Poly(Vinyl Alcohol)-Hydrogel Microparticles with Soft Barrier Shell for the Encapsulation of Micrococcus luteus

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The encapsulation of bacteria in polymers results in hybrid materials that are essential for the long-term biological activity of bacteria and formulations in practical applications. Here, the problem of bacterial escape and the exchange of metabolism products from hydrogel microparticles within an aqueous environment are addressed. Bacteria are encapsulated in chemically cross-linked poly(vinyl alcohol) (PVA) hydrogel-microparticles followed by their encapsulation in a pH-responsive and soft antibacterial shell of poly(N,N-diethylamino ethyl methacrylate) (PDEAEMA). This polymer shell acts selectively with regards to the mass transport in and out of the microparticle core and is affected by environmental parameters, such as pH and antibacterial effect. The pH-responsive PDEAEMA shell forms an open porous structure that accelerates nutrient transfer into the PVA core containing living Micrococcus luteus (M. luteus). Results show that the antibacterial effect of PDEAEMA retards the escape of bacteria up to 35 days when the shell is open. Additionally, the permeation of a small molecule into the gel, for example, methylene blue dye through the core/open-shell structure, certifies a flexible barrier for mass transport, which is required in the long term for the biological activity of encapsulated M. luteus.

1. Introduction

Bioremediation using bacteria is becoming an emerging field for reclaiming wastewater and decontamination of the ecosystem.[1–5] The use of bacteria for bioremediation affords various advantages over conventional methods, as it is environmentally friendly, non-toxic, inexpensive, and useful for the elimination of a wide variety of pollutants, among a broad spectrum of bacteria groups, for example, Bacillus, Pseudomonas, Streptomyces, Micrococcus, and Escherichia coli are potentially the most well-established for heavy metals’ bioremediation.[6] Micrococcus luteus (M. luteus) is an anaerobic, non-pathogenic, Gram-positive bacterium that has the capability of sequestrating heavy metals, such as copper (Cu), gold (Au), and strontium (Sr).[6–9]

Different environmental factors can negatively affect the physiological state of the bacteria. Such unfavorable impacts may be prevented by encapsulation in polymer matrices enabling their use in bioremediation.[1,6,10–12] Encapsulation of these bacteria provide not only protection against harsh environmental conditions but also provides other advantages, such as prolonged storage time, the stability of physiological activity, and ease of handling.[6,11,13] In particular, encapsulation in polymeric matrices endows protection to bacterial cells to retain their biological functionality. Natural and synthetic polymers are used for encapsulation purposes. Commonly used natural polymers are profitable and are characterized by biocompatibility, hydrophilicity, low cost, and some of them are biodegradable.[1] Chitosan and alginites are typical examples of such polymers. Despite their benefits, the low mechanical and chemical resistance of natural carriers limits their functionality for encapsulation purposes. Synthetic polymers show a range of properties, differing in porosity, polarity, and wetting behavior. Poly(vinyl chloride), poly(vinyl alcohol) (PVA), polystyrene, and polypropylene belong to such a group of synthetic polymers.[6]

