fluid flow are necessary to transport nutrients to the organoids and facilitate waste removal to stimulate differentiation. The creation of a mature human vascular network in organoids is crucial in the context of modeling diseases that affect the vascular system and for studying the interplay between endothelial–epithelial compartments. Such platforms, however, do not yet exist, but will aid the development of vascularized millimeter-scale tissues with a mature phenotype.

2.4. Sequential iPSC Expansion and Differentiation Inside 3D Hydrogels

To grow larger millimeter-scale tissue models, iPSCs are usually expanded and differentiated before being embedded inside hydrogels for 3D cell culture. Here, the challenge is that many differentiated cells do not have high proliferation capabilities and thus hinder the formation of dense tissues. For example, cardiomyocytes have a low growth rate below 3%, and even at very high cell density seeding, it is difficult to form dense functional tissue.[97] In addition, the differentiation of stem cells on 2D or 2.5D surfaces polarizes the cells in the *z*-direction, which does not mimic the in vivo environment.[98–100] On the other hand, iPSCs grown inside 3D hydrogels quickly lose their self-renewal capacities, and thus their proliferation capacity, and start differentiating in an uncontrolled manner due to the cues they sense from the surrounding material, highly influencing their cellular processes.[101] To bridge this gap, stem cells could be expanded into denser cultures before differentiating them inside the same construct in a spatially and temporally controlled manner. However, only a few research groups have designed and employed 3D hydrogel systems to study whether both processes can be combined in one and the same material system.[98] In one successful example, single iPSCs were seeded in thermoresponsive hydrogels formed via hydrogen bonds, which allowed the cells to move more freely toward each other and assemble into growing aggregates that maintained their stem cell phenotype and ability to differentiate into all three germ layers, likely by enhanced cell–cell contacts.[102] Alternatively, progenitor cells that already tend to differentiate into a specific cell type but still have high proliferation ability have been mixed in 3D hydrogels.[103] In the case of neural progenitor cells, it was demonstrated that their 3D expansion is significantly increased in fast-degrading gels, independent of their initial stiffness.[104] Both reports support the hypothesis that a crucial criterion to expand stem cells and maintain a stem cell phenotype inside 3D hydrogel constructs is to ensure their cell–cell interactions during expansion while tightly controlling cell–material interactions, which is not supported in most conventional hydrogels. Therefore, materials are required that offer the optimal matrix, growth factors, and physiological environment, providing sufficient space and the right signals to enable iPSC interaction, expansion, differentiation, and organization into structured and functional human tissue-like models.

3. Toward Millimeter-Scale Tissue Models

Native tissues consist of various cell types and extracellular matrix components that are integrated into complex spatial patterns to build up organs and ultimately whole organisms.[105] Therefore, tissues are versatile and unique in their cellular composition, architecture, and overall complexity. Depending on their function, as well as cellular and structural complexity, tissues can range from simple flat structures with predominantly one cell type to solid organs, which consist of multiple cell types that are precisely organized into specific structures.[106] Disruption of these structures often leads to diseases, such as muscular dystrophies,[107] fibrosis,[108] or cancer.[109] Thus, for 3D tissue models, it is important to mimic tissue architectures adequately. Besides selecting tissue inherent cell types, it is crucial to develop transformative materials that recapitulate the tissue organization and architecture to introduce proper mechanical and biochemical cues in a spatiotemporally controlled manner that is required to support tissue development.[110] This is already demonstrated in relatively simple, bidimensional structures, like the cornea, where the orthogonal fiber arrangement has to be mimicked to ensure optical transparency and mechanical endurance.[111] In the case of more complex bulk organs, the development of physiologically relevant tissue models becomes even more sophisticated. For example, when aligned anisotropic building blocks with human cardiac cells were bioprinter, it was shown that the contractile force and conduction velocity could be improved compared to spheroidal controls.[112] Also in in vitro epithelial tissue models, it has been shown that it is important to mimic the complex 3D architecture of epithelium in close connection with its underlying tissue.

