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Polyelectrolyte Microcapsule Arrays: Preparation and Biomedical Applications

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Abstract In the need of development of versatile and flexible platforms for sensing and other biomedical applications, micro- and nanostructured particle arrays attract strong scientific interest. In this review we focus on fabrication of arrays of polyelectrolyte layer-by-layer assembled microcapsules and bio-related applications of such arrays. A cargo encapsulated in the microcapsules can be released on demand, thus opening perspectives for biosensing, diagnostics, controlled drug delivery, and tissue engineering. Here, we also consider a new composite system—microcapsules embedded into polymeric film—both components are made by the LbL technique. Fabrication approaches and perspectives in the preparation and in the use of the microcapsule arrays are addressed.

Keywords Microcapsule · Array · Pattern · Layer-by-layer · Release · Biomedical applications

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1 Introduction

At present, an attention of researchers is attracted by the development of novel multifunctional structures with hierarchical ordering. Such low-dimensional functional materials could be applied in various fields of science and engineering. Therefore, one of the major research focus is a formation of the ordered structures with defined packing density consisting of solid micro- or nanoparticles self-assembled on the gas–liquid interfaces or solid surfaces [1–6]. Even more attractive research area is related to fabrication of the polymer microcapsule arrays. The microcapsules are the hollow microsized containers (usually of spherical shape but not necessarily [7]), which can be made by coating of sacrificial nano- and micrometer-sized particles by the Layer-by-Layer (LbL) assembly [8] and subsequent dissolving the sacrificial particles under conditions which do not alter the shell properties [9–12]. Microcapsules can have a variable composition and a tuned shell thickness [10–12]. Besides, they can be loaded with various active substances and objects, including living cells, preserving cellular functions [13]. This provides certain attractive functional applications for the microcapsules. For instance, recent work [10] demonstrates formation of unique multicompartamental LbL microcapsules (SiO_2-core)/($PS_nP_2VP_n$/PSS) based on highly branched pH-sensitive polystyrene-poly(2-pyridine) star-shaped block copolymer ($PS_nP_2VP_n$) and poly(styrene sulfonic acid) (PSS). Such star molecules have hydrophobic core and hydrophilic corona, so that the thin highly porous shells (about 25 nm in thickness) consist of interconnected hydrophobic domains, hydrophilic polyelectrolyte matrix, and nanosized water-filled pores [10]. This composition diversity might facilitate the ability for simultaneous loading of hydrophobic, hydrophilic, and charged species in different compartments of a single shell [10]. Larger arm star copolymer (22 arms) with stretched conformation showed a higher increment in shell thickness due to the effective ionic complexation.

The role of such a microcapsule expected for biological applications is to provide a proper environment for molecules and nanoparticles and to protect them from enzymatic degradation, e.g., when microcontainers travel through the tissues to the targeted site [11]. An important feature of these microstructures is the possibility to change their shell properties in response to environmental conditions leading to a release of encapsulated molecules [11]. An ability to control the assembly of the microcapsules to achieve well-defined hierarchical structures offers new opportunities for their ultimate applications, especially in biomedicine [11, 12, 14–21]. There are applications that require an effective immobilization of the microcapsules onto a solid support. These approaches are linked for instance to the integration of loaded microcontainers as elements into a chip in order to fabricate sensitive (micro)systems for detection, diagnosis, and analysis of various chemicals [22, 23]. Very interesting approach is related to potential application of the microcapsule arrays for theranostics and for development of a new generation of scaffolds for tissue engineering. Effective application of the filled microcapsules immobilized on solid surfaces requires the development of the 2D patterning. There is a limited number of publications focused on formation of the microcapsule arrays, most probably due to its implementation difficulties [24]. To our opinion, it is of high interest to summarize recent studies towards this topic which present in this review.

2 Patterning of the Microcapsules

The attention to the microcapsule arrays fabrication has been attracted by the first article published (Fig. 1). The promising direction of this field is based on patterning of spheres on modified surfaces by solution casting technique [22, 25–27], Langmuir–Blodgett (LB) technology [28, 29], heating the assembled microcapsule suspension [30], printing technology [22, 31–46], electron beam irradiation [25], capillary bond [22, 39, 44], electrochemical deposition [26, 28, 39], dropping of the suspension, and self-assembly [22, 25, 31–34, 37, 38, 47–52]. Such capsule arrays are kept assembled on the substrates due to covalent binding, electrostatic forces [31–33],

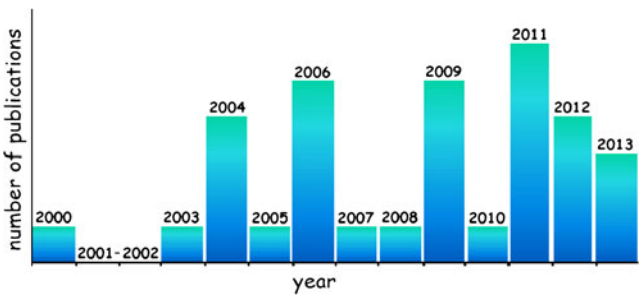

Fig. 1 Dependence of the publication numbers related to "microcapsule patterning" on years

biological affinity [34, 37, 38, 47–50, 53], hydrophilic/hydrophobic interaction [25, 45, 49], or gravitation only [22, 26–30, 35, 36, 39–44, 46, 51, 52].

