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Controlled release of α-amylase from microchamber arrays containing carbon nanoparticle aggregates

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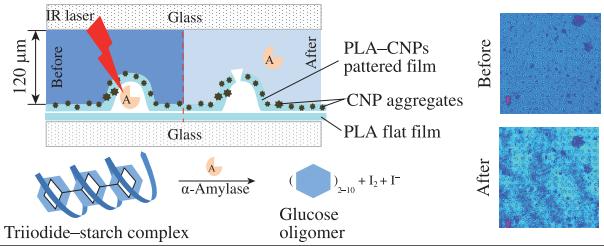
Microchamber arrays have been prepared from a composite of polylactic acid and carbon nanoparticles. Due to the presence of the nanoparticles, the microchambers are sensitive to infrared laser irradiation. α-Amylase has been encapsulated in the microchambers and released under controlled laser irradiation at 1064 nm with retention of the enzyme activity.



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The development of drug delivery and controlled release systems is a promising area of research, since it can significantly improve a therapeutic effect and reduce adverse actions of pharmaceuticals on the healthy body tissues.[1,2] As a rule, such compositions are sensitive to external factors, which ensures the release of drugs directly into pathological tissues and thus provides the local therapy.[3,4] Among these systems, the most promising are nanoparticles, micro- and nanocapsules,[5] hydrogels,[6] microgels[7] as well as microchamber arrays (MCAs). The MCAs represent thin polymer films with ordered microcontainers allowing the encapsulation of substances like polysaccharides,[8] fluorescent dyes,[9] neurotransmitters,[10] hormones[11] or growth factors.[12] The use of MCAs for medical purposes can be realized by their introduction during surgery as a coating for implants or stents[13] as well as in dermal wound healing.[14] Enzymes encapsulation is typically a challenging procedure because many factors affect their stability and activity. The creation of polymeric MCAs assumes negligible effect on the active substance, since the encapsulation occurs at the last step and involves only drying at room temperature and sealing the microcontainers at 50–60 °C for 15–30 s.[8,10–12] An inclusion of additional components like nanoparticles into the polymer shell can make MCAs sensitive to external stimuli, such as ultrasound,[11] magnetic field,[15,16] laser irradiation,[8,10,12,17] changes in pH[18] and temperature as well as enzymatic reactions,[19] which can prompt the release of substances from an MCA. The infrared laser irradiation is the most promising for the release of encapsulated substances due to its deep penetration into biological tissues.[20–22] To impart the required sensitivity to irradiation, various light-absorbing agents are employed in the design of MCAs, which convert the energy of photons into heat with the following local destruction of heat-sensitive materials and the release of substances from their carriers. Examples of the light-absorbing agents are gold[8,12,23] and copper[17] nanoparticles, carbon nanotubes[24,25] as well as graphene.[26,27] Along with highly ordered carbon structures, carbon nanoparticles (CNPs) and in particular their aggregates can also be used for the light absorption.[8] The main scientific focus on CNPs is aimed at their emission,[28–31] though their other properties can be employed in composite materials.[30,31] In this work, we use the CNP ability to absorb the light energy and convert it into heat.

The initial CNPs were synthesized by a hydrothermal method based on the technique of treatment of a carbon raw material solution in a closed volume, developed in our group.[31] For this, an aqueous solution of dextran sulfate sodium salt (DSS) was transferred into a Teflon pot with a tight-fitting lid and then heated in a stainless steel autoclave at 200 °C for 3 h (for the preparation details as well as absorption and fluorescence spectra of the CNPs, see Online Supplementary Materials).

To obtain a composite of CNPs and polylactic acid (PLA), an ethanol solution of CNPs was dropped into a Petri dish and dried at 50–60 °C for complete evaporation of the solvent. The resulting CNP powder was placed in a glass vessel, then chloroform and a weighed portion of PLA corresponding to a 1% solution were added and mixed until PLA had been fully dissolved (for details, see Online Supplementary Materials).

To obtain an MCA, the PLA–CNPs composite was placed onto the surface of a polydimethylsiloxane (PDMS) stamp with a relief in the form of ordered microsized cylindrical wells of a 10 μm diameter, a 5 μm depth and a pitch equal to 20 μm [Figure 1(*a*)]. As a model enzyme, α-amylase was encapsulated into the MCA using drying from its aqueous solution drop. Particularly, 1 μl of the 50 mg ml^{-1} enzyme solution was placed onto the patterned PLA–CNPs films and spread over the surface. After the evaporation of water, α-amylase crystallized in the microwells forming open microcontainers. To seal the microwells, the imprinting technique was employed, namely a PDMS stamp with the patterned PLA–CNPs film loaded with α-amylase was applied to a flat PLA film followed by heating at 50–60 °C for 15–30 s [see Figure 1(*a*)]. The polymer flat film melted and fused with the patterned film, as a result the microcontainers with the cargo were tightly sealed.