Polymer particles are broadly used for the encapsulation of sensitive materials in a wide variety of applications. Polymer particles are prepared by several methods, including emulsion and suspension polymerization, evaporation, and supercritical fluid technology.[14,15] Most of these methods
are organic solvent-based. Therefore, water-soluble polymers like biopolymers are not the desired choice for the aforementioned methods due to the low solubility in organic solvents. Contrarily, spray drying has no limitation for the production of polymer particles from water-soluble polymers. Spray drying is a rapid one-step technique for manufacturing dry particles and encapsulation since the early 1940s.[16] In this method, a polymer solution (emulsion or suspension) is atomized with an atomization nozzle, followed by exposure to a hot gas stream. Evaporation of the solvent results in the formation of dried polymer particles. As the evaporation process takes place in a few seconds, a minimal loss of encapsulated material is achieved, resulting in their ability to encapsulate heat-sensitive materials such as catalysts, enzymes, bacteria, pharmaceuticals, antibiotics, and vitamins.[15,17,18] Reich et al. reported the encapsulation of two different strains of bacteria in polymer particles prepared by spray drying.[19] Despite this, the uncontrolled release of bacteria from the particles is still a challenge, as is the uptake of nutrients for the bacteria in the particles. Indeed, bacteria were released from pure PVA particles immediately after contact with aqueous nutrient media. Suppression of the release of bacteria was achieved previously by the encapsulation of the bacteria in hydrophobic shells of poly(vinylidene fluoride) and poly(p-xylene).[20] The bacterial release was retarded significantly by wrapping the particles in a shell of poly(methyl methacrylate) by polymerization. It is possible that this hydrophobic shell blocks the diffusion of nutrients into the bacteria-containing particles.[6]

The limitations of hydrophobic shells for the encapsulation of hydrogel particles containing bacteria could be overcome by the use of shells made of responsive polymers. The release of the encapsulated material, therefore, is dependent on the composition of the outer shell. A dense layer of a responsive polymer permeable to water and nutrients or other small molecules, for example, metal ions or dye molecules, can be manipulated to release compounds by changing the pH or temperature, or medium. Chen et al. successfully established a method for drug release with pH-responsive polymeric micelles in tumors since the tumor pH is different from normal physiological pH.[21,22] Poly(N,N-diethylamino ethyl methacrylate) (PDEAEMA) is a well-known pH-responsive polyelectrolyte with pKₐ 6.9.[23] In a basic pH range, PDEAEMA coagulates and forms a closed pore membrane. A pH below 6.5 results in open pores in the shell due to the protonation of the amine group and steric repulsion of the chains in the membrane (Scheme 1A).

Herein, we report on a concept for the encapsulation of M. luteus in microparticles covered by an additional shell to prevent the uncontrolled release of M. luteus and provide the potential for the release and uptake of small molecules, including nutrients. Following Scheme 1B, M. luteus was encapsulated in PVA microparticles (M. luteus/PVA microparticles) using spray drying. M. luteus/PVA microparticles were chemically cross-linked by glutaraldehyde. Subsequently, the surface of the hydrogel microparticles was functionalized with an initiator for atom transfer radical polymerization (ATRP). This macrominitiator initiated the polymerization of N,N-diethylamino ethyl methacrylate (DEAEMA). The final result of this process was PVA hydrogel microparticles with a shell of the pH-responsive PDEAEMA. We explored the effect of this PDEAEMA cover on the release of M. luteus from the hydrogel PVA microparticles. In particular, at low pH, where the shell is quasi-open (the PDEAEMA is in its quaternized stage), it is antimicrobial for M. luteus.[24–27] Further, we analyzed the permeation through the shell at low pH using the dye methylene blue as a model for small molecules.

2. Result and Discussion

The PVA microparticles without M. luteus were prepared by spray drying a 2.5% PVA solution in phosphate-buffered saline (PBS) (sample 1). These spherical microparticles ranged from a few hundred nanometers to ≈2.8 μm in size (Figure 1A and Figure S1A, Supporting Information). Following this experiment (see also Scheme 1B), we encapsulated colloidal M. luteus, which has a spherical shape in a size range of 0.5–1 μm (Figure 1B). The encapsulation by spray drying yielded microparticles ranging from 2.64 ± 0.96 μm in size (sample 2, Figure 1C, and Figure S1B, Supporting Information). The successful encapsulation of live M. luteus in the PVA microparticles was proven using agar plate tests and observation of characteristic yellow colonies. In our previous work, we showed the encapsulation of bacteria in PVA microparticles by advanced characterization method, such as Raman-AFM.[28] Since some of these PVA microparticles also contained dead M. luteus, we analyzed the survival of M. luteus in additional steps using the live/dead analysis test and by differentiating the live and dead cells (microparticles with dead M. luteus cells [sample 3] were used as control samples for live/dead test).