The first attempt of fabrication of the polyelectrolyte microcapsule assemblies was demonstrated in 2000 by X.L. Chen and co-workers [51]. This work reports the synthesis and supramolecular self-assembly of a novel "rod (A)-coil (B)-rod (A)" triblock copolymer architecture. It was found that the new quinoline–styrene–quinoline (QSQ) triblock copolymers can spontaneously form robust microcapsules (or spherical vesicles) in aqueous solution. An interesting feature of these triblock copolymer microcapsules is their tendency for self-organization into highly ordered closely packed hexagonal arrays. This is a minimum elastic deformation (at the area of contact) during arrays formation from these microcapsules. The authors of the work [51] suggest that such high order assemblies of hollow spheres or vesicles could be used to prepare photonic crystals or being used as a model of a biological tissue. But other attempts of such investigation have not been reported up to now.

The following attempt of the polyelectrolyte microcapsule array fabrication was described by M. Nolte and A. Fery [32] and also by J. Feng and co-authors [33]. In these works, the capsule arrays are formed on solid substrates using electrostatic forces (Fig. 2). For this purpose, the authors have created surfaces with periodically negative and positive charged areas (by deposition of the oppositely charged polyelectrolytes using microcontact printing; Fig. 2). The microcapsules were prepared on the surface of melamine formaldehyde (MF) particles by the LbL assembly followed by dissolution of MF particles. In the first case [32] poly(sodium styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) were used for the shell formation; in the second case [33], PSS, PAH, and poly(diallyldimethilammonium chloride) (PDADMAC) were applied. Suspension of hollow microcapsules (with the PAH [32] or PDADMAC [33] as the outermost level) was deposited onto charged patterned substrates. Nanostructured capsules were preferentially adsorbed on oppositely charged (PSS-areas) surface only through electrostatic interaction [32, 33]. It was concluded that the well-orientated capsule array on the oppositely charged regions (circles and stripes) can be fabricated [32, 33]. But the coverage percentage on the pattern regions is not high and the patterned microcapsules can be detached by high ionic strength or extreme pH [32]. Closely packed microcapsule array was finally obtained by Feng and co-workers [33] on the substrate modified by $(PDADMAC/PSS)_3$ multilayers. Embedding of nano- and microsized particles including polyelectrolyte microcapsules into soft LbL-assembled film has been studied by D.V. Volodkin and co-workers [54–57] for better understanding of the capsule–film interaction. The capsules were spontaneously immobilized into the thick micrometer-sized multilayer films of biopolymers poly(L-lysine) (PLL) and

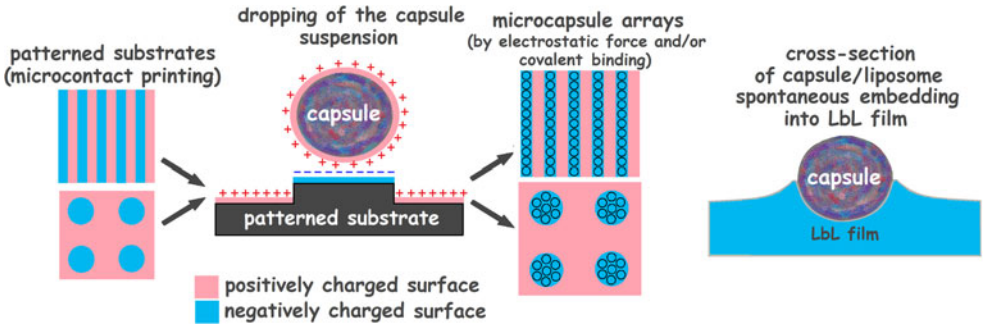

Fig. 2 Schematics of the microcapsule array fabrication based on electrostatic force and/or covalent binding

hyaluronic acid (HA). It was found that microcapsules were embedded into the LbL film to a half of its diameter (as shown in Fig. 2) by the same mechanism as smaller metal nanoparticles that is driven by electrostatic interactions. It is important that these embedded capsules can be loaded by active substances and the following LbL film decomposition leads to formation of free-standing capsules; this gives an option to prepare anisotropic microcapsules [56, 57].

The paper [25] is one of the first works in the field of the polymeric capsule layers (on the strongly hydrolyzed substrates) fabricated by the solution casting technique and laser radiation (Fig. 3). Some areas of the capsule layers were irradiated with electron beam (through the mask with different geometry). Then substrate with capsule assemblies was processed with detergent solutions under sonication. Finally, the irradiated capsules remain on the surface, non-irradiated have been removed. Thus, the laser beam causes a significant increase of the capsule adhesion to the substrate [25]. At the same time, a close regular packing of hollow capsules (on irradiated areas) has not been reached that might be due to the rather low concentration of the initial capsule solutions, polydispersity, and empty state of the capsules [25].

Another method of formation of polymeric capsule arrays is the use of the balance in hydrophilic/hydrophobic interactions that determines a surface wetting [45]. The described approach is based on preparation of physically heterogeneous solid surface (without actually changing its flat morphology) consisting of highly hydrophilic spots surrounded by highly hydrophobic area (Fig. 4). This allows one to precisely position small droplets of aqueous solutions without their spontaneous spreading over the boundaries of the modified regions [45]. The capsule arrays are created by placing drops of suspension onto sample surface and following drying during 1 h. Authors were prepared two types of microcapsules: the hollow (PAH/cirate)/(PAH/PSS) spheres and capsules containing YF_3 inside. Both kinds of capsules were successfully deposit on the modified substrates. The affinity of the hollow capsules to the surface is rather high (no removal by ultrasonic treatment) due to light weight and flexibility. The capsules containing YF_3 are more heavy and inflexible and were suffered from the poor stability on the surface.

