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Multi-layer microcapsules: fresh insights and new applications

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1. Introduction

Delivery of biologically active compounds remains a topic of intensive research in the last decade. A number of delivery systems have been proposed to enable effective entrapment, targeting and to prolong circulation of drugs with a reduction of side effects [1]. In the late 1990-ies, an important development in colloidal engineering was to apply layer-by-layer (LbL) assembly of oppositely charged polyelectrolyte and other charged species which had typically been used on planar surfaces, to coat micron and submicron sized colloidal particles. Coating of colloid particles evolved into fabrication of microcapsules with walls made of polyelectrolyte multilayers, and whose properties could be defined. Intensive research on multilayer microcapsules in the next decade underpinned the ability to bring different functionalities to this delivery system. Indeed, incorporation of responsive polymers or nanoparticles in the microcapsule wall can endow more functionality. There are now hundreds of publications on multilayer capsules demonstrating responsiveness to a range of stimuli including temperature, pH, enzyme activity, sugar, light, magnetic fields and ultrasound [2]. The use of stimuli that are already utilized in clinical medicine, such as magnetic fields, ultrasound and/or light to control delivery from microcapsules is a particularly attractive aspect of microcapsule use and could facilitate their development in biomedicine. Versatile use and ease of tailoring properties such as size, permeability, responsiveness and encapsulated cargo are major advantages of these multilayer capsules.

There are still some obstacles such as permeability to small molecules (e.g. anticancer drug – doxorubicin, fluorescent markers – rhodamine B or fluorescein, siRNAs) that limit the wide spread application of multilayer capsules but recent developments have started to address these limitations. In this article we will outline the potential of LbL microcapsules in biomedicine and review recent developments that overcome limitations.

2. Therapeutics that Benefits from Microcapsule Permeability

The meshwork of polymers formed in layer construction results in a structure that is permeable to small molecules. Depending on the proposed application this permeability can be advantageous or a disadvantage. An obvious use of microcapsules is as a delivery vehicle, and their permeable structure means they can act as a depot for release. Release kinetics will be sustained if the drug has an affinity with a microcapsule component, as we have shown with the antibiotic Doxycycline which interacts with dextran sulphate or Daunorubicin that interacts with poly(styrene sulfonate) (PSS) positioned within the layers and/or core of the capsules [3]. Depot release from microcapsules will also be optimal with less water soluble drugs so that they remain within the capsule for longer. Other modifications such as heat shrinkage and outer lipid layers have been employed to promote sustained release. Permeability of small molecules is also advantageous when enzymes are incorporated into the microcapsule structure so that small molecule substrate gains access through capsule layers. Larger polymer cargoes such as the entrapped enzyme will be retained, trapped in the core or integrated into the microcapsule layers. In order for larger polymers to be released from the microcapsule, the capsule structure will need to be degraded or physically disrupted.

3. Utilization of Impermeable Microcapsules with a Novel Hybrid Structure

In many applications it will be ideal that drugs are delivered in a targeted manner or in response to a specific trigger in these situations it is ideal that there is no non-specific drug release from permeable microcapsules. Indeed, most of the drugs being marketed by the pharmaceutical industry have relatively small molecular weight (below 1000 Da), which limits the application of PE microcapsules for their delivery [4]. Attempts to use hydrophobic polymers such as polylactic acid (PLA), for multilayer shell build-up in non-aqueous solution could reduce the permeability, but probably not enough to retain small molecules. Alternatively, introduction of inorganic structures into LbL microcapsules can generate impermeable capsules with high mechanical strength [5], such hybrid microcontainers combining the physicochemical properties of organic and inorganic components can extend their applications in drug delivery systems (DDSs). Recently, Sukhorukov, Parak and colleagues introduced sol-gel chemistry for modification of PE microcapsules [6]. The sol-gel approach is based on hydrolysis and poly-condensation of tetraethyl orthosilicate (TEOS) in the presence of an ammonium hydroxide catalyst. It produces nanoscale silica particles, which can easily cover the surface of PE microcapsules, forming a solid composite shell, composed of organic building blocks and silica nanostructures (Figure 1A, B). The resulting composite microcapsules were robust and maintained their spherical shape even after drying, indicating enhanced mechanics. The silica surface can adsorb different small molecules and prevent the leakage of low molecular weight chemicals from microcapsules. In addition, silica is non-toxic and can be dissolved in biological environments [5]. These hybrid microcapsules with the ability to encapsulate small molecule drugs can also be synthesised through LbL assembly of PE, inorganic nanoparticles (INPs) and graphene oxide (GO) (Figure 1C, D) [7,8]. As shown, GO has good colloidal stability and dispensability in water and simultaneously extremely high drug loading capacity [7]. Besides, the excellent mechanical

properties of microcapsules with inorganic nanoparticles the improved mechanical strength of the capsule shell provides the possibility for controllable permeability.