Next, we studied the morphology of non-cross-linked and cross-linked PVA microparticles as well as the effect of cross-linking on M. luteus in the microparticles with live (sample 4) and dead bacteria (sample 5). Cross-linking of the microparticles was done by glutaraldehyde. The amount of glutaraldehyde was below its minimum bactericidal concentration/minimum inhibitory concentration (MBC/MIC) for M. luteus (MBC = 0.7 mg L⁻¹ and MIC = 0.001 mg L⁻¹). The amount of glutaraldehyde varied in the range of 0.03–0.12 [glutaraldehyde]/[M. luteus]/PVA microparticles) g g⁻¹ to study the swelling behavior of the PVA microparticles to achieve low swelling of PVA microparticles in the water at the lowest possible amount of glutaraldehyde. As seen from Figure S2A, Supporting Information, the concentration ratio of 0.06 glutaraldehyde to PVA microparticles was used to ascertain the toxicity of glutaraldehyde toward M. luteus while attaining minimum swelling of the PVA microparticles. According to scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) analysis (Figure 1D,E), the swelling of the M. luteus/ cross-linked PVA microparticles (sample 4), the average size of the microparticles increased from 2.64 ± 0.96 to 5.28 ± 1.91 μm after 24 h at room temperature at a concentration of 0.1 wt% in water. The swelling ratio of cross-linked microparticles decreased with a larger amount of glutaraldehyde (Figure S2A, Supporting Information), while the soluble residue of non-cross-linked PVA (Figure S2B, Supporting Information) increased with decreasing amount of glutaraldehyde. The shape of the microparticles did not change significantly using
the ratio of 0.06 glutaraldehyde to PVA microparticles (Figure S2C,D, Supporting Information).

Next, we studied the grafting of the cross-linked PVA micro-particles loaded with live *M. luteus* and dead *M. luteus* by DEAEMA, as well as the grafting of microparticles containing methylene blue (Scheme 1B, samples 6–9). The encapsulated *M. luteus* in PVA hydrogel microparticles survive harsh conditions, such as treatment with organic solvents in comparison to the unprotected *M. luteus* as shown in our previous work.[12] The surface grafting of DEAEMA on the microparticles was accomplished by ATRP at room temperature. The results of these grafting reactions were cross-linked microparticles with a shell of PDEAEMA. The grafting of PDEAMA was confirmed by FT-IR (Figure S3, Supporting Information). The IR-spectra showed a band at 3500 cm\(^{-1}\), which corresponds to the amine group of PDEAEMA. The average diameter of the microparticles (100 particles were counted) with encapsulated *M. luteus* increased from 2.64 ± 0.96 µm for bare *M. luteus*/PVA microparticles (sample 2) to 3.25 ± 1.22 µm for sample 6 (see Figure S4, Supporting Information, for size distribution). The shell of PDEAEMA did not increase the size of the microparticles significantly according to SEM analysis (Figure 1F). It is obvious that SEM is not an appropriate method to conclude about the influence of the macromolecular layer on the microparticle diameter, notably due to the very broad distribution of the particle diameter and a very thin layer of the shell. Therefore, the PDEAEMA shell thickness of sample 6 was determined by transmission electron microscope (TEM) measurement. The PDEAEMA shell was stained using uranyl acetate, followed by embedding in epoxy resin and microtome sectioning, resulting in a shell thickness of ≈500 nm (Figure 2A).

To test the fitness of the encapsulated *M. luteus* over the whole process of spray drying, synthesis, and storage, we applied a live/dead analysis test. In this method, live *M. luteus* were indicated by green and dead by red color in a confocal laser microscope (Figure 2B). Sample 7 was also probed with staining of dead *M. luteus* to ensure the red and green dots are not an artifact of confocal microscopy (Figure S5A, Supporting Information). The survival of *M. luteus* was also confirmed using the agar plate test and SEM imaging of the microparticles after disassembly of the core/shell morphology (Figure S5B,C, Supporting Information).

