Nanotechnology in the Life Sciences

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Green Nanoparticles

Synthesis and Biomedical Applications

Springer

Nanotechnology in the Life Sciences

Series Editor

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Nano and biotechnology are two of the 21st century's most promising technologies. Nanotechnology is demarcated as the design, development, and application of materials and devices whose least functional make up is on a nanometer scale (1 to 100 nm). Meanwhile, biotechnology deals with metabolic and other physiological developments of biological subjects including microorganisms. These microbial processes have opened up new opportunities to explore novel applications, for example, the biosynthesis of metal nanomaterials, with the implication that these two technologies (i.e., thus nanobiotechnology) can play a vital role in developing and executing many valuable tools in the study of life. Nanotechnology is very diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale, to investigating whether we can directly control matters on/in the atomic scale level. This idea entails its application to diverse fields of science such as plant biology, organic chemistry, agriculture, the food industry, and more.

Nanobiotechnology offers a wide range of uses in medicine, agriculture, and the environment. Many diseases that do not have cures today may be cured by nanotechnology in the future. Use of nanotechnology in medical therapeutics needs adequate evaluation of its risk and safety factors. Scientists who are against the use of nanotechnology also agree that advancement in nanotechnology should continue because this field promises great benefits, but testing should be carried out to ensure its safety in people. It is possible that nanomedicine in the future will play a crucial role in the treatment of human and plant diseases, and also in the enhancement of normal human physiology and plant systems, respectively. If everything proceeds as expected, nanobiotechnology will, one day, become an inevitable part of our everyday life and will help save many lives.

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ISSN 2523-8027 ISSN 2523-8035 (electronic)
Nanotechnology in the Life Sciences
ISBN 978-3-030-39245-1 ISBN 978-3-030-39246-8 (eBook)
https://doi.org/10.1007/978-3-030-39246-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Advances in nanotechnology and engineering have revolutionized the materials used in our daily lives, and on which our societies and economies are based. While various nano-based products are increasingly used in different economic sectors, there is an increased awareness regarding the environmental and biological safety related to their preparation and uses. Green nanotechnology is certainly the most important division of nanotechnology, which uses 12 principles of green chemistry to promote sustainability and minimize health risks. This book, *Green Nanoparticles: Synthesis and Biomedical Applications*, outlines how green nanotechnology has been used to produce more efficient, reliable, and eco-friendly products and devices for biomedical, food, and agricultural applications. This volume certainly represents an important source of information for scientists who want to learn more about the current status and future perspectives of the use of green nanotechnology to create the next generation of products which could solve current and future challenges faced by the biomedical, food, and agricultural fields, as well society in general. The book contains 20 chapters covering topics related to the application of nanotechnology in the development of topical delivery systems, stimuli-responsive nanocarriers, biosensors, and the treatment of neglected tropical diseases. It also describes the uses of plants to produce green nanoparticles and evaluates the potential toxicity of nanomaterials. In this way, we believe that this book can provide knowledge to different sectors such as academia, industry, stakeholders, and anyone who has an interest in the improvements of green nanotechnology.

We are indebted to the authors who contributed in this book. We wish to thank Dr. Emmy Lee, Associate Editor, Springer Nature Korea Limited, for her generous assistance and persistence in finalizing the edited volume. Special thanks are due to our valued fellow colleagues and university authorities for their kind support and continuous inspiration throughout the task.

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Chapter 1
Biomedical Applications of Stimuli-Responsive Hydrogels

Anderson Ferreira Sepulveda, Roger Borges, Juliana Marchi, and Daniele Ribeiro de Araujo

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1 Introduction

A copolymer is a macromolecule derived from more than one species of polymers, consisting of two or more blocks of different polymers chemically bonded to one another. Among the considerable number of copolymer types, one of the most used is polyethylene glycol (PEG)-based polymers, including their blocks (with two or

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J. K. Patra et al. (eds.), *Green Nanoparticles*, Nanotechnology in the Life Sciences, https://doi.org/10.1007/978-3-030-39246-8_1

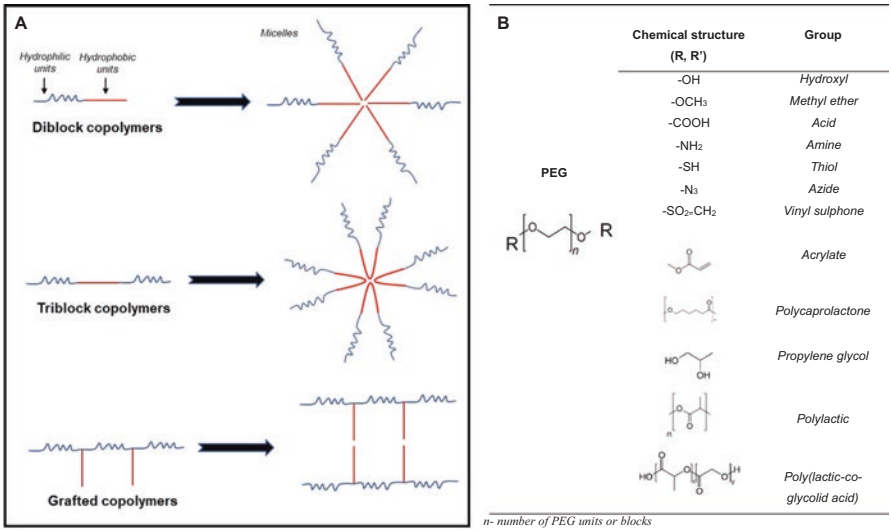

Fig. 1.1 Schematic representation of diblock, triblock, grafted copolymers, and their self-assembly in polymeric micelles (diblock and triblock) and their aggregates (grafted) (**a**). Polyethylene glycol (PEG) structure and some chemical groups used as its functional radicals on R and/or R' positions (**b**)

three and graft copolymers) (IUPAC 1997). PEG is a relatively hydrophilic and linear polymer, synthetized by polymerization of ethylene oxide units resulting in molecular weights ranging from 0.4 to 100 kDa and arrangements, such as micelles, according to the conjugation with hydrophobic polymers.

