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Concise Review: Fabrication, Customization, and Application of Cell Mimicking Microparticles in Stem Cell Science

NICHOLAS R. LABRIOLA [a], AHARON AZAGURY,[b] ROBERT GUTIERREZ,[a] EDITH MATHIOWITZ,[a,b,c] ERIC M. DARLING[a,b,c,d]

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[a]Center for Biomedical Engineering, [b]Department of Molecular Pharmacology, Physiology, and Biotechnology, [c]School of Engineering, [d]Department of Orthopaedics, Brown University, Providence, Rhode Island, USA

Correspondence: Eric M. Darling, Ph.D., Brown University, 175 Meeting Street, Box G-B397, Providence, Rhode Island 02912, USA. Telephone: 401-863-6807; e-mail: Eric_Darling@brown.edu

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ABSTRACT

Stem and non-stem cell behavior is heavily influenced by the surrounding microenvironment, which includes other cells, matrix, and potentially biomaterials. Researchers have been successful in developing scaffolds and encapsulation techniques to provide stem cells with mechanical, topographical, and chemical cues to selectively direct them toward a desired differentiation pathway. However, most of these systems fail to present truly physiological replications of the in vivo microenvironments that stem cells are typically exposed to in tissues. Thus, cell mimicking microparticles (CMMPs) have been developed to more accurately recapitulate the properties of surrounding cells while still offering ways to tailor what stimuli are presented. This nascent field holds the promise of reducing, or even eliminating, the need for live cells in select, regenerative medicine therapies, and diagnostic applications. Recent, CMMP-based studies show great promise for the technology, yet only reproduce a small subset of cellular characteristics from among those possible: size, morphology, topography, mechanical properties, surface molecules, and tailored chemical release to name the most prominent. This Review summarizes the strengths, weaknesses, and ideal applications of micro/nanoparticle fabrication and customization methods relevant to cell mimicking and provides an outlook on the future of this technology. Moving forward, researchers should seek to combine multiple techniques to yield CMMPs that replicate as many cellular characteristics as possible, with an emphasis on those that most strongly influence the desired therapeutic effects. The level of flexibility in customizing CMMP properties allows them to substitute for cells in a variety of regenerative medicine, drug delivery, and diagnostic systems. STEM CELLS TRANSLATIONAL MEDICINE *2018;7:232–240*

SIGNIFICANCE STATEMENT

This article discusses the various fabrication and customization methodologies capable of producing cell mimicking microparticles (CMMPs), as well as which of these techniques is optimal for particular applications or compatible with specific materials. Current and potential applications in tissue engineering/regenerative medicine, drug delivery, and diagnostic tools are described. Also included are expected timelines as to when such applications are likely to be adopted and suggestions on where future development of this technology should be focused for producing more accurate cell mimics. Readers should be able to easily identify the pros and cons of the various fabrication procedures as they relate to different applications, as well as how the customization methods can enhance CMMP-based experiments or therapies.

INTRODUCTION

Microparticles have long been used in research and clinical applications. Recently, research has focused on creating microparticles that resemble aspects of living cells, termed cell mimicking microparticles (CMMPs), to improve their performance in regenerative medicine, drug delivery, and basic research systems. CMMPs have been fabricated to mimic the mechanical, topographical, and morphological characteristics of cells, and can be further modified to recapitulate the surface coatings of cells or their release of biological compounds. These types of particles can serve as scaffolds and stimulants for use in three-dimensional (3D) culture systems, enabling increased control and directivity over stem cell

differentiation in more physiologically relevant morphologies. While two-dimensional (2D) mimicking strategies have had success [1, 2], tissue constructs organized as a 3D structure allow for more cell-cell contacts compared with monolayer culture, providing special advantages when using compliant materials that are known to influence stem cell differentiation [3–7]. Although several studies have used microparticles to investigate stem cell differentiation in 3D [8–13], their performance and integration with cultured constructs could be further improved by mimicking aspects of living cells. CMMPs are designed to simulate cell characteristics such as surface proteins, mechanical properties, morphology, size, and/or secreted factors, eliciting tissue regeneration responses similar to live-cell therapies [8, 13–17]. CMMPs with these characteristics have the potential to incorporate into 3D microtissue constructs, influence multi-cellular organization, and alter gene and protein expression to achieve moderate control of cell behavior and lineage-specific differentiation responses. Applications of CMMPs extend well beyond regenerative medicine and include drug delivery and diagnostic systems as well. Some modifications such as surface coating and mechanical property tuning can improve tissue-specific targeting and penetration of micro-to-nano-sized particles into tissues or cells for enhanced drug delivery or screening. More generally, CMMPs lend themselves to use as calibration and test particles in devices that manipulate, characterize, retain, or pass-through cells, as they more accurately replicate cellular adhesive and deformation behavior compared with unmodified, rigid particles.

