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Challenges and opportunities for the next generation of cardiovascular tissue engineering

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Engineered cardiac tissues derived from human induced pluripotent stem cells offer unique opportunities for patient-specific disease modeling, drug discovery and cardiac repair. Since the first engineered hearts were introduced over two decades ago, human induced pluripotent stem cell-based three-dimensional cardiac organoids and heart-on-a-chip systems have now become mainstays in basic cardiovascular research as valuable platforms for investigating fundamental human pathophysiology and development. However, major obstacles remain to be addressed before the field can truly advance toward commercial and clinical translation. Here we provide a snapshot of the state-of-the-art methods in cardiac tissue engineering, with a focus on in vitro models of the human heart. Looking ahead, we discuss major challenges and opportunities in the field and suggest strategies for enabling broad acceptance of engineered cardiac tissues as models of cardiac pathophysiology and testbeds for the development of therapies.

The field of tissue engineering emerged about four decades ago through the convergence of engineering and the life sciences, with the overarching goal of providing biological substitutes that can replace or regenerate native tissues lost to injury or disease[1]. Cardiac tissue engineering (cTE) has been a major focus of the field, as heart disease remains the leading cause of mortality worldwide with over 650,000 annual deaths in the United States alone[2]. Because the adult heart lacks the ability to regenerate, efforts have been geared toward recovering the damaged myocardium by implantation of cells and bioengineered contractile cardiac patches[3].

In parallel, rapid development of microfluidics and soft lithography converged with advances in human induced pluripotent stem cell (iPSC) biology to give rise to an entirely new area of 'organs-on-a-chip' that aims to establish controllable, minimally functional units capable of recapitulating specific organ-level functions in vitro[4]. These developments have been particularly transformative for cTE, where animal models are of limited use due to major differences in animal and human cardiac physiology, in both health and disease. Advancements in human iPSC-derived 'heart-on-a-chip' (HoC) systems, together with 'top-down' developmental models such as cardiac organoids (COs)[5,6], now offer a fast-track opportunity for tissue engineering to advance both basic cardiovascular research and precision medicine[7–10].

Despite substantial optimism, however, major biological and technical challenges remain to be addressed before engineered cardiac tissues can reach their full potential[11–13]. Many of these challenges are inherently coupled to each other, and this coupling suggests that discovering groundbreaking solutions to one problem may provide potential keys to solving another.

In this Perspective, we provide a brief overview of the state-of-the-art methods in the field and discuss some of the major challenges that will shape the next decade, with a focus on in vitro platforms. Rather than providing a retrospective summary of engineered tissue models introduced to date, we discuss common objectives shared by the community, and offer suggestions for future directions for research and commercial translation.

Finding the balance between throughput and tissue maturity

As with most design problems, there exists an inevitable trade-off between the operational throughput and biological complexity in cTE[12,14] (Fig. 1). A principal challenge for the field is thus to clearly define the minimal set of essential structural and functional features that need to be recapitulated[15], and to determine how to benchmark these readouts against clinical data[10]. Do all tissue models require extensive microvasculature, mature transverse tubules and well-aligned intercalated discs? How mature is 'mature enough'? If, for example, the objective is to perform a large-scale compound library screen based on one or two functional readouts, an array of >96 COs[16] might be better suited than a more sophisticated HoC system. On the other hand, studies that aim to model the phenotypes of a genetic variant of uncertain significance that are too subtle to observe in two-dimensional (2D) culture, might benefit from choosing platforms that provide a range of exogenous biophysical stimuli[17] to maximize structural, metabolic and functional maturation of the tissue. The common saying that 'all models are wrong, but some are useful' is therefore apt when considering cTE systems. Selecting from a diverse array of tissue platforms is likely to become increasingly context dependent and problem dependent[18], and researchers will be challenged more than ever to think about and rationalize which approach would be best suited to address their specific questions.

In the long run, the scalability and throughput of engineered tissues will undoubtedly need to be improved with continued optimization, automation, standardization and eventual widespread commercialization of fabrication and testing methods. Beyond technological advancements, a deeper understanding of developmental biology will ultimately need to serve as the guiding light.