The presence of hydroxyl end groups allows the formation of covalent bindings with a variety of chemical groups (polylactic acid, poly(amino acid), polycaprolactone, poly(lactic-co-glycolic) acid, acrylate, acetylene, etc.) (Fig. 1.1), resulting on particular physicochemical features and different self-assembly mechanisms and biological properties, such as interaction with proteins and peptides as well as the formation of biocompatible hydrogels matrices for drug delivery, tissue regeneration, and diagnosis platforms (Zhu et al. 2010; Boonlai et al. 2018; Qureshi et al. 2019). In fact, the association of hydrophobic and hydrophilic groups into the PEG-based chemical structure evokes the formation of self-assembled aggregates such as micelles and/or hydrogels, in response to concentration and environmental conditions such as temperature, UV light, pH, and ionic strength.

Hydrogels are defined as polymeric networks capable to absorb a significant water content, forming highly permeable matrices with several biomedical applications including small molecules and proteins carriers and scaffolds for cells growing and tissue regeneration. In fact, the main property of those materials is their capability for presenting as highly viscous materials with gradual dissolution after application associated with their ability for responding upon certain environmental conditions (such as temperature and pH), according to the polymeric composition

and concentration (Zhang et al. 2014; Moeinzadeh and Jabbari 2015; Kong et al. 2017; Chen et al. 2019).

In this context, PEG-based hydrogels have been used for several biomedical purposes considering their biocompatible, non-immunogenic, high purity, adequate physicochemical stability, and rheological properties. In special, this chapter focuses on PEG-based temperature and pH-sensitive hydrogels presenting their composition, mechanical properties, supramolecular structure, and self-assembled mechanisms, as well as highlighting their progress as biocompatible matrices for biomedical applications.

2 PEG-Based Temperature-Sensitive Hydrogels: Structural and Physicochemical Properties

Among temperature-sensitive materials, PEG-based polymers are considered one of the most used materials, especially for biomedical applications, which allows the incorporation of cells, drugs, and other biocompatible polymers into their porous matrix. In fact, the ability to form hydrogels is observed when this material is delivered in solution and, in response to the physiological temperature, their viscosity is instantaneously changed, reaching the sol-gel transition temperature and adequate mechanical properties for in situ depot systems. The main vantage attributed to those systems is that there is no need of chemical agents for the hydrogels formation, which results in materials with low biological toxicity (Klouda 2015). For this reason, these hydrogels have been pointed as one of the main matrices for delivery of drugs and other bioactive molecules. Also, the conjugation of PLGA and PCL with PEG have gained attention because of their biodegradability and biocompatibility. On the other hand, other polymer types, such as PEG-poly-N-isopropylacrylamide (PNIPAAm), produce hydrogels by chemical cross-link, and the chemical initiator must be removed from the systems for reducing the formulation toxicity. In this sense, physical methods are preferred for hydrogels formation, resulting on the formation of gels with adequate mechanical strength and capability to modulate the drug release rate for a long period of time (Alexander et al. 2014).

One of the most accepted mechanisms for explaining the thermogellification phenomenon concerns the interaction between the copolymer units. The monomers of these copolymers, at concentrations above the critical micellar concentration (CMC), are organized in micelles in aqueous medium in order to minimize free energy. As the temperature increases, the equilibrium between micelles and unimers is favored in the direction of micellization due to the dehydration of the hydrophobic units, conferring to the system new structural organization, observed by the formation of polymeric networks. As an example, copolymers composed of PEG-PPG-PEG are self-assembled in micelles, at the critical micellar concentration (CMC), and then organized as different supramolecular structures that assume lamellar, hexagonal, and cubic and the coexistence among them (Oshiro et al. 2014;

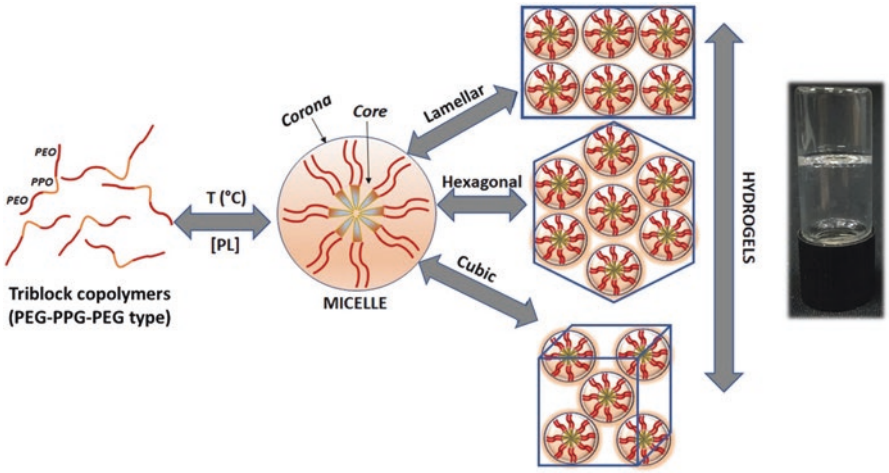

Fig. 1.2 Representation of triblock copolymers (e.g., PEG-PPG-PEG) thermoreversible self-assembly in micelles and hydrogels. In detail, the hydrogels supramolecular structures (lamellar, hexagonal, and cubic) are shown. Polyethylene glycol (PEG), propylene glycol (PPG)

Nascimento et al. 2018; Mariano et al. 2019) (Fig. 1.2). This phenomenon is reversible and below the sol-gel transition temperature (Tsol-gel), the hydrophobic chains are rehydrated, and the micelles restructured in solution (Jeong et al. 2002; Ur-Rehman et al. 2010).

Several reports have studied the thermogelation mechanisms, using a sort of techniques for characterizing the hydrogels. In special, the main employed techniques are rheology and small-angle X-ray scattering (SAXS) for determining the sol-gel transition temperature, mechanical properties, and the phase organization behavior, establishing relationships among composition, architecture, and hydrogels biological performances. Some of those studies are discussed throughout the next section.

