Biomaterials 32 (2011) 8562–8573

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Dendronized iron oxide nanoparticles for multimodal imaging

Giuseppe Lamanna [a], Marie Kueny-Stotz [a], Hind Mamlouk-Chaouachi [a], Cynthia Ghobril [a], Brice Basly [a], Annabelle Bertin [a], Imen Miladi [b], Claire Billotey [b], Geneviève Pourroy [a], Sylvie Begin-Colin [a], Delphine Felder-Flesch [a,*]

[a] IPCMS, UMR CNRS-Université de Strasbourg 7504, 23 rue du loess, BP 43, 67034 Strasbourg cedex 2, France
[b] LPCML/UCBL, UMR 5620, Service de Médecine Nucléaire Pavillon B, 5 place d'Arsonval, 69437 Lyon cedex 03, France

ARTICLE INFO

Article history:
Received 16 June 2011
Accepted 8 July 2011
Available online 23 August 2011

Keywords:
Dendrimers
Iron oxide nanoparticles
Phosphonates
MRI contrast agent
Biodistribution
Multimodal imaging
Vectorization

ABSTRACT

The synthesis of small-size dendrons and their grafting at the surface of iron oxide nanoparticles were achieved with the double objective to obtain a good colloidal stability with a mean hydrodynamic diameter smaller than 100 nm and to ensure the possibility of tuning the organic coating characteristics including morphology, functionalities, physico-chemical properties, grafting of fluorescent or targeting molecules. Magnetic resonance and fluorescence imaging are then demonstrated to be simultaneously possible using such versatile superparamagnetic iron oxide nanocrystals covered by a dendritic shell displaying either carboxylate or ammonium groups at their periphery which could be further labelled with a fluorescent dye. The grafting conditions of these functionalized dendrons at the surface of SPIO NPs synthesized by co-precipitation have been optimized as a function of the nature of the peripheral functional group. The colloidal stability has been investigated in water and osmolar media, and *in vitro* and *in vivo* MRI and optical imaging measurements have been performed showing encouraging biodistribution.

1. Introduction

The continuous growth of nanotechnology has brought challenging innovations in medicine, revolutionizing the field of diagnosis and therapy. In particular, nanomedicine has emerged as a particularly powerful interdisciplinary branch [1] and it raises new therapeutic hopes, for example for the tumours targeting through magnetic oxide nanoparticles (NPs) [2]. Also, progress in using iron oxide nanoparticles for biological applications [3–6] has advanced rapidly thanks to the tremendous work achieved in the synthesis and functionalization of these materials [7–11]. In all these applications, it is mandatory to engineer the surface of SuperParamagnetic Iron Oxides (SPIO) NPs not only to improve biocompatibility, solubility and stability in physiological media but also to ensure a small particle size distribution (below 100 nm) after decoration and to preserve appropriate magnetic properties, e.g. a high saturation magnetization. Furthermore the coating is more and more designed to bring bioactive multifunctions ensuring stealth, targeting and therapeutic care.

To be used *in vivo*, functionalized iron oxide NPs have to be stable in biological solutions at pH close to the physiological blood pH (7.4) and close to the plasmatic isoosmolarity (320 mosmol/l). Indeed, the isoosmolarity condition is essential to obtain for *in vitro* cellular marking. To avoid aggregates is mandatory for intra-venous injection (risk of pulmonary embolism in case of macro aggregates or of coagulation disorders in case of micro aggregates, causing the animal death). Furthermore, upon entering the blood circulation, NPs are subjected to opsonisation, the non-specific fouling of plasma protein at the NPs surface, and subsequent uptake by reticuloendothelial system (RES).