While the subject of microparticles has been extensively reviewed elsewhere with regard to drug delivery and tissue engineering applications [18–20], this Concise Review will focus on the design, fabrication, and use of particles that mimic the properties of living cells, with special attention to their stem cell-related applications. To begin, a brief summary of CMMP history is provided, highlighting their more general applications in the field of regenerative medicine. Additional details include the applicable fabrication and customization techniques used prominently by researchers, as well as how these modifications lend themselves to regenerative medicine, drug delivery, and diagnostic applications in the context of cell mimicking strategies.

Current and Potential Applications of CMMPs

CMMPs can serve as tools for regenerative medicine/tissue engineering therapies, enhancing drug delivery, monitoring intratissue stresses and strains, and elucidating the behavior of cells in flow-based devices. These effects and capabilities are driven by the cell mimicking nature of the particles—in some cases this means presenting bioactive molecules to the local environment and in others adopting deformability characteristics similar to living cells. As a nascent offshoot of the more established field of biomimicry, the extent to which CMMPs can be applied is yet to be determined.

Regenerative Medicine

CMMPs have been proposed for diverse applications in regenerative medicine, including use as a means of alleviating hypoxia and improving homogeneity by improving the diffusion of gases and soluble growth factors, respectively, as a local microenvironment sensor, and as a unique scaffold to provide structural support with tethered bioactive molecules, soluble factors, and/or specific mechanical cues to neighboring cells to encourage processes like vascularization and stem cell differentiation [21, 22]. Studies focused on mimicking the surface characteristics of specific cell types to elicit regenerative responses have coated poly(lactic-co-glycolic acid) (PLGA) nanoparticles with cell membranes extracted from red blood cells [23, 24], platelets [25], bone marrow stem cells and smooth muscle cells [26], leukocytes [27], and even cancer cells [28] (Fig. 1). Building from this work, a recent article by Tang et al. described their success mimicking the surface proteins and secretome of cardiac stem cells by attaching portions of their plasma membranes to PLGA microparticles, as well as incorporating cell secreted proteins into the polymer network during the fabrication process [17]. This work revealed that CMMPs replicating just these two aspects of cardiac stem cells can yield tissue regeneration responses similar to living cardiac stem cells used to repair damage due to myocardial infarction.

Microparticles in general have been used to address key issues of 3D tissue constructs, such as limited diffusion caused by the lack of vasculature and formation of gap junctions at cell-cell contacts—an issue that can complicate the delivery of oxygen, nutrients, and chemical induction factors through the extracellular space of these constructs [8, 13]. Integrating cell-sized and larger particles, while not truly cell mimicking, can prevent gap junction formation and allow waste and nutrients to diffuse more easily through the entire construct. Another issue with 3D constructs is the observed radial heterogeneity in stem cell differentiation response, where osteogenesis occurs more readily in the center and outskirts of stem cell spheroids while most adipogenic differentiation has been reported just under the surface [3]. Incorporating microparticles into stem cell spheroids can result in more homogeneous differentiation responses throughout microtissue constructs [8]. Microparticles have also been doped with growth factors or drugs to controllably deliver these factors to stem cells that would otherwise be more isolated deep within the microtissues [10, 29]. Loading microparticles with this kind of cargo yielded improved differentiation responses and regenerative capacities compared with blank microparticles by providing more direct delivery of soluble factors [30–32]. Although drug-doped microparticles have demonstrated great promise, there remains the possibility of microparticles being sensed as foreign, in which case it may be advantageous to mimic the characteristics of neighboring cells to limit fibrous encapsulation and maintain the free release of loaded cargo.

