Hydrogels belong to the most promising materials in polymer and materials science at the moment. As they feature soft and tissue-like character as well as high water-content, a broad range of applications are addressed with hydrogels, e.g., tissue engineering and wound dressings but also soft robotics, drug delivery, actuators, and catalysis. Ways to tailor hydrogel properties are crosslinking mechanisms, hydrogel shape, and reinforcement, but new features can be introduced by variation of hydrogel composition as well, e.g., via monomer choice, functionalization or compartmentalization. In particular, multicompartment hydrogels drive progress toward complex and highly functional soft materials. In the present review the latest developments in multicompartment hydrogels are highlighted with a focus on three types of compartments; micellar/vesicular, droplets, and multilayers including various subcategories. Furthermore, several morphologies of compartmentalized hydrogels and applications of multicompartment hydrogels will be discussed as well. Finally, an outlook toward future developments of the field will be given. The further development of multicompartment hydrogels is highly relevant for a broad range of applications and will have a significant impact on biomedicine and organic devices.

1. Introduction

Hydrogels play an important role in contemporary polymer and materials science. Due to their soft and tissue-like character as well as high water-content, major applications include tissue engineering\cite{1} and wound dressings\cite{2} but they are also employed in soft robotics,\cite{3} drug delivery,\cite{4} actuators,\cite{5} and catalysis.\cite{6} There are numerous ways to form hydrogel networks, e.g., via covalent bonds,\cite{7,8} dynamic covalent bonds,\cite{9} mechanical bonds,\cite{10} and supramolecular chemistry,\cite{11} giving rise to various properties ranging from durability to adaptability and self-healing. One of the important features to look out for is mechanical properties that are a determining factor for the targeted application. Mechanical properties can be tailored via various routes including crosslinking density, monomer type but also reinforcements like multiple networks,\cite{12} introduction of nanoparticles,\cite{13,14} or fibers.\cite{15} Also, the morphology of hydrogels has to be considered both from the length scale of the material but also the overall shape, for example, in the area of microgels,\cite{16} hydrogel cell culture scaffolds\cite{17} or hydrogel films.\cite{18} Especially, introduction of 3D printing in hydrogel formation has had a considerable impact for the formation of hydrogel materials with well-defined morphologies.\cite{19,20}

The present review deals with another way to change the properties of hydrogels apart from type of bonding, shape, and mechanical properties, namely, via composition. A variation of composition can be introduced via functionalization (also with spatial control),\cite{21,22} for example, but also by introduction of compartments. The variation of hydrogel composition puts hydrogels on a new level of complexity giving rise to novel applications and tuning of hydrogel properties. Especially, compartmentalization drives complexity of hydrogel structures, as it introduces specific areas/phases in hydrogel materials with designed properties, for example different mechanical properties,\cite{23} providing space for cargo encapsulation by introducing hydrophobic phases,\cite{24} including barriers between phases to tailor cargo release\cite{25} or as environment for chemical reactions.\cite{26} As such, a plethora of functions can be implemented into hydrogels or from the viewpoint of incorporated entities, specific features of a molecular system can be turned into a hydrogel material.

Multicompartment hydrogels open up new avenues toward complex and highly functional soft materials. In here, the formation of three types of compartments in hydrogel structures will be discussed, e.g., micellar/vesicular, droplets or multilayers (Figure 1) including various subcategories. In addition to types of compartments, morphologies of compartmentalized hydrogels and applications will be discussed as well. Finally, an outlook toward future developments will be presented.

2. Types of Multicompartment Hydrogels

In order to form multicompartment hydrogels, several mechanisms and techniques can be applied. The avenues for compartmentalization in hydrogel formation include three general categories: hydrogels with micellar or vesicular compartments,
Micelles formed from surfactants or amphiphilic block copolymers can be used to introduce compartments into a hydrogel matrix (Figure 2a). In terms of cargo delivery applications, the hydrophobic core of the micelle acts as reservoir for hydrophobic cargo, while the hydrophilic shell facilitates stability of the compartment in the hydrogel. As such drugs can be incorporated for single delivery or codelivery as well as cells for cell delivery.

Zhao and co-workers described agarose-based hydrogels with micelle loading. Micelles were formed from polyethylene glycol)-b-poly(2-(N,N-dimethylamino)ethyl methacrylate) (PEG-b-PDIPEMA), where the amino functional block served for pH stimulus response. Nile Red was used as a model for a hydrophobic drug and the release from the micelles was studied with respect to the pH of the surrounding medium. A hydrogel based on cellulose derivatives was presented by Tong and co-workers who utilized carboxymethylcellulose (CMC) as starting material for functionalization with amino and aldehyde groups (Figure 3). Crosslinking took place by imine formation and micelles from PEG-b-PDIPEMA were included in the hydrogel. To facilitate injectability, the two components were injected separately forming the hydrogel at the desired location. Minimal cytotoxicity of the hydrogels was observed in cell viability tests. The micelles were loaded either with Doxorubicin (DOX) or Nile Red and release studied via environmental (pH) trigger.

2.1.2. Micelles as Crosslinker

Micelles can act as a compartment to include cargoes but also as a way to crosslinkers in hydrogel formation or even both. The formation of micelles via the interaction of hydrophobic moieties allows the formation of supramolecular crosslinking points. For example, Okay and co-workers synthesized a copolymer of acrylamide (AAm) and dodecyl acrylate or stearyl methacrylate in the presence of a surfactant. The hydrophobic monomers form micelles together with the surfactant, namely, sodium dodecyl sulfate (SDS), and polymerization crosslinks these micelles via PAAm chains. As such, hydrogels with supramolecular micellar crosslinking points were obtained that could withstand elongation up to 3600% and showed self-healing properties with efficiencies around 100%. Later on, the self-healing properties of similar hydrogels based on AAm and n-octadecyl acrylate were studied in more detail. In-depth analysis of damaged hydrogels via atomic force microscopy showed a two-step process. At first, trenches and protrusions originating from gel damage reshape into circular holes and islands without an effect on depth and height. This reshaping process took place due to the intralayer mobility of mixed micelles. In the second step, complete healing of the hydrogel was induced by interlayer mobility.

Micellar crosslinking can be installed by using amphiphilic multiblock copolymers as well, where the hydrophilic block will form the strands in the network and the hydrophobic blocks join in the formation of a micellar core acting as crosslinking point. A straightforward way to obtain a micellar crosslinking is via utilization of an ABA block copolymer, where the A blocks form micelles and B blocks act as hydrophilic bridges for net-
work formation (Figure 2b). In the early stages, hydrophobic endgroups were utilized to drive hydrogel formation.François and co-workers introduced alkyl (i.e., C_{12}H_{25}) endfunctionalized PEG to form hydrogels via hydrophobic association,\[^{41}\] while Hogen-Esch and co-workers utilized a PEG backbone with perfluoroalkyl (C_{8}F_{17} and C_{10}F_{21}) functionalization on both ends.\[^{42}\] Block copolymers were utilized as well in a similar approach, e.g., poly(lactic-co-glycolic acid)-b-PEG-b-poly(lactic-co-glycolic acid) (PLGA-b-PEG-b-PLGA),\[^{43}\] poly(3-sulfopropylmethacrylate potassium salt)-b-PEG-b-poly(3-sulfopropylmethacrylate potassium salt) together with a cationic surfactant (i.e., dodecylamine hydrochloride),\[^{44}\] various oligoethylene glycol-based monomers in combination with PEG,\[^{45,46}\] poly(n-butyl methacrylate)-b-poly(2-dimethyamino)ethyl methacrylate)-b-poly(n-butyl methacrylate) (PnBMA-b-PDEAEMA-b-PnBMA),\[^{47}\] poly(lactic acid)-b-PEG-b-poly(lactic acid) (PLA-b-PEG-b-PLA),\[^{48,49}\] poly(styrene oxide)-b-PEG-b-poly(styrene oxide),\[^{50}\] or poly(2-methyl-2-oxazoline)-b-poly(2-phenylethyl-2-oxazoline)-b-poly(2-methyl-2-oxazoline).\[^{51}\]

An ABA block copolymer approach including stimuli responsive blocks was described by Armes and co-workers, who used various combinations of blocks to induce hydrogel formation. Two pH responsive ABA block copolymers, namely, poly(2-(diethylamino)ethyl methacrylate)-b-poly(2-methacyryloxyethyl phosphorylcholine)-b-poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA-b-PMPC-b-PDEAEMA) and PDIPEM-b-PMPC-b-PDIPEMA, were introduced.\[^{52}\] There, hydrophilic micellar domains were formed at pH above 8 due to the protonation of the amine functional block and elevated concentrations.

In another example, poly(N-isopropylacrylamide)-b-PMPC-b-poly(N-isopropylacrylamide) (PNIPAM-b-PMPC-b-PNIPAM) was utilized.\[^{53}\] The PNIPAM blocks feature a lower critical solution temperature (LCST) behavior, which induces a shift from the coil to the globule state at elevated temperatures. As such, the blocks can be triggered from a hydrophilic to a hydrophobic state leading to micelle formation at temperatures above the cloud point. In the case of an ABA block copolymer, hydrogel formation was observed above 37 °C. The concept was expanded later with various other combinations of polymer blocks, e.g., PNIPAM-b-poly(N,N-dimethylacrylamide) b-PNIPAM (PNIPAM-b-PDMA-b-PNIPAM),\[^{54,55}\] poly(diacetone acrylamide-co-DMA)-b-PDMA-b-poly(diacetone acrylamide-co-DMA),\[^{56}\] PNIPAM-b-PEG-b-PNIPAM,\[^{57}\] or poly(ethoxydi(ethylene glycol) acrylate-co-acrylic acid)-b-PEG-b-poly(ethoxydi(ethylene glycol) acrylate-co-acrylic acid).\[^{58}\] The concept of thermogelation was further expanded toward ABA block copolymers formed from protein central blocks and PNIPAM outer blocks for shear-thinning hydrogels by Olsen and co-workers.\[^{59}\] It should be
noted though that the ABA block copolymer approach is susceptible to network defects via dangling chains and loop formation, i.e., both A blocks from an ABA block copolymer enter the same micelle, which is highly sensitive to polymer concentration.

In contrast to the frequently used amphiphilic ABA block copolymers, more complex ABC block copolymers have been introduced as well, where the hydrophobic blocks, i.e., B and C, do not mix (Figure 2c).[60–62] This concept was used for triblock terpolymers based on nBMA-based, oligoethylene glycol-based and DMAEMA-based blocks, for example.[63,64] As such, the formation of loop-based network defects is suppressed.[65,66] A polypeptide-based ABC triblock terpolymer was introduced by Tirell and co-workers.[67] There, hydrogels formed from ABC triblock terpolymer were studied regarding their erosion stability, which is an important feature for applications like controlled release and cell encapsulation. A significantly lower tendency for erosion was found for the ABC block copolypeptide compared to the corresponding ABA and CBC architectures. Lodge and co-workers studied the formation of micellar crosslinks in hydrogels extensively as well. In particular, the authors studied the ABC triblock terpolymer poly(ethylene-alt-propylene)-b-PEG-b-PNIPAM (PEP-b-PEG-b-PNIPAM) including a thermoresponsive gelation mechanism.[68] The triblock terpolymer contained a constantly hydrophobic polyolefin block, a hydrophilic PEG block and a thermoresponsive PNIPAM block that could be switched from hydrophilic to hydrophobic via heating of the sample. As such, hydrogel formation was induced at elevated temperatures. In further studies, the microscopic features of hydrogel formation were elucidated in detail. Cryo-scanning electron microscopy (cryo-SEM), cryo-transmission electron microscopy (cryo-TEM), and small angle neutron scattering (SANS) were employed to prove the formation of two micelle types via the heat stimulus (Figure 4).[69] SANS measurements confirmed the formation of two types of micelles in the gel state, one for the PEP block and one for the PNIPAM block, as observed by the two distinct scattering peaks at \( q \) of around 0.01 and 0.02 Å\(^{-1}\).