Despite the ability for encapsulation of small drugs, such hybrid microcapsules should comply with the modern demand for "smart" DDSs, which is now significantly increased. Various types of stimuli-responsive carriers have been designed with controlled cargo release when exposed to single trigger which minimizes their therapeutic efficacy. Nowadays, combining several stimuli-responsive mechanisms in one intelligent capsule can be beneficial in some drug delivery situations offer effective strategy to enhance the therapeutic efficacy of many remedies. For example, we reported the preparation of bifunctional ultraviolet-ultrasound responsive TiO2/PE composite microcapsules that could be applied in cosmetics for delivery of bioactive substances on the skin epidermis or protecting the skin from UV light [9]. Such dual-responsive microcontainers are disintegrated under UV light or ultrasound irradiation (Figure 1E). By employing these physical stimuli, it allows us to control the rate of cargo release from seconds to hours providing different therapeutic effects.

Other triggers could also be harnessed for drug release such as alternate magnetic fields and near infrared (NIR) laser irradiation which also produce local hypothermia and this can have synergetic effects with toxic drugs and overcome drug resistance whilst reducing unfavorable side effects of chemotherapy. An example of such hybrid microcapsules was recently reported by L. Deng *et al.* [7]. They developed an easily assembled DDS platform consisting of Fe_3O_4-decorated GO deposited onto alginate/chitosan microcapsules (Figure 1F). Such dual-responsive triggers induced by NIR and magnetic hyperthermia (MH) generated synergistic effects to efficiently kill cancer cells, increasing the therapeutic performance in antitumor therapy.

Another example of multifunctional microcapsules for synergetic cancer treatment was described

by H. Chen *et al.* [10], they used folic acid (FA)-modified hollow microcapsules loaded with gold nanorods. These constructs undergo thermal degradation under NIR light and elicit photothermal therapy and controlled chemotherapy providing a synergistic cancer treatment. Such unique features of hybrid microcapsules support the controlled release of Doxorubicin (anticancer drug), potentially increasing the efficiency of such a system in cancer treatment.

3. Harnessing Multifunctional Hybrid Microcapsules in Cell Engineering

Various biomaterials, including LbL microcapsules, with outstanding physicochemical features have shown low effectiveness for *in vivo* administration due to their suboptimal size and surface chemistry. Therefore, the search for universal vehicles, which can increase the potential effectiveness for *in vivo* targeted delivery is of high importance. Previous investigations have demonstrated that Mesenchymal stem cells (MSCs) possess inherent tumor-tropic and migratory properties, which allow them to serve as vehicles for the *in vivo* delivery and treatment of isolated tumors [11]. The development of a biological platform based on stem cells and internalized biocompatible drug carriers can significantly improve the efficiency of *in vivo* delivery and establish a new approach for the cell functionalization. Some progress has been made in the design of stem cell engineering using mesoporous silica nanoparticles (MSNs) [11]. Interesting results published by X. Huang *et al.* demonstrated the idea of modification of MSCs with multifunctional MSNs for tumor targeting [11]. They combined tumor tropism of MSCs and multimodality imaging of MSNs coated with hyaluronic acid-based polymer (HA), along with FITC, dye ZW800 and Cu^{64} (HA-MSN-Cu^{64}) as imaging agents for optical, magnetic resonance (MR) and positron emission tomography (PET) (Figure 2A). MR images in Figure 2B, in comparison with the pre-injection image, showed an obvious increase in T1 signal in tumors 24 h

after injection, which confirmed successful targeted delivery of the MSCs to the tumor. The successful tumor homing of MSCs labeled with HA-MSN-Cu^{64} was also confirmed by PET (Figure 2C). These engineered MSCs offer great potential for effective tumor homing *in vivo* and future delivery of therapeutics.

LbL microcapsules have great potential in MSCs functionalisation compared with MSNs alone because of the unique properties of LbL microcapsules, they have the capacity for multi-encapsulation and controlled release of low or high molecular weight compounds under different external or internal stimuli. These unique features of microcapsules can potentially extend the properties of MSCs if such capsules can be internalized.

