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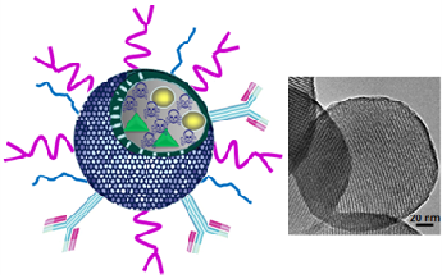

Description of some recent advances in the use of mesoporous silica nanoparticles as smart drug carriers for antitumor therapy

Biomaterials Science

REVIEW

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Since 2001, when our research group proposed for the first time MCM-41 as drug release system, the scientific community is devoting growing interest to mesoporous silica nanoparticles (MSNs) for their revolutionary potential in nanomedicine. Among the diverse pathologies that can be treated with MSNs, cancer has received increasing attention. MSNs can be loaded with large amounts of therapeutic cargoes and once intravenously administrated preferentially accumulate at solid tumours via enhanced permeation and retention (EPR) effect. Herein we report the recent developments achieved by our research group as a pioneer into this field, highlighting: the design of sophisticated MSNs as stimuli-responsive drug delivery systems to release the entrapped cargo upon exposure to a given stimulus while preventing the premature release of highly cytotoxic drugs before reaching the target; transporting non-toxic prodrugs and the enzyme responsible for its conversion into cytotoxic agents into the same MSN; improving the selectivity and cellular uptake by cancer cell by active targeting of MSNs; increasing the penetration of MSNs within the tumour mass, which is one of the major challenges in the use of NPs to treat solid tumours.

1. Introduction

Nanoparticles (NPs) capable to host, protect, transport and release therapeutic agents at target tissues, constitute one of the more exciting areas in modern nanomedicine.[1-4] Among the different pathologies than can be treated with NPs, cancer has received outstanding attention.[5] One of the main reasons relies on the fact that, once NPs are injected in the bloodstream, they preferentially accumulate in solid tumours due to the enhanced permeation and retention (EPR) effect. [6, 7] This phenomenon involves that macromolecules greater than 40 kDa or NPs intravenously administrated can leak out from the tumour blood vessels but not from healthy vessels. Normal vessels exhibit a well-organized branching hierarchy from large vessels into smaller vessels that feed a regularly spaced capillary bed. Contrarily, tumour blood vessels are abnormal, with wide interendothelial junctions, a high number of fenestrations, and transendothelial pores of several hundreds of nanometers.[8] Therefore, when NPs are injected in the bloodstream they might extravasate through the fenestrations present in the tumour vessels and accumulate in the tumour interstitium. Besides, the extravasated NPs are retained within the tissue for long times because of an ineffective lymphatic drainage as a consequence of the fast growth of the tumour cells. On the contrary, conventional antitumor drugs are small and then they can pass through the vessels both in tumour and healthy tissues. Hence, they are rapidly eliminated from the bloodstream, and therefore high doses are needed to achieve therapeutic levels. Both, the need of high doses and the lack of selectivity of highly cytotoxic antitumor drugs usually provoke severe side effects in the patient that may compromise the efficacy of the therapy or even endanger the patient's life.

The encapsulation of antitumor drugs into NPs offers several advantages, including: *i)* their capability to improve the administration of hydrophobic drugs; *ii)* improving the pharmacokinetic release profiles; *iii)* capability to protect drugs against environmental aggressions (enzymatic degradation, pH variations, etc); *iv)* allowing high local concentrations; *v)* capability to transport more than one drug simultaneously; *vi)* capability to cross epithelial or endothelial barriers; *vii)* capability to monitor NPs trafficking and therapeutic efficacy using labelled NPs; and *viii)* selective accumulation into the tumour mass thanks to the EPR effect. It is worth of mention that the specific accumulation of NPs can be enhanced by grafting on their surface entities (antibodies, aptamers, or small molecules) capably of being selectively recognized by the surface of tumour cells.