An in-depth investigation on block copolymer-based gel formation was performed by Georgiou and co-workers, who studied twelve different terpolymers with various features, e.g., thermoresponsive and pH responsive.[70] Several effects were observed, \( pK_a \)s and \( T_g \)s were not affected by the block copolymer architecture but by hydrophobicity of the side groups. Cloud points and hence sol–gel transition points correlated with length of alkyl side group and polymer architecture. In a follow up work, the effect of PEG side group length was investigated as well.[71] Overall, it was shown that the sol–gel transition temperature and the transition kinetics can be finely tuned via the block copolymer composition.

More complex architectures were investigated in recent years, for example, even more complex multiblock copolymers were introduced, e.g., pentablock polymers,[72] or linear blocks in the ABC block copolymer were exchanged with bottle brushes to enhance the stiffness of the connecting hydrophilic chain. Johnson and co-workers utilized ring-opening metathesis polymerization (ROMP) to synthesize ABC bottlebrush triblock terpolymers.[73] In particular, exo-norbornene terminated PEG,
of micelle crosslinked hydrogels formed by a copolymerization of acrylic acid (AA) and n-octadecyl acrylate was investigated by Okay and co-workers. Therefore, micellar hydrogels were formed employing SDS as surfactant. Due to the absence of covalent crosslinks the melting temperature of the micellar domains could be utilized to switch to a processable state at elevated temperature, e.g., for introduction into molds. Cooling below the melting temperature and removal of SDS from the hydrogel resulted in a material with permanent shape. The hydrogel materials featured high compressive strength of 90 MPa, a Young’s modulus of 26 MPa and cut damages could be removed via temperature induced healing. A water-triggered shape-memory hydrogel was described by Guo and co-workers, which was achieved by micelle-forming macromonomers that featured acylates as end groups and were copolymerized with AAm. Specific shapes could be formed in the hydrated state that could be recovered after drying and hydration.

A combination of micelle-based crosslinking and another supramolecular mechanism was described by Loh and co-workers. At first, micelles with hydrophobic PLA core and poly(oligo ethylene glycol methacrylate)-b-poly(2-(dimethylamino)ethyl methacrylate) (POEGMA-b-PDMAEMA) shell were formed. In the next step, the oligo ethylene glycol units were crosslinked via complexation with α-cyclodextrin (α-CD) and crystal formation. The hydrogel properties could be varied via external changes of pH or temperature, which affects the PDMAEMA segments or α-CD-POEGA complexes, respectively. A similar design was described by Yao and co-workers, who used the combination of micellar and α-CD/PEG crosslinking for the fabrication of injectable hydrogels. These were loaded with DOX and release of DOX was investigated under physiological conditions. Dong and co-workers combined a polypeptide, namely poly(γ-glutamic acid) (PLG), with PEG as precursor for micellar hydrogels. Again, complexation with α-CD was utilized as crosslinking mechanism in addition to micelle formation. The formed hydrogel structures could be tailored via pH, i.e., due to the polyelectrolyte character of the PLG block either PLG core micelles or PEG/α-CD cores could be formed before gelation.

A metal-complexation-based crosslinking of micelles was described by López and co-workers. Therefore, a triblock elastin-like polypeptide was synthesized comprising of a hydrophobic block for the micelle core, a hydrophilic block for the micelle shell and a short block for Zn²⁺ complexation, which was inspired by metalloproteins. At first, micelles were formed that turned the dispersion into stiff hydrogels after Zn²⁺ addition. Due to the supramolecular nature, the gels featured efficient energy dissipation upon compression as well as self-healing properties. Yang and co-workers presented a hydrogen-bond based hydrogel. The ureidopyrimidone motif was utilized to crosslink micelle cores from hydrophobic alkyl chains (C6 or C8), while the corona was formed from PEG that was endfunctionalized with amino groups. These micelles were further crosslinked via aldehyde endfunctionalized PEG via imine bond formation. As such, a multiresponsive hydrogel was formed, where the micellar crosslinks were not only stabilized by hydrophobic forces but hydrogen bonding as well. The hydrogels featured considerable nonswelling behavior under physiological conditions and a compressive strength of 4 MPa as well as quick self-recovery.
and injectability. Furthermore, good cytocompatibility was observed, where L929 cells showed good viability in the hydrogels for 14 days. Considerable tissue adhesion and degradation was observed in phosphate buffered saline solution over 2 months.

Hydrogel formation of micelle containing dispersions using the micelles as crosslinkers can be performed as well, meaning that micelles are formed in the first place and then the hydrogels are formed using the micellar entities as crosslinkers. A chitosan (CS)-based hydrogel was described by Liu and co-workers that was loaded with micelles from starch derivatives (dialdehyde starch, DAS), grafted with vinyl phenylboronic acid (VPBA) and sulfobetaine (SB) (Figure 5). Due to Schiff-base formation the micelles were incorporated into the CS-hydrogel scaffold. The micelles were loaded with insulin and Nattokinase and could release the cargo depending on surrounding glucose concentration. Studies of in vitro thrombolyis showed effective solvation of blood clots after 30 min of incubation.

Guo and co-workers utilized Pluronic-based micelles as crosslinkers for chitosan. Therefore, Pluronics were end functionalized with aldehyde groups to react with amino groups on chitosan and form hydrogels that were used as antibacterial injectable adhesives. The strategy of Pluronic micelle-based crosslinking of chitosan via the reaction of aldehydes and amines was utilized by Zhang and co-workers as well. In this work, dopamine units were introduced as well to enable interactions with MoS$_2@$MnFe$_2$O$_4$, which was exploited for photodynamic therapy. Another option is to add micellar crosslinking points to an existing covalently crosslinked network, which enables further supramolecular features. For example, Yin and co-workers formed hydrogels from aldehyde functionalized maltodextrin and carboxymethyl chitosan. Furthermore, drug-loaded micelles formed from maltodextrin were embedded loosely or integrated into the network. An injectable formulation was fabricated via injection of both components from different channels at the same time. The drug-release could be tailored by the incorporation mechanism of micelles in the hydrogel, i.e., faster drug release was observed from loosely incorporated micelles and slower release for micelles incorporated in the network. Furthermore, improved mechanical properties were observed for hydrogels with micelles incorporated in the network that act effectively as additional crosslinking points.

It is also possible to include more than one type of micelles into a hydrogel. For example, Matsumura and co-workers formed micelles from poly(l-lysine)-b-poly(l-phenylalanine) (PLys-b-PPhen) and poly(l-glutamic acid)-b-PPhen that were loaded with curcumin and amphotericin B, respectively. Next, the PLys-based micelles were crosslinked via Genipin to obtain a hydrogel network. The hydrogels featured independent and tailored drug release from the different compartments. Zhao and co-workers crosslinked two different types of micelles into one network via temperature induced assembly. Therefore, micelles from poly(e-caprolactone-co-1,4,8-trioxao[4.6]spiro-9-undecanone)-b-PEG-b-poly(e-caprolactone-co-1,4,8-trioxao[4.6]spiro-9-undecanone) and
PEG-b-poly(2-(perfluorobutyl) ethyl methacrylate) were formed. The formation of two different micellar compartments was ensured via utilization alkyl- and fluoro-based hydrophobic moieties and was proven via Förster-resonance electron transfer. PTX and DOX could be loaded and released independently, which was studied in vitro as well as in vivo. In the chemotherapy of the Bacap-37 tumor, superior performance compared to single drug treatment was observed.

The formation of micellar crosslinking points also implies supramolecular features, which enable a dynamic network formation with crosslinking points in an equilibrium state. As such, crosslinking points can be cleaved and reformed leading to self-healing properties as well as degradability. Hsu and co-workers described the synthesis of a self-healing hydrogel based on aldehyde endfunctional Pluronics F127 and CS via imine bond formation. Furthermore, the thermostressive nature of Pluronics was exploited to form micelles in the hydrogel structure at elevated temperature and to tailor hydrogel stiffness on demand. Slater and co-workers described a self-healing hydrogel formed from methacrylic acid (MAA), benzyl methacrylate, and stearyl methacrylate, where micellar domains formed from the hydrophobic monomers acted as crosslinking points. The mechanical properties of the hydrogel could be tailored via polymer concentration and ionic strength. Self-healing was achieved by hydrophobic associations across fractured surfaces without any external intervention.

2.1.3. Vesicles

In addition to micelles, vesicles can be incorporated into hydrogels as well (Figure 2d,e), e.g., liposomes based on lipids, niosomes based on nonionic surfactants, vesicles from cellular origin, vesicles based on ionic surfactants, protein–polymer conjugates or polymersomes based on block copolymers. A feature of vesicular structures is the membrane that separates the interior aqueous medium from the exterior aqueous medium, which contains a hydrophobic barrier. Therefore, permeability of vesicles is a significant property that has to be considered in the choice of vesicle types, especially when cargo release is targeted. In addition, mechanical properties/stability also rely on membrane structure/composition.

Incorporation of liposomes into carbop or hydroxymethyl cellulose hydrogels was described by Antimisiaris and co-workers. The liposomes were loaded with calcine and griseofulvin. The release behavior was tailored via liposome type (i.e., rigidity) as well as matrix hydrogel type. The incorporation of drug loaded liposomes into hydrogels enables another dimension of control over drug loading. Webb and co-workers formed liposomes that were crosslinked by magnetite nanoparticles and embedded in a hydrogel. To achieve crosslinking of the vesicles, the utilized lipids were partially equipped with affinity ligands toward metal ions. Then the formed assemblies were immobilized within an alginate hydrogel matrix. Due to the magnetic features of the assemblies, magnetic field induced pattern-assembly study of the self-assembly process, for example, fluorescent lifetime imaging microscopy (Figure 6b), cryo-TEM, differential scanning calorimetry as well as measurement of critical gelation concentration and sol-to-gel transition temperatures. 1,3,5-Triamide cis,cis,cyclohexane derivatives and dibenzoyl cysteine, bis(leucine) oxalyl-amide, monourea ethyl serine gal- tor, and gemini tartrate surfactant. These LMGWs were combined with phosphatidylcholine-based phospholipids and the assembly process studied. A variety of methods was employed to study the self-assembly process, for example, fluorescent lifetime imaging microscopy (Figure 6b), cryo-TEM, differential scanning calorimetry as well as measurement of critical gelation concentration and sol-to-gel transition temperatures. 1,3,5-Triamide cis,cis,cyclohexane derivatives and dibenzoyl cysteine showed orthogonal self-assembly, while the other examples revealed interactions of both entities going as far as coassembly. The authors found prerequisites for orthogonal interactions of the respective entities which were indicated by high melting temperatures (strong

Figure 6. a) Preferential incorporation of fluorescent phospholipids in liposomes and 1,3,5-triamide cis,cis-cyclohexane derivative LMWG in the self-assembled fibers and b) as evidenced by fluorescent lifetime imaging microscopy based on lifetime discrepancies ($\lambda_{em} = 385$ nm, $\lambda_{em} > 405$ nm, green reflects fluorescence lifetimes below 7 ns, red above 7 ns). Reproduced with permission. Copyright 2016, Royal Society of Chemistry.
interactions), a distinctive set of interactions of both entities and the shielding of hydrophobic stacks of the LMWG by hydrophilic groups.