The escape of *M. luteus* from non-cross-linked and cross-linked microparticles was investigated with and without PDEAMA shell. The escape of live *M. luteus* cells was analyzed by keeping the microparticle samples in a nutrient culture...
medium, followed by an agar plate test and detection of colonies. As expected, the escape of *M. luteus* from the non-cross-linked microparticles was observed within a few hours.[6] This occurred due to the swelling of the microparticles in water. The same result was observed for the cross-linked microparticles (Table S3, Supporting Information). In contrast to this, the escape of *M. luteus* was significantly retarded for the cross-linked microparticles with the PDEAMA shell. Therefore, we studied the microparticles with the PDEAMA shell in more detail. Sample 6 was suspended in a buffer solution of different pH values, and the diameter of the swollen microparticles was determined by SLS measurement (Figure 2C). Although the SLS measurement showed a significant increase in size of the microparticles by decreasing pH to acidic region, due to the broad size distribution, confocal laser microscopy was further used to confirm this effect. The confocal laser microscope image of sample 6 in aqueous media with pH = 1 is shown in Figure S6, Supporting Information. The diameters of 100 microparticles were counted and averaged (7.51 ± 3.99 µm). The average diameter of sample 6 particles in the dry state was 3.25 ± 1.22 µm as determined from SEM (Figure 1F).

The PDEAMA shell significantly reduced the escape of *M. luteus* out of core/shell microparticles even when the shell of PDEAMA is in its open state at pH = 5 (Table 1). To quantify this, the release of *M. luteus* was examined for the sample using grafted PDEAEMA shell from the surface of *M. luteus*/PVA microparticles (sample 6) and compared to the sample without a PDEAEMA shell (sample 4, Table S3, Supporting Information). The same study for the non-cross-linked PVA microparticles was shown in the work of Knierim et al.[6] Samples were kept separately in a nutrient medium (pH = 5), and the leaching of *M. luteus* was quantified by taking samples from the nutrient medium at weekly intervals. The release of bacteria was proven by the presence of yellow colonies on agar plates (Table 1). As yellow colonies appeared only after five weeks, it is likely that the release of *M. luteus* was significantly delayed by the presence of the PDEAEMA shell. In contrast, *M. luteus* from the microparticles without the shell (sample 2) was evident after 72 h in pH = 5.

The microparticles with the open shell of PDEAMA should allow the mass transfer of small molecules easily. To probe the mass transfer, we have used methylene blue as a model for small molecules. As the mass transport via the PVA hydrogel microparticles (cross-linked microparticles without PDEAEMA shell) occurs immediately (due to the swelling behavior), the dye transfers (in and out) were studied for the sample with the shell. This experiment was carried out by loading the core/shell microparticles (sample 8) with methylene blue at pH = 5 (open pore structure was formed by protonated PDEAEMA shell) (Figure S7, Supporting Information). The extra amount

![Figure 1. SEM image of A) PVA microparticles obtained by spray drying (sample 1). B) ESEM of *M. luteus* in PBS solution. C) SEM image of PVA microparticles with encapsulated *M. luteus* (sample 2). D) SEM micrographs of *M. luteus*/cross-linked PVA microparticles using the ratio of 0.06 glutaraldehyde to PVA microparticles (sample 4) after 24 h in water. E) ESEM image of *M. luteus*/cross-linked PVA microparticles (sample 4) in water. F) SEM image of core/shell microparticles (sample 6).](https://www.advancedsciencenews.com/figure1)
of methylene blue on the surface of the microparticles was washed with water, and samples were kept in pH = 9 (closed-shell) for 48 h to keep the methylene blue inside the microparticles. The release of the dye was quantified in acidic pH = 5, by absorption measurement with UV/vis. These measurements reveal that 10 wt% of the methylene blue leached within the first 24 h. The experiment proves sufficient nutrient transfer toward the suspension of core/shell microparticles with encapsulated *M. luteus* and vice versa.