Pavlov *et al.* demonstrated an interesting strategy to use magnetic microcapsules for the magnetic guidance of live cells (Figure 2D) [12]. This strategy was based on the internalization of magnetically-responsive capsules by cells (Figure 2E, G). Magnetic microcapsules can be targeted with a magnet and when they are engulfed by cells it is possible to navigate the engulfing cell. This is an alternative use of capsules, to reside inside cells for cell tracking and in particular, for cell navigation via an external magnetic field. This ability of cells to respond to a magnetic field was demonstrated with a number of cell lines. Such an innovative application of LbL microcapsules in cell functionalisation can be effectively used to design a cell-based multifunctional platform which, enables manipulation of cell behavior. It may also increase the effectiveness of targeted delivery of MSCs to the designated tissue or organ, resulting in significant improvement of tumor therapy. Additionally, the presence of magnetite nanoparticles in LbL microcapsules provides the possibility for MR imaging.

4. Perspective on the Microcapsule Application in Biomedicine

LbL microcapsules have many attributes that lend to their application in biomedicine. They are assembled under native conditions so that biologically active molecules are not chemically altered or inactivated. Using biodegradable polymers such as polyarginine or dextran sulphate in the fabrication of microcapsules permits degradation of the microcapsule structure. This process can be explored by working with microcapsules in isolation [13] but is also evident when microcapsules are introduced into live cells where enzymes within phagocytic structures access core contents [14]. In our studies we have been able to monitor this process in cells through the use of plasmid DNA in the microcapsule core, we know this cargo must be liberated and access the nucleus before gene transcription can occur [13]. Plasmid DNA was also released when it formed an outer or sub-outer microcapsule layer but when it was integrated into the structural layers of the microcapsule (a middle layer) no release (monitored via expressed luciferase enzyme) was observed. In this middle layer location it would seem that the plasmid DNA was subjected to degradation before its liberation [13]. If this so called 'hidden' middle layer could be liberated in a way that retained biological activity it could present a stealth approach for molecule delivery.

Unlike the use of biodegradable polymers in preparation of capsules for cargo release, the microcapsule structure can also be used to protect biomacromolecules when synthetic polymers are utilised in the microcapsule structure as these are non-degradable. The extent of protection seems to differ in studies with isolated microcapsules [13] and when engulfed by cells where cargo molecules in the core are protected from degradation [14]. There are few alternative approaches that physically protect biological molecules but it will be important to determine the biocompatibility of such synthetic structures.

At the present time the typical 3-4 micron size of microcapsules, similar in size to platelets, would preclude their clinical use by the intravascular route, but it does not preclude their direct injection into tissues where they will be rapidly cleared by phagocytosis, indeed it is this feature that has enabled their most successful biomedical application *in vivo* - antigen delivery. In *in vivo* studies they are similarly efficient as other particulate carriers for immunisation [15]. Potentially other components could also be introduced into microcapsules to further enhance the immune response. At the same time, the scalable fabrication of smaller nanosized multilayer capsules is important for the advancement of microcapsule applications *in vivo*.

LbL microcapsules can be endowed with switches to trigger release, target their delivery, enable remote navigation, permit the simultaneous delivery of similar or different types of molecules within cells, act as sensing-responding biosensors and even cellular organelles. As these abilities are refined and developed the full potential of microcapsules in biomedicine will be achieved.

5. Expert opinion

Microcapsules are often studied in isolation and have also been extensively used in fabrication of bioartificial organs with cells particularly the bioartificial pancreas. However, if PE microcapsules are to be utilised in medical application this will typically mean that they are engulfed by cells. Within cells the fate and function of the microcapsules is largely dependent upon the structural polymers used in their construction.

Progress needs to be made in determining the biocompatability of the polymers used in microcapsule structures. In biomedical applications repeat administration is likely and toxicity could be an issue with some polymers. The majority of work on microcapsules has stemmed from the use of $CaCO_3$ cores which results in capsules that are typically 3 to 4 microns in

diameter a similar size to platelets. In order to improve their functionality and increase their applications more work needs to be done with smaller cores. At present aggregation during synthesis is a major problem especially if their size is smaller than 300 nm as required for intravenous injection. Indeed, surface coating to stabilize capsules in serum remains a problem. At the present time the typical 3-4 micron size of microcapsules would preclude their clinical use by the intravascular route.