3.1. Multicomponent In Vitro Epithelial Tissue Models

Epithelial tissues separate the inside from the outside of the body and fulfill fundamental functions during development. In vitro epithelial tissue models are, therefore, of paramount medical interest to understand stress and trauma responses, multiple genetic diseases, and carcinogenesis. The most prominent and unique feature of epithelial cells is their propensity to form tightly coupled cell collectives that are connected by abundant cell–cell contacts. The resulting epithelial sheets are far from homogenous with complex differentiation patterns both within monolayers and in multilayers. Understanding epithelial physiology and pathology requires the analysis of cell collectives that are exposed to grossly different external and internal environments. Technical solutions include the formation of epithelial spheroidal acini and the growth of monolayers on defined material supports, exposing them to different environments at their apical and basal surfaces.[113] These setups can be used to study epithelial barrier dynamics in various biochemical and mechanical paradigms and how these affect invasions of the basal extracellular matrix, apical extrusion, and epithelial barrier breaching by microbes or immune cells during infection and inflammation or by embryonic trophectoderm cells during implantation. Questions that can be addressed with in vitro epithelium models are how and to what degree biochemical and mechanical stimuli affect epithelial differentiation and function. However, challenges remain to establish in vitro models, which not only consist of epithelial cells but can mimic the complex 3D architecture. The use of primary

epithelial cells and their limited proliferation and differentiation capacity in vitro complicates a reproducible and standardized production of these models.

As an example for in vitro epithelial models, airway epithelial cells are commonly cultured at an air–liquid interface with defined cell culture media.[114] Nevertheless, these simple 2D models neglect cell–cell interactions between the epithelium and underlying tissues. Thus, complex 3D models of the airway mucosa based on fibrin hydrogels were developed, which show a more relevant mucociliary differentiation of the epithelium with beating cilia as compared to their 2D counterparts.[115,116] The addition of fibroblasts recapitulates the cell–cell interaction between the epithelium and underlying tissues enhancing epithelial differentiation[116] It was observed that epithelial cells could even improve in vitro vascularization (Figure 2b-i). These in vitro models of respiratory mucosa can be used to evaluate treatment options and to replace or at least refine animal experiments.

Alternatively, 2D skin models have been slowly replaced by multicomponent 3D human skin equivalents (HSEs) presenting a more anatomical and physiological system. The major problem in these HSEs has been their short life, which ranges between 7 and 14 days as cell growth becomes unstable and tissue degradation ensues. Recently, an HSE model was developed and maintained for up to 28 days by introducing a dynamic flow culture bioreactor that provides a more physiological environment to better promote nutrient exchange and prevent byproduct accumulation. In addition, dynamic flow cultures showed faster healing after laser irradiation injury than static controls, extending the importance of dynamic perfusion during in vitro tissue development (Figure 2b-ii).[117] Alternatively, in a gastrointestinal tract model, Caco-2 cells cultured in scaffolds with villus topography and under flow, show cell proliferation, apoptosis patterns, and glucose uptake much more similar to in vivo tissue compared to static cultures.[118] The knowledge obtained from these models will be instrumental for the next step toward creating millimeter-scale tissue models using stem cells rather than cell lines and to improve tissue architecture and cellular composition using novel transformative materials in a standardized and controlled approach.

4. High-Throughput Production of mm-Scale Tissue Models

The implementation of automated high-throughput workflows for the production and analysis of growing human millimeter-scale tissue models is inevitable for enhanced optimization of the models and their successful translation toward industrial applications in drug development and toxicity screening. High-throughput screening (HTS) approaches rely on parallelized and efficient testing of a large range of material parameters, with standardized cells and culturing conditions in academic research; in the pharmaceutical industry, they allow for target screening of large compound libraries to identify possible new drug candidates.[125] Constituting a key module of HTS platforms, automated liquid handling systems enable the parallelized production and cultivation of 3D tissue models in 384 and 1536 microwell plate formats,[125] aiding to reduce run-to-run variabilities, the influence of human errors and the consumption of materials.[126] Simultaneously, the reproducibility and comparability between experiments are improved.[125] Over the last five years, 3D cell cultures inside biomaterial constructs have been achieved with automated liquid handling devices relying on the crosslinking of synthetic polymers in aqueous solutions to form the hydrogels around the cells. This led to the automated production of human kidney organoids for compound screening[86,127] and the maintenance, expansion and differentiation of different cell types.[128] These examples indicate promising approaches for fully automated high-throughput production of 3D human tissue models to be employed in compound screening for early drug development in the future. Challenges remain to achieve multidimensional tissue organization inside these cultures and to grow vascularized mini tissues to better mimic tissue function.

