Microgels

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Small but Smart: Sensitive Microgel Capsules

*Sebastian Seiffert**

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Microgel capsules are micrometer-sized particles that consist of a cross-linked and swollen polymer network complexed with additives. These capsules can be actuated by external stimulation if they are formed from sensitive or supramolecular polymer networks. To make this truly useful, it is crucial to control the microgel size, shape, and loading; this can be achieved by droplet-based microfluidic templating.

Microgels: Soft, Small, Superb

Microgels are micrometer-sized particles that consist of a cross-linked polymer network swollen by solvent, typically water.[1–3] This composition entails elastic moduli in the range of 0.1–100 kPa; as a result, microgels are a prime example of soft matter. Microgels are useful to host-sensitive payloads, including drugs, proteins, nucleic acids, and living cells.[4–9] Such soft capsules serve for both basic research and practical applications. In basic research, immobilization of payloads within microgels allows them to be studied in a state of three-dimensional confinement. In practical applications, microgel encapsulation serves to shield a payload from the exterior to temporarily inactivate it or to deliver it to a desired site. A central aspect in both classes of application is to selectively actuate the microgel capsules with a view to toggling their mechanical rigidity or to degrading them. Both can be achieved through the use of stimuli-sensitive polymer networks.[10,11]

In addition to controlling the sensitivity of microgel capsules, it is crucial to control their size and shape. The original use of the term microgel referred to particles with dimensions of a few micrometers to the sub-micrometer scale, even down to single, intramolecular cross-linked polymer coils.[1,2] Recent contributions have extended its use to larger particles with a size of tens to thousands of micrometers.[3,12] Whereas sub-micrometer-scale capsules are attractive for delivery and controlled release applications that rely on cellular uptake,[13] larger microgel capsules are attractive for the opposite purpose of encapsulation of cells within the microgel.[9] Established experimental techniques to fabricate sub-micrometer-scale microgel capsules include precipitation polymerization,[14–19] emulsion and miniemulsion templating,[20–24] and template-supported layer-by-layer assembly[25–33] as well as grafting.[34–37] The fabrication of larger microgel capsules, however, is challenging. A suitable technique that provides exquisite control in this endeavor is droplet-based microfluidics,[3,12,38–41] which is the focus of this Minireview. The principle of this technique is to use micrometer-scale channels to create a stream of a microgel precursor solution. Periodic break-up of this stream can be induced by flow-focusing with an immiscible carrier fluid. This process is controlled by the viscosities, polarities, and flow rates of the fluids; as a result, microfluidic devices produce uniform droplets with great control over their size, shape, and monodispersity.[42,43] Subsequent gelation of the droplets retains this uniformity and yields monodisperse microgel particles.[3,44]

This Minireview highlights some milestones in the use of droplet-based microfluidic techniques to tailor sensitive microgel capsules with sizes in the 10–1000 μm domain. The focus is on two classes of sensitivity. One class comprises microgels that consist of polymer networks with critical polymer–solvent miscibility, thus allowing the microgels to be selectively swollen and deswollen; this principle enables the mechanical constraint on an encapsulated payload to be varied (Figure 1, left). A second class comprises microgels that consist of reversible supramolecular rather than permanent chemical polymer networks; this allows the microgels to be degraded and their payload to be released (Figure 1, right). Both classes of microgels can be fabricated with different structures. A simple type of structure is bulk microgels that consist of a polymer network spanning the particle and entrapping additives within its mesh (Figure 2A). Another type of structure comprises core–shell morphologies that host additives within their interior (Figure 2B).

[*] Dr. S. Seiffert
Institute of Chemistry and Biochemistry, Freie Universität Berlin
Takustrasse 3, 14195 Berlin (Germany)
and
F-ISFM Soft Matter and Functional Materials
Helmholtz-Zentrum Berlin
Hahn-Meitner-Platz 1, 14109 Berlin (Germany)
E-mail: seiffert@chemie.fu-berlin.de
sebastian.seiffert@helmholtz-berlin.de
Homepage: http://www.seiffert-group.de

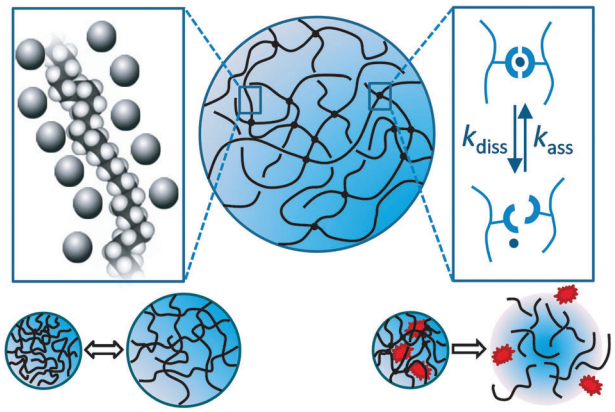

Figure 1. Schematic representation of a sensitive microgel particle and its actuation. Left: microgel sensitivity by critical polymer–solvent miscibility, thereby allowing the microgel to be swollen and deswollen. Right: microgel sensitivity by reversible cross-linking of the polymer, thereby allowing the microgel to be degraded and an encapsulated payload to be released.