It is possible to fabricate polyelectrolyte microcapsule assemblies using highly specific and strong biological recognition instead of rather weak electrostatic attractive forces [34]. The $(PAH/PSS)_5$ capsules (made on $CaCO_3$ cores) were biotinylated using the reactive biotin-NHS molecules, which was covalently coupled to the amino groups of PAH. Based on the specific recognition of avidin–biotin pair, capsules can be immobilized on the pattern area of the flexible polymer substrates (Fig. 5). The individual microcapsule arrays were obtained when the ratio between the capsule diameters and the pattern sizes were approximately in the range from 1:2 to 3:4 [34]. The as-prepared capsule arrays could survive treatments by ultrasonication, concentrated salt, acid, and base, as well as elevated temperature. However, the fabrication process is rather complicated, and unspecific adsorption of the capsules on the undesired areas is not easy to avoid. Further, these arrays were used by the authors of the work [34] as microreactors for synthesizing ZnS quantum dots.

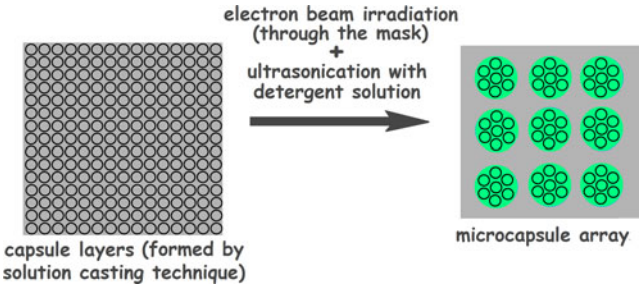

Fig. 3 Schematics of the microcapsule array fabrication based on solution casting technique and electron beam irradiation

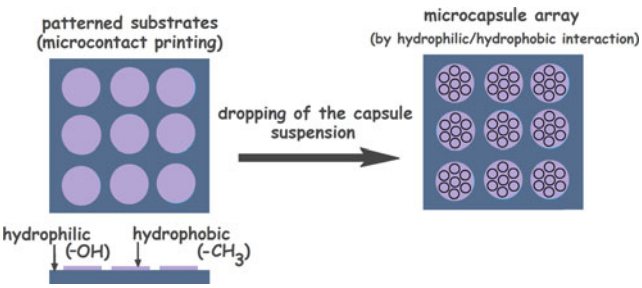

Fig. 4 Schematics of the microcapsule array fabrication based on hydrophilic/hydrophobic interaction

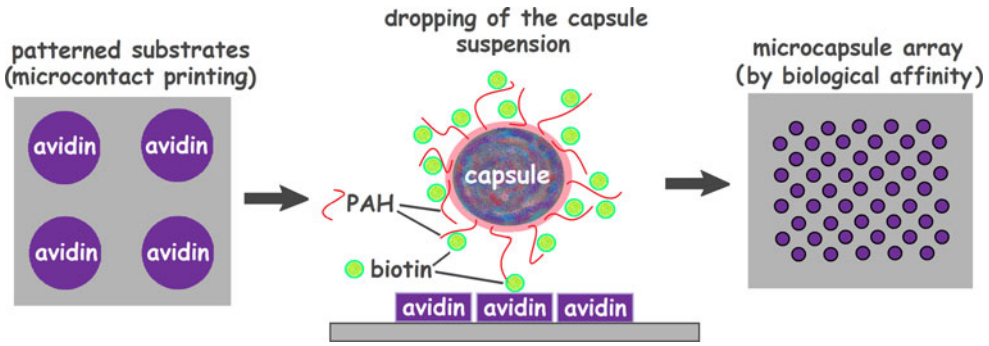

Fig. 5 Schematics of the microcapsule array fabrication based on biological affinity

The authors in [39] reported the fabrication of square metal microcapsules using patterned silicon (Si) membranes (with square-shaped micropores $30\times30\times150$ μm^3). Firstly the pores of the Si mold are modified with a polymer component, such that a gap is created along the wall of the channel. Subsequent electrochemical deposition creates square metal tubes (within this structure). They can be converted to floating square microcapsules (a maximum wall width is 1 μm) by polishing both sides of the metal array (while it is still within the membrane) that seals the ends of the tubes. These hollow metal capsules float on the surface of water and have interesting assembly properties. Capillary bond works to drive the assembly of these square capsules at the interface. Capsules oriented "edge-down" on the surface are readily assemble end-to-end. The oriented "face-down" in water capsules have a tendency to assemble along their long faces.

The report [26] presents an electrodeposition synthesis of two-dimensionally ordered nickel (Ni) spheres arrays based on polystyrene (PS) beads colloidal monolayer (produced by sintering on the ITO-glass substrate by spin-coating method and heating) with subsequent removal of this PS cores (Fig. 6). It is necessary to electrodeposit Ni under a constant current for a long time (90 min) to obtain arrays of metal hollow spheres; or for a short time to obtain ordered bowl-like pore arrays. The formation of hollow sphere arrays is attributed to preferential nucleation on the interstitial sites between PS in the colloidal monolayer and substrate, and growth along the PS surface. Formed Ni arrays only partially contact with the substrate (i.e. possess weak adherence force between them) leading to its integral transferability in water that makes it posible to fabricate such ordered sphere arrays on substrates of almost any shape.

