Recent Progress in Nano-biotechnology: Compartmentalized Micro- and Nanoparticles via Electrohydrodynamic Co-jetting

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Compartmentalized particles enable co-presentation of dissimilar sets of properties, thereby offering a broad design space for multifunctional particles. Electrohydrodynamic co-jetting is a simple, yet versatile fabrication technique that can be used to prepare such multicompartmental particles and fibers. Processing conditions are summarized for co-jetting of aqueous and organic polymer solutions as well as nanoparticle suspensions. Because particles can comprise distinct polymers in different compartments, selective surface modification becomes possible. The latter can result in unidirectional interactions with cells or may pave new routes towards targeted drug delivery.

1. Introduction

Multifunctional particles with orthogonal sets of properties may lead to complex hierarchical assemblies needed for a diverse array of applications, such as photonic bandgap materials, multiplexed bioassays, vehicles for multiple therapeutic modalities, biological sensors, biohybrid materials or miniaturized energy storage and conversion devices.

Thus, nano- and microparticles may be considered fundamental design elements in a variety of research topics related to nano-biotechnology.[1] Recent progress in particle engineering has continuously increased versatility, tunability, and structural flexibility of colloidal particles.[2] This progress is, at least in part, based on the advent of a variety of fabrication techniques, which have been developed to precisely engineer nano- and microparticles with respect to shape, size, surface chemistry, and mechanical properties.[3,4] Moreover, control over internal (bulk) and external (surface) architectures are

quickly gaining importance as additional design parameters.[5] Once dissimilar materials can be introduced into independent compartments of the same particle, the term "multifunctionality" may take on an entirely new meaning.

2. Compartmentalization of Colloids

The ability to independently control individual compartments on the same particle allows for inclusion of multiple materials and material combinations with dissimilar sets of properties that can give rise to orthogonal properties (**Figure 1**). A good example would be a Janus particle with different types of surface chemistries present on different
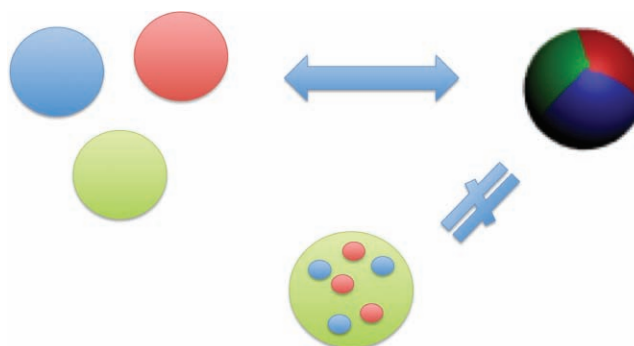
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Figure 1. Multicompartmental particles (right) can display the combined set of properties associated with the individual components. This architecture is distinctly different from geometries based on simple mixing of materials (bottom).

hemispheres, which then allow for selective modification of each compartment independent of the other. In this context, addressing individual compartments with orthogonal chemistries can result in "patchy" particles, which, in its own right, are of interest for self-assembly, photonic materials, or drug delivery.[6] A particle can thus exhibit bipolar characteristics, such as simultaneously being hydrophilic and hydrophobic. The bicompartmental architecture may also allow for release of multiple drugs with independent release kinetics (provided that each drug is loaded in another compartment), presentation of multiple ligands in defined surface patches, or combination of fully decoupled modalities for imaging and therapy. Further extension of the work with multicompartmental particles may be based on refined generations of multicompartmental particles with controlled sizes, shapes, or porosities. In principle, these design criteria can apply to the entire particle, i.e., changing the particle diameter from 100 nm to a few micrometers, or to individual compartments. In the latter case, particles may be designed that have large and small compartments, where the larger compartment may be loaded with a drug and the smaller compartment carries an imaging agent.

3. Microstructured Colloids

For Janus particles, a number of fabrication methods have been recently established, such as matrix embedding, Pickering emulsion polymerization, and glancing angle deposition.[7] In addition, direct UV writing[8] and maskless photopatterning methods[9] have been adopted for spatially selective functionalization of colloids.

Enhanced throughput is a major challenge in this area of particle preparation, although higher yields may be realized by taking advantage of the so-called "Pickering emulsions" for the preparation of Janus particles.[10] When micro- and nanoparticles are dispersed in nonmiscible liquids, the particles can self-assemble into clusters at the interface of nonmiscible solvents. To restrict the surface modification only to the surface area exposed to one liquid phase, Pickering emulsions rely on delicate thermodynamic control of the interface and precise control of the thermodynamics can result in a diverse range of Janus particles. Once wax–based colloidosomes are prepared, several surface modification approaches may be pursued including grafting polymerization, click-chemistry-based polymerization and surface-initiated free-radical polymerization.

