Human-Organoid Models: Accomplishments to Salvage Test-Animals

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Abstract

Late stage attritions in drug discovery are costly and consuming. Improbable response of test molecules acquired in non-human systems is attributed to be the major cause of clinical failures. While conventional *in vitro* methods of drug discovery do not truly represent the human system, the animal models used for *in vivo* validation are also genetically and phenotypically distant from humans. However, recent developments in organoid culture are motivating and elevate hopes for replacing test animals with artificial human tissue models. Possibility of creating functional tissue *ex vivo* has a potential to revolutionize the way human therapeutics is perceived. Not only will it bridge the gap between drug development and its clinical efficacy but also help strategizing regenerative medicine. Successful human-tissue surrogates would liberate test animals or at least minimize their use for research purposes. Potential drug candidates tested on human-tissue equivalents are expected to generate clinically much more relevant data. Here we deliberate upon the options and possibilities of accomplishing human organoid models for *in vitro* testing and their significance in therapeutics.

Keywords: Organoid culture; Extra cellular matrix; Humanoid tissue; 3D scaffold; Tissue engineering; *Ex vivo* models; Human tissue equivalents; Regenerative medicine

We sought our queries in 2D for what always existed in 3D; through evolutionarily distant organisms as substitute to humans, no wonder we piled up more clinical failures than successes.

Introduction

It takes multi million dollars to develop a new drug as an estimated 1 out of 10,000 chemicals that enter the discovery cycle (Figure 1) ever reaches the market [1]. The rising percentage of late-stage clinical failures (50% in phase 3) is also alarming [2]. The imperative reason for such low success is our inability to represent human tissue in laboratory. Test models like flat surface cell-culture, virtual computational methods and small animals cannot replicate human system; as a consequence the outcome has not been clinically valuable most of the time. Cells accustomed to spatially dynamic microenvironment are conventionally studied in isolation; mostly as homogeneous cultures must not be expected to display bona-fide behavior. Engagement of cells with immediate extracellular matrix (ECM) and neighboring cells has been overlooked while evaluating their response to peripheral stimulus; be it in the form of drug or toxin or intrinsic physiological entities like enzymes and hormones. Nevertheless, lack of appropriate ECM milieu has impacted least on *in vitro* studies related to intracellular molecular-machinery saving us from getting fundamentally wrong.

Non-availability of a flexible 3D system is attributed to be the major obstacle in establishing new standards through organotypic cell culture [3]. Nevertheless, commercial availability of scaffolds like Ultra-Web®, Extracell®, ECM-analog®, BD-Matrigel®, Corning-Matrigel®, Alvetex®, BioVaSc®, Algimatrix® and spheroids of 3D-Biotek® that allow organotypic culture and especially the ones available in conventional plate formats are expected to change the scene.
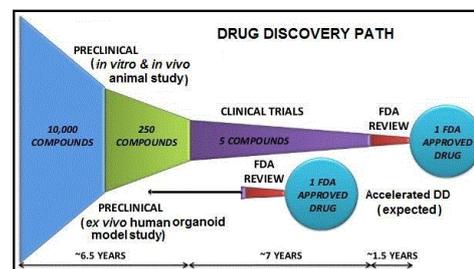

Figure 1: Drug discovery and development path conventionally followed (adapted from drug development approval process by Flavio Guzman http://pharmacologycorner.com) in comparison to potentially accelerated path expected after the availability of human-organoid models.

A dynamic and reciprocal exchange of information between cells and ECM contributes significantly in tissue specific gene expression regulating its morphology and physiology [4]. The hierarchy of ECM-mediated signaling in tissue differentiation and physiology is elegantly demonstrated by Bissell et al. [4] through *ex vivo* modeling of milk secreting mammary glands using Matrigel [5] a tumor derived ECM. We need an efficient cell-interactive scaffold of benign origin, having good shelf-life and stability, for studying cell-ECM dynamics in an experimental micro-environment [6]. Adapting organotype culture could reveal true behavior of cells in real tissue like layout and also elaborate on contextual cell-cell and cell-ECM dynamic relation. Cell-ECM dynamics being at the helm of fundamental understanding of normal *vs.* abnormal-cell response could thus provide an altogether new meaning to our approach towards therapeutics [7]. Improved comprehension of bi-directional relation of cell with its surrounding milieu has a potential to create a new line of drug design focused on

empowering and restoring the natural microenvironment to revert the unhealthy or diseased condition.