2.1 Hydrogels Mechanical Properties and Phase Organization Studied by Rheological Analysis and Small-Angle X-Ray Scattering (SAXS): Implications on Drug-Controlled Release

Rheological studies and mechanical properties characterization are important tools to analyze hydrogel behavior, as elastic and viscous materials. It allows interpreting microscopic or internal structural changes under gradual heating or continuous shearing. The oscillatory (or dynamic) experiments are accomplished to study rheological behavior, where a sinusoidal shear is applied to the sample. Linear visco-

elastic properties can be determined by time-dependent response of soft matter in small-amplitude oscillatory shear experiments (Barbucci et al. 2002).

Elastic behavior is the material ability to restore its original shape when the external force is removed, normally referred to as elastic modulus, also known as storage modulus (G'). The viscous modulus (or shear loss modulus G") is a property that shows how any deformation ceases when there is no more an external force. In general, thermosensitive hydrogels are viscoelastic materials, which display a temperature- and concentration-dependent phase angle.

In general, hydrogels can be more elastic (non-Newtonian or Hookean material) or viscous liquid (Newtonian material), depending on value G"/G' ratio. These properties are related by the systems molecular configuration. Under the micellization temperature, unimers are dispersed in solution, which confers a sol phase. At micellization temperature, unimers begin to self-assemble in isolated micelle structures, but the system continues in sol-gel state with low G' and G" values until they reach the sol-gel transition temperature (Tsol-gel). At Tsol-gel, it starts to agglutinate micelles in more complex structures, increasing G' values, and the material is then converted in a gel state with a G' > G". Furthermore, the copolymer structure is affected by the degree of entanglement, and, if this structure is symmetric, it ensues the maximum elasticity and viscosity (Lee et al. 2009) by higher degree of solvation of micelle shell than unimers chains (Prud'homme et al. 1996).

For thermosensitive materials, the viscosity in sol phase declines slowly on warming. This is ascribed to an increase in micellization and lowered solvent viscosity. However, it is observed a steep increase in viscosity when it is near to sol-gel point transition, what can be attributed to the reduction of intermicellar space and micelle entanglements (Yokaichiya et al. 2017). As it has been seen, the rheological properties of a thermosensitive hydrogel are controlled by the micellar phase organization that is studied by small-angle scattering techniques.

Small-angle scattering (SAS) techniques characterize macromolecular structures and dimensions by the incidence of X-ray or neutron beams on electronic cloud (SAXS) or atomic nucleus (SANS), respectively (Svergun, 2010; Jacques and Trewhella 2010). These beams generate coherent secondary waves after suffering interference. Resulting waves can be destructive or constructive, which allows the formation of diffraction patterns, usually described in the form of intensity I as a function of scattering vector amplitude q:

$$q = \frac{4\pi \sin\theta}{\lambda} \tag{1.1}$$

where λ is the wavelength of incident radiation and 2θ is the angle between incident and reflected beam. SAS techniques are known as low-resolution ones because it is not possible to determine atomic coordinates (like X-ray crystallography), just the shape and size of analyzed structure.

SAXS and SANS allow the observation of the supramolecular structure and the interactions between the different functional chemical groups, since wavelengths on

the nanometer scale of X-rays and neutron beams allow the observation of interatomic interactions (Putnam et al. 2007; Imae et al. 2011). SAS techniques are ideal to study soft gel materials, since it does not require crystal form and elaborated preparations, like X-ray crystallography. Therefore, they are being used to clarify different hydrogel structures. It has been pointed out that the organization of micellar aggregates in the cubic phase exhibits gel properties; however, depending on copolymers concentrations, hexagonal and lamellar phases may be formed, although they are characteristic of anisotropic molecular ordering (Chaibundit et al. 2007; Newby et al. 2009; Ulrich et al. 2012; Basak and Bandyopadhyay 2013; Nascimento et al. 2018; Mariano et al. 2019).

Rheological alterations are also related to micellar supramolecular organization. A possible mechanism for the micellar rearrangement to occur is "hard sphere crystallization" under cubic, hexagonal, or lamellar phase, due to packing of spherical micelles, what can be evidenced by small-angle X-ray scattering (SAXS) or small-angle neutron scattering (SANS) experiments and identification of Bragg diffraction peaks (Prud'homme et al. 1996; Artzner et al. 2007; Oshiro et al. 2014). However, it is well known the concentration and temperature role on phase organization type for each copolymer type. SANS results point that micelle shells are overlapped as enhancing PL concentration. Using SANS technique is possible to follow structural transition of hydrogels, suggesting that micelles initiate to agglutinate when PEG monomers (which are in micellar corona) become more hydrophobic and break hydrogen bonds with water following the temperature increased.

Hydrogels are thixotropic materials, showing a reversible transition on their structure because of viscosity alteration induced by temperature and/or pH changes. This property is important to define the therapeutic efficacy of the hydrogel formulations to pharmaceutical purposes, due to their ability to extend retention time at the application site and to enhance the systemic bioavailability of some drugs (Ricci et al. 2005; Lee et al. 2009; Akkari et al. 2016). Sol-gel systems, which have non-Newtonian behavior, present yield values that are required to break down the semi-solid structure and to initiate the plastic flow. Then, since these yield values are increased, it is possible to indicate a gradual strengthening of the three-dimensional network structure.

The body fluid elements, mainly water, are the major factors in controlling the yield value, altering the systems structure. These elements can be diffused into the hydrogel matrix, affecting the number of cross-links formed and the hydration level. It has been demonstrated that enhancing cross-links and reducing the hydration level is possible to change the release rate of the encapsulated drugs. As viscous matrices, hydrogels are barriers to the drug release, since high viscosity hydrogels with swelled micelles tend to retain incorporated molecules for a long period of time. Thus, the PEG-based copolymer type, its molecular weight, and the addition of high-viscosity polymers into PEG-based hydrogels, forming hybrid systems with high molecular weight natural polymers (such as cellulose derivatives, hyaluronic acid), for example, can be differential factors to change materials properties for a specific biomedical purpose.