4. Electrohydrodynamic Co-jetting

In addition to anisotropic surface modification, i.e., patchiness, particles with multifunctional bulk properties in terms of both chemical and physical attributes are of specific interest for a number of applications. In these cases, multicompartmental particles may offer a simple solution to design needs that require inclusion of several, often dissimilar, properties in the same particle. A growing body of research emphasizes the potential of droplet-based strategies for the preparation
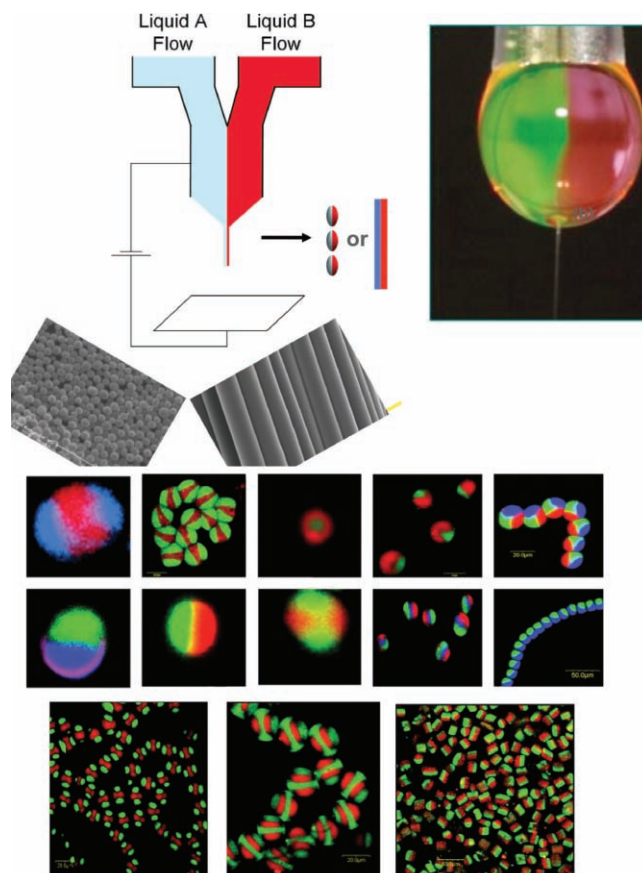

Figure 2. Top left: Schematic description of the electrohydrodynamic co-jetting process, which can lead to either particles (electrospraying) or fibers (electrospinning). Top right: Digital Images of a typical Taylor cone encountered during electrohydrodynamic co-jetting. The sharp interface between the different fluid flows is evident. Bottom: Examples of particles and fibers made by electrohydrodynamic co-jetting.

of this type of multifunctional particles.[11] Compartmentalized particles have been fabricated by either microfluidic processes[12] or electrohydrodynamic co-jetting (**Figure 2**).[13] In its simplest form, the electrohydrodynamic co-jetting process involves a side-by-side dual capillary needle system that allows for two miscible polymer solutions to move under laminar flow. Thus, there is no convective mixing and a very stable