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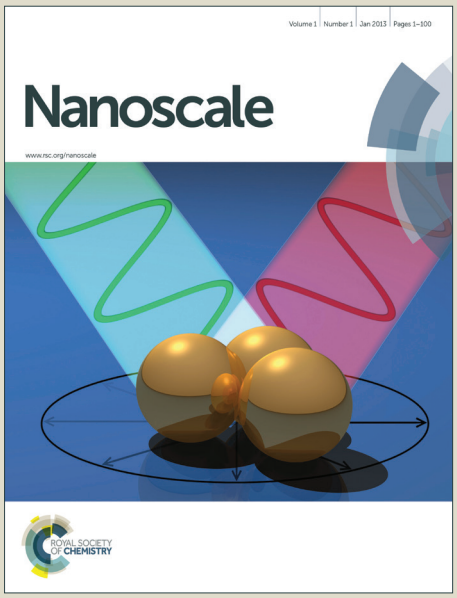

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Nanoparticle-cored dendrimers: functional hybrid nanocomposites as new platform for drug delivery systems

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Nanoparticle-cored dendrimers (NCDs) are now offering themselves as versatile carriers because of their colloidal stability, tunable membrane properties and ability to encapsulate or integrate a broad range of drugs and molecules. This kind of hybrid nanocomposite aims to combine the advantage of stimuli-responsive dendritic coating, in order to regulate the drug release behaviour under different conditions and improve the biocompatibility and *in vivo* half-time circulation of the inorganic nanoparticles. Size, surface chemistry and shape are key nanocarrier properties to evaluate. Here, we have reviewed the most recent advances of NCDs in drug delivery systems, compared their behaviour with non-dendritic stabilized nanoparticles and highlighted their challenges and promising applications in the future.

Introduction

Nanoparticles (NPs) are an intriguing class of highly tunable nanoscale objects, and are receiving particular attention due to their wide range of applications.[1-3] NPs usually exhibit size-related characteristics that differ significantly from those observed in bulk materials. Thus, their importance is attributed to the fact that they represent a critical link between current technologies and future applications due to their small size, large surface-to-volume ratio and size dependent properties. Metal and semiconductor NPs, as well as hybrid structures using polymeric material, have been synthesized and are part of the manufacturing of our daily life products for more than a decade (scratchproof eyeglasses, crack- resistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain-repellent fabrics, self-cleaning windows and ceramic coatings for solar cells).[4] Today, nanocomposites[5-7] with high-performance applications in numerous fields, such as electrochromic devices[8] and energy storage,[9, 10] make these hybrid materials of prime importance.

Gold nanoparticles (AuNPs) have been among the most extensively studied nanomaterials over a century because of their remarkable optical properties related to their plasmon absorption and are now heavily utilized in chemistry, biology, engineering, and medicine because of their unique optical, chemical, electrical, and catalytic properties.[11, 12] AuNPs have attracted the interest of scientists especially in the areas of photothermal therapy,[13] biosensing,[14] imaging,[15] and drug delivery.[2] Magnetic nanoparticles (MNPs) have generated considerable interest in the scientific world especially in the area of biomedicine and technology, particularly in magnetic storage media,[16] biosensing,[17, 18] inks and paints,[19] drug delivery and contrast agents in magnetic resonance imaging.[20] NPs are generally synthesized in either aqueous or organic solutions and thus require sophisticated coating for stability.[21] The aggregation processes occur frequently in the colloidal systems of metal or semiconductor NPs, and severely restrict their utilization in different applications. Thus, in order to prevent or slow the aggregation, NPs are usually functionalized with a thin shell of monomeric stabilizers, (thiols, carboxylates, phosphates or sulfates), synthetic and natural polymers (dextran, polythylene glycol (PEG), polyvinylpyrrolidone, polyethylene oxide or chitosan), inorganic material (silica), liposomes and another class of emerging molecules such as dendrimers and dendrons. Organic coatings for biomedical applications must contain several bioactive functions that ensure biocompatibility, targeting and possible therapeutic care, and also prevent the nanoparticles from agglomeration in a physiological environment thus favouring ideal biodistribution and bioelimination.[22]

Dendrimers are well-defined, highly branched macromolecules that can mimic certain properties of micelles and liposomes or even highly organized building blocks of biological systems. These properties make dendrimers suitable for many important application areas, particularly as drug carriers.[23] Crooks *et al* reported for the first time the synthesis and characterization of dendrimer-encapsulated nanoparticles.[24] These highly organized structures are able to encapsulate different metal ions and serve as a template for the formation of a wide range of metallic nanostructures including Cu, Ag, Au, Pt and Pd.[25] The development and research of dendritically

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stabilized NPs have been an exciting field of investigation due to the potential to combine the properties of nanosized core with the permeable network of the dendritic branches. Besides, the use of these discrete building blocks for biomedical purposes is a successful area of research, primarily due to their precisely defined structure and composition as well as their higher tunable surface chemistry.

The use of dendrimers and dendrons as stabilizing agents instead of the commonly non-dendritic ligands provides an interesting advantage, however, to date there are few reports about it. One reason could be the higher costs associated with their synthesis and purification. Schlüter *et al* [26] have shown that dendritic molecules present several advantages: (I) the dendron structure and size can be accurately controlled during synthesis; (II) the classical conical-like dendritic structures are of particular interest for ultra-small NPs with very high curvature and it is expected to improve steric resistance to macromolecular adsorption and particle agglomeration; (III) end-group-functionalized dendritic structures have the potential for exceptional and quantitatively controlled arrangement of (bio)ligands at high surface densities; (IV) the presence of hyperbranched arms increases affinity or avidity in multivalent (bio)specific interactions relevant to biosensing applications; (V) small molecules, such as drugs, could be incorporated into the dendritic framework of dendron-stabilized NPs making this system attractive as a potential drug release system and (VI) stabilization of small NPs with dendrons results in dendronized NPs with a relatively thin organic shell and, therefore, a small size in the range of 10-30 nm. This latter property is really important because it gives them interesting and promising material for biomedical applications such as drug delivery systems, efficient cell uptake of the NPs, improved tissue diffusion, and particularly for targeting tumors via the enhanced permeability and retention effect, exploiting the nanoporous nature of blood vessels in cancerous tissue.[22] Pan *et al* [27] have also demonstrated the effectiveness of the dendrimers for systematic control of nanoparticles spacing and developed a "bricks and mortar" strategy, in which the colloidal NPs serve as the bricks, while dendrimers serve as the mortar. These findings confirmed that the assembly process provides control over the resulting aggregates, allowing a versatile route to new material systems.