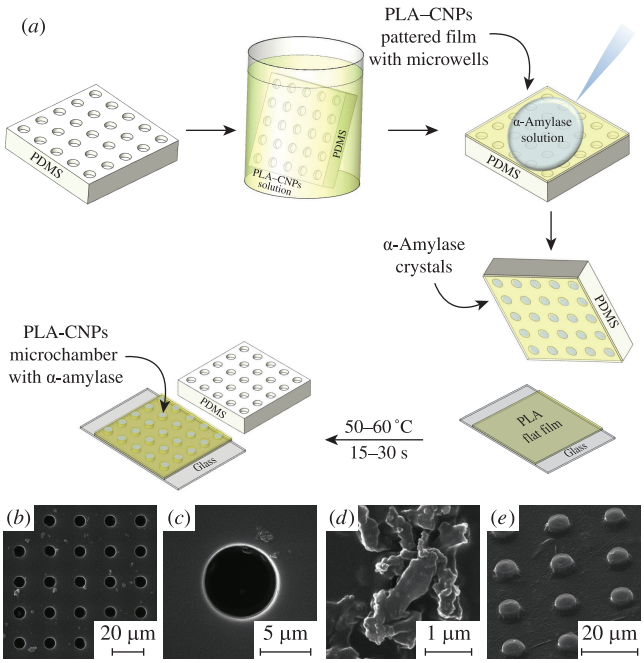

Figure 1 (*a*) MCA preparation from the PLA–CNPs composite with α-amylase as a cargo. (*b*)–(*e*) SEM images of PLA–CNPs films, namely (*b*) the film patterned with microwells, (*c*) a microwell, (*d*) CNP aggregates and (*e*) empty microchambers.

SEM images of the patterned PLA–CNPs film on a PDMS stamp and the empty microcontainers are shown in Figure 1(*b*)–(*e*). According to the SEM data, the arrangement of CNP aggregates in the patterned film is not uniform, the film contains the aggregates of submicron as well as micron size.

To track the release of α-amylase from the MCA, we choose the colorimetric method, which allows one to assess an enzyme reaction kinetics quantitatively using photometry and/or qualitatively from visual changes.[32] The colorimetric method employed was based on the starch hydrolysis by α-amylase as a glycoside hydrolase with formation of oligosaccharides. In the presence of iodine and the starch substrate, a dark blue triiodide–starch inclusion complex was observed, while hydrolysis of starch made it inaccessible for the formation of the colored complex,[32–34] the interaction scheme being shown in Figure 2(*a*).

In general, the presence of CNPs in a patterned PLA film can affect the MCA permeability for α-amylase. In this regard, we first carried out a colorimetric assessment of the spontaneous enzyme release from the microwells without any external influence. The MCAs designed from the PLA–CNPs composite with α-amylase as a cargo were covered with a 120 μm layer of an aqueous triiodide–starch complex solution similar to the arrangement in Figure 2(*b*), while thickness of the layer was controlled by an adhesive removable hydrophobic barrier (Secure-Seal Spacer, ThermoFisher Scientific). When the microchambers came into contact with the complex, no color change occurred, indicating an absence of the spontaneous enzyme release. After that, the sample was exposed to ultrasound as a typical external stimulus[11] and the gradual visual color change was observed. Finally, after 15 min of sonication the dark blue color completely disappeared. Thus, there was no spontaneous release of α-amylase from the MCA and the encapsulated enzyme retained its activity.

