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Review

Manufacturing Cell Therapies Using Engineered Biomaterials

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Emerging manufacturing processes to generate regenerative advanced therapies can involve extensive genomic and/or epigenomic manipulation of autologous or allogeneic cells. These cell engineering processes need to be carefully controlled and standardized to maximize safety and efficacy in clinical trials. Engineered biomaterials with smart and tunable properties offer an intriguing tool to provide or deliver cues to retain stemness, direct differentiation, promote reprogramming, manipulate the genome, or select functional phenotypes. This review discusses the use of engineered biomaterials to control human cell manufacturing. Future work exploiting engineered biomaterials has the potential to generate manufacturing processes that produce standardized cells with well-defined critical quality attributes appropriate for clinical testing.

Trends

Recent developments in several fields, including somatic cell reprogramming and precise gene editing, are leading to novel cell-based therapies.

Inherent heterogeneities in cell populations and methods for cell extraction, culture, and processing produce cell therapy products with considerable interbatch and intrabatch variability, hampering clinical translation and adoption of therapies.

Standards and critical quality attributes need to be developed and verified to have clear benchmarks for the development of cell therapy products.

Biomaterials could be engineered to reduce the root causes of heterogeneity, enhance functionality of cell-based therapies, and develop new standards for manufacturing.

Cell Therapies in the Clinic

Successful translation of cell therapies into clinical trials and on to the clinic has been enabled by the convergence of several fields of research, including **biomaterials** (see Glossary), stem cell and developmental biology, immunotherapy, **reprogramming**, and **gene editing**. There are currently thousands of active clinical trials for cell therapies [1], including therapies for cancer, cardiovascular disease, and neurological disorders. **Stem cell** and gene editing-based cell therapies are a rapidly growing sector within the cell therapy industry. Stem cells, with their ability to self-renew *in vitro* and differentiate into specialized cell lines, are an already attractive cell source for cell- and tissue-engineered therapies. The ability to manipulate stem cell genomes creates additional opportunities for modifying cell behavior, functionality, and clinical utility.

Engineering patient cells to impart new functionality shows promise for targeted therapies, as exemplified by the recent prominence of cancer immunotherapy using engineered T cells [2]. **Autologous** T cells have been genetically engineered to express chimeric antigen receptors (CARs) targeting the patient's own cancer cells, and have demonstrated positive outcomes in clinical trials against blood malignancies resistant to currently available therapeutic options. For example, Qasim and colleagues [3] recently reported leukemia remission in infants using **allogenic** CAR T-cell transplantation. Companies and researchers in this field are starting to apply synthetic biology methods to further engineer T cells to add new functionalities to therapies [2].

Apart from genetic engineering, cellular reprogramming using nonintegrating genetic engineering tools to obtain pluripotent cells that self-renew in culture can be used to generate a rich source of somatic cells for transplantation as well as for disease modeling 'in a dish' [4]. Induced pluripotent stem cells (iPSCs) are being used as precursors to manufacture both progenitor and differentiated somatic cells in ongoing clinical trials [5] for age-related macular degeneration, Parkinson's disease, spinal cord injury, and other diseases [6]. In age-related macular degeneration, which involves the progressive loss of the retinal epithelium monolayer, iPSC-derived

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retinal pigmented epithelium has been generated [7] that has been shown to partially repopulate the macula [8]. iPSC-derived pancreatic β-cell progenitor cells have also been deployed in clinical trials for type 1 diabetes [9].

Alternatively, the use of adult stem cells sidesteps some of the potential translational issues with pluripotent stem cells including extended differentiation procedures and possible teratoma formation. Adult stem cells, including hematopoietic, neural, and mesenchymal stem cells (MSCs), are currently being assessed in multiple clinical trials [10]. Neural stem cells are being used in several clinical trials targeting degenerative neural diseases, central nervous system damage, stroke, and ischemia [10]. As a prominent example, adult mesenchymal stromal cells that exhibit multilineage potential [11] can potentially be used in an autologous manner, are easy to isolate and expand, and they show reparative effects in clinical models [12]. *In vivo*, MSCs exert therapeutic effects through multiple pathways including secretion of cytokines and growth factors into the extracellular space [13], immunomodulation [14], and possibly differentiation, although this is still unclear. Injected MSCs have been tested as therapies targeting cardiac [15], bone/cartilage [16], and neural [17] regeneration and disease treatment [18]. MSCs can be 'primed' or preconditioned to increase their efficacy using physiological, chemical, molecular, or genetic manipulation [19]. Moreover, MSC homing properties can be utilized for the delivery of growth factors via transgenic modification [20], potentially in a differentiation-dependent manner [21].