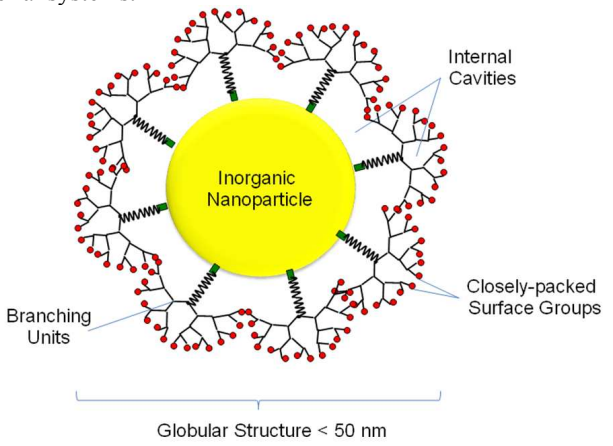

Figure 1: Scheme of a Nanoparticle-Cored Dendrimer

The exclusive use of dendrons as stabilizer gives rise to a new class of material: Nanoparticle-cored dendrimers (NCDs). NCDs are core-shell materials that possess nanometer-sized inorganic clusters at the core surrounded by a shell of dendrons of different generations which are attached radially to the core allowing their stabilization and therefore, impeding aggregation (see Figure 1). In this case, the NPs are structural units of the dendrimer and are not trapped or encapsulated in dendritic pockets.[28] The high stability of NCDs protected by dendrons with a thiol group was a clear improvement over that of dendrimer encapsulated NPs.[29]

Synthesis, characterization and relevant aspects of NCDs

One of three different synthetic methodologies has been generally applied to the generation of NCDs (see Figure 2).[30] The direct method employs the reduction of a metal cation in the presence of dendrons bearing suitable moieties at their focal point. The functionalities used for this purpose are thiols and disulfides groups for the synthesis of NCD with metal-sulfur bonds. In recent years a new approach of the direct method, which implies the simultaneous reduction of the metal cation in the presence of dendrons with a diazonium group at the focal point, has been developed. This methodology leads to the formation of NCDs with metal-carbon bonds.[31] In contrast, the ligand-place exchange method is an indirect method comprising two-step reactions: the synthesis of monolayer-stabilized NPs followed by the replacement of thiolate dendrons. This method has the advantage of keeping the core size unchanged during the ligand substitution reaction, but the main limitation is, in many cases, the difficult access to the thiol-containing dendrons. Lastly, the convergent approach is also used, in which single or multistep reactions are employed to build dendritic architectures onto monolayer-protected NPs. The size and material properties of the NCDs were thoroughly investigated using a variety of experimental techniques, such as spectroscopy, microscopy, electrochemistry, photochemistry, etc. The most synthesized NCDs have Au in the core and aliphatic or aromatic thiol dendrons in the shell. However, dendritic ligands have also been used in the preparation of NCD based on magnetic material in the core, particularly magnetite, Pd or Ag among other metals, or even semiconductors such as CdSe. Concerning the nature of the branching ligands, poly(amidoamine), polyarylether and polypropyleneimine dendritic wedges are probably the most widely used for building NCDs. Some of the novel synthetized NCDs are listed in Table 1.

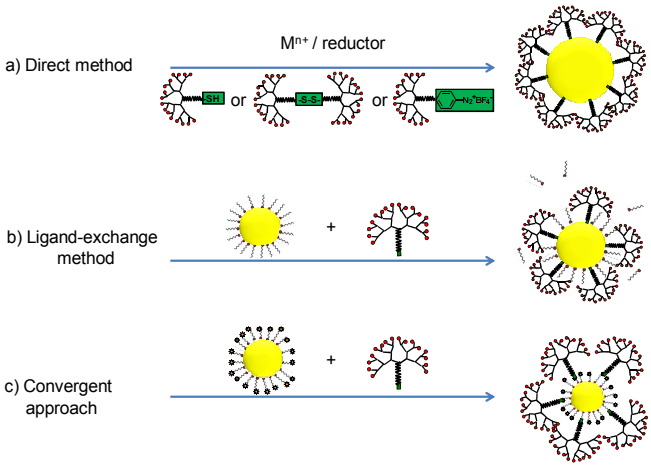

Figure 2: Schematic illustration of the three general synthetic strategies

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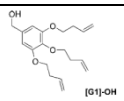
The simple synthetic tuning of the dendritic architecture, that is the chemical functional group and the extent of the dendritic branching, has an important control over the final NCDs properties. By using aromatic ether dendrons with either thiol or 4-pyridone units at the focal point, Kim [32] and Zheng [33] have clearly demonstrated that the dendritic generation could control the dimensions of the NCDs.[34] Zhong *et al* [35] have reported that unique structural properties of the dendritic arenethiol capping molecules not only enable the ability to control size growth of AuNPs but also ready surface exchange reaction for surface derivatization. Newkome-type ligands as capping molecules used for AuNPs synthesis also determine the solubility and stability of the NCDs, as well as the characteristics of the core through a dendritic control of the size.[36] Fox *et al* [28] using Frechet-type dendrons to stabilize the surfaces of gold NPs have shown that as dendritic generation increased, the density of dendrons on the NPs decreased as a consequence of the steric hindrance of the branches. Therefore, for the higher NCD generation, a large fraction of the gold surface was not passivated and thus available for the catalytic reaction.[28] Kumar *et al* also reported the synthesis of Pd NCDs using diazo-funcionalized Frechet-type dendrons. These core-shell materials were successfully used for catalytic applications.[37] Smith *et al* [34] have reported that using a branched ligand leads to smaller and better defined particles rather than using an analogous non-dendritic one. In addition, building blocks which have 'long' and 'short' branches might be expected to provide a less well-packed protective monolayer on the surface of the metal core hence exposing vertices and defect sites to the etchant solution.[34] Astruc *et al* have developed a sensor device by using dendrons with ferrocenyl-ended groups onto AuNPs, indicating how functionality can be introduced into NCDs giving specific properties and applications.[38] Moreover, a dendronization strategy can also be employed to control the interparticle spacing and the optical properties of the NCDs aggregates.[39]

