Self-Propelled PLGA Micromotor with Chemotactic Response to Inflammation

Jiamian Wang, B. Jelle Toebes, Adelina S. Plachokova, Qian Liu, Dongmei Deng, John A. Jansen, Fang Yang,* and Daniela A. Wilson*

Local drug delivery systems have recently been developed for multiple diseases that have the requirements of site-specific actions, prolonged delivery periods, and decreased drug dosage to reduce undesirable side effects. The challenge for such systems is to achieve directional and precise delivery in inaccessible narrow lesions, such as periodontal pockets or root canals in deeper portions of the dentinal tubules. The primary strategy to tackle this challenge is fabricating a smart tracking delivery system. Here, drug-loaded biodegradable micromotors showing self-propelled directional movement along a hydrogen peroxide concentration gradient produced by phorbol esters-stimulated macrophages are reported. The drug-loaded poly(lactic-co-glycolic acid) micromotors with asymmetric coverage of enzyme (patch-like enzyme distribution) are prepared by electrospraying and postfunctionalized with catalase via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide coupling. Doxycycline, a common drug for the treatment of periodontal disease, is selected as a model drug, and the release study by high-performance liquid chromatography is shown that both the postfunctionalization step and the presence of hydrogen peroxide have no negative influence on drug release profiles. The movement behavior in the presence of hydrogen peroxide is confirmed by nanoparticle tracking analysis. An in vitro model is designed and confirmed the response efficiency and directional control of the micromotors toward phorbol esters-stimulated macrophages.

1. Introduction

Inflammation is associated with many pathological disorders, including infections, cancers, atherosclerosis, neurodegenerative diseases, and arthritis.[1] When these disorders are not cured fast enough, chronic inflammation develops. Chronic inflammation can lead to tissue death, muscle degeneration, and the thickening and scarring of tissue.[2] One of the examples is periodontitis, a chronic inflammation surrounding the teeth and originated from pathogenic bacteria in the mouth that infect the periodontal tissues. Worldwide, 11.2% of adults are affected by its severe forms, and it has been considered as the major cause of tooth loss in adult humans.[3] Chronic periodontitis has also been linked to multiple debilitating diseases, such as diabetes, rheumatic, and cardiovascular diseases.[4] Treatment of periodontitis involves mechanical debridement to eliminate the pathogenic microflora of sites in periodontal pockets. However, the mechanical treatment cannot always produce the contamination-free periodontal environment in susceptible patients due to the rapid recolonization of biofilm, which induces the recurrence of uncontrolled inflammation.[5] This constant biofilm recolonization, together with the susceptibility is the major problem in managing periodontitis. Besides that, periodontitis does not have a linear progression, and each burst in the disease manifested as increase in the pathogenic microflora and increase in pocket depths, is associated with irreversible tissue loss. Therefore, the adjunctive treatment with antimicrobial or anti-inflammatory agents for long-term local application in addition to mechanical instrumentation could prevent such irreversible tissue loss and have already been advocated to minimize the surgical requirement.[6]

Local delivery therapeutics can provide higher drug concentrations and diminish the adverse effects compared to systemic...
delivery.\(^7\) However, their application requires frequent visits at the dental office, and more precise drug delivery due to the complicated geometry of the periodontal pocket, which is unique in each patient. In addition, these systems have to compete with the crevicular fluid, which continually flushes out the pocket and does not allow any agent to stay there for a long time to induce an effect. There are different delivery systems to transport the therapeutic agents sustainably inside the periodontal pocket, e.g., chips,\(^8\) gels,\(^9\) and particles.\(^10\) Compare to the others, drug-loaded polymeric particles can be injected into the periodontal pocket exhibiting longer release time, offering good patient compliance, and minimizing systemic complications.\(^11\) Yet, the administration of the particles inside deep, curvy, and narrow periodontal pockets still involves a passive trajectory, making it hard to reach the deepest part of the periodontal pocket where usually has severe inflammation. Therefore, autonomous drug carriers being able to move directionally to the diseased tissue facilitating an active delivery can better address the current limitations.