In recent years a great number of NPs have been reported for biomedical applications.[9-12] Among them, mesoporous silica NPs (MSNs) are promising candidates owing their unique properties such as large surface areas (700-1000 m^2 g^{-1}) and large pore volumes (0.6-1 cm^3 g^{-1}), which provide high loading capacity, tuneable size (50-300 nm), shape and pore diameter (2-6 nm), robustness and easy functionalization.[13-26] The features offer exceptional opportunities to host different therapeutic agents.[18, 27, 28] On the other hand, MSNs can be

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endocytosed by a huge number of mammalian cell lines, which depends on surface functionalization, size and morphology of NPs.[29-32] *In vitro* toxicology of MSNs indicated that they were well-tolerated by different cell lines at dosages <100 μg mL^{-1},[33] and only transient metabolic alterations were produced.[34, 35] *In vivo* biocompatibility of MSNs indicated that dosages <200 mg kg^{-1} were well-tolerated.[36] The good he hemocompatibility, which is a requirement for intravenous administration, has been also demonstrated.[37, 38]

Since 2001[13] when our research group proposed for the first time MCM-41 mesoporous materials as drug release system, an impressive number of innovative advances into MSNs' field have been reported worldwide. Herein we overview in a rational fashion the recent advances achieved by our research group as pioneer into the field of MSNs for nanomedicine.

2. Stimuli-responsive drug delivery systems

Drug release from conventional nanocarriers relays on the biodegradation of the nanoparticles *in vivo*, which makes very difficult to control the release of the drug. In this sense, stimuli-responsive drug delivery systems permit tailoring the release profiles with spatial, temporal and dosage control.[4, 25]

MSNs can act as drug carriers thanks to the high specific surface area that present the mesoporous silica matrix. The diameter of the mesopores can be tuned in order to retain different therapeutic compounds from two to five nanometers, allowing the transportation of compounds of very different nature from small molecules to proteins, among others.[39] However, if the transported compounds present high toxicity, as in the case of antitumor drugs, it is compulsory to design a strategy to avoid their premature release before reaching the target zone. As it has been mentioned above, MCM-41 type MSNs exhibit a unique pore network consisting in independent parallel pores without any connections between them. Thus, it is possible to obtain zero-release devices placing different moieties on the entrance of these pores which act as gate-keepers hampering the drug departure by steric hindrance or electrostatic repulsive forces.[40] Obviously, the attachment of these caps should be carried out in a reversible way for allowing the drug release once the nanodevice is in the diseased zone. This attachment should be performed through breakable bonds or employing moieties that might exhibit conformational changes which allow the drug departure.[41] In our research group, several stimuli-responsive MSNs have been designed in order to response to externally applied stimuli such as magnetic fields, light or ultrasounds which are described in detail below.

Magnetic-responsive MSN

The use of a magnetic field for triggering the drug release process presents interesting advantages for clinical applications due to its low toxicity and excellent penetration in living tissues. In order to exploit this stimulus a carrier able sensitive to the presence of magnetic fields should be designed. Thus, the most employed strategy consists in the encapsulation of superparamagnetic iron oxide nanocrystals (SPIONs) of 5–10 nm of diameter within the silica matrix. The exposition of SPIONs to alternating magnetic fields induces a significant temperature increase in the surroundings mainly due to two different mechanisms: Brownian relaxation, which implies the rapid rotation of the particles themselves and Neel relaxation, that is caused by the fluctuation of the magnetic moment within the particle.[42] The efficient encapsulation of SPIONs within mesoporous silica matrices can be achieved using different strategies such as aerosol techniques[43] or sol-gel process,[44] among others. Therefore, the presence of SPIONs inside MSNs allows the use of magnetic fields as trigger stimulus by placing thermosensitive moieties on the pore outlets which can be capable to suffer physic-chemical changes in response to temperature increases. The first temperature-responsive gatekeeper which was employed in our research group was DNA.[45] When two complementary single DNA strands are in contact, they form a stable double helix structure thanks to the attractive intermolecular interactions between the base pairs adenine-thymine (AT) and guanine-cytosine (CT) in a process known as hybridization. This process is reversible and the two DNA strands can be separated at certain temperatures due to the rupture of these intermolecular forces producing the dehybridization of the double helix. The temperature at which this process takes place is called melting temperature, and it can be tuned by modifications in chain length, GC content, or controlling the density of oligonucleotides grafted on the particle surface.[46] Our magnetic-responsive device was based on MSNs which contained SPIONs trapped within the silica matrix (Magnetic Mesoporous Silica Nanoparticles, MMSNs). MMSNs were decorated in their external surface with 15 base pairs DNA single strands designed to exhibit a melting temperature of 47^0C that is located in the upper limit of hyperthermia range.[45] This device was capped with iron oxide nanoparticles decorated with the complementary DNA single strands through the hybridization between the two oligonucleotide chains (Fig. 1). This device can be loaded with drugs before the pore closure, displaying a zero-release performance at room temperature once the system is sealed. The application of an alternating magnetic field of 24 kA m^{-1} and 100 kHz induces a significant temperature increase in the particle surroundings up to reach the melting temperature, which produces the dehybridisation of the double helix and the subsequent drug departure. One interesting advantage of this system is its reversibility, because once the magnetic field is ceased, the DNA strands are self-assembled again leading to the pore closure. Thus, this device exhibit an on-off behaviour being capable of releasing the transported therapeutic agents in a more precise and controllable way.