Authors in [27] described a two-step replication strategy for 2D ordered polymer hollow spheres and fabrication of solid convex structure arrays. The fabrication approach is suitable also for the most soluble polymer materials, which can solidify when they are concentrated. As in the previous paper [26] these capsule arrays can be made on polystyrene colloidal monolayer (and on FeO(OH) inverse opal also) received by spin-coating method. The hollow or truncated hollow sphere arrays can be obtained, depending on the morphology of the 2D FeO(OH) ordered pore arrays, which is controlled by concentration of its precursor. It is possible to form a small hole on the top of the hollow spheres by the decrease of the concentration of polymer precursors. Such holes could be important for fabrication of microreactor devices, micro-acetabula, selective permeability, nutrient and drug deliver, biotechnology, and even study of black-body irradiation in micro- or nanospace.

The self-assembly based on hydrogen-bonding is used in the study [52] for the formation of hollow nanoparticles based on amphiphilic block copolymer polystyrene-b-polyvinylpyridine (PS-PVP). Initially the authors of this work have formulated the nanoparticle assemblies, and then they have removed the (hydroxybenzeneazobenzoic acid) cores to obtain arrays of the hollow shells. The nanoparticles were stable and monodisperse (narrow size distribution with standard deviation of <7 %) at an average diameter of 35 nm. Therefore, the hollow nanocapsules form the large areas of ordered hexagonal arrays.

J. Yang and C. Gao [31] described the microcapsule patterning method based on covalent binding between the capsules and a substrate. According to this approach polyelectrolyte "core/shell" particles (formed on $CaCO_3$ templates using the LbL assembly) are treated with glutaraldehyde and covalently immobilized onto substrate chemically patterned with amino groups by microcontact printing (Fig. 2). Periodical 50-μm circles of fluorescein isothiocyanate-poly(allylamine hydrochloride) (FITC-PAH) on the surface have been

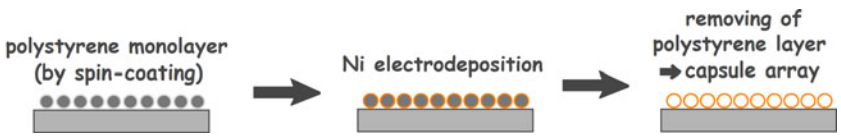

Fig. 6 Schematics of the microcapsule array fabrication based on spin-coating and electrodeposition techniques

obtained. Microparticle suspension was deposited on the substrate; and beads were attached to these areas. Then stable microcapsule arrays were obtained by core dissolution. The coverage of the particles on the PAH circles was reached around 70 %, but the particle arrangement in each circular region was not hexagonal close-packed. It is because the $CaCO_3$ particles have rather wide range of size distribution (4 to 6 μm) and there is an electrostatic repulsion of the particles of the same charge (therefore gaps among them are formed). Covalent binding gives a stable interaction (less sensitive to environment impact than electrostatic forces). Thus the prepared microcapsule arrays have good stability against salt, high and low pH.

The work [22] presents the approach to fabricate stable arrays of highly ordered patterns of polyelectrolyte multilayer microcapsules consisting of alternating polyelectrolyte layers of PAH and PSS (Fig. 7). The substrate composed of ethylene and tetrafluoroethylene film was pre-patterned by nanoimprint lithography (made using silicon mold with $1.5\times1.5\times1.5$ μm^3 cubic pillars, with a period spacing of approximately 5 μm) and dried. Then an aqueous suspension of MF microparticles coated with polyelectrolyte multilayers were placed on the patterned surface. During the water evaporation, the "core/shell" microparticles (diameter is 2 μm) are effectively driven into the hydrophobic surface cavities (depth is 1.2 μm) by means of both gravity and capillary forces, so patterned particles protruded out of the film surface by about 0.7 μm. Then HCl solution was added on the patterned film surface in order to dissolve the core template and thus form array of hollow polyelectrolyte microcapsules. The fill factor of microwells is 98 %. Different substances may be incorporated in the capsule shells.

2D ordered microcapsule arrays can be fabricated at a liquid–air interface via a self-assembly method [30] followed by a transfer onto solid substrates (Fig. 8). The method of patterning consists in heating of the LbL-assembled microcapsule suspension [30]. The beaker with capsule suspension was incubated in the oven at 120 °C (for defined times) and the suspension began to crystallize when the capsule concentration exceeded $3\ 10^6$ ml^{-1}. The capsules at the interface are inclined to cluster and arrange tightly in hexagonal order to reduce the surface energy. The hollow microcapsule arrays were withdrawn from the suspension surface through gently vertically immersing round substrate (such as a cover slips of 10 mm in diameter) into the suspension and picking up the floating colloids membranes. The dimensions of the polyelectrolyte ordered microcapsule array can be controlled through tuning a temperature and a time of treatment. This method requires very small quantities of the capsule suspension (200 μl) that allows producing well-ordered 2D hollow sphere arrays over large areas (1 cm^2) in time scale of minutes and does not require any special apparatus.

It is possible to create the organized multilayer structures of capsules (or different surface-active substances and nanoparticles) at the air/water interface using Langmuir–Blodgett technique with sequential deposition onto solid surfaces [14, 15]. The authors of the work [29] form the ordered arrays of stable pure protein (hemoglobin) microcapsules in the size range from 0.1 to 0.3 μm. Monolayer of the capsules is formed at the interface air/buffer with pH=6.8 (isoelectric point of the protein) at 24 °C. For the transfer of the capsule monolayers onto the substrate (Formvar/carbon coated copper grids, mesh size is 200 μm), the surface pressure is maintained constant at π=20 mN/m. A change in the surface area during dipping was monitored. A horizontal transfer method was used to prepare the films. The transfer ratio was around 0.75 for all the monolayers.

The interesting approach to create the ordered 2D capsule array is proposed in the work [28]. A patterned asymmetrical Janus microcapsule array is made of multi-walled nanotubes (MWNTs) and a conducting polymer by combining of the two methods: (a) particle self-assembly process by LB method (with subsequent bead dissolution) and (b) conductive polymer film electrodeposition using colloidal template.