2.2 *Biomedical Applications of Thermosensitive PEG-Based Hydrogels: From Structural Organization to Biopharmaceutical Use*

Several in situ PEG-based hydrogels have been synthetized considering the insertion of biodegradable polyesters, showing to be good matrices for drug delivery systems and tissue repair. A possible disadvantage of these systems is the incorporation of thermolabile drugs, since the hydrogels preparation must be performed at low temperatures. On the other hand, PEG conjugation with high crystallinity and hydrophobicity polymers, such as PLGA and PCL, increases the drug incorporation percentage and changes the hydrogels structural organization, their morphology, and degradation rate (Deng et al. 2019). Regarding the biocompatibility, other PEG-based copolymers, PEG-PPG-PEG-based copolymers (such as poloxamers and poloxamines), different safety studies in clinical practice have reported their approval by FDA and use as pharmaceutical excipients (Cho et al. 2012). In this section, it will be discussed the influence of structural and composition parameters on PEG-derivatives hydrogels biomedical applications and implications when associated with other biodegradable polymers (hyaluronic acid, poly(N-(2-hydroxypropyl)) methacrylamide mono-/dilactate) and/or forming hybrid systems with laponite, gold nanoparticles, and liposomes among other nanocarriers. Although the formulation and physicochemical characterization of PEG-based hydrogels have been reported by several studies, the relationships between chemical modifications on PEG molecule and its biomedical application have been discussed on few studies.

The synthesis of PEG-PCL-PEG hydrogels as delivery systems for timolol maleate was reported by Mishra et al. (2011). In this study, comparisons with PVA showed more pronounced sol-gel transition temperatures and low cytotoxic effects in rabbit corneal epithelial culture cells when compared to PVA. In fact, the gelation of PEG-PCL-PEG polymers is dependent on the length and molecular weight of the PCL units, since hydrophobic interactions are the main driving forces observed on reversible sol-gel transitions (Deng et al. 2019). In a similar study, PEG-PCL-PEG hydrogels were reported as insulin delivery system. The rheological characterization was strictly related to the formulation injectability, since it was observed a Newtonian flow behavior with low viscosity (for 20% and 25% PEG-PCL-PEG) and a shear rate-dependent flow, as also observed for PEG-PPG-PEG hydrogels (such as poloxamer 407) (Payyappilly et al. 2014).

In attempt to observe the impact of structural parameters (molecular weight and the ratio of PEG-PCL blocks) on sol-gel transition, hydrogels formulations were also tested as scaffolds on highly porous surface for cell attachments obtaining promising results related to the maintenance of chondrocytes morphology, enhancing the cartilage regeneration, and providing a mechanically functional extracellular matrix (Deng et al. 2019). For other copolymer types, Alexander et al. (2014) described the preparation of PEG-PLGA-PEG-based hydrogels compared with PEG-PPG-PEG, regarding the dissolution rates, since PEG-PPG-PEG are promptly

removed from the site of injection, reducing their performance as in situ depot formulations related to PEG-PLGA-PEG. Other important advantage attributed to those polymers is the high PEG blocks biocompatibility, while PLGA blocks provide the molecule biodegradability due to the presence of ester links (Zentner et al. 2001).

The association between PEG and PLGA was also used as other triblock architectures, PLGA-PEG-PLGA. In special, a recent work showed the release of collagenase and trastuzumab controlled by those hydrogels looking forward antitumor efficacy in breast cancer (Pan et al. 2018). Also, the authors stated that the peritumoral administration is a potential strategy for the modulation collagen-rich extracellular matrix in solid tumors, provided by the enhancement of the interstitial transport after collagenase administration and the antibody efficacy. Other interesting result from this study was the comparison with clinical treatment regimens by the evaluation of the pre-formulation pharmacological effects in relation to hyaluronidase in combination to trastuzumab, since the hydrogel was able to trigger the intra-tumoral collagen degradation (Pan et al. 2018).

In this sense, the conjugation of cyclized succinyl ester groups into a PEG hydrogel matrix was proposed as bioadhesive medical sealant device for in vivo hemostasis, with the advantage of easy removal without causing tissue damage by mechanical debridement or surgical excision, enhancing the hemorrhagic control after administration in patients treated with anticoagulants (Bu et al. 2019). The use as implants was also investigated for infection prevention by Casadidio et al. (2018), when reported the development of hydrogels composed of vinylsulfonated triblock-PEG copolymers cross-linked with thiolated hyaluronic acid. The system was proposed for daptomycin local delivery in the management of implant-associated infection with the additional capability to reduce the drug chemical degradation, controlling the release rate and enhancing the in vitro antibiofilm activity against *S. aureus*. The association between vinylsulfonated triblock-PEG copolymers and thiolated hyaluronic acid was investigated as hydrogels for intra-articular injection in the management of osteoarthritis (Agas et al. 2019).

In other reports, hyaluronic acid was incorporated into poloxamer-based (PEG-PPG-PEG) hydrogels, organized as binary systems composed of poloxamer 407 and its more hydrophilic analog, poloxamer 338 aiming intra-articular therapy (Nascimento et al. 2018). The main observation from this study was the influence of hyaluronic acid on hydrogels phase organization, since SAXS patterns revealed transitions from lamellar to hexagonal phase and structural changes from cubic to gyroid and/or cubic to lamellar but maintaining the hydrogel-thermosensitive properties. Furthermore, the hybrid systems hyaluronic acid-PEG-PPG-PEG reduced in vitro cytotoxic effects, pointing their possible application as intra-articular drug delivery systems. In a similar report, the thermoreversible supramolecular assembly was observed for alpha-cyclodextrin incorporated to PEG-betulinic acid-hydroxycamptothecin, but the sol-gel transitions were determined by the length of PEG chains and the ratio between the drug-loaded micelles and alpha-cyclodextrin (Dai et al. 2017). This structural organization induced the sustained drug release,

enhanced the drug aqueous solubility, and showed appropriate micellar size for inducing a possible EPR effect.