interface can be established between the two jetting solutions. Application of an electric field to the compound droplet, which forms at the outlet of the two capillary needles, results in distortion of the droplet into a Taylor cone and subsequent formation of an electrified polymer jet. The application of a high electric field induces velocities on the order of 250 m/s;[14] comparable to a rocket launch. Rapid acceleration of the viscoelastic jet leads to a reduction in jet diameter by several orders of magnitude and simultaneous increase in surface area. This induces instantaneous evaporation of the solvent and results in solidification of the non-volatile components. Because solidification is extremely fast, surface-energy-driven reorientation of the polymers into a thermodynamically more stable core/shell configuration is restricted, and the initial, flow-determined configuration can be maintained. Thus electrohydrodynamic co-jetting uses
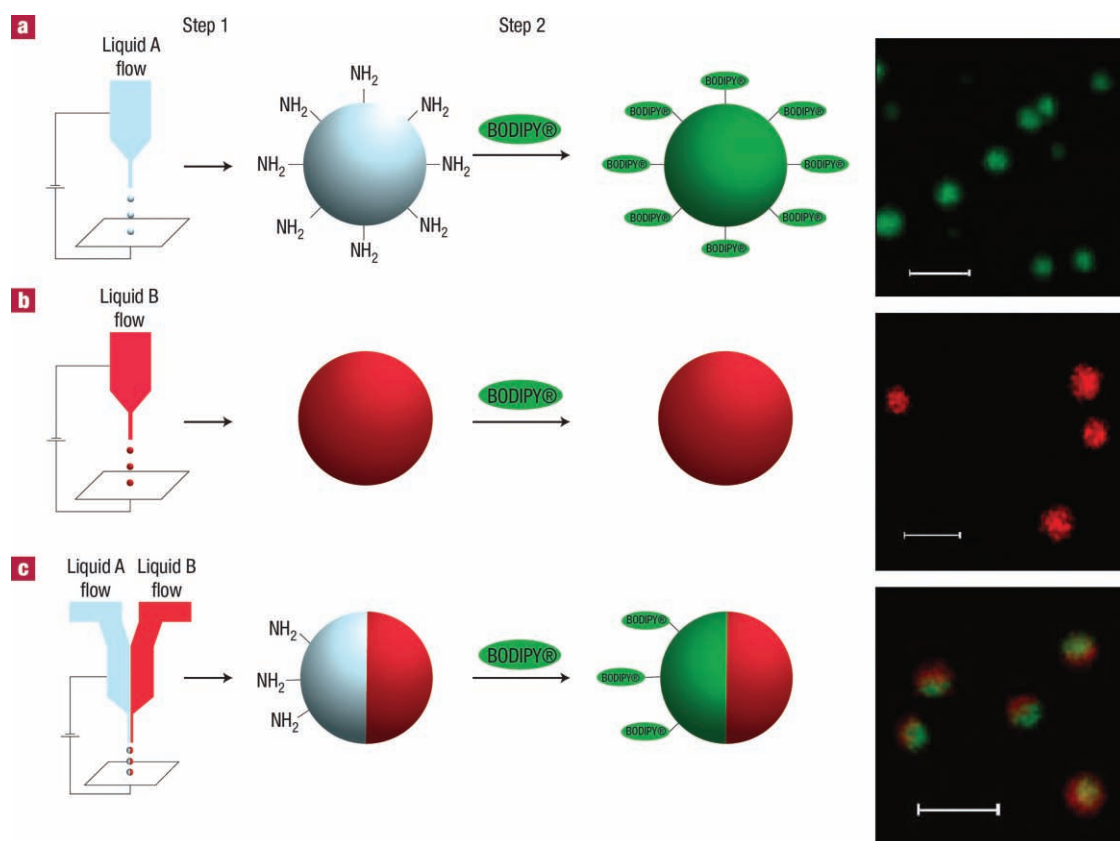
Figure 3. Selective surface modification of bicompartmental particles. Bicompartmental particles containing amino-functionalized polymer in only one single compartment are chemically modified with a green dye (reaction c). Control experiments with isotropic particles demonstrate high reactivity (reaction a) and low nonspecific binding (reaction b). Reproduced with permission.[13] Copyright 2005 Nature Publishing Group.