Emerging Cell Therapies

Increased accessibility and reliability of gene editing and cellular reprogramming have enabled the emergence of the next generation of cell-based therapies. Multiplexed gene editing is being used to improve **CAR T-cell (CAR-T) therapies** by enabling the use of allogenic cells [22], circumventing graft versus host disease [23], CTLA-4 inhibition or PD1 inhibition resistance [24], or combinations thereof, paving the way for much more feasible and effective therapy. **Epigenomic** transdifferentiation, or the conversion between cell types without passing through an intermediate pluripotent state, is an attractive option for generating desired cell populations while avoiding laborious reprogramming and differentiation processes [25]. This technique may additionally allow access to other healing modes, such as therapeutic transdifferentiation using either implanted cells [26] or small molecules *in vivo* [27]. Finally, combined gene editing and reprogramming technologies enable powerful expansion of cell replacement therapies and disease models through the introduction and correction of therapeutic mutations in wild-type or patient-derived cell lines, the ability to create gene knockouts/knock-ins, and various screening approaches [5]. However, despite these advances, human cell manufacturing is throttled by the lack of sufficient control over cell characteristics, especially after extensive manipulation and culture (Figure 1). Here, we review key issues facing **biomanufacturing** of human cells appropriate for clinical application, as well as novel biomaterials-based methods to address them.

Problem Poorly Characterized Cells Are Entering the Clinic

Epigenomic Heterogeneity in Human Cultures

A major roadblock in clinical translation is the donor-to-donor heterogeneity in cell populations. Heterogeneity can originate within the initial cell sources or be introduced through *ex vivo* processing, severely limiting the efficacy, ease of control, and quality of therapies [1]. Initial cell populations may vary based on parameters such as donor age and condition or cell source. T cells for immunotherapies, for instance, are often isolated from cancer patients undergoing chemotherapy. Chemotherapeutic treatments can deplete the host hematopoietic system and introduce variability in the expansion and cytotoxic efficiency of these cells. In addition, MSCs are harvested from various cell sources, and extracted populations are often functionally heterogeneous [28]. Some of the heterogeneities among donors can be traced to epigenomic

Glossary

Allogenic: cells or tissue obtained from an external donor.
Arginine–glycine–aspartic acid (RGD): a tripeptide composed of the amino acids Arg–Gly–Asp (RGD), identified from fibronectin, that is known to facilitate cell binding through integrin receptors.
Autologous: cells or tissue obtained from the patient himself or herself. Autologous transplantation does not require an external donor and, as the body recognizes the cells, helps avoids rejection.
Biomanufacturing: the use of living biological systems or their products to generate materials, devices, and biological products in a scalable, industrial manner.
Biomaterials: natural or synthetic materials with biological activity or functionality.
Cell therapy product: autologous or allogenic living cells used within the body for therapeutic benefit.
Chimeric antigen receptor T-cell (CAR-T) therapy: a type of immunotherapy where T cells are genetically modified with a synthetic receptor, which binds to tumors and potentiates a cytotoxic response within the T cell. T cells could be harvested directly from the patient in an autologous manner or be derived from allogeneic sources.
Critical quality attributes (CQAs): physical, chemical, or biological properties that are associated with or affect safety and efficacy of therapy products.
Epigenome: the set of chemical modifications to DNA and DNA-associated proteins, which regulate gene expression without modification of the DNA sequence. These modifications are heritable to daughter cells after cell division.
Extracellular matrix (ECM): the noncellular material external to cells, composed of structural and soluble molecules that can provide a rich biochemical and biomechanical signaling environment to cells.
Focal adhesions: multiprotein clusters containing integrins that act as links between the ECM and the cellular cytoskeleton, relaying mechanical forces and signaling to the cell.
Gene editing: controlled modification of DNA sequences through the use of engineered nucleases to induce double-stranded

variability [1], although other genomic, metabolic, and proteomic changes could also contribute to donor-to-donor variation.

Along with initial cell source, cell processing steps can introduce further epigenomic heterogeneity (Figure 1), as in the example of somatic cell reprogramming. This process is stochastic [29] and involves erasing source cell epigenetic marks, often resulting in cell populations with extensive functional, epigenetic, and gene expression variability [30,31]. Moreover, prolonged cell culture of pluripotent stem cells has been demonstrated to induce epigenetic abnormalities in these cells [32,33].

Prolonged cell culture is not solely an issue with iPSCs, as most cell types are cultured *in vitro* in environments dramatically different to those from their native environment, often resulting in altered cell state [30]. While *in vitro* culture is typically performed on tissue culture plastic, the *in vivo* **extracellular matrix (ECM)** is a very complex and rich environment [34], providing a heterogeneous mix of signaling cues from multiple components including fibrous structural proteins and proteoglycans. The ECM can present different ligands, apply mechanical forces, and dynamically interact with cells, instructing their behavior. Multiple ECM properties, such as fibrous structures, matrix composition and elasticity [35–37], geometry [38], nanotopography [39], and shear flow [40], have been shown to dramatically direct cellular behavior in the absence of other soluble chemical stimuli. The culturing and processing of cell populations *in vitro* within environments vastly different from their native niche can introduce large epigenomic and functional heterogeneities in these populations, often severely hampering clinical translation [41].