Conception of human organoid model for *ex vivo* data

Developmental biology has shown that lineage is not the only factor that governs or controls cell behavior, surrounding ECM and neighboring cells also contribute. They could regulate and deregulate tissue specific differentiation; and also that despite sharing identical genome, cell phenotypes could be different [8]. Physiologically active tissue consists of heterologous population of cells that consolidate to create uniformly patterned structural units while acquiring a distinct functionality. Multiple such units though variable in size exist in amenable relation with their surroundings and impact the physiological performance of the whole tissue. Each tissue is thus unique not only in its unit-morphology but also in commitment to a specific physiological function. Islets of beta cells in pancreas, glomerular units in kidney, acinus in liver, seminiferous tubules in testes are some of the fine examples. These functional units may or may not be confined by membrane but remain tightly packed with supporting or interstitial cells which remain closely aligned for feasible co-ordination. Non-linear but systematic functional co-ordination of this kind, among cells within each unit and with neighboring units demands connectivity through a responsive ECM. Concerted units in a defined tissue/organ format remain connected to other organs through vasculature that routes the paracrine and endocrine signals. Multiple functional units portray Nature's typical design advantage that allows efficient tissue function even in a state of accidental compromise e.g., due to energy shortage or other transient stress effects.

Feasibility of engineering functional tissue *ex vivo*

The complexity involved in creating human-tissue models *ex vivo* can be simplified by incorporating relevant intricacies while ignoring the extraneous at different levels in contextual manner. For example, *ex vivo* models need not to match the multiple unit profile of actual organ/tissue for their intended use in drug and toxicity testing. A small unit or micro-tissue model that can replicate the representative physiological function is adequate enough for such purposes [9]. They can as well be used as a graft for fixing and restoring (not replacing) the partly damaged or diseased tissues. *In vivo* use however, may require yardsticks like HLA matching as needed for organ transplant depending on the origin of the cells of the graft.

It is worth acknowledging that human tissues correspond to a range of cell-ECM ratio; for example cartilage, bones, retina like tissues are rich in ECM while heart, kidney, liver like tissues are rich in cells. Constructing a physiologically viable tissue demands fundamental understanding of cells *vis-a-vis* its surrounding ECM. Inherent characteristics of a tissue could possibly be acquired either through dissociating (Top-down) or constructing (Bottom up) the native complexity. Former involves separating organized network of cells and related extracellular molecules while ensuring minimal loss of information to get to the specifics. Latter on the other hand requires constructing ECM in a hierarchical manner starting from basic ECM molecules while adding cells and other tissue-specific complexity in a stepwise manner. 'Top-down' approach requires hydrolyzing elements including enzymes and may run the risk of losing important spatio-temporal information related to Cell-ECM and Cell-Cell association. The 'bottom-up' approach on the other hand involves assembling relevant molecules in a tissue-specific manner for acquiring typical function [10].

Architectural modeling, broadly defined by 3D scaffold and cellular arrangement within, is essential for creating human tissue models [11]. It includes simulation of texture, stiffness, size and shape of a tissue or organ to be modeled (Table 1). Overall size and shape of artificial tissue construct might be inconsequential for *in vitro* applications and tissue repair through grafting as long as physiological functions are duplicated and graft could be integrated efficiently. They may matter however, when total organ replacement due to accidental or pathological damage/atrophies is needed. Thus, feasibility of creating a functional human organoid model *ex vivo* heavily relies on structural and biochemical modeling of ECM for controlling the temperament of constituent cell-cluster. For which, we need to focus on few important aspects; (i) a cell responsive ECM mimicking 3D scaffold, (ii) an appropriate mix of cell population or pluripotent stem cells from apt source and (iii) effectively presented tissue specific factors and signaling molecules for orchestrated pact of cells. Oxygen supplements may also be needed where tissue size is an important contributor to the manifestation of physiological function or when micro-sized tissues need to be sustained for longer periods.