4.1. Synthetic Pipettable Building Blocks

To enable tissue production with an HTS device, the material building blocks must be pipettable and form a 3D construct inside the wells. This is based on a controlled phase transition from a fluid to a viscoelastic gel. Such materials can be characterized by shear-thinning properties because of reversible self-assembly capabilities, or in situ crosslinking of a molecular precursor solution with tunable gelation kinetics. Hydrogels based on natural materials are an obvious choice, as they exhibit important characteristics of the native extracellular matrix and thereby provide a suitable environment for cells to grow.[31] However, the biological and mechanical properties of natural materials are difficult to control and to modify further to steer tissue formation.[129,130] Natural materials, commonly obtained from biological sources, often show batch-to-batch variations, which are undesired when reliable tissue models are needed.[131] In addition, their chemistry cannot be easily adjusted to fine-tune the gelation kinetics, which is required for high-throughput production. Here, synthetic hydrogel precursors, such as polymers or peptides or a combination of both, are characterized by their superior batch-to-batch reproducibility, well-defined structure, and functionality, and represent an increasingly dominant platform for the generation of scaffolds for tissue development in vitro. As synthetic materials do not possess intrinsic biofunctionality, hydrogels have to be designed with selective cell attachment cues, degradation sites, and growth factor binding domains, while signaling cues can be programmed into the materials to be accessed by a specific cell population at defined locations and times.[132,133] This offers greater opportunities for customization to match the needs of the desired application and control cell–matrix reciprocity and cell behavior, as cells interact with and remodel their surrounding environment.[134] Importantly, due to the fibrous architecture of the ECM, native tissues possess nonlinear mechanics such as strain stiffening or stress relaxation as a result of cell contraction, which is known to influence cell spreading and proliferation.[135] Synthetic hydrogels, however, are commonly purely elastic and lack the nonlinear mechanical properties of the natural ECM resulting from their nonfibrous architecture. Therefore, macromolecules have been designed to form 3D hydrogels with strain stiffening and/or stress relaxation properties, which have been shown to influence cell spreading and govern stem cell fate, highlighting the importance of hydrogel dynamics on cell behavior.[135,136] To better match the viscoelastic properties of native tissues, supramolecular

hydrogels rely on physical crosslinks such as ionic or protein interactions, resulting in molecular networks or fibrous hydrogels based on, e.g., hydrazone crosslinks[137] or ureido-pyrimidinone (UPy),[138] respectively. These dynamic hydrogels allow cells to remodel their surrounding environment without the need for matrix degradation.[139] To mimic the strain-stiffening properties, polyisocyanide-based hydrogels have been developed, which also form a nanofiber structure that mimics the architecture and mechanical properties of biological hydrogels.[140] While the cell behavior is greatly affected by the hydrogel internal structure, mechanical properties, and degradation kinetics, the 3D environment remains isotropic and does not provide a heterogenous structure for the cells to mimic the compositional complexity of living tissues more faithfully. Here, microgel in hydrogel systems has been developed as multiphasic in vitro models to include cross-scale heterogeneity and directionality in matrix properties and/or cellular composition. This way, cells inside the microgels and cells within the bulk hydrogel matrices can be presented with different material properties and mesoenvironmental characteristics independently, which was demonstrated with a reductionistic in vitro model of vascularized prostate cancer tissue. As microgels are sufficiently small to be injected by themselves or in combination with hydrogel precursors,[141] these novel biomaterial building blocks are compatible with HTS.

4.2. Introducing Directionality Inside Pipettable Hydrogels

To overcome the limitations of conventional isotropic bulk hydrogels made from synthetic materials, transformative materials are required to mimic the hierarchical organization of multiscale tissues.[142] As tissues, such as bone, tendon, muscle, heart, nerves, etc., are characterized by their anisotropic structure, which is paramount for their function, this orientation needs to be captured inside in vitro tissue models.[110] In this regard, the Anisogel technology is developed to combine pipettability with creating directionality inside the hydrogel to align cells. To achieve this, a hybrid solution was developed consisting of micrometer-scale, magnetoresponsive, rod-shaped microgels or short fibers that can be aligned along an external magnetic field, and a surrounding hydrogel precursor solution that crosslinks around the oriented rods, thereby fixing their position in the hydrogel matrix and enabling the removal of the magnetic field.[24,143] It has been demonstrated that the Anisogel directs and aligns cell growth with unidirectional neurite extension (Figure 2c-i). Both components of the Anisogel can be varied in mechanical and physical properties and biofunctionalization, depending on the target cells and tissue.[144–146] Recently, a multidirectional Anisogel was created, in which the angle of microgel alignment inside the external magnetic field can be preprogrammed by prealigning ellipsoidal maghemite nanoparticles inside the rod-shaped microgels during their production.[147]