From Drops to Particles

The key to controlling the microgel geometry in droplet-based microfluidic templating is to control that of the pre-microgel droplets. One class of microfluidic techniques that provides such control is based on glass microcapillaries; in this approach, microfluidic devices are constructed by coaxial alignment of glass capillary tubes with cylindrical or square cross-sections.[42] Another class of microfluidic techniques is based on photo- and soft lithography; in this approach, elastomer-based devices are replicated from master molds.[45] Pumping immiscible fluids through either of these devices produces droplets by hydrodynamic flow focusing.[46,47] The droplet size can be tuned by the fluid flow rates and is monodisperse. In addition to simple bulk shapes, microfluidic devices can fabricate sophisticated droplet morphologies. A particularly useful morphology is multiple emulsions: these are drops that host additional smaller drops inside.[42,47–49]

The shape and uniformity of these different types of droplets can be retained by droplet solidification, including gelation.[3,44] This process yields monodisperse microgel particles that can be removed from the templating emulsion and swollen in a suitable medium, primarily water. In terms of microencapsulation, both classes of microgel morphology have their specific strength. Bulk microgels can entrap additives within their polymer-network meshes (Figure 2A), thereby subjecting them to mechanical constraint. In contrast, core–shell capsules can host additives within their hollow interior (Figure 2B), thus ensuring chemical and mechanical separation of the capsule and the additive. The first arrangement can be used to study the impact of confinement on the microgel payload, for example, to mimic and investigate artificial extracellular matrixes. The second type of conditions is attractive if sensitive payloads need to be isolated and shielded from the exterior.

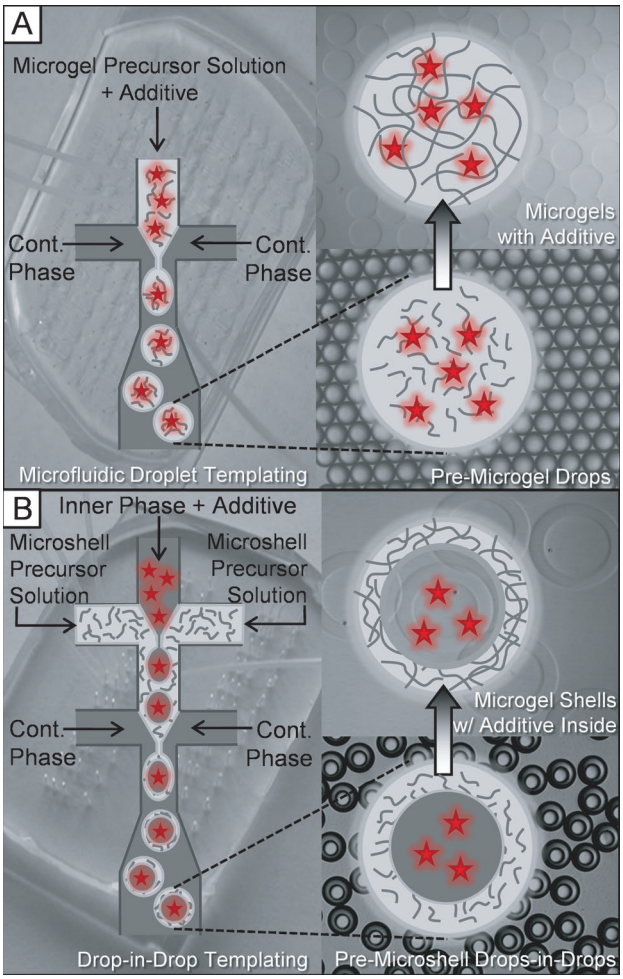

Figure 2. Droplet-based microfluidic production of microgel particles that encapsulate additives. A) Single emulsification of a microgel precursor solution with additives forms bulk microgel capsules that entrap the additives within the meshes of their particle-filling polymer network. B) Double emulsification forms drop-in-drop templates, thereby yielding core–shell microgel capsules that can host additives within their core. Copyright 2013 Wiley-VCH.[44]

Adding Function: Microgel Sensitivity

To truly benefit from the encapsulation of additives it is necessary to add function to the scaffolding microgel. For example, if the microgel capsules are to be used to study the effect of mechanical constraint on their payload, it is necessary to tune this parameter. A way to achieve this is to

Sebastian Seiffert, born in 1979, is a junior research group leader at Helmholtz-Zentrum Berlin and FU Berlin. His research focuses on the physical chemistry of polymers, particularly sensitive gels. After obtaining his PhD from Clausthal University of Technology, he spent two years at Harvard as a research fellow of the German National Academy of Sciences Leopoldina. He is now a Liebig fellow of the Fund of the Chemical Industry in Germany.

use microgel particles with tunable degree of swelling (Figure 1, left). Alternatively, if the microgel capsules are to be used for the controlled delivery and release of active substances, it is necessary to reverse the polymer cross-linking and degrade these capsules on demand. A way to achieve this is to employ reversible microgel gelation (Figure 1, right).

