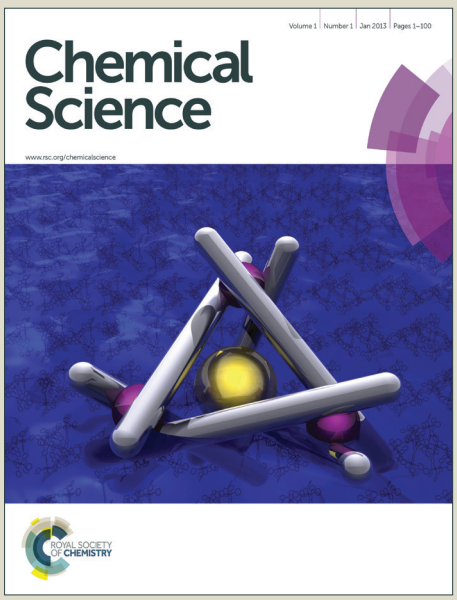
Chemical Science

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemicalscience

Journal Name

RSCPublishing

EDGE ARTICLE

"Self-repairing" nanoshell for cell protection

Cite this: DOI: 10.1039/x0xx00000x

Nan Jiang,[a] Xiao-Yu Yang,*[,a] Guo-Liang Ying,[c] Ling Shen,[a] Jing Liu,[a] Wei Geng,[a] Ling-Jun Dai,[a] Shao-Yin Liu,[a] Jian Cao,[d] Ge Tian,[a] Tao-Lei Sun,[a] Shi-Pu Li,[a] and Bao-Lian Su*[,a,b]

Received ooth January 2014,
Accepted ooth January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

Self-repairing is a nature's way of protecting living organisms. However, most of single cells are inherently less capable of self-repairing, which greatly limits their wide applications. Here, we present a self-assembly approach to create a nanoshell around cell surface using nanoporous biohybrid aggregations. The biohybrid shells present self-repairing behaviour, resulting in high activity and extended viability of the encapsulated cells (eukaryotic and prokaryotic cells) in harsh micro-environments, such as UV radiation, natural toxin invasion, high-light radiation and abrupt pH-value changes. Furthermore, an interaction mechanism is proposed and studied, which is successful to guide design and synthesis of self-repairing biohybrid shells using different bioactive molecules.

Introduction

Self-repairing is a common and wonderful phenomenon of living organisms to adapt constantly changing environments through the long term evolution.[1] However, it is rare to be seen in single cells, which might be a result of evolution of unicellular organisms to multicellular organisms having more advance environmental adaption and self-protection capability. There is therefore a great interest to endow the single cell with self-repairing behaviour. Cell-in-shell structure without complicated genetic manipulations is currently regarded as the most non-biogenic efficient way to cell protection and functionlization.[2-11] The nanostructured shell materials with tunable physico-chemical properties provide indispensible media to actively control[12] and offer functionalities to cells,[13-25] such as magnetic cell-in-Fe_3O_4 shell,[15,16] thermally durable cell-in-SiO_2 shell[17] and cell-in-SiO_2/TiO_2 shell,[18] electrically conductive cell-in-Au/Ca/graphene shell,[19] UV-resistant cell-in-$LnPO_4$ shell,[20] pH-responsive cell-in-poly(methacrylic acid)-*co*-NH_2 shell.[21] However, traditional nano-structured shells do not satisfy the increasing demands of modern applications because these synthetic shells not only disturb cell proliferation and life cycle, but also are inability to re-assemble onto cell surface after the process of encapsulation.[26] Once shell structures are broken that often caused by cell division, the decrease of stability and loss of functionalities of encapsulated cells and/or daughter cells will occur.[27] A path to self-repair the functional nano-scale shell is mostly preferred, whereby the preformed precursor could self-assemble onto the cell surface during cell division.

Natural amino acids can non-covalently bind with cell surface due to their bioactive groups (e.g. amino group, carboxyl group and/or thiol group etc.). It could, therefore, readily self-assemble onto the cell surface to form the nanothin meso-scaled layer. Also as the most basic biomolecules which do not require complicated synthetic procedure, amino acids possess similar physico-chemical properties of small biomolecules of cell, and therefore can be good candidates to form nanoshells. However, only amino acid molecules may transport through cell membrane/walls, suggesting that the amino acid molecules are not stable onto the cell surface as shell materials.[28] As known, amino acid molecules can interact with gold nanoparticles which have been successfully introduced to shells materials.[29-31] In this study, biohybrid aggregations, composed of Au nanoparticles and L-cysteine molecules have been successfully developed to fabricate the nanoshell around the cell surface and endow the encapsulated cell with self-repairing property. This self-assembled Au@L-cysteine biohybrid nanothin shell presents a worm-like porous structure due to properties and nano-effect of Au nanoparticles. Interestingly, the nanoporous biohybrid shell would not only allow fast mass exchange, and increase cell activity and stability in synthetic environments, but also offer the encapsulated cell with more functionalities to expand the applicability, such as protecting the cells against strong UV radiation, natural toxins, high light radiation, and abrupt pH changes. Most importantly, the self-assembled Au@L-cysteine hybrid aggregations dispersing in the culture solution can act as sol precursors to self-repair the broken shells and form integrated shells.

