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Magnetic halloysite nanotubes for yeast cell surface engineering

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ABSTRACT: Halloysite clay nanotubes are safe and biocompatible nanomaterials and their application in biomaterials is very promising. The microencapsulation of yeast cells in the shell of clay nanotubes modifying their properties was demonstrated here. Each cell was coated with a 200–300 nm-thick tube shell and this coating was not harmful for these cells' reproduction. Synthesis of magnetic nanoparticles on the surfaces of the nanotubes allowed for magnetic-field manipulation of the coated cells, including their separation. Providing nano-designed shells for biological cells is a step forward in development of 'cyborg' microorganisms combining their intrinsic properties with functions added through nano-engineering.

KEYWORDS: halloysite nanotubes, magnetic nanoparticles, yeast, cell-surface engineering, cyborg microorganisms, viability.

Halloysite clay nanotubes (Joussein *et al.*, 2005) have attracted significant attention from researchers worldwide as a promising nanomaterial suitable for industrial applications (Lvov *et al.*, 2014; Massaro *et al.*, 2015), drug formulations (Lvov *et al.*, 2016) and biomedical devices (Hughes & King, 2010). Good biocompatibility (Fakhrullina *et al.*, 2015) and effective adsorption of drugs into lumens of the nanotubes (Dzamukova *et al.*, 2015) allow for the use of halloysite in emerging areas where chemistry meets biology (Chiew *et al.*, 2016). In particular, the emerging discipline of cells-surface engineering (Fakhrullin *et al.*, 2012) might benefit from the use of halloysite clay tubes as structural elements of cell-deposited functional coatings. Cell-surface engineering aims to fabricate nanoscale biocompatible 'skin-like' coatings on the cell walls or membranes of biological cells, thus significantly altering the intrinsic functionalities of live cells, which in turn allows for use of such 'nanocoated' cells in a number of practical applications (Hong *et al.*, 2013). Recently, halloysite nanotubes (HNTs) have been applied as an element in the layer-wise assembled functional coatings built on the cell walls of live yeast (Konnova *et al.*, 2013) or bacteria (Fakhrullina *et al.*, 2014). The ability to load nutrients, enzymes and nucleic acids into the lumens of the halloysite nanotubes is the major advantage of using this nanoclay in cell-surface coatings. This potential can be further realized if one can tailor a magnetic functionality to halloysite nanotubes (Duan *et al.*, 2012), allowing the manipulation of surface-engineered cells bearing magnetic nanotubes on their outer surface in the same way as was reported for magnetic microbial (Safarik *et al.*, 2012) and human (Dzamukova *et al.*, 2013) cells. This paper reports, for the first time, the fabrication on magnetic halloysite-coated live yeast cells as a novel platform in cell-surface engineering. Clay nanotubes were functionalized with Fe oxide nanoparticles, rendering them with magnetic responsiveness (magnetic halloysite nanotubes, Mag-HNTs). Next, these Mag-HNTs) were deposited directly onto the viable yeast cells. This

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nanocoating was not found to compromise the viability and functional properties of yeast cells.

EXPERIMENTAL

A simple and straightforward procedure has been devised to fabricate magnetically labelled HNTs. Pristine halloysite (Fig. 1a) obtained from Applied Minerals (USA) (40 mg) was washed with deionized water and sonicated for 10 min using a Bandelin Sonoplus tip sonifier (40% output power) to disintegrate the clay aggregates, then 40 mg of halloysite was added to 4 mL of 2 M $FeCl_3$ (dissolved in aqueous 2 M HCl) and sonicated similarly for 10 min. Next, 1 mL of 1 M $FeCl_2$ (in aqueous 2 M HCl) was added, with further sonication. The mixture was heated to 75°C followed by addition of 30 mL of 1.5 M NaOH and subsequent heating/stirring for 30 min; the brown precipitate (containing the Fe oxide-doped halloysite nanotubes) was then isolated using a permanent magnet. After washing with water, the zeta-potential of the Mag-HNTs obtained was slightly positive (3.5 mV), whereas the pristine HNTs exhibited a negative zeta-potential (−25 mV). The morphology of the Mag-HNTs was then investigated using electron microscopy. As seen in transmission (TEM) and scanning (SEM) electron microscopy images, pristine HNTs (Fig. 1a, c) are effectively covered by nanosized Fe oxide particles. The magnetic nanoparticles can be assumed to be located both in the lumen and on the outer walls of HNTs. Free Fe oxide nanoparticles can also be found in the samples, though these can be removed gradually by filtration. Overall, the procedure of magnetization of HNTs appears to be very simple and rapid, resulting in magnetic halloysite comparable with the samples obtained using alternative approaches (Zhang & Yang, 2012).