3 Templates with Microchamber Arrays

Many works describe the process of microcapsule arrays formation using printing technologies [35, 36, 39–44, 46]. As an alternative to spherical microcapsules, the work [46] contains a new approach for encapsulation. Authors presented self-assembled/self-packed 3D arrays of monodisperse microtissues. These microcontainers consist of HepG2 cells that settle onto a micro-molded agarose gel and self-assembled within 24 h. Then the array (number of elements n is 822) of densely packed microtissues is encapsulated (in situ) using alginate and separated from the agarose micromold. Microtissue size, viability, and albumin secretion were all controlled by the number of cells seeded onto the original

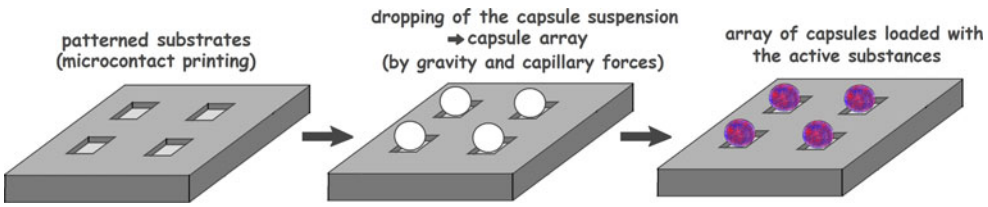

Fig. 7 Schematics of the microcapsule array fabrication based on capillary forces and gravity

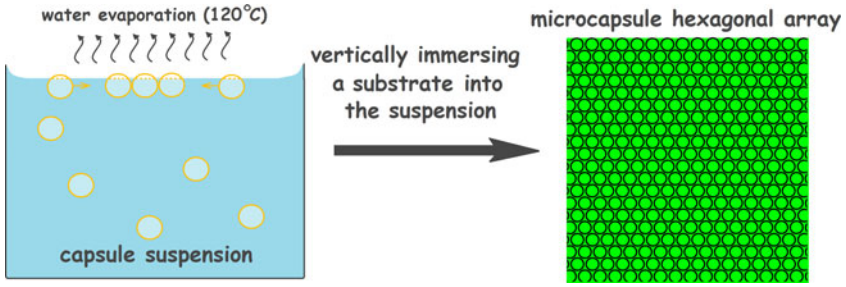

Fig. 8 Schematics of the microcapsule array fabrication based on heating of the capsule suspension

agarose micro-mold, and microtissue shape and spacing were controlled by the design of the mold. It gives a new design possibilities that can help to address certain limitations of spherical microcapsules in cell-based therapies.

Authors of the article [36] present the fabrication of hydrogen-bonded LbL microscopic rhodamine-encapsulated dot arrays. These patterned microcapsule structures are formed over large areas using inkjet printing. Microscopic dots (35–130 μm) with a controlled thickness ranging from 15 to 400 nm are formed from poly(vinylpyrrolidone)/poly(methacrylic acid) (PVPON/PMAA) components without an intermediate washing step and on both hydrophilic and hydrophobic smooth substrates. Capsules have relatively uniform surface morphology with a lower central region. These arrays of polymer dots can be utilized to encapsulate a variety of different components by delivery of small liquid droplets to the central area and further capping them with additional polymer multilayers.

Authors in [35] suggested the fabrication of arrays of microcapsules supported on elastomer substrates from buckled polymer thin films (it provides stretchability without defects) for controlled release by stretching.. Matrices of elongated containers were fabricated using microprinting technologies and tested (mechanical stretching). The amount of released molecules at each pumping event was adjusted by a strain degree.

The works [40–44] describe microcapsule arrays as a substrate with periodically patterned microcontainers, which can be loaded by various nanoparticles, oil micro-droplets. G.B. Sukhorukov and coauthors [40–43] demonstrated the fabrication of polyelectrolyte multilayer film as the array of microchambers using the LbL assembly of oppositely charged polyelectrolytes: PDADMAC [40, 42, 43], PSS [40–43], PAH [40, 41, 43]. The works [40–43] demonstrate a formation of polyelectrolyte multilayer patterned films with microchambers of different geometry (size, shape, aspect ratio) on templates with imprinted patterns of wells (by various silicon molds; Fig. 9). It is possible to create circle shaped or squared chambers from 2 to 25 μm in size [40–43]. Critical thickness of the stable freestanding film is about 400 nm [42]; if the film is thinner than the critical value, microstructures collapse upon dissolving the template. In the work [40] gold nanoparticles are incorporated in the microcontainers, which are sealed onto a glass slide precoated with an adhesive PDADMAC/PSS multilayer film. Hollow microcapsule arrays are suitable for loading a variety of active components of different nature for envisaged location and time controlled activation [40–43], which can be released by focused laser radiation [40, 42]. This issue is described in the last section of this review. There are different approaches to change a shell permeability: thermal treatment [58, 59], pH [60–63], laser radiation [18, 40, 42, 64, 65], and ultrasonication [66, 67]. Such remote activation requires the