As alternatives to magnetic fields, electrical fields, acoustic waves, and mechanical stress have been employed to induce cell alignment or patterning inside 3D hydrogels. For example, dielectrophoresis has been applied to pattern carbon nanotubes within gelatin methacrylate (GelMa) hydrogels. Therefore, the electric field induces dipole moments within the nanoparticles and forces them to align in the direction of the electric field, improving the mechanical properties and conductivity of the hydrogels. In this way, the differentiation and contraction of C2C12 myoblasts, when cultured on top of these hydrogels and being electrically stimulated are improved.[148]

Moreover, acoustic waves can generate a pressure gradient in a liquid solution that allows cells to pattern within hydrogels. This platform is used to locally increase the density of endothelial cells and MSCs to thereby pattern vascular structure formation in GelMa and fibrin hydrogels, which can be further tuned by changing the sound frequency and amplitude.[149] Alternatively, light can be employed to pattern hydrogels by creating, destroying or modifying chemical bonds in a biomaterial after injection, allowing for a wide range of changes in its structure and composition after the 3D construct is formed.[150] Shining light on the material will usually create a bulk modification, however, by using masks or focused light beams, such as those found in two-photon laser lithography, material modification can be precisely targeted into defined patterns. In this way, it is possible, for example, to selectively degrade the material to create channels, change its stiffness, or induce deformations to modify cell behavior.[151] Similarly, biomolecules or reactive groups, chemically protected to render them inactive, can be introduced into the materials to be later deprotected making them available to bind other molecules or to interact with cells. Biochemical patterns or gradients can thus be introduced to direct cell–material interactions,[84,152,153] which could be upscaled to HTS. This further demonstrates the important correlation between structure and function and how trigger-responsive materials offer new perspectives to develop advanced, hierarchically organized tissues in vitro.

4.3. Controlling Porosity in Injectable Matrices

Another major limitation of conventional synthetic hydrogels is their small pore sizes in the range of nanometers, which restricts cell infiltration and the formation of cell–cell interactions. Thus, the degradability of the hydrogel has to be introduced and needs to be precisely synchronized with tissue formation.[154] This controlled degradation rate is often difficult to achieve, preventing proper tissue formation when the rate of degradation is too slow or resulting in loss of mechanical stability and cell support when too fast.[18,124,125,155] To overcome these limitations, more porous hydrogels have been created by interlinking microgels into microporous annealed particle (MAP) scaffolds.[126,156] Here, the hydrogel precursor molecules are replaced by pre-crosslinked microgels that are approximately a factor of 10^4 larger but still pipettable. Crosslinking these larger entities together results in more interstitial space between the interlinked particles, improving cell infiltration.[18]

Most studies on MAP scaffolds are based on spherical microgels, where the pore sizes can be varied by changing the microgel diameter. This limits the dimensions of the pores and results in the formation of close-packed lattices with limited interconnectivity. Recently, it has been demonstrated that replacing spherical microgels with anisometric, rod-shaped microgels makes it possible to create microgel assemblies with increased porosity and pore sizes of up to 200 µm, thereby enhancing cell infiltration.[157] When rod-shaped microgels are chemically interlinked into MAP scaffolds, fibroblasts grow and fill up the pores, while

endothelial sprouting is observed as a first step toward vascularization (Figure 2c-ii).[120] As long as the microgels are present, they provide a path for the diffusion of nutrients, oxygen, and waste products, avoiding the formation of necrotic tissue. In the next step, growing vascular structures would need to take over this function so that microgels can degrade and a denser tissue can be formed. MAP scaffolds may facilitate vascularization compared to conventional hydrogels with a nanomesh, where cells have to work hard to remodel the environment and connect with each other. In the case of rod-shaped microgels, the porosity and pore sizes of the resulting MAP scaffold can be changed by varying the aspect ratio of the microgels. Variation of the aspect ratio between 5 and 20, using an in-mold polymerization technique, significantly increases the overall scaffold porosity without increasing the amount of synthetic material.[158]