relatively simple manipulation of liquid geometries followed by rapid solidification to provide access to solid nano- and microparticles with architectures that would be otherwise impossible, or at least extremely difficult, to achieve.[15]

5. Water-Based Jetting Systems

Electrohydrodynamic co-jetting was first demonstrated for the preparation of bi- and tricompartmental particles from aqueous solutions of poly(acrylic acid-*co*-acrylamide) (PAAm) and poly(ethylene glycol) (PEG) polymers.[16] In this case, the free surface energy of the solvent is relatively high and a nearly spherical droplet forms at the tip, from which the Taylor cone is formed as a virtual nozzle (Figure 2).

Water-based jetting systems are environmentally friendly and typically show low toxicity, which are important attributes for biomedical applications. However, the use of water-soluble polymers for the fabrication of biocompartmental particles also requires subsequent chemical modification to ensure stability of particles under physiological conditions. In principle, a number of crosslinking approaches are available for particle stabilization, including photoreactions, the use of chemical crosslinkers, or thermal crosslinking reactions. The later uses chemical groups already present in the polymers, but requires exposure of particles to elevated temperatures.

Thus, this approach is only suitable, if particles do not contain temperature-sensitive payloads, such as proteins. In the case of polyacrylamide-based particles, thermal imidization can result in water-stable particles that show controllable swelling.[17] Importantly, the particles maintain the bicompartmental character throughout the crosslinking process. With stable bicompartmental particles in hand, we conducted an initial biocompatibility study that indicated excellent biocompatibility up to particle concentrations as high as 1 mg/10^6 cells.[18] As mentioned earlier, bicompartmental particles offer the possibility of selectively modifying certain parts of the particle or simultaneously displaying multiple chemical moieties on the particle surface (**Figure 3**). A follow-up study suggests that non-specific binding of PAAm particles to the cell surface is substantial (**Figure 4**).[19] Thus, surface modification of the entire particle (i.e., both compartments) with PEG was necessary to reduce nonspecific binding. This was achieved by addition of acetylene-modified PAAm to both compartments followed by a click reaction of an azido-PEG. In addition, binding moieties had to be selectively introduced into one compartment to ensure specific interactions with cell-surface markers. Thus, a biotinylated PAAm derivative was added into one compartment only. Subsequent binding studies with selectively modified particles showed significantly increased binding of particles modified with specific binding moieties as compared to the nonspecific background.[19]
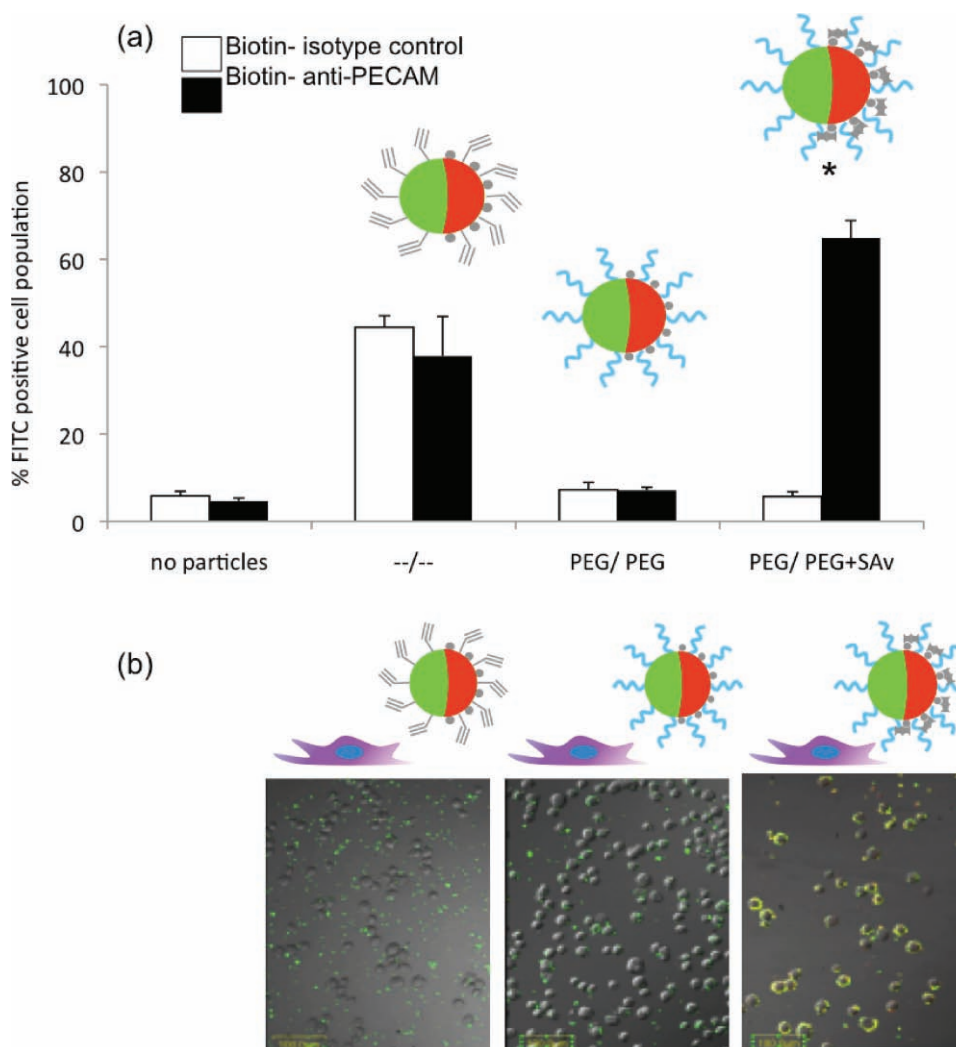
Figure 4. Specific cell binding of bicompartmental particles after selective surface modification of one hemisphere. Bicompartmental particles presenting PEG on the entire particle surface and biotin-functionalized polymer in only one compartment can bind selectively to human endothelial cells. Here, interactions are based on the biotin–strepdavidin interaction, where biotin is displayed on the particle hemisphere and strepdavidin has been introduced on the cell surface via a platelet/endothelial cell-adhesion molecule (PECAM, CD31) antibodies. Cell–particle binding was quantified by flow cytometry (top) and studied by fluorescence microscopy (bottom). Reproduced with permission.[19]

6. Organic-Solvent-Based Co-jetting

While this work demonstrates the potential of water-based polymer particles as building blocks in future biohybrid materials, the electrohydrodynamic co-jetting process is not limited to water-soluble polymers (**Figure 5**).[20] Recent demonstration of electrohydrodynamic co-jetting of organic solutions containing biodegradable polymers marked a significant extension of the earlier work and resulted in bicompartmental fibers and particles, which may find potential applications in drug delivery and tissue engineering.[21] Thorough investigation of the poly(lactic-*co*-glycolic acid) (PLGA) system revealed simultaneous control of compartmentalization, shape, and size of biodegradable particles by systematically varying different jetting solution and process parameters.[22]

