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Nano-labelled cells — a functional tool in biomedical applications

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Nanotechnology offers an unprecedented number of opportunities for biomedical research, utilizing the unusual functionalities of nanosized materials. Here we describe the recent advances in fabrication and utilization of nanoparticle-labelled cells. We present a brief overview of the most promising techniques, namely layer-by-layer polyelectrolyte assembly on cells and intracellular and extracellular labelling with magnetic nanoparticles. Several important practical application of nanofucntionalized cells, including tissue engineering and tumour therapy, are reviewed.

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Introduction

Nanosized particles of various morphologies and functionalities have been extensively employed in biomedical research and clinical applications [1]. Particularly, nanoparticles are regarded as promising drug delivery vehicles [2]. Among other strategies, labelling of cells with various nanomaterials, attracts the attention of researchers worldwide. The potential of cell labelling with nanoparticles is secured by the novel functionalities which nanoparticles render to the cells. This paper is aimed to briefly overview of the current progress within this novel and vibrant area of research.

The layer-by-layer (LbL) polyelectrolyte deposition [3,4], originally designed primarily for the fabrication of and colloidal microcapsules, has been successfully applied to modify and functionalize surfaces of biological cells [5] and since has been utilized in a number of biomedical applications [6]. First reports were focused on applying LbL assembly technique for the deposition of oppositely charged polymer thin films to modify surfaces of microbial and mammalian cells in order to fabricate polyelectrolyte microcapsules imitating the geometries of biological cells. The pioneering paper by Neu *et al.* demonstrated the polyelectrolyte LbL assembly for the effective fabrication of micron-sized multilayered polymer capsules template on red blood cells and bacteria [7]. Cells as templates are attractive as their morphology, if repeated by the resulting microcapsules, allows for the higher biocompatibility. The typical procedure of cell surface modification with polyelectrolytes is based on the sequential deposition of oppositely charged polymers onto cells, additionally, various nanomaterials, such as nanoparticles, nanotubes and nanosheets can be deposited between the polyelectrolyte layers [8]. Alternatively, several reports demonstrate the direct, single-step deposition of polymer-functionalized nanoparticles onto living cells [9,10]. These modifications can be performed using both viable and non-viable cells. Most of the researchers concentrated on fabrication of functional nanocoatings on viable cells, allowing for attenuation of functional properties of cells [11•]. Typically, the recent reports on LbL assembly of polymer shells on cells utilize the surface modification of microorganisms [5], while others are trying to investigate the toxicity of polymer shells towards the mammalian cells [12]. The toxic effects of the surface modification of mammalian cells are attributed to the impact of positively charged polyelectrolytes [13]. Nevertheless, several reports demonstrate the successful use of surface modification of mammalian cells with LbL nanofilms for the biomedical applications [14,15].

However, the current methodology of cells nanolabelling is not limited to the LbL modification. The other approaches include, but are not limited to the direct deposition of nanomaterials, based on fabrication of the layers of magnetic nanoparticles [10] or 'hard' mineral shells [16] on cells. In addition, the intracellular labelling of cells with nanoparticles (i.e. magnetic nanoparticles) is another promising way to functionalize the cells.

Layer-by-layer (LbL) cell surface modification for tissue engineering

Arguably, the most promising applications of LbL-based cell surface modification are attributed to tissue engineering, a novel therapy technique where the cells are controllably assembled in multi-layered tissue-like structures.

Matsusaki *et al.* have demonstrated an elegant and simple procedure for the preparation of up to four layers of mouse fibroblasts using the deposition of polyelectrolyte multilayer technique [14] which is an appropriate method to prepare nanometre-sized films on a substrate through the immersion into polymer solutions [15]. They have prepared multilayer films composed of biogenic polyelectrolytes fibronectin and gelatin for mimicking the extracellular matrix on the surface of the first layer of cells, which, in turn, provides an adhesive surface for the second layer of cells. This methodology can also be applied to fabricate xenogenic cellular multilayers. The blood vessels-mimicking bilayer clusters composed of human smooth muscle and endothelial cells were successfully prepared by Matsusaki *et al.* using polyelectrolytes as supporting coatings, while the absence of polymer nanofilms resulted in formation of a mixed monolayer of both types of cells [14]. Recently, another promising approach was reported, demonstrating a simple and rapid bottom-up approach for fabrication of artificial tissues, termed *cell-accumulation technique*, facilitated using the single cell coating with FN-G nanofilms [17]. This approach is based on induction of the three-dimensional cell to cell adhesion of all seeded cells at the same time induced by FN-G nanofilm which facilitates the recognition of the nanocoated cells by $\alpha5\beta1$ integrin receptor of the cellular membrane. As a result, the thickness of the obtained artificial tissue increased with increasing the volume of culture media. Currently, viable tissue constructs with maximum thickness $\sim$100 μm built up of more than 20 layers-structures were obtained. Another approach a more complex single cell nanocoating using the LbL technology was demonstrated by Borkowska *et al.* [18], who fabricated the LbL polymer shell doped with fullerenol. An *in vitro* and *in vivo* (in mice) study confirmed the low cytotoxicity of the shells fabricated. Furthermore, the authors observed that the membrane with incorporated fullerenol did not evoke NO production increase in human leukaemia cells. Potentially, the technology developed might find applications in cell therapy as an alternative for immunosuppression applied for supporting the transplant surviving [18].