### 3. Conclusion

The synthesis of the core/shell microparticle of PVA/DEAEMA was shown as a proof of concept for a system for the encapsulation of *M. luteus* in core/shell microparticles with a reduced bacterial escape but the potential for the mass transfer of small molecules. The pH-responsive design of the shell of these microparticles makes them of particular interest, for example, in wastewater treatment by bioremediation. The mild polymerization conditions for the grafting of the PDEAEMA shell by ATRP was very important for the fitness of *M. luteus* after the grafting reaction. The diameter of the *M. luteus*/PVA/PDEAEMA microparticles increased from 3.25 ± 1.22 µm at pH = 7 to 7.51 ± 3.99 µm in an aqueous medium at pH = 1, which indicated that the elasticity and ability of such core/shell structures also allowed bacterial growth, which is another important characteristic of a functional microbial system.

The efficiency of the core/shell microparticle morphology in terms of the physiological activity of encapsulated *M. luteus* was further investigated by measuring the release of *M. luteus* from the microparticles. The results are summarized in Table 1.

| Time [weeks] | Samples                                      |
|-------------|----------------------------------------------|
|             | *M. luteus*/PVA/PDEAEMA microparticles (sample 6) | no colony | no colony | no colony | no colony | colonies observed |

*Figure 2.* A) Microtome section embedded in epoxy resin of sample 6. B) Live/dead analysis of *M. luteus* using confocal laser microscopy, red: dead *M. luteus*, green: living *M. luteus*. C) Diameter change of core/shell microparticle (sample 6 in water) at different pH (pH = 1, 3, 5, 7, and 9), measured by SLS.
was demonstrated by performing a live/dead analysis test. The antibacterial effect of PDEAEMA was examined by suspending the microparticles in a nutrient medium and an agar plate test, retarding the release of \textit{M. luteus} up to 5 weeks. We conclude from the mass transfer experiment with methylene blue that mass transfer of nutrient and metabolism products will be possible from microparticles. As a result, the core/shell micro particles with encapsulated \textit{M. luteus} provide a promising living composite for a wide variety of applications since higher metabolism efficiency with retarded leakage of bacteria was observed.

4. Experimental Section

\textbf{Materials}: PVA (M\textsubscript{w} = 13000–23000 g mol\textsuperscript{-1}, 99% hydrolysis), glutaraldehyde (70 wt\% in H\textsubscript{2}O), 1,1,4,7,10,10-hexamethyl triethylenetetramine (HMTETA) (97%), \(\alpha\)-bromoisobutyryl bromide (98%), butyl acrylate (>99%); purchased from Sigma Aldrich, and ethyl-2-bromoisobutyr atoe (98%, Acros) were used as received. Copper bromide (CuBr) (98%, Acros) was flushed with argon before use. DEAEAMA (Sigma Aldrich, 99%) was distilled under vacuum and stored under argon. Dichloromethane (DCM), pyridine, tetrahydrofuran (THF), anisole, and acetone were distilled prior to use.

\textbf{Analytical Methods}: ESEM (QuantaTM FEG 250, EFI) was performed to investigate the morphology of microparticles in aqueous media (temperature \(= 2^\circ\)C; pressure \(= 220\) Pa). The measurements were carried out on a Wet-STEM, enabling control of humidity and temperature at an acceleration voltage of 1 kV. A gaseous secondary electron detector and circular backscatter detector detectors were used.

The particle size was measured by SEM; a Zeiss LEO 1530 (Jena, Germany) with a Schottky field emission cathode was used. The samples were adhered to a sample holder with double-sided adhesive tape and subsequently coated with 2.0 nm of platinum by a high-resolution circular backscatter detector detectors were used.