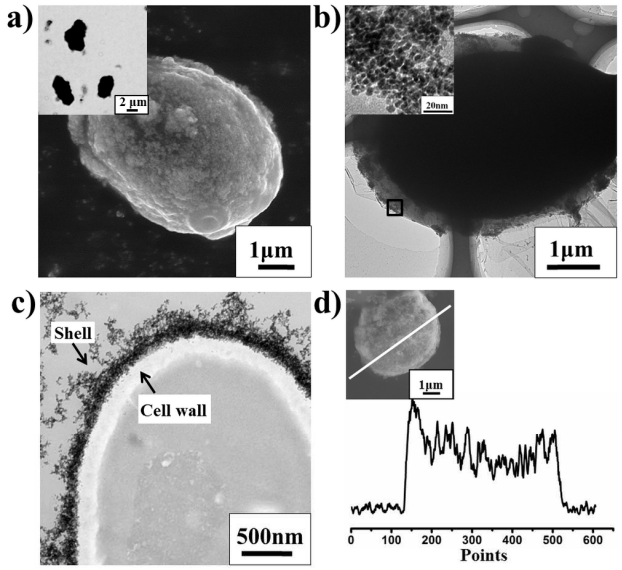


Fig. 1 Characterization of yeast cell-in-shell structure. a) SEM and OM in a visible light mode (inset) micrographs of yeast cells@biohybrid shells; b) TEM micrographs of yeast@biohybrid shell and the corresponding magnified micrograph of black square area (inset) show that the single cell is coated with nanoporous-structured biohybrid shell; c) Ultrathin section TEM images of yeast@biohybrid shell; d) EDX line profile for Au encapsulated yeast cells confirms the presence of biohybrid shells.

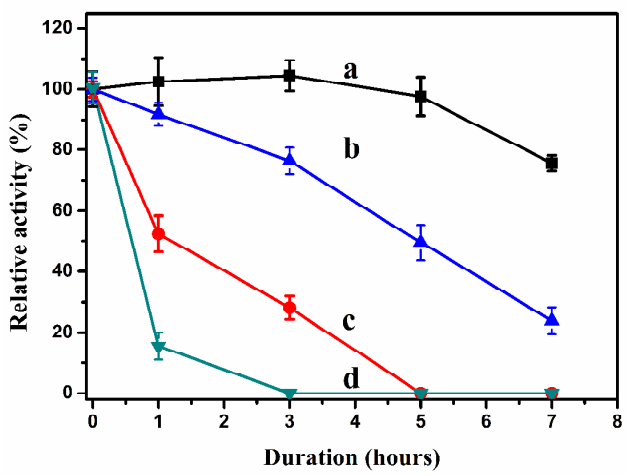


Fig. 2 Relative activities of yeast@biohybrid shell exposed under UVC radiation. Relative activities by yeast@biohybrid shell and native yeast exposed under UVC radiation in a) and c) fresh cultural medium, and b) and d) solution without cultural medium.

The formation of the biohybrid aggregations was first investigated. The L-cysteine is added to Au colloid (2-3nm in diameter from the Transmission electron microscopy (TEM) micrograph in Fig. S1a) at room temperature. Precipitates were characterized and after collection by centrifugation-washing steps. The TEM micrograph depicts that the biohybrid aggregations present nanoporous structure with 4-8nm of pore size (Fig. S1b). The Flourier transformed infrared spectroscopy (FTIR) and the X-ray photoelectron spectroscopy (XPS) spectrum confirms the formation of Au-S bond (Fig. Sa and b),[32,33] indicating that the interaction and structure of biohybrids are stable. The UV-vis spectrum shows that the Au@L-cysteine biohybrid not only can absorb excess high light (Fig. S2c), but also are stable in solution (Fig. S2d). It is safe to conclude that the nanosized Au@L-cysteine biohybrids are stable in solution and present nanoporous structure. These properties not only enable self-assembly and self-repairing of shells, but also facilitates mass communication.