Direct nanomodification of cells

Biomimetic modification of cellular membranes is believed to increase the viability of the cells and further expand their applicability [19]. Apart from the LbL deposition, a number of alternative approaches exist. Here we introduce the reader with the selection of technologies for the direct modification of cellular surfaces without the use of the polymer multilayers. One of such approaches is the bioinspired silicification of cells, in other words, fabrication of egg-like shells on cells, which normally bear no coatings on their membranes. This method was effectively applied for the coating of individual microbial cells [20], and later has been expanded to the titania (TiO_2) or TiO_2–SiO_2 coating of *Chlorella* and other microorganisms [21]. The modified microorganisms showed the increased resistance to external stressors. Recently, the bioinspired silicification was applied by to modify the viable mammalian cells [16]. Lee *et al.* described for the first time the technology of cytocompatible and cytoprotective nanocoating with silica of several types of mammalian cells with mechanically durable silica. This coating effectively protected the cells from different lethal environmental influences such as trypsin and poly(allylamine hydrochloride) effects. Furthermore, the silica nanocoating suppressed the cell growth and preserved the cells from the adhesion, yet keeping the cells viable for a relatively long time. Above mentioned bioinspired silicification approach might be used in single-cell studies as well as in biomedical fields (cell therapy, cell-based sensors, and cells-on-a-chip technologies) which require the long-term protection and preservation of living cells [22]. Another interesting approach was envisaged by Maheshwari *et al.*, who used of yeast cells as a template for electrochemical synthesis of ZnO nanorods [23]. Graphene oxide sheets-pretreated cells were applied for this procedure, while graphene acted as an electrical conductor on the cell surface required for the electrochemical synthesis. On later stages, the electrochemically reduced graphene oxide (ERGO) contributed to the surface potential of the cell wall allowing for the electrochemical growth of ZnO nanorods. This hybrid cell ERGO–ZnO system shows $\sim$50% increase in current due to photogenerated charge carriers which is $\sim$5 fold more than the same for the planar ERGO–ZnO system. Authors believe that this electrochemical process for synthesis of nanomaterials on the living cell surface will significantly expand the possible combinations of cell–nanomaterial hybrids leading to new applications as biochemical sensors and energy storage systems.

Magnetic labelling of cells

Among many others, we consider magnetic labelling of cells as an extremely important technology which will result in a number of practical applications in biomedicine. Currently, two strategies of magnetic functionalization of living cells exist, one is based on intracellular labelling with magnetic nanoparticles, while the other relies on cell surface engineering.

Intracellular labelling of cells

Currently, magnetically labelled cells are believed to be a promising tool for engineering of complex artificial tissue-mimicking structures. Magnetic functionalization is achieved via the labelling of cells with magnetic nanoparticles (MNPs). Obviously, there are two opposite strategies for magnetization of living cells: internalization of MNPs into living cells and extracellular coating with MNPs. For intracellular magnetic labelling of mammalian cells anionic magnetic nanoparticles were employed. Typically, iron oxide cores are stabilized by citrate [24•], dextran [25], or hyaluronic acid [26]. The iron oxide MNPs are routinely synthesized via co-precipitation of