Next, the Mag-HNTs obtained was used to fabricate the magnetically responsive coatings on the *Saccharomyces cerevisiae* yeast, chosen as a very simple yet biotechnologically relevant microorganism. The first experiments followed the previously

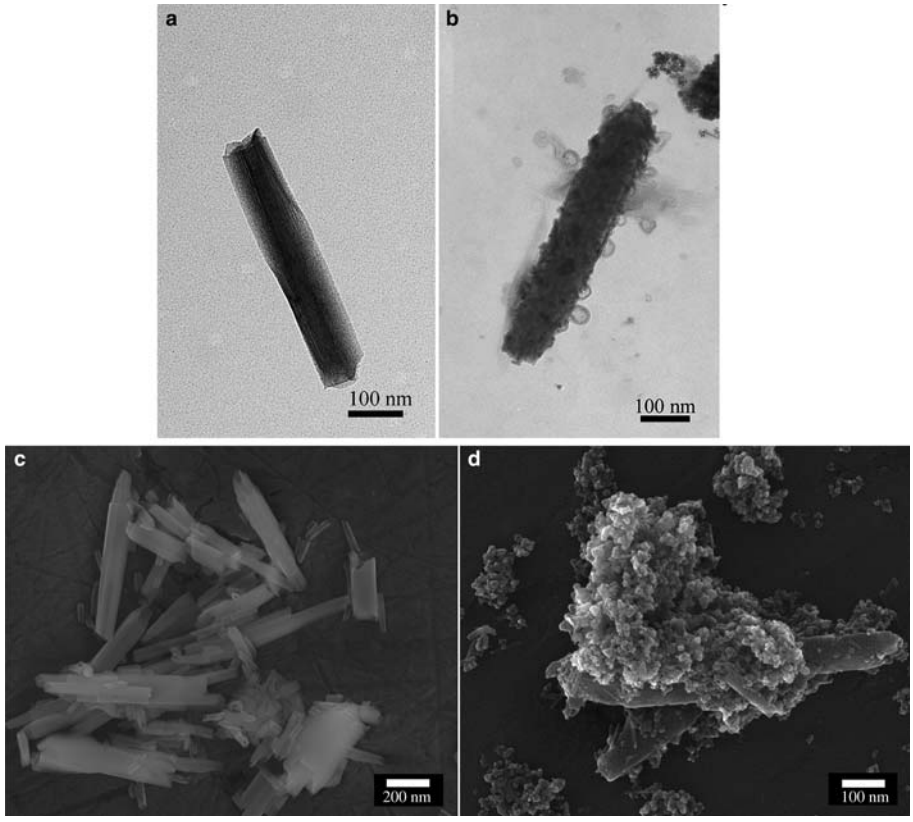


FIG. 1. TEM and SEM images of pristine HNTs (a,c) and magnetically modified Mag-HNTs (b,d).