Yeast cells *Saccharomyces cerevisiae* have been selected as our model eukaryotic cells to study self-repairing behaviours. In a typical preparation of self-repairing yeast cell-in-shell structure (Fig. S1), the biohybrid aggregations are firstly dispersed in phosphate buffer solution (PBS). Subsequently, the aggregations are added to the clean cell solution under gently shaking at room temperature. During the interaction between the native yeast cells (Fig. S1c) and the preformed precursors, native cells are gradually entrapped in the biohybrid shell formed by the condensation of the meso-structured Au@L-cysteine aggregations (Fig. S1d). The entrapped cells and excessive biohybrid aggregations are collected and re-dispersed into fresh medium for further incubation under ambient conditions. SEM (Scanning electron microscopy), OM (Optical microscopy) and TEM micrographs (Fig. 1) clearly depict that the yeast cells are individually and separately entrapped in dense shells and maintain their original morphologies, which strongly argue that such a self-assembly has no negative effect to biological morphologies of the cells. Biohybrid aggregations on cell surface form a worm-like nanoporous structure (inset in Fig. 1b), and the nanopore size is 2-6 nm which is slightly smaller than the TEM data of preformed precursors (Fig. S1b). Probably a little deformation of nanochannels occurs due to the interaction between the soft structured biohybrid aggregations and cell surface. Microtome-sliced TEM micrograph (Fig. 1c) shows that the cell is entrapped into the uniformly nanothin shell with 160 nm of thickness, and cell integrity is maintained. The EDX line profile of cell@biohybrid shell confirms that biohybrid aggregations containing Au element are uniformly coated on cell surface (Fig. 1d and inset). Furthermore, cell culture experiments show that the growth curve of encapsulated cells is similar to that of native cells, which indicating that the nanoshell has no obvious effect to cell division (Fig. S3). All together it can be concluded that 1) our procedure is facile and does not need numerous cycles of multilayers deposition in comparison with traditional layer-by-layer method, resulting that cell activity could be well maintained after encapsulation; 2) the biocompatible biohybrid aggregations can effectively and easily coat onto cell surface by self-assembly, which is beneficial for self-repairing while shell is broken; 3) nanothin and nanoporous structures of shells have been developed which would facilitate mass and energy transportation and cell division.

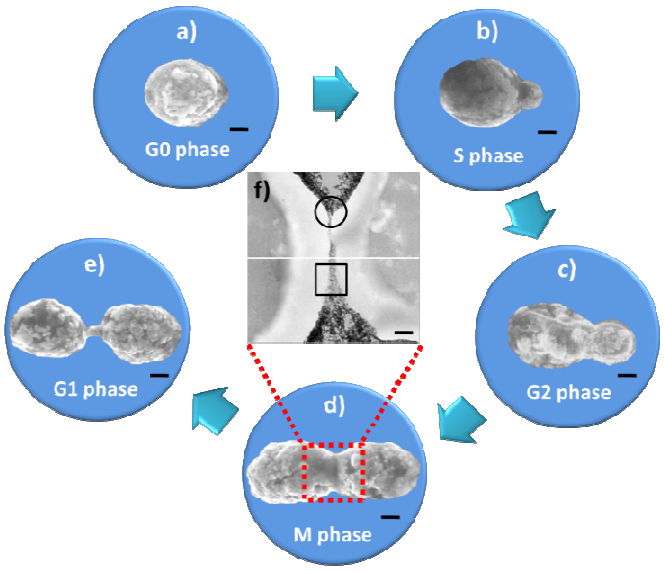


Fig. 3 Process of self-repairing biohybrid nanoshell in yeast cell division (a-e), (Scale bar: 1 μm). a) Encapsulated mother yeast cell (G0 phase); b) encapsulated mother cell and bud (S phase); c) encapsulated mother cell and growing bud (G2 phase); d) encapsulated mother cell and bud with the same size of mother's (M phase); e) encapsulated mother and daughter cells (G1 phase). f) merged magnified ultrathin section TEM micrographs of encapsulated dividing cell (Scale bar: 250nm). All original details are shown in Fig. S5.