ECM mimicking		Appropriate Cells	
Architectural simulation	Functional modeling	Primary	Transformed
i. 3D scaffold (external & internal topography)	i. Hydration characteristics (hydrophilic/hydrophobic)	i. Stem cells	i. Therapeutic cells
		ii. Adult cells	
ii. Constitution (fibrous/porous)	ii. Mechanical properties (Biomechanics)	iii. Xenogenic/Allogenic/Autologous	
iii. Strength			
iv. Shape	iii. Biochemical cues and signaling entities (Covalently integrated) or (Imposed through surface adsorption via ionic or co-ordinated interaction)		
v. Size			

Table 1: Basic requirements for engineering Human-organoid models ex vivo.

Extracellular matrix (ECM) mimicking

Engineering human tissue necessitates an ECM-mimicking 3D scaffold of optimal attributes [12,13]. Architecturally it should allow the cells to migrate into the interiors and populate the scaffold [14]. Spatial arrangement of encrypted signaling molecules in the biocompatible scaffold must be suitably instructive to the cells. Tissue

specific cues contributing to their physiological performance could be non-covalently absorbed or covalently integrated with the scaffold or appropriately supplied in culture medium.

Tissues like bone, cartilage and cornea represent ECM-rich, structural tissues where impact of genetic mutations reflects more on morphology than physiology of the tissue. On the other hand cell-rich tissues where functionality is dependent on orchestrated metabolic activity of cells, genetic mutations could be fatal. Unlike ECM-rich the cell dominant tissues like heart, lungs, CNS need peripheral ECM, only to retain the cell-cluster-morphology critical for acquiring functionality. ECM in these tissues is like glue that contributes to adjoin and bring co-ordination among the affiliated cells. Scaffolds for their *ex vivo* construction should therefore favor intercellular connections while permitting easy remodeling so that cells can rearrange in tissue like dense format. Independent studies with scaffolds of varying biomaterials have highlighted the importance of mechanical and geometrical cues for cell adhesion and proliferation. Cells are mechano-sensitive and found to respond to force-induced changes in protein conformation and geometry-dependent interactions around their local vicinity [15]. In multicellular organisms, cell-ECM and cell-cell interactions establish the polarity and cells cultured in conditions that do not facilitate these interactions normally remain unpolarized and die by anoikis or develop into a tumor [16]. Having considerable physiological implications the scaffold design needs to be matched with the corresponding tissue as stiffness of ECM has a decisive influence on tissue functionality [17]. Even stem cell lineage is found to be influenced by scaffold elasticity [18]. It is observed that the Linear Elastic Modulus (LEM) or stiffness of mammalian tissues span over three orders of magnitude [19] where each tissue falls in certain specified range. This understanding is incredibly helpful in providing primary guideline for the design and fabrication of scaffold for targeted tissue.

A 3D scaffold optimized to match the native tissue ECM in terms of its constitution (fibrous or porous or mixed), elasticity and compressive mechanical strength is thus a starting point for functional tissue modeling while cell interactivity, interconnected porosity, bio-compatibility and controlled degradation for swapping with neo-ECM remain other essential features. Cell response is also influenced by the size and extent of porosity of 3D scaffold [20,21] that needs to be optimized for different tissues [22].

Numerous scaffolds constituted from varying mix of homo, hetero or co-polymers of natural and synthetic origin have been explored for organoid culture [23]. Hydrogels that grossly match with natural ECM in hydration characteristics are also recommended [20,24]. Collagen, fibrin, gelatin, albumin, alginate are some of the commonly used natural polymers while polyvinyl alcohol, polyethylene glycol, poly-(lactic acid-glycolic acid) copolymers are the most customary synthetic polymers [25,26] tested for the purpose. Hydroxyapatites and tri-calcium phosphate like bio-ceramics are recommended for engineering artificial bone and cartilage [27]. However, both synthetic and naturally derived biopolymers have their own limitations. Natural biomaterials are cell-interactive but delicate to handle, difficult to reproduce and limited by source. Sterility issues are also associated with them. Synthetic materials on the other hand are sturdy and reproducible but unable to impart cell interactivity and tissue specific cues beyond physical framework. Most often they also exhibit unacceptable degradation kinetics or products. Choice of biomaterial depends not only on the tissue to be engineered but also governed by the technique utilized for creating the scaffold [28].

