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Review

Biomaterial-assisted scalable cell production for cell therapy

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ABSTRACT

Cell therapy, the treatment of diseases using living cells, offers a promising clinical approach to treating refractory diseases. The global market for cell therapy is growing rapidly, and there is an increasing demand for automated methods that can produce large quantities of high quality therapeutic cells. Biomaterials can be used during cell production to establish a biomimetic microenvironment that promotes cell adhesion and proliferation while maintaining target cell genotype and phenotype. Here we review recent progress and emerging techniques in biomaterial-assisted cell production. The increasing use of auxiliary biomaterials and automated production methods provides an opportunity to improve quality control and increase production efficiency using standardized GMP-compliant procedures.

1. Introduction

Live cell therapies can achieve more integrated and complex functions than small molecule drugs or biologics, resulting in more effective disease treatments. In 2018, there were 18.1 million new cases of cancer and 9.6 million cancer deaths [1], and roughly 50 million people suffering from Alzheimer's disease worldwide [2]. Cell-based therapy offers a new strategy for treating these and other intractable diseases. Stem cells have been used clinically with great success. In 2007, the first HIV/AIDS cure was achieved (the 'Berlin patient') by transplanting allogeneic HSCs [3], and a decade later, a second HIV/AIDS cure was achieved (the 'London patient') also using a blood stem cell transplant, demonstrating the tremendous potential of stem cell therapy [4]. In 2009, the world's first hESC clinical trial was approved by the FDA, and the development of hESC cell therapies has since accelerated [5]. In Japan, a MSC therapy for spinal cord injury was approved by the government [6], and iPSCs were used for the first time in a cornea transplant [7]. Clinical stem cell research has also emerged in China, where there are now 51 registered ongoing clinical stem cell research projects and 104 clinical stem cell research institutions [8]. In 2018, the global market for stem cell therapy grew to roughly $280 million [9].

In addition to stem cells, primary immune cells such as tumor infiltrating-lymphocytes, DCs, and genetically-modified T cells have demonstrated powerful anti-cancer effects and are quickly being adopted for cell therapies [10]. CAR T cells are genetically engineered to target human CD19 and initiate an immune response against cancer cells. CAR T cells have shown clinical efficacy against malignant tumors including ALL and non-Hodgkin lymphoma [11]. In 2017, the FDA approved the CAR T-cell therapy, Kymriah (Novartis Pharmaceuticals, clinical trial number: NCT02228096), for treatment of ALL. Months later, another

Abbreviations: AIDS, acquired immune deficiency syndrome; ADSCs, adipose-derived stromal cells; ALL, acute lymphatic leukemia; bFGF, basic fibroblast growth factor; BM, bone marrow; BMP-2, bone morphogenetic protein 2; CAR, chimeric antigen receptor; DCs, dendritic cells; DMAEMA, N,N-dimethyl aminoethyl methacrylate; DMSO, dimethyl sulfoxide; EBiSC, European Bank for induced pluripotent Stem Cells; ECM, extracellular matrix; EGF, epidermal growth factor; ESCs, embryonic stem cells; FDA, Food and Drug Administration; GMP, good manufacturing practice; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; hMSCs, human mesenchymal stem cells; hNPCs, human neural progenitor cells; hPSCs, human pluripotent stem cells; HUVECs, human umbilical vein endothelial cells; HSCs, hematopoietic stem cells; iPSCs, induced pluripotent stem cells; LCST, lower critical solution temperature; mESCs, mouse embryonic stem cells; MSCs, mesenchymal stem cells; NiPAAm, N-isopropylacrylamide; NK cells, nature killer cells; PCL, polycaprolactone; PDGF-BB, platelet-derived growth factor BB; PEI, polyethylenimine; PLGA, polylactic-co-glycolic acid; PLL, poly-L-lysine; PNiPAAm, poly(*N*-isopropylacrylamide); PS, polystyrene; RAFT, reversible addition-fragmentation chain transfer; SCF, stem cell factors; siRNA, small interfering RNA; UCB, umbilical cord blood

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CAR T-cell therapy, Yscarta (Kite Pharma, clinical trial number: NCT02926833), was approved by the FDA. In 2018, the FDA accepted an application by Celyad to start a clinical trial of a new CAR T-cell therapy (CYAD-101, clinical trial number: NCT03692429), the first allogeneic CAR T-cell therapy candidate to use non-gene editing methods [12]. The global market for all cell manufacturing is predicted to reach $11 billion by 2030 (assuming an average growth rate of 14.9%) [13].

A single dose of therapeutic cells for transplantation in adults is 10^8–10^9 cells [10], a quantity which requires massive cell amplification. To obtain sufficient therapeutic cell products, living cells derived from an individual patient (autologous), from a donor (allogeneic), or from another species (xenogeneic) must undergo isolation and purification, genetic modification, and large-scale expansion before being harvested for downstream processing or banked for future use. Nowadays, considerable challenges to meeting clinical demands for cell products still remain. Strict control of the activity, efficacy, safety, consistency, reproducibility, and quantity of therapeutic cell products is required [14,15]. Transmission of any causative agents from donors must be avoided, and the entire manufacturing process must be kept free of contamination [16]. The quality attributes of cell products must also be strictly controlled. For stem cell products, undesired differentiation during large-scale production must be avoided. For example, clinical-grade hiPSCs must show expression of pluripotent markers in > 70% of cells [17]. Therapeutic MSCs must show expression of positive markers CD105, CD73, and CD90 in > 90% of cells, and of negative markers CD34, CD45, and CD14 in < 5% of cells [14]. Stem cell tumorigenicity, toxicity, and immunogenicity must also be evaluated [15]. While for primary cells, increasing the efficiency of amplification while maintaining cell functions is a challenge. Donor selection is also critical since genotype and phenotype influence cell functions [18]. Quality control, process standardization, and cost reduction must also be considered, as reviewed recently by K. Roy et al. [10].

Biomaterials can facilitate scalable cell production by providing reliable solutions to above-mentioned obstacles. Biomaterials can recapitulate *in vivo* microenvironments to provide a defined niche for cultured cells, resulting in cell phenotypes that are similar to those *in vivo* [19]. Some biomaterials can support specific cell behaviors such as homing and directed differentiation. Cells are responsive to ECM properties such as material components, chemical bonds, stiffness, shape, and topography, and engineering approaches are used to recreate the ECM to regulate cellular behavior [20]. One example demonstrating the potential of biomaterials in cell therapy is HOLOCLAR [21]. HOLOCLAR is a mature stem cell product that integrates cells with biomaterials by seeding limbal epithelial stem cells onto fibrin scaffolds, and has been approved for treatment of limbal stem cell deficiency and damaged corneas by the European Union (clinical trial number: NCT02577861).

Biomaterials can used to improve cell amplification efficiency while maintaining therapeutic cell quality. With the rapid developments occurring in materials science, biomaterials are now becoming involved in all aspects of scalable cell production. In this review, we discuss recent scientific findings and technological innovations in biomaterial-assisted, large-scale cell production processes for cell therapy (Fig. 1). As a typical example, we discuss how mass production of HSCs has been aided by applying advanced biomaterials science and associated technologies.

2. Therapeutic cell isolation and purification

Scalable production of therapeutic cells begins with cell isolation and purification. Many therapeutic cells including chondrocytes, hepatocytes, and ADSCs are derived from primary tissues in which cells and ECM are highly intertwined. Proteolytic enzymes such as trypsin and collagenase are used to treat tissue and dissociate the desired cells from the ECM. Agents that interfere with cell-cell adhesion such as ethylenediaminetetraacetic acid are also commonly used. In tissues such as blood and BM in which cells are more loosely attached to ECM, gentler cell isolation methods such as agitation or pipetting are used.

After dissociating the target cells from ECM, the cells are purified using a variety of methods. Centrifugation is used to separate cells, taking advantage of differences in cell size or sedimentation properties. Preferential attachment of certain cell types such as MSCs to materials such as glass, plastic, or hydrogels can be used to separate these cells from other cells that adhere less strongly [22]. Antibodies that bind target cell antigens can be coupled to matrices such as polysaccharide beads to physically separate cells; such antibodies can also be coupled to fluorescent dyes to separate cells using fluorescence-activated cell sorting.

With the enormous success of CAR T cells in cancer treatment [23], T cell isolation and purification has become a highly active area of investigation. Immunomagnetic separation is a commonly used T cell purification method, and is an example of the used of biomaterials in cell therapy [23]. T cells are enriched by pushing pretreated patient blood through a density-gradient by centrifugation, bound to antibody-modified magnetic microbeads, and collected using a magnetic field [23,24]. Griwatz et al. used magnetic beads coupled with peptide-major histocompatibility complex multimers to isolate and amplify epithelial T cells [24]. Riethdorf et al. obtained peripheral blood mononuclear cells from patients by leukapheresis, then used Dynabeads® Human T-activator CD3/CD28 magnetic beads to purify and activate the T cells [25]. After obtaining a uniform T cell population, the cells can be subjected to genome modification, expansion, or differentiation, or can be banked for future use.