Table 1 Some examples of novel synthetized nanoparticle-cored dendrimers

Core	Dendritic arm	Remarkable aspects	Reference
Au	G2-poly(benzyl ether) dendrons	Non-spherical shape and faceted size: 3-25 nm (average size = 12 nm)	40, 41
	G1,G2 and G3 	Superior control of the critical molecular design using dendritic functionalization	42
	Disulfide-focal pi-conjugated dendrons	Electrical bistability due to a charge transfer interaction between Au and dendrons	43
	G1 Frechet-type dendrons	Size-controlled NPs depend on the mole ratio (*r*) of G1: Au. Average size (nm): 0.9 (*r*= 3), 2.8 (*r* = 1) and 5.1 (*r* = 0.33)	44
	G1, G2, G3 and G4 diazo-funcionalized Frechet-type dendrons	Average size (nm): 4.7-5.5 NCDs with metal-carbon bond. Highly stable	31
	G1, G2 and G3 Dihydroxy fatty acid –base dendrons	Long-lived nano-hybrids for biological application	45
	G1 and G2 cystamine-focal Newkome-type dendrons	Average size (nm): G1= 15.2, G2= 17.4 Relative surface coverage: G1 =0.38, G2=0.19	46
	Thiol-focal thiophene dendrons	NPs size = 3 - 5 nm Size distribution, and energy-transfer efficiency depend sensitively on both alkyl-chain length and dendron size	47
	G4 Cystamine core polyamidoamine dendron	Support for the covalent attach of tyrosinase, useful for cathecol biosensing	48
	G1 hybrid dendrons containing carborane and polyethylene glycol	Average size= 3.4 - 7.6 nm Water soluble and biofunctional NCDs could provide a biocompatible platform in therapeutical investigation	49
	Thiol focal, carbosilane dendrons	Average size = 2-3 nm NCDs are valuable in catalytic processes due to the difficult elimination of the dendron shells	50
	Newkome-type dendrons and L-lysine derivatized dendrons	Effect of dendritic structure on NCDs chemical stability	34, 51
γ-Fe_2O_3	G1 Fluorescein-modified dendrons D1 hydrophobic: alkyl chain and aromatic rings; D2 hydrophilic: polyether chain and polyamidoamine chains	The fluorescence measurements of these nanoparticles confirm the ability of dendronized molecules to increase the surface functionalization, in contrast to the linear analogues.	52
Fe_3O_4	G2 polyphenylenpyridyl dendrons	Magnetically recoverable catalysts	53
	G1 phosphonic acid -focal, hydrophilic oligo ethyleneglycol-derivatized dendrons D1: three biocompatible tetraethyleneglycol chains; D2: two tetraethyleneglycol chains and one carboxylic acid-ended octaethyleneglycol chain	NCDs did not induce any cytotoxicity. *In vivo* and *in vitro* Magnetic Resonance Imaging showed that the contrast enhancement properties of the NCDs were higher than those obtained with commercial polymer-coated NPs.	22, 54
CdSe	Poly(benzyl ether) dendrons	Biocompatible platform in therapeutical applications	55, 56
	Oligothiophene dendrons	NCDs are very soluble and stable in non-polar solvents. They exhibit energy transfer, surface plasmon resonance effects, and	57, 58

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		photoinduced charge transfer interactions (good photovoltaic cell)	
CdSe/CdS	G1 and G2 Tomalia type, thiol-focal poly(amidoamine) dendrons and phosphine -focal poly(alkyl ether) dendrons	G2 dendrons provide adequate surface dense packing to protect the NCDs against aggregation for up to 6 months. Useful as biological labels due to residual fluorescence intensity	59, 60
Pd	G1, G2 and G3 amino-focalized poly(benzyl ether) dendrons	Average size (nm): G1= 4.7, G2 = 3.2, G3=2.8 The steric hindrance affects the sizes in the microreactor, contrary to the batch tendency	61
	G1, G2, G3 and G4 diazo-funcionalized Frechet-type dendrons	NCDs with metal-carbon bond. Pd-G_1 can catalyze the Suzuki, Stille and Hiyama coupling reactions.	37
Ag	G3- anthracenyl-focal poly(amido amine) dendrons	Spherical (15-20 nm) polygonal (20-35 nm)	62

Performance of NCDs in bionanomedicine and as nanocarriers in controlled release

Nanotechnology provides a gateway of possibilities for scientists to explore their ideas in the biomedical field.[63, 64] Iron oxide nanoparticles,[65, 66] quantum dots,[67] carbon nanotubes,[68] gold nanoparticles,[69] and silica nanoparticles,[70] have previously been thoroughly investigated in the imaging setting and are candidate nanoplatforms for building up nanoparticle-based theranostics. Thus, theranostic nanomedicine is emerging as a promising therapeutic paradigm having both imaging and therapeutic agents that execute simultaneously diagnostic tests, targeted therapy, and monitor the therapeutic response. The resulting nanosystems play a significant role in the emerging era of nanomedicine and plenty of research effort has been devoted towards that goal.

An advantage of constructing such function-integrated moieties is that many nanoplatforms are already, themselves, imaging agents. Their well-developed surface chemistry makes it easy to load them with pharmaceutics and promote them to be theranostic nanosystems.[71, 72] *In vivo* and *in vitro* MRI measurements showed that the contrast enhancement properties of the dendronized NPs were higher than those obtained with commercial polymer-coated NPs (Figure 3).[22]

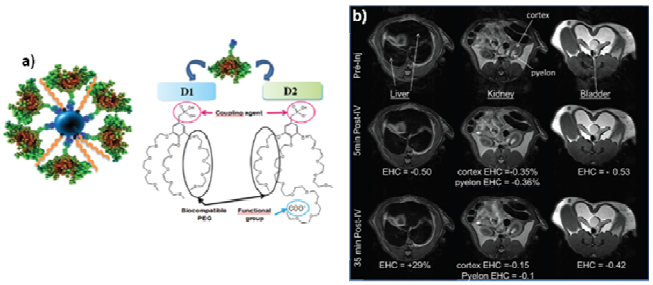

Figure 3: a) Synthetic scheme of NCDs, b) Spin echo T2 weighted axial images sequentially acquired before and after an injection of 650 µl of NCDs at 7T with a small animal dedicated MRI (Biospin – Brücker®). Reproduced from Ref. [22] with permission from The Royal Society of Chemistry.

Mesoporous silica nanoparticles (MSNs) have been extensively studied and proposed as promising candidates for numerous biomedical applications.[73, 74] Recently, Gu *et al* [75] have reported the design, preparation, characterization and biosafety evaluation of peptide dendron functionalized MSNs. These nanohybrids were prepared by surface modification of MSNs via click reaction of azido-MSNs with alkynyl peptide dendrons and *in vitro* cytotoxicity and *in vivo* toxicity of the nanohybrid were evaluated suggesting that the peptide functionalized NPs possessed good biocompatibility due to the non-observed significant side effects to normal organs of healthy mice.[75] Other nanoparticles, such as iron oxide and gold nanoparticles

are also the most useful platforms to build theranostic nanocarriers which combine both therapeutic and diagnostic functions within a single nanostructure. Nevertheless, their surface must be functionalized to be appropriate for *in vivo* applications.[76] Surface functionalization provides binding sites for targeting ligands and for drug uploading.

Several crucial factors need to be considered when developing a nanomedicine based platform:[77] I) identification of a specific molecular target, (II) choice of a suitable coating candidate, (III) design of the nanocomponent delivery system, (IV) characterization of the nanoform, and (V) *in vitro* and *in vivo* biological activity and pharmacological evaluation. Therefore, the surface state of the functionalized NPs largely depends on their synthesis routes, dictates the strategies used for functionalization and makes them suitable or not for future medical applications.