At the site of inflammation, including periodontitis, reactive oxygen species (ROS) are generated by immune cells, such as macrophages, to kill pathogens.\(^12\) Hydrogen peroxide (H\(_2\)O\(_2\)) is the precursor for the production of most ROS and is therefore found at elevated levels in inflamed tissue, up to 50–100 \(\times 10^6\) M.\(^13\) In the last decade, several groups have developed various autonomous micromotors responsive to the presence of H\(_2\)O\(_2\) to achieve a relevant biological mission.\(^14\) The first example was micromotors used for the transport and release drugs, which demonstrate the catalytic nanowire shuttles are capable of picking up drug-loaded particles or liposomes and transporting them in a predetermined route.\(^15\) Another typical attempt is to produce Janus multilayer capsule motor with partially coated dendritic platinum nanoparticles. The motors showed a prominent drug delivery capacity and had stimuli-response for controllable encapsulation and release of model drugs. The movement were recoiled by the bubbles, which is produced by the decomposition of hydrogen peroxide as fuel.\(^16\) Some other designs, equipping a polymer-based Janus motor with a thin gold layer on one side, followed by chemical immobilization of catalytic enzymes for targeted drug delivery. The motor can be navigated to a cell layer by an external magnetic field and release drugs by a near-infrared light trigger.\(^17\) A similar micromotor design for targeted drug delivery was later developed using a biodegradable bovine serum albumin/poly-l-lysine multilayer rocket, followed with the incorporation of a heat-sensitive gelatin hydrogel containing gold nanoparticles, drugs, and catalase. The system showed efficient delivery to cancer cells and near-infrared light-controlled release. The movement is created by a combination of catalytic bubble propulsion and magnetic guidance.\(^18\) Various other designs have been reported.\(^12b,19\) Although all these micromotors can be loaded with drugs to achieve drug delivery, their design is laborious, difficult to upscale while it is still a challenge to apply these motors in a biological-relevant H\(_2\)O\(_2\) environment that is produced by cells or resident bacteria. To create a micromotor which can respond to a biological H\(_2\)O\(_2\) concentration, a bottom-up design is needed. There are vast differences in construction, building materials (e.g., silica, polymers, or metals) and the type of catalyst used. For biomedical applications, in particular drug delivery, particles with biodegradability and biocompatibility, which can control drug release time would be ideal. For the catalyst, often metals are used such as platinum,\(^20\) silver,\(^21\) and molybdenum oxide.\(^22\) The recent trend, however, is to use enzymes, as these natural catalysts are efficient, sensitive to a low concentration of H\(_2\)O\(_2\), and biocompatible.\(^23\)

To engineer autonomous drug carriers and achieve the function of desired released drug doses, we combined catalase with poly (lactic-co-glycolic acid) PLGA microspheres, which could modulate the drug release from days to months. Catalase is a common enzyme, which can decompose H\(_2\)O\(_2\) into water and oxygen. It has been shown that this reaction can be used to propel nano- and micromotor systems.\(^24\) Besides its ability to fuel the motion of nano- and micromotor systems,\(^24\) the gradients of H\(_2\)O\(_2\) were demonstrated to sustain directional movement of the nanocarriers including of enzymes toward higher concentrations of fuel, thus making catalase a promising candidate as the catalyst for our microsphere carriers.\(^25\) Oxygen produced from the active particles moving in a gradient has another potential positive effect: it could change the original oral microenvironment, which is essential for the treatment of periodontal disease. In this system, doxycycline hyclate (DOX), a common drug for the treatment of periodontitis, was incorporated as a model drug.\(^26\)