During electrohydrodynamic processing of multiple polymer solutions, the parameters that can be simultaneously controlled constitute a truly multidimensional design space. Jetting parameters are either associated with the actual jetting solutions and may include surface tension, dielectric constant, electrical conductivity, density, or vapor pressure of the jetting fluids. Another critical factor is the viscosity of the solutions, which is mainly a function of molecular weight and concentration of the polymer. In contrast, process-related parameters are fluid flow rates, applied electrical current and separation distance between tip and counterelectrode, or the diameter of the capillaries used for fluid processing. In addition, there are a number of environmental variables, such as temperature, pressure, and humidity that can influence critical processes associated with electrohydrodynamic co-jetting. Simultaneous control over these variables may provide access to a multitude of particle types with defined sizes, shapes, surface morphologies, and porosities. Delineation of specific transitions between different particle properties is often challenging, but has been previously achieved
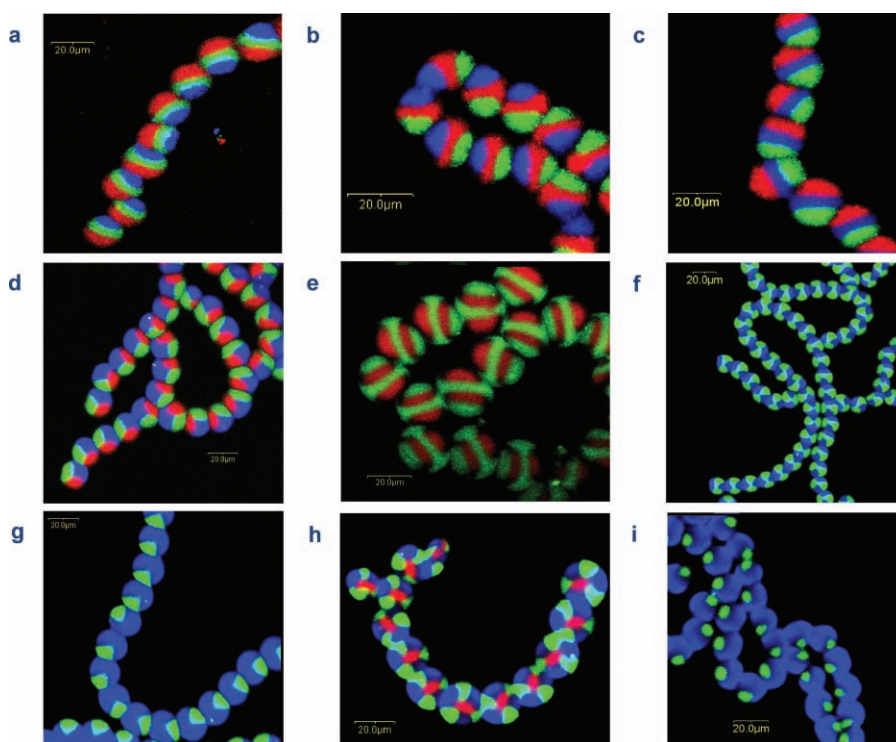
Figure 5. Electrohydrodynamic co-jetting of multicompartmental microfibers with up to seven distinct compartments. a–d) Cross-section of different "isomers" of tricompartmental microfibers; e) tetracompartmental microfibers; f) tetracompartmental microfibers resulting from a tetragonal arrangement of outlet streams; g) tetracompartmental microfibers with threefold larger compartments; h) heptacompartmental microfibers; i) heptacompartmental fibers with one compartment being six times larger than the other. The insets indicate number and spatial configuration of the jetting system. All scale bars are 20 μm. Reproduced with permission.[23] Copyright 2009, American Chemical Society.

for a PLGA system through systematic investigation of selected jetting parameters under otherwise constant settings. Focusing on polymer concentration and flow rate, we realized a number of defined shape transitions in bicompartmental PLGA particles (**Figure 6**).[22] Increasing either polymer flow rate or polymer concentration results in larger particles. On the other hand, polymer concentration directly influences particle shape by affecting solution viscosity, which in turn influences jet break-up tendency, and evaporation rate. Since solutions of higher viscosities resists jet breakup, these conditions tend to favor fibers. For a given solvent system, individual or simultaneous control of flow rates and concentrations may be an adequate means for obtaining particles of different sizes and shapes.[22]

Apart from the precise control over particle characteristics, the potentially most apparent advantage of the electrohydrodynamic co-jetting process may be the ability to fabricate particles with more than two compartments by simply increasing the number of capillaries, from which the solutions are processed.[23] This applies equally to microparticles and
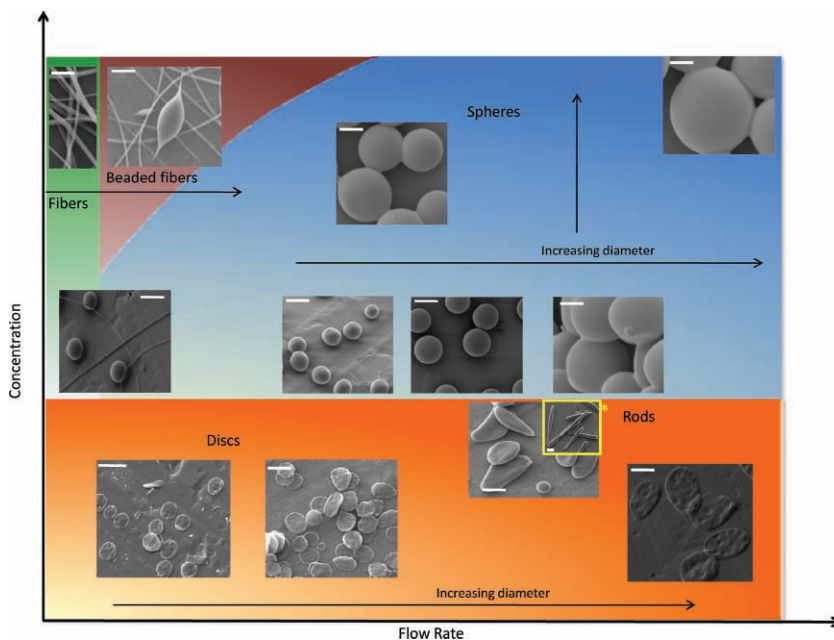