To date, pipettable microgels for MAP scaffold formation are mainly produced via microfluidics, which is characterized by its low throughput and limits the particle shape to spheres or rods, the latter with diameters determined by the channel width. Therefore, parallelized step emulsification[159] and compartmentalized jet polymerization[160] were established to scale up the production of spherical and produce thinner rod-shaped microgels, respectively. On the other hand, stop-flow lithography, where a polymer solution inside a microfluidic device is selectively irradiated using a photomask, has emerged as a facile fabrication method for arbitrarily shaped, soft polymeric microgels. Due to their variable shape, mechanical properties, and porosity, such microgels have tremendous potential as building blocks for in vitro tissue models.[161]

4.4. Introducing an In Vitro "Gym" to Stimulate Tissue Maturation

In addition to flow, other mechanical forces exerted during the culture of in vitro tissue models, such as tensile, compression, and extension forces, are important factors to consider that could affect cell behavior. For example, stretching devices can regulate a wide range of biological processes, including cell morphology, differentiation, cell migration, and induced polarization.[162] For the local control of the actuation of soft hydrogels, a temperature responsive hydrogel with embedded gold nanorods is photoactuated around its volume phase transition temperature of ≈37 °C in media via near-infrared laser-induced plasmonic heating and cooling. Depending on the frequency, amplitude and actuation protocol, cells are mechanically affected in their migration, adhesion, differentiation, and production of extracellular proteins (Figure 2c-iii).[123,163] Combining culture conditions with mechanical actuation in vitro, therefore, opens new opportunities to differentiate and mature the forming tissues.

5. Bioprinting of Tissue Models

Bioprinting technology is a powerful tool that enables a spatial prearrangement of different cell types in an anatomical 3D manner inside hydrogel-based constructs, which can significantly improve the effectiveness and duration of tissue maturation in vitro. However, while it offers precise and reproducible manufacturing of individualized, standardized, and scalable cell-laden scaffolds at low costs,[164–167] printing-related shear stress and hydrostatic pressure can limit cell viability, especially in the case of vulnerable stem cells.[168–170] Therefore, depending on the employed bioprinting method, the process can have an effect on cell behavior, which must be carefully monitored. Inkjet bioprinting and microextrusion bioprinting have both been comprehensively investigated to estimate the shear stress that occurs in the nozzle and how this affects the printed cells.[168,171] For example, bioprinting-related shear stress and hydrostatic pressure influence the angiogenetic potential of endothelial cells.[172] In addition, bioprinting-associated pulsatile hydrostatic pressure can elicit a proinflammatory response in cells.[173] Therefore, new printing technologies are being developed to enhance cell survival and integrity, such as drop-on-demand and nozzle-free 3D acoustic bioprinting techniques based on the principle of acoustic droplet ejection (Figure 2d-i),[121] or based on laser-induced forward transfer.[174] Drop-on-demand 3D bioprinting, which employs an electromagnetic microvalve for the precise delivery of bioink droplets with tunable volume and speed, was used to produce functional mimetic 3D corneal models with optical properties similar to those of real corneal stromal tissue using a bioink composed of collagen and agarose incorporating corneal stromal keratocytes.[175] In nozzle-free acoustic droplet ejecting, the droplet size can be varied over three length scales only by modulating the transducer frequency enabling the printing of cell clusters, as well as single cell droplets. Integrating such novel printing methods into automated 3D bioprinting systems has the potential to bring the means of obtaining 3D structures with complex features and enable the standardized and reproducible production of 3D in vitro models.[176]

Besides continuous improvement of the bioprinting hardware, novel bioinks are required to introduce new ways to increase the resolution, achieve specific architectures at a lower size scale, and create stimuli-responsive constructs to improve tissue formation. Therefore, the injectable materials described above can be further adapted to function as part of a bioink for 3D bioprinting. In general, bioinks should gel quickly to maintain shape fidelity after printing.[177] The most commonly used bioink is gelatin methacrylate (GelMa), which crosslinks via UV-triggered free-radical polymerization. As a semisynthetic biomaterial, GelMa combines the intrinsic biocompatibility of natural materials, including integrin cell-binding and protease-cleavage sites, with the reproducibility and tunability of synthetic polymers.[178] Currently, industrial efforts are made to produce GelMa according to good manufacturing practice and acquire approval for clinical use in body medical applications. However, ideally, the printing process should be nontoxic for cells, avoiding harmful initiators, chemistries, and UV light to crosslink the bioink. In one example, the complex 3D architecture of the aortic valve is generated by employing a gelatin-alginate bioink.[179] Low-pressure printing of this bioink prepared in culture media significantly improves the long-term cell viability inside 3D bioprinted valvular interstitial cell (VIC)-laden scaffolds.[167] An ECM-based bioink consisting of collagen and natively derived decellularized ECM components is being developed to improve the cell growth and viability of VICs.[179] Importantly, bioinks that are based on natural components can suffer from batch-to-batch variability. While it is often difficult to control their gelation rate, mechanical,