Indeed, the development of PEG-based hybrid systems seems to be a tendency in the last years, since different reports discussed the formation of nanocomposites by associating laponite with PEG-PLGA diblock copolymers (Maeda et al. 2019). However, the insertion of laponite reduced the sol-gel transition temperature and caused a thermoresponsive concentration-dependent effect due to the adsorption of PEG-PLGA micelles on the laponite surface, as observed by SANS analysis. On the other hand, the incorporation of poly(allylamine)-grafted gold nanoparticles into PEG-PPG-PEG hydrogels did not show remarkable structural changes but demonstrated pronounced wound healing properties upon topical application for antibacterial activity (Mahmoud et al. 2019). Similar results were also obtained for hybrid systems composed of liposomal doxorubicin and PLGA-PEG-PLGA hydrogels (Cao et al. 2019), implying that the adequate rheological properties and viscosity allowed the use of this system for peritumor injection, which will improve the drug therapeutic effect and reduce its systemic toxicity.

Despite the promising biomedical applications as drug delivery systems or injectable cell scaffolds, PEG-PLGA-based triblock copolymers (or their derivative PLGA-PEG) show water solubility dependent on PLGA content compared to PEG, indicating an ideal PEG/PLGA ratio of 0.56 for obtaining a thermoresponsive hydrogel with appropriate aqueous solubility for injectable administration (Maeda et al. 2019). Other important feature is that during the synthesis process, molecular weight among cross-links should be studied by rheology in order to produce polymers with adequate elastic/viscous (G'/G") moduli relationships for gel or fluid formulations, since low viscosity at high shear rate is critical for painless injection (Payyappilly et al. 2014; Bu et al. 2019). In this context, some essential characteristics for adequate hydrogels biomedical performance and applications are summarized in Fig. 1.3.

2.3 pH-Sensitive PEG-Based Hydrogels: Theoretical Principles in pH-Sensitive Delivery Systems

Among the different chemical issues that influence a drug delivery system, the organ's pH arises as a critical property. For example, for a drug to come into the stomach, if it is taken orally, the drug should be able to withstand the alkaline salivary pH and then reach the stomach that, on the contrary, has an acidic pH. Most of the drugs are sensitive to pH and may lose their folding and therapeutic effect when in an inappropriate chemical environment. In this case, drug delivery systems are designed to hold the alkaline salivary pH and to deliver the drug into the stomach as it reaches an acidic pH (Liu et al. 2017). These systems are so-called as pH-sensitive or pH-responsive.

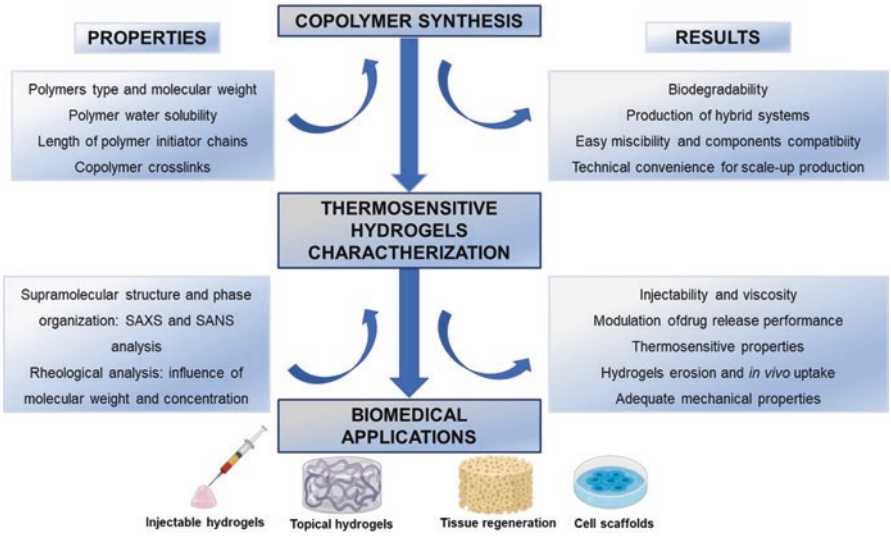

Fig. 1.3 Scheme of the main properties and results obtained for thermosensitive hydrogels synthesis and physicochemical characterization before their biomedical performance evaluation

In order to overcome the challenge of producing materials capable of resisting changes in the chemical environment in the human body, different pH-sensitive systems have been proposed in the literature (Liu et al. 2014, 2017). Currently, pH-sensitive drug delivery systems are designed by using inorganic, organic, or hybrid materials (a mixture of inorganic and organic materials) (Fig. 1.4).

Regarding inorganic materials, bioceramics like calcium phosphate (amorphous or crystalline hydroxyapatite) and zirconium oxide (ZrO_2) nanoparticles have been proposed as promising pH-sensitive carriers because of their high dissolution kinetics in acidic pH. It means that these ceramics can withstand alkaline pH, and delivery drugs at acidic pH, when they are degraded (Banerjee et al. 2011).

The class of pH-sensitive organic materials includes mostly polymers, liposomes, and micelles. If, on the one hand, inorganic materials become pH-sensitive because of their dissolution kinetic, organic materials, on the other hand, become pH-sensitive due to specific ionizable chemical groups found in their structure. Not all the organic molecules or polymers can become pH-sensitive, but as long as some ionizable chemical groups are grafted or functionalized into their structure, they can do so. Some of these ionizable chemical groups are carboxylic acids, amines, and phosphoric acids, among others. The fact that these chemical groups are ionizable means that they can be negatively or positively charged by donating or accepting protons, respectively (Shriver and Atkins 1999). Such property is related to their acid dissociation constant (Ka, which is more commonly referred to as pKa that, in turn, is its logarithmic representation), which consists of the equilibrium constant for a dissociation reaction in the context of an acid-base reaction (Shriver and Atkins 1999). When these organic molecules are either protonated or deprotonated due to