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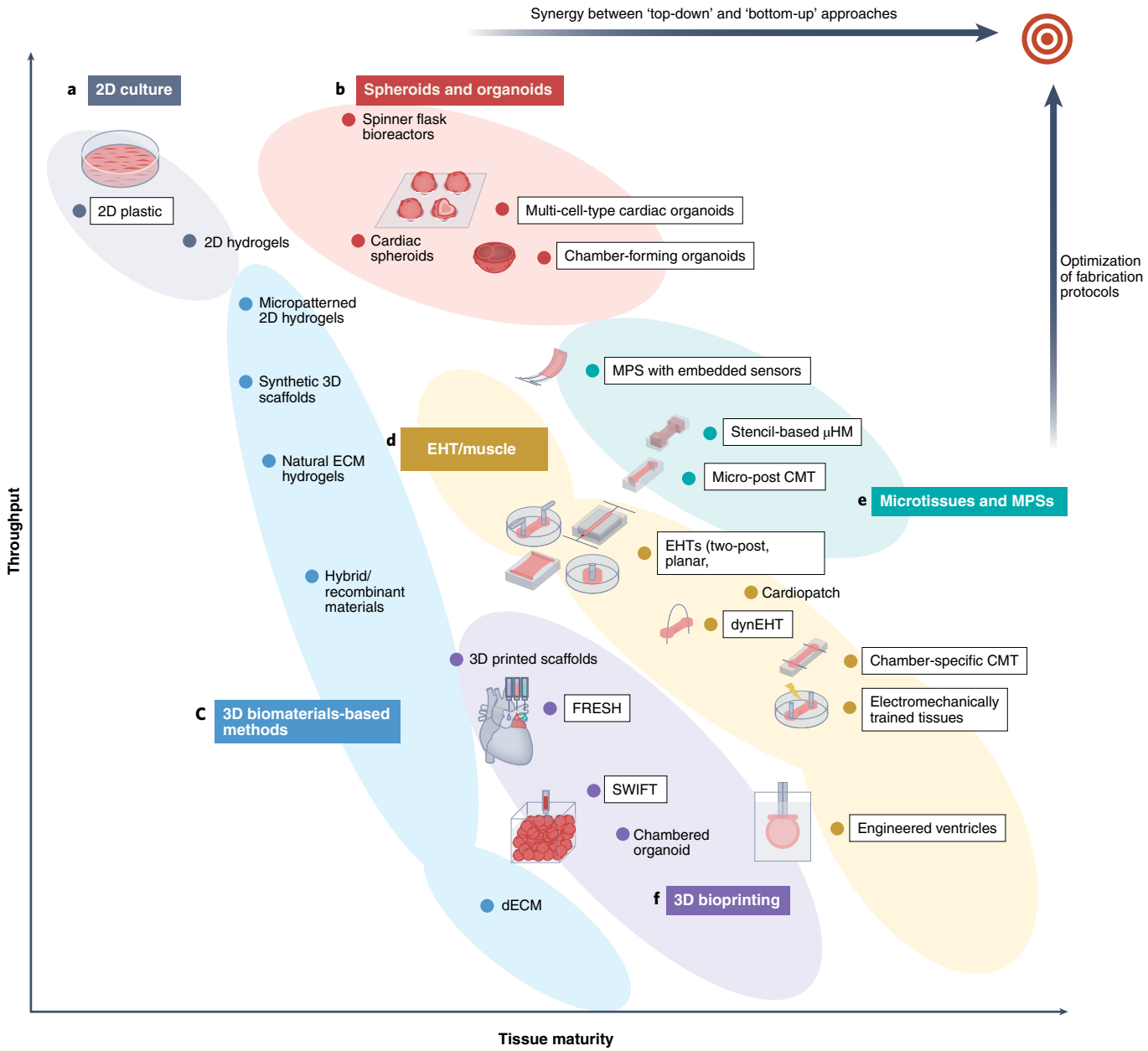

Fig. 1 | A scatterplot depicting the trade-off between throughput and maturity in cardiac tissue engineering. a–f, Models reported to date can be broadly categorized based on the fundamental methodologies: (**a**) traditional 2D models and hydrogel-based systems[72]; (**b**) spheroids that rely on self-aggregation of differentiated cells or organoids generated by spontaneous co-differentiation of cells in 3D aggregates[16,19,20,56,73,74] (red); (**c**) early methods that have focused primarily on 3D biomaterial scaffolds for cell seeding (blue); (**d**) engineered tissues of varying geometries and sizes that provide exogenous structural and/or electromechanical maturation cues to promote physiological tissue architecture[75–82] (yellow); (**e**) miniaturized microphysiological systems and microtissues[59,68,83,84] (green); and (**f**) 3D bioprinting-based methods that enable arbitrary structural control at the macroscale[29,85–87] (purple). The most common and relatively recent categories of techniques are highlighted in black boxes with an accompanying illustration. Note that 'tissue maturity' shown on the *x* axis is meant to be an abstract, qualitative representation, placing more weight on the structural, mechanical and electrophysiological maturity of the tissue as it pertains to the organ's primary function (that is, contractile beating), than on 'biological complexity', which takes into consideration how many physiological features are recapitulated by the model (for example, the number of cell types incorporated and the extent of vascularization). MPS, microphysiological system; μHM, micro-heart muscle; CMT, cardiac microtissue; dynEHT, dynamically loaded EHTs; dECM, decellularized ECM; FRESH, freeform reversible embedding of suspended hydrogels; SWIFT, sacrificial writing into functional tissue.