An example of niosome encapsulation was described by Díaz and co-workers.[116] Therefore, biohydrogels based on mixtures of κ-carrageenan and gelatin were synthesized and encapsulated with niosomes containing resveratrol. Thixotropic and rigid hydrogels were obtained that showed sustained resveratrol release, which could be tuned via the biopolymer formulation. The encapsulation of resveratrol into niosome-hydrogels did not only enable release but also facilitated protection of the drug from photodegradation in order to maintain the biologically active trans-isomer in higher concentration.

Hydrogels with polymersome loading were described by Nolte and co-workers.[117] Poly(styrene)-b-poly(isocyanoolamine(2-thiophene-3-yl-ethylamide))-based polymersomes were combined with hyaluronic acid (HA) hydrogels. The hydrogels were formed via copper(I) catalyzed azide–alkyne cycloaddition from azide and alkyne functionalized HA. Furthermore, enzymes were loaded into the polymersomes to obtain nanoreactors immobilized in the hydrogel matrix. As such, multiple cycles of catalysis reactions could be performed without loss of enzyme on the way. The nanoreactor-in-hydrogel concept could be further advanced to cascade reactions involving multiple enzymes by stacking the corresponding hydrogel scaffolds. Hydrogels containing polymersomes with antibacterial properties were investigated by Du and co-workers.[118] A hydrogel was formed via imine formation from PEG with two aldehyde endgroups and amino groups from CS, as discussed above. The polymersomes were formed from poly(ε-caprolactone)-b-poly(lysine-stat-phenylalanine) (PCL-b-P(Lys-stat-Phen)) (Figure 2f) that were incorporated into the gel network as well via the lysine repeating units. The hydrogels facilitated a dual antibacterial activity via the cation containing polymersome shell but also via loading with penicillin G to combat Gram-positive and Gram-negative bacteria.

Supramolecular crosslinking of β-cyclodextrin (β-CD)-based vesicles was presented by Ravoo and co-workers.[119] Therefore, β-CD-based vesicles were prepared via conjugation of dodecyl chains on one face of the β-CD ring and OEG chains on the other face of the β-CD ring. The vesicles were then crosslinked via host-guest complexion with adamantyl-functionalized hydroxy ethyl cellulose. The mechanical properties of the hydrogels could be tuned via the concentration of the components as well as shear-thinning and self-healing features were shown. Furthermore, due to the supramolecular nature of the crosslinking process the hydrogel could be cleaved via addition of additional guest or host molecules. Mann and co-workers introduced proteinosomes into hydrogels for actuation.[120] Helical Ca$^{2+}$/alginate based hydrogel filaments were loaded with proteinosomes, namely a bovine serum albumin–PNIPAM conjugate, via a microfluidic approach. In order to form actuators, enzymes were introduced into the semipermeable proteinosomes. The enzyme urease in the proteinosomes produced carbonate ions after addition of urea from the outside. As such, a competitive complexation of the carbonate with Ca$^{2+}$ was induced, which lead to elongation of the helical filaments. Control experiments of nonhelical filaments did not reveal elongation. Thus, it was concluded that the decomplexation released mechanical energy, which was stored during the helix formation. The approach was further turned into a reversible process via addition of another enzyme, i.e., glucose oxidase (GOx), acting as an antagonist to urease by producing gluconic acid and releasing Ca$^{2+}$ from CaCO$_3$ upon addition of glucose.

### 2.2. Emulsion Droplet and Coacervate Droplet Compartments

The incorporation of droplets into hydrogels offers a versatile avenue to include specific fluid compartments. Droplet-based compartments can be either based on an emulsion-like approach or coacervate droplets (Figure 7). Emulsions are metastable dispersions that separate into two phases under equilibrium conditions without addition of an appropriate stabilizer. Depending on dispersed phase and dispersion medium, emulsions are classified as oil-in-water (O/W) emulsion, where water is the continuous phase, or water-in-oil (W/O) emulsion.[121] Emulsions of increased complexity, e.g., W/O/W or O/W/O, can be formed by dispersing W/O or O/W emulsions in oil or water.[122] Furthermore, water-in-water (W/W) emulsions are accessible by using two compounds that form immiscible aqueous solutions as well.[123–125] Similar to vesicles, permeability of droplet surfaces is a major feature that needs to be considered with respect to release properties.[126–128]

#### 2.2.1. Oil Droplets/Emulgels

The most frequently described droplet compartment in hydrogels is fluid oil droplets based on O/W or W/O emulsions, which are also called emulgels (Figure 7a).[129–131] In particular O/W emulgels are frequently utilized in drug-delivery especially in dermatology, where the fluid oil droplets contain the drug and the hydrogel makes contact with the tissue that needs treatment.[129,132] As such, bioavailability of hydrophobic drugs can be enhanced considerably, not only the solubility of hydrophobic compounds can be increased that way but also the overall loading. Emulgels are further used in other areas of drug-delivery based on CS, 2-hydroxyethyl methacrylate (HEMA) and NIPAM,[133–136] as well as based on CS or alginate in food.[137,138] In the area of food, the introduction of lipids in the digestive tract further stimulates release of digestive compounds, e.g., bile salts and lipase, to support the emulsification of lipids and their digestion products by transferring lipid molecules to the aqueous phase.[139] Yang and co-workers described the formation of a n-dodecyl emulsion embedded in a hydrogel (Figure 8).[140] Interestingly, the emulsion stabilizer, i.e., 6-((1R,4aS)-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydro-phenanthrene-1-carboxamido)-N,N-dimethyl-hexan-1-amine oxide (R-6-ΑΟ), was used as LMWG at the same time, which can gelate water at concentrations as low as 0.18 wt% via fiber formation. Oil contents could be stabilized in a remarkable volume fraction range from 2% to 98%. Due to the molecular structure of R-6-ΑΟ no inclusion of the oil component was observed in the fiber structure as the surfactant stabilizes the oil droplets via steric stabilization.

Cellulose-based emulgels were employed by van de Ven and co-workers.[141] Therefore, dialdehyde cellulose was synthesized...
Figure 7. Droplet and coacervate compartments in hydrogels: a) emulgel, b) bigel, c) bijel, d) W/W emulsion hydrogel based on dextran/PEG, e) free coacervate droplets, and f) coacervate droplets as crosslinking points.

Figure 8. Top: Structure of R-6-AO. Middle: Cryo-TEM images of R-6-AO fibers in $5 \times 10^{-3}$ m aqueous solution (A), after addition of ethanol (20 wt%) (B), O/W hydrogel emulsion (50 wt% oil) (scale bars 100 nm) (C). Bottom: Confocal fluorescence images of $n$-dodecane droplets in an R-6-AO hydrogel matrix ($20 \times 10^{-3}$ m) with different oil volume fractions after 7 days stabilized by R-6-AO (scale bars: 50 μm, the aqueous phase was labeled with rhodamine B). Reproduced with permission. [140] Copyright 2020, Wiley-VCH GmbH.
and used for hexadecane emulsification. In the next step, CS was added to crosslink with the aldehydes via imine formation. The hydrogel was further used for β-carotene release, where 20% of the lipophilic compound were released at an acidic stomach pH and more than 50% were released in 4h under intestinal conditions. As such, a slow release could be obtained in a system based on polymer materials from sustainable resources, which could be useful for a variety of lipophilic drugs. Martínez-Sanz and co-workers described emulgels based on agar or κ-carrageenan with the target of generating tissue mimics. In the stabilization of ricepseed oil droplets, Agar performed better forming smaller and more uniform droplets. In the Agar hydrogels oil contents up to 50 wt% could be realized that also had a reinforcing effect, i.e., increment of the storage modulus (\(G'\)) was observed for oil loaded hydrogels. In this way, fat/low-water tissue mimicking hydrogels were obtained according to permittivity and conductivity measurements. Recently, Filonenko and co-workers described direct ink writing of an emulgel. Therefore, a Pickering emulsion of sunflower oil in water was formed, which was stabilized by cellulose nanocrystals. Addition of PEG and \(\alpha\)-CD led to gel formation of the continuous aqueous phase. The hydrogels featured shear-thinning behavior that was ultimately exploited to enable direct ink writing.

More complex hydrogel systems loaded with oil droplets can be formed as well. Helgeson and co-workers described the formation of oil loaded hydrogel capsules. The capsules were formed in a multistep procedure. At first, silicone oil was dispersed in an aqueous continuous phase containing PEG diacrylate stabilized via Tween 20 surfactant. The O/W emulsion was further emulsified in cyclohexane to form an O/W/O emulsion. Next, the PEG diacrylate was crosslinked via UV irradiation and the cyclohexane removed to reveal O/W hydrogel capsules with sizes in the nanometer region (30 nm oil droplets in 200–300 nm capsules). In addition, biocompatibility was probed via MTT essay using MDA-MB-231 triple negative breast cancer cells. An oil loaded hydrogel as model for artificial cells was presented by Sapra and co-workers. Bulk hydrogel cubes were formed from agarose, melted and injected with silicone oil/hexadecane mixture stabilized by the lipid 1,2-diphytanoyl-sn-glycero-3-phosphocholine. Subsequently, an aqueous solution was injected to form aqueous droplets inside of the oil phase. These aqueous droplets resided at the edge of the oil droplets just separated from the hydrogel layer via the surfactant membrane. To include artificial cell properties, ClyA membrane pore proteins were included and ion transport studied. Finally, the electrical communication between different aqueous droplets placed at the edge of oil droplets that were separated by a narrow hydrogel layer (100–200 μm) was investigated electrochemically.

A W/O emulsion approach toward compartmentalized droplet hydrogels was described by Bayley and co-workers. Basically, microgels were formed from water droplets stabilized by a lipid dispersed in an oil. A compartment structure was obtained by placing the precursor containing water droplets with a volume of 50 μL next to each other via microsyringe. Adhesion of the droplets occurred via the lipid stabilizer. As such, various droplets with different compositions could be placed next to each other deliberately to obtain a patterned array of droplets. In order to make use of the pattering approach, various stimuli responsive moieties were included in the droplets, e.g., temperature, magnetic or light response. The combination of stimulus and non-stimulus responsive droplets allowed the formation of droplet-derived actuators capable of transporting cargo.

While emulgels consist of a solid matrix hydrogel phase and a fluid oil phase, a compartmentalized system with both phases, oil and water, crosslinked to form hydrogel and organogel can be produced as well. These types of gel are called bigel consisting of a hydrogel phase and a semi fluid organogel phase (Figure 7b). As such, bigels give an additional handle to vary the overall gel properties, as now two structured phases are present, which can be used to tune the rheological and release properties further. A common gelator for the organogel in bigels are stearic acids/steartes that dissolve in oil at elevated temperature and gelate the oil upon cooling. A comparative study of emulgel and bigel was performed by Pal and co-workers. Mechanical measurements of stress relaxation showed a faster dissipation of stress in the emulgel probably due to the more fluid nature. Dewettinck and co-workers described a bigel based on fumed silica in vegetable oil and locust bean gum/κ-carrageenan. It was found that rheological properties of the synthesized bigels were considerably different from the gels formed by the individual components. The bigels featured a comparatively higher strength, while the structure recovery behavior was weaker than in the organogel. The composition of the bigel had a significant influence on the rheological properties, which presents an easy way to tailor the properties of bigels.