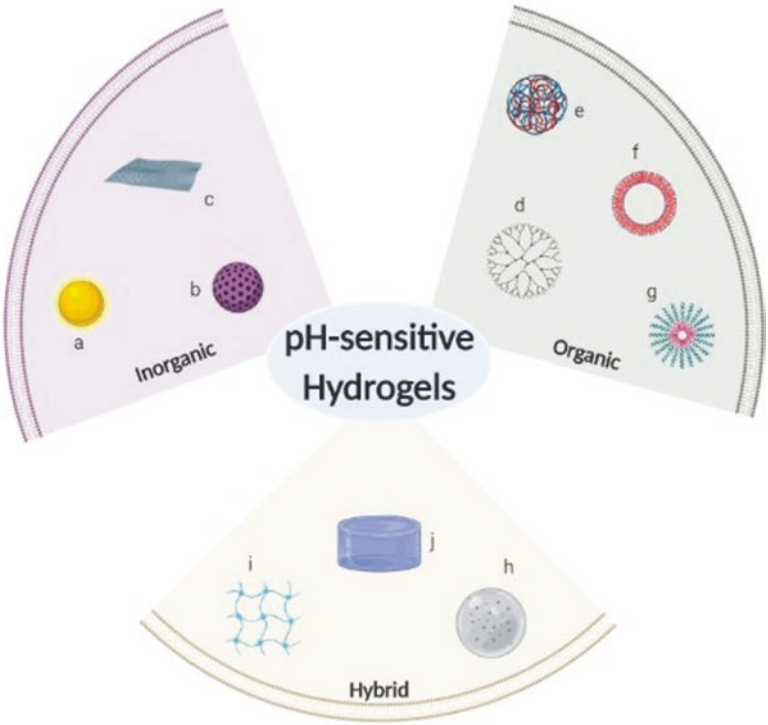

Fig. 1.4 The three main classes of materials used as pH-sensitive systems: inorganic (a, gold nanoparticles; b, zirconium oxide; c, hydroxyapatite delivery), organic (d, dendrimers; e, polymeric nanoparticles; f, liposomes; g, polymeric micelles), and hybrid systems (h, nanoparticles in hydrogel matrix; i, polymeric cross-linked hydrogels; j, micellar hydrogels)

their pKa and the pH of their environment, they can undergo three different conformational changes: (1) dissociation, (2) destabilization (by collapsing or swelling), and (3) changes in the partition coefficient between the vehicle and drug (Liu et al. 2014). Therefore, if these organic molecules are used to carry a drug into a specific site, they can deliver the drug when they suffer any of these conformational changes, releasing the drug into the desired environment.

Regarding polymeric materials, when such ionizable chemical groups are present in their structure, these polymers can either become cationic or anionic polymers. The names "cationic" or "anionic" polymers rely on the ability of the organic macromolecule to be ionizable at more acidic or basic pH, respectively. Also, there are some specificities about what type of ionizable chemical group can be found in these polymers. Usually, amino groups are used to produce cationic macromolecules, while carboxyl groups are used to produce anionic ones.

Cationic polymers with amino groups are more degradable in aqueous solution at acidic pH than in basic ones. As an illustration, aminoalkyl methacrylate copolymer (Eudragit E) is a Food and Drug Administration (FDA)-approved cationic

polymer having high solubility below pH 5. On the other hand, anionic polymers with carboxyl groups are more degradable in basic aqueous solution than in acidic pH. For example, poly(methacrylic acid-co-methyl methacrylate) (Eudragit L, S, and F), hydroxypropylmethylcellulose phthalate (HPMC-P), and HPMC acetate succinate (HPMC-AS) are conventional anionic polymers.

However, some polymers are neither cationic nor anionic, but it is not a limitation. Other strategies commonly employed to produce pH-sensitive polymers include functionalization and block copolymers. As long as there are innumerous polymers used in drug delivery applications, of course, the range of possibilities concerning pH-sensitive materials is extensive. Therefore, in the next sections, we shall keep our focus only on PEG-based pH-sensitive drug delivery systems. Despite all the almost infinite polymer candidate for pH-sensitive drug delivery systems, PEG has some advantages compared to other polymers, besides being an FDA-approved polymer and well-known behavior in the human body. Also, PEG is not an ionizable polymer, but some polymeric engineering techniques are used to make it pH-sensitive. We will explain why PEG is a proper polymer to be used in such delivery systems, as well as show what kind of applications are enabled when drug delivery systems based on PEG are employed.

2.4 Strategies to Make PEG pH-Sensitive: Chemical Modifications and Their Biomedical Applications

When referring to a pH-sensitive drug delivery system, PEG is often used in micellar assemblies constituted of a core-shell structure (Fig. 1.5). The main advantage of using PEG in such systems is the fact that PEG is highly hydrophilic, enabling enhanced permeability and retention (commonly referred to as EPR effect) in the bloodstream (Kale and Torchilin 2007; Lang et al. 2019). The EPR effect is responsible for making the core-shell structures to flow in the bloodstream for longer times, which increase the possibilities to the delivery the drug into the specific target. Also, PEG is not recognized as a foreign body by macrophages of the immune system, which enables it to keep in the bloodstream for extra time compared to other polymers (Zambanini et al. 2017). By being held in the bloodstream for a longer time, the drug delivery system can reach the target organ and be even absorbed by cells of a specific tissue. Besides, such stability in the bloodstream enables the usage of PEG-based drug delivery as an injectable system.

As aforementioned, PEG is not a pH-sensitive polymer by itself, which means that its chemical structure lacks in ionizable functional groups. However, three different strategies can be used to transform PEG-based systems into pH-sensitive ones:

(a) Produce copolymers containing anionic or cationic polymer chains bonded to PEG chains.
(b) Add chemical modifications into the PEG structure.