We expect that the next generation of engineers will increasingly rely on harnessing the cell's innate ability to self-organize and pattern into complex structures. Recent advancements in spontaneous chamber-forming organoids are early examples of how the cells themselves can be nudged to engineer their own tissue microenvironment with only minimal exogenous signals[19,20].

Going forward, we anticipate continued development of cTE protocols focusing on precisely timed developmental cues (for example, programmable gene perturbations[21], morphogen gradients and chemical pulses) in combination with exogenous biophysical cues to enable more efficient, directed assembly of mature organoids and HoC models. Systematic benchmarking of the

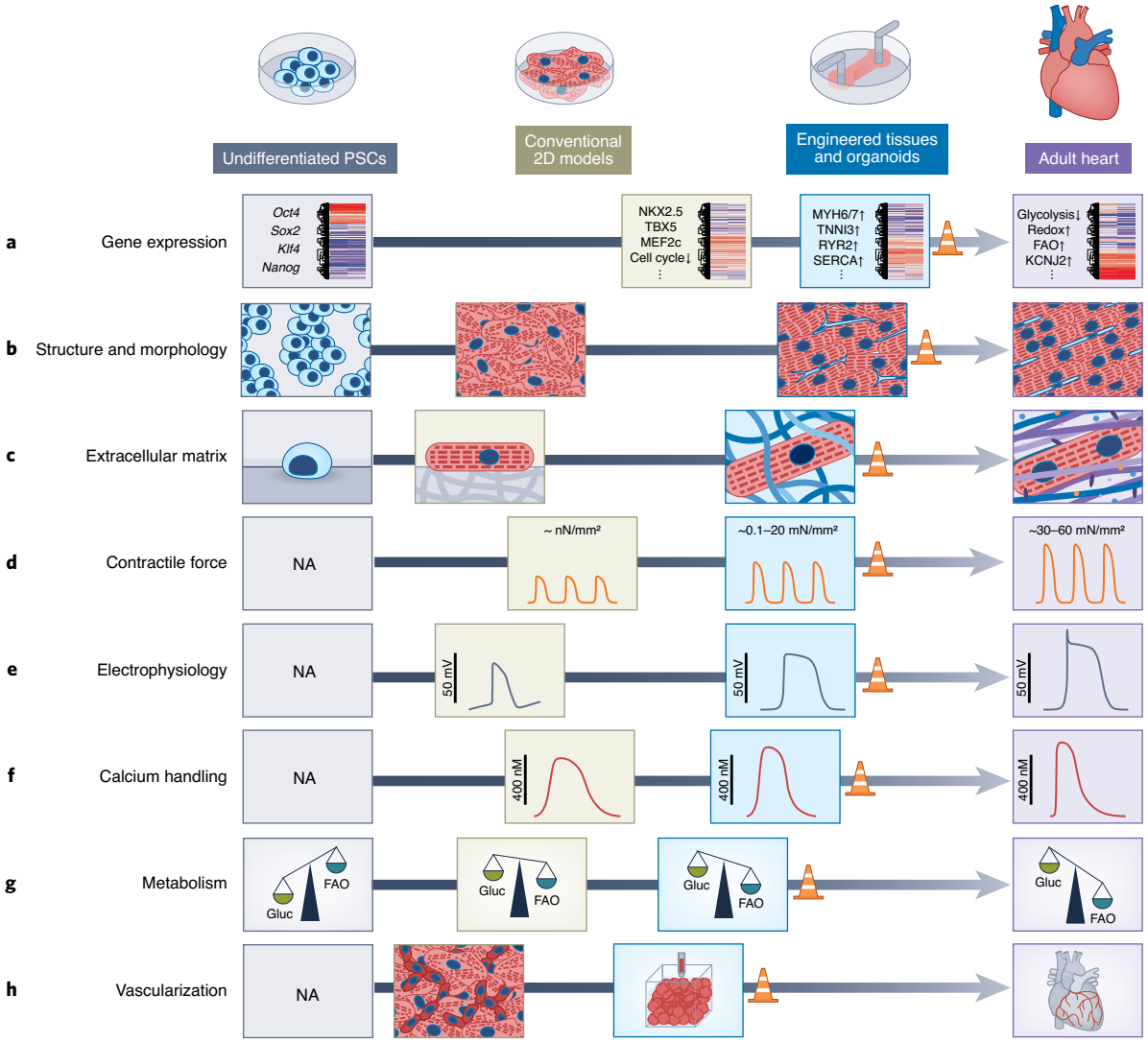

Fig. 2 | Illustration of tissue maturity levels that can be achieved by current tissue engineering models. a–h, The levels of maturity are categorized based on major readouts: gene expression profiles (**a**), tissue structure and morphology (**b**), ECM composition and mechanics (**c**), contractility (**d**), electrophysiological properties (**e**), calcium handling (**f**), metabolism (**g**) and vascularization (**h**). Cones and faded gray arrows are abstract representations of current roadblocks and how far the best-available tissue models today are from reaching adult myocardium-like maturity levels for each category. Many of the readouts are inherently intertwined (for example, metabolism can directly regulate gene expression and vice versa), suggesting major advancements in one area can indirectly contribute to overcoming obstacles in another. Gluc, glucose metabolism; FAO, fatty acid oxidation; NA, not applicable.