Figure 6. Effect of concentration and flow rate on size and shape of bicompartmental PLGA particles (note that the bicompartmental architecture is not visible in the SEM images). At lower concentrations, bicompartmental discs are observed. As the concentration is increased, the shape is somewhat affected resulting in increased flatness of the particles. Rods are observed at a critical concentration (3.5 wt%) and flow rate (0.9 mL h⁻¹), but only upon the addition of triethylamine. As concentration is increased, spheres are observed and further increasing the polymer concentration or flow rate leads to larger spheres. Fibers are observed at even higher concentrations. For both, higher concentrations and higher flow rates, and transition from fibers to beaded fibers is thus observed. All scale bars represent 5 μm. Reproduced with permission.[22]
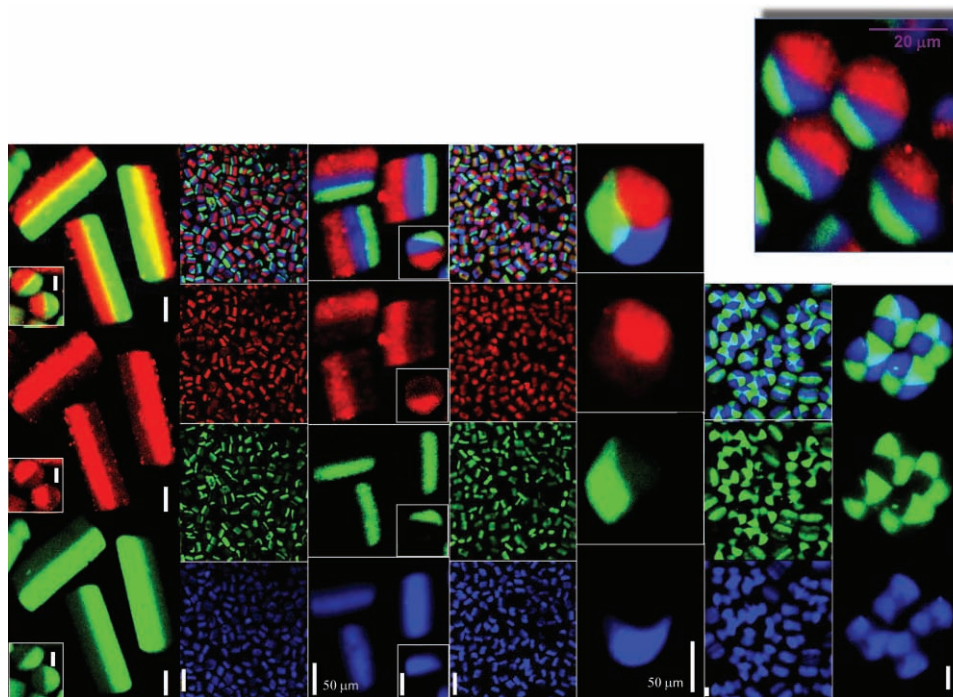
Figure 7. Multicompartmental microcylinders prepared by electrohydrodynamic co-jetting and subsequent microsectioning. Both overlays and the individual fluorescence images are shown. Scale bars are 10 μm, unless indicated otherwise. Reproduced with permission.[24]

microfibers. For instance, electrohydrodynamic co-jetting of aqueous PAAm solutions with a three-needle setup can result in tricompartmental particles with diameters between hundreds of nanometers and a few micrometers.[16] Similarly, multicompartmental fibers with as many as seven distinct compartments can be prepared by electrohydrodynamic co-jetting of PLGA solutions.[23] The higher solution viscosity encountered in the case of electrospinning adds further stability to the jetting process and thus enables not only control over the number of compartments, but also compartment sequences. Figure 5a–d shows the possible "isomers" of tricompartmental fibers, i.e., the sequences red/blue/green, red/green/blue and green/red/blue, as well as a pie-shaped compartmentalization.