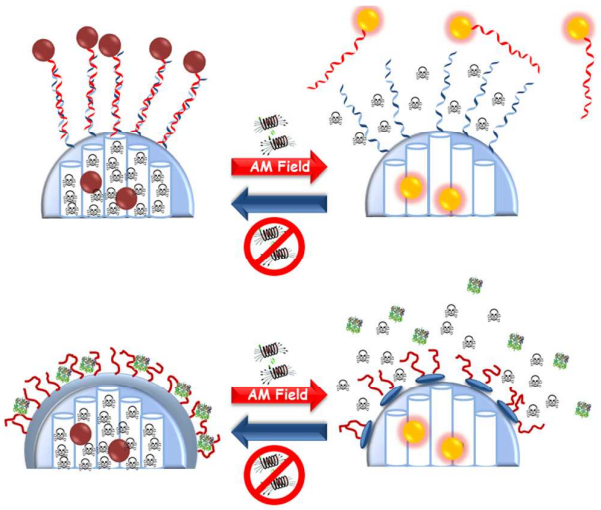

Fig. 1. DNA-gated nanodevices (top) and thermosensitive-co-polymer coating (blue shell corresponds to PNIPAM and red chains correspond to PEI) (bottom).

Thermosensitive polymers have been widely employed in nanomedicine both for the synthesis of temperature-responsive organic nanoparticles,[47] as well as for the creation of temperature-responsive gate-keepers placed on the surface of inorganic porous materials.[48] Poly(*N*-isopropylacrylamide) (PNIPAM) has been one of the most employed systems among the temperature-responsive polymer family. When this polymer is dissolved in water it presents a linear state at temperatures below 32^{0}C whereas it suffers shrinkage if the temperature exceeds this value. The temperature at which this transition takes place is known as lower critical solution temperature (LCST) and it can be tuned modifying the composition of the polymer chain. Thus, the co-polymerization of NIPAM with hydrophilic monomers such as acrylamide, acrylic acid or *N*-hydroxymethyl acrylamide (NHMA), among others, provokes an increase in their LCST due to the more favoured polymer-water interactions caused by the presence of these hydrophilic monomers.[49] Therefore, through changes in the polymer composition it is possible to obtain PNIPAM co-polymers in which the transition temperature is set around the hyperthermia range (42-45^{0}C).[50] In our research group, an engineered block co-polymer based on PNIPAM and polyethylene imine (PEI) has been grafted on the surface of MMSNs in order to provide two interesting properties: first, to act as temperature responsive gatekeeper able to control the release of drugs loaded within the pore network due to the presence of the PNIPAM backbone and, on the other hand, to retain proteins or enzymes within the polymer shell by electrostatic and hydrogen bond interactions between the housed macromolecules and PEI chains (Fig. 1). The presence of PEI increases the hydrophilic nature of the polymer chain rising the transition temperature up to *ca.* 40^{0}C. Our device demonstrated its capacity to transport two species of different nature: fluorescein, as a small molecule model and Soybean Trypsin Inhibitor, similar to growth factors, as a protein example. The polymer shell collapses once the system is exposed to an alternating magnetic field producing the release of the loaded compounds. Recently, we have anchored on the MMSNs surface a random thermosensitive PNIPAM copolymer doped with 10% of NHMA in order to obtain a precise drug release triggered by magnetic fields when the temperature reaches 43^{0}C.[51] More importantly, this device was able to release the housed drugs under alternating magnetic exposition even keeping the local temperature at physiological conditions (37^{0}C) placing the sample into a refrigerated chamber. This behavior proves that the presence of SPIONs within the silica matrix under magnetic field produces enough heat in the particle surroundings to induce the polymer collapse without the necessity to achieve a global temperature increment. This fact is of paramount importance for the future clinical application of magnetically triggered devices.