2. Results and Discussion

2.1. Preparation and Characterization of PLGA Motors

Here, DOX loaded PLGA particles were first prepared by electrospray technique (\textbf{Figure 1}). The PLGA was dissolved in acetone and mixed with DOX solution. The weight ratio of PLGA to DOX is 100: 7.5. Acetone is one of the Class 3 solvents (having less toxic and lower risk to human health) according to the guideline of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and well accepted in pharmaceuticals.\(^27\) During the electrospraying process, the organic solvents evaporated, leading to particle formation of around \(1.8 \text{~µm}\) (\textbf{Figure S1}, Supporting Information). The DOX loaded particles were collected in a water bath with 1% poloxamer to prevent aggregation. As the particles contained carboxyl end groups, they can be used as handles for surface functionalization of the PLGA particles. In our case, we coupled the enzyme catalase to the surface of the particles. The grafted catalase serves as the catalyst to decompose H\(_2\)O\(_2\) into oxygen and is responsible for the propulsion of the entire system. The movement behavior of functionalized PLGA particles was tested with the H\(_2\)O\(_2\) that is produced by inflammatory cells. \textbf{Figure 1} summarizes the experimental procedure for preparation by chemical linkage of catalase on grafted PLGA particles and the response to inflammatory cells. The encapsulation efficiency of DOX into the particles demonstrates the successful loading of the drug and no significant drug loss during the functionalization process (\textbf{Figure S2}, Supporting Information). After the encapsulation and the functionalization, the drug release was tested. The particles showed a long release profile of the drug over 150 h, as shown in \textbf{Figure 2}. Grafted PLGA particles (\textbf{Figure 2B}) showed a similar drug release profile compared to the original PLGA
particles (Figure 2A), also in the presence of H₂O₂ at different concentrations, indicating the functionalization with enzyme did not affect the drug release behavior. After functionalization, less burst release could be observed. Both reduced burst release and long-term drug delivery behavior are ideal for a periodontal application. It can prevent a reinfected pocket that often happened in periodontitis patients. The activity of DOX released from both groups were tested against \textit{Aggregatibacter actinomycetemcomitans} (\textit{A. actinomycetemcomitans}), an exogenous bacterium occurs in 90% of localized aggressive periodontitis. The released DOX from both groups showed similar minimum inhibitory concentration (MIC) as freshly prepared DOX (Table 1), indicating the preparation procedure does not affect the drug activity. As expected, the change was observed in morphology of the particles through the functionalization process, as shown with scanning electron microscopy (SEM) (Figure 2C). Blank PLGA particles have a smooth surface, whereas enzyme functionalized particles showed a rough asymmetric surface, due to uneven distribution of catalase on the surface (Figure 2D; and Figure S3, Supporting Information). Catalase grafted on the surface formed asymmetrical domains, which is essential for an autonomous propelling system. These patch-like catalases asymmetrically distributed in a random fashion. It can be attributed to a few amounts of randomly distributed acid end groups of PLGA on the surface of particles after the preparation process. Energy-dispersive X-ray spectroscopy (EDX) was...
used to show the element distribution of functionalized PLGA motors (Figure 2E), in which the distribution of nitrogen is asymmetric. Elements spectra of EDX indicate the appearance of nitrogen after the functionalization process (Figure 2F), in which the atom proportion on the surface of the particle increased from 0% in PLGA particles to 18.02% (Table S1, Supporting Information) in functionalized PLGA particles. After functionalization, the size of PLGA particles increased from ≈1.8 to ≈2 μm (Figure S1, Supporting Information). The particles may fall within the size range favorable for phagocytosis by immune cells. On the one hand, this might contribute to an insufficient amount of drugs reaching the deep target tissue. On the other hand, this may help to target the intracellular pathogens inside macrophages which are very difficult to eliminate.[30] Furthermore, the dispersity of particles in phosphate buffer saline (PBS) also increased after functionalization (Figure S4, Supporting Information). This might be due to the increased z-potential (≈−7.4 to −15.3 mV) and hydrophilicity of the grafted PLGA particles. Quantification of the catalase after binding was done via inductively coupled plasma mass spectrometry (ICP-MS). A calibration curve for iron in samples with different concentrations of catalase was made, after which the sample with catalase grafted PLGA particles was measured. The particle concentration was determined with nanoparticle tracking analysis (NTA) and from these data the catalase binding was done via inductively coupled plasma mass spectrometry (ICP-MS). A calibration curve with different concentrations of catalase was made, starting from 0.1 to 4 U mL⁻¹ of DOX for A. actinomycetemcomitans.