3. Genomic modification of therapeutic cells

Genetic engineering of primary cells prior to large-scale expansion can be used to modify specific cell functions for desired therapeutic purposes. Recent examples include inducing somatic cells to differentiate into iPSCs, inducing DCs to express CD40 ligands, and introducing CAR or T cell receptor genes in T cells [26,27]. To perform genome modification for cell therapy, gene expression vector systems must be efficient, safe, easy-to-produce, and cost-effective. Viral vectors such as retroviruses, lentiviral, adenovirus, and adeno-associated viruses have been reviewed elsewhere [28,29]. Viral vectors are effective gene therapy tools due to their efficient gene integration and stable gene expression; viral vectors can deliver high levels of genes to correct a pathogenic gene. Lentivirus-based CAR T-cell therapy is safe and efficient, with a low risk of oncogenic transformation [30]. Although viral vectors are efficient for transgenic delivery, they face challenges including cytotoxicity, immunogenicity, packaging capacity, and production problems [31]. Nonviral vectors have several advantages over viral vectors, including better safety, larger cargo capacity, and more cost-effective manufacturing [32]. The use of biomaterials as nonviral delivery systems is rapidly growing.

Nonviral vectors include plasmid DNA, mRNA, miRNA, and siRNA [33]. During gene delivery, extracellular and intracellular barriers that might reduce efficiency must be considered [34]. Extracellular barriers include undesired immune responses and non-specific binding, which may prevent gene delivery systems from reaching target cells. Intracellular barriers include issues with cell uptake, endosomal escape, and nuclear localization [31,35]. Considerable effort has been devoted to developing reliable nonviral delivery systems that avoid degradation of genetic materials, with the goal of applying gene modification to cell therapy [35]. CRISPR/Cas9, a programmable small guide RNA targeting the endonuclease system, has been used to eradicate human papillomavirus genes in human cervical cancer cells. Although CRISPR gene editing nucleases are powerful genetic modification tools, efficient delivery of nonviral nucleases requires further investigation [36]. Various nonviral delivery systems have been developed based on lipids, polymers, carbon nanostructures, peptides, or inorganic materials [36].

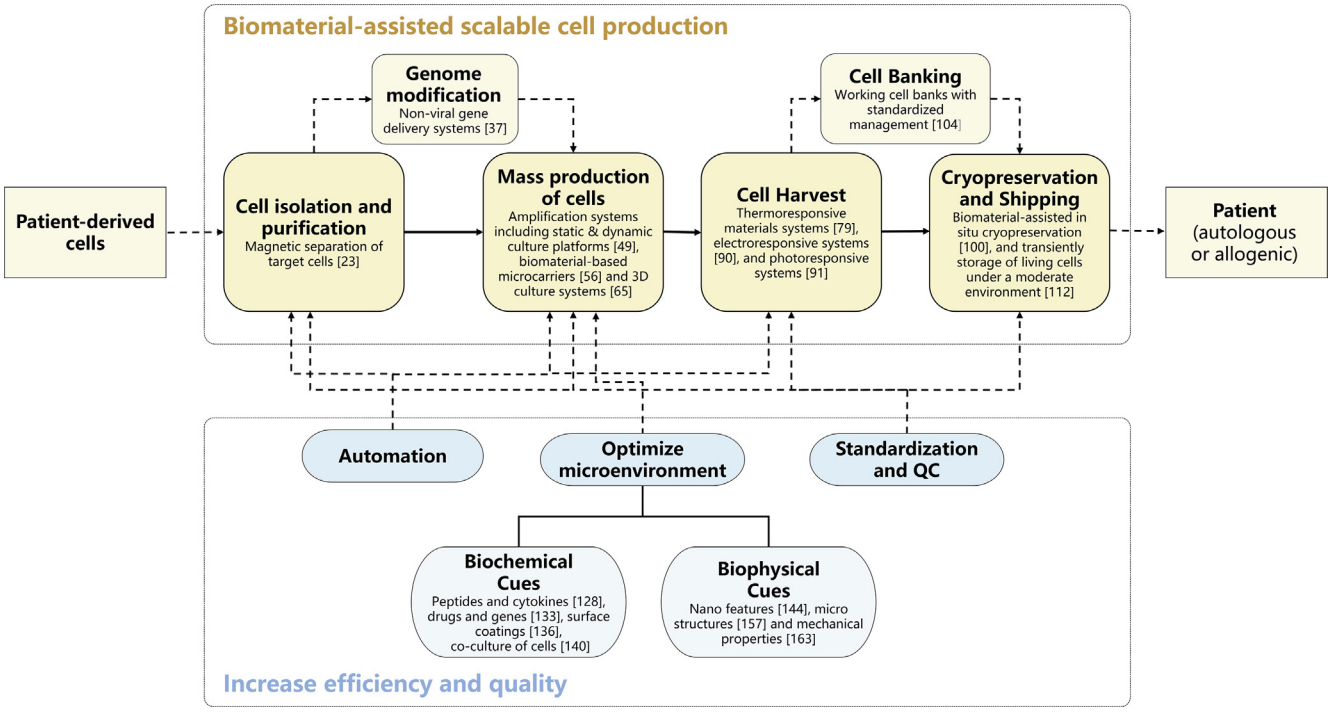

Fig. 1. Biomaterial-assisted scalable production of therapeutic cells.

Of these, polymer-based delivery systems are the most promising and most extensively investigated.

Polymeric gene delivery systems can be fabricated using natural or synthetic materials. Chitosan, a natural cationic polymer, has been studied extensively for gene delivery due to its safety and biocompatibility. Chitosan derivatives with improved solubility and charge properties have been developed for siRNA vectors [37]. PLL was one of the first synthetic polymers developed for gene delivery, and is non-toxic and non-immunogenic [38]. DMAEMA and poly (β-amino esters) are also non-toxic and non-immunogenic, and are gene carrier candidates [39,40]. PEI is another promising synthetic delivery system due to its superior nucleic acid binding [41], though it can suffer from low transfection efficacy and weak targeting ability [42]. Novel natural/synthetic hybrid systems have been developed to address these obstacles. For example, PEI-grafted chitosan showed greater MSC plasmid transfection efficiency and reproducibility than PEI alone [43]. Magnetic-silk/PEI core-shell nanoparticles have been used to achieve greater oligodeoxynucleotide uptake efficiency by human breast cancer cells with reduced cytotoxicity [44]. PLGA has been used to deliver peptides, nucleic acids, and DNA for gene modification in hematopoietic progenitor cells [45]. The properties and advantages of the biomaterials most commonly investigated for nonviral gene delivery are listed in Table 1.

4. Mass production of therapeutic cells

Conventional planar cell cultures poorly mimic the *in vivo* cell niche and often result in loss of normal cell functions and unwanted cell differentiation [46]. This problem is compounded by the large dose of therapeutic cells required for transplantation (~10^9 cells for an adult dose), making it difficult to meet the rigorous clinical requirements [10]. Large-scale cell production with high efficiency and quality control is crucial for obtaining sufficient therapeutic cells from autologous or xenogenous sources [47].

Mass production systems are developing rapidly and can be divided into two broad categories of culture platforms: static and dynamic (bioreactors). Static culture platforms maintain cell functions using a diffusion transport mechanism. These platforms are applied widely due to their simplicity, small space requirements, low cost, and efficient timing. Commercially available static culture platforms include Nunc Cell Factory Systems (Thermo Scientific Nunc®) and the CellSTACK® System (Corning). However, static culture platforms are limited in their ability to provide physiological levels of nutrients, growth factors, and oxygen, and are limited in their ability to remove waste metabolites, both of which are indispensable for obtaining reproducible and homogeneous large-scale cell products [10]. Dynamic culture platforms (bioreactors) have been extensively studied to address these deficiencies. Bioreactors such as perfusion culture platforms (E-Cube™, Corning and Xpansion System, Pall Life Sciences), wave bioreactors (Xuri cell expansion system, GE Healthcare), stirred tanks (ambr 15™, TAP Biosystems and Finesse, Thermo Fisher), and rotating wall vessels (RCCMAX, Synthecon) have been explored for large-scale production of therapeutic cells including DCs, NK cells, and CAR T cells [48].

In these bioreactor systems, biomaterials are often used as a semi-permeable membrane that promotes diffusion of dissolved gas, nutrients, and waste products, to create an appropriate culture environment [49]. Hollow-fiber bioreactors, for example, feature a fibrous membrane structure [50]. In these dynamic culture platforms, a hollow, porous fiber membrane with diverse filtration rates, fabricated from semipermeable hydrophilic polysulfone, serves as the core of a hollow fiber cartridge for substance exchange. Commercially available hollow-fiber bioreactors include FiberCell Hollow Fiber Cell Culture Bioreactor (FiberCell Systems) and Quantum Cell Expansion System (Terumo BCT). FiberCell is currently used for mass production of monoclonal antibodies, recombinant proteins, viruses, and cells (HSCs and placental MSCs) [51]. The FiberCell cartridge has a surface area of 3000 cm^2 and can support the simultaneous culture of 2×10^9 cells [52]. With the Quantum Cell Expansion System, MSCs derived from 25 mL of BM can be reproducibly expanded to ~6.6×10^8 cells over 21 days [53]. The Quantum system was used with a fibronectin coating to achieve clinical-scale MSC expansion in 5 days of culture starting from a BM volume of 18.8–28.6 mL, while retaining desired cell functions and differentiation capacity [54]. Biomaterials have also been used as culture scaffolds in 3D perfusion bioreactor systems. Zhao et al. designed a bioreactor using non-woven PET fibrous matrices which achieved a high cell density (4.2×10^7 cell/mL, more than 50-fold expansion)

Table 1
Properties of biomaterials used for nonviral gene delivery.