The first class of sensitivity is realized in polymer–solvent systems with critical miscibility. As the entropy of polymer solutions is considerably lower than that of mixtures of low-molecular-weight components, it is the enthalpy that has to favor mixing.[50–53] If the enthalpy is sensitive to thermodynamic parameters such as temperature or the composition of the system, variation of these parameters induces swelling and deswelling of the polymer coils. At the affine limit, the individual coil swelling and deswelling translates to the swelling and deswelling of cross-linked polymer networks, often referred to as the volume-phase transition.[54,55] One of the most prominent materials that undergoes such a transition is poly(*N*-isopropylacrylamide).[56]

The second class of microgel sensitivity is realized by noncovalent supramolecular polymer cross-linking.[57–59] In contrast to covalently jointed and cross-linked polymers, supramolecular polymers and networks can be assembled and disassembled by alteration of parameters such as temperature and building-block concentration.[57] Thus, supramolecular cross-linking can be reversed by external triggering.[60]

Sensitivity + Shape = Service

Both types of polymer gel sensitivity can be used in droplet-based microfluidics to tailor sensitive microgel capsules with either simple bulk or sophisticated core–shell structures. As a result, four different subcategories of sensitive microgel capsules can be discussed.

Environmentally Sensitive Microgels with Bulk Structure

One of the earliest examples of microfluidic-assisted fabrication of microgel capsules was reported by Weitz and co-workers:[12] these researchers used glass microcapillary devices to template temperature-sensitive particles complexed with fluorospheres, quantum dots, or magnetic nanoparticles (Figure 3 A). The thermosensitivity of the constituent polymer network is retained in this approach, because the different payloads are mechanically entrapped within the microgel capsules but not chemically linked to them. The same research group also encapsulated colloidal crystals into sensitive microgels.[61] Tuning the temperature-dependent degree of microgel swelling tunes the wavelength of the photonic band activity (Figure 3 B).

Following up on these examples, Kumacheva and co-workers reported a microfluidic strategy for simultaneous encapsulation of two types of additives with different polarities.[62] This approach is based on the bulk generation of a primary oil-in-water emulsion that is emulsified again by droplet-based microfludicis. Droplet gelation yields composite microgel particles that can host nonpolar additives within their oil compartments and polar additives within their hydrogel polymer network (Figure 3 C). Stimuli-sensitive deswelling of these microgels releases both additives.

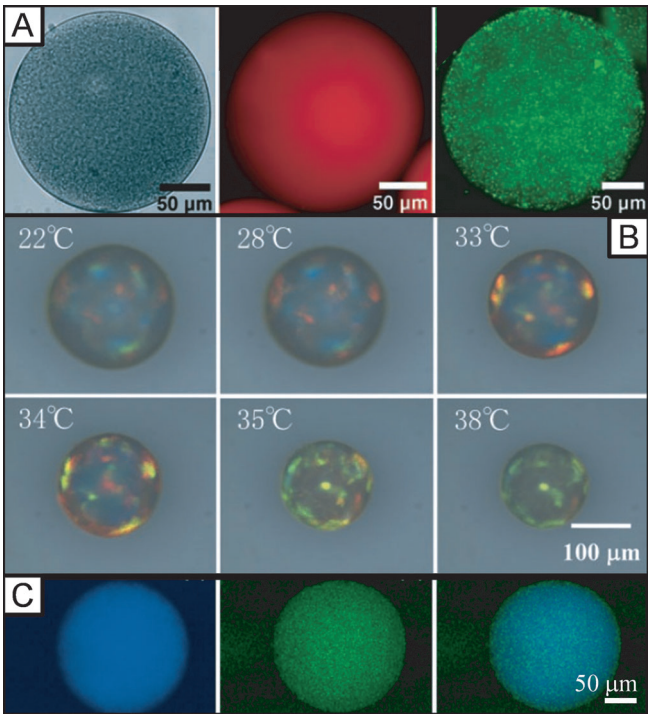

Figure 3. Environmentally sensitive microgels with embedded additives. A) Poly(*N*-isopropylacrylamide) complexed with magnetic nanoparticles (left), quantum dots (middle), and fluorescent polystyrene beads (right).[12] B) Colloidal crystals immobilized within poly(*N*-isopropylacrylamide) at different temperatures.[61] C) Poly(*N*-isopropylacrylamide) containing hydrophobic compartments complexed with Nile Red as well as hydrophilic 4′,6-diamidino-2-phenylindole (DAPI) molecules trapped within the hydrophilic hydrogel.[62] Left: Nile-Red fluorescence; middle: DAPI fluorescence; right: overlay. Copyright 2007, 2010, 2011, Wiley VCH.