Generally, researchers have been able to direct cell behavior by controlling external stimuli that dictate cellular adhesion, migration, proliferation, morphology, gene expression, and differentiation in 3D, biomimicking environments to produce tissue constructs for implantation or promote the regeneration of existing tissues [5, 6, 17, 33–39]. The recent development of CMMPs provides a new approach to delivering cues capable of directing stem cell fate while also addressing some of the limitations of current tissue engineering practices. CMMPs can be designed to match the size, morphology, surface coating/roughness, mechanical properties, and protein release profiles of living cells [16, 40–42]. These characteristics allow for passive and active incorporation into tissues and engineered constructs and can directly influence the behavior and biology of local environments. From a practical perspective, CMMPs are also compatible with fluorescent stains, making them an incredibly versatile tool for tissue engineering applications and general research [16]. CMMPs can be loaded with drugs or therapeutics and tuned to have specific

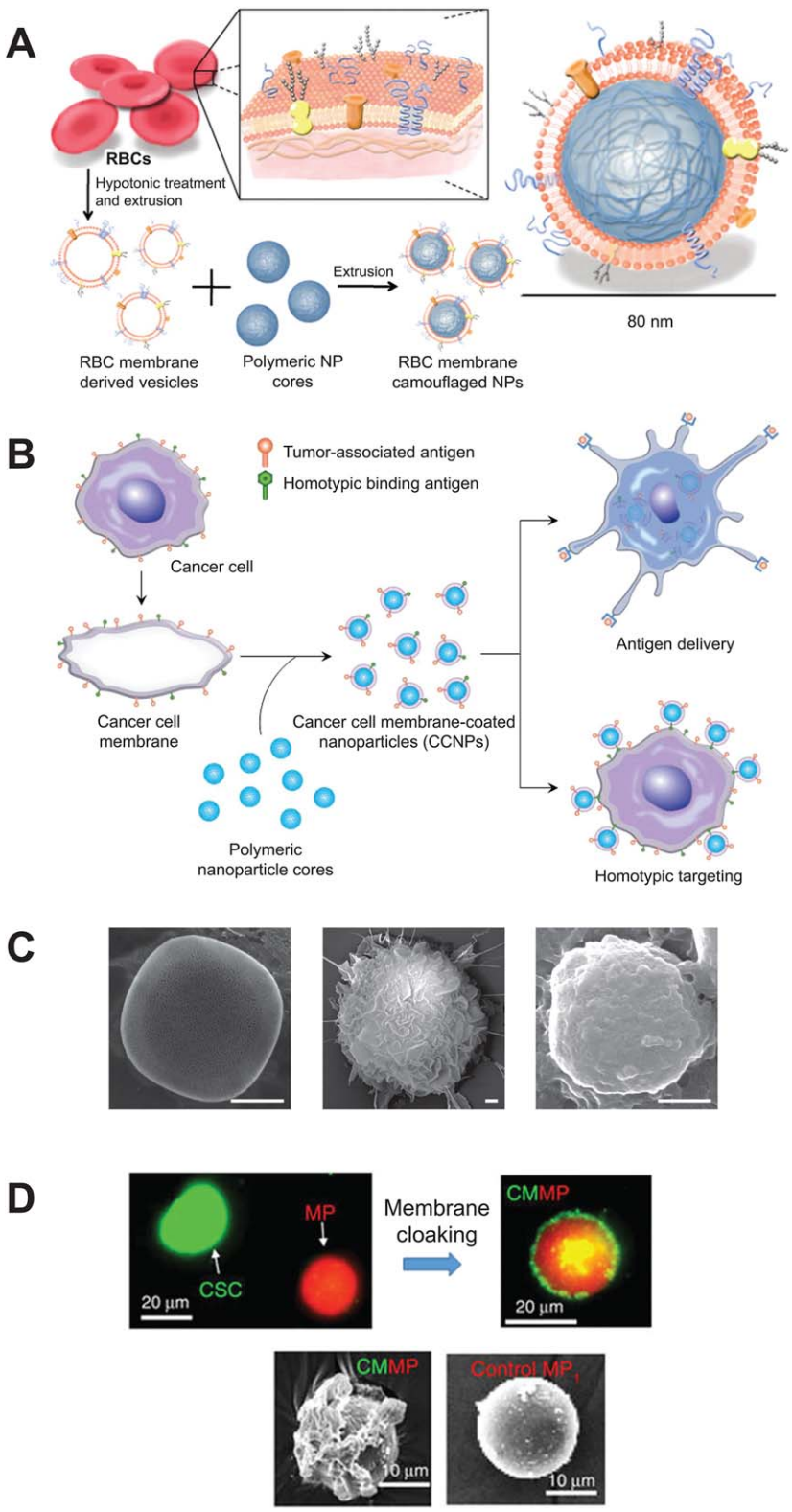

Figure 1. Membrane cloaking/ghosting/camouflage of polymer particles. NPs have been coated with **(A)** RBCs membranes to extend circulation time in the body (adapted from [23]) and **(B)** cancer cell membranes to both increase antigen delivery to dendritic cells and target source cancer cells (adapted from [28]). **(C):** SEM image of unmodified nanoparticle, leukocyte, and nanoparticle camouflaged with leukocyte membrane (adapted from [27]). This coating can be used to extend circulation time by avoiding uptake by the phagocyte system. Scale bar = 1 μm. **(D):** MPs (red) can also be cloaked with membrane fragments (adapted from [17]). Green fluorescent DiO-labeled CSCs are used to form the layered CMMP (red particle with green coat). Scale bar, 20 μm. SEM of control MP and cloaked CMMP showing the presence of CSC membrane fragments. Scale bar, 10 μm. Adapted and reproduced with permission. Abbreviations: CMMPs, cell mimicking microparticles; CSCs, cardiac stem cells; MPs, microparticles; NPs, nanoparticles; RBCs, red blood cells.

release profiles for administering treatments to damaged or diseased tissues via diffusion, post integration [15, 43, 44]. Compared with bulk biomaterials that encapsulate cells or rely on their infiltration into pores, neotissues composed only of cells and CMMPs allow for more natural formation of cell-cell and cell-CMMP contacts, making cell arrangement and interaction more dynamic than traditional scaffolds [16]. Recent innovations have allowed for the creation of CMMPs that can mimic the size and mechanical properties of stem cells for incorporation into self-assembled cell spheroids [16], as well as the size, shape, and stiffness of red blood cells to investigate how these properties influence movement through capillary-like channels [40, 43, 45]. Although early CMMPs have shown great promise for improving tissue-based therapies, their efficacy can potentially be enhanced by combining surface coatings, mechanics, drug loading, and morphological control of the particles.

Drug Delivery