Biomaterial	Target cell(s)	Biomaterial properties	Advantages for nonviral delivery	Reference(s)
Chitosan	HepG2 human liver cancer cells	Safe, non-toxic, good biocompatibility	Enhanced cell uptake of siRNA	[37]
PDMAEMA/DNA	Human ovarian cancer cell line (OVCAR-3), monkey kidney cell line (COS-7)	Low cytotoxicity, good stability	High transfection efficiency	[39]
Poly(β-amino esters)	Monkey kidney cell line (COS-7)	Biodegradable, safe	High transfection efficiency	[40]
PLL	Retinal pigment epithelial cells	Biodegradable, non-immunogenic, superior thermal stability	High biological activity, high cell uptake and transfection efficiency	[38]
PEI	Human breast carcinoma cell line, C2C12 murine myoblast cell line	Non-toxic and low immunogenicity	Overcomes lysosomal barrier, efficient gene delivery	[41,42]
PEI-grafted chitosan	Rat bone marrow-derived MSCs, human lung carcinoma cell line	Non-degradable, high pH buffering capacity	High transfection efficiency, reproducibility	[43]
Magnetic-silk/PEI	Human breast cancer cells	Lower performance charge, low cytotoxicity, stability	Simple operation and low cost	[44]
PLGA	Human hematopoietic stem/progenitor cells	Biodegradable, good biocompatibility	Efficient delivery of peptides and nucleic acids	[45]

after 40 days of culture [55]. One concern during the bioreactor culture process that should be noted is that excessive shear stress can cause irreversible cell damage, leading to unwanted cell differentiation and altered cell phenotype [49].

The use of microcarriers is another biomaterials approach to large-scale cell production. Microcarriers are used in suspension conditions to increase the surface area for cell attachment, enabling faster cell amplification [10]. Microcarriers can be composed of either natural or synthetic materials that have good biocompatibility, low cytotoxicity, and sufficient mechanical stability for dynamic culture [56]. Microcarrier size, shape, surface coating, and charge are all important for the maintenance of cell functions [56]. Different types of microcarriers are available and can be classified as nonporous or macroporous. It is essential to choose a suitable microcarrier for a specific cell type [57]. Nonporous microcarriers allow easier cell seeding and harvesting than porous microcarriers. Commercially available nonporous microcarriers include Cytodex® 1 (a cross-linked dextran matrix with positive-charged diethylaminoethanol groups), Cytodex® 3 (a cross-linked dextran matrix with denatured collagen bound to the surface), and Synthemax® II (United States Patent Class VI PS material). MSCs cultured on Cytodex 1 microcarriers showed 80% cell adherence at 3 h after seeding, and can be induced to differentiate into osteogenic and chondrogenic lineages (Fig. 2A) [58]. mESCs have been expanded nearly 200-fold on Cytodex 3 microcarriers while maintaining pluripotency (Fig. 2B) [59]. hMSCs have been expanded over 1000-fold in 40 days on Synthemax II microcarriers while maintaining the desired cell phenotype, karyotype, and stemness (Fig. 2C) [60].

Macroporous microcarriers provide a larger surface area for cell adherence and migration than nonporous microcarriers. Macroporous microcarriers include CultiSpher-S (a cross-linked gelatin matrix), and Cytopore (porous cellulose with N,N-diethylaminoethyl groups) [61]. Using CultiSpher-S, researchers achieved a 439-fold expansion of ESCs to a cell density of $> 3.5 \times 10^6$/mL in 6 days while maintaining pluripotency (Fig. 2B) [59]. Microcarriers are often used together with bioreactor systems to protect cells from shear stress caused by the stirring impeller, thus preventing undesired differentiation and improving product consistency [48]. Using CultiSpher-S microcarriers within a spinner flask, hMSCs were expanded 10^3-fold in 30 days – a fast expansion due to the enhanced cell contact between the cells and microcarrier surfaces. The harvested cells retained their defining features after several passages [57]. When cultured on CultiSpher-S in wave bioreactors, MSCs reached high (> 90%) seeding efficiencies, and achieved ~15-fold expansion in 7 days of culture [62]. In addition to large-scale cell expansion, microcarrier suspension culture is also used for directed differentiation of stem cells. hiPSCs cultured on matrigel-coated DE-53 microcarriers (Whatman) reached 20-fold expansion in stirred spinner flasks and were directly differentiated to hNPCs with 78–85% efficiency (Fig. 2D) [63].

3D culture matrices can be used to create biomimetic microenvironments that promote cell adhesion, proliferation, migration, and activation [64]. 3D culture matrices have porous structures and interconnected pore networks that can facilitate diffusion of nutrients and oxygen and removal of waste, making them good candidates for improving *in vitro* cell expansion [65]. Natural materials including collagen, chitosan, hyaluronic acid, gelatin, fibrinogen, vitronectin, and decellularized ECM [66–70], and synthetic materials such as PCL, PLGA, PLL, polyurethane, and PS [71–76], have been used to fabricate 3D porous scaffolds of different formats for large-scale cell production. Several commercial platforms are available, including the AlgiMatrix 3D Culture System (Thermo Fisher), HydroMatrix™ (Sigma), Bio-scaffold (Tantti), and JET CellSCAFFOLD™ (JET BIOFIL); these commercial platforms use gelatin, synthetic peptides, collagen, and PS, respectively. 3D scaffold variants have been investigated extensively to achieve higher cell populations in less time. A robust vitronectin-derived peptide was designed for long-term expansion of hNPCs, and the cultured cells maintained specific morphology, stemness, and hNPC marker