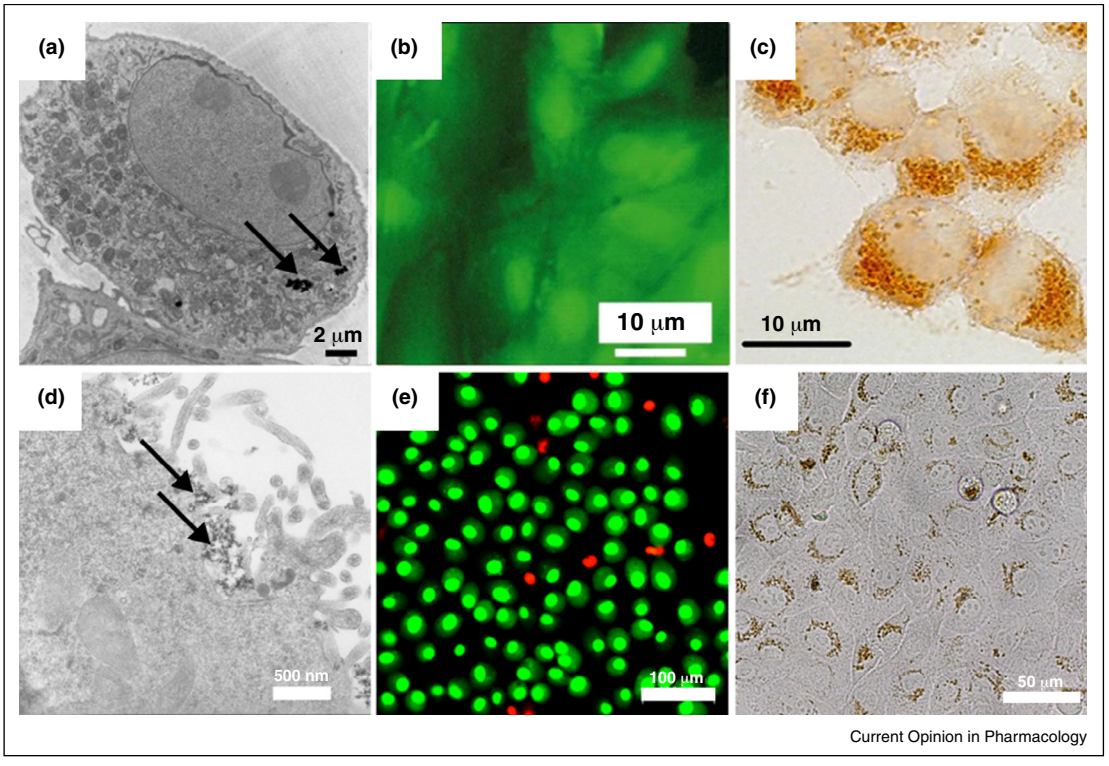
Fe^{2+} and Fe^{3+} ions in aqueous media accompanied by addition of alkali, followed by washing and stabilization. Next, the MNPs are mixed with culture media and then the cells are co-incubated in the presence of MNPs for prolonged periods of time [27]. Typically, the uptake of MNPs during the incubation is dose-dependent, which can be visualized using different appropriate methods, particularly high-resolution microscopy techniques (such as transmission electron microscopy) [28], flow cytometry [27] and Prussian Blue staining [24•].

It is believed that anionic MNPs are taken up by cells via endocytosis [28–31]. MNPs attached to the cell membrane assemble into the larger clusters, which induce the invagination of the membrane resulting in formation of densely packed endosomes. The localization of MNPs inside cells can be monitored microscopically (Figure 1a–c). Next, the endosomes migrate into the cytoplasm and undergo the typical endocytosis pathway (early endosome, late endosome and lysosome, respectively) [32]. The concentration of MNPs inside the cells directly affects the magnetic responsiveness of the labelled cells; however the viability of cells can be affected by the nanoparticles in cytoplasm. Low levels of acute toxicity of citrate-stabilized MNPs were demonstrated (Figure 1b), resulting in unaffected morphology, growth rate, surface markers synthesis and differentiation capacity of MNPs-labelled human mesenchymal stem cells [24•]. However, high MNPs intracellular concentrations significantly affected chondrogenesis and decreased chemokine-induced migration in magnetically labelled cells [24•]. Alternatively, intracellular magnetic labelling of human cells can be performed using cationic MNPs stabilized with polycations [33] or incorporated into cationic magnetic liposomes [34]. For example, MNPs stabilized using poly(L-lysine) (PLL) were recently applied in magnetic cell-labelling of mesenchymal stem cells [33].

Extracellular labelling of cells

Another approach is based on the surface functionalization (cell surface engineering) of mammalian cells via a single-step deposition of poly(allylamine)-stabilized biocompatible MNPs (PAH-MNPs). We have elaborated this rapid magnetic functionalization method for mammalian cells (Figure 1d–f). Our approach is based on magnetic modification based on the electrostatic interaction of biocompatible polymer-stabilized MNPs with the cell surface. Noteworthy, the deposition of cationic nanoparticles is

Figure 1



(a) Electron microscopy image indicating the internalization of MNPs nanoparticles in human fibroblasts (reproduced with permission from [30]); **(b)** viability staining of immortalized primary human fibroblasts after functionalization with dextran-coated MNPs (reproduced with permission from [28]); **(c)** bright-field microscopy image indicating the localization of aminodextran coated MNPs in endocytic compartments (reproduced with permission from [31]); **(d)** external localization of PAH-coated MNPs on the surface of human lung carcinoma cells (A549); **(e)** live/dead staining of A549 cells coated with PAH-MNPs; and **(f)** bright-field microscopy image of extracellular deposition of MNPs on A549 cells.