Results and discussion

To test whether the biohybrid shells protect cells in harsh conditions during cell proliferation, relative activities of encapsulated yeast cells have been carried out with native yeast cells as a comparison. It has been proven that cells keep their ability to proliferate in fresh media (Fig.S4). Therefore, the encapsulated yeast cells are cultivated in fresh liquid media and in normal solution (without fresh medium) under short wave ultraviolet radiation (UVC, strongest ultraviolet band of sunlight for destroying genetic structure of cells[34]), respectively (Fig.2). Encapsulated yeast cells maintain higher activities under UVC radiation. For example, even after 5 hours radiation, 98% (±6%) of initial activities of encapsulated cells in fresh medium remain (Fig. 2a), while native cells in medium (Fig. 2c) quickly lose their activity within 5 hours. When in normal solution without medium, yeast cell@biohybrid shell (Fig. 2b) also shows higher activities than yeast cell within silica (Fig. S4) and native yeast cells (Fig. 2d) in normal solution. Its lower activity, comparing with the encapsulated cell in fresh medium, should be attributed that cells abilities to divide are inhibited in absence of the fresh medium. Furthermore, the test of nanosized natural toxin (lyticase)[35] invasion shown in Fig. S5 can also prove that the shells can protect the cells against the natural toxin invasion.

It is also necessary to point out that the encapsulated cell can easily break its shells during cell division since the shell is very thin and soft. That seems to easily cause loss of functionalities and decrease of stabilities. However, the encapsulated yeast cells above-mentioned still show high

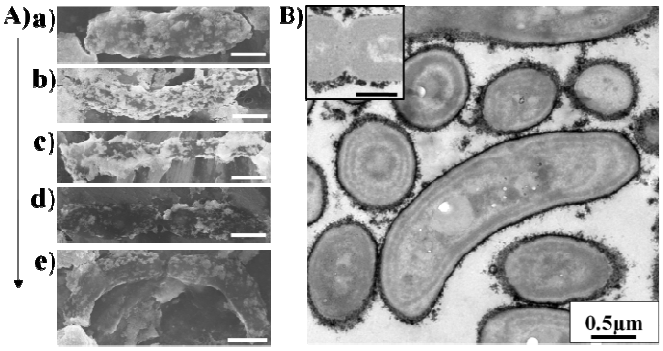


Fig. 4 A) Process of self-repairing biohybrid nanoshell in cyanobacteria division (a-e); B) Microtome-sliced TEM micrograph and magnified micrograph (inset) of encapsulated cyanobacteria (Scale bar: 0.5μm).

stability in proliferation. Therefore, there should be a presence of other protection mechanism. The surfaces and morphologies of the yeast cells during division are observed by SEM micrographs in Fig. 3 (a-e) and Fig. S6A. The micrographs show that the generation of yeast cell is typical budding reproduction, and the morphologies of cells during the budding are normal and no shrink. Notably, nanoparticles aggregated on the cell surfaces do not show significant decrease in 5 stages in life cycle (G0, S, G2, M and G1 phases, see Fig. 3 and Fig. S6A). The shell thickness of dividing cell (around 140 nm, Fig. 3f) becomes slightly thinner, compared with that of the single cell (around 160 nm, Fig. 1C). Because, the enlarged surface area during cell division will make the shell become thinner. It is surely impossible to cover both of mother cells and daughter cells only by the original biohybrid shells, in spite that the shells are soft to allow deformation. The excessive biohybrid aggregations could play an important role to self-assemble onto the naked buds and/or daughter cells. Because the only possibility is that the excessive biohybrid aggregations in solution self-repair the thinner shells and/or broken shells.

Direct evidences of self-repairing are observed in square and circle area in Fig. 3f and Fig. S7B. There is no difference of shell thickness between the mother cell and the bud (circle area in Fig. S7B (b)). This means that the biohybrid aggregations can self-assemble onto the naked surfaces of both mother cell and the bud. More interestingly, biohybrid aggregations have been found in the new generating interface between mother cell and the bud (square area in Fig. S7B (a)). This suggests that the generating interface is subsequently filled by original biohybrid shell aggregations or excessive nano-aggregations after cell division. It is a very unique phenomenon, which seems that the broken shell can be self-repaired by self-assembled biohybrid aggregations, and also a very excellent advantage, indicating that cells are protected and functionalized by biohybrid shells in life cycle. In contrast, the self-assembling phenomenon can hardly be found in yeast cell@polymer matter (Fig. S8a and b) and cell within inorganic matter (Fig. S8c and d) in the presence of excessive shell materials. The results confirms that traditional polymeric or inorganic shell cannot be self-repaired, even in the presence of excessive shell precursors, directly

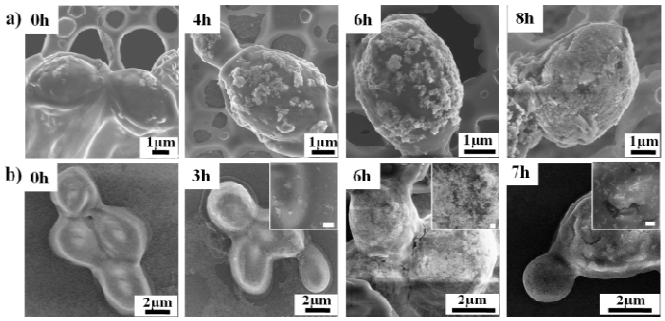


Fig.5 SEM images of yeast cell a) in biohybrid solution, and b) on silicon substrate surfaces coated by biohybrid aggregations in various times. Insets are magnified images, scale bar: 200 nm.