Microcapsule structures are quite stable and for biomedical applications it will be important to determine how long functionalities are retained. An important aspect for this will be to use high purity and sterile reagents and conditions for their construction. Scalability is another issue in the transition to biomedical and other commercial applications. Development of optimal methods for large scale synthesis appears to be an area that needs considerable input. There are some developments to accelerate the process of multilayer capsule production including filtration, microfluidics and fluid bed coating, but the process remains time consuming and less scalable as compared with other conventional particles/capsule preparation approaches.

Multifunctionalisation offers great potential for delivery but great attention must be paid toxicity of nanoparticles both in cells and in *in vivo* applications.

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Declaration of interest

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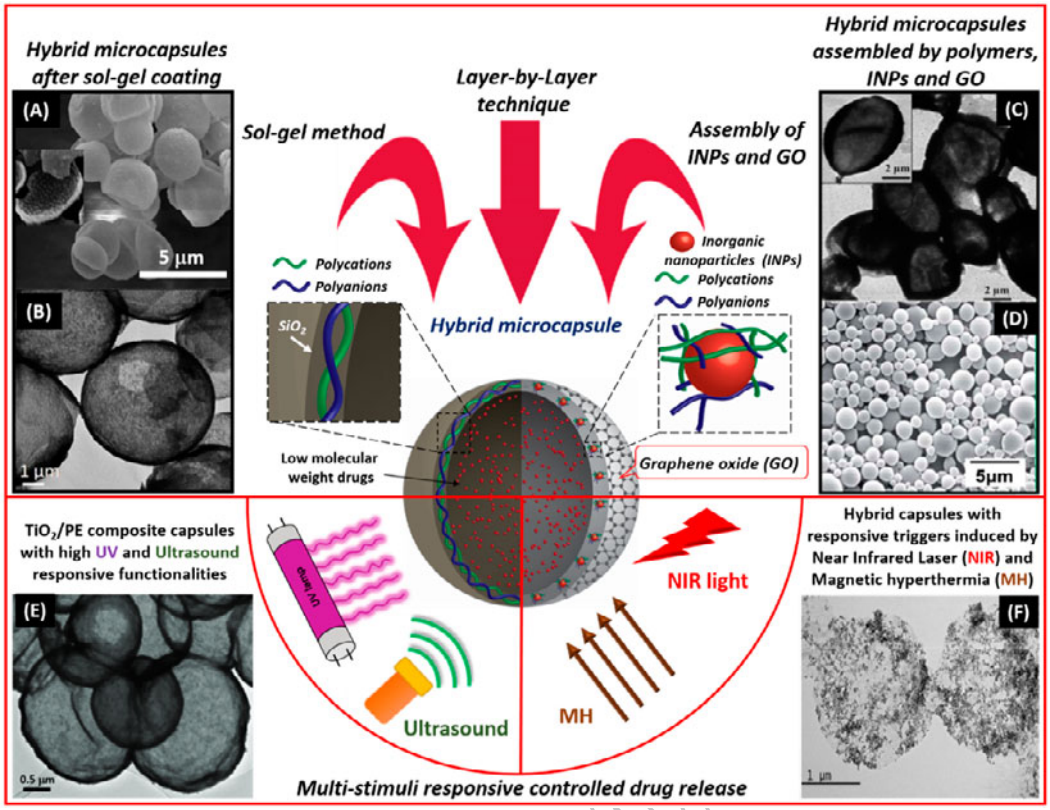

Figure 1. Schematic illustration of hybrid microcapsules fabricated via the combination of layer-by-layer technique with sol-gel method and assembly of polyelectrolytes with inorganic nanoparticles (INPs)/graphene oxide (GO). SEM and TEM images of SiO2-coated PE microcapsules (A, B) and hybrid microcapsules assembled by polymers, INPs/ GO (C, D). Multi-responsive mechanisms of triggered drug release from hybrid microcapsule. SEM image of TiO2/ PE composite capsules (E) that could be triggered upon ultrasound or/and UV irradiation. SEM image of hybrid Fe_3O_4-decorated GO LbL microcapsules (F) possessing dual-responsive triggers induced by near infrared laser (NIR) and magnetic hyperthermia (MH). Figure 1A, B. Reproduced from [6] with permission of Wiley publishing group and American Chemical Society. Figure 1C, D. Reproduced from [7] with permission of Royal Chemical Society and American Chemical Society. Figure 1E, F. Reproduced from [8, 9] with permission of American Chemical Society and Royal Chemical Society.