Appropriately defined cell traction

Suitable combination of synergistic cells, nurtured in tissue specific microenvironment only, could arrange and mature as functional tissue. While scaffold should provide essential traction to the cells for organization, both the cell types and the scaffold biomaterial need to be in tune for engineering a tissue *ex vivo*. Synchronized degradation, assimilation or reorganization of scaffold is essential for giving way to the neo-ECM and growing cell mass. Functional duplication of a tissue is hence possible only if the biomechanical properties of scaffold can facilitate the seeded cells to attach, proliferate and subsequently differentiate [29]. Topological cues of scaffold have more significance in constructing ECM dominant tissues where ECM contributes structurally whereas terminally differentiated cells remain mostly in quiescent state. In complex, cell-rich tissues where physiology of tissue relies more on co-operative metabolic activity of cells, holding them together and mediating swift co-ordination through specific signals is the major function of ECM. It is understood that cell-ECM balance in native tissue is developmentally regulated and beyond certain age secretion of neo-ECM or proliferation of cells is triggered through environmental signals only. Neo-ECM generation for example ensues in response to injury or tissue damage and the signals normally come from immune response pathway, which often ends up as a scar/keloid.

Importance of cell traction is also recognized by the fact that asymmetric orientation of plasma membrane caused by irregular organization of constituting proteins and phospholipids can influence cell arrangement [30]. Furthermore, cell-cell connection can also reorient nucleus and other organelles thereby changing cell polarity, a requisite for their concerted physiological response [31].

Thus, not only a concerted survival but a symbiotic and coordinated association of cells is desired to yield functional tissue *ex vivo*. Engineering a physiologically active artificial tissue therefore needs cells that are well defined for their state, level of expression and relevance [7,32]. It also demands a balanced nutritional input along with fitting biochemical supplements for sustaining the heterogeneous cell population in tissue format. Approximating an optimum combination of functionally committed cells is challenging not only for their feasible source but also for our limited knowledge about de-differentiation and trans-differentiation like phenomena. Adult or differentiated cells of autologous origin may be an option in some cases but satisfactory or acceptable characterization and validation would be an issue. Pluripotent stem cells having a capacity to differentiate in any type of cell are therefore considered the best option [33]. Intuitively, if microenvironment is appropriate in terms of elasticity and biochemical cues, pluripotent stem cells may be sufficient to form an organoid tissue albeit factors that can guide them to commit to a desired pathway are known [19,34]. Nevertheless, pluripotency and plasticity of stem cells provide a window to persuade them to specific differentiation pathway [35]. Chemokines being potent factors for homing and mobilization could also be used for recruiting endogenous stem cells [36]. They may also be employed for inducing and amplifying *in situ* tissue regeneration. Sufficient evidence exists for scaffold elasticity acting as a differentiating factor to which pluripotent stem cells are found to be even more sensitive [37]. They could possibly be committed to differentiate into desired type by altering biophysical and biochemical cues in their microenvironment. Controlled differentiation of mesenchymal stem cells (MSC) is found to be very promising [38]. Embryonic stem cells (ESC) being pluripotent are also considered worthy of TE. However, their innate tendency to remodel ECM makes them susceptible to create teratoma

or tumorous growth through trans-differentiation [39]. Source of cells for human organoid culture may be a concern for *in vivo* applications but not for testing efficacy and toxicity *ex vivo* where validated cell lines or cells from mammalian donor or cadaver could serve the purpose. Immune-privilege and immune-independent issues like cornea and cartilage could also be modeled using either donor cells or cell lines. Autologous cells however remain valuable when customized graft or implant is needed.