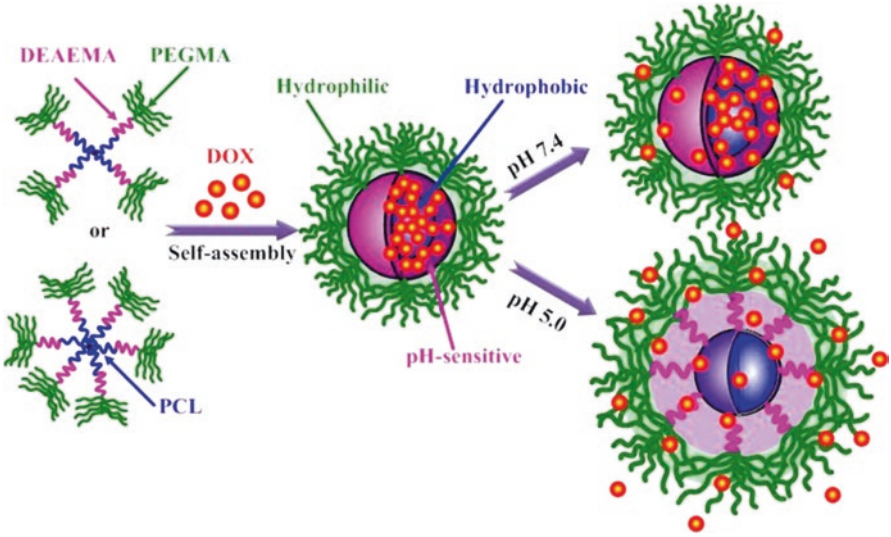

Fig. 1.5 Example of a core-shell structure that contains PEG in a triblock-polymer structure with PCL (poly(ε-caprolactone)) and PDEAEMA (poly (2-(diethylamino)ethyl methacrylate)). This later portion is pH-sensitive. The amino groups in the PDEAEMA structure, the core-shell complex, become pH-sensitive at low pH, when the PDEAEMA structure is destabilized, and the drug is released. Note that in the core-shell complex, the PEG portion is kept in the outer part, enabling the system to take advantage of all of the PEG biological properties. (Yang et al. 2013)

Regarding pH-sensitive PEG-based copolymers, peptides are often used as the blockchain containing the ionizable chemical. Peptides are made of amino acids, while amino acids are organic molecules containing an amine ($-NH_2$) and carboxyl groups (-COOH) in their structure. Then, because of the deprotonation of the amine and the carboxyl groups at different pH, peptides may display a more cationic or anionic polymer behavior depending on their structure. For example, arginine, histidine, and lysine are positively charged amino acids, and consequently, their polymers counterparts display a cationic polymer role in copolymer structures. On the other hand, aspartic acid and glutamic acid are negatively charged amino acids, and their polymers counterparts display an anionic polymer role in copolymers.

There are several works which employed PEG-based copolymers employed as block, as grafted, or even as a combination of block and graft. For example, in work carried out by Lim et al. (2019), both strategies – graft and block copolymer – were used to produce a carrier system to deliver DOX (doxorubicin) and chlorin e6 into cancer sites. To do so, they produced a drug delivery system based on an ionomer polymer that was a result of complexation of two copolymers. The first was composed of PEG-PLL(-g-Ce6) [chlorin e6 grafted poly(ethylene glycol)-poly($_L$-lysine)], and PEG-PLL composed another system (-g-DMA)-PLA [2,3-dimethylmaleic anhydride grafted poly(ethylene glycol)-poly(L-lysine)-poly(lactic acid)]. Note that both copolymers are made of a diblock or triblock main chain grafted with another block. Because of the ionizable chemical bonds found in

the PLL (poly-l-lysine) – that is, a cationic polymer – the carrier system was able to collapse at pH around 6, where the chemical bond between the dimethyl maleic anhydride and the PLL is broken, and the drug is released.

While the block bonded to PEG is responsible for triggering the delivery of a drug, it is not only the unique property related to the drug release kinetics. Note that the pH sensitivity is an ability only related to the chemical structure of the host block, but it will not govern the release kinetics. The drug delivery kinetics will depend on the length of the host block and its chemical composition. Such effect was very clear in a work carried out by Mostoufi et al. (2019), who studied the release kinetics of paclitaxel from series of hybrid diblock copolymers methoxy-poly(ethylene glycol)-b-poly(γ-benzyl-L-glutamic acid) (PEG-PBLG) and triblock copolymers of poly(ethylene glycol)-b-poly(L-glutamic acid-co-NULL-leucine) (PEG-PGA-PLeu). In such study, the glutamic acid chains are ionizable at acidic pH; the authors changed the length of the leucine chain, which is not ionizable. It was noted that a higher pH responsiveness was correlated to the longer hydrophobic non-ionizable segment, Pleu (Fig. 1.6).

The chemical modification enables the adding of functional chemical groups or ionizable molecules in a polymer structure. It is a strategy commonly used to turn non-ionizable polymers into pH-sensitive by introducing ionizable species

Regarding functionalization, the structure of PEG can be modified by adding COOH or NH_2 group at the end of the PEG chain (Zhang et al. 2019). The pH-sensitive hybrid system, composed of graphene oxide (GO) and PEG-COOH as a coating agent, showed to be an effective drug delivery system to carrier doxorubicin (Zhang et al. 2019).

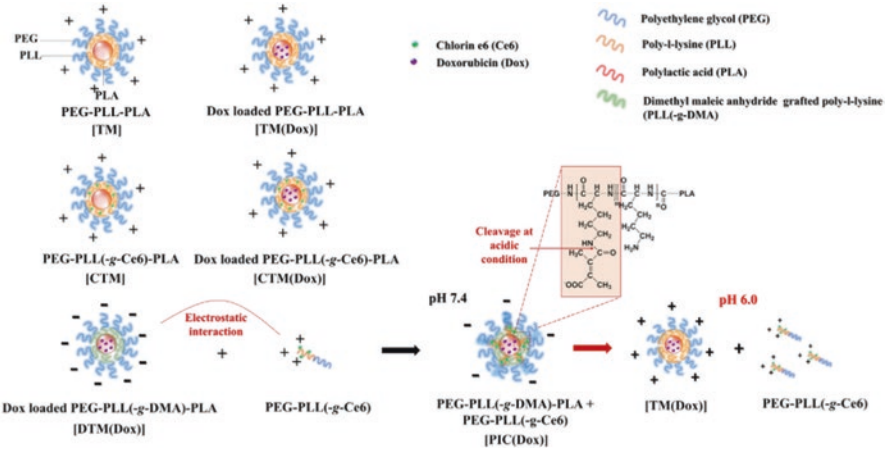

Fig. 1.6 Ionomer polymer composed of two copolymers PEG-PLL(-g-Ce6) and PEG-PLL(-g-DMA)-PLA. The copolymers were prepared in separate and then complexed together to carry doxorubicin and Chlorin e6. When the complex reaches a pH lower than the normal pH of the human body (7.4), the chemical bond between the DMA and PLL is broken, which results in a collapse of the complex structure. Afterward, the drugs are released in the target site. (Lim et al. 2019)

Concerning chemical modification using molecules, usually, ionizable molecules are used to crease acidic or basic liable bonds, which are bonded to a specific drug. Then, when the drug delivery system reaches the target tissue, the drug is released. For example, in work carried out by Chen et al. (2019), the author produced a drug delivery system based on monomethoxypoly(ethylene glycol)-poly(L-lysine)-graft-dimethyl maleic anhydride (PEG-PLL-DMA). The chemical bond between DMA and PLL is liable at slight acid condition, leading to the formation of an NH^{3+} species when the system reaches pH around 6.5 and DMA is also released in the medium.