Micro- and nano-sized particles have long been the primary approach for drug and gene delivery purposes [46], although only recently have properties such as complex surface coatings and mechanical properties been taken into consideration for improving aspects such as tissue-specific accumulation and circulation time. By loading microparticles with biological compounds, these systems can mimic the release profiles of cells and organs, although they lack the feedback mechanisms that living cells possess [15, 44]. The characteristics of the particles are integral to how an organism interacts with them. Particle size and morphology also play important roles in their tissue distribution [47]. For example, the number of spherical particles in a given tissue/organ will decrease monotonically as size increases; however, a disproportionate fraction of particles will always accumulate in the reticuloendothelial system organs [48, 49]. Discoidal particles have been observed to accumulate in most tissues to a greater extent than spherically, quasi-hemispherically, or cylindrically shaped particles. Also in regard to intracellular delivery, rod shaped particles have been reported to undergo increased phagocytosis when compared with spherical microparticles [50]. These alternative shapes are particularly relevant to CMMPs that replicate unique cell types like discoidal red blood cells. Particle size is also integrally related to the loading and encapsulation efficiency of drugs, with larger particles correlating with greater efficiency. As with any other particle-based drug delivery approach, chemically loaded CMMPs would typically exhibit a burst release of their cargo [51], although material and processing modifications can ameliorate this effect to achieve more controlled/sustained release [52]. The strategies currently used to accumulate drug delivering microparticles in a specific organ or area can also be applied to CMMPs [53]. These types of drug delivery systems are often intended for use in cancer treatment and target the diseased tissues through an enhanced permeability and retention effect, mainly through size-based mechanisms [54, 55]. The integration of various ligands on the surface of microparticles is another means of accomplishing targeted delivery and has been demonstrated with $\alpha_v\beta_3/\alpha_v\beta_5$ integrin-binding RGD peptides [56], as well as alendronate and aspartic acid peptides [57]. Another key component to consider for CMMP-based, drug delivery applications is circulation time. Researchers have been able to increase the circulation time of both polymeric and liposomal microparticles by adding polyethylene glycol (PEG) to the surface or altering the mechanical properties and size of the particles. The former approach is so widely used that it even has its own term: PEGylation [58]. The enhanced retention/circulation time is attributed to the fact that PEGylation reduces renal clearance [59], which in turn may affect cellular uptake and intracellular trafficking [60]. As this field continues to develop and innovate, researchers striving to increase circulation time by mimicking other circulatory cells will need to incorporate important features such as highly compliant mechanical properties and coatings that disguise particles as native cell types.

