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Polymeric hollow micro and nanospheres for biotechnological applications: A focused review

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ABSTRACT

The rapid advancement of nanotechnology in the last ten years opened in medicine a real perspective of a significant breakthrough in the ability of therapeutics and imaging contrast agents to reach exactly the desired targets, with marginal or no collateral damages. Nanotechnologies based on the self-assembly of molecules resulting in nano-sized complexes play a key role in this advancement. In fact, molecular assemblies promise a tremendous potential for developing new diagnostic and therapeutic tools, as genuine 'nano-devices', able to interact with biological systems at molecular levels and with a high degree of specificity. However, the strong expectations raised by this approach, despite the great effort in terms of research and investments, have been since now frustrated by the rather low efficiency of the existing vectors. Nevertheless, taking into account the difficulty of the challenge, results are encouraging, and considering the importance of the stake, research in this field continues to be intensive. In this review we focus on a particular class of "micro/nano-devices", i.e. hollow spheres, that appear to be particularly useful as "carriers" of active substances in biotechnological applications.

1. Introduction

The potential uses of hollow micro/nanospheres in medicine were envisioned soon after the discovery of liposomes in the 1960s. In mid 1970s, pioneering papers [1,2] indicated liposomes as potential drug carriers. Few years later [3] it was suggested that liposomes could have been used to localize tumoral cells, and at the end of the 1970s sufficient work was done to produce a review on potential applications of liposomes in drug delivery for cancer treatment [4]. A review on biomedical uses of liposomes was written in 1995 by Gregoriadis [5], and a lot of work has been made on these systems since then. Small sizes (which limit the amount of drug that can be carried as well as the efficacy of liposomes as contrast agents) and short lifetime in the blood-stream (due to interaction with the RES, the reticulo-endothelial system) limit the efficacy of liposomes for these kinds of applications. However, both these obstacles can be partly removed by appropriate liposome engineering [6].

Polymeric hollow micro/nanospheres (MnS) have been introduced in the late 1980s [7,8] as a possible alternative to liposomes. Compared to liposomes, MnS can be realized over a much wider range of sizes and shell materials, allowing for a fine tuning of their properties. In particular, the research has been focused on the realization of carriers able to target specific cells or tissues. The simplest carriers (so-called "first-generation") target the diseased site by passive mechanisms. Simply, their small size (50–100 nm), allowing extravasation through gaps in tumor neovasculature, favors their accumulation in tumor tissues, provided a sufficiently prolonged circulation time that can be obtained by a "stealth" coating (for example polyethylene glycol) reducing their uptake by phagocytes. Surface charge density and morphology are other features that might convey specificity towards certain targets. In more sophisticated carriers (second generation) additional functionalities allow for molecular recognition of the target tissue (active targeting), or for the active or triggered release of the nanoparticle "payload" (the pharmacologically active molecules carried by the vector). Remote controls have also been devised for triggering the drug release by external stimuli, such as electromagnetic radiation, ultrasound, magnetic fields, or heat, that act on sensitive components integrated within the carrier. More recent advancements concern a third generation of nanoparticles. These are multicomponent and multifunctional systems where different nanoparticles are nested into a single construct. In these systems the functions of biorecognition, toxicity and biological barrier avoidance are decoupled, however they act in sequence and in synergy. The realization of multiple liposomes assembled into larger multicompartment micro/nanoparticles [9,10] is a

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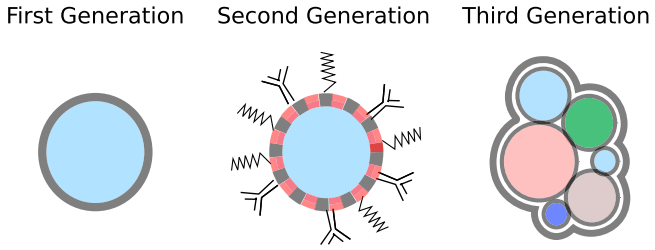

Fig. 1. Evolution of nanocarriers through years. First generation nanocarriers were simple particles containing a drug or a contrast agent. A second generation of carriers consists of particle whose surface is functionalized with different kinds of macromolecules to make the particles flow more efficiently towards the desired target and/or react to external stimuli to release their cargo only when requested. A third generation of carriers is proposed: multicompartment carriers could be used to realize the theranostic paradigma and/or to simultaneously deliver different drugs to the same target.

promising step towards such third generation nanovectors (see Fig. 1).

The huge interest in nanovectors is witnessed by the very large amount of papers that appeared in the last few decades, which make a comprehensive review of all present results almost impossible. A large number of reviews on selected aspects of the realization and use of MnS for biological applications appeared in the past few years. We try here to furnish a global view on the wide topic of micro/nanospheres for biotechnological applications, referring the reader for technical details to the cited reviews and selected papers.