The applications of pH-sensitive polymers are often focused on the delivery of drugs into tissues that exhibit pH different from that of the physiological fluid (White et al. 2017). In this case, the most used application is on cancer treatment. The cancer cell has low extracellular pH and, consequently, higher intracellular pH compared to healthy cells. Then, many types of research have used pH-sensitive systems to delivery doxorubicin, paclitaxel, and azoreductase, among other drugs (Chen et al. 2019; Cui et al. 2019; Ma et al. 2019; Mostoufi et al. 2019; Yang et al. 2019). In addition, because of the higher specificity of pH-sensitive drug delivery system, they are often used allied to other therapies like photothermal therapy and chemotherapy or used with luminescent molecules that enable the combination of therapy and diagnostic (also known as theranostic) (Liu et al. 2019; Pei et al. 2019; Zhang et al. 2019). For example, in a work carried out by Pei et al. (2019), they produced a drug delivery system based on emulsion copolymerization of glycidyl methacrylate (GMA), poly(ethylene glycol) methyl ether methacrylate (PEGMA), and N-rhodamine 6G-ethyl-acrylamide (Rh6GEAm) with N,N-bis(acryloyl)cystamine) (BACy) as disulfide cross-linker, followed with conjugating DOX via an acid-labile hydrazone linkage (Fig. 1.7). The acid-labile hydrazine linkage enables

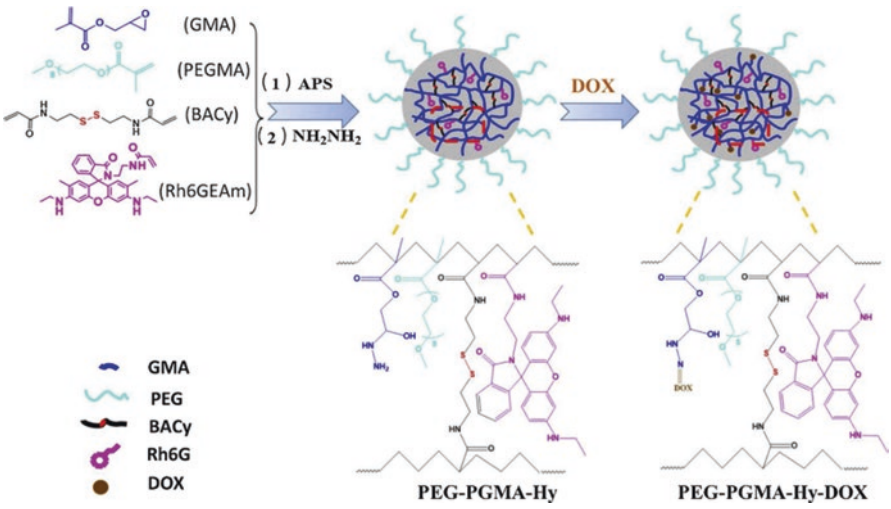

Fig. 1.7 Schematic representation of PEG-PGMA microspheres and PEG-PGMA-Hy-DOX prodrug microspheres. (Pei et al. 2019)

the release of DOX only at acid pH, while Rh6G emits fluorescence only at acidic pH. Then, such drug delivery could be used as a theranostic due to its ability to treat cancer and produce detectable fluorescence.

Another exciting application of pH-sensitive drug delivery systems includes the utilization of a system with bactericidal properties. Bacteria biofilms usually exhibit pH different that of the physiological body fluid, which can be used as a strategy to target them. For example, Zhao et al. (2019) studied a carrier system to delivery chlorhexidine (CHX) in cariogenic biofilm. The drug delivery system was based on cationic poly(ethylene glycol)-block-poly(2-(((2-aminoethyl)carbamoyl)oxy)ethyl methacrylate) (PEG-b-PAECOEMA), and PAECOEMA was modified by citraconic anhydride (CA), forming negatively charged PEG-b-PAECOEMA/CA. The citraconic amides of PEG-b-PAECOEMA/CA block copolymer cleave in acidic medium and accomplish negative to positive charge conversion in a short time. The drug delivery system showed to be effective against *Streptococcus mutans*, and the cytotoxicity of CHX was reduced because of the micellar structure.

3 Conclusion and Prospects

PEG-based materials are one of the most investigated matrices in the fields of thermo- and pH-sensitive systems. Important advances have been achieved particularly on the development of drug delivery systems and tissue regeneration. However, the systems components choice and their synthesis control are the driving conditions for obtaining appropriate structural organization and mechanical properties considering the biomedical applications proposed. The most used techniques for characterizing those systems are small-angle scattering (X-ray and/or neutrons) and rheology. By controlling the hydrogels phase organization and viscoelastic properties, it is possible to obtain systems capable to modulate the drug release rate, dissolution kinetics, bioadhesion, and the in vivo sol-gel transition process. The conjugation of PEG with different synthetic polymers described in this chapter allowed the production of hydrogels systems responsive to physiological (as injectable temperature-sensitive hydrogels) or physiopathological conditions (e.g., pH-responsive hydrogels proposed as therapeutic strategies for acid biological environment). Other exciting potential for the use of PEG-based hydrogels is its approval by FDA, highlighting the potential safety of PEG-derivatives copolymers in biomedical applications. Additionally, all matrices developed must be controlled regrading the synthesis process, physicochemical characterization, components compatibility, and local or systemic toxicity, being of high interest in the fields of biotechnology, biomedicine, engineering, and medicine.

Acknowledgments The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, finance code 001), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2014/14457-5), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 309207/2016-9, 402838/2016-5, 303946/2018-0).

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