Light-responsive MSN

Light was one of the first stimuli employed for triggering the drug release in MSNs. Tanaka *et al.*[52] grafted UV-light sensitive coumarin derivatives on the pore walls in order to control the drug departure process. The use of light presents several advantages as low toxicity, easy application and fine focalization in the target place. However, the main drawback is its poor tissue penetration. Despite this fact, a wide number of MSNs have been functionalized with light-sensitive moieties on their external surface in order to control the drug release by the exposition of light with different wavelengths, from UV to near infrared radiation.[53] Our research group has recently reported the use of a responsive biocompatible protein shell able to act as light-cleavable gatekeeper which avoids the premature drug release. Additionally, the proteins attached on MSNs surface are capable to work as targeting agent enhancing the uptake by the tumour cells. This carrier is based on MSNs decorated with biotin molecules grafted on the surface through a light-sensitive cross-linker which suffer cleavage when is irradiated UV light at 366 nm (Fig. 2). Once the carrier is loaded with different cytotoxic compounds (doxorubicin or oxalilplatin), a solution of biotynilated transferrin and streptavidin seals the system thanks to the self-recognition capacity of the biotin-streptavidin bridges. Transferrin is the protein in charge of iron transportation in human body. Many different tumour cell lines overexpress transferrin receptors in order to capture this valuable oligoelement.[54] Therefore, the presence of transferrin on the external surface of these carriers not only act as sensitive cap of the housed drug but it also significantly enhances the particle uptake by the tumour cells up to ten times more than the naked particles. Once the carriers are internalized within the tumour cell, irradiation with UV light during a short period of time, less than 5 minutes, provokes the cleavage of the protein caps and then, the drug release. Surprisingly, these smart nanocarriers achieved a great cytotoxicity provoking that 100% of the cell population entered into apoptotic state using doses so low as 0.01 μg mL^{-1}. The samples maintained in darkness conditions or the samples irradiated with UV light, but without particles, presented a same survival ratio than the controls. These results confirmed the capacity of these nanocarriers to destroy tumour cells in a controlled manner.

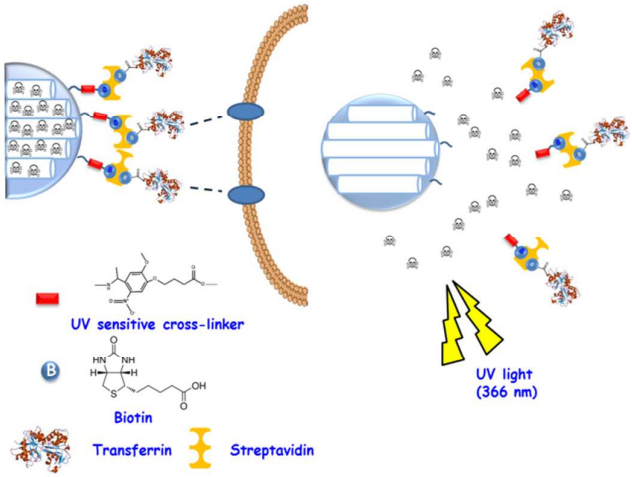

Fig. 2. Light-responsive protein shell in MSN which act as targeting agent and capping system at the same time.

Ultrasound-responsive MSN

Ultrasounds (US) represent an external stimulus that allows spatiotemporal control of the drug release at the desired site with very interesting features, such as: non-invasiveness, absence of ionizing radiations, cost effectiveness, and easy regulation of tissue penetration depth depending on frequency, cycles and exposure time. What is attractive of US in the field of nanomedicine is that high-frequency US can penetrate deep into the body with focused beams, which permits local therapies avoiding any damaging on healthy tissues.[55]

Ultrasound waves propagate through living tissues provoking several physical effects that can be used as triggers for ultrasound-mediated drug release. In this sense, US inducing thermal and/or mechanical effects have been employed to trigger the release of drugs form different nanocarriers, such as liposomes and micelles.[55]

The development of an US-triggered drug nanocarrier should be designed to respond to those physical effects generated by the US radiation. In this sense, mechanophores, chemical bonds that can be cleaved under US radiation,[56] can be employed in the design of responsive NPs. This is the case of 2-tetrahydropyranyl methacrylate (THPMA), a hydrophobic monomer with an acetal group sensitive to US, than can be easily be converted into hydrophilic methacrylic acid (MAA).[56, 57] This hydrophobic to hydrophilic phase transformation under US irradiation has been employed in the development of responsive MSNs using those moieties as gatekeepers of the pores.[58] Additionally, those monomers were combined with thermoresponsive monomers, such as (2-(2-methoxyethoxy)ethyl methacrylate, MEO_2MA, to yield dual-responsive copolymers, that is, copolymers sensitive to US and heat. Grafting that copolymer on the surface of mesoporous silica nanoparticles allows:

(1) loading the drugs into the already prepared NPs at low temperature (4°C), since the thermosensitive polymer presents an open conformation under these conditions. This is one of the great advantages of this system, because conventional MSNs load their cargo simultaneously to the functionalization process, which leads to low efficiency on loading and functionalization.