Diagnostic Tools

Potential applications of CMMPs extend well beyond regenerative medicine and drug delivery to use as calibration or test particles for flow cytometry and microfluidic devices, force measurement probes, and tools for toxicology screening, among other possibilities. Any system that involves cells could substitute CMMPs for preliminary testing purposes. Particle sizers, automated cell counters, flow cytometry, and fluorescence activated cell sorting (FACS) are common techniques used to analyze or sort cell populations through the detection of fluorescence or light scattering to determine either the presence of specific proteins/genes or the size and complexity of the cell/particle passing through an interrogation point [61]. These devices are a regular tool for the assessment of stem and other cell types. However, the polystyrene and latex particles used to calibrate these systems exhibit mechanical moduli 5–6 orders of magnitude higher than those of living cells, resulting in substantially different deformation behavior when flowing at high speeds in small channels [62, 63]. Mechanically matched CMMPs should behave more similarly to cells in regard to their locations in streamlines, deformation/elongation, and rotation in flow. These highly compliant particles should vastly improve the utility of forward and side scatter (FSC and SSC) measurements, providing a more accurate assessment of cell size in these ubiquitous devices. More generally, use of CMMPs as a stable, off-the-shelf substitute for cells in product testing could potentially save researchers (and companies) significant time and money normally devoted to maintaining and handling biohazardous cell cultures. Although CMMPs with stable, physiologically relevant, mechanical properties have been produced successfully [16], none to date have been fabricated in ways that incorporate internalized structures with the purpose of matching light diffraction caused by the cytoplasmic contents of living cells.

Microfluidic devices are another tool being developed to characterize and/or sort cells for high-throughput assessment of cell populations or the detection of rare cell types [64–66]. Such devices have potential applications in cancer/rare cell diagnostics, general research purposes, and cell-based medicine. The mechanophenotype of cells has been recognized as a powerful biomarker that correlates with the metastatic potential of cancer cells [67] and the lineage-specific differentiation potential of stem cells [68]. As such, microfluidic devices that can characterize and sort cell populations by their mechanical properties can be used for cancer diagnostics or to isolate subpopulations of stem cells with the greatest potential for the desired tissue type, potentially resulting in major improvements to current tissue engineering techniques that use more heterogeneous cell populations. Similarly to flow cytometry or FACS, calibration particles that more closely resemble the characteristics of cells would provide obvious advantages over nondeformable particles for modeling cellular behavior in these types of flow fields. CMMPs could substitute for cells during pilot work, optimization of flow rates, and

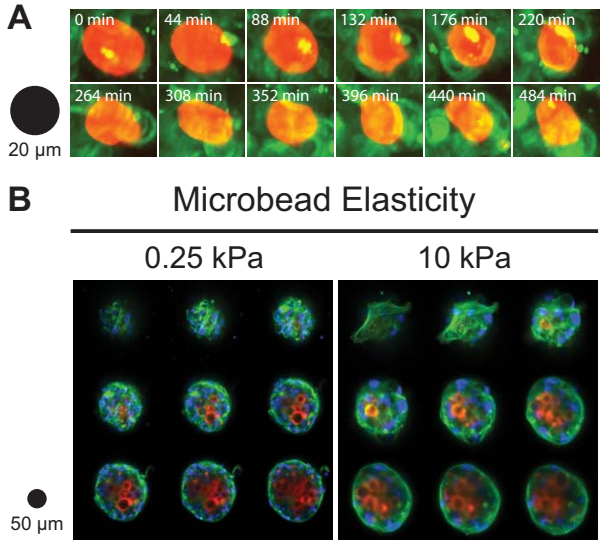

Figure 2. Cell mimicking microparticles (CMMPs) within self-assembled, stem cell spheroids can serve as force probes by monitoring their shape. **(A):** These three-dimensional projection images from the Darling Lab at Brown University illustrate how highly compliant, 0.25 kPa CMMPs (red) deform in response to the contractile and adhesive forces of surrounding cells (green). Theoretically, an accurate reporting of the in situ stresses could be calculated based on the known mechanical properties of the CMMPs and their deformation from an original, spherical shape. **(B):** These two montages of confocal images (∼60 μm thickness, 7 μm steps) demonstrate that both 0.25 kPa (left) and 10 kPa (right) microbeads (red) are shuttled to the center of cell spheroids when coated in collagen. Cell nuclei (blue) and actin cytoskeletal structures (green) were stained with 4′,6-diamidino-2-phenylindole (DAPI) and Alexa Fluor 488 phalloidin, respectively. Magnification: ×40.

determination of device accuracy and precision. Particles used for this purpose should be very stable and thus should not use biodegradable materials to minimize potential changes in mechanical properties and size.