Allometric growth and differentiated state

Organs and tissues in mammals including humans demonstrate an allometric growth pattern. Despite having identical genomes different cell types within the same multi-cellular organism acquire variable size and shape [40]. Optimizing growth to stay within limits of desired size, while attaining appropriate differentiated state is an important issue in tissue engineering. To replicate human physiology especially while creating ADME models with multiple tissue types on single plate or chip it is critical to maintain correct relative size [41].

Taking care of oxygen needs

Cells packed to form a tissue need perpetual supply of oxygen and in absence of optimum vascularization, are not expected to survive for long. Individual organs and cells of a healthy mammalian system differ considerably in their sensitivity to hypoxia [42] and this information might have direct bearing on their differential oxygen needs during *ex vivo* development [43]. Cells multiply and grow much more vigorously in 3D therefore their endurance especially in a state of transition from cell-mass of few microns to metabolically functional tissue unit often poses a limitation. Frequent or continuous perfusion with fresh medium is found useful and could possibly suffice for smallest functional unit which is sufficient for *in vitro* testing purposes. However, when prolonged culture or a specific macro-tissue dimension is critical, superfluous oxygen supply or vascularization might be essential. Interestingly, hyperbaric oxygen i.e., 100% oxygen above absolute atmospheric pressure is found helpful in skin-wound as well as bone-fracture healing. It stimulates proliferation of human dermal fibroblasts [44] as well as osteoblasts [45] suggesting that high oxygen pressure might be effective for engineering certain tissues. Adequate use of biocompatible oxygen supplying agents could be another way of handling the extra oxygen needs [46]. Covalent or non-covalent adsorption in and around the scaffold without compromising the oxygen carrying ability of these agents might be helpful. Inducing angiogenesis through smartly designed scaffold is also a way; BioVaSc°, generated from decellularized porcine small bowl segment with preserved tubular structures of capillary network for example, inherently takes care of vascularization *ex vivo* [47]. Human vasculature has also been modeled by generating multilayered tubes of smooth muscle cells and subsequent luminal seeding of endothelial cells [48]. It is further developed into a microfluidic chip to be used as a tool for studying specific physiological phenomena like inflammation, tumor growth, metastasis and degenerative diseases [49]. It would be worthwhile however; if these cellular microtubes could be fittingly integrated with other scaffold systems for *ex vivo* tissue maturation as they might help supplying oxygenated nutrient medium to the interiors of engineered tissue analogous to blood supply *in vivo*.

Functional viability and tissue efficiency

Cell mass grown *in vitro* cannot be called a tissue unless it demonstrates the physiological activity characteristic to that tissue [50]. It is observed that cells organized to acquire differentiated phenotype comparable to their native tissue counterpart have a tendency to exhibit functional activity as well [51,52]. However, challenges in recapitulating different tissues are as unique as the tissue itself. For example, the spectrum of human tissues ranges from flat (skin), tubular (urethra), vacuolous (lungs), villious (intestine), viscous (brain), mucilous (vagina), compounded (liver, kidney, testes), fibrous (muscles), elastic (tendons) to flexibly tough (cartilage) and hard (bone) [10]. Such wide tissue morphology and physiology encompass variety of cells and associated microenvironments. Understandably, the tools/techniques required to engineer them *ex vivo* would also vary widely. Most importantly, we have yet to comprehend and control the 4th dimension which an engineered tissue could acquire in consequence to the growing/multiplying cells and their dynamics with the surrounding scaffold in 3D. Physiological competence of an artificially grown tissue depends on the co-ordination among the cells and also on the geometrical, structural and biochemical cues offered by their ECM. Nonetheless, it is essential to establish standard and representative characteristics of engineered *vis-à-vis* native healthy tissue through two or more specific markers. Extent of expression in healthy tissue could be used as baseline reference. Literature, pre-existing standards and first-hand experience with healthy, unhealthy and infected tissues in clinics would help in creating such baseline standards. Thus, an engineered human organoid model needs to be defined through specific markers or a biochemical assay representative of their physiological activity before it can be classified as a model tissue [53].