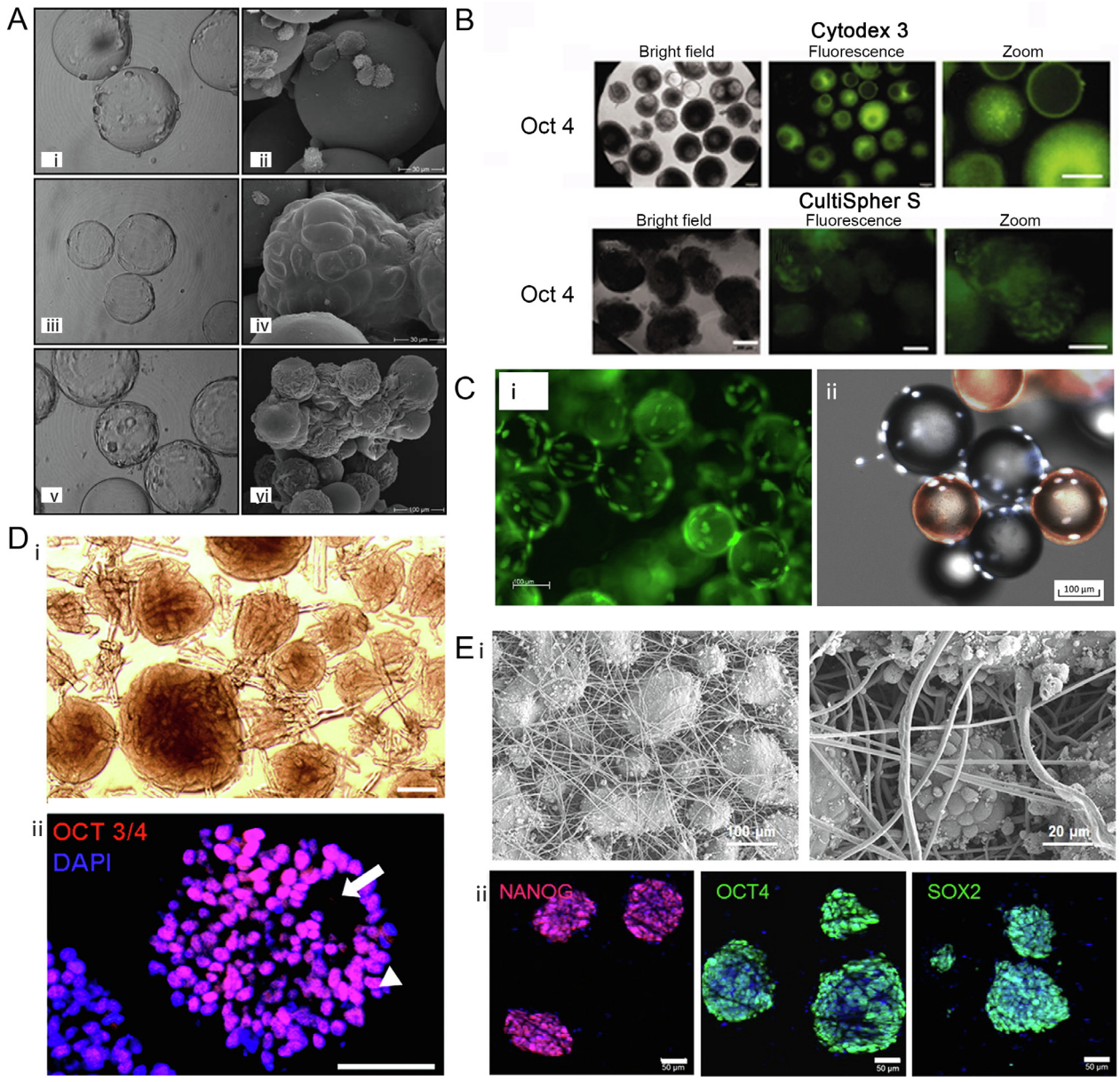

Fig. 2. Biomaterials-assisted large-scale cell expansion systems. A) Light microscopy (i, iii, v) and SEM (ii, iv, vi) images of adherent MSCs on Cytodex 1 microcarriers on day 0 (i, ii), 14 (iii, iv), and 28 (v, vi). Adapted with permission from Wiley Online Library: [Biotechnology Progress], copyright (2008) [58]. B) OCT4 staining of hESCs loaded with Cytodex 3 or CultiSpher-S microcarriers. Adapted with permission from Wiley Online Library: [Biotechnology Progress], copyright (2011) [59]. C) Images of hMSCs after 40 days of culture on Synthemax II microcarriers. i) Live cells stained with calcein AM (green). ii) Cells with nuclei stained with DAPI (white) migrated from existing microcarriers (grey) to newly added microcarriers (red). Adapted with permission from PLOS: [PLOS ONE], open-access (2014) [60]. D) Images of iPSC clumps on microcarriers in a spinner flask. i) Images of iPSC clumps on microcarriers (transparent rods). ii) Pluripotent marker expression on day 7. Adapted with permission from Liebert: [Tissue engineering Part C], open-access (2013) [63]. E) Images of an electrospun PS scaffold with adherent hiPSCs, and long-term culture of hiPSCs. i) SEM image after 6 days of culture. ii) Pluripotent marker expression after 10 passages. Adapted with permission from Elsevier: [Acta Biomaterialia], copyright (2016) [76].

expression [69]. Cheung et al. developed a novel scaffold system composed of mesoporous silica microrods and a fluid lipid bilayer. Mimicking natural antigen-presenting cells, this scaffold provides T cells with membrane-bound cues and soluble paracrine cues, and achieves 2- to 10-fold greater primary T cell expansion than commercial cell expansion beads (Dynabeads) [77]. Havasi et al. developed an electrospun PCL scaffold with an increased hNPC proliferation rate versus standard plate cultures [71]. In a comparison of a series of electrospun xeno-free PS scaffolds with different porosities, a larger number of hiPSC 3D aggregates were observed on more porous scaffolds (Fig. 2E), and after 10 consecutive passages the cultured hiPSCs retained their pluripotency. Differentiated neuron-like cells can also be cultured on this PS scaffold for up to 18 months [76]. Although some of these 3D culture systems have not reached clinical-scale cell expansion, they have increased the cell proliferation rate while maintaining cell functions, which is crucial for use in cell therapy. We summarize the key features of biomaterial-based systems for mass production of therapeutic cells in Table 2.

5. Harvesting cells after mass production

After large-scale expansion, target cells must be harvested effectively for downstream processing. Harvesting cells without cell damage and while preserving desired cell phenotypes and functions remains a

Table 2
Summary of biomaterials-based systems for mass production of therapeutic cells.

Mass production system		Commercial system or biomaterial type	Cell type(s)	Cell functions	Amplification efficiency	References
Bioreactors	Hollow fiber bioreactors	FiberCell	HSCs, Placental MSCs	Specific surface marker expression	1×10^6 cells extracted every 2–7 days	[51,52]
		Quantum Cell Expansion system	BM-derived MSCs	Better colony formation than flask cultures; specific tri-lineage potential, expression of CD73, CD90, and CD105	6.6×10^8 cells from 25 mL BM in 21 days of culture	[53]
		Quantum Cell Expansion system coated with fibronectin	MSCs	Specific tri-lineage potential; extended surface maker phenotype	1×10^8 cells from 18.8 to 28.6 mL BM in 5 days	[54]
	Perfusion bioreactor system	Non-woven PET fibrous matrices	MSCs	Maintain potential to differentiate into adipogenic and osteoblastic lineages	> 50-fold expansion in 40 days	[55]
Microcarriers	Nonporous	Cytodex 1/cross-linked dextran matrix	MSCs	Maintain potential to differentiate into osteogenic and chondrogenic lineages	Proliferation rate varies among donors	[58]
		Cytodex 3/cross-linked dextran matrix, serum-free medium	mESCs	Maintain pluripotency	193-fold expansion in 5 days	[59]
		Synthemax II/PS	MSCs	Retain normal hMSC phenotype, karyotype, and stemness	> 1000-fold expansion in 40 days	[60]
	Porous	CultiSpher-S/cross-linked gelatin matrix, serum-free medium	ESCs	Maintain pluripotency	439-fold expansion of ESCs in 6 days	[59]
Microcarriers in bioreactors		CultiSpher-S, spinner flask	MSCs	Retain multilineage differentiation potential and specific surface markers after several passages	1000-fold expansion in 30 days	[57]
		CultiSpher-S, single-use bioreactor systems	MSCs	Remain mesodermal differentiation potential and express specific cell surface markers	~15-fold expansion in 7 days	[62]
		DE-53, spinner flask	hiPSCs	Maintain pluripotency and possess normal karyotype over 10 passages; can differentiate to neural hNPCs with 78–85% efficiency	20-fold expansion in 7 days	[63]
3D culture systems		Vitronectin-derived peptide	hNPCs	Maintain specific morphology and stemness; express hNPC markers	5×10^5-fold expansion after 10 passages	[69]
		Biomimetic natural antigen-presenting cell scaffolds	T cells	Can realize antigen-specific expansion of T-cells; present similar efficacy in xenograft lymphoma model.	2- to 10-fold greater than commercial cell expansion beads (Dynabeads)	[77]
		Electrospun PCL scaffold	hNPCs	Not mentioned	~5-fold expansion in 3 days	[71]
		Electrospun PS scaffolds, xeno-free medium	hiPSCs	Remain pluripotency for up to 10 passages	~9-fold expansion in 6 days of culture	[76]

challenge. During conventional harvesting procedures, cells are detached from their substrates by treatment with recombinant proteases, which can cause irreversible damage to cell membrane proteins and receptors [78]. Thermoresponsive systems have been developed to address the drawbacks of enzymatic detachment. These polymer-based systems allow cells to be harvested without proteases by altering cell adhesion using the reversible transition of thermoresponsive polymers from hydrophilic to hydrophobic when temperature is reduced below their LCST [79]. The most commonly used thermoresponsive material is PNiPAAm. Numerous cell types including human corneal endothelial cells [80], muscle cells [81], human ADSCs, and ESCs [82] have been harvested using PNiPAAm or a NiPAAm copolymer fabricated using plasma immobilization, electron beam polymerization, plasma polymerization, or RAFT polymerization. Schmaljohann et al. fabricated PEG-grafted NiPAAm thin films using low-pressure argon plasma, and detached adherent fibroblasts by reducing the temperature from 37 °C to 32–35 °C. The harvested cells demonstrated the desired proliferation and adhesion properties [83].

There have been extensive efforts to optimize thermoresponsive materials for specific cells with different adhesive properties. Akiyama et al. modified the thickness of grafted PNiPAAm layers using electron beam polymerization and found that ~20 nm is optimal for endothelial cell adhesion and detachment [84]. Takahashi et al. optimized PNiPAAm graft density and chain length to tailor the thermoresponsive material to specific cell types [85]. A stiffness-controlled thermoresponsive hydrogel was synthesized by crosslinking NiPAAm and PEG. Fibroblasts cultured on this hydrogel were harvested and maintained their mechanical characteristics, which are important for *in vivo* wound healing applications [86]. A hybrid polydopamine/PNiPPAm film demonstrated high efficiency cargo delivery and cell harvesting while retaining cell viability. Various cell types including difficult-to-transfect mouse embryonic fibroblasts, HUVECs, and mouse DCs can be edited and harvested using this method [87]. Thermoresponsive materials with advanced structures and specialized properties have also been developed. A nano-brush surface composed of three copolymers was developed using RAFT polymerization [82] and enabled continuous harvesting of hESCs and hiPSCs while maintaining cell pluripotency [88]. Tang et al. used comb-type grafted PNiPAAm hydrogels, which contain PNiPAAm graft chains with freely mobile ends, to achieve an increase in the rate of detachment of bovine aortic endothelial cell sheets [89]. From tuning LCST via copolymerization to fabricating nanostructures, current research on thermoresponsive materials is focused on specialized functions and higher efficiency cell harvesting. Increasingly sophisticated thermoresponsive biomaterial structures will meet the demands of scalable cell harvest (Fig. 3).

Electroresponsive and photoresponsive systems have also been developed for harvesting cells. Electroresponsive systems mediate cell attachment and release through an electrical trigger. Ligands tethered to electroactive self-assembled monolayers are released when electroactive molecules are oxidized by an applied electrical potential [90]. Electrically switched cell adhesion or detachment can be achieved using appropriate peptide ligands. For example, the RGD-containing oligopeptides CGRGDS and CCRRGDWLC were used to harvest fibroblasts by peptide desorption from the substrate under an electrical stimulus [90]. Photoresponsive systems allow cell harvesting by switching substrate wettability between hydrophilic and hydrophobic under light illumination. The metal oxides TiO_2 and SiO_2 were used to harvest cell sheets while retaining cell viability [91]. Cheng et al. immobilized RGD onto TiO_2 nanodot films and achieved cell adhesion and proliferation, short detachment time, and a high level of pan-cadherin expression [92].