\textbf{Encapsulation of Micrococcus luteus in Poly(Vinyl Alcohol) Microparticles Using Spray Drying; Cultivation of \textit{M. luteus}}: A mixture of meat extract (lysogeny broth [LB] culture medium) (Roth) in extra pure water provided by a Milli-Q Plus system (conductivity \(= 0.072 \mu S\) cm\textsuperscript{-1}, pH \(= 7\)) was used as a nutrient medium for cultivating \textit{M. luteus} (DSM-No. 20030, DSMZ Braunschweig). The mixture was sterilized with an autoclave at 121 °C. Utilizing encapsulation, \textit{M. luteus} bacterial cells were harvested after 72 h of growth in LB culture medium at 37 °C. The cell pellet was achieved by centrifuging at 4000 rpm for 10 min. Subsequently, the bacterial cells were washed with PBS (pH \(= 7.4\)).

Agar plates were prepared by mixing 15 g LB culture medium and 12 g agar-agar (Roth) in 750 mL water, followed by sterilizing the mixture.

In all the steps of the encapsulation and synthesis, a survival test was done by incubating the \textit{M. luteus} on agar plates for 72 h at 37 °C. The growth of yellow colonies revealed the fact that \textit{M. luteus} is biologically active.

\textbf{Encapsulation and spray drying}: In general, a solution of 2.5 wt\% PVA in PBS, pH = 7.4, was prepared and sterilized in an autoclave (121 °C). The bacterial pellet was washed and resuspended in the PVA solution at room temperature (Scheme 1B). Dry PVA microparticles with encapsulated \textit{M. luteus} were prepared by spray drying, using a mini spray dryer b290 (Büchi, Switzerland). The atomization gas was set to 600 L h\textsuperscript{-1} (50 mm) with an inlet temperature of 110 °C. A mixture of PVA solution with \textit{M. luteus} was delivered to the device with a feeding rate of 2.5 mL min\textsuperscript{-1}. The experiment was carried out under an ambient atmosphere, using an open-loop system, in which the atmospheric air was proceeding to the device and heated for evaporation of the solvent. Parameters used for spray drying are listed in Table S1, Supporting Information. Products (sample 2, Table 2) were collected and stored at 4 °C.

Two control samples were manufactured (samples 1 and 3, Table 2): sample 1 was prepared by spray drying of PVA microparticles without

| Samples | Cores | \textit{M. luteus}/PVA microparticles [g g\textsuperscript{-1}] | Diameter [µm] |
|---------|-------|---------------------------------------------------|--------------|
| 1       | PVA particle | —                   | 1.68 ± 1.12  |
| 2       | Alive \textit{M. luteus}/PVA microparticle | 1/20            | 2.64 ± 0.96  |
| 3       | Dead \textit{M. luteus}/PVA microparticle | 1/20            | 2.64 ± 0.96  |
| 4       | Alive \textit{M. luteus}/cross-linked PVA microparticle | 1/20            | 2.64 ± 0.96  |
| 5       | Dead \textit{M. luteus}/cross-linked PVA microparticle | 1/20            | 2.64 ± 0.96  |
| 6       | Alive \textit{M. luteus}/cross-linked PVA/PDEAEMA microparticle | 1/6            | 3.25 ± 1.22  |
| 7       | Dead \textit{M. luteus}/cross-linked PVA/PDEAEMA microparticle | 1/6            | 3.25 ± 1.22  |
| 8       | PVA/PDEAEMA microparticle | 1/6            | —            |
| 9       | Methylene blue/PVA/PDEAEMA microparticle | 1/6            | —            |

For the shell characterization, an elastic bright-field TEM (Zeiss 922 Omega EFTEM, Jena, Germany) at a voltage of 200 kV was used.

A confocal laser microscope was performed using a Leica TCS SP8 (Model DMI 6000, HyD Hybrid-Detector; PMT-Detector) with an argon laser (488 nm).