Significance of Human Organoid Models

Pragmatic data directly relevant to humans

Deficiencies of *in vitro* cellular systems for toxicity data applicable to humans is recognized in early nineties and the need to use human or an animal tissue that corresponds to human condition with fidelity was emphasized [54]. Unreliable data on toxicity of new oncology drugs is said to be a major impediment to efficient and successful drug development. Likewise data on toxicity and skin irritant chemicals generated on test animals is also not very reliable and often misleading. For example glass fibers could be linked with cancer and declared carcinogenic only after a human study [55]. Safety or lack thereof, is an important consideration in regulatory decisions that may even lead to withdrawal of a drug from the market place [56]. Data generated on human organoid models being more realistic and of direct relevance to humans can prevent such mistakes. Important signaling pathways like mitogen-activated protein kinase (MAPK) are found to be regulated differently in 2D *vs* 3D systems [57] making organoid modeling even more relevant. Organoid models of liver and gastro-intestine are particularly suggested for toxicity studies [58]. Thus, the level of confidence in extrapolating the toxicity and efficacy data generated on human organoid models for human applications would certainly be more.

Enhanced accuracy

Animal models are universally accepted for preclinical efficacy and toxicity testing but an ideal test species for humans is human.

Interspecies variation in metabolism and physiology of test animals hinder getting accurate information and thwart its human applicability. Lethal dose (LD-50) measurement for scaling toxicity represents the most unethical attempt where test animals are ingested with maximum possible dose that results 50% of them to be dead. To minimize this practice a sensible attempt is made subsequently with *in vitro* culture plate methods using specific cells and cell lines. However, the convenience and too much dependency on flat surface devices led to several unrealistic assumptions. Application and success of 3D spheroid models in identifying most effective anticancer treatment has already established the importance of 3D organoid over 2D culture plate methods.

Availability of 3D culture techniques allow *ex vivo* construction of organoid tissue models using human derived cells/cell lines that can increase the test accuracy multifold while averting undue animal sacrifice. These models by virtue of being human tissue-mimics are expected by far any means to provide more accurate and valid results. Incorporating convenience and simplicity of *in vitro* testing can add to their usefulness as an alternate model to animals.

Accelerated drug discovery

Course of drug discovery and development (DD) conventionally involves identification and *in vitro* testing of potential drug candidates and proving their efficacy in animal models before tagging them ready for clinical trials in human. Candidate molecules are usually selected on the basis of past experience with established structure-activity correlation, traditional knowledge or computational methods like Bioinformatic tools (Figure 2). New molecules may be incremental, a new derivative of well known, clinically proven active entity. After establishing the chemical structure, stability and solubility they are tested for desired functional impact that includes initial pharmacology and mechanistic analysis and safety studies like cytotoxicity, sterility, immunogenicity. Success of these analyses makes the basis for *in vivo* experiments designed in suitable test animals. Choice of animals largely depends upon appropriateness of model for the target disease and/or condition. Test animals therefore play an important and major role in the DD by providing a link between *in vitro* experimental data and estimated outcome in humans. Quite often the animal study is done in small animal models (mostly rodents) first and then validated in larger animals like monkeys and dogs having little more physiological closeness with humans. Once proved effective these molecules are put to test in humans under three distinct clinical trial phases using successively rigorous parameters (Figure 2).
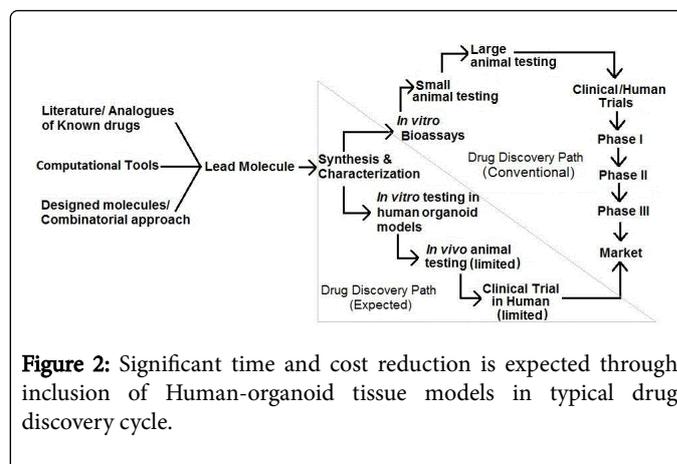

Figure 2: Significant time and cost reduction is expected through inclusion of Human-organoid tissue models in typical drug discovery cycle.