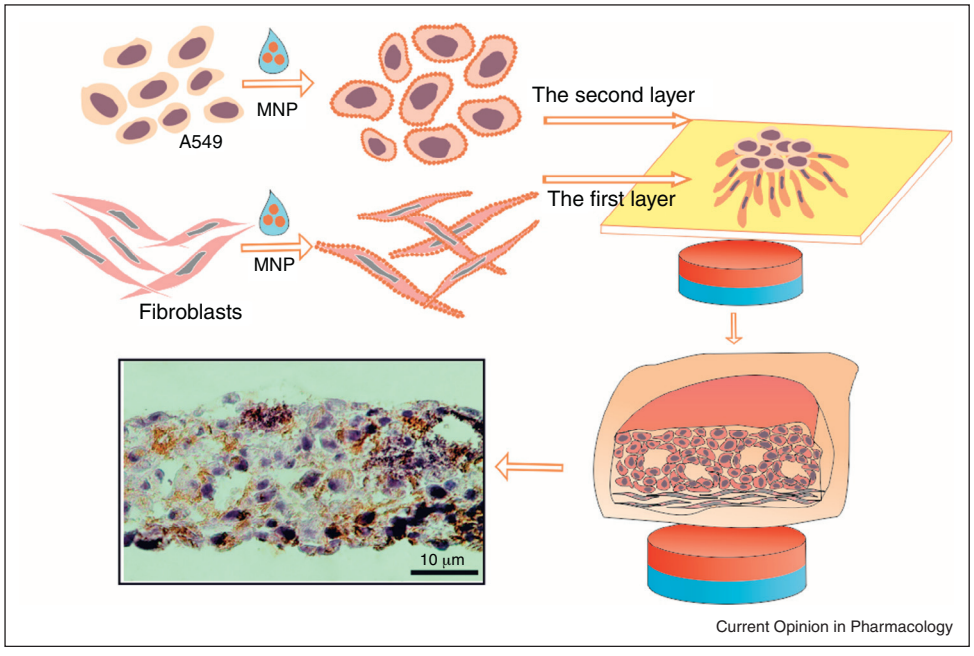
based purely on electrostatic interactions and does not require any specific ligand-receptor interaction of nanoparticles with membranes. As an example, HeLa cells were efficiently coated with a uniform layer of magnetic nanoparticles arranged exclusively on the outer surfaces of cell membranes [10], occurring due to the preferential attachment of the positively charged MNPs to anionic carbohydrates constituting the outer layer of cellular microvilli. Biocompatibility of PAH-MNPs was investigated via the simultaneous assessment of membrane integrity, enzymatic activity, and ability to grow and colonize substrates. Therefore, we conclude that PAH-stabilized SPIONs are nontoxic to HeLa cells, which we attribute to the previously reported low toxicity of both iron oxide nanoparticles [27,35] and PAH [36]. In another study [37], we found that PAH-MNPs have no significant toxic effect on the magnetically labelled cow embryo lung epithelial cells within the wide range of concentrations (0.0125–0.05 mg ml^{-1}). Moreover, PAH-MNPs-modified cells are still able to actively proliferate, the apparent growth rates of MNPs-coated cells was even faster than that of the intact ones [38•].

Magnetically functionalized cells in tissue engineering

Tissue engineering currently represents one of the fastest growing areas of biomedicine. It aims at the development of strategies for the formation of different tissues *in vitro* and their application *in vivo*. Among challenges that transplantology faces, the impossibility of allogenic donor organ selection that exactly matches the antigenic status of the recipient is one of the most crucial [39,40]. Construction of heterotypic 3D culture is an essential step for creation of tissues and organs [41]. Interaction between various types of cells is very important for the activation of cellular functions [42]. The use of 'magnetic cells' in the tissue engineering and regenerative medicine can be employed to tackle these challenges. Labelled cells become magnetically responsive and can be coordinated and positioned by an external magnetic field to create complex tissue structures which cannot be obtained using conventional cell culture techniques. MNPs are considered as a promising tool to assemble the magnetically modified cells into artificial tissue prototypes [41,43,44] from patient's own cells. Tissue engineering based on magnetically functionalized cells offers the scaffold-free arrangement of cells thus minimizing the use of supporting scaffolds [41] and to fabricate artificial tissue constructs with precise morphologies resembling the natural ones [45].