(2) closing the pore entrances when temperature was raised to 37°C, since the thermosensitive polymer collapses at that temperature. This permits to the nanoparticles to carry the drugs avoiding any premature release, which is of tremendous importance when transporting cytotoxic drugs towards tumours,

(3) releasing the drugs when irradiated with ultrasounds, since the US-sensitive polymer changes from hydrophobic to hydrophilic. This effect leads to a coil-like conformation of the copolymer, and, therefore, opens the gates of the pores allowing the release of their cargo.

This ultrasound-responsiveness technology have been evaluated with different model molecules, fluorescein and the fluorescent ruthenium complex $[Ru(bipy)_3]^{2+}$, observing that nanocarriers respond efficiently to the stimulus independently of the nature of cargo transported.[58] Additionally, these nanocarriers were observed to be non-cytotoxic and after internalisation into LNCaP cells, they retained their ultrasound responsive behaviour in the cytoplasm of those cells. When loading those MSNs with doxorrubicin and incubating with LNCaP cells, they induced cell death only once they were exposed to US, which means that the employed cytotoxic was only released after being triggered with the external stimulus, avoiding any type of premature release (no cell death) before US stimulation.[58]

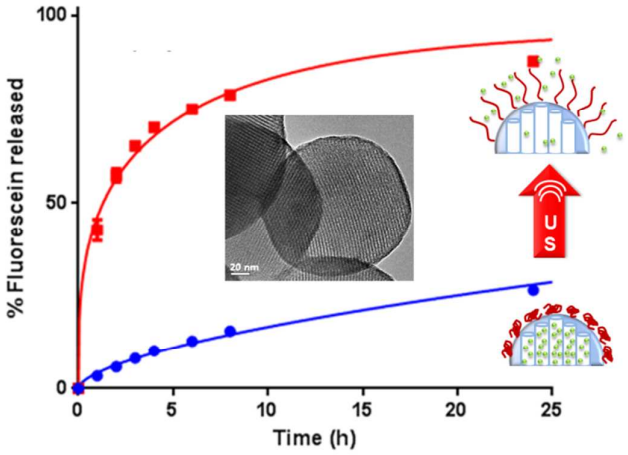

Fig. 3. Release profiles of fluorescein from MSNs functionalised with an US sensitive copolymer in PBS solution over time after 10 min of US irradiation (top) and without US (bottom).

3. *In situ* cytotoxic drug generation.

As it has been detailed above, the transportation of highly cytotoxic drugs, which is the common case of the nanocarriers with oncological application, demands the design of zero-release devices, *i.e.*, systems able to retain the cytotoxic species until reaching the target tissue or cell. This is the case

of the stimuli-responsive nanodevices mentioned in the previous section. The construction of these smart devices introduces in many cases high complexity that hampers their clinical application because the approval by regulatory agencies, which requires the extensive evaluation of all their components.[59] Other alternative to address this issue consists in the development of systems able to transport the cytotoxic species in an inactive state and, once accumulated within the tumour area, activate them generating the toxic compounds only in the affected tissue. This strategy is exploited by the enzyme-prodrug approach which is usually based on the administration of harmless prodrugs which are activated by certain enzymes overexpressed by the tumour tissue.[60] Unfortunately, this strategy is limited in many cases by the low concentration of activating enzyme in the target tissue. Additionally, many prodrugs lack for a natural activating enzyme being necessary to co-administer them or to introduce the gene which encode them in the target cell.[61] In our research group we have developed a nanocarrier able to generate highly toxic species within tumour cells transporting a non-toxic prodrug retained within the silica pore network and the enzyme required for its activation covalently attached on the particle surface.[62] This system is composed by MSN functionalized with amino groups on the pore walls in order to retain by electrostatic interactions the prodrug, in this case indol-3-acetic acid (IAA). Horseradish peroxidase (HRP) transform IAA into indole-3-carbinol generating cytotoxic compounds in the process, mainly hydroxyl and reactive oxygen species (ROS), which are capable to destroy tumour cells by membrane and DNA damage.[63] Enzymes are really labile materials which present low stability in living tissues due to the presence of proteases, among other aggressive agents. In our device, HRP were previously coated with a polymeric capsule designed to facilitate its grafting on MSNs surface whereas preserving its activity during longer times (Fig. 4). IAA is slowly released and activated by the HRP once the carrier is internalized within the tumour cells producing enough cytotoxic compounds to provoke the cell destruction.