Previous research by Labriola et al. has demonstrated that hyper-compliant CMMPs (<1 kPa) deform substantially within microtissue constructs in response to the contractile forces of surrounding cells (Fig. 2A) [16]. Since the material properties of CMMPs can be tightly controlled, it is feasible for them to be used as a tool for measuring intratissue forces that cells exert within 3D constructs. Such a tool can allow for researchers to probe changes in contractile forces generated by cells within microtissues resulting from changes in the lineage commitment of stem cells, metastatic potential of cancer cells, or changes in cadherin/integrin binding and cytoskeletal structures resulting from drug treatments. Not only would this technique provide force measurements from within complex microtissues, an advantage over other mechanical characterizing techniques such as atomic force microscopy, but these measurements could also be obtained from fluorescent images without the use of more expensive and complicated equipment. Complex morphologies would likely complicate the calculation of these forces, making simple, spherical particles advantageous for this type of application.

Additionally, CMMPs may be used as a tool for toxicology screening. CMMPs can be theoretically loaded with a drug of interest and delivered into microtissue constructs to test various doses or release profiles. This technique provides advantages over traditional toxicology experiments that use 2D culture systems or rely on diffusion of drugs/soluble factors through 3D tissue constructs by providing a high throughput platform that can provide more information on the localized effects of the cargo within the more physiologically relevant 3D microtissue constructs [3, 69]. Furthermore, the CMMPs can be fabricated to mimic smaller structures, such as bacterium or other pathogens, to study phagocytic uptake by cells or macrophages to determine the effects of drugs when delivered directly to the cytosol.

Fabrication Methods

A variety of fabrication methodologies exist for producing microparticles, and hence CMMPs, each with their own advantages and limitations (Table 1). Most of these fabrication techniques use polymers or fatty acids/amphiphilic materials (e.g., liposomes) to produce either homogenous spheres or core-shell structured microcapsules, respectively [70]. Self-assembly and phase separation are the driving mechanisms for many of these methods, including: solvent evaporation, emulsion polymerization and in situ/interfacial polymerization, salting-out, and phase inversion nanoencapsulation [71–74]. Highly monodisperse particles with custom-designed morphologies can be produced using Particle Replication In Non-wetting Templates (PRINT); however, templates need to be entirely redesigned to produce particles of different morphology or size, which can be expensive and time consuming [75, 76]. Another fabrication technique that allows for morphological control is layer-by-layer (LBL) deposition. This method involves depositing layers of a selected material on template seed particles that possess the desired morphology to produce shells that maintain the original, irregular shape [77, 78]. Once shell particles are obtained they can be porated and infiltrated with hydrogels to alter the material of the microparticles [79]. Microfluidic/capillary-based approaches can form highly monodisperse populations of microparticles but are less high-throughput by the nature of their setups [80, 81]. Beyond these general methodologies, there are also preparation techniques and self-assembly driven systems specific to liposomes that produce microparticles through: mechanical agitation (e.g., sonication, vortexing, micro fluidizers, French press, etc.), solvent replacement, detergent removal, size transformation, and fusion [82–86]. Emulsion droplet size is controlled, most simply, by adjusting the level of mechanical agitation during production or through filtering once the particles are formed.

Material Considerations

Multiple types of synthetic and natural materials have been used to generate CMMPs. A subset of materials fabricated as microparticles and used with stem cells include: PLGA, agarose, gelatin, chondroitin sulfate (CS), hyaluronic acid (HA), and polyacrylamide (PAAm) [8, 11, 16, 17, 87]. While all of these materials can be fabricated in ways that replicate cell properties in regard to size, protein coating, and topography, only a few are suitable for achieving physiologically relevant mechanical properties (i.e., Young's modulus < 10 kPa). Cell mimicking stiffness has been demonstrated using CS [88], HA [89], gelatin [90], agarose [91], and PAAm [16], while materials such as PLGA are orders of magnitude stiffer than cells, even when hybridized with more compliant materials [92]. Characteristics such as biodegradability and biocompatibility can be incorporated into all of these materials through chemical modification or copolymerization; however, the simplicity of these