engineered constructs against native tissue samples and sections will be key (Fig. 2) and would likely start with characterization of cell types and gene expression profiles. For a dynamic and structurally complex tissue such as heart, however, quantitative morphological and biomechanical metrics are likely essential beyond mere compositional analyses. In all cases, the availability of real-time, noninvasive methods for evaluation of structural and functional features at subcellular, cellular and tissue levels will be critical to enabling dynamic longitudinal studies and to minimizing experimental variability. Implementation of such rigorous quality-control mechanisms, in conjunction with continued refinement of fabrication protocols, likely holds the key to striking the elusive balance between throughput and tissue maturity (Fig. 3a).

Establishing vascularization and perfusion

Dense tissue constructs with sizes above the diffusional penetration depth (>100 µm in the shortest dimension) often form a characteristic necrotic core due to poor supply of oxygen and nutrients[22] (Fig. 3b). Oxygen levels in development, at birth and afterwards vary considerably and affect the differentiation and function of several cardiac lineages including cardiomyocytes (CMs)[23], which rely heavily on fatty acid oxidation for contractile beating. Beyond nutrient penetrance, the absence of vasculature also neglects critical cross-talk among multiple cell types within the myocardium (for example, angiocrine signaling[24]), which limits the ability of engineered tissues to faithfully model diseases in vitro or test drug toxicity and efficacy. A longstanding goal in the field has thus been to develop ways to efficiently vascularize and perfuse cardiac tissue at various scales. Vascularization remains a major obstacle in tissue engineering (Fig. 2), but one that also presents perhaps the greatest potential for the next breakthrough.

Several intriguing strategies have been developed in recent years to better vascularize cardiac tissues, including the use of microfluidic systems, sacrificial materials and micropatterning technologies[25–27]. One approach that seems particularly promising for larger constructs is the use of embedded three-dimensional (3D)