Animal experiments demand conditional breeding, testing and ultimately disposing them off as pathogenic or hazardous waste. Each animal study involves at least 3-4 weeks' time and minimum 4-5 animals in each group to gather statistically relevant data for one single dose. Not only it is harsh on animals but also involves cost, space and man-hours to handle them. Housing and handling animals requires special arrangements and training. In spite of all these efforts the success rate for a potential candidate to reach the market remains 0.01% [59].

A successfully developed human organoid model can cut short the need of animals for establishing the preclinical relevance of a drug candidate. Limiting the number of animal study and associated expenditure is expected to reduce significantly the overall cost and time involved in drug development.

Better simulation of pathological conditions

One of the major reasons of clinical failures during DD is the genetic and physiological gap between humans and the animal models chosen for their evaluation. Modeling a human disease in animals could not reflect the same severity and/or symptoms. This difference could be minimized by cultivating functional human-tissue units *ex vivo*. Such human tissues once standardized for their characteristic function could provide a cost-effective, simple to use *in vitro* models with inherent convenience of using 4-5 replicates to arrive at statistical consensus. Drug sensitivity to tumors and cancers is generally estimated by culturing biopsies from patients. It has been well proven now that spheroid/organoid cultures are better representation of tumors and therefore more helpful in choosing the correct drug. Organoid cultures are also being employed to identify cancer phenotypes [60,61]. Development of complex 3D *in vitro* systems and disease models including that for kidney are already underway [12]. Modeling of *Helicobactor pilori* infection *in vitro* using gastric primary culture is recently reported by Schlaermann et al. [62]. These disease models being in 3D environment are expected to unravel novel pathways and disease markers helping in the identification of new targets for treatment.

Human organoid and disease models make it possible to compare natural physiological *versus* pathological/diseased state response *ex vivo* [12,53]. Cell growth and differentiation pattern of tumor cells versus healthy cells in organoid models can highlight discriminating markers that can subsequently be used as drug targets. For example study related to gene responsible for expression of islet-neogenesis

associated protein (INGAP) which is lost in 2D culture conditions but critical for studying the regulation of diabetes could be feasible only in 3D organoid model [63]. It is also expected that an engineered tissue in combination with genomics can create a bridge between traditional cellular and *in vivo* gene expression studies.

Feasibility of micro-physiological systems

ADME (adsorption, distribution, metabolism and elimination) analysis that helps understanding the pharmacokinetics and pharmacodynamic behavior of a potential drug typically involves animals. Organoid models once established could be evolved into a human physiology mimic, a second generation model through co-culture, making it feasible to evaluate ADME *ex vivo*. In absence of relevant co-culture system where different organs could be represented simultaneously, the pharmacokinetics or ADME analysis had to rely on hepatic metabolism. Till recently new molecules were first screened in 2D cultures of hepatocytes, followed by testing the promising candidates in animals. 3D culture techniques and organoid models have enabled exploration of natural tissue complexity in micro scale range [64,65].

ADME is requisite for any efficacy and toxicity study including side effects of a drug on long term use. It also helps in deciphering possible pathways and mechanisms involved in tissue homeostasis and prognosis of a disease. How does a physiological imbalance or a microbial infection lead to pathologies? Or what are the factors or signals involved in metastasizing tumorous growth? Such questions can be easily explored through new generation organoid models for ADME. The gaps and time frames between an infection and visible symptoms can also be estimated using these models. They can also be utilized for exploring the physiological path of chronic ailments. Conditions leading to autoimmune disorders could also be investigated using them.