### Table 1. MIC50 values (μg mL⁻¹) of DOX for A. actinomycetemcomitans.

| Groups    | Fresh DOX | DOX released from PLGA particles | DOX released from grafted PLGA particles |
|-----------|-----------|----------------------------------|------------------------------------------|
| MIC 50    | >0.3      | >0.2                             | >0.3                                     |

2.2. Motion Characterization of PLGA Micromotors

After confirming the morphology and drug release ability of grafted PLGA particles, we tested their response to H₂O₂. NTA was used to record the movement of the particles in presence and absence of H₂O₂. Videos of 60 s were taken, and the x and y coordinates of the particles were used to calculate and plot the mean square displacement (MSD) (Figure 3). The MSD plot of functionalized PLGA particles in PBS buffer showed a linear relation to the corresponding step time interval, indicating an isotropic random movement or Brownian motion. In presence of H₂O₂, the particles moved faster by showing an increase in the diffusion constants (Table S2, Supporting Information). The moving speed of particles increased with increasing amounts of fuel. The same behavior is found for the particles in cell medium in presence of H₂O₂, showing no interference effects of the surrounding medium (Figure 3D). Blank particles showed no changes in moving speed when exposed to H₂O₂ solution (Figure S7, Supporting Information). Similar micromotors with asymmetric enzyme distribution were detailed demonstrated in previous research.[31]

2.3. Chemotaxis Behavior of PLGA Micromotors in an Inflammatory Periodontal Model

Finally, we created a chemotaxis model to simulate the inflammatory environment of periodontitis to test the chemotactic behavior of these particles in vitro (Figure 4A). In an inflammatory spot, H₂O₂ is produced by immune cells through respiratory burst.[32] The respiratory burst of phagocytes generates ROS that contribute to antimicrobial defense.[33] In our in vitro model, macrophage cells of Raw 264.7 were seeded on one side of the chamber and incubated for 3 d. In the experimental groups, phorbol-12-myristate-13-acetate (PMA) was added to the medium to trigger cells, which lead to the expected cell morphologic change, as shown in Figure 4B,C.[34] The produced H₂O₂ of the cells before and after the PMA treatment was found to be 0 and 6.78 × 10⁻⁶ M, respectively, indicating the respiratory burst of the activated macrophages (see data, Supporting Information). Those cells were used to simulate the immune cells

![Figure 3. A) MSD values of 40 grafted PLGA particles obtained at various H₂O₂ concentrations in PBS. B) Trajectories of grafted PLGA particles in H₂O₂. C) Trajectories of grafted PLGA particles in PBS. D) MSD values of 40 grafted PLGA particles obtained at various H₂O₂ concentrations in cell medium.](image)
in a deep, narrow periodontal pocket which produce a higher concentration of H₂O₂. Compare to our experimental condition, at a chronic inflammation spot, H₂O₂ can be produced by different cells, such as monocyte, neutrophils, and macrophages. The large number of cells in the tissue contribute to a higher concentration of biological H₂O₂. After 3 d incubation, fresh medium was added to the other side to form the gradient of H₂O₂. Several hours later, small amounts of particles were added to the same side (position 0 (P0) in Figure 4A). The chamber was left to rest for half an hour to minimize possible flows. Two control groups were also tested, one involving the cells without PMA treatment and the other containing particles without catalase functionalization (Table 2). To test the moving efficiency of the particles, the moving behavior of particles was measured at 2 locations by bright field microscopy: 1) position 0 (P0) where the fresh medium was added; 2) position 1 (P1) where it was the edge of the cell cluster with a high concentration of H₂O₂ produced by triggered Raw 264.7 (Figure S8, Supporting Information). An H₂O₂ gradient was expected to form between these two locations due to the convection of H₂O₂. Videos of blank and functionalized particles were taken at both positions for 10 min. The gradient field resulted in directional movement of the functionalized PLGA particles toward the triggered cells, as demonstrated by several hundred micrometers displacement of particles in the chemotactic channel (Figure 4). The similar trajectories have been reported by previous research which showed a chemotaxis behavior of micromotors. Brownian motion was observed

![Figure 4. A) Schematic overview of the chemotactic model. Raw264.7 cells before B) and after C) triggered by PMA (green: actin cytoskeleton, blue: cell nuclei). The trajectories of PLGA particles at different positions over time. D) The catalase-grafted PLGA particles performed a directional movement in response to the gradient of H₂O₂ at P0, a randomly fast movement at P1. E) The movement behavior of PLGA particles without enzyme in a gradient medium at P0 and P1. F) The movement behavior of grafted PLGA particles in a medium with untriggered cells at P0 and P1.