Genomic Heterogeneity

Regardless of epigenomic changes, permanent genomic changes can also occur in cultured cells. Culture-associated point mutations, copy number variants, and chromosomal translocations within cell populations can be generated with extended culture and can seriously influence clinical outcomes. For example, sources of genetic changes through the process of cell reprogramming [31] include background mutations in parental cell lines [42], selection protocols that amplify specific mutations (especially those that may confer survival advantages) [43], or during reprogramming and subsequent culture [44]. Together, these factors make genomic stability an important consideration when characterizing cells during manufacturing processes.

Genomic heterogeneity has also been observed upon genome editing, an issue made more pressing with the increased application of gene editing using the clustered regularly interspaced short palindromic repeats-Cas9 (CRISPR-Cas9) system. Uncontrolled subcellular delivery and the imprecise stoichiometry of nuclease components within cells can cause differences in editing efficiencies and outcomes amongst cell populations. The use of powerful site-directed nucleases that induce breaks in DNA can cause unintended local or large-scale genomic alterations, due to the mechanics of DNA repair. DNA breaks can be repaired through imprecise nonhomologous end joining, or more precise homology directed repair [45]. Nonhomologous end joining can introduce insertions, substitutions, or deletions at the cut site (indels), often in a stochastic manner, causing genetic heterogeneity in edited populations. This imprecise mutation correction generates broad mutagenic spectra that can be detected by next-generation sequencing, ranging from single-nucleotide polymorphisms to frameshift mutations resulting from indels. Nucleases can also cut at 'untargeted' sites causing off-target editing. Off-target editing can lead to unintended activation or deactivation of off-target genes, or large-scale genomic alterations such as translocations or inversions [46]. Finally, introducing sequences novel to patients may generate neoantigens ultimately provoking an immune response. This could be an important concern when using mixed cell populations, large gene edits, or repeated dosing of **cell therapy products**.

breaks in DNA. Zinc finger nucleases, Tal-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) technology are popular methods of editing genes within human cells.

Identity: specifications of cell-based therapies to confirm the desired characteristics.

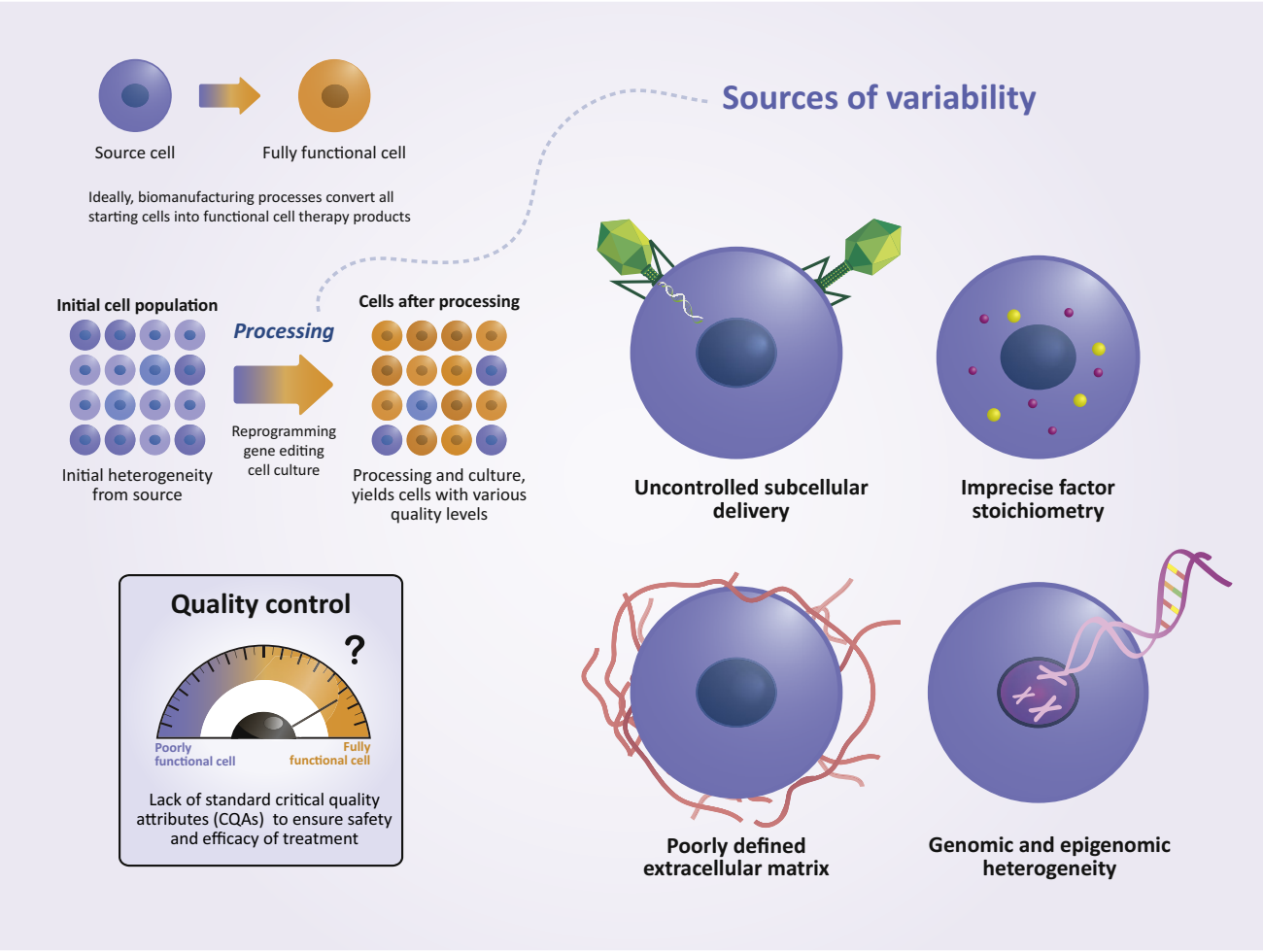
Organoid: a self-assembling 3D structure generated from stem cells, containing organ-specific cell types that may incorporate some features of the represented organ.