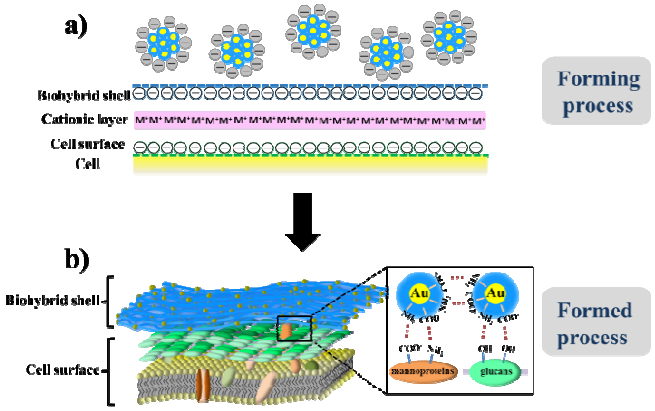


Fig.6 Formation mechanism of self-repairing biohybrid shell on yeast cell surface. a) ionic interactions proposed between cell and nano-aggregations during forming process, where M^+ is cationic ions in solution; b) interaction between the cell surface and biohybrid shell after formation.

attributing to easily polymerization of the traditional bulky polymeric precursors during the cell encapsulation procedure. The aggregation behaviours of typical polymeric nanocomposites are also evidenced (Fig. S9 a and b). TEM images clearly evidence that Au@PAH (poly allyamine hydrochloride) (Figure 2a below) and Au@PLL (poly L-lysine) (Figure 2b below) nanocomposites are easily polymerized to very large particle aggregates or large scale net-like aggregates. As well known, these large aggregates or precipitates are difficult to self-assemble onto cell surface or self-repair the broken shell. In contrast, small amino acid molecules-based nano-aggregations (Au@L-cysteine biohybrids) (Fig. S9c) could easily achieve the goal of self-repairing property due to nano-effect and their surface abounding functional groups. This is a big superiority of small amino acid molecules in self-repairing of shells.

Such a self-repairing phenomenon is not only limited to eukaryotic cell. It could also be extended to prokaryotic cell system. Cyanobacteria, which play a major role in global carbon cycle,[36] have been picked as one of the typical examples of prokaryotic cells. The self-repairing process of encapsulated cyanobacteria is also observed by SEM and TEM images (Fig. 4A and B). Fig. 4A shows that the reproduction of cyanobacteria is a typical binary fission, and morphologies of cells are normal and without shrink, implying that the shells do not limit the encapsulated cells division. The nanoparticles are clearly observed to aggregate on cell surfaces in whole division cycle. Compared with yeast cells, the shells of the encapsulated cells show thinner thickness (around 100 nm, Fig. 4B). This indicates that the interaction between the biohybrid aggregations and the cyanobacterium is possible weaker than the yeast cell, attributed to the difference bio- and physicochemical properties of cells surface. Furthermore, the self-repairing biohybrid shell could also act as a safeguard toprotect cyanobacteria from harsh conditions, such as high light and strong UV radiation, and abruptly pH changes (Fig. S10).

All the protection in UVC and high-light radiation, natural toxin invasion and abruptly pH change should be attributed to the biohybrid shell, for example, the strong absorption of UVC and high-light (in wavelength of 190-280 nm (Fig. S11), and 450-700 nm (Fig.S2d)), strong interaction between the amino acid and nature toxin (Table S2), and good buffering capacity of amino acid molecules. It is notable that the cell protection during proliferation would be due to the self-repairing behaviour of the encapsulated cells. Self-assembly between nano-aggregations and cell surface is the critical factor of this self-repairing. It is reasonable to consider self-repairing behaviours caused by self-assembly. We cultivate yeast cells in biohybrid solution (Fig. 5a) and on the biohybrid-coated silicon substrate (Fig. 5b), respectively. SEM images clearly show that with the time prolonged, the biohybrid aggregations on cell surfaces gradually increase from the loose structure to the dense structure under the two conditions. There is no obvious difference on cell surface after 10 hours compared with that after 8 hours in the case of the cells in biohybrid solution (Fig. S12), indicating that the thickness of shell cannot unlimitedly increase with time prolonged. Similarly, in the case of the cell on the biohybrid coated silicon substrate, the shell grows uniformly from bottom to up. These results point to a clear demonstration that biohybrid aggregations could actively self-assemble onto cell surface to form a dense shell, even cultivating the cells on the biohybrid coated silicon substrate, which is the direct reason why the nano-aggregations can self-repair the shells.