Hybrid multifunctional systems with sizes typically ranging between 1–100 nm which may deliver the bioactive agent at the targeted site with improved therapeutic activity over the free form of a bioactive agent is usually named as "nanocarrier".[77] In particular, NPs have been developed as an important strategy to deliver conventional drugs, proteins, vaccines or nucleic acids like DNA or RNA. Drug delivery nanosystems should provide positive attributes to a 'free' drug by improving solubility, *in vivo* stability, and biodistribution.[78, 79] AuNPs are capable of delivering large biomolecules without restricting themselves as carriers of only small molecular drugs. Tunable size and functionality make them a useful scaffold for efficient recognition and delivery of biomolecules. AuNPs and other colloidal drug-delivery systems modify the kinetics, body distribution and drug release of an associated drug.[80-82] Usually, they are of similar size to typical cellular components and proteins, and thus may bypass natural mechanical barriers possibly leading to adverse tissue reaction.[83] In particular, positively charged AuNPs are of interest in terms of cellular uptake.[84] These properties from their cationic nature induce electrostatic interactions with negatively charged entities such as cell membranes, plasmid DNA, siRNA, or antibodies.[85] While clinically-approved nanoparticles have consistently shown value in reducing drug toxicity, their use has not always been translated into improved clinical outcomes. This has led to the development of "multifunctional" NPs, where additional capabilities like targeting and image contrast enhancement should be added.[86] AuNPs are generally considered nontoxic and biocompatible, but Pan *et al* [87] have reported that AuNPs of diameter 1.4 nm capped with triphenylphosphine monosulfonate are much more cytotoxic than 15 nm nanoparticles of similar chemical composition. These results highlight the importance of an optimal size of the nanocarriers. Stobiecka *et al* [88] have shown that the main advantage of poly L-lysine coatings in AuNPs is no toxicity related to the ligand

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release in gene delivery processes in contrast to the thiol-functionalized AuNPs. Papp *et al* [89] have demonstrated that a sialic-acid-terminated glycerol dendron when chemically functionalising small AuNPs provides highly stable NPs that show high activity towards the inhibition of influenza virus infection. As the binding of the viral fusion protein hemagglutinin to the host cell surface is mediated by sialic acid receptors, a multivalent interaction with sialic-acid-functionalized NPs is expected to competitively inhibit viral infection.[89]

The diverse functional possibilities of NCDs allows for a variety of approaches for nanocarriers design (see Figure 4). Fox *et al* reported the synthesis of water soluble Au NCDs that exhibit micellar properties capable of encapsulating pinacyanol chloride.[90] These properties make NCDs ideal candidates as nanocarriers. Hydrophobic drugs can be loaded onto nanocarriers through non-covalent interactions within the hydrophobic interior requiring no structural modification to the drug for drug release.[91, 92] Likewise, covalent conjugation to the NCDs through cleavable linkages can be used to deliver prodrugs to the cell and the drug can then be released by external or internal stimuli. Significantly, the internal stimuli operate in a biologically controlled manner, whereas the external stimuli provide spatio-temporal control over the release.[45] Regardless of the approach used, the tunability of the NCDs is crucial for internal or external release. Commonly, a covalently conjugated drug to a nanocarrier is better suited for specifically targeted drug delivery, while a drug as a nanocarrier inclusion complex is readily released and active *in vitro*.[93] For example, direct conjugation of functional peptides onto AuNPs provides a number of tunable properties that can modulate their functions inside living cells. The intracellular distributions of the particles changed from the nucleus to the endoplasmic reticulum by increasing the peptide density at a constant nanoparticle diameter or by increasing the particle diameter at a constant peptide density which leads to less cellular uptake.[94, 95]

There is a great interest in the development of drug delivery systems that could allow an efficient and site-specific transport of drugs to the target tissues affected by a disease. A broad spectrum of synthetic polymers with structural and architectural variations, including linear, star-like, dendritic, and hydrogels is currently under investigation. A comparison of dendrimer and linear polymer features shows that dendritic polymer architecture is advantageous for delivery applications.[96] An interesting example is described by Contantino *et al*,[97] who have presented a general method for incorporating target moieties in a well-defined arrangement onto the surface of biocompatible nanoparticles of polyester poly(D,L-lactic-co-glycolic acid) (PLGA) material using Lin´s amine dendrons. In this way, it is possible to obtain NPs with a varying degree of surface coverage. This new strategy has been successfully applied to the preparation of peptide- and b-D-glucose-covered NPs. This application is based on the discovery of NPs with a regular architecture of a dendrimer able to cross the blood–brain barrier after systemic administration. Gold nanoparticle-cored tetraethylene glycol spacer-poly(propyleneimine) dendrimers, synthetised by Daniel *et al*,[98, 99] have shown great stability in phosphate buffer solutions and they were found to protonate at pH around 4.5 for their internal tertiary amines, which play a crucial role in lysosomal escape during drug delivery. Moreover, the primary amine termini provide great convenience for direct derivatization with moieties such as drugs or targeting entities, for the formation of multifunctional drug delivery systems with high payloads.[99]