Potency: existence and effectiveness of the desired biological functionality.

Purity: lack of undesired components (contaminants) in the final product.

Reprogramming: modifying the epigenomic state of cells to change their identity.

Stem cells: cells that can self-renew and differentiate into multiple different cell types. Pluripotent stem cells can differentiate into any cell type in the adult organism, and iPSCs can be generated through reprogramming somatic cells. Adult stem cells are present in multiple organs and are more restricted in the range of cell types they can differentiate into. They can be found in bone marrow (hematopoietic and mesenchymal stem cells), the brain (neural stem cells), and several other organs.



Trends in Biotechnology

Figure 1. Variability in Cell Therapy Products Can Be Introduced during Biomanufacturing. In addition to the initial heterogeneity present in starting cell populations, cell culture and processing introduce additional variability in cell populations through poorly defined extracellular matrix, uncontrolled subcellular delivery, and stoichiometry of delivered factors, as well as genomic/epigenomic heterogeneities. Variability creates a challenge for quality assurance during clinical application, as one or more critical quality attributes for such variable cell therapy products need to be well defined. Purple cells delineate harvested, unprocessed cells that may have low functionality, while orange cells delineate cells after processing to generate a functional cell therapy product.

A Need for Better Characterization and Standards

To harness the full potential of cell-based therapeutics, cellular heterogeneity and stochasticity in processing should give way to high-quality, potent cellular products and reliable, reproducible, and scalable processing. Thus, for the rational design of cell therapies, defining **critical quality attributes (CQAs)** is imperative [47]. CQAs such as cell **identity**, **purity**, and **potency** could be rigorously defined and controlled for throughout the biomanufacturing process. The characterization toolkit could encompass cell and nuclear morphology, genomic, epigenomic, and phenotypic assays, as well as metabolomics and proteomics readouts. Currently, international standards are being established and disseminated to identify, characterize, and standardize CQAs [48] (Box 1).

In the next section, we discuss ways in which novel biomaterials can resolve some of the challenges facing emerging cell therapies, including their use for tackling genomic and

Box 1. International Standards for Biomaterials and Cell Therapy Products

Several organizations are working toward developing standards for cell therapy products [49]. Currently, in the United States, the National Institute of Standards and Technology Biosystems and Biomaterials Division is working to develop standards and measurement assurance for regenerative medicine biomanufacturing including several biomaterials standards (https://www.nist.gov/resources/guides-protocols). ASTM International Committee F04 on Medical and Surgical Materials and Devices (https://www.astm.org/COMMIT/SUBCOMMIT/F04.htm) also publishes several standards relevant for cell therapy products and biomaterials characterization. Finally, ISO Technical Committee 276 (Biotechnology) also produces standards, which are generally less specific to regenerative medicine and wider in scope. These are all works in progress: CQAs and benchmarks will require deep collaboration among academic, industrial, regulatory bodies, and standards organizations as new biomaterials enter this field.

epigenomic heterogeneity, cellular and subcellular delivery, and the reliable scaling and quality control of biomanufacturing processes.