For diagnostic or therapy applications, the non-specific uptake by the macrophages can be used in precise cases such as hepatic or ganglionic tumoral imaging. But except this specific case, the biodistribution must be favourable, with little non-specific tissue uptake (mainly hepatic), and a quasi complete elimination of the non-uptaken nano-objects, knowing that the most effective ways of elimination are the urinary and the hepato-biliary pathways. *in vivo* imaging techniques are essential tools to study these processes, in particular those allowing a whole body imaging study, as well as the *ex vivo* signal measurement at every organ level. In order to prevent NPs opsonisation and increase the ability to evade the RES, not only the organic coating and it's anchoring at the NPs surface

* Corresponding author. Tel.: +33 388107163; fax: +33 3881072446.
E-mail addresses: Sylvie.Begin@ipcms.u-strasbg.fr (S. Begin-Colin), Delphine.Felder@ipcms.u-strasbg.fr (D. Felder-Flesch).

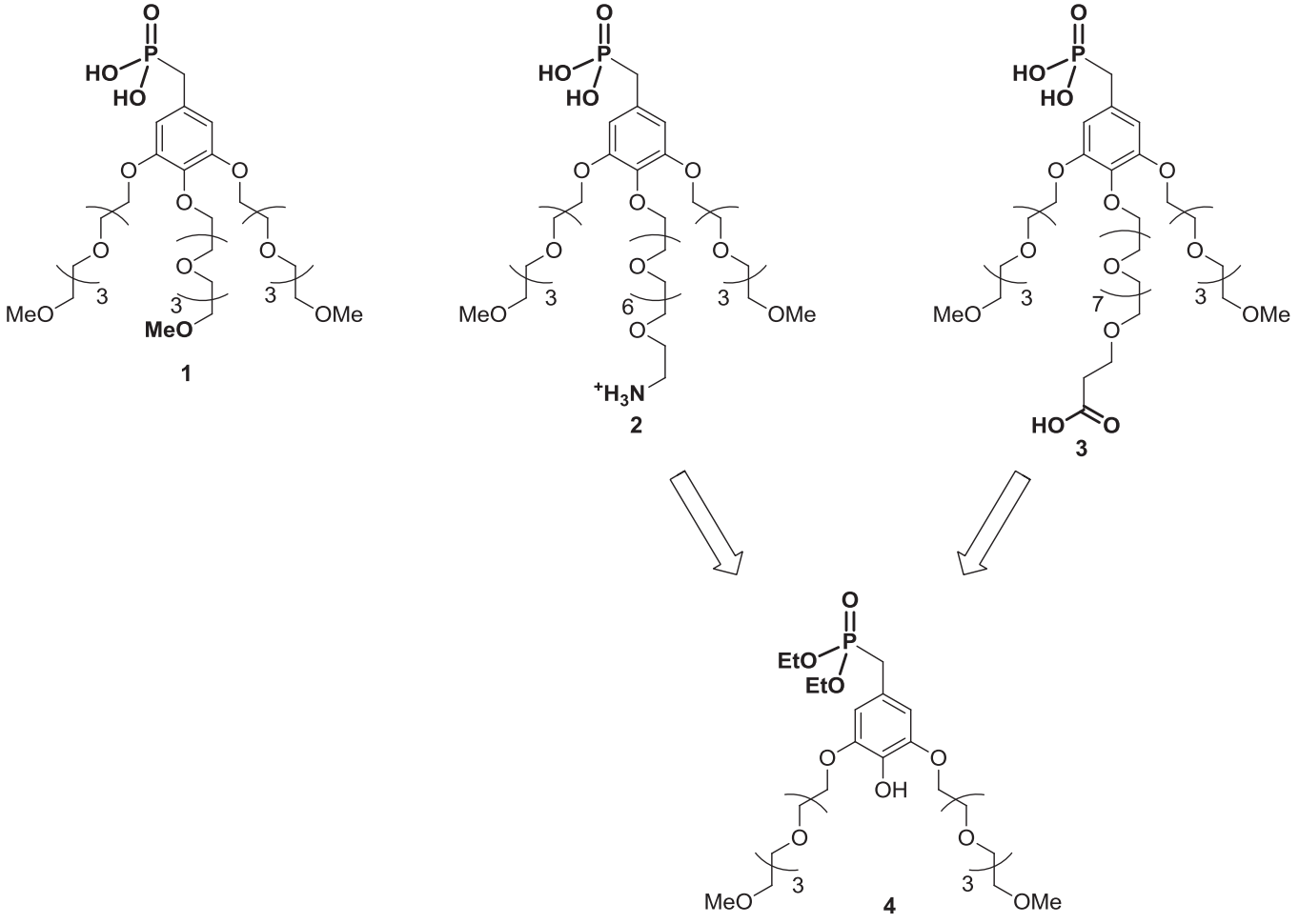
0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.07.026