Other types of responsive systems for harvesting cells gently include pH-responsive systems [93] and magnetic systems [94]. pH-responsive systems are limited to certain cell types because most cells perform normal functions in a small pH range (typically 6.8–7.4). For cell attachment and release using magnetic force, target cells are labeled with magnetic nanoparticles, a requirement which may limit the application of this approach to cell therapy [95].

6. Cryopreservation, banking, and shipping

After harvest, therapeutic cells are supplied as off-the-shelf products for clinical applications, requiring cryopreservation, storage, and shipment. These procedures must all be carried out under strict GMP-compliant conditions to guarantee product safety and efficacy.

During cryopreservation, cryopreservation media is used to prevent freeze-induced cell damage. DMSO is the most commonly used cryoprotectant, but is potentially toxic to therapeutic cells [96]. Thus, alternative methods including using serum-free cryoprotectants [97], reduced DMSO concentration [98], and computer-controlled multistep slow freezing have been developed [99]. Another approach is to use biomaterials that protect cells during freezing. For example, emulsion-based carriers with small drops of alginate (internal phase) embedded in larger drops of light liquid paraffin (membrane phase) were used to encapsulate cells [100]. The encapsulated cells showed high viability (> 90%) and nearly the same doubling time as cells before cryopreservation. Microfibers have also been developed for cell encapsulation. Lu et al. fabricated alginate-chitin fibers using interfacial electrostatic interactions. The repeating units of this polymer are similar to those in glycosaminoglycans, which have proven effective in regulating cell adhesion and self-renewal [101]. Cryopreservation of hPSC cultured in this alginate-chitin microfiber resulted in > 17-fold greater cell viability than cryopreservation of hPSC cultured on planar Matrigel [102]. A cryopreservation system consisting of a supramolecular gel constructed using self-assembly of small-molecular-weight gelators reduced cryopreservation-associated neural cell damage [103]. This gel system confines the growth of ice crystals, thus reducing the freezing point of the cryopreservation system and reducing cell damage during thawing, leading to improved cell viability and function.

After cryopreservation, cell products are stored for banking and/or distribution. As cell therapies are globalized, it is essential to establish effective cell banks with standardized donor registries and to regulate product labeling for efficient operation of the supply chain. hESCs are an ideal source for cell transplantation, but differentiated HLA types may cause graft rejection. Creating a hESC bank with known HLA types can help overcome this obstacle [104]. iPSCs, which are induced from adult somatic cells, reduce ethical concerns, circumvent immunologic rejection, and offer unlimited sources for stem cell therapy [105]. Shinya Yamanaka at Kyoto University plans to create an iPS cell bank with a standardized iPSC array for cell therapy purposes that matches 80% of Japan's population by 2020 [106]. In Europe, the EBiSC has been working on establishing a standardized framework for hiPSC since 2014. EBiSC works with academic and industrial partners to advance the basic and translational usage of hiPSCs [107]. BioOutsource has expanded its services to manufacturing and banking cells, and provides reliable cell banks for producing quality-controlled biological products [108]. Saetersmoen et al. have proposed that engineered iPSC-derived NK cell therapy will provide next-generation immunotherapy with enhanced persistence, homing, and functional potential, thus improving off-the-shelf cell therapy [109].

Shipping cryopreserved cell products to clinics while maintaining quality control, cell viability, and biofunctions during transfer presents another critical challenge. Current shipping practices have been adapted from the blood bank industry and organ transfer protocols [110]. Adult stem cells cryopreserved using slow freezing methods (< 1 °C/min) are shipped on dry ice, since vitrified cells must be transported below the de-vitrification temperature [111]. Recently, attempts have been made to transiently store living cells under a more ambient environment. For example, human ADSCs encapsulated in 1.2% calcium alginate can maintain viability for up to 72 h at a hypothermic temperature (4°C–23 °C) [112]. Spheroidal hMSCs can survive under ambient conditions much longer than conventional monolayer culture, with > 90% viability after 7 days [113]. These

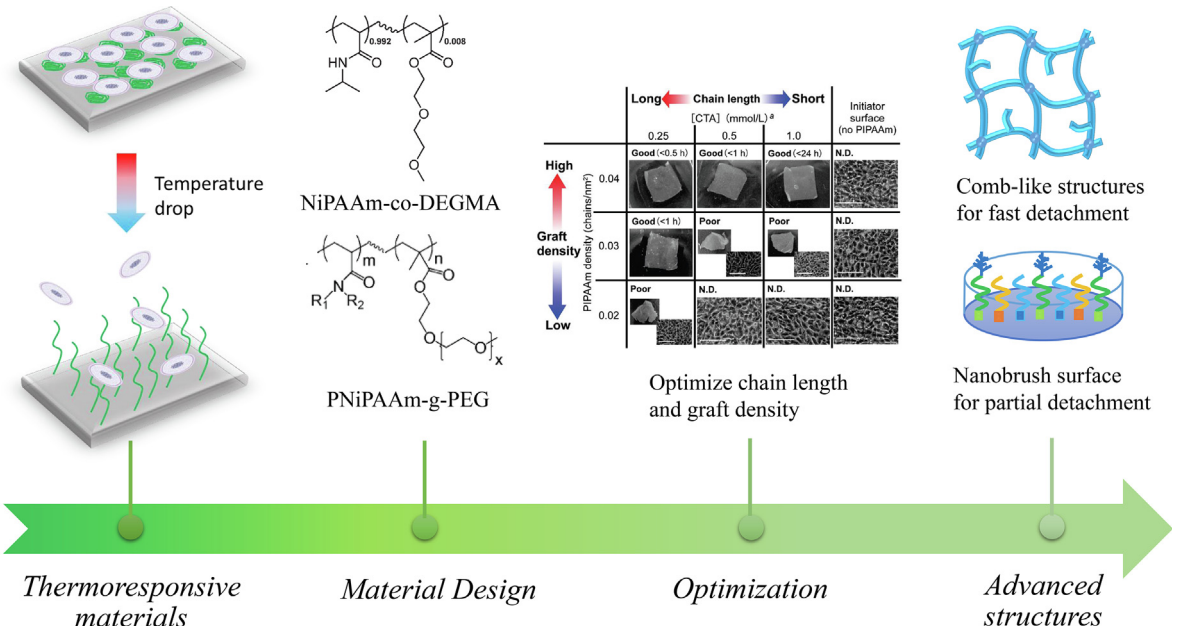

Fig. 3. Thermoresponsive materials for harvesting cells without cell damage. Thermoresponsive materials release cells when temperature is reduced. These materials are designed using copolymers to tune the LCST or other physical properties. Adapted with permission from Wiley Online Library: [JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART A], copyright (2006), and from (Biomacromolecules). Copyright (2003) American Chemical Society [80,83]. The PNiPAAm chain length is altered to optimize cell attachment and detachment. Adapted with permission from (Biomacromolecules). Copyright (2010) American Chemical Society [85]. Cutting-edge research is focused on developing advanced material structures with specialized functions.

advancements in biomaterial science may allow development of improved methods for regulation and standardization of cell storage and transportation.

7. Automation and real-time monitoring of therapeutic cell production

Therapeutic cells are living products that are highly fragile and easily modified. Therapeutic cell production is a complex process with multiple steps from isolation to cryopreservation, each with specialized requirements, in-process control, and quality control. To meet the rigorous criteria required for GMP-compliance bioproduction, automation and standardization are crucial for avoiding flaws in conventional manual cell culture processes, for reducing human error, and for increasing production quality and efficiency [114].

Commercial devices are available that perform partial cell culture procedures with minimal human labor. For example, the COBE®2991 Cell Processor (Terumo BCT, USA) enables automated washing and concentrating of cell components, and high quality isolation and purification of red blood cells, leukocytes, mononuclear cells, and islet cells [115]. G-Rex® (Wilson Wolf, USA) is a powerful cell production platform that provides a controlled culture environment and minimizes the complexity of downstream processing. G-Rex was created for the production of immune cells such as T cells, NK cells, and hematopoietic cells [116] (Fig. 4A). The Xuri W5 system (GE Healthcare) (Fig. 4B) achieves automated control of temperature, dissolved oxygen, and pH during cell culture, which increases the efficiency of cell expansion. The Xuri W25 system (which is based on the Xuri W5 system) (Fig. 4C) has new functions including long-range control and regulation [117]. More recent developments have focused on integrating multiple culture platforms, simplifying the production process, and improving automation. The CliniMACS Prodigy system (Miltenyi Biotec) is an integrated platform which automates the entire cell production process from cell preparation, enrichment, transduction, plasma harvesting, magnetic separation, and cell cultivation to formulation of the final product, using a GMP-compliant one-off tubing set (Fig. 4D) [118]. In addition to reducing human error, lowering cost, and reducing labor, some automatic platforms are equipped with real-time monitoring and adjustment modules which help guarantee the quality, safety, and consistency of cell products. For example, the Scinus cell expansion system (Scinus, Holland) automatically controls temperature, dissolved oxygen concentration, and pH, and even adds microcarriers during culture.