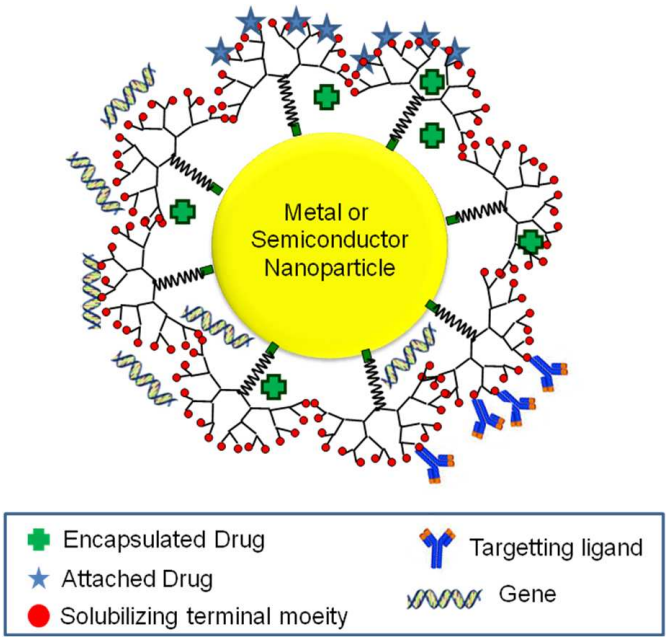

Figure 4: Cartoon depictions of some possibilities for NCDs as nanocarrier

In addition, one of the most popular modifications of the surface of nanocarriers is PEGylation which offers many advantages in addition to cytotoxicity reduction such as improved bioavailability/oral delivery application related to improved biodistribution and pharmacokinetics, enhanced solubility, increase in drug loading, sustained and controlled delivery of drugs, better transfection efficiency, and tumor localization.[100] Bergen *et al* have reported how different physicochemical properties, such as size, shape, PEGylation, or the ligand, regulate non-specific versus target-specific uptake.[101] Hackley *et al* [85] have reported the development of a cationic dendron with spherical carboxylic groups esterified with PEG and then, functionalized with quaternary ammonium end groups. These dendrons were used for the preparation of a positively charged gold nanoparticle-dendron conjugate and they were evaluated by *in vivo* and *in vitro* studies. Overall, the investigation has confirmed the successful development of stable NCDs with excellent stability in biologically relevant test media containing proteins and electrolytes, and with a shelf life exceeding 6 months. The excellent aqueous stability and apparent lack of toxicity for this chemical modification enhance the potential use as a test material for investigating interactions between positively charged NPs and biocellular and biomolecular systems, or as a vehicle for drug delivery. Thus, NCDs are promising candidates for a nanoscale test material for cellular assays or for use as a drug carrier in nanotherapeutics. Rotello *et al* [102] have demonstrated that the lysine dendron-functionalized gold nanoparticle was 28 times more effective and potent vector than condensing DNA. Most importantly, these aminoacid-functionalized NPs showed no cytotoxicity when used as transfection agents. These materials were also responsive to cellular glutathione level during *in vitro* transfection, providing insight into their mode of activity as well as being a potential tool for orthogonal control of

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transfection.[82] Recently, dendronised AuNPs have been used as a vector for siRNA delivery suppressed β-gal expression by ~50% with minimal toxicity.[103] In latter studies, G2-AuNP vectors possess the benefits of polymeric delivery vehicles such as polyethylenimine, while minimizing toxicity through the use of a non-toxic core functionality.

Due to their physical properties, superparamagnetic iron oxide nanoparticles (SPION) are being extensively studied as a part of diagnostic and therapeutic strategies in cancer treatments. They can be used as agents for *in vitro* or *in vivo* imaging, for magnetic drug targeting and/or thermal therapy.[76, 104] The tendency of NPs to aggregate limits their therapeutic use *in vivo*; but surface modifications to produce high positive or negative charges can reduce this tendency.[105] Thus, magnetic nanoparticles as the core in NCDs have recently emerged as a simple solution to build novel nanocarriers suitable for controlled release. One example is the results described by Gunduz *et al*.[106] They have reported the immobilization of 1-methylimidazole, Polyinosinic-Polycytidylic acid (Poly (I:C)) onto different generations of PAMAM dendron-coated magnetic nanoparticles (G2 to G7, Dc-MNP) which can be targeted to the tumor site under magnetic field. They have demonstrated that systems having more functional groups at the surface, like higher generations (G7, G6, and G5) of Dc-MNPs were found more suitable as a delivery system for Poly (I:C) (Figure 5). Further *in vitro* and *in vivo* analyses of Poly (I:C)/PAMAM magnetic nanoparticles may provide new opportunities for the selective targeting and killing of tumor cells.

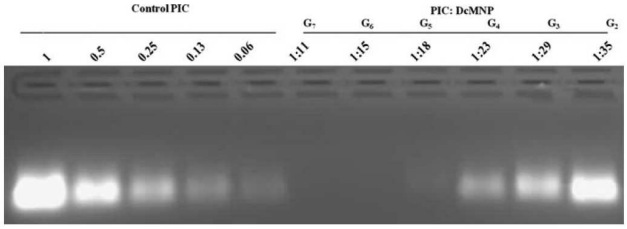

Figure 5: Agarose gel electrophoresis of loading optimization Poly (I:C) on G_7,G_6,G_5,G_4,G_3, and G_2DcMNPs at pH 6. Wells 1–5 demonstrate the control Poly (I:C) and different dilutions. Wells 6–11 show the PIC:DcMNP ratio of on G_7,G_6,G_5,G_4,G_3, and G_2DcMNPs. 1 in the ratio represents 4 μg of Poly I:C or DcMNP, reprinted with kind permission from ref. [106] Copyright 2013, Springer Science and Business Media.

Schlüter and coworkers[26] have reported the stabilization of magnetite NPs by dendritic and linear ethylene-glycol-based stabilizers with varying generation and chain length, respectively. Dendron-stabilized NPs were found to provide excellent colloidal stability, despite having a smaller hydrodynamic radius and lower degree of soft shell hydration when compared to linear PEG analogues. Moreover, for the same grafting density and molecular weight of the stabilizers, PEG dendron-stabilized NPs show a reversible temperature-induced aggregation behaviour, in contrast to the essentially irreversible aggregation and sedimentation observed for the linear PEG analogues. This class of dendritically stabilized NPs is believed to have a potential for future biomedical use and other applications, in which stability, resistance to (or reversible) aggregation, ultra small size (for crossing biological barriers or inclusion in responsive artificial membranes), and/or high corona density of (bio)active ligands are key for many applications, which range from particle-reinforced materials to targeted *in vitro* and *in vivo* diagnostics. Magnetite NPs modified with the thiol functionalized PAMAM-dendron were also successfully used to DNA recovery by magnetic separation.[107] Martin *et al* [108] have aimed at modulating the uptake of SPIONs by cells for *in vitro* labeling using polyester dendrons having multiple peripheral guanidine groups. This dendritic guanidine was found to have good penetrating capability upon conjugation with SPIONs and also enhanced the cellular uptake (Figure 6). Zhu *et al* [109] have also employed dendronized SPION to load doxorubicin. The anticancer drug was conjugated to the peripheral functional groups of a poly (L-glutamic acid) dendron with pH sensitive hydrazine linkers to construct magnetic drug nanocarriers.

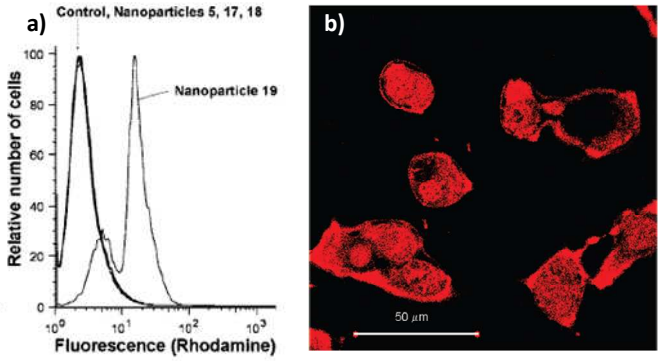

Figure 6: Cells after 2 h incubation with NCDs (nanoparticle 19). a) Flow cytometry analyses, b) Confocal laser scanning microscopy image. Adapted with permission from Ref. [108] Copyright 2008. American Chemical Society.