has to be tailored but also the particle size distribution of the functionalized NPs must be optimised. Long blood-circulation time would maximize the possibility to reach target tissue: suspensions of particles with average hydrodynamic sizes of 10–100 nm are optimal for *in vivo* delivery, as smaller ones (<10 nm) are rapidly removed by renal clearance while bigger ones (>200 nm) are quickly sequestered by the RES.

The design of hybrids for biomedical applications is therefore challenging. Various kinds of organic systems have been investigated to coat NPs [12]. Most developed molecules are biocompatible polymers made of lipids, proteins, gelatin, alginate [13], or such as dextran [14,15], chitosan [16], pullulan, PEG, poly(ethylene- *co* -vinyl acetate), poly(vinylpyrrolidone) (PVP), PLGA, or poly(vinyl alcohol) (PVA) [17–21] and poly(acrylic acid) (PAA) [22]. Other types of molecules, called linkers or coupling agents, were also investigated, such as bifunctional 2,3-dimercaptosuccinic acids (DMSA) [23], dopamine [24], phosphonate [25–30], carboxylates [31] and silanes [32], to ensure grafting at the inorganic surface. Dopamine (through its catechol function) and phosphonate functional groups were found to be stable and strong anchors on the IO NPs surface [33,34]. Silanes were employed to exchange hydrophobic ligands on ferrite magnetic nanostructures [35]. Indeed, the silanes end groups, including isocyanate, acrylate, thiol, amino and carboxylic functions, offer extensive chemistry for the modification of these nanostructures. Dopamine and silane often combined with poly(ethylene glycol) or other polymers offer long-term NP stabilization in biological solutions [36].

Besides, dendrimers or dendritic architectures [30] are more and more developed for biomedical applications due to their precisely defined structure and composition, and also high tuneable surface chemistry [37]. Indeed a clear input is brought by the dendritic molecules as they are discrete and monodisperse entities which relevant characteristics like their size, hydrophilicity, molecular weight and biocompatibility can easily be tuned as a function of their generation [38,39]. Furthermore a dendritic shell allows versatile and reproducible polyfunctionalization at its periphery which could lead to, multimodal imaging probes through dye or fluorophore grafting, theranostics through specific drug anchoring. Current studies show that small-sized dendrons may have an impressive future in the functionalization of magnetic nanoparticles [25–30] thanks to their highly controlled molecular structure and high tuneability leading to biocompatible, polyfunctional and water-soluble systems. Dendronized iron oxide nanoparticles using a phosphonate or hydroxamic acid anchor were shown to display very good colloidal properties and high relaxivity values [27,28,40]. Such anchoring groups induced a strong binding [40], and, in the case of phosphonic anchors, were also demonstrated to preserve NPs' magnetic properties.

To further demonstrate the dendron architecture versatility, the control of its grafting, once bearing multifunctions allowing coupling to bioactive species, is mandatory. Herein we first describe the synthesis of small-size dendrons displaying either no functional group or carboxylate or ammonium groups at their periphery (Scheme 1) and we refine their grafting at the NPs surface with the



Scheme 1. Retrosyntheses of final dendritic phosphonic acids.