Current automated cell manufacturing systems are used for commercial clinical cell amplification. The X-series™ (ThermoGenesis Corp.) was launched in 2018 and received wide attention for its ability to adapt to the requirements of bioproduct supply chains with flexible cell manipulation modules and automatic control. CAR-TXpress™ is another multi-device platform that provides solutions for CAR T- and CAR NK-cell therapies (Fig. 4E) [119]. UniCAR-Therapy Biomedicine Technology Company (Shanghai, China) is collaborating with Terumo BCT to automate cell manufacturing processes for CAR T-cell therapies [120].

Although biomaterials are not yet commonly involved in commercial automation systems for cell therapy, tissue engineering products of automation devices have demonstrated the promise of biomaterials. Using ADAPT® technology, a flexible collagen bioscaffold called CardioCel® (Admedus) was produced to repair congenital heart deformities or other heart defects. With excellent biocompatibility, these durable scaffolds incorporate into normal heart tissue without calcification for 9 years after implantation [121]. Heart patches with embedded stem cells made using automated methods are now moving to the clinic [122].

8. Optimizing the microenvironment for cell production

Biomaterials have been widely used to create biomimetic microenvironments that provide structural support for cells [123,124]. Biomaterials can also promote cell attachment, proliferation, and differentiation through biochemical and biophysical cues [125,126]. Although many biomaterials-based systems are still in preclinical studies, biomaterials have shown great potential for use in large-scale cell production [127]. With a biomimetic microenvironment and appropriate inductive cues, cells proliferate and retain function with less unwanted differentiation during cell amplification.

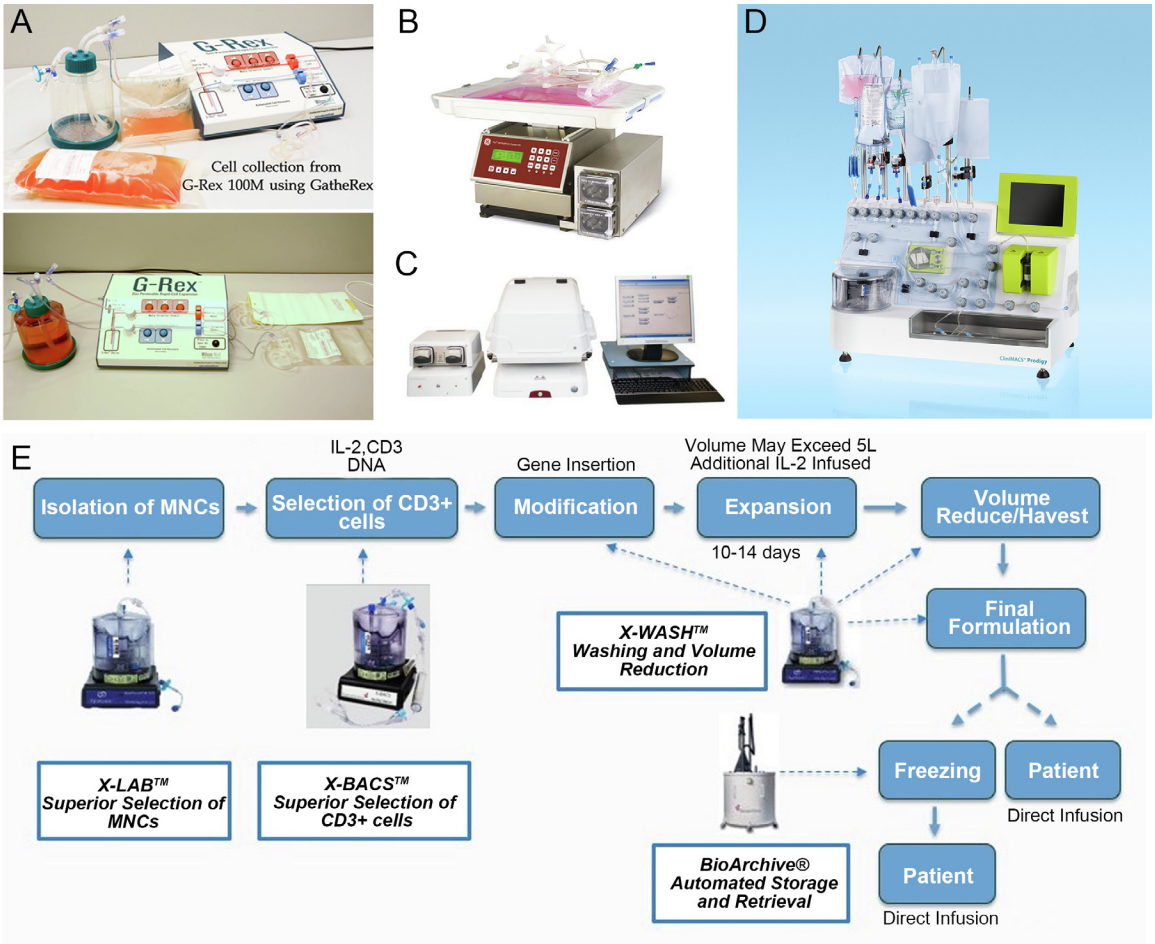

Fig. 4. Automated systems for large-scale cell production. A) The G-Rex® (Wilson Wolf, USA) system is used for automatic cell manipulation [116]. B) The Xuri W5 system, and C) improved Xuri W25 system with long-range control and regulatory functions [117]. D) The CliniMACS Prodigy system (Miltenyi Biotec) can manage the entire process of cell production [118]. E) Process schematic of CAR T-cell manufacturing using the multi-device platform CAR-TXpress™ (ThermoGenesis Corp.) [119].

Biomaterials can be used to present natural biochemical cues such as ligands that typically connect cells and ECM, and to provide soluble factors. Biomaterials are versatile and can be modified to perform a wide range of functions. Modification approaches include immobilization of peptides or growth factors, encapsulation of drugs or genes, surface coating, and co-culture of support cells. Polymers tethered with oligopeptides such as RGD have been shown to improve the adhesion, migration, and proliferation properties of fibroblasts and hMSCs [128,129]. More complex molecules with specific functions, such as bFGF [130] and EGF [131], have been tethered to coiled-coil fusion proteins to promote the proliferation of HUVECs and MSCs (Fig. 5A). Growth factors not only promote proliferation, but also help maintain stemness during stem cell expansion. Mahadik et al. reported that PEG-functionalized SCF can be tethered to methacrylamide-functionalized gelatin and retain its native bioactivity for 7 days [132]. Matrix-immobilized SCF showed better effects on lineage specification than soluble SCF. Biomaterials also serve as delivery vehicles that release high molecular weight components such as drugs or genes in a spatially- and/or temporally-controlled manner [133]. For example, an antibiotic and anti-inflammatory drug was encapsulated in a multi-layer structure composed of dextran sulfate and gelatin to achieve controlled drug release during neuron cell culture [134]. A bottom-up strategy was developed to synthesize drug-loaded inorganic nanomaterials composed of antimicrobial drugs and silica, which showed enhanced drug loading capacity and long-term drug release (Fig. 5B) [135]. Because cells interact only with the surface of nonporous biomaterials, surface coating is an effective and economical strategy to synthesize biomaterials. Cell behaviors regulated by functional coatings include collagen-facilitated HUVEC attachment [136], laminin-enhanced osteoblast-like cell adhesion [137], and chitosan-modulated inflammation by activating macrophages and DCs [138]. Growth factors such as BMP-2 and PDGF-BB [139], and drugs such as minocycline hydrochloride [134], can also be encapsulated in coating materials to improve cell functions (Fig. 5C). Target cell growth can also be promoted by using paracrine stimulation from co-culturing multiple cell types in specially structured materials (Fig. 5D) [140–142]. Andrejecsk et al. found that functional paracrine signals enhanced the functions of target cells when distributing target cells and particles embedded with supporting cells into a matrix [143].

In addition to biochemical cues, biophysical cues incorporated into biomaterials can also regulate cell adhesion, viability, proliferation, and differentiation. These biophysical cues include nanofeatures, microstructure properties such as porosity, hydrophilicity, and injectability, and macro-mechanical properties such as mechanical stability and stiffness. Unique nanostructures on the biomaterial surface including groove shape [144], crates [145], needles [146] and pores [147], can modulate cell behavior (Fig. 5E). Nanopatterns can be generated on the surface of bioceramics by using electron beam lithography [144], nanoparticle sandblasting [146], coating and sintering of nanoparticles [147], or laser irradiation [148]. Biocompatible nanomaterials such as graphene have been used as substrates to promote osteoblast cell proliferation and to induce osteogenic differentiation of MSCs [149,150].