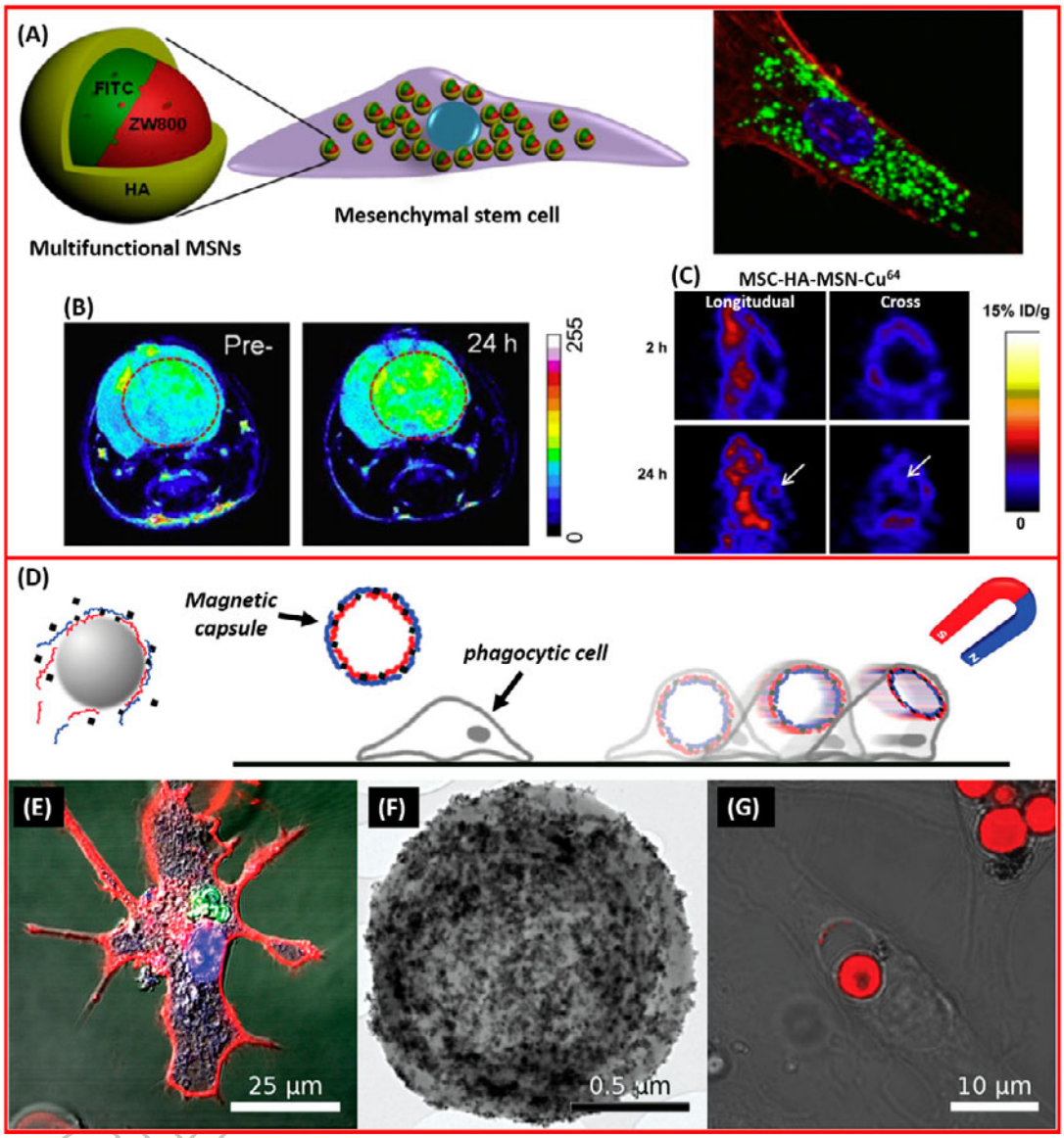

Figure 2. Schematic illustration and characterization of a mesenchymal stem cell-based multifunctional cell platform (MSC-platform) (A). MR imaging demonstrated the increased signal at tumor site (circle) after MSC-platform administration for 24 h compared with pre-injection (B). PET imaging of the tumor targeting of the MSC-platform at the indicated time points (C). Internalization of magnetic capsules by phagocytic cells and possible response of

cells towards applied magnetic field (D). Confocal microscopy image of microcapsules internalized by dendritic cells (E). TEM image of the magnetic microcapsule (F). Confocal microscopy image of microcapsules internalized by phagocytic cells (G). Figure 2A – C. Reproduced from [11] with permission of Elsevier. Figure 2D – G. Reproduced from [12] with permission of Wiley publishing group.