In summary, NCDs have emerged as promising drug delivery carriers. To accomplish this purpose, NCDs should exhibit high water-solubility and drug-loading capacity, low toxicity, favorable retention and biodistribution characteristics, specificity, and proper bioavailability. One of the key advantages that arise from the dendritic architecture is access to the spatial-control over the bioactive-ligand presentation so that the targeting capability of the carrier could be optimized. The well-defined interior void space between the core and the dendritic arms can be used to encapsulate small molecular drugs, meanwhile the terminal surface groups of NCDs are ideal for bioconjugation of drugs or targeting moieties. The use of NCDs can also increase the stability of the payload. Careful consideration of the surface modification approach is needed to avoid any agglomeration after dendronization and yet still retain a capability to deliver the payload. Based on our understanding of what is known about NCDs characteristics, the following strategies may be useful in future studies for their use as nanocarriers: (a) focusing on diameters ranging between 10-30 nm; this size appear to be more likely to maintain an equilibrium between the blood circulation and the extracellular space, thereby increasing circulation time and facilitating organ clearance; (b) identifying organ specific endothelial cell surface markers and target cell specific markers and design specific affinity peptides or antibodies for these targets and (c) designing dendron coatings to functionalize NPs with hydrophilic shells with cationic character keeping hydrodynamic diameter below the critical size for effective clearance. These attributes can be exploited to provide an

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effective and selective platform to obtain a targeted intracellular release of some substances.

Conclusions

Here, we have reviewed the most recent advances in the use of NCDs in drug delivery systems and highlighted the great importance of the dendritic stabilized nanoparticles. These hybrid (organic/inorganic) nanoparticles have emerged as a simple solution to build "theranostic" systems that are currently under intensive investigation for the buildup of novel carriers.

We expect this platform to offer a superior performance, especially for targeting applications where multivalent interactions are the key for specific medical therapies that involve the crossing of tissue barriers; a crucial factor in the exploitation of the EPR (enhanced permeation effect). The higher cost of the synthesis of dendrons is clearly justified by this reason. Topics such as surface modification, branched ligands, and drugs delivery of this type of nanocarriers have been discussed. A precise control of the amount and distribution of functional groups on the internal and external surfaces of NCDs would help increase the loading of hydrophobic drugs and control the release timing of the loaded drugs. Undoubtedly, progress and development on the design and application of NCDs in biomedicine, will shorten the gap to obtain successful results in biomedicine in the near future.

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References

1. A. Lapresta-Fernandez, A. Salinas-Castillo, S. Anderson De La Llana, J. M. Costa-Fernandez, S. Dominguez-Meister, R. Cecchini, L. F. Capitan-Vallvey, M. C. Moreno-Bondi, M. P. Marco, J. C. Sanchez-Lopez and I. S. Anderson, *Critical Reviews in Solid State and Materials Sciences*, 2014, 39, 423-458.
2. I. Fratoddi, I. Venditti, C. Cametti and M. V. Russo, *Journal of Materials Chemistry B*, 2014, 2, 4204-4220.
3. F. Viñes, J. R. B. Gomes and F. Illas, *Chemical Society Reviews*, 2014, 43, 4922-4939.
4. P. H. M. Hoet, I. Bruske-Hohlfeld and O. V. Salata, *Journal of Nanobiotechnology*, 2004, 2:12.
5. V. K. Thakur, D. Vennerberg and M. R. Kessler, *ACS Applied Materials and Interfaces*, 2014, 6, 9349-9356.
6. V. K. Thakur, M. K. Thakur, P. Raghavan and M. R. Kessler, *ACS Sustainable Chemistry and Engineering*, 2014, 2, 1072-1092.
7. V. K. Thakur, E. J. Tan, M. F. Lin and P. S. Lee, *Polymer Chemistry*, 2011, 2, 2000-2009.
8. V. K. Thakur, G. Ding, J. Ma, P. S. Lee and X. Lu, *Advanced Materials*, 2012, 24, 4071-4096.
9. V. K. Thakur, M. F. Lin, E. J. Tan and P. S. Lee, *Journal of Materials Chemistry*, 2012, 22, 5951-5959.
10. V. K. Thakur, E. J. Tan, M. F. Lin and P. S. Lee, *Journal of Materials Chemistry*, 2011, 21, 3751-3759.
11. O. Pluchery, H. Remita, P.-F. Brevet and S. Roux, *Gold Bulletin*, 2013, 46, 211-212.
12. A. Majdalawieh, M. C. Kanan, O. El-Kadri and S. M. Kanan, *Journal of Nanoscience and Nanotechnology*, 2014, 14, 4757-4780.
13. S. Hwang, J. Nam, J. Song, S. Jung, J. Hur, K. Im, N. Park and S. Kim, *New Journal of Chemistry*, 2014, 38, 918-922.
14. Y. Lin, J. Ren and X. Qu, *Advanced Materials*, 2014, 26, 4200-4217.
15. J. Lee, D. K. Chatterjee, M. H. Lee and S. Krishnan, *Cancer Letters*, 2014, 347, 46-53.
16. F. Bensebaa, in *Interface Science and Technology*, 2013, vol. 19, pp. 429-479.
17. M. Martin, P. Salazar, R. Villalonga, S. Campuzano, J. M. Pingarron and J. L. Gonzalez-Mora, *Journal of Materials Chemistry B*, 2014, 2, 739-746.
18. S. Sagadevan and M. Periasamy, *Reviews on Advanced Materials Science*, 2014, 36, 62-69.
19. L. He, M. Wang, J. Ge and Y. Yin, *Accounts of Chemical Research*, 2014, 45, 1431-1440.
20. R. Banerjee, Y. Katsenovich, L. Lagos, M. McIintosh, X. Zhang and C. Z. Li, *Current Medicinal Chemistry*, 2010, 17, 3120-3141.
21. S. Dietrich, S. Chandra, C. Georgi, S. Thomas, D. Makarov, S. Schulze, M. Hietschold, M. Albrecht, D. Bahadur and H. Lang, *Materials Chemistry and Physics*, 2012, 132, 292-299.
22. B. Basly, G. Popa, S. Fleutot, B. P. Pichon, A. Garofalo, C. Ghobril, C. Billotey, A. Berniard, P. Bonazza, H. Martinez, D. Felder-Flesch and S. Begin-Colin, *Dalton Transactions*, 2013, 42, 2146-2157.
23. D. A. Tomalia, A. M. Naylor and W. A. Goddard Iii, *Angewandte Chemie - International Edition in English*, 1990, 29, 138-175.
24. M. Zhao, L. Sun and R. M. Crooks, *Journal of the American Chemical Society*, 1998, 120, 4877-4878.
25. R. M. Crooks, M. Zhao, L. Sun, V. Chechik and L. K. Yeung, *Accounts of Chemical Research*, 2001, 34, 181-190.
26. T. Gillich, C. Acikgoz, L. Isa, A. D. Schluter, N. D. Spencer and M. Textor, *ACS Nano*, 2013, 7, 316-329.
27. B. Pan, F. Gao, L. Ao, H. Tian, R. He and D. Cui, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2005, 259, 89-94.
28. K. R. Gopidas, J. K. Whitesell and M. A. Fox, *Journal of the American Chemical Society*, 2003, 125, 6491-6502.
29. Y. S. Shon, in *Advanced Nanomaterials*, 2009, vol. 2, pp. 743-766.
30. J. I. Paez, M. Martinelli, V. Brunetti and M. C. Strumia, *Polymers*, 2012, 4, 355-395.
31. V. K. R. Kumar and K. R. Gopidas, *Chemistry - An Asian Journal*, 2010, 5, 887-896.
32. M. K. Kim, Y. M. Jeon, J. Woo Sung, H. J. Kim, H. Seung Gab, P. Chan Gyung and K. Kim, *Chemical Communications*, 2001, 667-668.
33. R. Wang, J. Yang, Z. Zheng, M. D. Carducci, J. Jiao and S. Seraphin, *Angewandte Chemie - International Edition*, 2001, 40, 549-552.
34. C. S. Love, I. Ashworth, C. Brennan, V. Chechik and D. K. Smith, *Journal of Colloid and Interface Science*, 2006, 302, 178-186.
35. H. Yan, C. Wong, A. R. Chianese, J. Luo, L. Wang, J. Yin and C. J. Zhong, *Chemistry of Materials*, 2010, 22, 5918-5928.
36. J. I. Paez, V. Brunetti, E. A. Coronado and M. C. Strumia, *Current Organic Chemistry*, 2013, 17, 943-955.
37. V. K. R. Kumar, S. Krishnakumar and K. R. Gopidas, *European Journal of Organic Chemistry*, 2012, 3447-3458.