Ito *et al.* have demonstrated the fabrication of artificial tissue prototypes based on magnetically labelled cells, developing a novel methodology of three-dimensional heterotypic magnetically facilitated co-culture system. Complex structures consisting of heterotypic layered cells were obtained using magnetically labelled rat hepatocytes and human aortic endothelial cells. Accumulation of cells was mainly observed at the sites where magnets were positioned and formed tissue-mimicking clusters, where the heterotypic cells were layered and formed

Figure 2

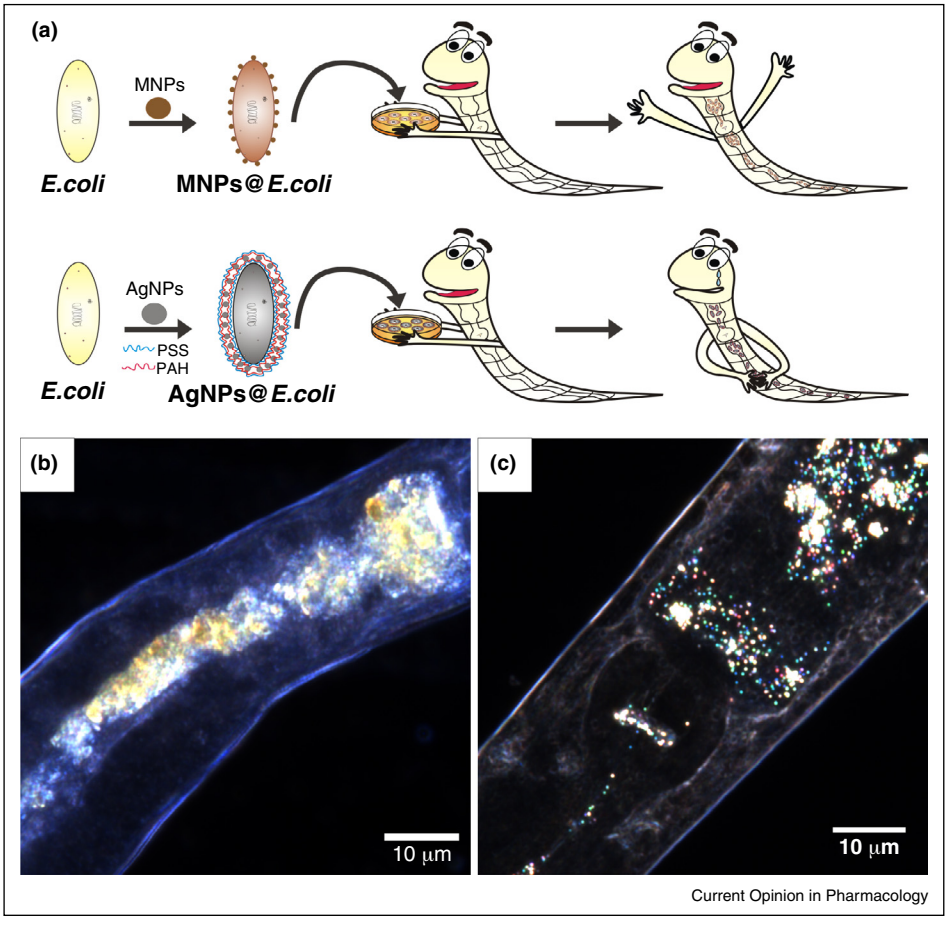


A scheme illustrating the method of tissue-like lung-mimicking construct fabrication using MNPs-labelled surface-functionalized human cells (fibroblasts and lung carcinoma cells).

intracellular contacts. Magnetically driven cell clusters were fully functional, preserving thesecretion of albumin by hepatocytes [43]. Further, magnetically driven tissue engineering was later employed for the *in vitro* fabrication and harvesting of cell sheets containing human hepatocellular carcinoma (HepG2) cells and immortal mouse embryonic fibroblast (NIH3T3) cells [41]. Magnetically labelled cells were utilized fabricate more elaborate tubular tissue structures template over using cylindrical [44]. Using this technique two types of artificial tissues were produced — urothelial cell layers and vascular tissue consisting of three types of cells (endothelial cells, smooth muscle cells, and fibroblasts). Multi-layered cell sheets were also fabricated using magnetically labelled cells, next the tissue constructs were transplanted subcutaneously into mice, and the histological examination of transplants after 14 days of culturing in mice bodies revealed that the sheet grafts produced vascularized tissues characterized with substantial mass, thickness, and cell density [34]. The on-going search for the new methods to label cells with magnetic nanoparticles facilitates the fabrication of more complex, yet functional carriers of nanoparticles based on the LbL microcapsules taken up by cells *via* endocytosis [46]. This approach allows for the direct control over the cell mobility, which may find important applications in tissue engineering and cell delivery.