In recent years, bicontinuous interfacially jammed emulsion gel (bijel) was introduced that consists of a structurally stable biphasic bicontinuous emulsion and as such of a multicompart-ment structure (not to be confused with bigels) (Figure 7c). The stability of the emulsion is facilitated by jamming nanoparticles at the interface between the immiscible liquids forming the two-phase system during spinodal decomposition. Unlike typical emulsions, the structures in bijels do not undergo coarsening over time due to the rigidity provided by the interfacially jammed nanoparticle layers. A variation in the biphasic domain size can be introduced via tailoring of particle size or concentration. Lee and co-workers described a bijel as environment for enzymatic catalysis. The bijel was formed from water and diethylphthalate and stabilized by silica nanoparticles as well as cetyl ammonium bromide via the solvent transfer-induced phase separation process to form fibber bigels. In particular, bijel was employed to improve the conversion of hydrophobic substrates due to increased interfacial area between hydrophobic and hydrophilic phase. The authors used hydrolysis of tributyrin via lipase as a model reaction in a batch mode and showed a four-fold increase of reaction rate in the bijel compared to a stirred two-phase medium.

Mohraz and co-workers studied bijel rheology in detail. The system water/2,6-lutidine was stabilized by silica nanoparticles, where at first a single phase system was fabricated at low temperature that was turned into the biphasic bijel via heating. The bijels showed gel character in the two-phase region, i.e., the storage modulus (\(G'\)) exceeded the loss modulus (\(G''\)). It was found that stronger bijels were formed at increased concentration of stabilizing particles and deeper temperature quenches. Furthermore, advanced processing of the gels toward microporous scaffolds was investigated, i.e., polymerization/crosslinking of one phase and removal of the remaining non-crosslinked material. The...
The combination of polymerization with bijels enables the formation of scaffolds that are closer to common compartmentalized hydrogels and can be utilized for cell delivery, which was studied by Mohraz and co-workers (Figure 9) as well. Again, the system water/2,6-lutidine/silica nanoparticles was utilized to form the bijel and the aqueous phase crosslinked by employing poly(ethylene glycol) diacrylate (PEGDA). As such, a continuously interpenetrating PEG hydrogel scaffold was fabricated that was further filled with a cell encapsulating fibrin matrix (Figure 9b). Next, the scaffold was loaded with normal human dermal fibroblast (NHDF) cells (Figure 9c) and delivery of the cells to initially acellular fibrin gels studied. Imaging via confocal laser scanning microscopy (CLSM) confirmed radial cell density growth in the surrounding acellular fibrin gel over the course of 8 days demonstrating cell delivery from bijel derived scaffold. A microfluidic approach for the processing of bijels was described by Haase and co-workers. Twisted bijel fiber bundles were formed with pitch lengths from 500 to 2400 μm and helix angles from 10° to 36°. This way soft fibers with elastic moduli below 1 MPa and yield strengths ranging from hundreds of Pa to MPa were obtained. Furthermore, selective polymerization of the network phases enabled formation of high tensile strength fibers.

Another way to form hydrogels via compartmentalized precursors is the utilization of high internal phase emulsions (HIPE). These are emulsions with an internal phase comprising more than 74% of the volume. Crosslinking of the HIPE phases leads to hydrogels with intriguing properties. For example, Silverstein and co-workers synthesized compartmentalized HIPE-based hydrogels from styrene/divinylbenzene and AAm/methylenebisacrylamide (MBA). As such, bicontinuous material was obtained that could be reversibly dried or hydrated. Furthermore, the mechanical properties and pore structure could be tuned via the locus of polymerization (in the aqueous phase, oil phase or in both phases). The same group described the formation of HIPE-based hydrogel employing styrene sulfonate/MBA and 2-ethylhexyl acrylate/divinylbenzene in order to form hydrogels containing elastomeric particles. Furthermore, HIPEs can be employed as template for hydrogels as well, i.e., the oil phase is removed after polymerization to obtain a highly porous hydrogel scaffold.

2.2.2. Water Droplets

In recent years, W/W emulsions have gained increased interest. These emulsions are often based on two different immiscible polymers, e.g., dextran/PEG, guar/amylopectin/gelatin, gelatin/dextran or high molecular weight poly(acrylamides). Commonly such emulsions are stabilized by nanoparticles, i.e., a Pickering emulsion approach is used, which is required due to the low interfacial tension in an aqueous two-phase system (ATPS) as well as the broad interface of both phases. W/W emulsions constitute an ideal option for compartmentalized hydrogels, as they are based on aqueous environments completely. This facilitates a compartment structure without the need of hydrophobic barriers or hydrophobic compounds.

A transition of W/W emulsion-based compartment hydrogels and bijels was introduced by Sabapathy and co-workers. The authors employed a new way of stabilizing the phases via assembly of oppositely charged LUDOX nanoparticles. Depending on mixture composition (ratio of charged particles) and respective polymer concentration (PEG and dextran), a bicontinuous bijel phase or droplets were generated. Both types of droplets (PEG or dextran containing) could be obtained that way. Zhang and co-workers exploited W/W emulsions as template for 3D bioprinting. An ATPS of PEG and gelatin methacryloyl (GelMA) was utilized, where the GelMA phase was loaded with cells. In the subsequent step, the emulsion was printed into a scaffold for cell growth via crosslinking of the GelMA phase. Then, the PEG phase could be removed from the scaffold leaving pores behind. As such, improved cell viability, spreading, and proliferation compared to common hydrogel scaffolds was induced. All-aqueous-based multicompartment hydrogel particles were described by Chen and co-workers. The parent droplets were deposited on a superhydrophobic surface and a
two-step hydrogel formation performed, i.e., gelation of agarose via cooling of heated droplets and gelation of alginate via CaCl₂ addition. Multicompartment structures were obtained by utilizing superhydrophobic tweezers for the placement of gelated droplets into nongelated droplets. In this way, considerable control over droplet structure was implemented albeit at the expense of throughput. Furthermore, the complex droplet structures could be developed into light responsive entities via incorporation of poly(pyrrole).

Rousseau and co-workers formed W/W compartment hydrogels with the gelatin/maltodextrin system. A solution of both polymers (6 wt% respectively) was heated to 90 °C and cooled to ambient temperature, which led to phase separation and gelation. As such, the droplets were not stabilized by particles but held in place via the hydrogel network. The characteristics of the hydrogel structures, i.e., droplet sizes, could be tailored via the thermal treatment (temperature and time). In addition, microorganisms were introduced in the hydrogels as well, which had a profound effect on the phase separation kinetics and phase microstructure. For example, Lactobacillus delbrueckii subsp. Bulgaricus and Saccharomyces Cerevisiae yielded bicontinuous bi jel-like structures, while droplet environments were preserved in the presence of chlorella and spirulina cells. Furthermore, the incorporation of microbes varied the rheological properties of the compartment hydrogel. LMWG were employed by van Esch and co-workers to gelate a W/W emulsion. Similar to the report described above, no stabilizer was used in the emulsion formation. The gelation process itself was employed to lock the droplets in place. As such, multicompartment hydrogel systems comprising dextran/poly(vinylpyrrolidone) (PVP), dextran/poly(2-ethyl-2-oxazoline) and dextran/Ficoll were obtained, where the location of the individual polymer types could be assigned via the respective ratios of the constituting polymers (Figure 10). Most strikingly, the movement of molecules through the hydrogel was studied. For example, fast diffusion of small molecule dyes was observed as well as for the enzyme dextranase. The movement of dextranase was correlated with the hydrolysis activity toward the dextran component of the hydrogel and the related breakdown of dextran-based droplets. Finally, the authors advanced the system further by addition of a third polymer (i.e., PEG) to form an

Figure 10. CLSM images of hydrogels formed from a–c) dextran/poly(2-ethyl-2-oxazoline), d–f) dextran/poly(vinylpyrrolidone) and g–i) dextran/Ficoll ATPS using different volume ratios of the polymer phases (dextran was labeled red with rhodamine B and the gel network green with fluorescein; scale bars: 50 μm). Reproduced with permission. ©2017, Wiley-VCH GmbH.
aqueous three phase system and hence more complex multicomponent hydrogels derived thereof.

An approach to gelate W/W emulsions via α-CD/PEG complex formation was described by Zhang et al. First, an emulsion of dextran-based droplets in PEG-based continuous phase was fabricated via poly(dopamine) particle stabilization. Next, α-CD was added as gelator for PEG in order to form supramolecular inclusion complexes of PEG and α-CD in a rotaxane fashion that crystallize via hydrogen bonds to form a crosslinked network (as described above). Thus, the continuous phase was gelated. The persistence of dextran-based droplets inside of the supramolecular hydrogel was verified via optical microscopy and CLSM with fluorescently labeled PEG. As the network was based on supramolecular interactions it featured dynamic properties and could be altered or cleaved via external stimuli. Hence, a competing guest molecule, i.e., anthranilic acid, was added to the hydrogel that shifted the equilibrium toward anthranilic acid/α-CD complexes due to utilization of excess anthranilic acid and a higher association constant of anthranilic acid compared to PEG. As a second trigger, dilution of the hydrogels was utilized to break the structure as well. Interestingly, the system could be broken in two steps. At first the hydrogel was cleaved due to a shift in the equilibrium via competing guest addition keeping the gelation itself intact. Furthermore, dilution resulted in a complete dissolution of the system.

The introduction of microfluidics into W/W droplet systems offers additional opportunities to improve the homogeneity of the formed droplets or to include additional functionality, e.g., in hydrogel microparticle formation also with complex structures, e.g., Janus particles, bucket-shape, and half-spheres, the fabrication of colloidosomes with hydrogel core or for complex droplet morphologies. As such, increased complexity of W/W droplet-based hydrogels can be achieved via utilization of microfluidics as well, which allow improved control over droplet size and composition. For example, different streams of fluid enable the combination of different polymer types in single droplets. Weitz and co-workers utilized alginate/Ca2+ crosslinking to form alginate-based droplet hydrogels that were loaded with various cargoes. In this example no classical compartments were formed but rather areas in the hydrogel were differentiated via spatial control of cargo loading. Therefore, streams of alginate solution containing different cargo were combined in single droplets in a continuous oil phase via microfluidics and rapidly transferred into aqueous medium after pHE-change induced crosslinking. The formed microgels were loaded with biological cells and also specifically loaded with cells in different compartments for defined cell coculture experiments, i.e., to study cell–cell interactions. Hydrogel microcapsules were described by Ono and co-workers. At first, a W/O emulsion was formed in a microfluidic chip. In due course, the aqueous droplets underwent phase separation leading to a dextran core and tetra-arm-PEG shell. In order to form crosslinked structures, tetra-arm PEG with amino or N-hydroxy succinimide functionality were employed, leading to hydrogel formation via amide coupling. Microgels with PEG shell and dextran core with adjustable size and core/shell ratio were obtained that way.

Multicomponent microgels were described by Qin and co-workers as well. A stream of dextran in water and PEG in water generating droplets of dextran containing phase in PEG containing phase were combined with a continuous oil phase. Crosslinking of the PEG phase was performed via incorporation of sodium alginate and ethylenediaminetetraacetic acid complexed Ca2+. Release of Ca2+ was triggered via acid incorporation in the oil phase. The microfluidic approach allowed incorporation of several droplets with different loading selectively, which was exploited for studies on the coculture of Hep G2 (a human liver cancer cell line) cells and human umbilical vein endothelial cells. Liang and co-workers described the formation of compartmentalized hydrogel fibers in a microfluidic approach. A continuous phase of PEG and alginate was combined with oil droplets and additionally with dextran-based aqueous droplets. In order to form a hydrogel structure and embed the droplets in a stable way, the stream was extruded into a CaCl2 solution leading to immediate crosslinking of the continuous phase. The fibers were used as scaffold for cell culture and as environment for Bacillus subtilis for the application of nitrite removal from sewage. In a similar way, Qin and co-workers fabricated hydrogel fibers loaded with aqueous droplets. Therefore, the dextran/PEG ATPS was employed to form dextran-based droplets in a PEG continuous phase via microfluidics. The outer PEG phase was crosslinked via two streams of PEG solution containing alginate and Ca2+, respectively. The density and distance of dextran droplets could be adjusted via the microfluidic setup. Furthermore, the fibers were used as scaffold for organoid generation. Islet organoids were cultured from pancreatic endocrine progenitor cells from human induced pluripotent stem cells in the dextran droplets. Good cell viability and retention of islet functions were observed by high expression of islet-specific genes and sensitive glucose-stimulated insulin secretion.