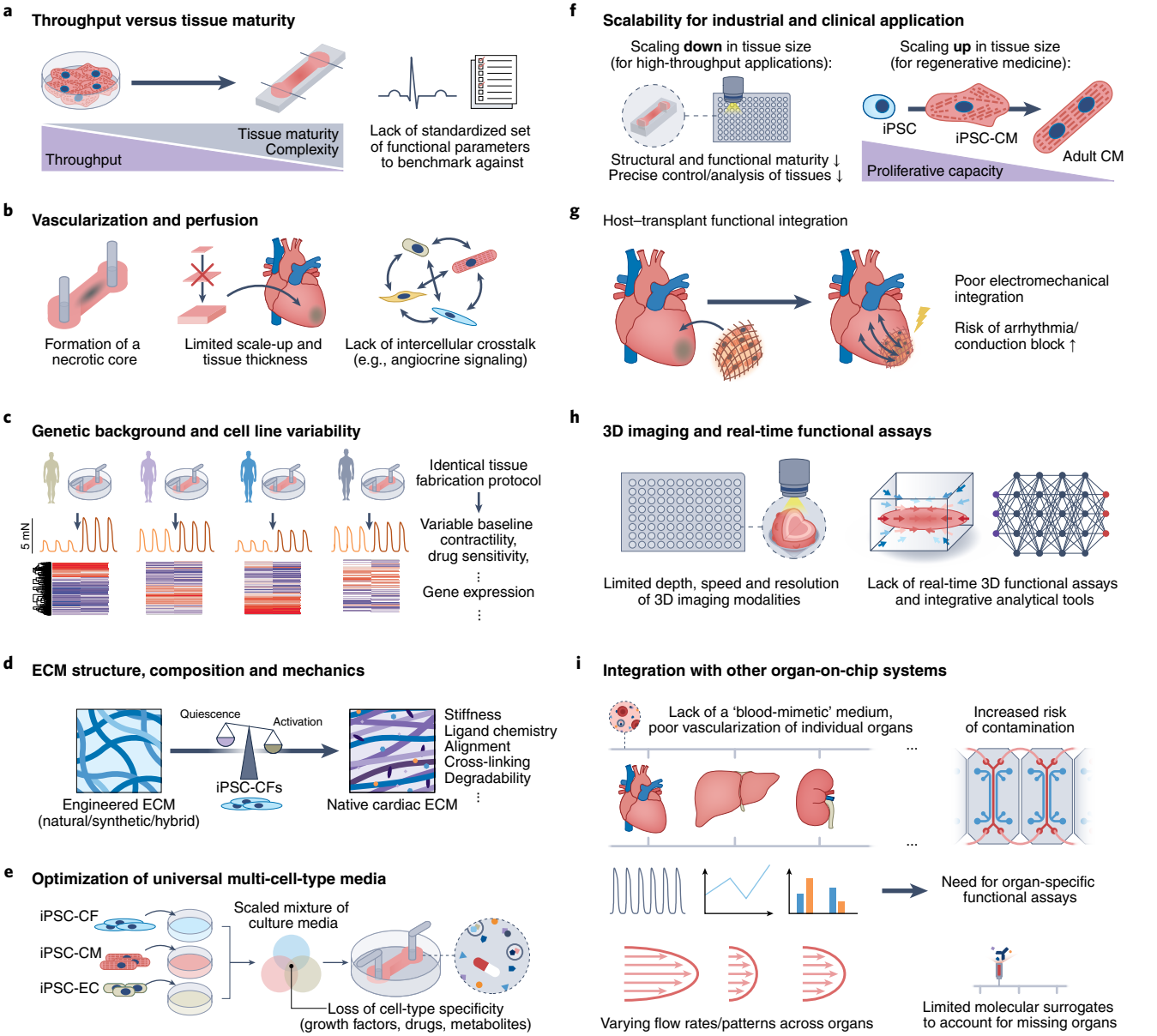

Fig. 3 | Major obstacles and opportunities in cardiac tissue engineering. a, Enhancing tissue maturation to near-adult myocardium levels without substantially compromising throughput. **b**, Efficient vascularization of tissues for improved nutrient delivery, scale-up, long-term maintenance, and modeling of physiological cell–cell cross-talk mechanisms. **c**, Addressing problems of reproducibility and variability across cell lines and across batches of the same line. **d**, Engineering an ECM that mimics the compositional diversity, structural alignment and mechanical robustness of native cardiac ECM. **e**, Optimization of universal multi-cell-type culture media. **f**, Improving scalability for both industrial and clinical applications. **g**, Enhancing electromechanical integration of transplanted tissue to the host myocardium. **h**, Development of improved 3D imaging technologies and real-time functional assays. **i**, Integration with multiple organ chips for systems-level modeling of diseases and drug responses.

bioprinting[28] to directly print vascular channels of arbitrary size and shape within tissues[29]. We anticipate that such methods will evolve rapidly over the next few years with continued optimization of bio-ink formulations, sub-50-μm printing resolutions and tissue compaction protocols. For smaller microtissues and organoids, recent 'top-down' approaches have also successfully yielded microvascularized organoids[20,30] as well as entire 'blood vessel organoids'[31] that develop capillary networks by self-organization of endothelial cells (ECs) and pericytes. One could thus envision tissue constructs that incorporate a hybrid of the two approaches: fabrication and priming of large vessel structures (for example, by embedded bioprinting) followed by induction of microcapillary branching by the addition of well-defined angiogenic factors. Fusing blood vessel organoids with COs at the appropriate developmental stage could also be a promising strategy for producing densely vascularized cardiac tissue. We anticipate increased development of such combinatorial methods in the future.

Accounting for genetic background and variability across cell lines

An important lesson from the past two decades of cTE is that both tissue formation and maturation can vary vastly depending on the iPSC line used, even when using identical protocols (Fig. 3c). Discrepancies across cell lines of varying genetic background and

demographics (for example, sex, age and race) have long been evident in human iPSCs[32], mouse embryonic stem cells[33], as well as differentiated cell types including neurons[34] and CMs[35] in 2D monolayer format, with substantial differences in key signaling pathways (for example, Wnt/β-catenin). Such differences are unsurprisingly even more pronounced in higher-order, self-organizing tissue structures, as additional variables are introduced into the fabrication process. For example, comparative analyses of ten different control human iPSC lines (from both commercial and academic sources) in the engineered heart tissue (EHT) format have revealed widely varying baseline contractile forces, kinetics and rates, as well as sensitivities to drugs[36]. The issue of line-to-line variability is further compounded with concerns of batch effects, as variable tissue phenotypes (morphology, beating frequency, contractile force, contraction and relaxation rates) can be observed even across technical replicates of the same cell line[36,37]. This poses substantial challenges for ensuring reproducibility and for establishing causality in disease modeling and drug screening studies.