We employed surface-engineered magnetically labelled human cells for development of 3D-tissue constructs [38•]. Magnetically functionalized viable human lung carcinoma cells (A549) and human skin fibroblasts (HSF) cells were used to fabricate scaffold-free lung tissue mimicking multicellular clusters. We used strong cylindrical magnets positioned under the culture wells for directed concentration of MNPs-coated cells around the magnets. Magnetically functionalized HSF (first layer) and A549 cells (second layer) were assembled layer-wise (Figure 2) and after that the magnets were removed, the resulting multi-cellular two-layered clusters could be easily defoliated from the bottom of wells. Brown aggregated MNPs were dissociated in the extracellular space allowing for magnetic manipulation with the clusters. The morphology of the artificial lung tissue-mimicking clusters obtained exhibited morphological similarity with

Figure 3



Nano-labelled cells as nanoparticle delivery vehicles: **(a)** a sketch demonstrating the uptake on 'nanobaits' by *C. elegans* nematodes (reproduced with permission from [47]); dark-field microscopy of distribution of **(b)** MNPs and **(c)** silver NPs delivered using 'nanobaits' inside the microworms.

the human lung tissue with both relatively large mature and smaller emerging pores. This approach is can be applied for fabrication of artificial tissue from MNPs-functionalized cells of other types, as we have shown earlier with HeLa cells [10].

The use of surface-fucntionalized cells in biomedical applications can be further extended into more complex systems, where the nanocoated 'cyborg' cells act as nanoparticles delivery vehicles. This concept was demonstrated by employing silver and magnetic nanoparticles-coated cells for the directed delivery of nanoparticles into *Caenorhabditis elegans* intestines [47]. As schematically shown in Figure 3, 'nanobaits' are ingested by microworms and facilitate the delivery of nanoparticles inside the worm's bodies, which can further be utilized in nanotoxicity studies.

As demonstrated by dark-field high-resolution microscopy (Figure 3b and c), nanoparticles are localized inside the intestines of the worms, thus the nanocoated cells can be regarded as a microscopic 'pills' used to effectively deliver the nanoparticles. This approach can potentially expand the use of microscopic model organisms in nanotoxicology.

In conclusion, in this paper we outlined the major directions of research in the field of nanoparticle-functionalized cells. We focused primarily on magnetically labelled cells, demonstrating the novel approaches for therapy, tissue engineering and delivery vehicle fabrication based on nanoparticle-functionalized cells. We believe that this emerging direction will result in the nearest future in a considerable advancement of biomedical research.

Conflict of interest

None declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC: **Nanoparticles in medicine: therapeutic applications and developments**. *Clin Pharmacol Ther* 2008, **83**:761-769.

2. Medina C, Santos-Martinez MJ, Radomski A, Corrigan OI, Radomski MW: **Nanoparticles: pharmacological and toxicological significance**. *Br J Pharmacol* 2007, **150**:552-558.

3. Shutava TG, Pattekari PP, Arapov KA, Torchilin VP, Lvov YM: **Architectural layer-by-layer assembly of drug nanocapsules with PEGylated polyelectrolytes**. *Soft Matter* 2012, **8**:9418-9427.

4. LvovY, Pattekari P, Shutava T: **Making aqueous nanocolloids from low soluble materials: LbL shells on nanocores**. In *Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials.* Edited by Decher G, Schlenoff J. Wiley-VCH; 2012: 151-170.

5. Diaspro A, Silvano D, Krol S, Cavalleri O, Gliozzi A: **Single living cell encapsulation in nano-organized polyelectrolyte shells**. *Langmuir* 2002, **18**:5047-5050.

6. Ai H: **Advanced layer-by-layer capsules for magnetic resonance imaging and drug delivery**. *Drug Deliv Rev* 2011, **63**:772-788.

7. Neu B, Voigt A, Mitloehner R, Leporatti S, Gao CY, Donath E, Kiesewetter H, Moehwald H, Meiselman HJ, Baumler H: **Biological cells as templates for hollow microcapsules**. *J Microencapsulation* 2001, **18**:385-395.

8. Fakhrullin RF, Lvov YM: **"Face-lifting" and "make-up" for microorganisms: layer-by-layer polyelectrolyte nanocoating**. *ACS Nano* 2012, **6**:4557-4564.

9. García-Alonso J, Fakhrullin RF, Paunov VN: **Rapid and direct magnetization of GFP-reporter yeast for micro-screening systems**. *Biosens Bioelectron* 2010, **25**:1816-1819.

10. Dzamukova MR, Zamaleeva AI, Ishmuchametova DG, OsinYN, Nurgaliev DN, KiyasovAP, Ilinskaya ON, Fakhrullin RF: **A direct technique for magnetic functionalization of living human cells**. *Langmuir* 2011, **27**:14386-14393.

11. • Lee J, Yang SH, Hong S-P, Hong D, Lee H, Lee H-Y, Kim Y-G, Choi IS: **Chemical control of yeast cell-division by cross-linked shells of catechol-grafted polyelectrolyte multilayers**. *Macromol Rapid Commun* 2013, **34**:1351-1356.
A mussel-inspired cytocompatible encapsulation method for achieving a 'cell-division control' with cross-linked layer-by-layer (LbL) shells from catechol-grafted polyethyleneimine and hyaluronic acid is demonstrated. Cell division is controlled by the number of the LbL nanolayers and cross-linking reaction.