View Article Online
DOI: 10.1039/C4NR04353

38. M. C. Daniel, J. R. Aranzaes, S. Nlate and D. Astruc, *Journal of Inorganic and Organometallic Polymers*, 2005, 15, 107-119.
39. J. I. Paez, E. A. Coronado and M. C. Strumia, *Journal of Colloid and Interface Science*, 2012, 384, 10-21.
40. G. Jiang, L. Wang, T. Chen, H. Yu and C. Chen, *Materials Chemistry and Physics*, 2006, 98, 76-82.
41. G. Jiang, L. Wang and W. Chen, *Materials Letters*, 2007, 61, 278-283.
42. Y. S. Shon, D. Choi, J. Dare and T. Dinh, *Langmuir*, 2008, 24, 6924-6931.
43. C. K. Kim, W. J. Joo, H. J. Kim, E. S. Song, J. Kim, S. Lee, C. Park and C. Kim, *Synthetic Metals*, 2008, 158, 359-363.
44. D. Li and J. Li, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2005, 257-258, 255-259.
45. K. Leonard, M. Kawashima, H. Okamura and J. Kurawaki, *Materials Letters*, 2010, 64, 2240-2243.
46. T. Joon Cho, R. A. Zangmeister, R. I. MacCuspie, A. K. Patri and V. A. Hackley, *Chemistry of Materials*, 2011, 23, 2665-2676.
47. S. Deng, T. M. Fulghum, G. Krueger, D. Patton, J. Y. Park and R. C. Advincula, *Chemistry - A European Journal*, 2011, 17, 8929-8940.
48. R. Villalonga, P. Díez, S. Casado, M. Eguílaz, P. Yánez-Sedeño and J. M. Pingarrón, *Analyst*, 2012, 137, 342-348.
49. N. Li, P. Zhao, L. Salmon, J. Ruiz, M. Zabawa, N. S. Hosmane and D. Astruc, *Inorganic Chemistry*, 2013, 52, 11146-11155.
50. F. G. De Rivera, L. I. Rodríguez, O. Rossell, M. Seco, N. J. Divins, I. Casanova and J. Llorca, *Journal of Organometallic Chemistry*, 2011, 696, 2287-2293.
51. C. S. Love, V. Chechik, D. K. Smith and C. Brennan, *Journal of Materials Chemistry*, 2004, 14, 919-923.
52. K. Heuzé, D. Rosario-Amorin, S. Nlate, M. Gaboyard, A. Bouter and R. Clérac, *New Journal of Chemistry*, 2008, 32, 383-387.
53. N. V. Kuchkina, E. Y. Yuzik-Klimova, S. A. Sorokina, A. S. Peregudov, D. Y. Antonov, S. H. Gage, B. S. Boris, L. Z. Nikoshvili, E. M. Sulman, D. G. Morgan, W. E. Mahmoud, A. A. Al-Ghamdi, L. M. Bronstein and Z. B. Shifrina, *Macromolecules*, 2013, 46, 5890-5898.
54. B. Basly, D. Felder-Flesch, P. Perriat, C. Billotey, J. Taleb, G. Pourroy and S. Begin-Colin, *Chemical Communications*, 2010, 46, 985-987.
55. Y. Park, P. Taranekar, J. Y. Park, A. Baba, T. Fulghum, R. Ponnapati and R. C. Advincula, *Advanced Functional Materials*, 2008, 18, 2071-2078.
56. J. Y. Park, Y. Park and R. C. Advincula, *Soft Matter*, 2011, 7, 5124-5127.
57. R. C. Advincula, *Dalton Transactions*, 2006, 2778-2784.
58. J. Locklin, D. Patton, S. Deng, A. Baba, M. Millan and R. C. Advincula, *Chemistry of Materials*, 2004, 16, 5187-5193.
59. B. Huang and D. A. Tomalia, *Journal of Luminescence*, 2005, 111, 215-223.
60. B. Huang and D. A. Tomalia, *Inorganica Chimica Acta*, 2006, 359, 1961-1966.
61. K. Torigoe, Y. Watanabe, T. Endo, K. Sakai, H. Sakai and M. Abe, *Journal of Nanoparticle Research*, 2010, 12, 951-960.
62. C. Hirano, T. Imae, Y. Yanagimoto and Y. Takaguchi, *Polymer Journal*, 2006, 38, 44-49.
63. A. Kumar, F. Chen, A. Mozhi, X. Zhang, Y. Zhao, X. Xue, Y. Hao, P. C. Wang and X. J. Liang, *Nanoscale*, 2013, 5, 8307-8325.
64. A. Kumar, X. Zhang and X. J. Liang, *Biotechnology Advances*, 2013, 31, 593-606.
65. G. Liu, J. Gao, H. Ai and X. Chen, *Small*, 2013, 9, 1533-1545.
66. K. Turcheniuk, A. V. Tarasevych, V. P. Kukhar, R. Boukherroub and S. Szunerits, *Nanoscale*, 2013, 5, 10729-10752.
67. T. R. Pisanic Ii, Y. Zhang and T. H. Wang, *Analyst*, 2014, 139, 2968-2981.
68. A. Battigelli, C. Menard-Moyon, T. Da Ros, M. Prato and A. Bianco, *Advanced Drug Delivery Reviews*, 2013, 65, 1899-1920.
69. E. C. Dreaden, A. M. Alkilany, X. Huang, C. J. Murphy and M. A. El-Sayed, *Chemical Society Reviews*, 2012, 41, 2740-2779.
70. K. C. W. Wu and Y. Yamauchi, *Journal of Materials Chemistry*, 2012, 22, 1251-1256.
71. J. Xie, S. Lee and X. Chen, *Advanced Drug Delivery Reviews*, 2010, 62, 1064-1079.
72. J. Xie, G. Liu, H. S. Eden, H. Ai and X. Chen, *Accounts of Chemical Research*, 2011, 44, 883-892.
73. X. Li, Y. Chen, M. Wang, Y. Ma, W. Xia and H. Gu, *Biomaterials*, 2013, 34, 1391-1401.
74. S. B. Hartono, M. Yu, W. Gu, J. Yang, E. Strounina, X. Wang, S. Qiao and C. Yu, *Nanotechnology*, 2014, 25.
75. D. Pan, C. Guo, K. Luo and Z. Gu, *Chinese Journal of Chemistry*, 2014, 32, 27-36.
76. J. Gautier, E. Allard-Vannier, K. Herve-Aubert, M. Souce and I. Chourpa, *Nanotechnology*, 2013, 24.
77. M. Calderon, M. A. Quadir, S. K. Sharma and R. Haag, *Advanced Materials*, 2010, 22, 190-218.
78. B. Duncan, C. Kim and V. M. Rotello, *Journal of Controlled Release*, 2010, 148, 122-127.
79. P. V. Patel, T. G. Soni, V. T. Thakkar and T. R. Gandhi, *Micro and Nanosystems*, 2013, 5, 288-302.
80. S. Parveen, R. Misra and S. K. Sahoo, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 2012, 8, 147-166.
81. P. Ghosh, G. Han, M. De, C. K. Kim and V. M. Rotello, *Advanced Drug Delivery Reviews*, 2008, 60, 1307-1315.
82. P. Ghosh, X. Yang, R. Arvizo, Z. J. Zhu, S. S. Agasti, Z. Mo and V. M. Rotello, *Journal of the American Chemical Society*, 2010, 132, 2642-2645.
83. Y. Pan, S. Neuss, A. Leifert, M. Fischler, F. Wen, U. Simon, G. Schmid, W. Brandau and W. Jahnen-Dechent, *Small*, 2007, 3, 1941-1949.
84. L. Dykman and N. Khlebtsov, *Chemical Society Reviews*, 2012, 41, 2256-2282.
85. T. J. Cho, R. I. MacCuspie, J. Gigault, J. M. Gorham, J. T. Elliott and V. A. Hackley, *Langmuir*, 2014, 30, 3883-3893.
86. Z. Cheng, A. Al Zaki, J. Z. Hui, V. R. Muzykantov and A. Tsourkas, *Science*, 2012, 338, 903-910.
87. Y. Pan, A. Leifert, D. Ruau, S. Neuss, J. Bornemann, G. Schmid, W. Brandau, U. Simon and W. Jahnen-Dechent, *Small*, 2009, 5, 2067-2076.
88. M. Stobiecka and M. Hepel, *Biomaterials*, 2011, 32, 3312-3321.
89. I. Papp, C. Sieben, K. Ludwig, M. Roskamp, C. BÃ¶ttcher, S. Schlecht, A. Herrmann and R. Haag, *Small*, 2010, 6, 2900-2906.