Batch effects can be addressed to some extent by incorporating large numbers of technical replicates, and line-to-line variability can likewise be accounted for—albeit much more inadequately—by inclusion of multiple cell lines from diverse demographic backgrounds. Ultimately, however, to fully understand the sources of line variability, the tissue engineering community will need to investigate systematically how genetic variation impacts function at the whole-genome level, beyond a specific mutation or element of interest, including both protein coding and noncoding regions. This will first require comprehensive characterization of the downstream consequences of genome perturbation (for example, large-scale CRISPR screens to generate 'catalogs' of genotype-to-phenotype correlations), followed by computational and bioinformatics analyses to evaluate causal relationships. Efforts are already in place to perform such large-scale studies using conventional 2D models (led by the Impact of Genomic Variation on Function Consortium) and are soon expected to be extended to 3D tissues.

For cardiac tissues specifically, the most critical steps for such genotype–phenotype studies will be to determine which functional readouts to focus on, to develop high-throughput assays for the selected readouts and to devise strategies to meaningfully integrate and analyze the different types of data (molecular, structural and functional). Importantly, the quantitative relationship (or lack thereof) between clinical parameters measured in patients (for example, resting heart rate and QT interval) and parallel readouts measured in iPSC-derived tissue models (for example, spontaneous beating rate and action potential duration) would need to be rigorously characterized. To achieve this, we anticipate increased use of high-content, high-throughput optical phenotyping instruments that can record multiple parameters simultaneously in organoid or tissue format (for example, contractile motion, calcium handling and electrophysiology). Given the scale of such functional screens, we also expect deep learning algorithms to play a key role in predicting, for example, the functional phenotype(s) of tissues derived from a given cell line, based on its whole-genome sequencing data. This is a challenging long-term endeavor that will require sustained, coordinated efforts of molecular biologists (genome perturbations), tissue engineers (fabrication and phenotyping) and computational biologists (deep learning and bioinformatics analyses).

Optimizing the composition, structure and biomechanics of extracellular matrix

While previously considered to be a largely static and inert scaffold, the cardiac extracellular matrix (ECM) is now understood to be a highly dynamic and responsive signaling hub in homeostasis, disease, development and a variety of chronic and acute injuries[38]. Providing cells with a more physiological microenvironment than the highly unnatural tissue culture plastic is one of the major advancements that bioengineers have achieved over the years. Still, challenges remain in engineering an ECM that precisely recapitulates the structural intricacy, biochemical complexity and mechanical robustness of native cardiac ECM (Fig. 3d).

To date, engineers have focused primarily on 'bottom-up' approaches that mimic physiological ECM by mixing cells with a broad range of natural, synthetic and hybrid biomaterials. Alternatively, decellularized matrices from explanted heart tissue have been widely used as scaffolds to better mimic the structure and composition of native ECM[39]. However, such methods have largely been hampered by inefficiencies in recellularization and cell seeding. In parallel with continued developments in biomaterials design, we expect future methods for engineering tissues and organoids to focus increasingly on exploiting the ability of the cells themselves to build and remodel their own ECM. While most cardiac cell types including CMs secrete matrix, especially during early development, cardiac stromal cells (for example, most importantly cardiac fibroblasts (CFs)) are the primary source of ECM in the mature myocardium. One major challenge will thus be to promote ECM synthesis and organization by CFs while maintaining them in a quiescent, nonactivated state (Fig. 3d), as CFs are known to spontaneously transdifferentiate into myofibroblastic cells in stiff microenvironments (for example, rigid plastic or stiff hydrogels) or under mechanically stressed conditions (for example, cyclical stretch)—even in the absence of exogenous cytokine activation[40,41]. Given that ECM abundance (particularly that of fibrillar collagens[42]) is a major determinant of tissue stiffness[43], mechano-activation of CFs can result in a vicious cycle wherein increased ECM secretion and crosslinking activates CFs and in turn results in further stiffening of the tissue. Innovations are thus needed to suppress, or perhaps even reverse, CF transdifferentiation in the tissue format, in which cells bear constant, cyclical mechanical load. Molecular inhibition of cellular mechanotransduction pathways has shown some promise in these areas[44] and could help strike the elusive balance between active ECM deposition and pro-fibrotic CF transdifferentiation.

