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Amphiphilic poly(2-oxazoline) copolymers as self-assembled carriers for drug delivery applications

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ABSTRACT

The potential of poly(2-oxazoline) containing copolymers for drug delivery is discussed in this feature article. Advances in synthetic routes for 2-oxazoline copolymerization and chemical modification of biocompatible poly(2-oxazoline) copolymers allow the control of amphiphilic poly(2-oxazoline) copolymers at the molecular level. Thus macromolecular design dictates self-organization of poly(2-oxazoline) copolymers at the nano-scale into morphologically diverse, functional nanostructures in aqueous solutions, able to respond to changes caused by external stimuli and showing fine-tuned interactions, loading and release properties of encapsulated hydrophobic drugs and biological macromolecules. The latest advances in the use of poly(2-oxazoline) containing copolymers as carriers of hydrophobic drugs, therapeutic proteins/peptides and nucleic acids are presented through representative examples from recent literature. It can be safely concluded that nanocarriers based on poly(2-oxazoline) containing block copolymers show a great potential for applications in the biomedical field in the near future.

1. Introduction

Macromolecular nanostructures have attractive properties for the field of bio-applications, pharmaceutics and food industry [1]. Thermo-responsive properties [2] and the ability to tune interactions with proteins [2] and other biological molecules at the nano-level are key factors that make macromolecular soft-matter functional in cooperation with biological matter. Besides manipulating interactions in bulk aqueous solutions modifications on surface properties [3] may induce biocompatibility or tune adsorption of proteins and cells. Additionally hydrogels [4] are capable of carrying and releasing drugs in various responsive ways or act as templates for tissue engineering applications.

In the last decades, oligo- and poly(2-oxazoline)s have attracted growing interest due to the ease of copolymers synthesis. Different architectures such as star-shaped polymers [5], with a variety of molecular weights and functional groups have been synthesized and are able to form various structures such as micelles, and vesicles in solutions. Till today many poly(2-oxazoline)s have been synthesized and numerous articles have referred their uses [6]. Despite this extensive interest, studies still should be carried out to certify the poly(2-oxazoline)'s ability in fields such as biotechnology (including gene transfer, drug delivery, tumor therapeutics), food and cosmetics industry. Poly(2-oxazoline)s (POxs) are promising components for biomaterials because they are biocompatible and their chemical functionality and architecture can be modified within a great range [6]. They have been produced by (living) cationic ring-opening polymerization of 2-oxazolines [6]

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and their synthesis can be assisted by microwave irradiation [7–9] and/or can be based on renewable sources [10,11]. Homo- and co-poly(2-oxazoline)s have been characterized in solution and in bulk, as far as their thermal and self-assembly properties are concerned [12,13]. Both homo- and co-poly(2-oxazoline)s have been described as bio-inspired smart materials, in the biological and biotechnology field (including gene transfer, drug delivery, tumor therapy). Recently, further studies have combined different copolymers with poly(2-oxazoline)s in order to succeed in facing complex challenges [6]. This feature article is focused on the potential of polymers containing poly(2-oxazoline) sequences and their self-assembled nanostructures (including micelles, physical hydrogels, mixed polymeric systems and bioconjugates) for applications with biomedical interest. Poly(2-oxazoline) based polymers only recently have began to be explored more intensively towards utilization in biomedicine. Recent examples of their use mainly as nanocarriers will be discussed with special attention given to encapsulation studies involving hydrophobic drugs [14].

2. Amphiphilic poly(2-oxazoline) copolymers for drug delivery

Poly(oxazoline)s have been used as versatile adhesives in the field of painting restoration and conservation [15] and ligands for catalysis and other applications [16]. Generally, as bio-materials, POxs can be used in different formulations including bioconjugates [17], fibrous scaffolds [18] and non-viral vectors [19]. Poly(2-oxazoline)s present properties similar to poly(ethylene glycol)s (PEG)s, such as biocompatibility, protein repellency and stealth behavior against the mammalian immune system. Also, POxs are structural isomers of polyacrylamides and polypeptides, showing low toxicity. So it is believed they can be useful in many biotechnological applications [20]. Concerning amphiphilic poly(2-oxazoline)s copolymers, their easy synthesis and their assembly into micelles and other nanostructures made them useful as nanocarriers for many applications [21] including delivery of hydrophobic low molecular weight drugs. Several examples can be found in recent literature.