Table 1. Advantages and disadvantages of common microparticle fabrication methods

Technique	Pros	Cons
Solvent evaporation	Scalable Easy to use Hydrophobic encapsulation	Uses organic solvents High polydispersity Only spherical particles No hydrophilic encapsulation
Emulsion polymerization and in situ/ interfacial polymerization	Scalable Easy to use Good compatibility with high compliance materials	Uses organic solvents Medium polydispersity Only spherical particles
Salting out	Hydrophobic/hydrophilic encapsulation	Can disturb sensitive biologics High polydispersity Only spherical particles Limited versatility
Phase inversion nanoencapsulation	Scalable Easy to use Hydrophilic encapsulation	Uses organic solvents Medium polydispersity Only spherical particles Requires large volumes
Particle replication in non-wetting templates (PRINT)	High monodispersity Morphological control High loading/encapsulation efficiencies	Low yields Difficult to scale process Relatively complex
Layer-by-layer (LBL)	Morphological control High loading/encapsulation efficiencies Hydrophilic/hydrophobic encapsulation	Low yields Difficult to scale process Relatively complex
Micro/capillary fluidics	Easy to use High monodispersity High loading/encapsulation efficiencies	Low yields Only spherical particles

chemistries depends on the molecular composition of the materials. Furthermore, special attention must be given to how the new material behaves mechanically, if this is a property that is being mimicked.

CMMP Customization

Polymer microparticles can be customized using well-established techniques to more closely mimic additional properties of cells, including: morphology, surface molecules, protein secretions, mechanical properties, and more. Particle morphology modification has recently become significantly easier to achieve. Previously, most (if not all) polymeric particles were spherical in shape since existing production methods typically used self-assembly and colloidal mechanisms, governed by the laws of thermodynamics. Now, the morphology of microparticles can be controlled through techniques that make use of templates, such as PRINT or LBL, through the careful design of microfluidic devices [80, 93], or through the physical modification of spherical microparticles produced by other methodologies [94, 95].

Surface molecules are a critically important cell characteristic that should be considered for CMMP coatings since they play a primary role in how the particle interacts with biological systems. From a delivery/homing standpoint, coatings can be used to extend circulation time (PEGylation) or allow targeting of specific cells/organs, for example, by the addition of tissue-specific membrane receptors [59, 96]. In a broader sense, coating with cell adhesion molecules will allow for a range of CMMP-cell and CMMP-material interactions that would otherwise not occur with an inert polymer [16]. Researchers can use this approach to investigate how specific integrins, cadherins, or other binding molecules influence the organization and movement of CMMPs within a cell-dense structure. This could be an important factor for controlling the dispersion of CMMPs in tissues since recent reports show a tendency for collagen-coated PAAm microparticles to aggregate in the center of stem cell spheroids. (Fig. 2B) [11, 16]. Alternatively, surface coatings can be passive elements whose function is only triggered by a change in the environment, for example, releasing active enzymes as the sacrificial outer layers degrade [97].

Another key characteristic of cells is their mechanical properties. While the vast majority of microparticles are made from rigid materials that are 5–6 orders of magnitude stiffer than a living cell, recent advances have demonstrated that hydrogel materials can be used to fabricate CMMPs exhibiting physiologically relevant sizes and elasticities (5–40 μm, 0.1–5 kPa, respectively), [16]. Substrate material stiffness, in both 2D and 3D culture systems, dramatically influences stem cell morphology, mechanical properties, and differentiation response [36]. Although adjusting the crosslinking density of a polymer is the most prevalent means of tuning microparticle mechanical properties [98, 99], other possibilities exist and are associated with specific fabrication methods. For example, microparticle stiffness can be adjusted for LBL by controlling how many layers are deposited [100], and techniques that generate core/shell structures can choose shell materials with defined elastic moduli [101, 102]. In general, most of these approaches are limited to use with high-modulus materials outside of the physiologically relevant range. The majority of published studies that modulate microparticle crosslinking do so to control the release rate of encapsulated drugs, rather than mimicking cellular properties [103]. In this nascent direction, hydrogel microparticles offer the best range of mechanical properties to achieve accurate mimics. While not compatible with all fabrication techniques, these materials provide unique advantages in the area of biomechanics compared with other, primarily solid materials. Apart from directing cell behavior or altering molecular release kinetics, the mechanical properties of microparticles have

also been shown to influence their uptake by cells and tissues as well as circulation and clearance time in an organism [104]. Stiffer particles exhibit increased uptake compared with their softer counterparts while the more compliant particles remain in circulation longer.