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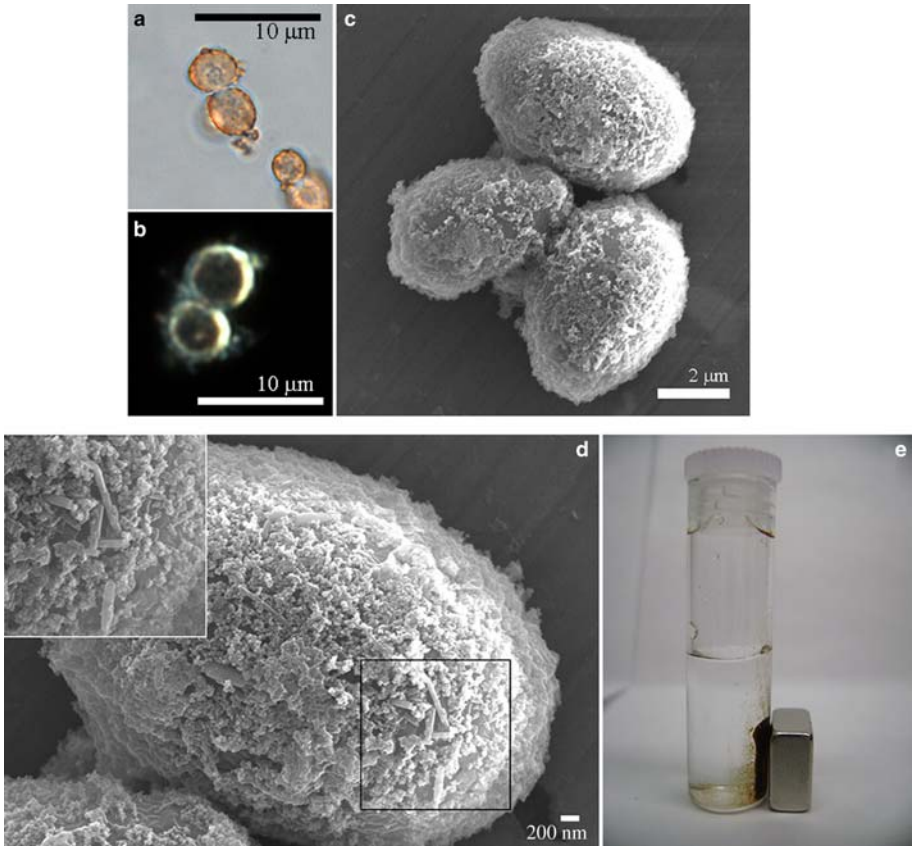


FIG. 2. Optical bright field (a); dark field (b) and scanning electron (c,d) microscopy images of Mag-HNTs; (e) magnetic responsiveness of Mag-HNTs attracted by a permanent magnet positioned near the vial.

published procedure (Konnova *et al.*, 2013) by depositing Mag-HNTs *via* the layer-by-layer sandwiching in between polyelectrolyte layers. This approach, although effective, is time-consuming, so the possibility of direct deposition of Mag-HNTs was evaluated, bearing in mind the positive surface charge (opposite the negative surface charge of yeast cells), and the extraordinary absorbent properties of halloysite. Yeast was added to 1 mL of Mag-HNTs (at 1 or 2.5 mg mL^{-1}), incubated on a shaker for 15 min; next the cells were separated using a permanent magnet and then washed several times with 0.9% aqueous NaCl. Figure 2 illustrates the formation of Mag-HNT shells on the cells. Bright-field optical microscopy (Fig. 2a) indicates that the coated cells obtained the characteristic brown colour of Mag-HNTs. Dark-field microscopy of Mag-HNTs@yeast *in situ* (Fig. 2b) shows the typical hedgehog-like appearance of HNTs attached to the cell wall. The nanoscale surface of Mag-HNTs@yeast was then investigated with SEM; a typical image shown in Fig. 2c confirms that a uniform layer of Mag-HNTs covers the whole cell evenly, resembling the surface coating of pristine HNTs sandwiched between polymers on yeast cells (Konnova *et al.*, 2013). In addition, the cells were magnetically responsive and could be attracted or rotated by a strong permanent magnet (Fig. 2e).