The precise control of compartments, diameters, and the high degree of alignment typically encountered in these systems led to follow-up work: by using electrohydrodynamic co-jetting to create well-defined bundles of multicompartmental fibers and subsequent sectioning using an automated microsectioning device, we were able to create monodisperse microcylinders with controllable aspect ratios.[24] **Figure 7** highlights examples of multifunctional microcylinders with a range of different compartments. Because of the extremely stable jet observed under the jetting conditions that resulted in fiber bundles, cylinder diameters can be considered monodisperse. In addition, the microsectioning can be done with such a precision that not only the diameter, but also the length are well controlled. Individual compartments can thus be selectively modified to create microstructured cylinders, or made of different polymers, as to selectively remove a compartment, resulting in "pacman"-like particles (**Figure 8**).[24]

7. Electrohydrodynamic Co-jetting of Suspensions

Beyond polymer solutions, recent work suggests that electrohydrodynamic co-jetting of nanoparticle suspensions is—at least under certain conditions—feasible (**Figure 9**).[25] Gold nanocrystals were selectively incorporated into only one compartment of anisotropic PAAm microparticles. After subsequent polymer crosslinking, nanocrystal-loaded polymer particles were optically visible and maintained a clear interface between compartments. Similarly, PAAm particles were prepared from two different nanoparticle suspensions: iron oxide and titanium dioxide nanoparticles. Chemical analysis verified the compartmentalization of the inorganic payload, and the application of magnetic fields resulted in selective orientation of the bicompartmental particles.[26] The ability to magnetize only certain parts of a particle will affect its response to magnetic fields and may be a key feature for future magnetophoretic displays.

8. Conclusion

As outlined throughout the course of this report, electrohydrodynamic co-jetting can be used with either organic or aqueous solutions of a wide range of different polymers. The resulting particles can combine orthogonal sets of properties in unusal ways. This substantially expands the design space for colloidal scientists and may enable applications in a range of different biological and biotechnological areas (**Figure 10**). Importantly, many of the polymers used so far, such as polyacrylic acid, polyethylene glycol, polylactic acid,
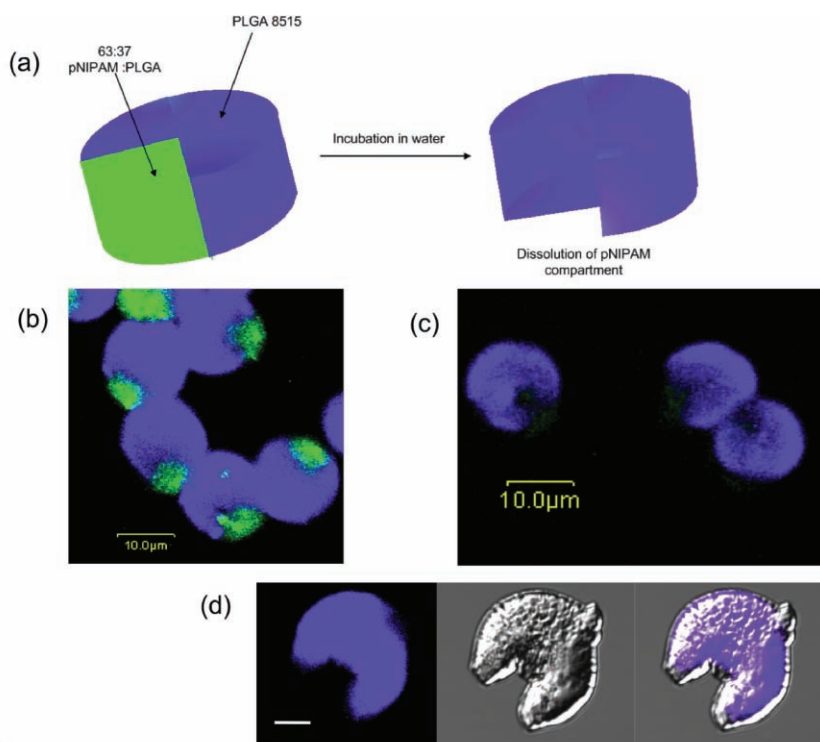
Figure 8. Bicompartmental PLGA microdisks prepared by electrohydrodynamic co-jetting and subsequent microsectioning. The smaller one-quarter compartment comprises a water-soluble polymer (poly(*N*-isopropylacrylamide), PNIPAM), which was selectively removed to yield "Pacman"-like particles. All scale bars represent 10 μm. Reproduced with permission.[24]

be used in self-assembly studies were they undergo directional interactions with each other or with human cells. In addition, compartments may not only be designed to present dissimilar properties in the same particle, but to equip particles with synergistic effects. For instance, selective changes in one compartment may trigger transitions in the adjacent compartments that may result in secondary effects.[30,31] One example could be a bicompartmental particle that is loaded with a nanoparticle formulation in one compartment and a temperature-sensitive polymer in the second. Inductive heating may induce local temperature gradients in the particle that serve as the trigger for phase transitions in the second compartment. These, and many other examples, are possible because of the advent of novel compartmentalized micro- and nanoparticles. Still, a much better understanding of the design space and its limitations is needed to realize the countless possibilities that are offered by electrohydrodynamic co-jetting. Finally, the short-term success of the technology will depend on our ability to identify core applications, where multifunctional design can provide distinctive advantages over conventional particles.