biological, and structural properties, and degradation rate to enable cells to grow, while still providing sufficient support. Therefore, synthetic bioinks are under development to overcome these limitations. For example, PEG-based bioinks that crosslink via photopolymerization[180] or Michael-type addition[181] are printed into complex shapes that can be biofunctionalized to support cell culture.

To enhance the resolution and structure of printed constructs and better recapitulate the extracellular matrix features found in vivo, such as anisotropy, bioinks can be combined with the alignment of anisotropic particles. This can be induced by wall shear stress during extrusion-based bioprinting or by magnetic fields. By shear-aligning anisometric organ building blocks, consisting of cardiomyocytes and fibroblasts, along the printing direction inside a surrounding collagen hydrogel, cardiac tissue with improved contractile functionality compared to isotropic controls could be engineered.[112] In addition, GelMa was recently combined with magnetoresponsive short fibers. The Anisogel-based bioink is printed with a magnetically assisted extrusion-based 3D bioprinter, enabling in-print orientation of human adipose-derived stem cells.[182]

The concept of 3D printed scaffolds presenting microstructures for improved cell growth has led to the development of a new generation of bioinks. These include microgel-based inks, capable of interlinking through physical or chemical bonds. These microgels are generally produced through microfluidic techniques, where different payloads, including cells, can be embedded. After physical jamming, the microgels are extruded using a bioprinter to create macroporous structures, similar to the MAP technology mentioned above.[183] By using microgels bound by physical interactions, the bioinks acquire unique characteristics, such as a shear thinning behavior, which is highly desired to achieve printability. By combining microgels of different materials and properties, it is possible to tailor the bioinks to specific needs; for example, a degradable fraction allows the creation of larger pores postprinting.[184] Other building blocks, such as entangled microstrands, have also been used to create unique inks.[185] These colloidal-based bioinks open the door to a wider range of material properties depending on the target tissues.

6. Bioreactors to Provide Native-Like Conditions

In addition to architecture, cell maturation plays a key role in growing functional tissues. As discussed before, organoids advance toward physiologic functionality but fail to mature beyond a fetal phenotype as they lack fully differentiated and specialized cell types, as well as cell organization, which is often limited by their short lifespan resulting from limited nutrient and waste exchange.[34] To improve organoid cultures, bioreactors that facilitate media exchange by stirring or similar means have been created, thereby allowing for better nutrient supply and oxygenation.[186] For example, long-term culture and large-scale production of stem cell-derived liver organoids in spinner flasks have been achieved, which significantly accelerates cell expansion and improves differentiation, as shown by upregulated expression of hepatocyte markers compared to static controls.[89] In addition, hollow fiber membrane bioreactors enable the large-scale production of freestanding tissues, which is normally restricted to tissue culture plastic with limited upscaling capacity. Functionalization of the membrane with thermoresponsive microgels allows for intact tissue harvesting without the need for proteolytic enzymes by lowering the temperature below the volume phase transition temperature of the microgels, which reverses cell adhesion (Figure 2d-ii).[122] Moreover, perfusion bioreactors are essential dynamic flow systems that mimic physiological mechanical stimulations and thereby can improve, e.g., vascular network formation.[187] To further investigate the effect of dynamic loading on cells, bioreactors for cyclic stretching of cells and hydrogels are developed. For example, such dynamic bioreactors can be used to align collagen fibers as an effect of defined cyclic stretching.[188] However, providing a controllable dynamic environment and being able to track cell activity at the same time remains a key challenge.[187] In this context, bioreactors that enable the online monitoring of barrier properties in cell layers are being developed.[187,189] This has led to the creation of a novel 3D printed bioreactor with a homogeneously distributed flow field enabling epithelial cell culture experiments and online barrier monitoring by integrated electrodes through electrical impedance spectroscopy. Therefore, the introduced bioreactor helps to understand the pathophysiology of various cellular barriers in a noninvasive manner, which is crucial for fundamental and pharmaceutical research.[190]