Developing multi-cell-type culture media

Engineered cardiac tissue models and organoids now routinely incorporate several distinct cardiovascular cell types, including CMs, ECs, CFs and in some cases immune cells (for example, tissue-resident and circulating macrophages) and co-emergent progenitors of other tissues (for example, gut)[30,45,46]. In conventional 2D culture, each cell type requires well-defined, specific media compositions (for example, nutrients, growth factors and small molecules), with varying timelines for efficient differentiation into their respective lineages[41,47–54]. This cell-type specificity is lost, however, when cells are assembled into 3D tissue either before or after differentiation (Fig. 3e). To date, cardiac tissues have been successfully supported by mixing cell-type-specific culture media in ratios proportional to the respective cell numbers, or by custom formulations using a common base medium supplemented by mixtures of cell-type-specific cytokines and growth factors[55,56]. Nevertheless, the impact of suboptimal media on tissue maturation and function remains poorly understood and is likely nontrivial.

Systematic studies are thus needed to determine optimal media compositions for multi-cell-type tissues. Ideally, formulations will be guided by comprehensive mass-spectrometry-based analyses of embryonic, fetal and adult plasma, but technical and logistical challenges currently hamper the routine procurement of such clinical samples. An alternate screening approach might therefore be more reasonable, in which individual components of culture medium are varied systematically followed by evaluation of their effects on key readouts for maturity. Initial characterization can be facilitated by double- or triple-fluorescence reporter systems for cell-type-specific marker genes (for example, TBX5-Clover2, NKX2-5-TagRFP)[57] that correlate with maturity (for example,

TNNT2-GFP for CMs, CDH5-mOrange for ECs and ACTA2-CFP for smooth muscle cells). Collection of these initial readouts can then be followed up by a secondary screen using high-content optical instruments that enable simultaneous measurements of multiple functional parameters including contractile motion, electrophysiology and calcium handling in organoid or tissue format. Such functional data can subsequently be coupled with single-cell sequencing to analyze the effects of varied media composition in a cell-type-resolved manner. Importantly, the precise timing of compositional changes will need to be optimized, given that virtually all soluble signals are dynamically regulated during embryonic and postnatal development. Because culture media can dictate cell metabolism, drug pharmacokinetics and cellular differentiation states, these large-scale studies are expected to have a profound impact on the field in the upcoming years.

Improving scalability for industrial and regenerative medicine applications

As cardiac tissue models become increasingly sophisticated, scaling down the tissue size (and hence the number of cells per tissue) while scaling up the operational and analytical throughput (moving to 384-well plates) becomes exponentially more challenging with additional biological and engineering considerations (Fig. 3f). From a biological standpoint, the structural (compaction, cell–cell alignment and ultrastructure) and functional maturity of tissues tend to decline with very small tissue sizes, and miniaturized dimensions also often limit opportunities for vascularization and innervation, despite substantial advantages in nutrient transport[58,59]. From an engineering standpoint, reduced tissue sizes make it increasingly difficult to precisely control electromechanical stimulation and to maintain low volumes of medium (for example, due to evaporation and fluctuations in pH) without sophisticated microfluidic systems[60]. Importantly, scaling down in size often presents compatibility issues with downstream assays (for example, histology, contractility analyses, conduction velocity measurements, and so on), thus requiring fabrication protocols and instrumentation to be adjusted accordingly for high-throughput analyses in 96-well or 384-well format.

On the other hand, scaling up in tissue size (for regenerative medicine applications) or in sample number (for high-throughput screening) presents an entirely different set of challenges that arise largely from the limited proliferative capacity of CMs and poor vascularization ('Establishing vascularization and perfusion'). Innovative strategies are being explored to trigger CM proliferation in vitro, including exogenous delivery and activation of cell cycle genes[61], the use of spinner flask bioreactors for massive expansion of COs[62] and modulation of CM–CM contact-mediated signaling[63]. We expect such methods to be increasingly adapted for generating large numbers of differentiated CMs required for both reparative cardiac patches as well as large HoC arrays. Parallel efforts to improve scalability—in both directions—will thus be critical to move the field toward true industrial and clinical application.

Enhancing functional integration between engineered tissue and host myocardium

Organs-on-a-chip in general, and HoC in particular, are also being considered for studies of regenerative therapies[64]. From a clinical standpoint, one of the major hurdles for cTE is poor electromechanical integration between the transplanted tissue and the host myocardium (Fig. 3g). Due to the limited structural and functional maturity of engineered patches (for example, misaligned CMs, poor vascularization, weak mechanical properties and low conduction velocity), transplantation often results in arrhythmia and disruption of the electrical wave front to and from the native myocardium. To minimize the risk of arrhythmia, conductive biomaterial scaffolds may offer a promising solution by facilitating host-tissue electrical propagation[65]. Alternatively, strategies to recruit native cells to transplanted patches[66] may enhance physical coupling at the interface and promote cross-talk among host cells and transplanted cells. Continued improvements in such methods will be needed, combined with rigorous evaluation of immunogenicity, to enable safe and constructive transplantation of engineered tissues for cardiac repair and regeneration. Importantly, HoC models allow systematic, quantitative studies of multiple factors that determine electromechanical integration between engineered tissue and host myocardium. For example, efforts are well underway to use perfusion-based HoCs to examine the role of immune cells and circulating factors in tissue maturation and integration, as recently reviewed[4].