2.2.3. Coacervate Droplets

Coacervates are multiphase systems consisting of a phase enriched and a phase depleted from a particular component formed via liquid–liquid phase separation. Most commonly, coacervates are synthesized by complex formation of two oppositely charged polyelectrolytes. These structures play a considerable role in the biology of the cell via the association of charged biopolymers like proteins or nucleic acids. These form organelles that are omnipresent in the nucleus and the cytoplasm of eukaryotic cells, e.g., the nucleolus, stress granules, and germ granules. In recent years, coacervate droplets have been investigated in the direction of compartmentalized water-based structures and in the realm of synthetic cells/protocells. Coacervate droplets can be easily combined with hydrogels forming permeable completely water-based and membrane-free compartments (Figure 7e).

Similar to micelle containing hydrogels, coacervate droplet-based hydrogels can be formed via multiblock polymers. As such, the coacervate domain acts as the crosslinker, e.g., when two triblock polymers are combined that feature a water-soluble middle block and outer polyelectrolyte blocks with opposite charge (Figure 7f). Hawker and co-workers described an intriguing example for this type of coacervate hydrogel. Oppositely charged ionic ABA triblock copolyelectrolytes with a PEG backbone were synthesized containing either sulfate, carbonate, ammonium or guanidinium groups in the A blocks. While ammonium con-
taining polymers did not form hydrogels in combination with sulfonate or carboxylate containing polymers, the combination of sulfonate and guanidinium led to strong hydrogels. The microstructure of the hydrogel could be varied systematically via the B block lengths as shown via small angle X-ray scattering measurements, leading to a shift in the spacing between coacervate domains. Furthermore, the hydrogel properties could be changed via addition of sodium chloride. The system was investigated later on in-depth with respect to the effect of polymer and salt concentration on network structure. The hydrogel microstructure ranged from disordered spheres to body centered cubic (BCC) spheres to hexagonally packed cylinders with increasing polymer concentration. Furthermore, mechanical properties were sensitive to polymer concentration as well, revealing a considerable increase in the modulus upon increasing the polymer concentration as the structure changed from disordered domains to an ordered BCC gel. Nevertheless, a decreased modulus was observed when hexagonally packed cylinders were the main structure. The coacervate hydrogels were robust to salt concentrations up to 0.25 M with a weakening in modulus with increasing salt concentrations. In additional studies, the authors could show a clear correlation between hydrogel microstructure and mechanical properties. The relaxation dynamics of such coacervate hydrogels were investigated by Choi and co-workers. It was shown that the relaxation time is hypersensitive to the length of the charged block, which was attributed to the interfacial energy barrier between the coacervate droplets and the aqueous hydrogel medium, even though low interfacial tension is present (∼1 mN m⁻¹). Klinger and co-workers used similar block copolymer precursors to form coacervate-based microgels. Microfluidics were employed to form water-droplets in a continuous oil phase that contained the hydrogel precursors as well as cargo molecules. The release of payloads could be tailored by the microgel size.

Another example of a triblock copolymer based coacervate hydrogel was described by Voets and co-workers. An ABA triblock copolymer was synthesized containing a central PEG and outer poly(potassium sulfopropyl methacrylate) blocks that was combined with poly(allylamine hydrochloride). As such, hydrogels were formed by association of both polymers in complex coacervate micelles that could be tuned by pH, concentration, temperature, and ionic strength. A CO₂ responsive coacervate hydrogel was described by Zhao and co-workers. Therefore, PMAA-b-PEG-b-PMAA was combined with PDMAEMA-b-PEG-b-PDMAEMA that formed complex domains depending on solution pH. In order to form a hydrogel, the balance between positive and negative charges as well as the polymer concentration had to be adjusted carefully.

The theoretical background of electrostatically crosslinked hydrogels was investigated by Holm and co-workers. Two types of the charged block, which was attributed to the interfacial energy barrier between the coacervate droplets and the aqueous hydrogel medium, even though low interfacial tension is present (∼1 mN m⁻¹). Klinger and co-workers used similar block copolymer precursors to form coacervate-based microgels. Microfluidics were employed to form water-droplets in a continuous oil phase that contained the hydrogel precursors as well as cargo molecules. The release of payloads could be tailored by the microgel size.

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of four arm star polymers with oppositely charged end blocks were investigated. It could be shown that too high salt concentrations hinder hydrogel formation due to steric repulsions in the polymer-rich phase and charge screening. Furthermore, the gelation relied strongly on the solution pH and the pKa of the polyelectrolyte blocks.

Coacervate droplets that are not formed from block copolymers can be included in hydrogels as well and they do not have to be the primary crosslinking points.\[217] Especially, biomedical applications have attracted interest for this type of hydrogel. For example, Davies and co-workers described the formation of degradable hydrogels as matrix for coacervates loaded with growth factors [Sonic hedgehog and cytokine interleukin-10] for preservation of heart function after myocardial infarction.\[218] A PEG-based hydrogel was formed by Michael addition between vinyl sulfone groups on derivatized PEG and thiols from bis-cysteine matrix metalloproteinase-1-sensitive peptides. These hydrogels were loaded with coacervates consisting of poly(ethylene arginylaspartate diglyceride) (PEAD) and heparin. The task of the hydrogel was the retention of coacervates in the heart wall, while coacervates led to improved hydrogel integration. As such a combined effect was obtained to achieve tissue repair in the infarction region. A similar goal was targeted by Wang and co-workers, who utilized protein loaded coacervates for cardiac treatment after infarction.\[219] For coacervate formation PEAD and heparin were utilized again that were loaded into a fibrin hydrogel matrix. As growth factors, fibroblast growth factor and stromal cell-derived factor 1-alpha were included in the coacervates, while metalloproteinase-3 (TIMP-3) was included in the fibrin hydrogel. As such, a two-phase treatment was achieved, where the TIMP-3 reduced matrix degeneration as well as scar expansion and the growth factors supported long-term tissue repair.

Cartilage regeneration was investigated by Kim and co-workers utilizing a coacervate of PEAD and heparin in an interpenetrating network matrix formed from thiolated gelatin/PEGDA.\[220] Insulin-like growth factor-1 was introduced into coacervates and adipose-derived stem cells were embedded in the hydrogel matrix. As such, a platform for osteochondral tissue regeneration was developed that benefited from the mechanical properties of the hydrogel for a long-term transplantation and the growth factor/stem cell combination for tissue regeneration. Another example was described by Alsberg and co-workers, who embedded coacervate droplets into hydrogels for growth factor delivery.\[221] For the coacervate formation, oxidized methacrylated alginate was combined with GelMA leading to coacervate droplets. In the next step, coacervates were formed directly in the presence of human mesenchymal stem cells and human bone morphogenetic protein-2 followed by photocrosslinking of the methacrylate groups. The protein growth factor partitioned into the coacervate droplets and was slowly released to the hydrogel environment. As such, sustained release of the growth factor and a growth medium for the cells was obtained in one step.

An enzyme based hydrogelation system was introduced by Liu and co-workers.\[222] Therefore diethylaminoethyl (DEAE)-dextran and double stranded (ds) DNA were combined to form a coacervate that was loaded with GOx and CaCO₃ powder. These coacervates were dispersed in sodium alginate solution. Finally, glucose was added and oxidized to gluconic acid. Gluconic acid in turn led to the dissolution of CaCO₃ powder and the diffusion of Ca²⁺ ions out of the coacervate droplets. Subsequently, the presence of Ca²⁺ induced alginate hydrogel formation and immobilization of the coacervates similar to extracellular matrix formation. A modular hydrogel microreactor based on enzymatic catalysis was described by Mann and co-workers.\[223] DEAE-dextran and dsDNA were combined to form coacervate droplets and then embedded in agarose hydrogels (Figure 12). Furthermore, a two-reaction cascade was localized inside of the compartment structure. Coacervate droplets were loaded with GOx, horseradish peroxidase (HRP) and Amplex red. Addition of glucose triggered the cascade reaction via formation of H₂O₂, which then led to HRP mediated oxidation of Amplex red to resorufin. Most notably, multilayer hydrogels were formed that contained coacervate droplets in the layers with spatial control (Figure 12). This concept was further advanced with photocatalysis to perform another cascade reaction (Figure 12g). An input module was produced that contained coacervate droplets containing photocatalysts (TiO₂/Ag) that formed H₂O₂ upon UV irradiation and downstream diffusive flow into the output module. There, subsequent formation of a red fluorescence readout took place via resorufin formation from Amplex Red with G4-hemin catalyst.

### 2.3. Multilayer Hydrogels

Multilayered hydrogels can be considered as multicompartiment structures as well (Figure 13). This type of hydrogel covers a broad range of morphologies and applications,\[224] e.g., in thin film layer-by-layer hydrogels,\[225,226] in microgels,\[227] in actuators,\[228,229] and biomedical applications.\[230,231] One of the challenges with multilayer hydrogels is the interconnection between individual layers, which depends considerably on the gelation mechanism. Supramolecular crosslinking enables layer connection by placing gel materials next to each other. In the case of covalent crosslinking, layer connection is more challenging to achieve. For example, in the case of free radical polymerization-based crosslinking, a careful addition of a second polymerization solution at the right time can lead to a covalent connection between the gel layers. One of the main features of multilayer hydrogels is the high permeability between layers, as only porous hydrogel layers are connected without a membrane and the like in between.