The formation of uniform nanoshell and shell thickness are possibly related to the surface charges and potentials of cells and biohybrids. Surface charges and potentials of cells and biohybrids have been measured by zeta potentials (ζ), where ζ(yeast cell) is -17.1±1.2 mV, ζ(cyanobacteria) is -14.2±0.8 mV, ζ(biohybrids) is -19.3±0.7 mV (Table S1). After shellization, zeta potentials of encapsulated cells are higher than native cells (ζ(yeast cell@biohybrid shell) is -18.5±1.5 mV, ζ(cyanobacteria@biohybrid shell) is -16.4±1.4 mV) (Table S1). This means that the encapsulated cells provide better dispersity than native cells, because the charged encapsulated cells repel one another and therefore overcome the natural tendency of cells to aggregate.[37] According to the simplified Grahame equation for low zeta potential ($\sigma = \varepsilon\varepsilon_0\zeta/\lambda_D$, where ε is

dielectric permittivity and λ_D is the Debye length),[38] surface charge (σ) is positive proportional to zeta potential. In our proposed model, the negatively charged cell surfaces are possibly attracted electrostatically to the ion pair (negatively charged biohybrid aggregations with cationic ions (M^+)) forming an electrical triple layer (Fig. 6a).[39,40] After self-assembling onto cell surface, biohybrid aggregations intimately coated with cell surface by hydrogen-bonds between amino groups/carboxyl groups of cysteine molecules in biohybrid aggregations and functional groups (such as amino groups and carboxyl groups of proteins and hydroxyl groups of polysaccharide) of cell surface (Fig. 6b). It is evidenced that pure cysteine molecules can also form a net-like aggregation on cell surface (Fig. S13a, the smooth native cell surface is the comparison in Fig. S13b), in spite of instability. These interactions might cause the deformation of nanopores of nano-aggregations, corresponding with the previous results shown in Fig. 1b and S1b. After encapsulation, moreover, yeast cell can attract more charged biohybrid aggregations due to its higher surface charge, compared with cyanobacteria surface, which gives reasons that the biohybrid shell on yeast cell surface is thicker than shell on cyanobacteria surface. This is also in very good agreement with the TEM results (Fig. 1c and Fig. 4B). With the increased thickness of shell, the surface charge of encapsulated cells would reach to a balance that biohybrid aggregations in solutions could not continue to assemble onto original biohybrid shell (Fig. 6a). When the shell is broken during the cell proliferation, the balance is broken and the encapsulated yeast cell would absorb and re-assemble the nano-aggregations to self-repair the shell. Such a self-repairing behaviour is analogous to a certain self-repairing way in living organisms, where the broken area is self-repaired by uptaking external precursors. For example, diatoms absorb silicon source from their living environment to re-build their silica shell during cell division.[41,42] These proposed models are in good agreement with the experimental results, which would be helpful to understand the self-repairing behaviour of encapsulated cells.

Furthermore, in our case, negatively charges encapsulation around the cells do not affect intrinsic characteristics of cell surface charge,[43] which avoid cell surface damages caused by traditional positively charged polyelectrolytes shell.[44] Different bioactive molecules can be therefore easily introduced to form self-repairing biohybrid shell, such as different amino acids and peptides. These shells can also be engineered onto cell surface to protect cells. For example, cyanobacteria within Au@L-lysine hybrid shell show higher photosynthetic activities under high light radiation (Fig. S14a). Cyanobacteria cells within Au@glutathione shell have clearly confirmed by the microtome-sliced TEM image (Fig. S14b).

Conclusions

To be concluded, we have described an experimental success of the biohybrid nanoshells in cell-in-shell encapsulation endow the encapsulated cells with self-repairing behaviour. These self-repairing shells present a structural superiority of nanopores and nanolayer, and provide the cells with excellent protection. The interaction mechanism has been investigated in detail, and guided the synthesis of the self-repairing biohybrid shells using different bioactive molecules. It is believed that our strategy is not limited to yeast and cyanobacteria, and should be applicable to higher eukaryotes, such as human cells, even multicellular organism.[25] Furthermore, other functional matter can also be used to enhance cell activity and introduce various functionalities. For example, bioactive proteins can be used to improve the selective activity; polymers can be used to design smart interface; oxides can be used to introduce magnetic, electronic, optical, and thermal properties. The self-repairing strategy developed here therefore offers a general, facile, and unique approach for the encapsulation of cells with long-term viability, extraordinary stability, high activity and multiple functionalization.