Environmentally Sensitive Microgels with Core–Shell Structures

A particular strength of droplet-based microfluidics is the ability to form multilayered emulsion droplets, which serve to template multilayered microgels. In a seminal paper, the Weitz research group demonstrated the application of this approach to fabricate thermosensitive microgel shells that encapsulate a multiphase core (Figure 4 A).[42] Thermosensitive actuation of these capsules ruptures their shells and releases their payload. Despite the elegance of this strategy, however, multiple-emulsion templating is limited to the formation of particle layers with alternating polarity. To overcome this limitation, a multistep strategy has been introduced.[63,64] In the first step, monodisperse, micrometer-sized hydrogel particles are created to serve as the core material. In the second step, these particles are wrapped with monodisperse, aqueous polymer shells by using a second

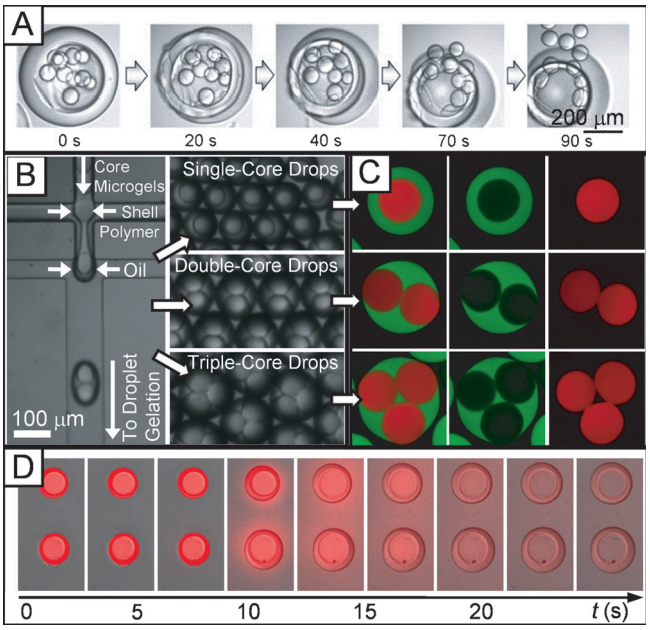

Figure 4. Environmentally sensitive microgel capsules for controlled release. A) Poly(*N*-isopropylacrylamide) hydrogel shell for the pulsed release of an encapsulated water-in-oil emulsion.[42] Upon an increase in the temperature, the hydrogel shell shrinks by expelling water. As both the core and the surrounding of the hydrogel shell are incompressible oils, a counterstress arises that ruptures the capsule, thereby releasing the encapsulated emulsion droplets. B–D) Core–shell microgels that consist of non-thermoresponsive polyacrylamide cores surrounded by thermoresponsive poly(*N*-isopropylacrylamide) shells. B) Particle templating by periodic injection of prefabricated core microgels into droplets of the shell precursor.[64] C) Resulting core–shell microgels, tagged with green and red fluorescence.[63] D) Controlled release of rhodamine B isothiocyanate–dextran (RITC-dextran; $M = 10000\ g\,mol^{-1}$) by temperature-triggered swelling of the microgel shell.[63] Copyright 2007, 2012, Wiley VCH; 2010, American Chemical Society.

microfluidic device (Figure 4B). When these structures are locked by gelling the shell, particles are obtained that consist of a hydrophilic polymer core nested within a hydrophilic polymer shell, both cross-linked and swollen with water, but both formed from different macromolecular precursors (Figure 4C). If these particles are formed with a stimuli-responsive polymer shell, they provide a controlled release system: additives are trapped within the microgel core when the microgel shell is deswollen and impermeable, and they are released from the capsules by swelling the shell (Figure 4D).

Supramolecular Microgels with Bulk Structure

Whereas environmentally sensitive microgel capsules can be swollen and deswollen by external stimulation, their selective degradation is limited to mechanical burst.[42] More elegant approaches to achieving this goal can be realized by reversible rather than permanent cross-linking of the polymer; a particularly elegant method is supramolecular cross-linking.[59]

Several research groups developed degradable microparticles by reversible cross-linking of natural polymers.[65–74] A well-established approach to achieve such reversible cross-linking is based on Ca^{2+}-mediated linkage of alginate polymers. The preparative use of this method traces back several decades,[71,72] with a particular focus on biomedical applications.[73,74] This approach was later refined by the use of controlled multiphase microfluidic templating to fabricate monodisperse, sub-millimeter-sized alginate hydrogel beads.[65] In this respect, Kumacheva and co-workers delimited three approaches to fabricate monodisperse alginate microgels: internal droplet gelation, external droplet gelation, and gelation by droplet fusion.[39,69] In internal gelation, the cross-linking agent Ca^{2+} is dissolved within pre-microgel droplets in an inactive form such as nanoparticulate $CaCO_3$ and then activated by influx of acetic acid from the continuous phase. In external gelation, Ca^{2+} itself diffuses into the drops from the continuous phase, preferably in the form of $CaCl_2$ or CaI_2. In the droplet-fusion approach, the alginate polymer and Ca^{2+} are encapsulated within separate precursor droplets, which are merged downstream.