The area of thin film multilayer hydrogels from layer-by-layer deposition of oppositely charged polyelectrolytes has found considerable attention in the past years. Such multilayers have been employed as template for thin-layer hydrogels, where one of the layers was crosslinked after deposition of a thin film and removal of the other polymer layer, as shown with the systems PVP/PMAA,\[232,233] poly(vinylcaprolactam)/PMAA\[234,235] or both.\[236] The combination of PV and PMAA with subsequent crosslinking was later also employed to form capsules with thin crosslinked polyelectrolyte complex shell.\[237] Trau and co-workers used an agarose microbead as template for coating with poly(allylamine) (PA) and poly(styrene sulfonic acid) via layer-by-layer process.\[238] The complexation of both polymers could be used to form hydrogel-like shells with tailored permeability. In a similar way, Shi and co-workers formed multilayer capsules from HA and CS starting from a CS core.\[239] Vilas-Vilela and co-workers formed thin films from polyelectrolyte complexes be-
Figure 12. Three-level stack prepared from sequential Ca^{2+}-mediated crosslinking of three alginate/agarose hydrogel modules (fluorescein labeled-GOx-loaded DEAE-dextran/dsDNA coacervate droplets (green), N-acetylcysteine-capped-CdTe quantum dots-loaded DEAE-dextran/dsDNA coacervate droplets (yellow), cyanine-5-DNA-loaded DEAE-dextran/dsDNA coacervate droplets (red)): graphic shows the a) idealized 3D arrangement of the stack, and b,c) optical and fluorescence microscopy images. Scale bars = 10 mm. d) Scanning confocal fluorescence microscopy image of the adhesion interface (dotted white line) between modules 1 and 2. The imaged area is denoted by the small rectangular white box shown in (c). Scale bar = 100 μm. e) Fluorescence microscopy images recorded 12 h after immersion of a linear stack of modules 1, 2, and 3 in water in the dark showing no changes in fluorescence intensities. Scale bar = 10 mm. f) As for (e) but in the absence of entrapped coacervate droplets showing ≈37% decrease in fluorescence intensity at the boundaries of the modules due to interzonal diffusion. Scale bar = 10 mm. Photocatalytic/peroxidation serial processing under flow conditions. g) Graphical representations of a linear arrangement of two hydrogel-based droplet microreactor modules connecting a photocatalytic/peroxidation cascade under flow conditions, and details of the upstream input TiO_{2}/Ag nanoparticles module, and downstream output C4-hemin module. Reproduced with permission. [223] Copyright 2020, Wiley-VCH GmbH.
Hydrogels consisting of AA, AAm, and MBA as well as adamantane/CD content and the extent of actuation was controlled by the time used for the polymerization reaction. Furthermore, nanoparticles and small molecule cargo could be embedded into the hydrogel layers. Lin and co-workers described the growth of a second hydrogel initiated from a first layer.[252] A thiol–ene-based click chemistry was used to form a covalently crosslinked hydrogel via multiple photopolymerization steps.[247] HEMA and diethylene glycol dimethacrylate were combined to form the multi-layer hydrogels. In this way, two processes happened: Diffusion of Ca^{2+} led to the formation of an alginate hydrogel layer, while diffusion of the photocrosslinker in the presence of UV light led to permanent crosslinking of GelMA. The time of the immersion/crosslinking process defined the hydrogel layer thickness. For example, removal of remaining non-crosslinked GelMA afforded hydrogel tubes with alginate outer and GelMA inner layer. An enzymatic-based polymerization to form multilayer hydrogels was used by Bowman and co-workers.[251] GOx was used to perform a redox reaction on the glucose molecules. This reaction was used to trigger the polymerization of the alginate solution. The authors formed a hydrogel tube and then polymerized additional layers on the surface of the cylinder. The layer thickness could be controlled by the time used for the polymerization reaction. Furthermore, nanoparticles and small molecule cargo could be embedded into the hydrogel layers. Lin and co-workers described the growth of a second hydrogel initiated from a first layer.[252] A thiol–ene-based crosslinking mechanism was used to form the first hydrogel via light mediated initiation with eosin-Y. Addition of further precursor solution around the first layer enabled further gelation due to diffusion of the initiator from the first layer. The layer thickness could be adjusted via irradiation time, initiator concentration, and monomer concentration.

A multilayer hydrogel formed via strain promoted azide–alkyne cycloaddition (SPAAC) was described by Kloxin and co-workers.[255] Therefore, eight-arm PEG functionalized with dibenzylcyclooctyne was combined with homotelechelic azide functionalized PEG. Multilayers could be formed in a layer-by-layer approach, where the different layers were loaded with different fluorescent proteins and in part also with azide functionalized proteins in order to achieve immobilization. Another avenue to multilayer hydrogels is open channel microfluidics as shown by Berthier and co-workers.[254] Open microfluidics were used to form multilayer hydrogels in a layer-by-layer approach. In such process, light intensity, monomer concentration, and cargo concentration were varied to introduce a gradient in mechanical properties and cargo loading. Finally, the gels were studied regarding cell culture and the migration of cells along the gradient in the hydrogel. A combination of electrospinning and photopolymerization was introduced by Koh and co-workers (Figure 14).[249] At first several layers of PCL fibers were formed via electrospinning. Next, the whole fiber mat was covered with PEGDA/photoinitiator solution and the solution covered with a photomask. After UV irradiation, hydrogel monoliths were formed in uncovered areas, while bare PCL fibers remained in the covered area that could be washed away with solvent. Finally, patterned hydrogel monoliths were obtained that contained layers of PCL fibers. Furthermore, the PCL fiber layers could be loaded with various compounds, e.g., quantum dots or antibodies.
Figure 14. a) Overview for the preparation of multicompartmental hydrogel microparticles via sequential electrospinning and photopatterning and b) CLSM image of three-compartment hydrogel microparticle (scale bar: 200 μm). Adapted with permission. Copyright 2015, Wiley-VCH GmbH.

a way, patterned hydrogels could be formed via designer shaped rails above the previous hydrogel layer that held the gelation solution in place via capillary forces. After gelation the next layer could be added via a rail with different geometry to add a different shape on top.

Mechanical properties belong to the most important properties of hydrogels, first of all to prove that a hydrogel was synthesized at all but also to have a material ready for the specified application. Adams and co-workers studied mechanical characterization of multilayer hydrogels and 3D printed hydrogels in detail. Three-layer hydrogels based on LMWG were formed with varying LMWG concentration in each layer. In particular, the difference between vane and plate/plate geometry in rheology measurements was investigated. It was shown that plate/plate geometry measurements are often dominated by the top layer, while vane measurements take all layers into account but also the contribution of each layer can be investigated. The study further showed that preparation via 3D printing had a significant effect on the hydrogel properties, which was attributed to the extrusion process. One of the target biomimetic structures for multilayer hydrogels is cartilage that has excellent mechanical properties. Zhou and co-workers worked on bilayer hydrogels to mimic high load capacity and lubricity of cartilage. First a hard hydrogel layer was formed by free radical polymerization of AA, AAm, and MBA that was further reinforced by complexation of AA moieties with Fe³⁺. Furthermore, an atom transfer radical polymerization initiator containing monomer was included in the first layer, which was subsequently used to graft potassium 3-sulfopropyl methacrylate or 2-(methacryloyloxy)ethyl(dimethyl(3-sulfopropyl) ammonium hydroxide on top of the hard hydrogel layer. The monomers diffused into the hard hydrogel layer leading to functionalization of the upper part of the hard layer as well. Low friction was observed for the top layer (friction coefficients below 0.025), which stayed in the low friction regime even with loads up to 10 MPa. In a similar way, Zhang and co-workers formed cartilage-inspired multilayer hydrogels from poly(vinyl alcohol) (PVA), HA, and PAA. An anisotropic multilayer system based on cellulose was presented by Jeon and co-workers. Therefore, anisotropic cellulose gels were formed by alignment of cellulose chains in organic solvent swollen cellulose gel films under shear stress. In the next step, cellulose gel films could be combined in a “welding”-like process. Films could be combined in various ways to tailor the mechanical properties, e.g., with orthogonal or parallel cellulose orientation. A multilayer hydrogel formed by adhesion of single Ca²⁺/alginate-based layers was presented by O’Reilly and co-workers (Figure 15). The authors studied the effect of poly(l-lactic acid) (PLLA) particles and their shape on mechanical properties of the individual hydrogel layers and on the adhesion between different layers. The adhesive energy differed significantly with particle shape, size and charge, e.g., platelets, small particles and cationic particles showed the strongest adhesive effect. Most likely, the platelet shape gave the best opportunity for the gel building blocks to connect due to the planar surface. Smaller particles were probably more effective due to a better interaction with the contours of the gel blocks, while cationic particles likely had an improved interaction with anionic alginate.

As with other hydrogel materials, the fabrication of multilayer hydrogels has been frequently performed via 3D printing. For example, Chang and co-workers utilized hydroxybutyl chitosan to form multilayered hydrogels. The crosslinking was performed in salt solution, which shifted the LCST of the polysaccharide and rendered it insoluble. In addition, the mechanical properties could be tailored with salt type and concentration.

Certainly, the implementation into bioapplications has been a focus in recent years with the utilization of multilayer hydrogels in bioprinting. Bashir and co-workers used stereolithography to form cell laden multilayered hydrogels. As monomer, PEGDA was employed to form multiple layers that were loaded with different cell types, e.g., NIH or 3T3. Mechanical properties of the formed gels could be varied via the molecular weight of the PEG-based monomers. A hydrogel containing fluidic channels was developed by Yoo and co-workers. A collagen hydrogel...
precursor was utilized as main phase, printed and subsequently crosslinked via sodium bicarbonate solution. Furthermore, gelatin was introduced into the printed structure as well to act as sacrificial phase for the fluidic channels. The scaffold was printed layer-by-layer in order to obtain a 3D hydrogel structure with internal substructuring. To study bioprinting and cell growth, dermal fibroblasts were introduced in the printing process and cultured in the scaffold, where the removal of the gelatin introduced channels for nutrient and oxygen transport. Duncan and co-workers fabricated a supramolecular multilayer hydrogel.\textsuperscript{[263]} Therefore, a polypeptide grafted with short DNA units was synthesized and crosslinked with complementary DNA strands in a 3D printing process. Next, hydrogels were printed including AT-20 cells in a multilayer approach. Due to the supramolecular crosslinking approach, the hydrogels showed self-healing properties and no boundaries between hydrogel layers were observed. A thiol–ene-based bioprinting approach was introduced by Zimmermann et al.\textsuperscript{[264]} The main component forming the hydrogel were 4 arm-PEGs with thiol and maleimide endgroups. Furthermore, maleimide-functionalized heparin was introduced to provide cell-instructive environment, while the pure PEG environment repelled cell adhesion. In such a way, cell growth could be localized in the hydrogel scaffold with high precision. The local hydrogel properties could be adjusted via the ratio of PEG to heparin in order to have additional control over cell fate. Projection-based bioprinting was performed by Kloke and co-workers.\textsuperscript{[265]} Notably, an HA-based bioink was developed that featured enzymatic degradation. Multiple bioinks, e.g., mixtures with PEGDA, GelMA, acrylated HA, and methacrylated HA, were utilized to form a compartmentalized system capable of forming vascular functions to support cell growth. As such, composite 3D scaffolds were formed from several digestible materials that could be individually removed after printing and even in a timed fashion.

Figure 15. Adhesion properties of Ca\textsuperscript{2+} alginate hydrogel blocks with each other containing PLLA-based nanoparticles of different morphologies: a) schematic representation, b) picture of connected hydrogel blocks with PLLA-b-PDMAEMA platelets (scale bar = 0.5 cm), c) bulk shear stress behavior comparing representative interfacial stresses for different particle shapes, d) adhesive energy of bulk shear of two Ca\textsuperscript{2+} alginate gel blocks adhered with water (control), platelets, cylindrical, or spherical micelles, e) schematic representation of end-to-end and bulk shear adhesion experiments performed with dynamic mechanical analysis to quantify adhesion, f) effect of size on adhesive energy (small: 372 × 223 nm, medium: 893 × 528 nm, and large: 1700 × 993 nm), and g) effect of charge on adhesive energy.\textsuperscript{[259]} Reproduced under the terms of the CC-BY license.\textsuperscript{[259]} Copyright 2020, the Author(s). Published by Springer Nature.
2.4. Morphologies of Compartmentalized Hydrogels

In addition to the type of compartments, various shapes of hydrogels can be applied leading to further complexity, e.g., bulk hydrogels, hydrogel particles or thin film hydrogels (Figure 13). Some of these morphologies have been mentioned in the previous sections. Nevertheless, the next section will summarize the common morphologies of compartmentalized hydrogels to give a general overview.

2.4.1. Compartmentalized Bulk Hydrogels

One of the most common hydrogel morphologies are bulk hydrogels, for example in the shape of cylinders or cubes (Figure 13a). These bulk hydrogels are macroscopic objects and can be handled easily as such. Specific shapes of hydrogels can be formed via designed molds or via photomasks. An avenue toward compartmentalization of bulk hydrogels is via post gelation photopatterning, i.e., a bulk hydrogel is modified internally via light irradiation. West and co-workers described such an approach. A PEGDA-based hydrogel was patterned with acryloyl-PEG conjugated to Arg-Gly-Asp (RGD) peptides via a photomask. The patterned structures were then used to guide cell adhesion. A reversible patterning approach was investigated by Anseth and co-workers, who introduced RGD peptides into a hydrogel matrix with spatial control. At first, a hydrogel was formed via SPAAC of a 4 arm star PEG end functionalized with tetracyclooctyne and a bis(azido) polypeptide conjugated with allyl groups. The allyl handles were then used for attachment of RGD peptides via photoinduced thiol–ene conjugation under visible light. Notably, the thiol functionalized RGD peptides were endowed with a 2-nitrobenzyl ester group between peptide and thiol. As such, the peptides could be conjugated under visible light and cleaved under UV light in a spatially controlled fashion. Thus, the bulk hydrogel could be compartmentalized on purpose for controlled cell adhesion.