The ultimate aim of cell-surface engineering is to produce viable and functional nano-coated cells; therefore the viability of Mag-HNTs-coated yeast was investigated, utilizing several independent viability tests. First, the live/dead stain (a mixture of fluorescein diacetate and propidium iodide dyes), which stained live cells green and dead cells red if observed under a fluorescence microscope, was applied. As shown in Fig. 3a,b, Mag-HNTs-coated cells (up to 2.5 mg mL^{-1}) exhibited the same ratio of live/dead cells as the intact cells. The MTT test based on using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was then employed,

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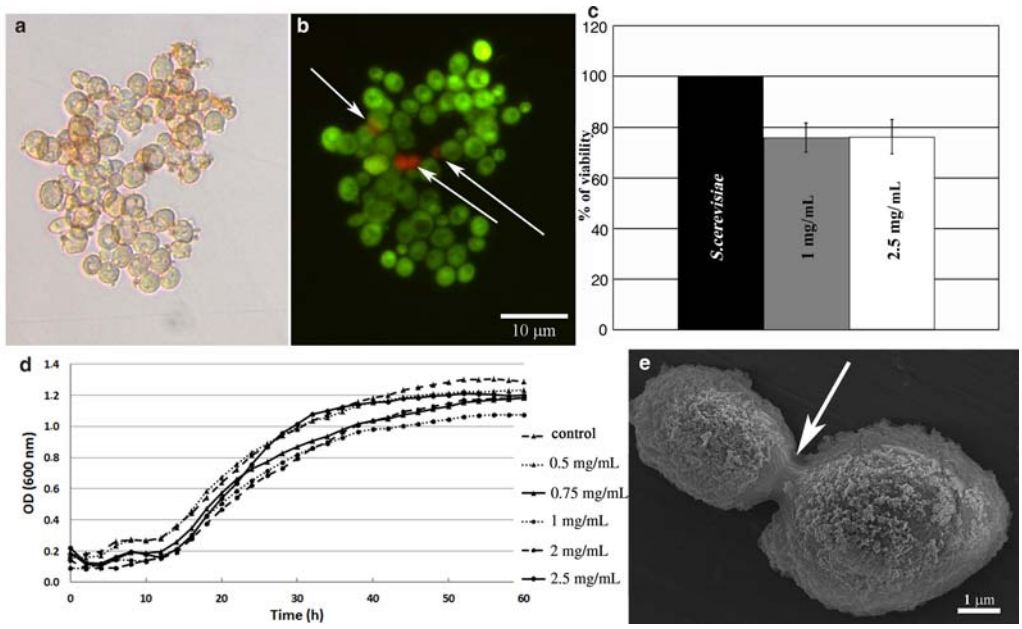


FIG. 3. Viability of Mag-HNTs@yeast: optical (a) and fluorescence (b) microscopy images of Mag-HNTs@yeast stained with live/dead dye (dead cells are indicated by arrows); proliferation of Mag-HNTs@yeast as monitored using the MTT test (c) and real-time growth monitoring (d); budding yeast cells partially removing Mag-HNTs (indicated by an arrow) (e).

characterizing the proliferation of yeast. The method is based on the reduction of the originally yellow dye into a purple product by mitochondrial enzymes, indicating the viability and proliferation of cells (Isa *et al.*, 2014). As shown in Fig. 3c, the Mag-HNT-coated cells demonstrated the same viability levels as intact (non-coated) cells. Next, the real-time growth curves of Mag-HNT@yeast was monitored using a microplate reader (Fig. 3d), demonstrating that the modification of cell surfaces did not affect the division of yeast. This method is based on photometric detection of yeast cells growth while the cells are incubated at 37°C under constant shaking. The graph shows the growth profiles of cells coated with increasing concentrations of Mag-HNTs. In addition, SEM was also employed to demonstrate that the samples of Mag-HNT@yeast contained several budding cells, where the magnetic halloysite coating was visibly removed during budding (Fig. 3e).

CONCLUSION AND IMPLICATIONS

The direct and rapid cell-surface engineering of yeast cells using magnetically modified halloysite has been demonstrated. Nanoclay can be further loaded with enzymes, nutrients and nucleic acids, thus serving as the 'microbial backpack' filled with nutrients. Magnetic functionality might be very important in magnetically facilitated separation or delivery of cells. In particular, such nanotube-carrier cells with the magnetic functionality may find practical applications in whole-cell biosensors or as effective adsorbents. It is likely that future work will demonstrate the full potential of halloysite nanoclay for modification of cell surfaces.

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