Roles for Engineered Biomaterials

In the last decade, there has been incredible growth in the manner in which materials have been used to manipulate, study, and interact with biological matter. Synthetic and natural biomaterials have a wide range of tunable properties. They offer the ability to observe, probe, and direct cell behavior, sometimes in ways not accessible by regular *in vitro* culture or even *in vivo*, allowing distinct opportunities for cell-based therapies (Figure 2, Key Figure). The ability to interact with specific cell types, specifically engineered cellular interactions, and environmental responsiveness can enable these materials to behave in a smart manner.

Engineering Tunable Scaffolds

Biomaterials have been engineered to both recapitulate many aspects of *in vivo* environments and incorporate novel functionality. Several studies have shown that cells are most similar to their *in vivo* phenotype when they are cultured in matrices and scaffolds resembling their native physiological niche [50]. For example, MSCs can be directed to differentiate toward adipogenic, myogenic, or osteogenic lineages *in vitro* through culture on substrates with elasticity similar to fat, muscle, or bone tissue, respectively [35]. While 2D and 3D culture substrates coated with engineered biomaterials are commonly used for adherent cells, even suspension culture can employ synthetic beads with tunable biomaterials to control signaling to expand particular cell types, such as activated T-cell subsets [51].

Matrix properties such as shape, stiffness, composition, force, and topography are sensed by cells through membrane and transmembrane receptors, although there is very limited scope for manipulating these factors through traditional *in vitro* cell culture methods. By controlling these interactions, biomaterials can be engineered for a range of desired functionalities. Integrins, for instance, mediate cell adhesion and signaling through particular peptide motifs, such as **arginine–glycine–aspartic acid (RGD)** peptides. By modifying scaffold surfaces to incorporate RGD peptides, otherwise nonadhesive surfaces enable cell attachment and growth [52]. By varying peptides or adhesion ligands presented to cells, different cell-specific integrin interactions can be engineered. For example, biomaterials tailored for specific integrin interactions promote adhesion of select cell types and activate downstream kinase signaling to control MSC differentiation [53] or iPSC pluripotency [54]. Similarly, materials can be designed to control multiple ECM properties. Matrix composition can be established by adsorption or chemical conjugation of matrix proteins, peptides, or other moieties onto synthetic scaffolds. Alternatively, ECM components such as collagen, decellularized matrices, or cell-derived matrices can be utilized to form more 'organic', albeit less defined, scaffolds. Photo-lithography, microcontact printing, and other patterning techniques can be used to pattern single cell shapes or multiple cell aggregates [55,56]. Other sophisticated fabrication techniques such as block co-polymer micelle nanolithography allow subcellular, nanoscale ligand patterning, and control over parameters such as ligand spacing, presentation, and **focal adhesion** size [57].

Key Figure

Roles for Engineered Biomaterials in Reducing Variability and Enhancing the Efficiency and Quality Control of Cell Therapy Products