Acknowledgements

This work was supported by a Chinese Ministry of Education (NCET-11-0688, IRT1169, RFDP-20110143120006, 2012-YB-04), NFSC-51101115, NFSC-51472190. B.L.S. acknowledges "Program of Global Expert" and program of "Chang Jiang Scholar". X.Y.Y. acknowledges program of "Chutian Scholar". N.J. carried out all the experiments. X.Y.Y. conceived the project, provided the idea, and designed and guided the experiments. G.T. guided the synthesis of the precursor. G.L.Y., W.G. and G.T. analysed the data and edited the figures. L.S., L.J.D., S.Y.L. carried out precursor preparation. J.L. performed the TEM measurements. T.L.S. and S.P.L. analysed the data and support guidance of interface materials. B.L.S. conceived the project, and supported scientific and technological platform and guidance. N.J. and X.Y.Y. wrote and revised the paper. J.C. and B.L.S. revised the paper.

Notes and references

[a] *State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, and School of Materials Science and Engineering, Wuhan University of Technology, 430070 Wuhan, China Email: xyyang@whut.edu.cn and tian.ge@yahoo.com*

[b] *Laboratory of Inorganic Materials Chemistry, The University of Namur (FUNDP), B-5000 Namur, Belgium Email: bao-lian.su@fundp.ac.be*

[c] *School of Material Science and Engineering, Wuhan Institute of Technology, 430073 Wuhan, China*

[d] *Department of Chemistry and Biochemistry, University of California San Diego, CA 92037 La Jolla, USA. Email: jiancaoucsd@gmail.com*

† Electronic Supplementary Information (ESI) available: [Experimental section and additional data]. See DOI: 10.1039/b000000x/

1 J. F. Miller, *Genetic and Evolutionary Computation–GECCO 2004*. Springer Berlin Heidelberg, 2004, 129.

2 I. Drachuk, M. K. Gupta and V. V. Tsukruk, *Adv. Funct. Mater.*, 2013, **23**, 4437.

3 E. C. Carnes, D. M. Lopez, N. P. Donegan, A. Cheung, H. Gresham, G. S. Timmins and C. J. Brinker, *Nat. Chem. Bio.,* 2010, **6**, 41.