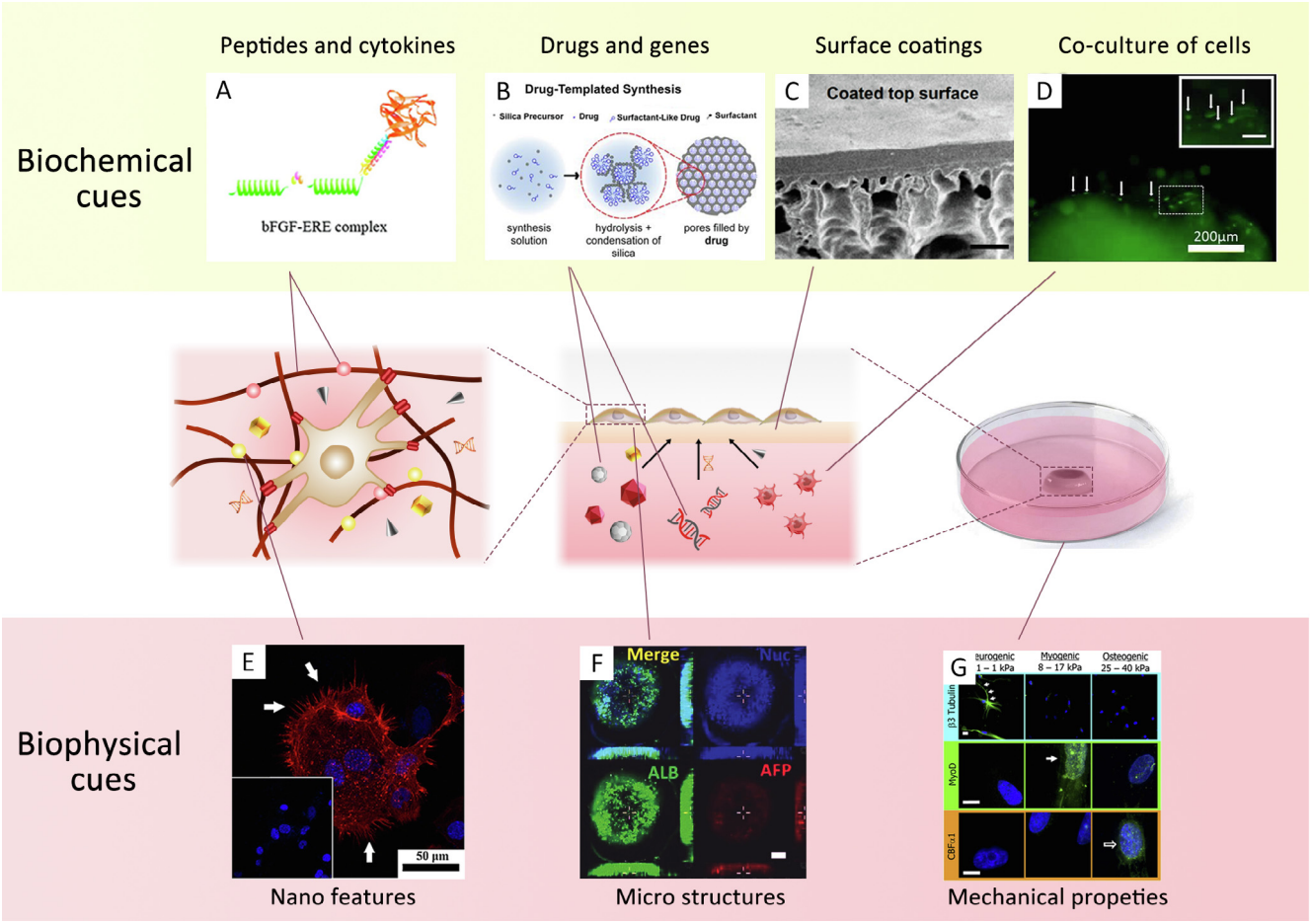

Fig. 5. Biomaterial-assisted microenvironment optimization can improve cell functions during proliferation. A) The structure of bFGF-tethered fusion proteins. Adapted with permission from (Bioconjugate Chemistry). Copyright (2011) American Chemical Society [130]. B) Schematic of drug-templated synthesis involving self-assembly of an antimicrobial drug and silica. Adapted with permission from Nature Publishing Group: [Scientific Reports], open-access (2018) [135]. C) A nano-layer coating composed of BMP-2 and PDGF-BB growth factors on a porous PLGA membrane. Adapted with permission from the National Academy of Sciences: [PNAS], copyright (2014) [139]. Scale bar is 2 μm. D) CD31 staining demonstrating HUVEC attachment and sprouting on a collagen coating layer. Adapted with permission from Wiley Online Library: [Biotechnology & Bioengineering], copyright (2012) [140]. E) Fluorescence images of osteoblast-like cells cultured on plate-like nanostructures; arrows indicate filopodia. Adapted with permission from Elsevier: [Colloids and Surfaces B: Biointerfaces], copyright (2016) [145]. F) Immunofluorescence staining of hESC-derived micro-livers. Adapted with permission from Wiley Online Library: [Small], copyright (2014) [158]. G) MSC differentiation influenced by matrix stiffness. Adapted with permission from Elsevier: [Cell], copyright (2006) [168].

Well-aligned nanotubes have been used to aid in the proliferation of neurons [151] and fibroblasts [152] with a particular orientation. As for micro-scale properties, open pores are essential for exchange of molecules throughout the biomaterial, which can affect cell growth kinetics [153]. Porosity can modulate cell adhesion, migration, proliferation, and differentiation because pore size, shape, density, and distribution impact the availability of ligands for cell adhesion and space for cell movement [154,155]. Modification of PLGA surface micro/nanostructure can alter the surface hydrophobicity to enhance MSC adhesion, elongation, proliferation, and osteogenic differentiation [156]. Mastoid microstructures with periodic spacing on polyurethane have shown altered hydrophobicity and improved hemocompatibility, thus reducing platelet adhesion and facilitating vascular endothelial cell culture [157]. Moreover, micro-stencil arrays have been developed to enable uninterrupted differentiation and proliferation of hESC-derived cells and to generate multilayered colonies that are composed of more mature and homogenous hepatocyte-like cells (Fig. 5F) [158]. Injectable biomaterials such as thermosensitive hydrogels [159], shape-memory scaffolds [160], microspheres [161], and layer-by-layer self-assembly spheroids [162], have been used for *in vivo* transplantation in addition to *in vitro* target cell expansion. These injectable biomaterials can increase cell viability and functions, facilitate tissue formation and remodeling, and potentially improve tissue repair and regeneration. Biomaterials must be sufficiently stable to support long-term culture of target cells [163]. Muller et al. reported that micro-breaks caused by distortion during biomaterial degradation can lead to cell death [164]. Because many biomaterials are biodegradable, their stability inevitably decreases during culture. The degradation speed may be in accordance with the speed of ECM production, which is needed to provide adequate space and mechanical strength for cell migration and proliferation [165]. Stiffness is another macro biophysical property that can regulate cell behaviors, as cells can sense resistance and mechanical signals from the matrix via the cytoskeleton [166]. Polydimethylsiloxane/Al_2O_3 composite substrates with tunable stiffness have been shown to regulate endothelial cell adhesion, morphology, and proliferation [167]. Stem cell differentiation can also be influenced by biomaterial stiffness. MSCs can be induced into neural (0.1–1 kPa), myogenic (8–17 kPa), or osteogenic (25–40 kPa) lineages using different biomaterial stiffnesses (Fig. 5G) [168]. Other cellular functions such as B cell activation and proliferation, T cell independent antibody response [169], or VEGF secretion from smooth muscle cells [170], can also be influenced by biomaterial stiffness.

Biomaterials can provide a biomimetic microenvironment during large-scale cell production, as described above. Chemical modification

Table 3
Summary of microenvironment optimization methods and effects on cells.

Microenvironment optimization types		Biomaterials/co-culture cells/specific features	Effects on cell functions	Cell types, references
Biochemical cues	Peptides and cytokines	RGD	Improve cell adhesion, migration, and proliferation	hMSCs [128] Fibroblasts [129]
		bFGF	Facilitate cell proliferation	HUVEC [130]
		EGF	Facilitate cell proliferation	MSCs [131]
		PEG-functionalized SCF	Facilitate cell proliferation; improve selectivity for maintaining HSCs	HSCs [132]
	Drugs and genes	Multilayer structure consisting of dextran sulfate and gelatin	Control drug release	[134]
		Co-assembly of antimicrobial drugs and silica	High drug loading; long-term diffusion drug release	[135]
	Surface coatings	Collagen	Facilitate cell attachment	HUVEC [136]
		Laminin	Enhance cell adhesion	Osteoblast-like cells [137]
		Chitosan	Modulate inflammation	Macrophages, DCs [138]
	Co-culture of cells	Alginate/pericytes	Simplify the isolation of target cells	HUVEC [143]
Biophysical cues	Nanofeatures	Grooves	Regulate selective cell migration	Mesenchymal cells [144]
		Crates	Modulate cell morphology, differentiation, proliferation and mineralization	Osteoblast-like cells [145]
		Needles	Enhance cell proliferation and bone formation	Osteoblast-like cells [146]
		Pores	Influence cell seeding efficiency and proliferation	Bone marrow stromal cells [147]
		Graphenes	Promote cell proliferation	Osteoblast cells [150]
			Induce cell differentiation	MSCs [149]
		Nanotubes	Aid cell proliferation	Neurons [151], fibroblasts [152]
	Microstructures	Micropores	Affect exchange of molecules	Fibroblast cells [153]
		Porosity of biomaterials	Modulate cell adhesion, migration, proliferation, and differentiation	MSCs, osteoblasts [154] ADSCs [155]
		Hydrophobicity of biomaterials	Enhance MSC adhesion, elongation, proliferation, and osteogenic differentiation	MSCs [156]
			Reduce platelet adhesion and improve suitability	Vascular endothelial cells [157]
		Micro-stencil arrays	Enable uninterrupted differentiation and proliferation	hESCs [158]
	Mechanical properties	Degradation rate	Provide adequate space and mechanical strength for cell migration and proliferation	[165]
		Stiffness	Regulate endothelial cell adhesion, morphology, and proliferation	Endothelial cells [167]
			Regulate cell differentiation	MSCs [168]
			Activation and independent antibody response of immune cells	B cells, T cells [169]
			Regulate VEGF secretion	Smooth muscle cells [170]

through functional binding or surface coating can endow biomaterials with unique bioactive properties. Biomaterials can also serve as controlled release systems that regulate cell performance. The micro/nanoscale design of these materials can also have an important impact on cultured cells. Microenvironment optimization using biomaterials and their effects on cell functions are summarized in Table 3.