dextran, or polymethylacrylate,[27] are already commodity materials in the hand of biomedical engineers. While the range of polymer candidates for electrohydrodynamic co-jetting is relatively broad, choice becomes narrower, when different pairs of polymers are considered for co-jetting. In general, electrohydrodynamic co-jetting works best, when key solution parameters of the different jetting solutions, such as conductivity, viscosity, and density, are comparable. Under certain conditions, change of process parameters can result in transitions between particle architectures. In one instance, co-jetting of aqueous PAAm/polyethyleneimine solutions under various conditions resulted in either core/shell or Janus-type particle geometries.[28]

In the future, a wide range of novel particles with previously unseen architectures will be prepared by taking advantage of new design tools associated with compartmentalized colloids. Inclusion of optically distinct materials may offer novel ways for particle manipulation,[29] and selective removal of certain compartments may lead to unique shapes. Microstructured particles with dissimilar surfaces patterns may
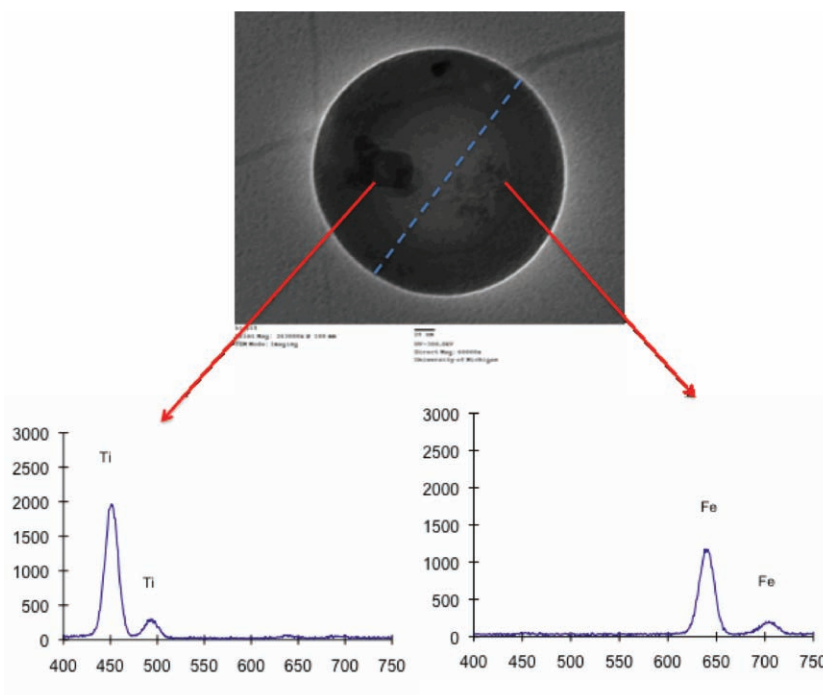

Figure 9. Bicompartmental particle prepared by electrohydrodynamic co-jetting of two nanoparticle suspensions: magnetite and titanium dioxide. The inorganic nanoparticles are compartmentalized as shown by energy dispersive X-ray (EDX) spectroscopy. In this case, co-jetting was facilitated by use of a viscous jetting solvent (ethylene glycol). The scale bar represents 40 nm. Reproduced with permission.[26] Copyright 2010, Royal Society of Chemistry.
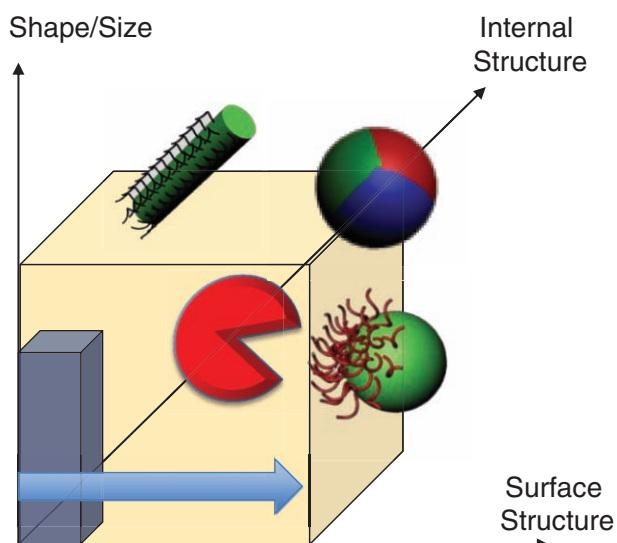
Expansion of design space

Figure 10. Multicompartmental particles offer independent design of surface microstructure, internal architecture, i.e., the distribution of matter in pockets of functionality, shape, and size. This novel type of particle architecture manifests a significant expansion of the design possibilities for multifunctional particles in the range from 100 nm to tens of micrometers.

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