4 N. Nassif, O. Bouvet, M. N. Rager, C. Roux, T. Coradin and J. Livage, *Nat. Mater.*, 2002, **1**, 42.
5 R. F. Fakhrullin and Y. M. Lvov, *ACS Nano*., 2012, **6**, 4557.
6 R. F. Fakhrullin, A. I. Zamaleeva, M. V. Morozov, D. I. Tazetdinova, F. K. Alimova, A. K. Hilmutdinov, R. I. Zhdanov, M. Kahraman and M. Culha, *Langmuir*, 2009, **25**, 4628.
7 V. Kozlovskaya, S. Harbaugh, I. Drachuk, O. Shchepelina, N. Kelley-Loughnane, M. Stone and V. V. Tsukruk, *Soft Matt.*, 2011, **7**, 2364.
8 S. H. Yang, S. M. Kang, K.-B. Lee, T. D. Chung, H. Lee and I. S. Choi, *J. Am. Chem. Soc.*, 2011, **133**, 2795.
9 A. I. Zamaleeva, I. R. Sharipova, A. V. Porfireva, G. A. Evtugyn and R. F. Fakhrullin, *Langmuir*, 2010, **26**, 2671.
10 S. R. Shin, H. Bae, J. M. Cha, J. Y. Mun, Y.-C. Chen, H. Tekin, H. Shin, S. Farshchi, M. R. Dokmeci, S. Tang and A. Khademhosseini, *ACS Nano*, 2012, **6**, 362.
11 R. F. Fakhrullin and R. T. Minullina, *Langmuir*, 2009, **25**, 6617.
12 J. Lee, S. H. Yang, S.-P. Hong, D .Hong, H. Lee, H.-Y. Lee, Y.-G. Kim, I. S. Choi, *Macromol. Rapid Commun.*, 2013, **34**, 1351.
13 H. Ai, M. Fang, S. A. Jones and Y. M. Lvov, *Biomacromolecules*, 2002, **3**, 560.
14 S. S. Balkundi, N. G. Veerabadran, D. M. Eby, G. R. Johnson and Y. M. Lvov, *Langmuir*, 2009, **25**, 14011.
15 R. F. Fakhrullin, J. García-Alonso and V. N. Paunov, *Soft. Matt.,* 2010, **6**, 391.
16 S. Y. Yang, T. Lee, E. Seo, E. H. Ko, I. S. Choi, B.-S. Kim, *Macromol. Biosci.,* 2012, **12**, 61.
17 G. C. Wang, L. J. Wang, P. Liu, Y. Yan, X. R. Xu and R. K. Tang, *Chembiochem*, 2010, **11**, 2368.
18 J. Lee, J. Choi, J. H. Park, M.-H. Kim, D. Hong, H. Cho, S. H. Yang, and I. S. Choi, *Angew. Chem. Int. Ed.*, 2014, **53**, 8056.
19 R. Kempaiah, S. Salgado, W. L. Chung and V. Maheshwari, *Chem. Commun*., 2011, **47**, 11480.
20 B. Wang, P. Liu, Y. Y. Tang, H. H. Pan, X. R. Xu, R. K. Tang, *Plos One*, 2010, **5**, e9963.
21 I. Drachuk, O. Shchepelina, M. Lisunova, S. Harbaugh, N. Kelley-Loughnane, M. Stone, and V. V. Tsukruk, *ACS Nano*, 2012, **6**, 4266.
22 X. Y. Yang, G. Tian, N. Jiang and B. L. Su, *Energ. Environ. Sci.*, 2012, **5**, 5540.
23 W. Xiong, Z. Yang, H. L. Zhai, G. C. Wang, X. R. Xu, W. M. Ma and R. K. Tang, *Chem. Commun.*, 2013, **49**, 7525.
24 J. T. Wilson, W. X. Cui and E. L. Chaikof, *Nano Lett.*, 2008, **8**, 1940.
25 R. T. Minullina, Y. N. Osin, D. G. Ishmuchametova and R. F. Fakhrullin, *Langmuir*, 2011, **27**, 7708.
26 A. Diaspro, D. Silvano, S. Krol, O. Cavalleri and A. Gliozzi, *Langmuir*, 2002, **18**, 5047.
27 S. Krol, A. Diaspro, R. Magrassi, P. Ballario; B. Grimaldi, P. Filetici, P. Ornaghi, P. Ramoino and A. Gliozzi, *IEEE Trans. NanoBiosci.*, 2004, **3**, 32.
28. A. Meister, *Science*, 1973, **180**, 33.
29 V. Berry and R. F. Saraf, *Angew. Chem.*, 2005, **117**, 6826.
30 V. Berry, S. Rangaswamy and R. F. Saraf, *Nano Lett.*, 2004, **4**, 939.
31 V. Maheshwari, D. E. Fomenko, G. Singh and R. F. Saraf, *Langmuir*, 2010, **26,** 371.
32 M. Koneswaran, R. Narayanaswamy, *Sens. Actuators B Chem.*, 2009, **139**, 104.
33 K. Uvdal, P. BodÖ and B. Liedberg, *J. Colloid. Interf. Sci.*, 1992, **149**, 162.
34 S. G. Nair and G. R. Loppnow, *Photochem. Photobiol.*, 2013, **89**, 884.
35 J. H. Scott and R. Schekman, *J. Bacteriol.*, 1980, **142**, 414.
36 R. Iturriaga and B. G. Mitchell, *Mar. Ecol. Prog. Ser.*, 1986, **28**, 291.
37 B. Heurtault, P. Saulnier, B. Pech, J.-E. Proust, J.-P. Benoit, *Biomaterials*, 2003, **24**, 4283.
38 H. J. Butt, K. Graf and M. Kappl, *Physics and chemistry of interfaces*. John Wiley & Sons, 2006.
39 P. Behrens, *Angew. Chem. Int. Ed.*, 1996, **35**, 515.
40 Q. S. Huo, D. I. Margolese , U. Ciesla, D. G. Demuth , P. Y. Feng, T. E. Gier , P. Sieger, A. Firouzi and B. F. Chmelka, *Chem. Mater.*, 1994, **6**, 1176.
41 E. Paasche, *Mar. Biol.*, 1973, **19**, 17.
42 V. Martin-Jézéquel, M. Hildebrand and M. A. Brzezinski, *J. Phycol.*, 2000, **36**, 821.
43 A. A. Eddy and A. D. Rudin, *Proc. R. Soc. Lond B Biol.*, 1958, **148**, 419.
44 D. Fischer, Y. X. Li, B. Ahlemeyer, J. Krieglstein and T. Kissel, *Biomaterials*, 2003, **24**, 1121.