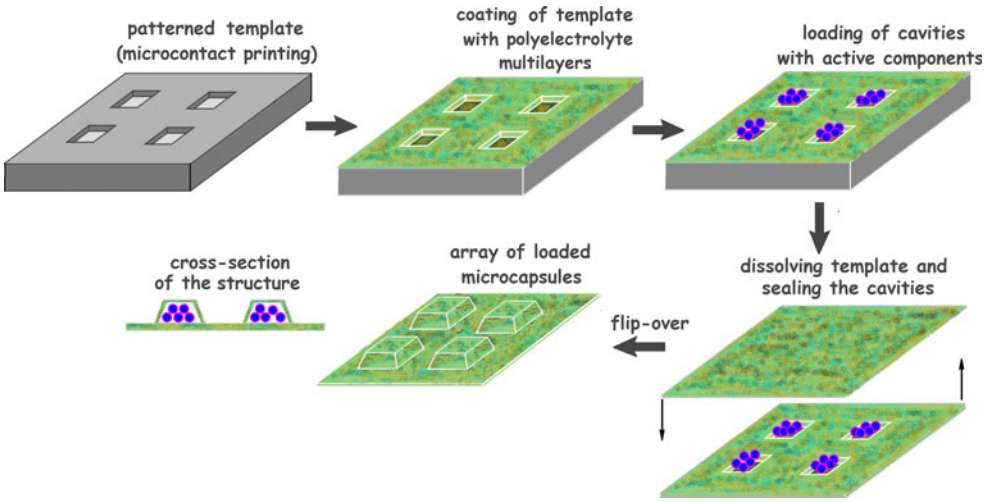

Fig. 9 Schematics of the microcapsule array fabrication based on LbL assembly and printing technology

shell functionalization by substances sensitive to these stimuli [14]. Dyes (absorbing in in a certain wavelength range) [12, 61, 64, 65, 68], plasmon-resonance, and noble metal (Au, Ag) nanoparticles are used to provide the capsule sensitivity to the light irradiation: the shell with Au nanoparticles is destroyed by IR irradiation [18]. Microcapsules sensitive to both magnetic field and laser irradiation have also been reported [69]. Disadvantage of laser opening of the microcapsules is a high power density required to open a single capsule. It was demonstrated, that for collective opening of many microcapsules by external stimulation the ultrasonic treatment can be applied at the power density higher [66] or similar to that which induces a cavitation [67]. The increase of the microcapsule shell sensitivity is may be achieved by a control over mechanical properties of microcapsule shell by changing a volume fraction of inorganic nanoparticles [67]. The other way of consists in hydrophobization of substrate surface by imprint techniques [70–72]. This approach can be expanded on the microcapsule arrays. The paper [44] reports a unique one-step fabrication method for the growth of hollow polymeric microchambers and microcapsules bound to hollow microchannels based on electric field-assisted capillary action. The experimental setup consists of a top patterned electrode (a nickel master) and a bottom electrode (Ti/Cu/Ti metal thin film deposited on a glass wafer), which is spin coated with a liquid polymer, so that an air gap exists between the top electrode and the polymer surface. Then the polymer is heated just above the glass transition temperature (in the work [44] polydimethylsiloxane was used). The electrostatic field between the two electrodes destabilizes the thin viscous polymer film and induces the microstructure growth upward toward the top electrode (from regions of low to high electric field) until it fully touches the top electrode [44]. Capillary forces become dominant and drive the liquid polymer into the microcavity such that the whole surface of the top nickel electrode is coated by the polymer [44]. Then the polymer thin membrane acting as a cap for the capsules is heated to solidify the hollow structure and released from the top electrode. This method demonstrates the manufacturing of self-encapsulated microstructures from polydimethylsiloxane of 100-μm height from an initial polymer layer of 22 μm. Microcapsule caps have shell thickness of several microns; the inner surface of the hollow microstructures is smooth. The authors showed that the capsules keep their shape under bending or delamination from the substrate [44].

4 Functional Liposome Arrays

Liposomes can be considered as carriers for biomedical application [53, 73–83], and they can be immobilized to solid surfaces by specific biological recognition (Fig. 5) [37, 38, 47–50]. For instance, in the work [47], liposomes were coupled on the array via oligonucleotide hybridization.

There are two main functional liposome types that can be used for the immobilization of membrane proteins such as receptors and ion channels onto biosensing platforms [53]. The first type is a pure liposome containing only the purified membrane proteins that have been reconstituted into lipids (either synthetic or isolated natural lipids). The second liposome type is a native liposome, which is isolated directly from the cell source and contain any membrane associated proteins expressed by that particular cell line [53]. This strategy minimizes the risk of denaturation of the membrane proteins. Such functional liposomes are immobilized on surface by antibody-directed strategies to produce arrays [49, 50]. Silin and coworkers [50] used intact liposomes (300±100 nm in diameter) as the lipid support of the chemokine CCR5 receptor utilizing the biotin-streptavidin and antibody–antigen interactions to effectively capture a functional CCR5 receptor. The successful liposome immobilization via this antibody–antigen pair was monitored using surface plasmon resonance. The extension of platforms developed for liposomes, which can be considered as simple models for biological cells, could prove beneficial for biosensing applications [53]. It would allow for the high-throughput screening of signaling systems within cells; different types of cells could be positioned in predefined areas on a chip [53].

The results obtained in the study [48] demonstrate a new approach for direct liposome immunoassay based on the gramicidin-induced enhancement of fluorescence with pH-sensitive dye-encapsulating liposome arrays immobilized on avidin slips (by avidin–biotin technique) or gramicidin channels. The advantages of the method are: (a) antibodies and antigens do not need to be labeled with fluorescent molecules and (b) separation of bound and unbound species is not necessary.