Table 2. Group set up of moving behaviors of PLGA particles.

| Groups            | Type of medium                          | Type of particles |
|-------------------|-----------------------------------------|------------------|
| Experiment group  | DMEM with 1 × 10⁻⁶ M PMA                | Grafted PLGA particles |
| Control group 1   | DMEM                                    | Grafted PLGA particles |
| Control group 2   | DMEM with 1 × 10⁻⁴ M PMA                | PLGA particles    |
for functionalized particles with nontriggered cells (Figure S9, Supporting Information). In a real periodontal pocket, the concentration of H$_2$O$_2$ is expected to be higher than the experimental condition because there exist much more immune cells. Correspondingly, the speed of the functionalized PLGA particles toward the lesions in deep periodontal pockets would be faster. In general, the “clinical” distance that these particles should cover/travel is 3–6 mm in case of deepest pockets. The motored particles could move around 1 mm in 30 min in the in vitro model. In the clinical situation, they may move even faster due to the higher concentration of H$_2$O$_2$ produced by the immune cells. Furthermore, the average width of the periodontal pocket is around 1 mm, which provide enough space for further motion of the particles. It is worth mentioning that the in vivo conditions are very complex and will be greatly different from the conventional culture condition, a more complex in vitro stimulation or in vivo experiments should be performed in future to validate the motor system further. After 2 h, the motored PLGA particles were observed at position 1, with trajectories showing typical Brownian motion. The control groups, PLGA particles without enzyme functionalization and functionalized particles with untriggered cells, showed very limited diffusional displacement due to lack of the catalyst and the fuel (H$_2$O$_2$), respectively. Instead of moving toward cells, a slight movement of particles toward the injection position was observed for the control groups, which could be caused by the counterforce created during the injection of particles. In the clinical situation, when the liquid is dropped into the periodontal pocket, a small counterforce will be formed because of existing surface tension from the saliva in the narrow route, which could lead to a small reflection of the particles. It is coordinated with the findings in our study.

3. Conclusion

In conclusion, we developed an active moving biodegradable particle intended for delivering drugs to inflammatory lesions by functionalized PLGA particles with the enzyme catalase. We showed that the functionalization process did not affect the drug release from the PLGA particles and provided the necessary asymmetry for enhanced motion by domains formation on the surface of the particles. The motored PLGA particles moved faster with increasing levels of H$_2$O$_2$. Finally, we demonstrated directional movement of the particles toward activated macrophages. This self-propelled drug carrier could be extended for use in the treatment of other lesions, such as those caused by rheumatoid arthritis and cancers, where an elevated level of H$_2$O$_2$ present.

4. Experimental Section

**Electrospraying Drug-Loaded PLGA Particles**: PLGA ($M_w$: 17 kDa, obtained from Purac) 1.6 g was dissolved in 10 mL acetonitrile. The solution of DOX in methanol 550 µL was added to the polymer solution at a ratio of 10:0.75 PLGA/DOX. The mixture was stirred for 1 h and transferred into a 10 mL plastic syringe with a 21 gauge needle. The solution was pumped at a flow rate of 10 µL min$^{-1}$ using a syringe pump. The voltage power source was 20 kV and the working distance between the needle and the liquid collector was between 17 and 20 cm. The liquid collector was filled with 1% Poloxamer 407 in Milli-Q and kept shaking during the electrospraying process to prevent aggregation.

**Attachment of Catalase to PLGA Particles**: 2 mg of PLGA particles were added to 0.5 mL 2-(N-morpholino)ethanesulfonic acid (MES) buffer (0.1 M, pH 5.5). The sample was sonicated for several minutes to suspend the particles. Afterward, 0.5 mL of a 400 × 10$^{-3}$ M ethyl(dimethylaminopropyl) carbodiimide with 100 × 10$^{-3}$ M N-hydroxysuccinimide solution in MES buffer (0.1 M, pH 5.5) was added. The mixture was mixed on a roller bench for 1 h at room temperature, after which it was centrifuged for 10 min at 2653 rcf. Several washing steps with 1 mL PBS buffer (0.1 M, pH 7.4) were performed to remove all unbound reagents. The particles were resuspended in 0.5 mL PBS buffer and then mixed with 0.5 mL catalase solution (2 mg, > 20 000 units per mg protein) in PBS. The coupling reaction was performed overnight on the roller bench at room temperature. After the coupling, the particles were centrifuged for 10 min. at 2653 rcf and subsequently washed several times with PBS buffer. The final solution was stored in the fridge until further use.