A mold-based formation of a compartmentalized organ-on-a-chip was described by Stefanini and co-workers. A lymph node microenvironment from collagen-based hydrogel was developed, which was basically a microfluidic device that replicates the flow characteristics of the lymph node. Various immune cell types were introduced with an in vivo-like spatial distribution, i.e., compartments, to simulate the lymph node. Another frequently used way to form compartmentalized bulk hydrogels is 3D printing. Malda and co-workers printed specimens containing areas with various bioinks for tissue formation as well as areas for mechanical support. In such a way, cell cocultures were formed giving rise to a layered distribution of collagens and glycosaminoglycans. The combination of articular cartilage-resident chondroprogenitor cell, bone marrow mesenchymal stromal cell and chondrocyte containing bioinks enabled to model articular cartilage with defined superficial and deep regions each with distinct cellular and extracellular matrix composition. He and co-workers utilized 3D printing via an injection process to form complex models of organs based on PEGDA and AAm as well as Carbopol microgels. For example, liver cancer models, human brain models or kidney models were fabricated. As such, models were formed for training of surgeons or simulation of surgeries.

2.4.2. Compartmentalized Thin Film Hydrogels

Thin hydrogel films comprise another frequently utilized morphology (Figure 13b) that is particularly interesting for applications in sensing and lubrication. A micelle-based hydrogel film was described by Kataoka and co-workers. Micelles from PEG-b-PLA were crosslinked with poly(allylamine) in thin films formed in a layer-by-layer process. To crosslink the structures, aldehyde functional micelles were formed and reacted with poly(allylamine) via reductive amination. Protein adsorption studies showed a controlled adsorption behavior depending on the top layer (micelle or poly(allylamine)). In addition, the hydrophobic PLA micelle cores were utilized for cargo loading. A combination of polymersomes and PVA hydrogel thin films was described by Städler and co-workers. The polymersomes were loaded with a small payload and included before the hydrogel formation in a salting out approach. Microtransfer molding was applied to yield surface-adhered microstructured hydrogel films with polymersome loading. Finally, the hydrogel film was coated with poly(dopamine) to improve adherence of myoblasts.
and the effect of cytotoxic payload on myoblast metabolic activity was probed. Fang and co-workers described the formation of hydrogel films containing liquid crystal loaded oil droplets.[281] Chitosan hydrogel films were formed by gelation with Ag⁺ in the presence of the surfactant tetradecyl sulfate sodium salt. The hydrogel was embedded with droplets formed by the liquid crystalline molecule 4-cyano-4´-pentylbiphenyl. Finally, the hydrogel films could be applied as selective sensors for bile acids in an easy label-free approach.

### 2.4.3. Compartmentalized Hydrogel (Micro)particles

The formation of microgels or nanogels enables dispersion of gel materials for a vast number of applications (Figure 13c), e.g., catalysis or cargo delivery.[282–285] Thus, hydrogel particles have attracted considerable attention in the past.[16] An incoacervate microgels as protocells.[293] At first, coacervated droplets of proteins into the microgels depending on protein size and shape. Furthermore, the gel network was conjugated with small molecules enabling the generation of defined multicore microgels. Rogach and co-workers utilized a microfluidic electrospray approach to synthesize compartmentalized microgels for cargo delivery.[290] Alginate-based hydrogels were formed from two precursor solutions in order to separate incompatible drugs/entries in different compartments and control their release, as shown for sample compounds methylene blue and tetracycline hydrochloride or CdTe quantum dots and poly(ethylene imine) (PEI) quencher. A microgel system based on alginate and chitosan was described by Xue and co-workers.[291] Emodin loaded micelles were formed from chitosan/disodium glycerol 2-phosphate complex and embedded into alginate hydrogel beads. Finally, pH dependent release was studied.

A core–shell microgel was investigated by Choi and co-workers.[292] The authors used a microfluidic triple emulsion approach including a sacrificial oil layer to separate two PEG-based hydrogel compartments. The emulsion technique allowed for a tailored shell and core–prepolymer composition. As such, uniform and tunable shell dimensions were available. Furthermore, the gel network was conjugated with small molecules and proteins, which was utilized to study the diffusion kinetics of proteins into the microgels depending on protein size and PEG–shell density. Liu and co-workers described coacervate-in-coacervate microgels as protocells.[293] At first, coacervate droplets were formed from poly(diallyldimethyl amonium chloride) and DNA, which were then coated with DEAE-dextran and CM-dextran in a layer-by-layer process. The structure was then used as environment for enzymatic catalysis. In order to show the effect of the compartment structure, two cases were investigated (Figure 17). In the first case, the core was loaded with HRP, GOx, and CAT (catalase) (case I). In the second case, the core was loaded with HRP as well as GOx and the shell was loaded with CAT (case II). Next the catalytic activity for the cascade reaction from glucose to gluconic acid/H₂O₂ and oxidation of Amplex Red to resorufin was monitored. CAT and HRP compete in the catalysis process, which leads to an enhanced activity of the HRP-based process in the case of CAT-loaded shell due to the physical distance of CAT and GOx (case II). As such, the catalysis process could be tailored via spatial control of enzyme placement in different compartments.

### 3. Applications

In the following sections some of the most frequently used applications of compartmentalized hydrogels are presented (Figure 18). The applications, i.e., drug delivery, cell culture, actuators, sensing, energy, and catalysis, are underpinned by illustrative examples.

#### 3.1. Drug Delivery

One of the most promising applications of hydrogels is drug delivery, which is mainly due to their water-based nature, biocompatibility, high porosity, and tissue like behavior.[294,295] As indicated in the previous sections already, compartmentalization endows hydrogels with a higher level of complexity for the release behavior (Figure 19a), e.g., when multiple drugs are encapsulated[296] or layers with different permeability are combined.[297,298] Voit and co-workers described the formation of an NIPAM/MBA/AA-based hydrogel incorporating a boronic acid monomer that was loaded with dendritic maltose endfunctionalized PEI.[299] The maltose units formed complexes with the boronic acids leading to immobilization of the maltose-PEI particles in the hydrogel. Furthermore, the maltose-PEI particles were loaded with adenosine triphosphate. Depending on pH either the small molecule could be released from the gel or the dendritic carrier loaded with the small molecule drug. A PVA-based hydrogel that used the formation or boronic esters of borax and hydroxyls of PVA as crosslinking points was described by Park and co-workers.[300] The structure was loaded via HEMA-based micelles obtained by free radical polymerization before gelation with borax. Furthermore, the hydrogel was loaded with Nile Red. The PHEMA micelles had two effects on the gel properties. On the one hand, PHEMA micelles acted as stress absorber and plasticizer improving the mechanical properties of the hydrogel. On the other hand, the PHEMA micelles improved the cargo release of Nile Red upon swelling of the hydrogel and expulsion of micelles in a controlled manner.

A supramolecular approach for hydrogels with drug-delivery applications was followed by Li and co-workers, who combined PEG-b-PCL with α-CD.[301] The PCL blocks acted as hydrophobic core for micelles and as container for the drug Diclofenac, while PEG and α-CD formed inclusion complexes for gel formation. In the course of the project in vivo tests were performed to prove the biocompatibility of the hydrogels in ocular environment and bioavailability on the corneal surface in a rabbit model was investigated. Gong and co-workers formed a hydrogel from PEG-b-PCL-b-PIC micelles.[302] The hydrogel was formed at temperatures around 40 °C and polymer concentrations above 20 wt%. Furthermore, the micelles were loaded with Dexamethasone, which could be released in a controlled manner. In vitro
studies showed high biocompatibility of the hydrogels and in vivo studies revealed a slow degradation of the hydrogels over 20 days. In a recurrent tissue adhesion animal model, the expected anti-tissue adhesion effect stemming from the Dexamethasone drug was observed.

An injectable hydrogel formed from PNIPAM-grafted alginate was described by Li and co-workers. At a concentration of 7.4 wt% and body temperature, the polymers formed a hydrogel, which incorporated PNIPAM micelles that could be loaded with DOX. A slow and sustained release of the drug was observed as well as cellular uptake of the drug into multidrug resistant AT3B-1 cancer cells. Duvall and co-workers described a reactive oxygen species (ROS)-sensitive release (Figure 19). Therefore, poly(propylene sulfoxide)-b-PDMA-b-PNIPAM (PPS-b-PDMA-b-PNIPAM) was synthesized by a combination of anionic and RAFT polymerization. The PPS block formed micelle cores in an aqueous environment, while the PNIPAM block was introduced to form hydrogels upon heating above the LCST to crosslink the polymers via additional micelle formation. The block copolymer micelles were loaded with Nile Red as model cargo and ROS-induced release was studied. The hydrogels showed minimal in vitro toxicity and in vivo studies of subcutaneously injected hydrogels showed a sustained release over at least 14 days. The same group enhanced the portfolio of triblock terpolymers later, e.g., PCL-b-PDMA-b-PNIPAM, and PLGA-b-PDMA-b-PNIPAM were investigated for hydrogel formation and stimulus induced drug delivery. In a similar way to before, drug containing micelles were formed at first that could be further transformed into hydrogels via heating above the LCST of PNIPAM, i.e., when heated to body temperature. In addition to the oxidation sensitive PPS block, hydrolytically susceptible blocks were introduced that could be degraded via changes in pH or enzymatically.
Schneider and co-workers formed a peptide-based fibrillar hydrogel and loaded it with Erlotinib (ERL), an epidermal growth factor receptor inhibitor, and with vesicles containing DOX.\textsuperscript{306} The system was designed in a way to deliver ERL and DOX sequentially, which was facilitated via the additional membrane barrier imposed by the vesicle for the release of DOX into the gel matrix before delivery could take place. As such, improved overall drug efficiency was achieved. In addition, the hydrogel material was injectable and showed an excellent synergistic effect of both delivered drugs on glioblastoma cells. An emulsion capable of drug release was described by Boekhoven and co-workers.\textsuperscript{307} The innovative part was the dissipative and dynamic assembly of the droplets in the hydrogel. The droplets were formed from succinic acid precursors that reacted with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to form a reactive O-acylurea intermediate, which reacted with a second carboxylic acid in the molecule to form a hydrophobic anhydride leading to oil droplets. This anhydride hydrolyzed in the presence of protons, e.g., from other succinic acid precursors. As such a cycle between precursors and oil molecules was established that relied on EDC as fuel to enable ongoing oil formation. The hydrophobic oil droplet compartment formation and duration of droplet presence could be tailored precisely, i.e., by the addition of specific amounts of EDC. Finally, an agar-based hydrogel was formed as matrix for the oil droplets and the oil droplets were loaded with model drugs. As such, zero-order release could be implemented and the release period of drugs controlled.