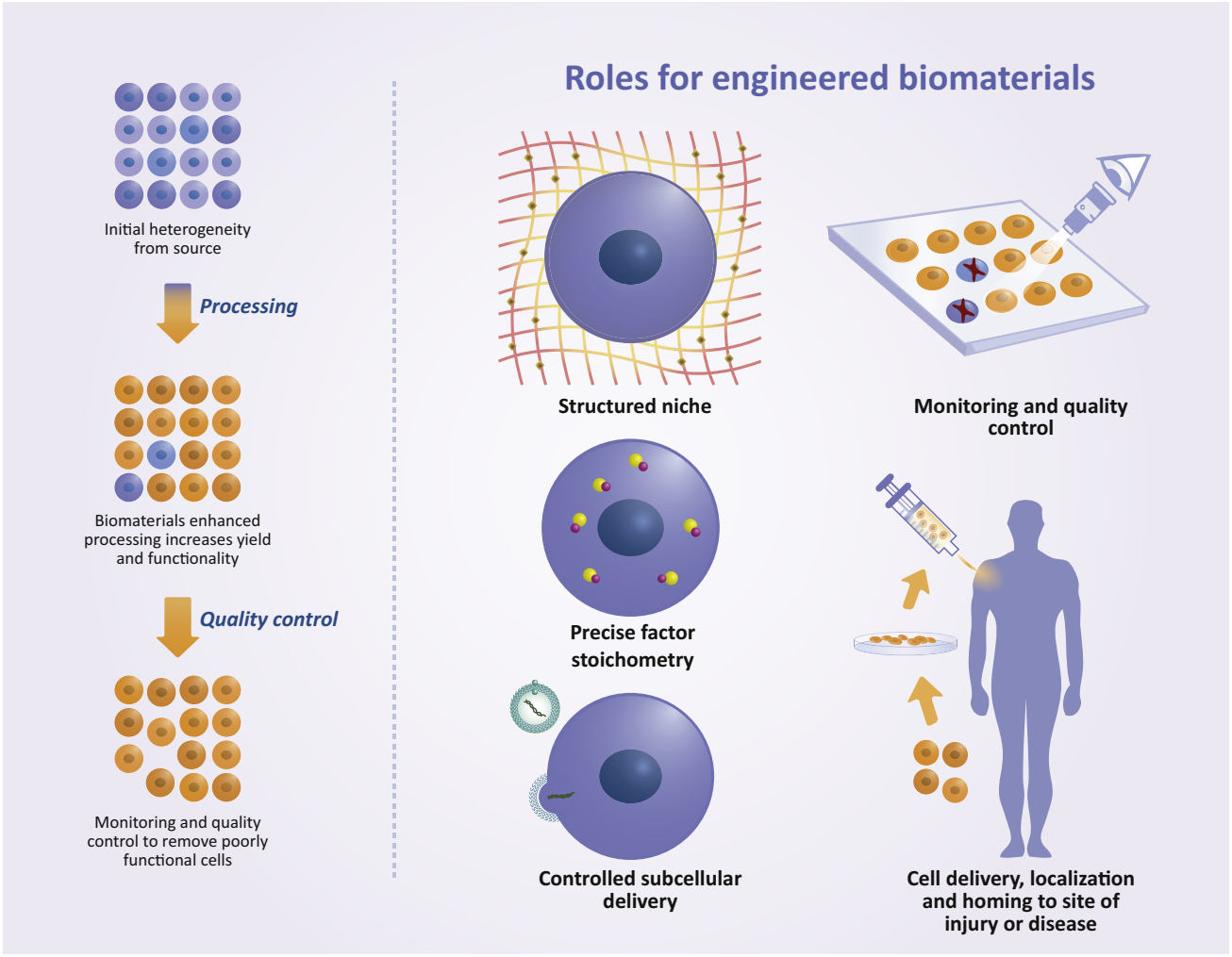


Figure 2. Potential roles for engineered and smart biomaterials include tailored cell–niche interactions, controlled subcellular delivery and stoichiometry of delivered factors (e.g., Cas9/sgRNA complexes or reprogramming factors – shown here in yellow/violet), cell delivery, and monitoring and quality control of cell therapy products. Purple cells delineate harvested, unprocessed cells that may have low functionality, while orange cells delineate cells after processing to generate a functional cell therapy product. sgRNA, single guide RNA.

The use of hydrogels and other tunable-stiffness materials enables access to a wide range of elasticity encountered physiologically [58] to modulate ECM elasticity and other mechanical properties in synthetic scaffolds. Using microfabrication and nanofabrication techniques to regulate matrix nanotopography permits manipulation of the physical architecture of the ECM [39]. Notably, for the aforementioned properties, differences between 2D flat culture and 3D biomimetic environments are well documented, usually suggesting that 3D cell culture

platforms may be preferred [59]. Finally, a wide variety of switchable materials have been developed that enable temporal control of an assortment of matrix properties including engineered surfaces with switchable adhesion to stiffening hydrogels [60].

Reducing Variability in Cell Populations

Precise control of the environment and presentation of a defined niche to all cells could reduce variability among cells and provide a reliable, consistent growth environment. This potentially allows for more stable and predictable epigenomic and phenotypic states, leading to more robust biomanufacturing processes. This is particularly useful with stem cells, which are extremely sensitive to ECM properties [61] and prone to differentiation *in vitro*. Fully defined synthetic scaffolds are now available for stem cell maintenance and culture *in vitro* [62,63]. Xeno-free microenvironments having a fully defined, reproducible niche may abrogate much of the epigenetic variation caused by uncontrolled or variable ECM during cell maintenance [64] and reduce batch-to-batch variation, an issue especially common with naturally derived components such as Matrigel.

Cellular Perturbation, Monitoring, and Screening

Cell–biomaterial interactions may be used to direct cells toward favorable functional states. Increased understanding of sophisticated biological processes and specific cell–ECM interactions enable engineering of a wide range of tailored matrix-mediated cellular perturbations. For instance, substrate-mediated epigenetic regulation of matrix properties can guide MSC differentiation [36] and secretion [65], as well as chromatin remodeling [56] and reprogramming efficiency of iPSCs [66]. iPSC differentiation and tissue or **organoid** formation may be enhanced through the use of biomaterials [67,68]. In these cases, organoid formation requires sophisticated matrix interactions to self-assemble and form functional units; mechanical, physical, and chemical properties and organization of the niche all affect form and function of organoids, making control of the matrix critical, especially if organoids are to be leveraged for therapeutic purposes [67]. Microparticles have been developed that provide stimulatory cues, such as interleukin-2, to enhance T-cell effector function and expansion [51]. Techniques such as micropatterning, which segregate populations into unique units, lend themselves well to high-content imaging, analysis, and screening [69], which enables facile assaying of phenotype and the selection of homogenous subpopulations. Previous studies demonstrated the utility of this *in situ* monitoring approach in evaluating heterogeneity in CRISPR-Cas9 delivery strategies [70] and editing of pluripotent stem cells [71]. Taken together, this body of work illustrates how biomaterials can provide a powerful platform for the guidance, monitoring, analysis, and quality control of cell populations.