12. Veerabadran NG, Goli PL, Stewart-Clark SS, Lvov YM, Mills DK: **Nanoencapsulation of stem cells within polyelectrolyte multilayer shells**. *Macromol Biosci* 2007, **7**:877-882.

13. Germain M, Balaguer P, Nicolas JC, Lopez F, Esteve JP, Sukhorukov GB, Winterhalter M, Richard-Foy H, Fournier D: **Protection of mammalian cell used in biosensors by coating with a polyelectrolyte shell**. *Biosens Bioelectron* 2006, **21**:1566-1573.

14. Matsusaki M, Kadowaki K, Nakahara Y, Akashi M: **Fabrication of cellular multilayers with nanometer-sized extracellular matrix films**. *Angew Chem Int Ed* 2007, **46**:4689-4692.

15. Tang Z, Wang Y, Podsiadlo P, Kotov NA: **Biomedical applications of layer-by-layer assembly: from biomimetics to tissue engineering advanced materials**. *Adv Mater* 2006, **18**:3203-3224.

16. Lee J, Choi J, Park JH, Kim M-H, Hong D, Cho H, Yang SH, Choi IS: **Cytoprotective silica coating of individual mammalian cells through bioinspired silicification**. *Angew Chem Int Ed* 2014, **53**:1-5.

17. Nishiguchi A, Yoshida H, Matsusaki M, Akashi M: **Rapid construction of three-dimensional multilayered tissues with endothelial tube networks by the cell-accumulation technique**. *Adv Mater* 2011, **23**:3506-3510.

18. Borkowska M, Grzeczkowicz A, Strawski M, Kawiak J, Szklarczyk M, Granicka LH: **Performance and detection of nano-thin polyelectrolyte shell for cell coating**. *J Nanopart Res* 2014, **16**:2488.

19. Drachuk I, Gupta MK, Tsukruk VV: **Biomimetic coatings to control cellular function through cell surface**. *Eng Adv Funct Mater* 2013, **23**:4437-4453.

20. Yang SH, Ko EH, Jung YH, Choi IS: **Bioinspired functionalization of silica-encapsulated yeast cells**. *Angew Chem* 2011, **123**:6239-6242.

21. Ko EH, Yoon Y, Park JH, Yang SH, Hong D, Lee K-B, Shon HK, Lee TG, Choi IS: **Bioinspired, cytocompatible mineralization of**

silica–titania composites: thermoprotective nanoshell formation for individual chlorella cells. *Angew Chem* 2013, **125**:12505-12508.

22. Michelini E, Cevenini L, Calabretta MM, Spinozzi S, Camborata C, Roda A: **Field-deployable whole-cell bioluminescent biosensors: so near and yet so far**. *Anal Bioanal Chem* 2013, **405**:6155-6163.

23. Hong D, Park M, Yang SH, Lee J, Kim Y-G, Choi IS: **Artificial spores: cytoprotective nanoencapsulation of living cells**. *Trends Biotechnol* 2013, **31**:442-447.

24. • Andreas K, Georgieva R, Ladwig M, Mueller S, Notter M, Sittinger M, Ringe J: **Highly efficient magnetic stem cell labeling with citrate-coated superparamagnetic iron oxide nanoparticles for MRI tracking**. *Biomaterials* 2012, **33**:4515-4525.

Stem cells intracellular labelling with anionic nanoparticles was investigated comparatively in terms of *in vitro* labelling efficiency, effects on stem cell functionality and *in vivo* magnetic resonance imaging.

25. Su H, Liu Y, Wang D, Wu C, Xia C, Gong Q, Song B, Ai H: **Amphiphilic starlike dextran wrapped superparamagnetic iron oxide nanoparticle clsuters as effective magnetic resonance imaging probes**. *Biomaterials* 2013, **34**:1193-1203.

26. El-Dakdouki MH, El-Boubbou K, Zhu DC, Huang XA: **Simple method for the synthesis of hyaluronic acid coated magnetic nanoparticles for highly efficient cell labelling and *in vivo* imaging**. *RSC Adv* 2011, **8**:1449-1452.

27. Wilhelm C, Gazeau F: **Universal cell labelling with anionic magnetic nanoparticles**. *Biomaterials* 2008, **29**:3161-3174.

28. Berry CC, Wells S, Charles S, Curtis AS: **Dextran and albumin derivatised iron oxide nanoparticles: influence on fibroblasts *in vitro***. *Biomaterials* 2003, **24**:4551-4557.

29. Fayol D, Luciani N, Lartigue L, Gazeau F, Wilhelm C: **Managing magnetic nanoparticle aggregation and cellular uptake: a precondition for efficient stem-cell differentiation and MRI tracking**. *Adv Healthc Mater* 2013, **2**:313-325.

The effects of magnetic nanoparticles labelling on stem cell differentiation are reported.