3.2. Cell Culture

Another direction of biomedical application of multicompartiment hydrogels is cell culture, which is a common application of hydrogels in general (Figure 18b).\textsuperscript{308,309} Compartmentalization enables an additional level of control in the cell culture process, e.g., regarding cell cocultures or the combination of various mechanical properties in one scaffold. Ma and co-workers synthesized compartmentalized hydrogel particles with various structures, e.g., core–shell, Janus or triple layers.\textsuperscript{310} The hydrogels were based on alginate, incorporated with extra cellular matrix and could be loaded with various cell types on demand. The authors showed culturing of small intestinal organoids as well as size-controlled tumor microtissues with the hydrogel particles. The coculture of a hepatocyte/stroma system showed an improved hepatocyte viability due to the presence of stromal cells. Zhao and co-workers used all-aqueous microfluidics to synthesize multicompartiment microgels for cell encapsulation.\textsuperscript{311} Core–shell microgels were formed in an aqueous multiphase system with PEG in water as continuous phase, alginate/dextran in water as shell phase and cells loaded in a CMC solution in water as core phase. During the culturing process 3D cell aggregates were formed with high cell viability. The approach allowed the performance of microfluidic derived 3D cell culture without the need of oil removal. A bacteria culturing approach was described by Alper and co-workers.\textsuperscript{312} Therefore, a bioprinter was used to print hydrogels containing bacteria, e.g., containing \textit{E. coli} or yeast, namely, \textit{S. cerevisiae}. These hydrogels could be lyophilized and rehydrated to allow a facile bioprocessing. The compartmentalization of the hydrogels to obtain areas with yeast or \textit{E. coli} enabled bacteria growth of each species without impeding the other. Even more so, a consortium of bacteria was formed to perform a synthetic task. An \textit{L-3,4-dihydroxyphenylalanine} (\textit{l-DOPA}) producing \textit{E. coli}-laden hydrogel was fabricated next to a yeath-laden hydrogel capable of converting \textit{l-DOPA} into betaxanthins—a food colorant. The hydrogel-based synthesis outperformed a control synthesis in the liquid phase considerably. In addition, the ink ratios in the 3D printing process could be used to control consortium dynamics and optimize the reaction progress.

3.3. Actuators

Actuators are another frequent application of compartmentalized hydrogels where mainly multilayer hydrogels are employed (Figure 18c).\textsuperscript{313,314} The combination of stimuli-responsive layers enables contraction or swelling of specific layers in order to induce motion of the whole hydrogel, for example, to grab objects.\textsuperscript{315,316} to mimic natural plants\textsuperscript{317} like the venus flytrap\textsuperscript{318} or to form muscle-like devices.\textsuperscript{319} A mimosas inspired hydrogel actuator was described by Chen and co-workers.\textsuperscript{120} Therefore, a hydrogel layer featuring an LCST polymer (PNIPAM) was combined with a hydrogel layer featuring an upper critical solution temperature polymer (P(AA-co-AAm)). Temperature changes allowed transfer of water from one layer to the other, which enabled actuation.
even in nonaqueous surrounding, e.g., under air or paraffin. A reversible actuation was obtained that could be used to grab and release cargo. A hydrogel actuator based on nanoclay crosslinking was investigated by Chu and co-workers.\textsuperscript{[321]} Macrosopic hydrogel building blocks were prepared from a mixture of NIPAM, AAm, laponite clay crosslinker, and photoinitiator. In order to realize actuation, the ratio of NIPAM and AAm was varied in different hydrogel blocks as blocks with different monomer ratio have different swelling characteristics. Finally, hydrogel blocks with different NIPAM/AAm ratio were assembled and connected via the supramolecular hydrogen-bonding derived crosslinking between Laponite and the polymers in the gel blocks. As such, a modular assembly of hydrogel blocks was possible, which allowed construction of a multitude of assembled architectures and in turn actuation features. Recently, Wang and co-workers fabricated a photoactive actuator via 3D printing (Figure 20).\textsuperscript{[322]} A thermoresponsive PNIPAM-based layer loaded with spinach-leaf-derived nanothylakoid was combined with a PAAlayer. The nanothylakoid was introduced to enable photothermal conversion and oxygen evolution. NIR laser energy could be converted to thermal energy by nanothylakoid and subsequently actuation was induced due to the contraction of the PNIPAM layer. In addition, the hydrogel actuator could transform H$_2$O$_2$ into O$_2$, and thus mimic plants in actuation and oxygen production.

3.4. Sensing

Compartmentalized hydrogels can be employed for sensing as well (Figure 18d). The compartments are used for example to combine various sensors that are incompatible with each other via a spatial separation. A combination of glucose and pH sensor was described by Onoe and co-workers, who formed Janus microgels.\textsuperscript{[323]} The Janus microgels consisted of sodium alginate, AAm, and crosslinker and were formed by immersion in CaCl$_2$ solution as well as UV light. The individual phases of the Janus particles contained either a glucose-responsive fluorophore or a pH-responsive fluorophore. As such the fluorescence readout could be used for pH calibration of the glucose sensor directly. Hao and co-workers described the utilization of a PEG in Dex W/W emulsion stabilized by crystalline nanocellulose for sensing.\textsuperscript{[324]} After emulsion formation in a mixture of water and glycerol the continuous phase was gelled via AAm/MBA to obtain a compartmentalized hydrogel with high stretchability. Furthermore, NaCl was introduced obtaining conductive gels that could be used as sensor for human movement. A 3D printing approach toward biosensing was introduced by Marquette and co-workers.\textsuperscript{[325]} For example, a hydrogel consisting of two compartments was formed, where one compartment consisted of a plain hydrogel and the other compartment of hydrogel containing

Figure 19. a) Schematic representation of micelle gelation at 37 °C and PPS-\textit{b}-PDMA-\textit{b}-PNIPAM structure, b) TEM confirmation of temperature dependent morphology switch of PPS$_{60}$-\textit{b}-PDMA$_{150}$-\textit{b}-PNIPAM$_{150}$ micelles at 25 and 37 °C, c) STEM-EDS element maps for sulfur (red) and oxygen (green) of PPS$_{60}$-\textit{b}-PDMA$_{150}$-\textit{b}-PNIPAM$_{150}$ core-shell compartments at 37 °C, d) sustained, local drug release in vivo via PPS$_{60}$-\textit{b}-PDMA$_{150}$-\textit{b}-PNIPAM$_{150}$ hydrogels (blue circle) over 12 days and dye-loaded diblock copolymer solution as control (green circle) diffusing rapidly, and e) cumulative in vivo drug release from the drug-loaded triblock terpolymer hydrogels and diblock copolymer micelles as control. Reproduced with permission.\textsuperscript{[304]} Copyright 2014, American Chemical Society.
anti-brain natriuretic peptide (BNP) antibodies. A sandwich assay was performed with addition of biotinylated anti-BNP antibody and labeled streptavidin, which led to a light signal readout. Zhang and co-workers utilized an emulgel for sensing.\cite{326} An emulsion of eicosane in water was gelated via free-radical polymerization of AAm and HEA in the water phase. The hydrogels showed significant stretchability (up to 1000%) and a high resistive sensitivity. Due to the melting/crystallization of the eicosane droplet phase the resistive sensitivity of the emulgels changes considerably. As the melting point of eicosane is at 36.8 °C, i.e., close to body temperature, the melting could be used to monitor body movements, which was shown in the sensing of the movement of the larynx happening during speaking.

3.5. Catalysis

Another application for compartmentalized hydrogels is catalysis, which allows the spatial separated placement of different catalysts in one hydrogel reactor or the formation of a crowded catalysis environment (Figure 18e). Multicompartment structures for catalysis have been in discussion with regard to synthetic cells/artificial cells that mimic the features of biological cells.\cite{327,328} There, a hydrogel could be a mimic for the cytoskeleton, for example.\cite{329-331} Chen and co-workers formed multicompartment hydrogel particles loaded with enzymes to perform a cascade reaction.\cite{332} The compartmentalized particles were formed via microfluidics and loaded with either GOx or HRP. In the next step, the particles were utilized to catalyze the reaction from glucose to $\text{H}_2\text{O}_2$ and Amplex red to Resorufin leading to a spectroscopic readout. Compared to the direct mixture of both enzymes a 23-fold increase in activity was observed. An olefin metathesis process coupled to enzymatic catalysis was studied by Patel and co-workers.\cite{333} An alginate-based hydrogel platform was used to form hydrogel beads with a core containing pig liver esterase and a shell containing Grubbs II catalyst. As such, ring-closing metathesis could be performed followed by an ester hydrolysis. The compartment structure allowed to increase catalyst selectivity by suppression of side product formation. An organocatalysis approach was described by Pich and co-workers.\cite{26} Microgels based on PNIPAM were synthesized that contained covalently bound l-proline as organocatalyst. The authors studied the aldol reaction of 4-nitrobenzaldehyde with cyclohexanone. To investigate the effect of spatial placement of the catalyst in the microgels, catalyst was introduced in the shell or in the core of the particles. In a homogenous methanol reaction medium, the particles with catalysts in the shell performed superior over molecular catalysts and particles with catalysts in the core, which was assigned to the diffusion of reagents. Interestingly, in a heterogeneous reaction medium consisting of water and methanol, no catalytic activity was observed for the molecular catalysts (as expected) but the microgel catalysts showed catalytic activity. In this case, microgels with catalysts in the core indicated superior activity. This was assigned to a local hydrophobic environment inside of the particles, which enables the aldol reaction and is most pronounced for particles with catalysts in the core.
3.6. Energy

Recently, multicompartiment hydrogels have been used in energy applications as well (Figure 18f). Stacked hydrogels were introduced as a power source by Mayer and co-workers (Figure 21).\textsuperscript{[334]} The authors designed a system that mimics the electric organ of an electric eel, which uses thousands of membranes containing ion channels to generate voltage and current. To mimic the electric organ of the eel, four different hydrogel lens shaped patches were synthesized, i.e., a hydrogel from neutral monomers containing concentrated NaCl (red in Figure 21), a cation selective hydrogel from negatively charged monomers (green in Figure 21), a hydrogel from neutral monomers containing diluted NaCl (blue in Figure 21) and an anion selective hydrogel from positively charged monomers (yellow in Figure 21). The hydrogels were patterned on a substrate in a way that mechanical contact would lead to ionic gradients due to alternating charge-selective hydrogel compartments. In order to achieve high potentials thousands of these ion gradients were printed and brought together. As such, potentials above 100 V were obtained as well as power densities of 27 mW m$^{-2}$ per cell of four hydrogels. Recently, the same group converted the design into a layered hydrogel approach.\textsuperscript{[335]}

4. Conclusion and Outlook

Hydrogels are a highly relevant type of materials with implications for a broad variety of applications going from cell culture to soft robotics. In particular, mechanical properties, biocompatibility, swelling properties, chemical durability or self-healing properties have been in the focus of researchers to tailor hydrogels. Another dimension to alter hydrogel properties is variation of composition, for example via functionalization or compartmentalization. Especially, compartmentalization opens up opportunities to alter hydrogel properties and functions.

A multitude of options for compartments exist, e.g., micelles, vesicles, oil droplets, water droplets, coacervate droplets, and
multilayers. These all vary in their properties regarding interface area, permeability, effect on mechanical properties, hydrophilic/hydrophobic environment. As such, compartments are employed to tailor hydrogel properties and applications. A prominent option is cargo encapsulation and delivery, where inclusion of compartments adds an additional level of control on release behavior. Just like compartments in hydrogel for cargo/drug release, compartmentalization can be used to increase complexity of hydrogel structures for all sorts of applications, e.g., catalysis, cell culture, sensing or even voltage generation.

As with hydrogels that have found more and more important applications, research on compartmentalized hydrogels will continue to grow. There are several directions of particular interest. First of all, tissue engineering and cell culture should be mentioned, where compartmentalization can be introduced and scope of catalysts that can be used. Finally, sensing should be mentioned, where compartmentalization can be introduced to improve sensitivity or to protect vulnerable sensing moieties.

Overall, compartmentalized hydrogels have a bright future ahead. The aforementioned applications are highly relevant and have a significant impact on development in biomedicine and organic devices.

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Conflict of Interest

The author declares no conflict of interest.

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