The reversible cross-linking of natural polymers allows microgels to host living cells.[9] For example, Nakajima and coworkers reported the encapsulation of human kidney cells in alginate microgels that are gelled by droplet fusion,[65] whereas Tan and Takeuchi encapsulated mammalian Jurkat cells within alginate microgels solidified by internal gelation.[66] Another study introduced cell-laden alginate microgels with anisotropic morphology, with the cell load hosted in one half, while the other half is complexed with magnetic nanoparticles to allow remote manipulation.[70] As an alternative to alginate, the Kumacheva research group formed microgels from thermoreversibly gelling agarose polymers to encapsulate mouse embryonic stem cells; the microgel elasticity was modified by on-chip adjustment of the precursor polymer concentration.[67] In a related approach, co-encapsulation of factor-dependent and responsive blood progenitor cell lines at varying ratios was achieved by on-chip mixing of separate cell-suspension fluids.[68]

Despite their accessibility from natural sources, natural polymers have disadvantages in terms of the rational design of microgel capsules.[75] Their composition varies from batch-to-batch because they are derived from several living organisms and not from just one individual.[76,77] Furthermore, they cannot be produced in large volumes, and the ability to modify and tailor them is limited.[78] To overcome these limitations, recent effort has focused on the use of synthetic polymers. Abell, Sherman, and co-workers prepared supramolecular microcapsules that consist of a polymer–gold nanocomposite cross-linked by ternary cucurbit[8]uril complexes.[79] These capsules are degradable by one-electron reduction of methyl viologen. Alternatively, iron(II) coordination to bipyridine-capped poly(ethylene glycol) can produce cleavable microgel capsules for the encapsulation of mammalian cells.[80]

Supramolecular Microgels with Core–Shell Structures

A particularly elegant combination of microgel functionality and microgel geometry comprises reversible particles with core–shell structure. The first and simplest way to form such particles is to obtain them from single-emulsion droplets that are solidified only in their rim. Following this strategy, the Kumacheva research group fabricated alginate microcapsules by the method of external gelation.[69,81] In this approach, aqueous alginate-laden droplets are subjected to diffusive influx of CaI_2 or $Ca(CH_3COO)_2$ from the continuous organic phase, thereby leading to droplet gelation that proceeds from the droplet rim to the core (Figure 5A). Tuning the Ca^{2+} concentration in the continuous phase and the time of droplet exposure to this phase controls the microcapsule morphology (Figure 5B and C).

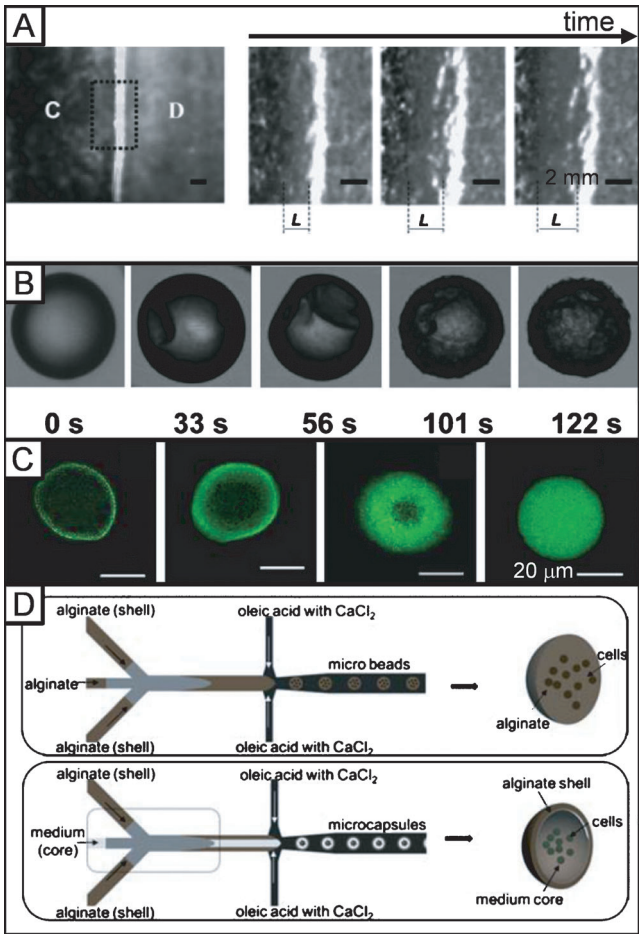

Figure 5. Supramolecular microgels based on natural polymers. A) Alginate microgel shells formed by external gelation: Ca^{2+} penetrates into the templating droplets from the continuous phase, thus leading to droplet gelation from the rim to the core.[69] B) Microgel morphologies of Panel A as a function of time of Ca^{2+} influx.[81] C) Alginate microgels obtained by emulsification of an aqueous alginate in a solution of CaI_2 in undecanol with concentrations of 0.05, 0.10, 0.20, and 0.25 wt%; gelling time 18 s.[81] D) Formation of alginate bulk and core–shell microgels for the ensapsulation of cells.[82] Copyright 2006, American Chemical Society; 2007, Wiley VCH; 2011 The Royal Society of Chemistry.