**Morphology and EDX Microanalysis**: The surface morphology of the DOX loaded particles and functionalized particles with catalase were evaluated using a field emission scanning electron microscope (FE-SEM; Sigma 300, Zeiss, Germany) with an electron accelerating voltage of 10 kV and a current of 10 mA and a JEOl 6330 Cryo FEM with an electron accelerating voltage of 3 kV and a current of 12 mA. Both samples were prepared by dispersing the particles on a silica slice and sputter-coating with a conductive 10 nm chromium layer. A working distance of 14–20 cm was used for scanning. For evaluation of compounds of PLGA particles, the sections were assessed using the Sigma 300 SEM equipped with an EDX detector.

**Size Distribution Measurement**: Dynamic light scattering measurements were performed on a Malvern Instruments Zetasizer (ZEN 1600), using Zetasizer Software (Malvern Instruments) for analysis of the data. Samples were loaded in Malvern disposable capillary cells. The average of three size measurements with 10 scans of 10 s was taken.

**Encapsulation Efficiency of Drug-Loaded PLGA Particles Before and After Functionalization**: Prior to the initiation of the drug release experiment, the loading of DOX in PLGA particles before and after functionalization were determined. First, 5.0 mg of drug-loaded particles were dissolved in 1 mL chloroform (Sigma-Aldrich, Germany). Once completely dissolved, 5 mL of Milli-Q was added to the mixture, then vigorously mixed for 1 h, after which the sample was left on the bench to obtain a clear layered solution. The aeous phase was analyzed for DOX content by measuring the absorption at 360 nm using high performance liquid chromatography (HPLC) (BioTek, synergy HTX, USA). The encapsulation efficiency of the DOX-loaded particles was calculated using the following formula.

\[
EE(\%) = \frac{\text{Detected amount of drug (mg)}}{\text{Theoretical amount of drug (mg)}} \times 100\%
\]

**In Vitro Drug Delivery Test**: 5 mg of drug-loaded PLGA particles before and after functionalization were added in 1.5 mL Eppendorf tubes (n = 4) and 500 µL of PBS with different concentrations of H$_2$O$_2$ (Gibco, Paisley, UK) was added to each tube. The tubes were incubated at 37°C on a rotating plate with a rate of 120 rpm. At each time point, the tubes were centrifuged at 9300 rcf for 2 min, the supernatants were obtained for further analysis, a fresh buffer was added into the tubes, and the particles were resuspended by vortexing, and then were put back in the incubator until the next release time point. The experiments were performed for up to 1 week.

**MIC**: To investigate the antibacterial efficacy of DOX released from PLGA particles and grafted PLGA particles, 5 mg of DOX loaded PLGA particles, and DOX loaded grafted PLGA particles were placed in 1.5 mL Eppendorf tubes (n = 3) and 500 µL of PBS was added to each tube. All the tubes were then placed on a shaking plate with a shaking rate of 120 rpm at 37°C. After 24 h incubation, the supernatants were collected...
and tested by HPLC. The supernatants were diluted to 5 μg mL\(^{-1}\). Same concentration of Fresh DOX solution at the same concentration was prepared as the positive control.

The MIC of each collected supernatant was evaluated using A. actinomycetemcomitans Y4. In brief, the overnight culture of A. actinomycetemcomitans was diluted to 1 \(\times 10^6\) CFU mL\(^{-1}\) in Brain Heart Infusion (BHI; BD Difco, Le Pont de Claix, France) broth supplemented with 0.02 M NaHCO\(_3\) and 1% glucose. The collected supernatants from 2 experimental groups, positive control group and growth medium (control group) were twofold serial diluted with PBS. The maximum dilution was 64-fold. A. actinomycetemcomitans culture was mixed with the twofold serial diluted samples in a 96-well plate. The optical density (OD) value of each well was recorded at the wavelength of 600 nm at 37 °C after 24 h in a SpectraMax i3 microplate reader (Molecular Devices, San Jose, CA). The average OD value of A. actinomycetemcomitans culture in the medium control group after 24 h was used as the reference for 100% growth. The MIC was determined as the concentration of the DOX that inhibited more than 50% of the bacterial growth.