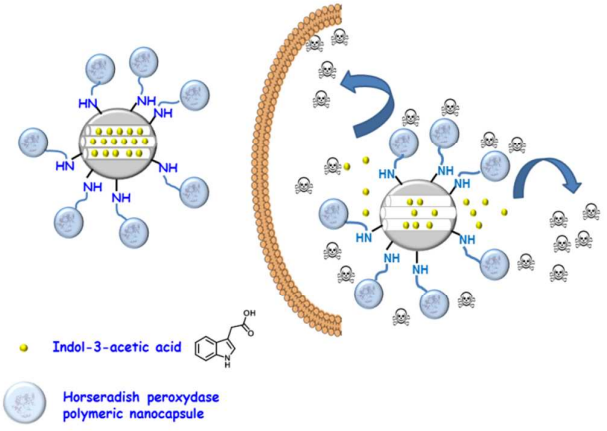

Fig. 4. *In situ* cytotoxic compound generation by prodrug-loaded MSN particles decorated with HRP nanocapsules.

3. Selective targeting against tumour cells

3.1. Active targeting

Nanoparticles injected into the blood stream tend to be accumulated in tumour zones thanks to the particular blood vessel architecture of these diseased tissues.[64] These vessels present high permeability as a consequence of the presence of large pores and fenestrations.[8] Additionally, the fast growing rate of the tumour cells usually compress the lymphatic vessels compromising the drainage of the liquid, waste products and death cells in the tumour place. This fact enhances the accumulation of the nanoparticles in the zone which may remain there up to a few weeks. The combination of high permeability and enhanced retention is the basic of EPR effect being one of the most important asset of the use of nanoparticles in oncology, as was mentioned in the introduction section.[65] However, tumour mass is not a homogeneous tissue composed only by tumour cells but it is a very complex tissue composed by many different cells.[66] Thus, if an efficient destruction of the malignant cells is required, it is necessary to provide the nanocarrier the capacity to distinguish between the diseased cells and the myriad of different cells which form the neoplastic tissue. One of the most employed strategies to achieve this goal is the active targeting which consists in the decoration of the external surface of the nanoparticles with molecules or macromolecules able to interact with specific membrane cell receptors overexpressed by the tumour cells. This selective affinity between the nanocarrier and the tumour cell enhances the particle uptake.[67] In our research group we have focused our attention initially in one particular type of cancer, neuroblastoma (NB). NB is the most frequent extracraneal cancer in pediatric oncology and it presents a poor prognosis despite the combination of several treatments as chemotherapy, radiotherapy, surgery and bone narrow transplant.[68] More than 90% of NB cells overexpress a membrane protein in charge of the reuptake of norepinephrine, the norepinephrine transporter (NET).[69] Certain synthetic molecules as *meta*-iodobencylguanidine (MIBG) interact with this transporter in a similar way that the natural epinephrine.[70] We have found that the attachment of a specific MIBG derivative, *meta*-aminobencylguanidine (MABG) through a cross-linker based on polyethylene glycol of 2000 Da of molecular weight (PEG_{2000}) enhances the nanoparticle uptake by the NB cells in more than four times improving the cytotoxic capacity of the nanocarrier against this disease.[71] However, only the *in vitro* evaluation of the efficacy of a targeting agent is not enough to prove its suitability within complex living system. A targeting agent may be completely masked once the nanocarrier enters in contact with the proteins present in the blood stream, which commonly form a protein corona on the particle surface.[72] Moreover, the fact that a certain targeting agents works well in *in vitro* conditions, even with particles suspended in multiprotein serum which mimic the blood composition, do not guarantee its suitability in a real situation, where the nanocarriers might be

accumulated in other organs such as liver, spleen, lungs, etc.[73] In order to conveniently evaluate our targeting device, we have employed a xenograft murine model in which the NB cells express luciferase which permits an easy visualization of the tumour mass by luminescence measurements. MSNs were labeled with a near infrared fluorophore (Cy7) for allowing the tissue localization of the NPs within the living organism. MSN functionalized with the targeting agent (group IV) showed a much higher accumulation within the tumour mass at 48 and 72 hours after the administration in comparison with the particles functionalized simply with PEG_{2000} and the naked particles (Fig. 5). In contrast, the PEGylated particles (group III) were mainly accumulated in the liver, probably due to the longer circulation time of these particles in contrast with the naked ones (group II) which were completely removed from the host. Moreover, the NPs functionalized with the MABG analogue remain in the tumour after 72 hours whereas the other ones are rapidly cleared from the zone.

