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Smart poly(2-hydroxyethyl methacrylate/itaconic acid) hydrogels for biomedical application

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ABSTRACT

pH- and temperature-sensitive hydrogels, based on 2-hydroxyethyl methacrylate (HEMA) and itaconic acid (IA) copolymers, were prepared by γ-irradiation and characterized in order to examine their potential use in biomedical applications. The influence of comonomer ratio in these smart copolymers on their morphology, mechanical and thermal properties, biocompatibility and microbe penetration capability was investigated. The mechanical properties of copolymers were investigated using the dynamic mechanical analysis (DMA), while their thermal properties and morphology were examined by thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). The morphology, mechanical and thermal properties of these hydrogels were found to be suitable for most requirements of biomedical applications. The *in vitro* study of P(HEMA/IA) biocompatibility showed no evidence of cell toxicity nor any considerable hemolytic activity. Furthermore, the microbe penetration test showed that neither *Staphylococcus aureus* nor *Escherichia coli* passed through the hydogel dressing; thus the P(HEMA/IA) dressing could be considered a good barrier against microbes. All results indicate that stimuli-responsive P(HEMA/IA) hydrogels have great potential for biomedical applications, especially for skin treatment and wound dressings.

1. Introduction

Hydrogels based on 2-hydroxyethyl methacrylate copolymers are of a great interest in biomedical applications because of their tunable chemical composition and three-dimensional network structure. They can be designed to have optimal water or biological fluid content in an aqueous medium without dissolution, good mechanical properties, permeability to oxygen, biocompatibility, shape stability and softness similar to that of the soft surrounding tissue. These materials are also interesting because of good chemical and biochemical stability, absence of extractables, and high permeability for water-soluble nutrients and metabolites (Safrany, 1997; Rosiak and Yoshii, 1999; Yu and Ober, 2003). Therefore, a thorough understanding of fluid–polymeric hydrogel interactions and related physicochemical and biomedical phenomena of such systems is critical for the development of these materials for biomedical applications. Intelligent or smart hydrogels have been developed as stimuli-responsive materials, which can undergo volume changes in response to changes in temperature, pH, pressure, when exposed to a biological target (antigen, nutrient, growth factor, receptor, antibody, enzyme, or whole cell) (Zhang et al., 2001). These unique characteristics are of great importance in wound healing, cell encapsulation, drug delivery, and tissue engineering (Sen and Guven, 1999; Tomić et al., 2007; Alarcon et al., 2005). Poly(2-hydroxyethyl methacrylate) (PHEMA) is also favorable because of its excellent biocompatibility and physicochemical properties similar to those of living tissues (Brahim et al., 2003; Lahooti and Sefton, 2000). It also exhibits good chemical and hydrolytic stability and good tolerance for entrapped cells. Because of these characteristics, PHEMA is one of the most extensively studied materials in tissue engineering (Brahim et al., 2003; Lahooti and Sefton, 2000). It has also been widely used as the backbone for synthesizing stimuli-responsive hydrogels (Young et al., 1998). Numerous studies have been conducted to modify PHEMA with the aim of improving its mechanical properties (Young et al., 1998; Gursel et al., 1998; Johnson et al., 2004), its electro-responsive properties (Guiseppi-Elie et al., 1998) and of eliciting better physiological responses (Kabra et al., 1991). Copolymers of HEMA with methacrylic (Garcia et al., 2004; Brazel and Peppas, 1999) and acrylic acid (Johnson et al., 2004; Basan et al., 2002; am Ende and Peppas, 1997), as pH sensitive components, and some itaconic acid mono alkyl esters (Barcellos et al., 2000) have been reported previously. On the other hand, the potential for substitution of acrylic and methacrylic acid in hydrogels with itaconic acid is high. Itaconic acid easily copolymerizes and

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provides polymer chains with carboxylic side groups, which are highly hydrophilic and able to form hydrogen bonds with corresponding groups. Unlike monocarboxylic methacrylic and acrylic acid, itaconic acid has two COOH groups with different pK_a (pK_{a1}=3.85 and 5.45) values, so that very small amounts of IA, smaller than those of acrylic acid, render good pH sensitivity and increase in the degree of hydrogel swelling (Tasdelen et al., 2004; Sen and Yakar, 2001; Tomić et al., 2005). In addition, incorporation of comonomers which can contribute to H-bonding can increase the mechanical strength of the hydrogel (Peppas et al., 2000). Furthermore, IA is very hydrophilic and is expected to show high biocompatibility because of its natural source. Coupling of temperature-responsive and pH-sensitive components allows a flexible control over the polymer phase behaviour, resulting in multifunctional smart materials such as P(HEMA/IA) copolymeric hydrogels.