2. Materials

Polymeric MnS can be realized using an huge class of materials. Specific realization techniques (see below) may require specific properties of materials. However, for medical application the major constraint is that the materials must be well tolerated by the human body. Aliphatic polyester polymers are a first, wide class of materials satisfying this requirement. Poly-lactic acid (PLA), poly-glycolic acid (PGA), poly-lactic-co-glycolic acid (PLGA) are widely used for their biocompatibility and biodegradability [11]. PLA/PLGA microspheres have been extensively studied for both vaccine [12] and drug [13] delivery.

Although biocompatibility is often the only requirement for biomedical applications of MnS, for specific applications some materials may present advantages over others. As an example, poly $\varepsilon-$caprolactone (PCL) possesses several interesting properties, including its high permeability to small drug molecules, and an exceptional ability to form blends with other polymers. Moreover, the slower degradation rate of the PCL homopolymer as compared to PLGA and polyglycolic acid-co-lactic acid makes it more suitable for long term delivery systems, extending to a period of more than one year. For an excellent and detailed review on the use of PCL based microspheres see Sinha et al. [14].

Chitosan (CS) is a natural polysaccharide similar to cellulose, possessing several interesting properties for microsphere realization. Among others, it is mucoadhesive, thus favoring the interaction with tissues, and may induce a relaxation of tight-junctions between the cells of epi/endothelium tissues, thus favoring drug absorption. Finally, it is charged, and can be neutralized by increasing the pH, leading to a transition from soluble to insoluble. Based on these characteristics, an innovative method to produce chitosan/DNA nanospheres aimed to gene delivery has been recently proposed [15]. A review on realization and use of chitosan-based microspheres has been recently written by Dash et al. [16].

Polyethylene glycol (PEG) is used as a "stealth" material to prevent unwanted adsorption of proteins (opsonins) [17], which in turn prompt the recognition of nanoparticles as "foreign objects" by macrophages, the first step of a process that ends with particle's clearance from blood or tissues. Polysaccharides have been proposed as other possible candidates to produce "stealth" nanoparticles [18].

Other materials have been widely used for functionalization of microspheres. Different materials can be used to reach different goals. Magnetic materials can be useful for driving the MnS to selected places [19], for hyperthermia therapy [20,21], for NMR contrast enhancement [22], or for all these tasks together. Functionalization with biological macromolecules (e.g. antibodies, carbohydrates or peptides) of the MnS surface has been shown to considerably increase adhesion of microspheres on specific sites (see Hernot and Klibanov [23] and references therein). In particular polypeptides, being the building blocks of proteins, can easily be connected to and/or made to interact with biological matter. In addition, peptide based materials can be selectively destroyed or further assembled by proper enzymes [24], thus allowing for controlled drug release [25]. Other "smart materials" whose properties can be controlled by external stimuli include redox-active compounds (e.g. poly(ferrocenylsilanes) (PFS)) [26], light-sensitive polyelectrolytes[1] [28] and thermoresponsive polymers such as poly(N-isopropylacrylamide) (PNIPAM).[2]

3. Realization techniques

Several methods have been proposed to produce MnS. All of them may present advantages and disadvantages for specific applications, so that the choice of the method to be used strongly depends on the application for which MnS are realized. Growth methods can be classified according to the growth process (self-assembly vs template coating), kind of template (gaseous, liquid or solid) or materials used (lipids, colloids, polymers). Encapsulation methods have been reviewed by Yow and Routh [31].

Fluid (gaseous or liquid) microbubbles have been the first template used to realize MnS. Microbubbles can be generated quite simply by a liquid emulsion process. The fluid–liquid interface (FLI) becomes then the seed for the formation of the microsphere (see Fig. 2, left panel). Microsphere walls can be build using colloids (which under particular conditions adsorb on the FLI), polymer precipitation by phase separation (dispersed monomers are induced to form stable polymers at the FLI) or polycondensation (monomers of different kinds are first dispersed in the two fluids forming the emulsion, and then made to react forming stable polymers at the FLI). Colloids allow to finely tune the shell permeability during the stabilization step that follows the adsorption of colloids onto the droplet/bubble surface. Polymer precipitation allows further fine tuning of the shell thickness. However, polycondensation has the disadvantage that requires monomers in both fluids, limiting the possibility of using this technique for encapsulation of organic materials.

All the above methods are based on the self-growth of the microsphere at a liquid–liquid interface. A major problem with this class of methods is the rather large number of conditions that must be met to achieve the final result: the two liquids must be chosen in such a way as to guarantee a given surface tension at the liquid–liquid interface; the material used to produce the shell must be selectively soluble in any or both of the liquids; materials

[1] In order to be usable in *in vivo* conditions, photosensitive polyelectrolytes must be sensitive to near-infrared wavelength, since for these radiations the human tissues are almost transparent [27].

[2] Due to its peculiar chain-to-globule transition at a lower critical solution temperature (LCST) close to 32 °C, PNIPAM has been studied as a possible candidate for selective drug delivery as the local temperature varies across LCST [29,30].