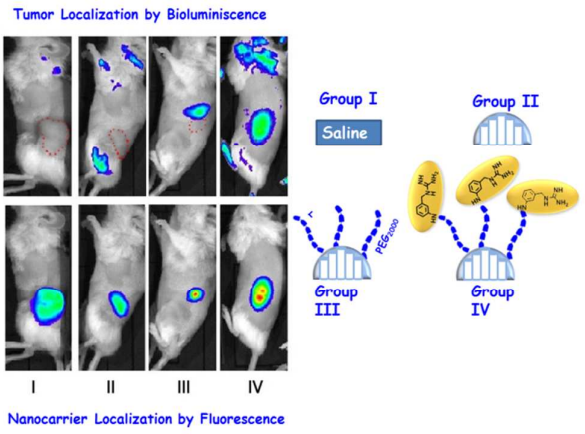

Figure 5. In vivo evaluation of the targeting efficacy of MSN decorated with the MABG derivative.

3.2. Highly penetrative nanocarriers in 3D tumour models

One of the most important problems of the use of nanocarriers to treat tumours is the poor penetration of the nanodevices within the tumour mass. As it was mentioned above, once the NPs reach the highly permeable tumour blood vessels, they pass through the pores presents in the vessel walls diffusing along the tissue. However, the diffusion rate of an object is ruled by the Stokes-Einstein equation which implies that the diffusion (D) is inversely proportional to the hydrodynamic radius (r) and the viscosity of the medium (η).[74]

$$D = kT/6\pi\eta r$$

NPs are big objects and therefore, their diffusion within the tumour is highly hampered. Moreover, the extracellular matrix of the tumour mass usually presents a high content of collagen, which makes it denser than the matrices present in healthy tissues. The higher density of the tumour tissues compromises even more the penetration of the nanocarrier. The administration in the tumour zone of proteolytic enzymes, as collagenase, able to digest the extracellular matrix prior to the NPs treatment enhances the penetration of the nanocarriers within the diseased zone and therefore, their therapeutic efficacy.[75] These enzymes have also been directly attached by covalent bonds on the nanocarrier surface.[76] However, as mentioned above, enzymes are very sensitive molecules which may suffer denaturalization or degradation in a high range of conditions. Our research group has gone one step forward developing pH-sensitive collagen nanocapsules which were attached on MSN surface in order to improve the particle penetration in 3D tissues.[77] In our device, collagen was coated by radical polymerization employing three different monomers: acrylamide (Am) as structural monomer, 2-aminoethylmethacrylate (Am) which provides amino groups on the capsule surface necessary for the capsule attachment on MSN surface and ethylene glycol dimethacrylate (EG) as pH-cleavable cross-linker which is the responsible for the rupture of the polymeric capsule and the subsequent collagenase release. Thus, MSNs transport these collagen nanocapsules attached on their surface (MSN-Col-nc) and, when the pH drops to mild acidic conditions that is typical for many different solid tumours as a consequence of the elevated glycolysis of the malignant cells,[78] the capsules are broken releasing the collagenase which degrade the collagen matrix and enhances the penetration of the nanocarrier. The protective role of the polymer nanocapsules was tested exposing the system to a high concentration of proteolytic enzymes showing that the enzymatic activity of the transported collagenase remains intact. This higher penetration capacity of this novel nanodevice was tested using 3D-collagen gel with Human Osteosarcoma cells embedded in order to provide a more realistic model which mimics a solid tumour. The results showed that MSN-Col-nc, labeled with fluorescein in order to achieve an easy visualization of the particles by confocal fluorescence microscopy, were capable to penetrate deeper within the tissue reaching zones which are not accessible for the naked particles (Fig. 6). Additionally, the complete system is completely biocompatible which pave the way for its future application in the clinical field.