3D imaging and real-time functional assays

Dense and dynamically beating cardiac tissue constructs are inherently difficult to image and characterize in 3D. Despite substantial progress in recent years, 3D imaging modalities are still far from reaching the standard of 2D systems in terms of optical depth, resolution, speed (real-time motion tracking capabilities) and throughput. This poses challenges not only for analyzing intricate cellular morphologies, but also for a wide range of imaging-based functional assays including real-time calcium and cell contractility measurements (Fig. 3h). Options are particularly limited for 3D organoids that only rarely incorporate sophisticated sensors or probes. While light-sheet microscopy offers promise, inaccessibility, needs for customization and difficulty to monitor tissue dynamics have limited its impact on cTE[67].

For improved phenotyping of engineered hearts, advancements are needed in three specific areas: (i) continued development of high-speed, high-resolution 3D imaging platforms, (ii) innovations in noninvasive, live-cell molecular probes (for example, fluorescence gene-reporter systems, protein tags and molecular sensors), and (iii) imaging-friendly design of engineered tissues (for example, sheet-like geometries). Concurrent with advancements in 3D imaging modalities, improvements in deep learning image analysis tools, quantitative real-time sensor technologies and electromechanical phenotyping assays will also be crucial. Some early examples include the incorporation of flexible embedded biosensors (for example, by 3D printing[68]) in HoC systems that enable noninvasive, real-time measurements of tissue-generated contractile forces. Developing such functional assays is arguably as important as building the tissue itself and is often the technical bottleneck in tissue engineering. Innovations in these areas will thus play a pivotal role in dictating where the field is headed.

Integration with other organ-on-chip systems

One of the long-term goals of tissue engineering is to investigate systems-level phenomena by integrating multiple organ systems on a single chip[4]. Connecting HoC platforms with other tissues such as liver, kidney and bone marrow, for example, could in principle enable more holistic physiological modeling of systemic diseases as well as drug pharmacokinetics, and could ultimately help bridge the gap between 2D culture and animal models in drug discovery. The promise of integrated 'body-on-chip' systems, however, will likely require many years before its full potential can be realized.

Linking multiple organs is not trivial and requires consideration of a wide range of factors including, but not limited to, the use of a common 'blood mimetic' medium, maintenance of sterility during the assembly and culturing processes, the need for extensive tissue vascularization within individual organs, and the incorporation of sophisticated microfluidic systems[69] that enable tight control of varying flow rates and patterns across the different organs (Fig. 3i). Any technical issues arising from individual organ chips will inevitably be compounded upon each other, and even the most complex body-on-chip platforms will necessarily be missing several organs that must somehow be accounted for (for example, by

continuous or periodic injection of immune and/or endocrine factors). Nevertheless, despite the steep biological and technical challenges faced by personalized body-on-chip platforms, the ability to investigate drug responses and diseases in multiple organs, sourced from isogenic cells, offers immense rewards for both basic and translational medicine. The first such examples of integrated organ chips are already being developed[4,70,71] and are expected to gain full momentum heading into the latter part of the 2020s.

Outlook

There is optimism that engineered cardiac tissues will one day outperform and replace traditional 2D models for disease modeling, drug discovery and cardiac repair. Since the very first EHTs were introduced more than two decades ago, the field has matured to the point where initial inflated expectations have now been tempered with a brief stall in progress, largely due to the challenges discussed in this Perspective. For the pace of innovations to recover and reach a productive plateau, these obstacles will need to be addressed systematically, through a sustained and orchestrated effort of the entire research community. These may not be the most sophisticated and scientifically rewarding endeavors but are critical to advance the field toward true clinical and industrial application. Looking ahead into the next decade, we are optimistic that substantial breakthroughs will ultimately be made in all areas covered in this paper, with refined tissue fabrication and maturation protocols, increased use of high-throughput optical phenotyping platforms, and improved synergy between 'bottom-up' engineering and 'top-down' 3D developmental approaches. It will take a collaborative effort by academic laboratories, the biotechnology and pharmaceutical industries and government agencies to help the field reach this point and to ultimately contribute to drug discovery and the development of new therapeutic strategies.

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Author contributions

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Competing interests

J.C.W. is a cofounder of Greenstone Biosciences and G.V.-N. is a cofounder of Tara Biosystems; however, the work presented here is independent. The other authors report no competing interests.

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