ABA triblock copolymers of the type poly[2-methyl-2-oxazoline-block-(2-isopropyl-2-oxazoline-co-2-butyl-2-oxazoline)-block-2-methyl-2-oxazoline], with two hydrophilic A blocks and one central thermoresponsive B block with different monomer unit ratios [22] were investigated regarding their thermoresponsive properties [23]. Copolymers were molecularly dissolved below their cloud point, while they formed micelles at higher temperatures. The phase transition was narrow and the cloud point temperature increased as the hydrophilic content of the polymer increased. A phenolic group was incorporated to allow radionuclide labeling with iodine. ^{255}I loaded micelles showed good labeling yield and *in vitro* stability. The toxicity of all the copolymers was considered essentially non-existing as proved by haemolitic activity studies. Hence the particular system shows potential for utilization in radiodiagnostic protocols. In another example, poly(2-isopropyl-2-oxazoline–co–2-butyl-2-oxazolines) has also been combined with pluronic F127 to produce thermoresponsive nanoparticles for potential use in solid tumor diagnostics [24].

Pluronic F-127 (PF-127) is known to act as an excellent drug delivery system for several routes of administration and to be compatible with numerous different substances [25]. In Bogomolova et al. [26] the authors combined the advantages of PF-127 surfactant with the thermoresponsive statistical poly[(2-butyl-2-oxazoline)-stat-(2-isopropyl-2-oxazoline)] copolymer [BuOx-co-iPrOx]. Both DLS and SAXS techniques were used to study these nanoparticles. The change of system parameters such as temperature and molecular weight point towards the conclusion that the statistical poly(2-oxazoline) copolymer was incorporated in PF-127 micelles. As a result these systems can be also used for radionuclide delivery.

The physicochemical characteristics of micellar taxane nanoformulations based on poly(2-oxazoline) were tested regarding their *in vitro* stability and *in vitro* cytotoxity in several cancer lines [27]. Drug loaded micelles were formed by the film hydration method (Fig. 1). Polymer structure (diblock, triblock or nature of hydrophobic block), chemical structure of the drug, drug to POx mass ratio and POx concentration critically affected drug content and size of the nanoparticles present within the formulations. The amphiphilic triblock copolymer that contained BuOx as a middle block had the highest stability in high drug content. Cytotoxicity of the formulations was found similar to the one of free drugs in a variety of cancer cell lines. The PMeOx-PBuOx-PmeOx triblock copolymers had balanced properties that provided high drug loading, good formulation reproducibility and colloidal stability [28]. Multidrug loaded micelles were found to act synergistically against several tumor models.

Gradient (or pseudo diblock) copolymers from 2-methyl-2-oxazoline and 2-phenyl-2-oxazoline were synthesized towards the formation of nanocarriers for hydrophobic drugs [29]. The resulting self-assembled structures in aqueous solutions were spherical micelles, vesicles or aggregates depending on the copolymer composition. By mixing with indomethacin (IND) it was shown that drug molecules act as strong hydrophobic aggregation centers. The original micelles increased in size, while aggregates of large dimensions appeared for all copolymers. This way IND could not only be loaded to the self-assembled nanostructures but also acted as an additive able to tune the aggregation state of amphiphilic copolymers.

The self-assembly of POx copolymers has a clear interest for pharmaceutical and biological applications. It is therefore of great importance to elucidate their morphology and interactions in solution. Poly(2-methyl-2-oxazoline-grad-2-phenyl-2-oxazoline) (MeOx-grad-PhOx) has been partially hydrolyzed [13] so that MeOx groups were transformed to ethylene imine (EI). The stability of the spherical aggregates in aqueous media and their complexation with DNA was investigated by light scattering and ζ-potential measurements. A SANS study [30] explored the internal morphology of the complexes of the hydrolyzed gradient copolymers with sodium dodecyl sulfate (SDS). In this case the hydrophobic segments form spherical hydrophobic domains that are hierarchically arranged within large fractal aggregates in the absence of SDS. Incorporation

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