30. Buyukhatipoglu K, Clyne AM: **Superparamagnetic iron oxide nanoparticles change endothelial cell morphology and mechanics via reactive oxygen species formation**. *J Biomed Mater Res A* 2011, **96**:186-195.

31. Villanueva A, Canete M, Roca AG, Calero M, Veintemillas-Verdaguer S, Serna CJ, del Puerto Morales M, Miranda R: **The influence of surface functionalization on the enhanced internalization of magnetic nanoparticles in cancer cells**. *Nanotechnology* 2009, **20**:115103-115112.

32. Thorek DL, Tsourkas A: **Size, charge and concentration dependent uptake of iron oxide particles by non-phagocytic cells**. *Biomaterials* 2008, **29**:3583-3590.

33. Babic M, Horák D, Trchová M, Jendelová P, Glogarová K, Lesný P, Herynek V, Hájek M, Syková E: **Poly(L-lysine)-modified iron oxide nanoparticles for stem cell labeling**. *Bioconjugate Chem* 2008, **19**:740-750.

34. Akiyama H, Ito A, Kawabe Y, Kamihira M: **Genetically engineered angiogenic cell sheets using magnetic force-based gene delivery and tissue fabrication techniques**. *Biomaterials* 2010, **31**:1251-1259.

35. Koshi T, Hatsugai S, Yamada T, Takemura Y: **Magnetic characterization of surface-coated magnetic nanoparticles for biomedical application**. *J Magn Magn Mater* 2011, **323**:1398-1403.

36. Huang J, Bu L, Xie J, Chen K, Cheng Z, Li X, Chen X: **Effects of nanoparticle size on cellular uptake and liver MRI with polyvinylpyrrolidone-coated iron oxide nanoparticles**. *ACS Nano* 2010, **4**:7151-7160.

37. Dzamukova MR, Naumenko EA, Hamzina EY, Dzamukov RA, Shilyagin PA, Iliinskaya ON, Fakhrullin RF: **Polymer-stabilised magnetic nanoparticles do not affect the viability of magnetically-functionalised cells**. *Cell Transpl Tissue Eng* 2012, **3**:52-56.

38. • Dzamukova MR, Naumenko EA, Lannik NI, Fakhrullin RF: **Surface-modified magnetic human cells for scaffold-free tissue engineering**. *Biomater Sci* 2013, **1**:810-813.

A novel magnetically facilitated scaffold-free assembly of lung tissue mimicking two-layered multicellular clusters is reported.

39. Vacanti JP: **Beyond transplantation**. *Arch Surg* 1988, **123**:545-549.

40. Russel IS: **Selective transplantation**. *Ann Surg* 1985, **201**:255-262.

41. Ito A, Jitsunobu H, Kawabe Y, Kamihira M: **Construction of heterotypic cell sheets by magnetic force-based 3-D coculture of HepG2 and NIH3T3 cells**. *J Biosci Bioeng* 2007, **104**:371-378.

42. Chia SM, Lin PC, Yu H: **TGF-beta1 regulation in hepatocyte-NIH3T3 co-culture is important for the enhanced hepatocyte function in 3D microenvironment**. *Biotechnol Bioeng* 2005, **89**:565-573.

43. Ito A, Takizawa Y, Honda H, Hata K, Kagami H, Ueda M, Kobayashi T: **Tissue engineering using magnetite nanoparticles and magnetic force: heterotypic layers of cocultured hepatocytes and endothelial cells**. *Tissue Eng* 2004, **10**:833-840.

44. Ito A, Ino K, Hayashida M, Kobayashi T, Matsunuma H, Kagami H, Ueda M, Honda H: **Novel methodology for fabrication of tissue-engineered tubular constructs using magnetite nanoparticles and magnetic force**. *Tissue Eng* 2005, **11**:1553-1561.

45. Ho VHB, Muller KH, Barcza A, Chen R, Slater NKH: **Generation of magnetic multicellular spheroids for biomedical engineering applications**. *Biomaterials* 2010, **31**:3095-3102.

46. Pavlov AM, De Geest BG, Louage B, Lybaert L, De Koker S, Koudelka Z, Sapelkin A, Sukhorukov G: **Magnetically engineered microcapsules as intracellular anchors for remote control over cellular mobility**. *Adv Mater* 2013, **25**:6945-6950.

47. Däwlätşina GI, Minullina RT, Fakhrullin RF: **Microworms swallow the nanobait: the use of nanocoated microbial cells for the direct delivery of nanoparticles into *Caenorhabditis elegans***. *Nanoscale* 2013, **5**:11761-11769.

A novel method to deliver nanomaterials into *Caenorhabditis elegans* nematodes using nanoparticle-coated microbial cells as 'nanobait', which are ingested by nematodes as a sole food source.