Through combining the various fabrication methods and customization techniques, ever more accurate CMMPs can be created. Ideally, these particles should be completely biocompatible while also mimicking the morphology and mechanical properties of the cells they are replicating. When needed, CMMPs should be designed to mimic the cellular release of proteins, steroids, growth factors, and other compounds to elicit desired biological responses. Interactions with neighboring cells can be encouraged by incorporating physiologically representative surface coatings and, depending on the specific application, CMMP degradation may be an important design factor to consider. For temporary use in the body, CMMPs should be biodegradable, lessening the chance of a negative, long-term response to any shed materials. Alternatively, use of CMMPs as calibration particles or cell substitutes for testing equipment would favor nondegrading materials that extend shelf-life and ease-of-handling.

Final modifications that can add versatility to CMMPs are fluorescent staining and nanoparticle incorporation. By adding a visual indicator, researchers are better able to track particle movement, interactions with cells, deformation, or assist with detection in various devices such as flow cytometers. Such dyes can be incorporated through covalent bonding, hydrogen bonding, intercalation, and so forth, making them compatible with many different polymer types and fabrication methodologies. Nanoparticle incorporation can serve a variety of purposes, including degradative release of drugs, light refraction, and magnetic control. This type of modification is not compatible with all fabrication approaches since the pre-formed nanoparticles are typically doped in during the formation phase of the microparticles. The ultimate function can be similar to a coated, solid particle; however, there is often more versatility in being able to add a variety of function-specific nanoparticles within a larger CMMP.

Conclusion

Although researchers in the nascent CMMP field have successfully mimicked a subset of cellular properties, they have yet to combine these characteristics in ways that produce CMMPs that can truly substitute for cells. By incorporating surface modifications, nanoscale topographies, bulk mechanical properties, and size restriction, CMMPs can be optimized for use in regenerative medicine or as replicas that can calibrate devices, deliver drugs, and measure forces. In vitro applications provide ample opportunity for the immediate use and study of CMMPs. Likewise, these novel particles can be used for early testing and troubleshooting of devices that normally would require biohazardous, live cells. CMMPs can also offer a new tool for basic research involving toxicology screening, drug/growth factor delivery, and force measurements within 3D microtissues. These applications would likely be early to mid-stage applications as they still involve in vitro experiments but seek to answer more complex mechanistic research questions. Late-stage uses of CMMPs would include more in vivo and clinical applications such as the delivery of drugs, growth factors, or topographical and mechanical signals to tissues within the body, or implanting microtissue constructs of stem cells whose fate has been optimally directed with the use of such particles. These types of applications will likely be implemented last as they will require the most accurate cell mimics to reduce immune responses, improve tissue integration, provide responsive biological feedback, and truly offer control over cell differentiation, which will require the combination of multiple customization techniques as well as extensive testing to elucidate side effects and obtain proper governmental approvals. CMMPs may be a useful tool for multiple facets of both research and clinical settings, but each specific application will require extensive customization to optimize CMMP performance in these unique systems. Future research should strive to replicate aspects of the cell that have yet to be synthetically recapitulated. Once these cell properties have been individually mimicked by CMMPs, combining the aforementioned fabrication and modification techniques should yield highly accurate, synthetic cell mimics that can be used in place of live cells for regenerative therapies or serve as a versatile research and diagnostic tool.

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Authors Contributions

N.R.L.: conception and design, literature review, manuscript writing; A.A. and R.G.: literature review, manuscript writing; E.M.: conception and design, financial support, manuscript writing; E.M.D.: conception and design, financial support, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

N.R.L. is an employee of MimicSphere, LLC, and has obtained a provisional patent on hypercompliant cell mimicking microparticles. E.M.D. is a founder and stockholder of MimicSphere, LLC, and an inventor of some of the patent-related material presented in this article. The other authors indicated no potential conflicts of interest.

Note Added in Proof

This article was published online on 9 January 2018. Minor edits have been made that do not affect data. This notice is included in the online and print versions to indicate that both have been corrected 29 January 2018.

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