Nanoscale View Article Online DOI: 10.1039/C4NR04438G MINIREVIEW

90. K. R. Gopidas, J. K. Whitesell and M. A. Fox, *Journal of the American Chemical Society*, 2003, 125, 14168-14180.
91. D. Ma, Z. H. Liu, Q. Q. Zheng, X. Y. Zhou, Y. Zhang, Y. F. Shi, J. T. Lin and W. Xue, *Macromolecular Rapid Communications*, 2013, 34, 548-552.
92. D. Ma, H. B. Zhang, Y. Y. Chen, J. T. Lin and L. M. Zhang, *Journal of Colloid and Interface Science*, 2013, 405, 305-311.
93. A. K. Patri, J. F. Kukowska-Latallo and J. R. Baker Jr, *Advanced Drug Delivery Reviews*, 2005, 57, 2203-2214.
94. S. Rana, A. Bajaj, R. Mout and V. M. Rotello, *Advanced Drug Delivery Reviews*, 2012, 64, 200-216.
95. S. Rana, Y. C. Yeh and V. M. Rotello, *Current Opinion in Chemical Biology*, 2010, 14, 828-834.
96. M. Calderon, M. A. Quadir, M. Strumia and R. Haag, *Biochimie*, 2010, 92, 1242-1251.
97. L. Costantino, F. Gandolfi, L. Bossy-Nobs, G. Tosi, R. Gurny, F. Rivasi, M. Angela Vandelli and F. Forni, *Biomaterials*, 2006, 27, 4635-4645.
98. H. Pan, M. E. Grow, O. Wilson and M. C. Daniel, *Tetrahedron*, 2013, 69, 2799-2806.
99. M. C. Daniel, M. E. Grow, H. Pan, M. Bednarek, W. E. Ghann, K. Zabetakis and J. Cornish, *New Journal of Chemistry*, 2011, 35, 2366-2374.
100. B. Yavuz, S. Bozdag Pehlivan and N. Unlu, *The Scientific World Journal*, 2013, 2013.
101. J. M. Bergen, H. A. Von Recum, T. T. Goodman, A. P. Massey and S. H. Pun, *Macromolecular Bioscience*, 2006, 6, 506-516.
102. P. S. Ghosh, C. K. Kim, G. Han, N. S. Forbes and V. M. Rotello, *ACS Nano*, 2008, 2, 2213-2218.
103. S. T. Kim, A. Chompoosor, Y. C. Yeh, S. S. Agasti, D. J. Solfiell and V. M. Rotello, *Small*, 2012, 8, 3253-3256.
104. J. Gautier, E. Allard-Vannier, E. Munnier, M. Souce and I. Chourpa, *Journal of Controlled Release*, 2013, 169, 48-61.
105. H. W. Yang, M. Y. Hua, H. L. Liu, C. Y. Huang and K. C. Wei, *Nanotechnology, Science and Applications*, 2012, 5, 73-86.
106. R. Khodadust, P. Mutlu, S. Yalcin, G. Unsoy and U. Gunduz, *Journal of Nanoparticle Research*, 2013, 15.
107. T. Tanaka, K. Shibata, M. Hosokawa, K. Hatakeyama, A. Arakaki, H. Gomyo, T. Mogi, T. Taguchi, H. Wake, T. Tanaami and T. Matsunaga, *Journal of Colloid and Interface Science*, 2012, 377, 469-475.
108. A. L. Martin, L. M. Bernas, B. K. Rutt, P. J. Foster and E. R. Gillies, *Bioconjugate Chemistry*, 2008, 19, 2375-2384.
109. R. Zhu, K. Luo, X. Xu, Y. Wu, B. He and Z. Gu, *Acta Polymerica Sinica*, 2011, 679-686.