Best substitute to test animals

Possibility of human tissue equivalents as substitutes to experimental animals in research could be the most profound achievement of *ex vivo* human organoid models. Low success rate of a drug candidate that reaches the clinical stage after years of efforts is due to our inability to represent human tissue in laboratory. Animals constitute major part of typical drug discovery cycle in terms of time, energy and investment (Figure 2). There is no guarantee that the drug exhibiting excellent efficacy in test animals would show same effect/potency in humans. Genetic and phenotypic diversity among the human race further contributes to the mixed and often complicated outcome of clinical trials. Availability of human organoid models would bring salvation to test animals by minimizing the need to engage them for research purposes. Human organoid models can potentially replace *in vitro* cell culture assays as well as *in vivo* animal studies undertaken to establish the efficacy of a new drug entity. Thus, easy accessibility to organoid models that can generate data valid for humans can potentially redeem experimental animals that are sacrificed for drug and toxicity testing.

Impact on tissue engineering and regenerative medicine

Success in engineering implantable bladder, skin and cartilage like tissues has been instrumental in understanding the structural and functional role of ECM. In fact they present an example of specified cell-dominant tissue-constructs where mimicking the morphology and material characteristics of matrix is sufficient to accomplish the model. Involving only one or two cell types these tissues are considered simple to emulate. Nevertheless successful engineering of such organs with inherently limited physiological activity have motivated the efforts for modeling other complex, more challenging tissues. A recent achievement in modeling various discrete, interdependent regions of human brain by pluripotent stem cells has opened up the possibility for similar interventions in considerably complex tissue like brain. It has been realized that efficient mimicking of ECM in 3D is the key element even for the survival of neurons the major constituent of brain and spinal cord [24].

Ex vivo creation of functional human-organoid models has set the stage for engineering whole organ and/or physiologically active tissue grafts for replacement of diseased, damaged or pathologically impaired organs. However, system integration, anastomosis, vascularization, reinnervation and restoring resident immune cells through lymphatics are other universal needs, to be taken care while implanting *ex vivo* engineered organs. The task would apparently be easier in repairing partially damaged tissues where these concerns could be taken care by the native healthy segment of the tissue once the reconstructed graft integrates successfully.

Future Trend

Age of 3D scaffolds and feasibility of growing cells in the environment physically and biochemically similar to the native has elevated our hopes to a level where test animals could be replaced with artificially created human tissue models.

Culturing functional tissues that can replicate physiological activity *ex vivo* can revolutionize the way human therapeutics is perceived today. Not only will it bridge the gap between drug research and the clinical outcome but also make it possible to correct certain malfunctioning tissues *in vivo* if integrated appropriately. For *ex vivo* testing, however only small functional units or micro-organoid tissues with representative physiological activity should be sufficient. Engineered tissues could thus be categorized as tier-1 and tier-2 models on the basis of incorporated complexity. Drugs and molecules tested in human organoid models created using allogenic, xenogenic or cadaveric cells would enable us to generate information much more legitimate in human context. Human cell lines could also be used if accessibility or sustainability of primary cells is an issue.

Significance of organoid models in the evaluation of underlying mechanisms particularly related to pharmacogenomics is also realized [64]. Modeling normal versus cancerous tissues in identical manner *ex vivo* can help in differentiating their survival needs and identifying more effective oncology drugs. The oncogenic transformation of gastrointestinal tissues in primary organoid culture is also demonstrated recently. However, developing high throughput methods with these 3D micro-organoid tissues will have an added advantage in toxicity and efficacy testing in statistically reliable and cost effective manner.

Though the real tissue like complexity is yet to be achieved for most of the tissues yet the pace with which research is progressing is very encouraging. This could be made possible due to the combined interdisciplinary efforts. Successive progress kindles hope for all animal lovers who support principals of 3Rs (replacement, reduction and refinement) in scientific research. Extensive use of animals in research not only for the drug but also cosmetics and other consumer product development over last many decades reflect humans in the

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light of most selfish creature on the planet. Replacement of animals with human organoid models in pre-clinical studies could be extended to ADME analysis by successive adoption of co-culture systems. Successful creation of human organoid models that can substitute animals while truly representing the human body may prove double edge tool. Not only it would accelerate the new drug development by bridging the knowledge gap between *in vitro* and *in vivo* data through an *ex vivo* model but also has a potential to be evolved for repair and replacement of complete tissue if need arise.

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