9. Clinically-relevant example: large-scale HSC production

HSCs play a critical role in the generation of blood cells such as erythrocytes and platelets and immune cells such as white blood cells. HSCs have extensive clinical applications in treating hematological malignancies and autoimmune diseases [171]. According to the World Marrow Donor Association, tens of thousands of new patients each year are diagnosed with leukemia and other blood-related diseases, with more than 50,000 patients receiving blood stem cell transplants each year [172]. Human HSCs are primarily found in the BM, and can also be isolated from UCB or peripheral blood [173]. Compared to peripheral blood, UCB is a more attractive source of HSCs because the cells have higher proliferative potential and reduced matching requirements [174]. Due to the tremendous clinical demand for HSCs, large-scale HSC production, microenvironment optimization, and computational-aided regulation of HSC culture conditions are being actively investigated. Standardization and quality control during HSC amplification as well as establishment of relevant policies and regulations must also be considered.

9.1. Large-scale HSC production

Hematopoietic cytokines have been used since the mid-1980s to amplify human HSCs. Gilmore et al. developed a combination of growth factors, *flt*-3 ligand, and thrombopoietin/*c-mpl* ligand to successfully amplify HSCs 90-fold in 16 weeks, a sufficient amplification for adult transplantation. Subsequent clinical phase I/II trials demonstrated the safety of this strategy [175]. In addition, small molecules such as pyrimidoindole derivatives [176], nicotinamide [177], aryl hydrocarbon receptor antagonists [178], and the RNA-binding protein Musashi-2 [179] have been identified as HSC expansion enhancers. A 330-fold expansion of UCB-derived HSCs was achieved using the aryl hydrocarbon receptor antagonist StemRegenin-1 [180]. Subsequent clinical transplantation into 17 patients demonstrated the feasibility and safety of this procedure [180]. Co-culture systems established with BM stromal cells [181], MSCs [182], endothelial cells [183], and fetal hepatocytes [184] have also shown promising results for enhancing HSC expansion. MSCs play an important role in regulating HSC behavior via cell-cell contacts and secretion of endogenous cytokines [180]. A phase I clinical trial in 31 patients demonstrated that co-culture with MSCs can significantly improve the engraftment of HSCs [185].

Various bioreactor systems have been employed for the massive amplification of HSCs using an engineering strategy for development and industrialization. Jaroscak et al. used the AastromReplicell System (Aastrom Biosciences, Inc., USA) to expand UCB-derived HSCs for a phase 1 clinical trial and verified the safety of the expanded cells (clinical trial number: NCT00498316) [186]. HSCs amplified using this production system facilitated functional hematopoietic recovery in two chronic myelogenous leukemia patients [187]. Other types of bioreactors (discussed in the mass production section) have also been used to amplify HSCs, with ongoing clinical trials to verify the safety and efficacy of these mass-produced HSCs [188,189].

9.2. Microenvironment optimization for HSC production

HSC fate is controlled by the surrounding BM microenvironment, particularly the hematopoietic niche, which is characterized by a specific local geometry and arrangement of stromal cells [190]. To construct a biomimetic microenvironment *in vitro*, biomaterials have been developed that imitate natural BM architecture and the matrix-based signaling network [191]. For example, 3D nano-scale PCL scaffolds coated with fibronectin have demonstrated greater HSC expansion with higher self-renewal properties than 2D culture conditions [192]. Ferreira et al. fabricated scaffolds with defined geometric parameters using four different biomaterials (PCL, PLGA, fibrin, and collagen), and found that $CD34^{+}$ cord blood cells cultured on fibrin reached the highest overall growth (3×10^7-fold expansion in 14 days) [193]. Encapsulating cells in hydrogels is another strategy for HSC production, because hydrogels can recapitulate the ECM and enable diffusion of soluble factors [194]. Natural polymers such as chitosan, gelatin, and HA/chitosan-gelatin hydrogel [195], and synthetic polymers such as polyacrylates [196] and poly(ethylene glycol) diacrylate [197], have been used to amplify HSCs.

9.3. Computational approaches to optimizing HSC culture conditions

Adjusting the combinations and concentrations of cytokines and/or chemicals to establish an ideal microenvironment and achieve the desired cell amplification *in vitro* is laborious and costly [198]. Emerging computationally-controlled processes have tremendous potential for use in optimizing cell culture conditions. Computational models based on cell signaling interactions were developed to predict how additional factors including SCF, thrombopoietin, and *flt*-3 ligand will impact the survival, duplication, and differentiation of HSCs after culturing for 8 days on a NANEX nanofiber surface (Arteriocyte, MA) [199]. Modeling of protein-protein interaction networks and functional module analysis were also performed to identify potential HOXB4/HOXC4 downstream effectors and elucidate regulatory mechanisms involved in HSC expansion [200].

Computational methods can contribute to bioreactor-based large-scale HSC expansion by reducing unwanted cell differentiation. Three feeding strategies were developed based on a fed-batch cultivation strategy and nutrient distribution analysis to optimize nutrient distribution and cell concentration in a microchannel perfusion bioreactor for HSC amplification while maintaining stemness [201]. Computational approaches are also being used to explore regulatory mechanisms during *in vivo* applications. Maclean et al. performed a computational analysis of 3D niche dynamics and HSC migration tracks, and showed that increased motility of HSCs during inflammation could help host animals better cope with deteriorating HSC niche microenvironments [202].

In conclusion, current approaches to amplify HSCs *in vitro* are based mainly on combining 3D scaffold/hydrogel structures with exogenous cytokines/small molecules or co-cultures with supporting cells to replicate the natural hematopoietic niche. To meet the demands of clinical applications, commercial bioreactors with automation and monitoring modules must perform quality-controlled large-scale expansion of HSCs in less time. To produce therapeutic cells with stringent quality control and to coordinate with subsequent preclinical trials, computational models can be used to predict cell proliferation, differentiation, and *in vivo* performance with high efficiency and accuracy.

10. Perspective

The entire process of therapeutic cell production—from cell isolation and gene modification to large-scale expansion, harvest, and the supply chain—has been advanced through the use of biomaterials. Biomaterials provide support for cell isolation, purification, attachment, and growth, and protect cells during cryopreservation and shipment. Biomaterials also regulate cell functions via specialized chemical modifications and nanostructures.

However, to keep pace with clinical demands and meet the requirements of cell production in a quality-controlled manner, challenges remain. First, natural biomaterials face problems such as batch differences and the potential influence of xenogenic components, which

can influence the stability and safety of the cell products [203]. These drawbacks of natural biomaterials may also impact the FDA approval process and slow down the clinical process. Second, biomaterials can provide biological and mechanical signals to cells that impact proliferation, migration, and differentiation. The mechanisms by which biomaterials regulate cell behavior require additional investigation [204]. A better understanding of the interactions of biomaterial with cells during cell production will help in the adoption of biomaterials in therapeutic cell culture processes. Third, determining which biomaterial is most appropriate for a certain cell type and more closely mimic the specific cell niche remains a challenge. Last, an important consideration when using biomaterials in therapeutic cell production is to ensure the purity of cell products by removing any residual biomaterial components.

Cell therapies are rapidly developing. Novel therapies like CAR T cells have progressed towards clinical applications, and research on stem cell therapies continues to accelerate and attract attention. Progress in cell dedifferentiation and transdifferentiation and the use of UCB will help overcome ethical issues related to cell sources. Genome modification will allow versatile cell engineering. However, it is still extremely important for governments, academia, and industry to work together to formulate professional standards and criteria for cell products. New GMP-compliant biomaterials should be developed and the production of these biomaterials should comply with strict standardization. The safety of any derivatives or embedded signaling molecules such as cytokines and functional peptides needs to be evaluated. The mechanisms by which biomaterials regulate cell behavior should continue to be investigated. A reliable evaluation system should be established to evaluate and monitor the impacts of biomaterials on the quality, safety, and efficacy of cell products. The increased use of biomaterials will undoubtedly improve therapeutic cell products and will accelerate related research and product development. Safer, more efficient, and more precise cell manipulation methods will provide desired target cells, which can then be amplified, stored, shipped, and banked for clinical use using reliable, automated, standardized methods, all leading to the more extensive application of large-scale, biomaterials-assisted cell production for clinical applications.

CRediT authorship contribution statement

Ruoyu Chen: Writing - original draft, Writing - review & editing, Visualization. **Ling Li:** Writing - review & editing. **Lu Feng:** Writing - original draft. **Yixue Luo:** Writing - original draft. **Mingen Xu:** Writing - original draft. **Kam W. Leong:** Writing - review & editing. **Rui Yao:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

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