SLS measurements were performed on an LS spectrometer of LS Instruments AG (Fribourg, Switzerland) using a HeNe laser (maximum 35 mW constant output at 632.8 nm) as a light source. Two APD detectors in pseudo-cross-correlation were used to detect the scattered light. The time average scattering intensities were measured at a scattering angle of 90°.

\textbf{Table 2.} Spray-dried PVA microparticles with encapsulated \textit{M. luteus} (non-cross-linked and cross-linked microparticles (using the ratio of 0.06 glutaraldehyde to PVA microparticles), and the synthesis of core/shell microparticles of PVA/PDEAEMA.
encapsulated M. luteus. For sample 3, dead M. luteus cells were encapsulated in PVA microparticles. To produce sample 3, M. luteus pellet was separated from the culture medium and washed with ethanol (for killing M. luteus) before encapsulation. Dead cells were mixed with 2.5% PVA solution before spray drying. The agar plate test resulted in formation of zero colonies, which reveal the encapsulation of dead cells in PVA microparticles.

Cross-linking and functionalization of M. luteus/PVA microparticles: PVA microparticles with encapsulated M. luteus (live and dead cells, samples 2 and 3) were chemically cross-linked with glutaraldehyde at (live and dead cells, PVA microparticles with encapsulated M. luteus (0.29 g) were added to the mixture and stirred for 4 h at RT. The cross-linked microparticles (samples 4 and 5) were filtered and washed with THF.

Synthesis of functionalized M. luteus/cross-linked PVA microparticles: M. luteus/cross-linked PVA microparticles were functionalized to be used as a macroinitiator (Scheme 1B). Cross-linked M. luteus/PVA microparticles (0.1 g) were dispersed in 25 mL DCM and 1.6 mL (0.02 mol) pyridine, α-bromoisobutyryl bromide (0.87 mL, 7 mmol) was added, and the mixture was kept stirring at RT while purging with argon for 24 h. The microparticles were filtered and washed with DCM and acetone.

Surface polymerization of PDEAEMA on M. luteus/PVA microparticles using ATRP: Core/shell microparticles (samples 6–9, Table 2) were synthesized using surface-initiated ATRP (Scheme 1B). The core/shell ratio was calculated by regarding the weight (g) of PVA microparticles and the feeding ratio of the DEAEMA monomer for surface polymerization. In the following, the surface polymerization of sample 7 was described, Table 2, as an example. The cross-linked and functionalized M. luteus/PVA microparticles (macroinitiator) (0.404 g) were dispersed in 20 mL anisole and hexamethylenetetramine (HMTETA) (0.55 g, 3.9 mmol). The polymerization was started by the addition of CuBr (0.2 g, 1.4 mmol). Mixtures were kept for 24 h while purging with argon at RT. The core/shell microparticles were filtered and washed thrice with acetone and DCM. In order to check the survival of bacteria, sample 6 was immersed in liquid nitrogen, filtered and washed thrice with acetone and acetone.

Staining with uranyl acetate: PVA microparticles (sample 1, Table 2) were added to a mixture of 0.01 g ruthenium oxide in 1 mL water. After 5 min, the microparticles were centrifuged and washed thrice with water. Staining with uranyl acetate: M. luteus/PVA/PDEAEMA microparticles (core/shell, sample 7, Table 2) were stained with uranyl acetate before embedding in epoxy resins. Uranyl acetate (0.04 g) was dissolved in 4 mL water. This was followed by the addition of 0.1 g of sample 10, followed by shaking, precipitation, and washing the microparticles thrice with water.

Live/dead analysis: The viability of M. luteus was tested with a confocal laser microscope (LSM510, Zeiss, Oberkochen, Germany) and by staining [BacLight Bacterial Viability Kit, Life Technologies [Eugene, OR]] of M. luteus (live/dead stain). The green color of Syto9 (green, fluorescent nucleic acid stain) showed living bacteria, and the red color of propidium iodide represented the dead bacterial cells.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability Statement
The data that supports the findings of this study are available in the supplementary material of this article.

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