**Enzyme Activity Assay:** The enzyme activity of catalase was determined by an Amplex red hydrogen peroxide assay. In this assay, the Amplex Red reagent competes for hydrogen peroxide to produce the fluorescent product, resorufin. A calibration curve with different concentrations of catalase was made, starting from 0.1 to 4 U. The formed fluorescent resorufin was measured by a Tecan Spark M10 plate reader (excitation at 550 nm and Emission at 590 nm). Catalase samples in buffers with pH 5, 7.2, and 8.6 were tested and compared to the calibration curve for their activity.

**ICP-MS:** A calibration curve was prepared by measuring catalase samples ranging from 1 to 1000 μg L\(^{-1}\). Each sample was acidified with 0.65% nitric acid and measured with kinetic energy discrimination with Helium. A standard iron solution (Merck 1.19781) and a Quality Control Multielement standard solution IV 1.11355 Merck were used as controls.

**NTA:** The analysis was performed on a Nano sight LM10 at 20x magnification. The technique used laser light scattering combined with a CCD camera (30 fps) to track individual particles in real time. The Stokes–Einstein equation \(D = \frac{k_B T}{6 \pi \eta d}\), where \(D\) is the particle diffusion coefficient, \(k_B\) is the Boltzmann constant, \(\eta\) is the viscosity, \(T\) is the temperature, and \(d\) is the hydrodynamic diameter, is used to correlate the coordinates of each particle with their size. In this experiment, the movement of catalase functionalized PLGA particles without and with addition of several concentrations of hydrogen peroxide was analyzed. Solutions containing blank and catalase functionalized particles were diluted in 1 mL PBS buffer/cell medium (0.05 M, pH 7). To these samples H\(_2\)O\(_2\) was added until a final solution was obtained of 0, 0.5, 1, or 10 \(\times 10^{-3}\) M H\(_2\)O\(_2\). The samples were injected in the cell block, after which videos of 60 s were recorded and processed by the NTA2.2 software. By analyzing the video, x and y coordinates of each particle were determined as a function of time intervals. Mean square displacements obtained for 100 frames by averaging over 40 particles were plotted versus the time intervals. The diffusion coefficient (D) of the PLGA particles at these concentrations were extracted from the linear fit of the MSD (\((r^2)\) versus the time (t)) according to the equation (2) \(D = 4D \cdot t\).

**Cell Culture:** RAW 264.7 macrophages were purchased from Sigma, USA. Cell lines were dropped in the T75 flask, Dulbecco’s modified eagle medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS, Sigma), penicillin (100 μg mL\(^{-1}\)), and streptomycin (100 μg mL\(^{-1}\)) at 37 °C in a humidified atmosphere with 5% CO\(_2\) and 95% air for 24 h prior to culture in the chemotaxis chamber. Experiments for H\(_2\)O\(_2\) generation were performed in the DMEM supplemented with PMA (1 \(\times 10^{-6}\) u) for 3 d in chemotaxis chamber.

The morphology of the cells before and after triggered with PMA were observed by Olympus FV1000 confocal laser scanning microscopy. The cells were cultured in 8-well slides (ibidi). Fixation of the cells was carried out for 15 min in freshly prepared 4% w/v paraformaldehyde. Then the samples were washed in PBS three times. Thereafter, they were stained with Phalloidin-Alexa 488 and 4′,6-diamidino-2-phenylindole (DAPI) for F-actin and cell nuclei, respectively.

**Movement Test of Grafted PLGA Particles in PMA-Activated Macrophages Model:** The chemotaxis chambers were used to model the process of targeting. The Raw 264.7 cells were seeded in different media (with or without 1 \(\times 10^{-6}\) u PMA) in one side of the chamber and incubated for 3 d. The other side was filled with the fresh medium. After incubation, small amounts of particles were added to the chamber side without cells. The displacement was observed under the microscope after certain time points. The videos were taken for 10 min for each group. The trajectories of each particle were analyzed with Fiji (a free program developed by NIH and available at https://fiji.sc/).

**Statistical Analysis:** The statistical data were plotted as mean ± standard deviation (SD) and analyzed with the software GraphPad InStat. Statistical analysis for the encapsulation efficiency of PLGA particles and the activity of catalase were performed by the Student’s t-test (n = 3). Differences were considered statistically significant at p < 0.05.

**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.

**Keywords**

biodegradable micromotors, drug delivery, inflammation inhibition, micromotors

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