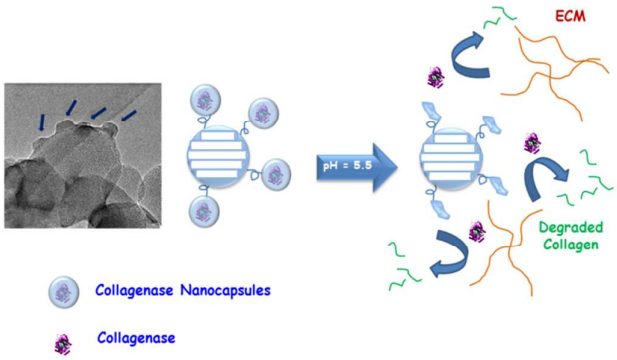

Fig. 6. Action mechanism of MSN-Col-nc. Blue arrows in the TEM micrograph correspond to collagenase nanocapsules attached on MSN surface.

3.3. Cellular vehicles of nanoparticles

As it was mentioned in the previous section, to achieve a deeper penetration of the nanocarriers within the tumour mass constitutes a great challenge. In this sense, human mesenchymal stem cells (MSCs) with migratory properties towards tumours have been successfully used as carriers of NPs.[79, 80] These cells are accumulated in the tumour tissues and once there, they can navigate within the diseased tissue reaching deeper areas. A particular type of these MSCs derived from the decidua of human placenta, DMSCs, are a very homogeneous population, very easy to obtain, and, more importantly, besides their migration properties towards tumours, they have been observed to inhibit the growth of primary tumours and the development of new tumours.[81]
Those DMSCs have been employed as carriers of MSNs towards tumour cells for cancer therapies.[82] Once potential toxicity of the MSNs over DMSCs was discarded, the effect of MSN surface chemistry over cellular uptake was evaluated, finding that positively charged MSNs were better internalised. The particles were located inside lysosomes just after internalisation, but after 3 days they escaped the lysosomes into the cytoplasm. The retention time of the MSNs into the cells was found to be 5 days or longer, time enough for the cells to migrate to tumours. The capacity of cells migration was evaluated both *in vitro* and *in vivo*, observing that DMSCs retained their homing capacity towards tumours when carrying MSNs. Additionally, DMSCs with MSNs loaded with doxorubicin were able to induce cell death in NMU cancer cells when co-cultured *in vitro*, which showed that this DMSCs represent a promising platform for cancer therapy as carriers of NPs loaded with anticancer drugs.

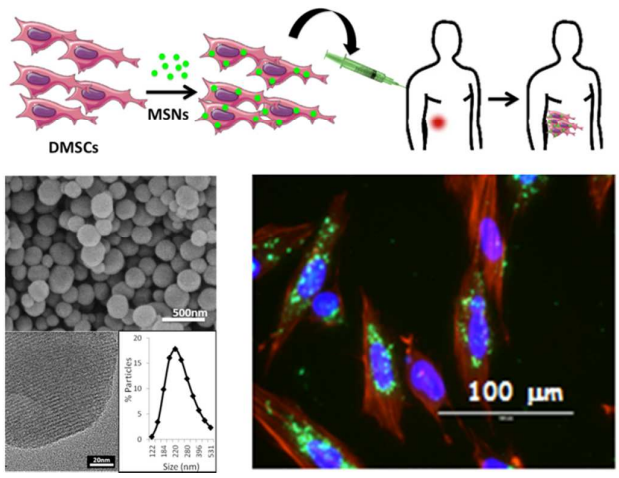

Fig. 7. Schematic representation of DMSCs as carriers of MSNs towards tumour tissue (top); SEM, TEM and DLS of MSNs (bottom left hand corner); and DMSCs with fluorescein-labelled MSNs internalized into their cytoplasm (bottom right hand corner).

4. Futures Prospects

In the last years, MSNs have become a powerful material in the war against cancer thanks to their interesting properties as high loading capacity, easy functionalization, robustness and low toxicity, among others. Many scientist along the world has extensively studied the use of this system generating smart stimuli-responsive materials and designing novel targeting strategies in order to improve its capacity to destroy tumour cells in a more controllable and accurate way. There are still different issues that are necessary to address in order to reach the real clinical applications, *i.e.*, it is necessary to study the long-term toxicity of these devices or in the case of the stimuli-responsive MSN, we should test their efficacy in more realistic conditions employing *in vivo* models or at least using *in vitro* models which mimics more properly the complexity of a living organism such as tumour spheroids, *ex ovo* chick embryo models or 3D cell co-cultures. In any case, the knowledge acquired during these years and the excellent results harvested until now are hopeful enough to think that in the coming years these smart nanocarriers will provide more efficient weapons to fight against this terrible disease.

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