Authors of the work [37] have immobilized polydiacetylene liposomes on glass substrates modified by the micro-contact printing technique to obtain the micro-patterned self-assembled monolayer of 3-aminopropyltriethoxysilane (APS). Squared (width 70 μm) and circle-like (diameter 15–50 μm) patterns have been fabricated. The amidization between surface amine groups of the pattern and N-hydroxy succinimide moieties of the liposomes allows to attach (covalent interaction) the polydiacetylene liposomes to the APS areas (square or circle) [37]. The similar study is presented by Jiseok Lee and coauthors [38] who described a highly selective blue polydiacetylene liposome-based sensor system to detect potassium ions (K+) even in the presence of sodium ions (Na+). The liposome surface contains tethered G-rich ssDNAs that provides selectivity by wrapping around K+ to form bulky quadruplexes, which repulse each other (due to the steric hindrance) and induce the ene–yne backbone perturbation of the liposomes. Such selective event initiates the color change (from the blue to the red phase) of the liposome and also produces red fluorescence emission.

Emulsion droplets synthesized using microfluidic technologies can be considered in the same way as microcapsules and liposomes. It is possible to fabricate the highly controlled monodisperse microparticles (including spherical core-shell structures and balls, nested multiple emulsions) with desired composition and structure using special capillary microfluidic devices [84–87]. In the case of multidroplets the external (bigger) droplet acts as the shell of the microcapsule, the inner and middle droplets are like a core of capsule (their number is controlled). Similar to polyelectrolyte LbL capsules, fabricated using microfluidic channels microstructures can be also released at a controlled manner, and the structural and chemical modifications allow the tuning of release rates [84–87]. The structure of fabricated using microfluidic techniques particles is determined by the flow properties in the devices. The chemical composition is dependent on selected fluids introduced into the microfluidic channels [84–87]. Biocompatible materials can be included in such highly controlled multidroplets and microcapsules that create opportunities to produce a wide range of the useful for advanced drug delivery microstructures [84–86]. Microfluidics allows creation of the ordered monodisperse multidroplet arrays (as with LbL capsules).

5 Biomedical Applications of Microcapsule Arrays

Polyelectrolyte LbL-assembled microcapsules [88–90] are carriers which holds encapsulated molecules and potentially should release the molecules on demand [91, 92]. External stimulation of the microcapsules is more convenient because the treatment with external stimuli such as light or magnetic field is less harmful if compared to direct administration of bioactive molecules by common delivery routes such as oral, intravenous, etc. In the last case, an efficiency of the delivery is rather low because of redistribution of the bioactive molecules in the whole body. Thus, the externally stimulated release is highly localized and, as a result, potentially more effective. Moreover very desirable would be to have an option of controlled release with a high precision in both space and time. In respect to this, light-stimulated release is especially attractive because of non-invasive character, well-defined spatial and temporal control over the light intensity as well as rather deep tissue penetration up to some cantimeters [93]. This has stimulated researchers to use light as a trigger for activating intracellular processes by controlled release from engulfed microcapsules inside a living cell [94]. Along this line, one may conclude that light-stimulated release of bioactive molecules from surface immobilized microcapsules has promising application for coating of medical devices including implants, catheters, etc. In addition, the externally triggered and controlled release might be an indispensible tool for tissue engineering applications where growth factors should be not only protected from enzymatic degradation but also delivered with a desired rate in a desired place [95, 96]. In this section of the review, we further focus on the light-triggered release from the microcapsule arrays.

Bioapplications of LbL-assembled polyelectrolyte films have attracted significant scientific attention leading to a number of comprehensive reviews [97–102]. The films play a role of reservoirs for biomolecules and govern cellular response as well [55, 97, 103, 104]. This demonstrates a high potential to employ the films for biomedical purposes. However, light-induced release of film-loaded biomolecules is rather difficult to trigger and just a few examples have been recently shown [18, 105, 106]. Alternatively, the films have been loaded with the microcapsules, which host encapsulated biomolecules and offer release opportunities by permeabilization of a capsule shell. Thus, this composite system—capsule-laden films, both assembled by the LbL approach—showed recently high promise for externally controlled release [18, 54, 106, 107].

Negatively charged $(PDADMAC/NP/PSS)_4$ microcapsules have been spontaneously embedded into the positively charged $(PLL/HA)_{24}$/PLL film (Fig. 10) [18]. The mechanism of the embedding is still not fully understood but the embedding is most probably driven by doping of mobile PLL to film surface and formation of cooperative interaction with the particles as mentioned in the section I of this review. The microcapsules have been modified with gold nanoparticles (NP) to achieve a light response. One capsule has been subjected to laser irradiation followed by release of its cargo—dextran–rhodomine molecules (Fig. 10a–c). The release mechanism is based on temperature induced localized permeabilization of the capsule shell as has been reported earlier [64,

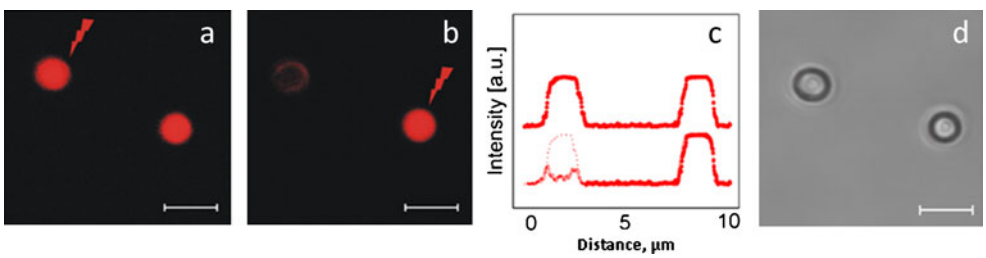

Fig. 10 CLSM images of dextran–rhodamine-loaded $(PDADMAC/NP/PSS)_4$ microcapsules immobilized into $(PLL/HA)_{24}$/PLL film before (**a**) and after (**b**) light irradiation of the capsule located on the left part of the images. Fluorescence profiles (**c**, *upper* for the image (**a**) and *lower* for the image (**b**)) and light transmission mage of the capsules (**d**). Adopted from [18]