7. High-Throughput Sensing and Advanced Data Acquisition

Advances in microsystem methods in combination with cell and tissue models have enabled the realization of physiological culture systems capable of recapitulating many aspects of human physiology in vitro. Microscopy techniques, as well as offline and downstream analysis methods, have been used as primary means of data acquisition and analysis of these tissue models. Thanks to an increase in imaging throughput (primarily fluorescent), it is now possible to measure the phenotypical response of large numbers of single cells or multicellular samples upon exposure to compounds. Recently, a high-throughput colorectal cancer spheroid platform was developed by optimizing an alginate-gelatin-based bioink that allowed rapid multiwell bioprinting with low cell numbers to achieve a highly automated microscopy-based screening system.[191] Such high-throughput systems generate large volumes of data requires new bioinformatic techniques to either identify those compounds that help achieve a desired phenotype or to create cell profiles describing the response upon exposure to compounds.[192] Such phenotypic profiles can later be integrated with omics data to better understand cell responses and the mechanism of action or be used in the creation of predictive in silico models (see Section 7.1) to strengthen drug development.[193]

Today, miniaturization enables the use of a large number of sensors and transducer principles, which can be integrated into such platforms. Integration of sensor devices into the microsystems permits online monitoring and a high-throughput analysis of cellular development. In particular, parallelization and online monitoring are two main arguments to develop and refine microsystem-integrated tissue models. In this regard, over the past years, different microsystems for cell analysis have been created. Microsystem-integrated analysis has been developed for human tissue models, such as the lung,[15] skin,[194] heart,[195]

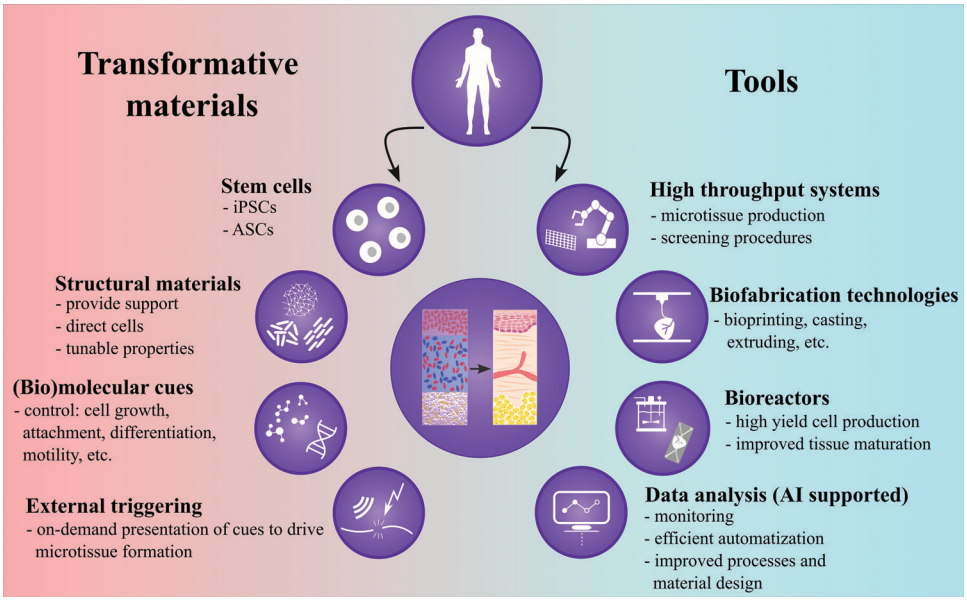

Figure 3. Synergy between transformative materials and the technological tools needed to propel the production of human microtissues in a reproducible manner.

kidney,[196] liver,[69] eye,[197] blood-brain-barrier,[198] physiology of the brain,[199] and for different types of cancer.[200,201] Focus has been placed on the integration of metal microelectrodes to record electrical signals within complex scaffolds[202] and as sensor elements inside microfluidic systems.[202] Biosensors and cell–surface adhesion sensors for the detection of cellular adhesion and biomolecules based on field-effect transistors and organic electrochemical transistors have been developed.[203,204] Here, it is instrumental to design electroactive elements, which would favor not only the mechanical stability at the cell–material interface but characterize the biorecognition mechanism often governed by the plasma membrane response and its curvature.[205] Electroactive elements can resemble typical electrogenic features[206] and functions[207] as in living tissue (i.e., neuronal tissue), as well as exploit multimodal sensing capabilities.[208] Here, the ability of the electroactive biomaterial to sense ions and electrons is fundamental for the monitoring of electrochemical processes, which govern cell–cell communication within the tissue.[209]