In an extension of this principle, Kang and co-workers fabricated cell-laden alginate microcapsules (Figure 5D).[82] In this study, a sharp microshell interface is formed by surrounding the cell-laden aqueous core flow with an independent aqueous alginate flow, followed by concurrent dispersion of both. Diffusive influx of $CaCl_2$ from the external oil phase leads to solidification of the droplets. The morphology of the resulting gel capsules can be tuned by the fluid viscosities and flow rates. It is also possible to form multi-shelled capsules by extension of the fluid-wrapping principle. In an application of this technique, monodisperse cell spheroids were grown within the microshell cores.[82]

Current Challenge: Numbering-Up

To make the utility and function of sensitive microgel capsules available outside of academic research it is necessary to produce large quantities of them. Depending on the rates of fluid flow and device geometry, a single microfluidic channel can produce up to a hundred grams of particles per day. This is not profitable. To overcome this limitation, the Nisisako research group used 256 microfluidic channels in parallel to fabricate isotropic and anisotropic microparticles at rates of about a hundred grams per hour.[83] For more complex core–shell structures, however, perceptible numbering-up is a persistent challenge. The Weitz research group has used 15 channels in parallel to form water-in-oil-in-water double emulsions at production rates of a kilogram per day,[84] whereas the Nisisako research group used 40 hydrophilic double-emulsion drop makers and 32 hydrophilic triple emulsion drop makers in parallel to mass-produce oil-in-oil-in-water double emulsions and oil-in-oil-in-oil-in-water triple emulsions.[85] On the industrial side, BÜCHI Labortechnik has commercialized a device to produce alginate, cellulose, gelatin, and other bulk and core–shell microgel capsules at production rates in the kHz regime (Encapsulator B-390/B-395 Pro). With these approaches, paths have been opened for sensitive microgel capsules to become a common material platform in chemical and biological laboratories as well as in fine-chemical industries.

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[1] W. Funke, O. Okay, B. Joos-Mueller, *Adv. Polym. Sci.* **1998**, *136*, 139–234.
[2] M. Das, H. Zhang, E. Kumacheva, *Annu. Rev. Mater. Res.* **2006**, *36*, 117–142.
[3] S. Seiffert, *Macromol. Rapid Commun.* **2011**, *32*, 1600–1609.
[4] J. B. Thorne, G. J. Vine, M. J. Snowden, *Colloid Polym. Sci.* **2011**, *289*, 625–646.
[5] V. Castro Lopez, J. Hadgraft, M. J. Snowden, *Int. J. Pharm.* **2005**, *292*, 137–147.