108–110]. The spherical shape of the light affected capsule is kept visually intact (Fig. 10d) indicating no mechanical distortion and showing a promise for release applications at mild conditions. It is of note, that for such composite system (LbL capsules immersed into the LbL film) one should focus on an effect of the film on the capsule permeability and also on the polymer exchange [111] between these two polymeric systems. This is especially of interest considering high polymer mobility in the soft and thick HA/PLL film [112–115], which plays a role of reservoir able to uptake even large micrometer-sized capsules.

Temperature related mechanism of a capsule shell permeabilization has been also reported for other carriers—liposomes—the phospholipid vehicles able to host and release by external stimulation [116–120] small molecules such as drugs or model fluorescent dyes. Immobilization of liposomes stabilized by polymer coating into LbL-assembled polyelectrolyte multilayers has been demonstrated [121, 122]. It was shown that the high integrity of the film-embedded vesicles is due to highly hydrated state of the film being a hydrogel with about 80 % of water. Liposomes integrated into the polyelectrolyte film respond to temperature increase above the lipid phase transition temperature demonstrate high potential for stimuli-sensitive biocoatings. Such composite liposome-film systems might have biologically relevant applications, for instance as antibacterial coatings [123] or light-triggered release if light-sensitive liposomes [124] would be used.

Recently light-triggered release of particle arrays formed on the solid surface with the particles trapped in the microchambers constructed by ustilizing the polyelectrolyte LbL self-assembly has been demonstrated [40]. The microparticles freely floating into the chambers can be released from the chambers by opening of a specific chamber with light (Fig. 11). This approach of micropatterning demonstrates an applicability of the surface-mediated light-triggered release not only for (bio)macromolecules and small drugs as mentioned above but also for large objects such as microparticles.

We believe that future applications of the microcapsule arrays based on composite capsule-loaded LbL films will be further developed towards biologically relevant drugs such as proteins with high local concentrations as often required to achieve a strong biological response. This might be achieved, for instance, by loading of the films with protein microparticles [125–129]. It is of note that in this composite system a binding of the released proteins to the nearby located LbL films should be taken into account because proteins interact with a polyelectrolyte complex, moreover in a controlled fashion [130–132]. Interaction of the release molecules may lead to a dual release giving additional options for adjustment of the release kinetics or the release profile.

6 Conclusions

This short review describes various approaches of the surface modification by microcapsule arrays. The microcapsules produced by different methods can have tuned shell properties adjusted for a specific application. The capsules can be loaded with different molecules of interest or other larger objects in that way, that variation of the environmental conditions or external stimulation lead to the shell permeabilization followed by the cargo release. It is possible to obtain the patterned microcapsule arrays on surfaces by different methods and approaches: covalent binding, electrostatic forces, biological recognition, an interplay of hydrophilic/hydrophobic interactions, LB technology, heating the assembled microparticle suspension, printing technology, electron beam irradiation, electrodeposition. Such capsule arrays have a defined order that allows implementing target release and controlled release of encapsulated substance (depending on the number of the opened capsules). The most uniform patterns of capsules can be formed using microprinting techniques and microfluidic systems. These approaches are the simplest for fabrication of the structured microcapsule arrays; the highly determined order of capsule matrixes prepared using these methods simplify the controlled release process. It is important to note also that the capsule matrixes formed using electrostatic interaction and by printing technologies are the most stability of all described systems. The ordered microcapsule arrays are designed for biomedical applications including diagnostics and biosensorics, externally controlled release, drug delivery, etc. Each and every application requires a certain conditions for

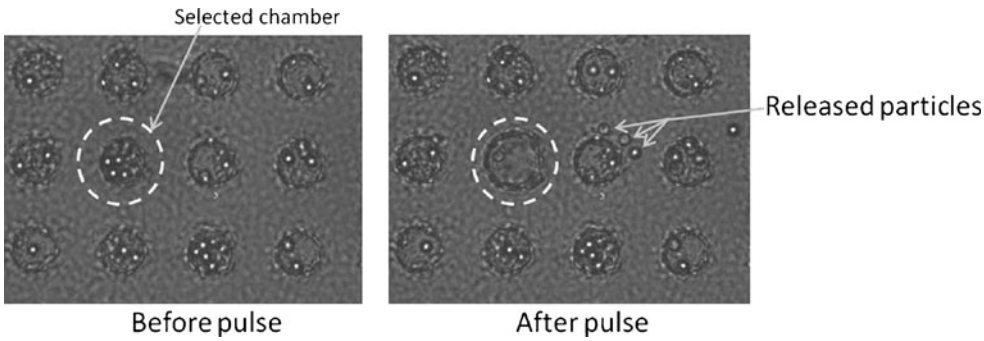

Fig. 11 Transmission optical microscopy images of LbL-assembled chambers filled with melamine formaldehyde particles before (*left*) and after (*right*) laser irradiation of the selected chamber. The particles are released from the chamber indicating its opening. Adopted from [40]

the arrays formation and the arrays properties, thus stimulating new approaches for the arrays formation and strong scientific interest to this field. Composite capsule-film system formed by spontaneous capsule loading into soft LbL films is a new platform for the array formulation with high potential impact in tissue engineering and controlled drug release applications.

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