In addition, microsystem-based combinations of human tissue models have been described, where the interaction between several interconnected organotypic microtissue models is studied.[196,210] Modern organ-on-chip technologies are often realized at the expense of throughput, industry-standard form factors, and compatibility with state-of-the-art data collection tools. Therefore, it is of paramount importance that microsystem-integrated tissue models not only maintain the cellular cocultures and create physiological microenvironments, but also contain sensing devices to monitor parameters, such as cell growth, barrier function, and metabolism. For the realization of high-throughput data collection capability, batch processing of many organ-on-chip devices in parallel is necessary. This has been exemplified by a microfluidic culture plate with 96 independent microfluidic-based tissue models, including micropumps and transepithelial electrical resistance electrodes as the main sensing method (Figure 2e).[124] For outcomes that are more relevant for humans, different human tissue models could be integrated into one system to study their interaction. For such visionary projects in high-throughput mode, however, technological limitations still exist, as the integration of the different models requires highly complex individually tailored systems. For platforms with multiple sensors, the modern trend is to evaluate the combined sensor data using artificial intelligence methods.[211]

7.1. In Silico Models

Engineering of 3D functional tissue can also be supported by data gained from in vitro experiments that can be integrated with mechanistic computational models, not only to improve our understanding of complex biological systems but also to elucidate and modulate the interaction between cells and biomaterials. Mechanistic models account for fundamental biological processes such as cell division, cell migration or regulatory pathways. With the help of computer simulations, these models allow us to investigate how observable phenomena arise from these basic principles. This can be used to simulate the impact of specific interventions on the behavior of a system, to test whether a hypothesized mechanism of action can quantitatively explain experimental observations, or to design experiments that are suitable to discriminate between competing hypotheses. Furthermore, such models allow us to estimate process parameters that cannot be measured directly (e.g., proliferation rates and differentiation probabilities of malignant or engineered cells). Aspects that have been studied using mechanistic models include the impact of hematopoietic growth factors on stem cell self-renewal, differentiation, and proliferation or the impact of cell culture protocols on the heterogeneity of mesenchymal stromal cell populations.[212,213] Once validated by experiments, computational models are an attractive tool to optimize existing procedures and generate novel hypotheses. As an example, it

was revealed by an in silico model, namely by a computational fluid dynamics (CFD) simulation, that the maximum shear stress during acoustic droplet ejection using acoustic bioprinting technique is approximately three times lower than using a microvalve-based bioprinting method.[121] Likewise using the in silico tool CFD, it could be shown that the impingement-related shear stress—that occurs when a 3D bioprinted cell-laden droplet hits the building platform—can even exceed the wall shear stress in the nozzle during microvalve-based bioprinting.[214] These are important findings as the shear stress can affect not only cell survival but also cellular differentiation behavior postprinting.

8. Outlook

The quest to grow functional human tissue models in vitro in a reproducible and automated manner is a truly interdisciplinary task combining inputs from medicine, cell biology, physiology, chemistry, engineering, and computer sciences. This fusion of expertise has generated a new wave of professionals capable of solving problems in a more holistic manner. These professionals are now proposing novel transformative materials with the aim of replicating their in vivo counterparts, an avenue that still requires further exploration. Toward this aim, interdisciplinary training of a new type of researcher with cross-disciplinary skills is of high demand. Producing life-like human tissues to be used for personalized medicine and therapy decisions requires nuanced ethical reflection on moral acceptability, including questions on risks and safety, or on clinical trial design, that evolve into more complex interrogates, such as ownership, the therapeutic promise, and the impact on human self-understanding in light of engineered life-like materials. These ethical aspects should be considered early in the research process. Nonetheless, to continue this path and tackle the major healthcare challenges of the 21st century, transformative materials need to be combined with other emerging technologies (**Figure 3**), such as iPSCs, genetic modification, artificial intelligence, advanced biofabrication, dynamic bioreactors, and nano- and microelectronics, to converge into automated high-throughput systems that will impulse diagnostics and therapeutics.

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Conflict of Interest

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