[6] S. V. Vinogradov, T. K. Bronich, A. V. Kabanov, *Adv. Drug Delivery Rev.* **2002**, *54*, 135 – 147.
[7] J. R. Retama, B. Lopez-Ruiz, E. Lopez-Cabarcos, *Biomaterials* **2003**, *24*, 2965 – 2973.
[8] S. Schachschal, H. J. P. Adler, A. Pich, S. Wetzel, A. Matura, K. H. van Pee, *Colloid Polym. Sci.* **2011**, *289*, 693 – 698.
[9] D. Velasco, E. Tumarkin, E. Kumacheva, *Small* **2012**, *8*, 1633 – 1642.
[10] M. A. Cohen-Stuart, W. T. S. Huck, J. Genzer, M. Mueller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V. V. Tsukruk, M. Urban, F. Winnik, S. Zauschner, I. Luzinov, S. Minko, *Nat. Mater.* **2010**, *9*, 101 – 113.
[11] D. Klinger, K. Landfester, *Polymer* **2012**, *53*, 5209 – 5231.
[12] J.-W. Kim, A. S. Utada, A. Fernández-Nieves, Z. Hu, D. A. Weitz, *Angew. Chem.* **2007**, *119*, 1851 – 1854; *Angew. Chem. Int. Ed.* **2007**, *46*, 1819 – 1822.
[13] Y. Yan, A. P. R. Johnston, S. J. Dodds, M. M. J. Kamphuis, C. Ferguson, R. G. Parton, E. C. Nice, J. K. Heath, F. Caruso, *ACS Nano* **2010**, *4*, 2928 – 2936.
[14] A. Pich, W. Richtering, *Adv. Polym. Sci.* **2010**, *234*, 1 – 37.
[15] W. McPhee, K. C. Tam, R. Pelton, *J. Colloid Interface Sci.* **1993**, *156*, 24 – 30.
[16] K. Kratz, T. Hellweg, W. Eimer, *Colloids Surf. A* **2000**, *170*, 137 – 149.
[17] S. Meyer, W. Richtering, *Macromolecules* **2005**, *38*, 1517 – 1519.
[18] S. Schachschal, A. Balaceanu, C. Melian, D. E. Demco, T. Eckert, W. Richtering, A. Pich, *Macromolecules* **2010**, *43*, 4331 – 4339.
[19] M. J. Kettel, F. Dierkes, K. Schaefer, M. Moeller, A. Pich, *Polymer* **2011**, *52*, 1917 – 1924.
[20] Ref. [8].
[21] D. Klinger, E. M. Aschenbrenner, C. K. Weiss, K. Landfester, *Polym. Chem.* **2012**, *3*, 204 – 216.
[22] Z. H. Cao, K. Landfester, U. Ziener, *Langmuir* **2012**, *28*, 1163 – 1168.
[23] Z. H. Cao, U. Ziener, K. Landfester, *Macromolecules* **2010**, *43*, 6353 – 6360.
[24] A. Ethirajan, K. Schoeller, A. Musyanovych, U. Ziener, K. Landfester, *Biomacromolecules* **2008**, *9*, 2383 – 2389.
[25] B. Städler, A. D. Price, R. Chandrawati, L. Hosta-Rigau, A. N. Zelikina, F. Caruso, *Nanoscale* **2009**, *1*, 68 – 73.
[26] A. L. Becker, A. N. Zelikin, A. P. R. Johnston, F. Caruso, *Langmuir* **2009**, *25*, 14079 – 14085.
[27] S.-F. Chong, R. Chandrawati, B. Staedler, J. Park, J. Cho, Y. Wang, Z. Jia, V. Bulmus, T. P. Davis, A. N. Zelikin, F. Caruso, *Small* **2009**, *5*, 2601 – 2610.
[28] S.-F. Chong, J. H. Lee, A. N. Zelikin, F. Caruso, *Langmuir* **2011**, *27*, 1724 – 1730.
[29] J. Cui, Y. Yan, Y. Wang, F. Caruso, *Adv. Funct. Mater.* **2012**, *22*, 4718 – 4723.
[30] D. Mertz, J. Cui, Y. Yan, G. Devlin, C. Chaubaroux, A. Dochter, R. Alles, P. Lavalle, J. C. Voegel, A. Blencowe, P. Auffinger, F. Caruso, *ACS Nano* **2012**, *6*, 7584 – 7594.
[31] D. Mertz, H. Wu, J. S. Wong, J. Cui, P. Tan, R. Alles, F. Caruso, *J. Mater. Chem.* **2012**, *22*, 21434 – 21442.
[32] O. Shimoni, Y. Yan, Y. Wang, F. Caruso, *ACS Nano* **2013**, *7*, 522 – 530.
[33] J. Tan, Y. Wang, X. Yip, F. Glynn, R. K. Shepherd, F. Caruso, *Adv. Mater.* **2012**, *24*, 3362 – 3366.
[34] L. Zha, Y. Zhang, W. Yang, S. Fu, *Adv. Mater.* **2002**, *14*, 1090 – 1092.
[35] J. Cui, Y. Wang, A. Postma, J. Hao, L. Hosta-Rigau, F. Caruso, *Adv. Funct. Mater.* **2010**, *20*, 1625 – 1631.
[36] M. Karg, S. Wellert, S. Prevost, R. Schweins, C. Dewhurst, L. M. Liz-Marzán, T. Hellweg, *Colloid Polym. Sci.* **2011**, *289*, 699 – 709.
[37] S. Wu, J. Dzubiella, J. Kaiser, M. Drechsler, X. Guo, M. Ballauff, Y. Lu, *Angew. Chem.* **2012**, *124*, 2272 – 2276; *Angew. Chem. Int. Ed.* **2012**, *51*, 2229 – 2233.
[38] S. Y. Teh, R. Lin, L. H. Hung, A. P. Lee, *Lab Chip* **2008**, *8*, 198 – 220.
[39] E. Tumarkin, E. Kumacheva, *Chem. Soc. Rev.* **2009**, *38*, 2161 – 2168.
[40] D. Dendukuri, P. S. Doyle, *Adv. Mater.* **2009**, *21*, 4071 – 4086.
[41] J.-T. Wang, J. Wang, J.-J. Han, *Small* **2011**, *7*, 1728 – 1754.
[42] L. Y. Chu, A. S. Utada, R. K. Shah, J.-W. Kim, D. A. Weitz, *Angew. Chem.* **2007**, *119*, 9128 – 9132; *Angew. Chem. Int. Ed.* **2007**, *46*, 8970 – 8974.
[43] A. Utada, A. Fernandez-Nieves, H. Stone, D. A. Weitz, *Phys. Rev. Lett.* **2007**, *99*, 94502.
[44] S. Seiffert, *ChemPhysChem* **2013**, *14*, 295 – 304.
[45] Y. Xia, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 568 – 594; *Angew. Chem. Int. Ed.* **1998**, *37*, 550 – 575.
[46] L. Martín-Banderas, M. Flores-Mosquera, P. Riesco-Chueca, A. Rodriguez-Gil, A. Cebolla, S. Chávez, A. M. Gañán-Calvo, *Small* **2005**, *1*, 688 – 692.
[47] A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone, D. A. Weitz, *Science* **2005**, *308*, 537 – 541.
[48] T. Nisisako, S. Okushima, T. Torii, *Soft Matter* **2005**, *1*, 23 – 27.
[49] M. Seo, C. Paquet, Z. Nie, S. Xu, E. Kumacheva, *Soft Matter* **2007**, *3*, 986 – 992.
[50] P. J. Flory, *J. Chem. Phys.* **1942**, *10*, 51 – 61.
[51] M. L. Huggins, *Ann. N. Y. Acad. Sci.* **1942**, *43*, 1 – 32.
[52] P. J. Flory, J. Rehner, *J. Chem. Phys.* **1943**, *11*, 512 – 520; P. J. Flory, J. Rehner, *J. Chem. Phys.* **1943**, *11*, 521 – 526.
[53] A. R. Shultz, P. J. Flory, *J. Am. Chem. Soc.* **1952**, *74*, 4760 – 4767.
[54] Y. Li, T. Tanaka, *Annu. Rev. Mater. Sci.* **1992**, *22*, 243 – 277.
[55] S. Hirotsu, *Phase Transitions* **1994**, *47*, 183 – 240.
[56] H. G. Schild, *Prog. Polym. Sci.* **1992**, *17*, 163 – 249.
[57] L. Brunsveld, B. J. B. Folmer, E. W. Meijer, R. P. Sijbesma, *Chem. Rev.* **2001**, *101*, 4071 – 4098.
[58] J. D. Fox, S. J. Rowan, *Macromolecules* **2009**, *42*, 6823 – 6835.
[59] S. Seiffert, J. Sprakel, *Chem. Soc. Rev.* **2012**, *41*, 909 – 930.
[60] E. A. Appel, X. J. Loh, S. T. Jones, F. Biedermann, C. A. Dreiss, O. A. Scherman, *J. Am. Chem. Soc.* **2012**, *134*, 11767 – 11773.
[61] T. Kanai, D. Lee, H. C. Shum, D. A. Weitz, *Small* **2010**, *6*, 807 – 810.
[62] D. Jagadeesan, I. Nasimova, I. Gourevich, S. Starodubtsev, E. Kumacheva, *Macromol. Biosci.* **2011**, *11*, 889 – 896.
[63] S. Seiffert, J. Thiele, A. R. Abate, D. A. Weitz, *J. Am. Chem. Soc.* **2010**, *132*, 6606 – 6609.
[64] S. Seiffert, *Macromol. Rapid Commun.* **2012**, *33*, 1286 – 1293.
[65] S. Sugiura, T. Oda, Y. Izumida, Y. Aoyagi, M. Satake, A. Ochiai, N. Ohkohchi, M. Nakajima, *Biomaterials* **2005**, *26*, 3327 – 3331.
[66] W.-H. Tan, S. Takeuchi, *Adv. Mater.* **2007**, *19*, 2696 – 2701.
[67] A. Kumachev, J. Greener, E. Tumarkin, E. Eiser, P. W. Zandstra, E. Kumacheva, *Biomaterials* **2011**, *32*, 1477 – 1483.
[68] E. Tumarkin, L. Tzadu, E. Csaszar, M. Seo, H. Zhang, A. Lee, R. Peerani, K. Purpura, P. W. Zandstra, E. Kumacheva, *Integr. Biol.* **2011**, *3*, 653 – 662.
[69] H. Zhang, E. Tumarkin, R. M. A. Sullan, G. C. Walker, E. Kumacheva, *Macromol. Rapid Commun.* **2007**, *28*, 527 – 538.
[70] L. B. Zhao, L. Pan, K. Zhang, S. S. Guo, W. Liu, Y. Wang, Y. Chen, X. Z. Zhao, H. L. W. Chan, *Lab Chip* **2009**, *9*, 2981 – 2986.
[71] M. Kierstan, C. Bucke, *Biotechnol. Bioeng.* **1977**, *19*, 387 – 397.
[72] J. Klein, J. Stock, K.-D. Vorlop, *Eur. J. Appl. Microbiol. Biotechnol.* **1983**, *18*, 86 – 91.
[73] O. Smidsrod, G. Skjakbraek, *Trends Biotechnol.* **1990**, *8*, 71 – 78.
[74] A. D. Augst, H. Joon Kong, D. J. Mooney, *Macromol. Biosci.* **2006**, *6*, 623 – 633.
[75] R. Perez-Castillejos, *Mater. Today* **2010**, *13*, 32 – 41.
[76] S. Fu, A. Thacker, D. Sperger, R. Boni, S. Velankar, E. Munson, L. Block, *AAPS PharmSciTech* **2011**, *12*, 449 – 449.