Engineering Smart Delivery Strategies

Traditional methods for delivery of nucleic acids, proteins, or drugs involve viruses, electroporation, or nonviral transfection agents. These strategies rely on either an integrating virus, which is efficient but may induce insertional mutagenesis, or a nonintegrating delivery, which typically suffers from lower efficiency and variable spatiotemporal expression profiles [72]. Biomaterial delivery strategies based on microparticles and nanoparticles can be designed for controlled release of factors at desired stoichiometry. This precision control is accomplished through chemically controlled molecular stoichiometry and degradation kinetics of particles. There are several technical limitations for the standardization of drug delivery to cells. However, by standardizing the components of drug delivery systems, biomaterial approaches such as high-density nanoneedles [73] are demonstrating more uniform and reproducible delivery. The regulated control of delivery strategies has several implications for cell therapies.

Standardizing Factor Delivery

Tunable properties of biomaterial carriers enable control of factor delivery and release and alleviate some of the stochasticity in the reprogramming process. These carriers have been shown to decrease variability and enhance efficiency in reprogramming outcomes across cell populations [74]. mRNA, with its ability to act from the cytoplasm, may present a compelling, safer alternative to DNA-based reprogramming strategies that could integrate elements into the host genome [75]. Non-nucleic acid-based techniques including controlled protein delivery can be effective and abrogate the need for genetic material entirely [76]. Vector free, microfluidics-based techniques are attractive potential alternatives for *in vitro* cell engineering as well [77]. In addition to transcription factor-mediated reprogramming, an emerging set of epigenomic regulators employing catalytically inactive Cas9 could also be delivered in a standard fashion to modify gene expression and reprogram cells efficiently to reduce cell population heterogeneity [78]. For CRISPR-Cas9 gene editing, tunable biomaterial carriers could provide efficient, temporally controlled delivery of Cas9 nuclease and guide RNA. Gene editing efficiency with nonviral methods remains a concern [79], especially with stem cells, which are notoriously hard to transfect [72]; thus, it is imperative to improve editing potency through enhanced delivery. Furthermore, more uniform delivery across cells could help reduce genetic heterogeneity resulting from gene editing processes.

Standardizing In Vivo *Delivery of Cells*

Once therapeutic cells are ready, they have to be delivered to the right spot *in vivo* and allowed to perform their therapeutic function. Effective *in vivo* cell delivery is a major challenge, as cell death, loss of injected cells, or changes in cell behavior occur upon transplantation [80]. Furthermore, it may be desirable to localize cells to a specific area or organ. In these aspects, biomaterials can be used to direct and tether cells to a specific location, protect them, control their behavior, or isolate them from the host, particularly the host immune system [81,82]. During cell delivery, scaffolds can anchor cells as well as provide an engineered microenvironment for optimal function [83]. For example, biopolymer carriers for cell delivery could support T-cell expansion and dispersal upon implantation [84]. Moreover, biomaterials can deliver cells less invasively, without surgery, through injectable cell carriers [85]. Microencapsulation or macroencapsulation of cells can be utilized for the efficient diffusion of growth factors and nutrients, while preventing attack on cells via antibodies or other immune system components.

End-to-End Use in Biomanufacturing

Taken together, biomaterials could be utilized to improve every step of the biomanufacturing and delivery of emerging cell therapy products (Figure 2). Biomaterials provide a well-defined, less complex, and more tunable environment than ECM-derived substrates or feeder cells (cell layers – typically fibroblasts – grown in the same dish as iPSCs to provide proteins, growth factors, and nutrients). Furthermore, their production is more amenable to scale-up [74] and the final products can be made xeno free. 3D biomaterial culturing of stem cells yields higher cell numbers than 2D systems and is inherently more scalable, although methods for facile cell extraction and resuspension need to be established [62]. Automation and *in situ* monitoring enable continuous improvement and implementation of lean manufacturing techniques for any biomanufacturing system. Microcarrier-based systems can be integrated into available bioreactor formats, while microfluidics would enable perfusable, continuously monitored culture systems [1]. Furthermore, successful biomanufacturing of cells will require reliable and scalable expansion procedures, especially in processes that introduce population bottlenecks (e.g., selection steps in reprogramming or gene editing).