Investigation of swelling, mechanical and thermal properties and morphology is only the first step in biomedical application of hydrogels. As a next step, it is necessary to perform a great number of *in vitro* and *in vivo* investigations in accordance with specific biomedical applications of such materials. High and unique requirements and characteristics are indispensable for application of such systems in drug delivery, cell encapsulation or tissue engineering. The long-term potency of any implied medical device and its functional efficiency ideally require its anatomical (in some cases even histological) and functional integration into the host tissues and/or organ with which it is put in relation. The clinical application of a biomaterial should not cause any adverse reaction in the organism and should not endanger the life of the patient; any material to be used as part of a biomaterial device has to be biocompatible. The definition of biocompatibility implies that the material has to be nontoxic, non-allergenic, non-carcinogenic, and non-mutagenic, and that it does not influence the fertility of the patient (Williams, 1986). In general, hydrogels have good biocompatibility (Montheard et al., 1992; Park and Park, 1996; Coleman et al., 1982), as evidenced by their prolific use in a wide variety of biomedical applications such as ophthalmic and vascular prostheses (Arora et al., 2004; West and Hubbell, 1996), drug delivery systems (Bos et al., 2004; Nam et al., 2004), soft tissue replacement (Woerly et al., 1996), wound dressings (Jones and Vaughan, 2005), and scaffolds (Nguyen and West, 2002). The interaction of hydrogels with tissue and body fluids is of interest because of their high application potential. Cells and proteins generally display a low tendency for adhesion to hydrogel surfaces because of the low interfacial free energy of the hydrogels when in contact with body fluids (ISO document, 1992a). It is well known that hydrogels show good tissue compatibility upon implantation and are non- or only slightly thrombogenic upon contact with blood. Also, tissue-like consistency of hydrogels minimizes frictional irritation upon implantation. However, cytotoxicity *in vitro* assay is the first test to evaluate the biocompatibility of any material for use in biomedical devices (ISO document, 1992a). Furthermore, investigation of hemolytical activity can also give valuable information about biocompatibility.

Hydrogel dressings could be considered good barriers against microbes and this characteristic is important, especially in protecting the wound from further infection, which may accelerate the healing of the wound (Nho and Park, 2002; Yu et al., 2007). Wound dressings based on polymeric hydrogels were first invented by Rosiak's group (Rosiak et al., 1989). This hydrogel system was based on simultaneous crosslinking and sterilization by radiation of the mixture of medical grade poly(vinyl pirrolidone), poly(ethylene glycol), and agar polymers. Prevention of the appearance of versatile microorganisms is very important not only for biomedical applications, such as burn bandages and wound dressings, contact lenses, artificial skin and in drug release systems, but also for some cosmetic and pharmaceutical applications (Rosiak and Yoshii, 1999; Rosiak et al., 1989). Early onset infections are generally caused by different bacteria that have entered in contact with tissue and/or body fluids during different biomaterial applications; for example, the majority of deep tissue implant infections are caused by *S. aureus*, *S. epidermidis*, and *P. aeruginosa* (Wilson et al., 1982; De Gevigney et al., 1995; Chastre and Trouillet, 1995; Potera, 1999). Therefore, antibacterial activity is one of the most valuable properties in the field of biomedical applications.

Radiation-induced synthesis of new copolymeric P(HEMA/IA) hydrogels with different IA contents, and investigation of their temperature- and pH-sensitive swelling behaviour and drug release properties were reported in our previous paper (Tomić et al., 2007). In the present study, these smart P(HEMA/IA) copolymers, with IA content up to 5 mol%, were investigated in order to evaluate their potential use in biomedical applications. Our studies were focused on mechanical and thermal properties and biocompatibility evaluation, as well as on the influence of IA content on these properties. The biocompatibility was investigated by determining the *in vitro* cytotoxicity and hemolytic activity. *In vivo* biocompatibility (skin irritation, sensitization, subcutaneous, etc.) studies on these materials (to be used in contact with skin) are currently in progress. Microbe penetration testing of P(HEMA/IA) hydrogels was performed as well, using Gram-positive (*S. aureus)* and Gram-negative bacteria *(E. coli)*.

2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) (Aldrich), itaconic acid (IA) (Aldrich) and ethylene glycol dimethacrylate (EGDMA) (Aldrich) were used as reactants in the synthesis of the hydrogels. Prior to use, the HEMA monomer was double distilled, under vacuum. Buffer solution was prepared using hydrochloric acid (La Chema), potassium mono and dihydrogenphosphate (Fluka) and sodium hydroxide (Fluka). Demineralized water was used for all polymerizations and the preparation of the buffer solution.

2.2. Hydrogel preparation

The P(HEMA/IA) hydrogels were synthesized by the γ-radiation-induced radical copolymerization (Tomić et al., 2007). The monomers were dissolved in 10 ml of a water/ethanol mixture. The IA mole fractions were 2.0, 3.5 and 5.0, and according to the monomer mole fraction samples were designated as P(HEMA/2IA), P(HEMA/3.5IA) and P(HEMA/5IA), respectively. The reaction mixture was degassed prior to polymerization and placed between two glass plates, sealed with a spacer. The monomer solutions were irradiated in a ^{60}Co radiation source, under ambient conditions, at a dose rate of 0.5 kGy/h, to an absorbed dose of 25 kGy. Further, the hydrogels were immersed in deionized water, which was changed every day, for 1 week, to remove any unreacted chemicals. The gels were cut into discs (5 mm in diameter, 1 mm thick) and dried at room temperature to constant mass.

2.3. Dynamic mechanical analysis (DMA)

Strain-frequency sweeps were performed on hydrogel discs using a Rheometrics 605 mechanical spectrometer, with parallel plates geometry (25 mm in diameter). The shear modulus was