Then we investigated the effect of CNPs on the opening of the MCA by pulsed infrared laser irradiation at 1064 nm with a 3 kHz frequency, a power of 1.5 W and a pulse duration of 2 ns. When the radiation was absorbed by the CNPs, local heating of the MCA surface occurred followed by its destruction. This led to the release of α-amylase and the manifestation of an enzymatic reaction, namely the discoloration of triiodide–starch complex

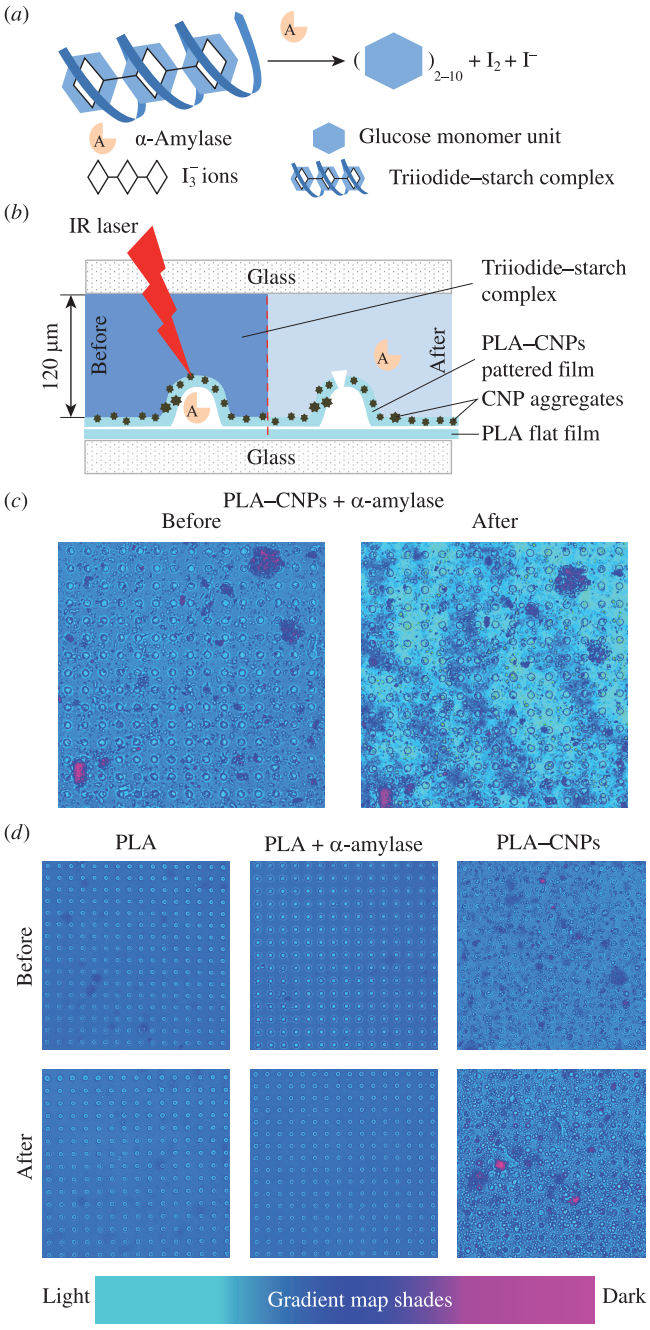

Figure 2 (*a*) Interaction of triiodide–starch complex with α-amylase. (*b*) Laser opening of MCA containing α-amylase as a cargo and the enzyme release into an environment with triiodide–starch complex. (*c*) Gradient maps of the MCA optical microscopic images before and after the laser exposure for the PLA–CNP sample with α-amylase as a cargo compared with (*d*) control samples.

[see Figure 2(*b*)]. An image of MCA from PLA–CNPs with α-amylase before and after the laser irradiation is shown in Figure 2(*c*).

To exclude the influence of the laser exposure and thermal effects on the color intensity of triiodide–starch complex or on the enzyme activity, control runs were performed with three samples, namely (i) an empty MCA from PLA, (ii) an empty MCA from PLA–CNPs and (iii) an MCA from PLA with α-amylase as a cargo [Figure 2(*d*)]. For all the samples, images were obtained using an optical microscope before and after the laser exposure and processed by the Adobe Photoshop SC6 Extended software employing the 'gradient map' function, which allowed one to replace the dark and light shades of the image with the chosen colors. The gradient maps obtained are shown in Figure 2(*c*),(*d*),

their counterparts in gray shades are presented in Figure S3 (see Online Supplementary Materials).

As a result of laser action on the MCA from the PLA–CNPs composite, uneven discoloration of triiodide–starch complex occurred [see Figure 2(*c*)], which indicated the release of the enzyme and the retention of its biological activity. For the control samples [see Figure 2(*d*)] under similar laser exposure these changes had not been observed.

Thus, we have demonstrated that an MCA based on a PLA–CNPs composite retains α-amylase tightly with no spontaneous release, whereas under the action of an infrared laser the enzyme is released from the MCA with preservation of its biological activity.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.11.034.

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