[77] M. J. Paszek, N. Zahir, K. R. Johnson, J. N. Lakins, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, D. Boettiger, D. A. Hammer, V. M. Weaver, *Cancer Cell* **2005**, *8*, 241 – 254.
[78] T. Ota, T. W. Gilbert, D. Schwartzman, C. F. McTiernan, T. Kitajima, Y. Ito, Y. Sawa, S. F. Badylak, M. A. Zenati, *J. Thorac. Cardiovasc. Surg.* **2008**, *136*, 1309 – 1317.
[79] J. Zhang, R. J. Coulston, S. T. Jones, J. Geng, O. A. Scherman, C. Abell, *Science* **2012**, *335*, 690 – 694.
[80] T. Rossow, S. Bayer, R. Albrecht, C. C. Tzschucke, S. Seiffert, *Macromol. Rapid Commun.* **2013**, *34*, 1401 – 1407.
[81] H. Zhang, E. Tumarkin, R. Peerani, Z. Nie, R. M. A. Sullan, G. C. Walker, E. Kumacheva, *J. Am. Chem. Soc.* **2006**, *128*, 12205 – 12210.
[82] C. Kim, S. Chung, Y. E. Kim, K. S. Lee, S. H. Lee, K. W. Oh, J. Y. Kang, *Lab Chip* **2011**, *11*, 246 – 252.
[83] T. Nisisako, T. Torii, *Lab Chip* **2008**, *8*, 287 – 293.
[84] M. B. Romanowsky, A. R. Abate, A. Rotem, C. Holtze, D. A. Weitz, *Lab Chip* **2012**, *12*, 802 – 807.
[85] T. Nisisako, T. Andoa, T. Hatsuzawa, *Lab Chip* **2012**, *12*, 3426 – 3435.