To overcome the current variability in cell therapy products, standards and CQAs must be developed and supplemented with high-throughput, reliable characterization methods [48] with functionally relevant outputs. Biomaterials offer a much wider parameter space compared with

Table 1. Examples of the Potential Use of Biomaterials for the Development, Monitoring, and Evaluation of CQAs for Three Types of Cellular Therapies

Cell type	CQA category	CQA	Potential role for biomaterials	Refs
iPSC-derived cardiomyocytes [90,91]	Identity	Mature cardiomyocytes (surface markers, morphology, multinucleation)	Phenotyping through micropatterning and high-content imaging	[70,71]
	Purity	Low number of cardiac fibroblasts ($CD90^+$), pluripotent ($Oct4^+$), and nonmesoderm ($Nestin^+$) cells	Label-free microfluidic enrichment	[92]
	Potency	Contraction force	Micropatterning and traction force microscopy	[93]
			Micropost arrays	[94]
		Calcium transients	Engineered heart tissue	[95]
		Electrophysiology	Nanoelectronic probes	[96]
			Nanostructured multielectrode arrays	[97]
MSCs for ischemic stroke [98]	Identity	Trilineage potential *in vitro*	Microfluidic-based enrichment	[99]
	Potency	Trophic effect (proangiogenic potential)	*In vitro* angiogenesis models	[100]
		Immunomodulation	*In vitro* hydrogel coculture models with immune cells	[101]
CAR T-cell therapy [102]	Identity	Effector T cells ($CD3^+$, CAR^+)	Affinity-based microfluidic cell sorting and expansion	[103]
	Purity	CD4/CD8 ratio		
	Potency	Antitumor efficacy	*In vitro* 3D model of tumor–T-cell interactions, high throughput	[104,105] [106]
		CAR-targeting efficiency		

traditional *in vitro* assays that may be more suitable for probing the wide range of properties required for the identification and characterization of CQAs. Engineered biomaterials can be utilized to characterize cells based on surface markers, proteomics, secretomics, physical properties, epigenetics, and functional behavior. Their tunable, well-controlled properties allow the determination and deconvolution of sources of variability. This can be done using multivariate design of experiments approaches to systematically examine the significance and interactions of process parameters, while reducing sample size and effort required compared with conventional methods. For example, integrating biomaterials with design of experiments methods will allow efficient optimization of biomaterial properties for factor delivery [70] or cell functionality [86]. Altogether, biomaterials are suitable as standardized reference materials that could be used as benchmarks in the biomanufacturing process. Table 1 provides examples of the use of engineered biomaterials for the establishment, characterization, and maintenance of CQAs in some representative cell therapies: iPSC-derived cardiomyocytes, MSCs for ischemic stroke, and CAR-T therapies. For all three of these cell types, biomaterials are particularly well suited for CQAs involving physical properties of cells and the ECM and those involving *in vitro* modeling.

Outlook

Sophisticated engineered therapies are likely on the horizon, including newer methodologies that will enable extended control over the cellular niche, allowing the manipulation of more dynamic elements *in situ*. Successes in cell engineering have encouraged the move from single gene editing to multiplexed editing and design of synthetic circuits to impart functionality [87,88]. However, to realize precise biomanufacturing of cell therapies, several challenges

need to be resolved (see Outstanding Questions). The development of efficient, precise genome editing techniques that can be delivered to desired somatic tissues; the derivation of high-quality, patient-derived iPSCs in a facile, cost-effective, and efficient manner; and the development of standards, measures, and quality control procedures that follow good manufacturing practice [41] are key bottlenecks. Reduction of the variability inherent to some of these processes would ease the way for clinical translation of cell-based therapies.

As cell-based therapies become more intricate and rationally programmed, biomaterials-enabled precise control will play a significant role in directing the process, from biomanufacturing to final delivery. Biomaterials have several features that lend themselves to improving biomanufacturing processes including a large parameter space for properties, reproducibility, and scalability. Biomaterials are particularly suited to applications where physical and mechanical properties are important, precise control over cellular environment is required, or when complex behaviors may be required *in vivo*. Recently, engineered nanocarriers could genetically edit T cells effectively *in situ* in mice, resulting in long-term remission [89]. The move to *in situ* cell engineering has the potential to simplify or even abrogate the need for *ex vivo* biomanufacturing. However, biomaterials-based platforms have their own limitations that need to be considered. Although synthetic biomaterials have become more sophisticated in recent years, they still do not rival the complexity of natural systems. Furthermore, as biomaterials get more complex, their behavior may become harder to predict and control. Biomaterials-based manufacturing workflows have different parameters from those currently in clinical and industrial settings, requiring more work to establish and test CQAs, standards, and scalable biomanufacturing processes. Nevertheless, in the next decade, engineered biomaterials are likely to be key players in establishing more potent, well-defined, and well-characterized cell therapy products.

Outstanding Questions

How much of the variability in current biomanufacturing processes can be eliminated through the optimization of biomaterials?

Can *in vitro* biomaterials-based assays meaningfully characterize cell therapy products with *in vivo* relevance, abrogating the need for more expensive *in vivo* testing?

Will rational design of cell therapies based on standards and CQAs accelerate clinical adoption of cell-based therapies?

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