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Effects of gamma rays and neutron irradiation on the glucose response of boronic acid-containing "smart" hydrogels

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ABSTRACT

If a biomaterial is to be implanted in the body, it must be subjected to a sterilization procedure which often involves gamma irradiation. We report results for the effects of γ-irradiation on the glucose response of a hydrogel with glucose-binding boronic acid moieties. This 'smart" hydrogel is of a type suitable for use in non-enzymatic glucose sensors. In addition, the effect of neutron irradiation on the glucose response of these hydrogels is also of interest, because the hydrogels could be used with minor modification to deliver boron to tumors during boron neutron capture therapy (BNCT). We show that the glucose response of the smart hydrogels is unaffected by exposure to neutrons in the dose range typical for BNCT. The effect of gamma rays on the glucose response depends on the method used to cure the smart hydrogel. If the hydrogel is cured with a thermal free-radical-initiator, then the hydrogel can be sterilized by gamma irradiation with no adverse effects upon the glucose response. However, if the hydrogel is cured with a UV-initiated free radical initiator, then the glucose response decreases in magnitude with increase in the gamma radiation dose.

1. Introduction

Radiation sterilization is a common means of microbial control and sterilization for single-use bioprocess systems and devices [1]. Gamma irradiation is the application of electromagnetic radiation emitted from radionuclides such as Cobalt 60 or Cesium 137 isotopes. This ionizing radiation damages the nucleic acids of microorganisms, and leaves no residual radioactivity within the biomaterial or device. In order to satisfy the sterility assurance level of the United States Pharmacopeia, the recommended gamma dose is 25.7 kGy or larger [1].

Boron neutron capture therapy (BNCT) is a treatment protocol for cancer in which non-radioactive boron atoms (isotopic ^{10}B) are delivered to tumors and then exposed to thermal neutrons, such as those produced by nuclear reactors [2]. The resultant fission reactions produce short-ranged alpha-particles that destroy all cells located within 10 μm of the ^{10}B nuclei. Currently, low molecular weight pharmaceuticals such as boronophenylalanine (BPA) or sodium borocaptate (BSH) are used to deliver ^{10}B nuclei to the site of a tumor. However, because the concentration of ^{10}B nuclei that can be delivered by BPA or BSH to tumor cells is limited to about 100 ppm, there is interest in increasing BNCT therapeutic effectiveness by using high molecular weight delivery agents that contain multiple boron atoms such as polymers, dendrimers, or micelles [3].

One possible type of high molecular weight agent for delivering high concentrations of boron atoms to tumors is a polymer hydrogel nanoparticle containing the monomer 3-acrylamido phenylboronic acid (3-APB), as synthesized by precipitation polymerization [4]. A hydrogel of this type is also glucose-responsive, meaning that it has a degree of equilibrium swelling that reversibly changes in response to the environmental glucose concentration [5–8], and thus can be used in a wireless non-enzymatic glucose sensor when coupled with a suitable transduction mechanism [7]. The hydrogel is glucose responsive because the phenylboronic acid moieties present in the hydrogel reversibly bind to glucose molecules. Enzymatic glucose-responsive hydrogels, on the other hand, employ the enzyme *glucose oxidase* to bind glucose, and cannot be exposed to sterilization protocols involving gamma irradiation, because the gamma rays will degrade the enzyme. Hence the first aim of the current study is to determine whether or not glucose-responsive hydrogels that employ phenylboronic acid moieties to reversibly bind glucose can be subjected to γ-sterilization without loss of glucose sensitivity. The second aim of the current study is to

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determine whether or not the same hydrogel retains its glucose sensitivity when subjected to simultaneous irradiation by both thermal neutrons and gamma rays, at the relative dose levels expected to be present during BNCT. In previous studies, the effect of gamma sterilization on the water content, swelling pressure, or drug release rate from polymer hydrogels has been studied Fernandez-Carballido et al. [9], Kanjickal et al. [10], Elijarrat-Binstock et al. [11], and Guenther et al. [12]. Generally, γ-irradiation is found to be less disruptive to hydrogel properties than competing sterilization technologies, particularly when γ−irradiation is performed on freeze-dried samples at lower temperatures [9,11]. Nonetheless, ESR [13] and ATR-FTIR measurements [14] clearly show that γ-irradiation produces free radicals which may either degrade covalent bonds such as amides [14] or induce chemical crosslink formation [15]. As far as we know, there have been no previous reports of the effects of γ-irradiation on boronic acid-containing smart hydrogels, nor of the effects of neutron irradiation on any type of hydrogel.

2. Materials and methods

2.1. Materials and hydrogel synthesis

The monomers used for preparation of the gels were obtained as follows: acrylamide (AAM, Fisher Scientific), *N,N*-methylenebisacrylamide (BIS, Sigma−Aldrich), 3-acrylamido phenylboronic acid (3-APB, Frontier Scientific, Logan, UT, USA), and *N*-(3-dimethylaminopropyl acrylamide) (DMAPAA). The monomers were used as received. 2-hydroxy-4'-(2-hydroxyethoxy)-2-methyl propiophenone (HHMP, Sigma−Aldrich), 1-vinyl-2-pyrrolidinone (V-pyrol, Sigma−Aldrich), N,N,N′,N′-tetramethylethylenediamine (TEMED; Sigma−Aldrich), ammonium persulfate (APS; Sigma−Aldrich), D(+)-glucose (Mallinckrodt Chemicals), dimethyl sulfoxide (DMSO, Sigma−Aldrich), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, Sigma−Aldrich), and Dulbecco's phosphate-buffered saline solution (1X PBS, Sigma−Aldrich) were also used as received.

A pre-gel solution was formed by gentle stirring at room temperature of the monomers AAM, DMAPAA, 3-APB, and BIS in the mole ratio 80:10:8:2, respectively in the mixed solvent HEPES buffer/DMSO. The amount of solvent in the pre-gel solution was 20 wt%; the amount of free radical initiator was 2 wt%. This is the same monomer composition that has previously been used with different free-radical initiators to prepare glucose-responsive hydrogels for use in glucose sensors [7,8]. In the current study, for gels that were thermally cured (12 h at 25 °C), we used APS and TEMED as the free-radical initiator and reaction accelerator, respectively. For gels that we cured by UV irradiation (3 min of UV exposure at wavelength 365 nm), we used HHMP as the free-radical initiator [16,17]. In order to enhance HHMP solubility, an amount of V-pyrol equal to 10 mol% of the HHMP was also added to the pre-gel solution. For both thermal and UV curing, monolithic gel films were synthesized by injecting the monomer solution into a mold of thickness 400 μm and then initiating free-radical crosslinking copolymerization [5,6]. After curing, the gels were washed multiple times in deionized water and PBS buffer to remove residual monomer and initiator. Precipitation polymerization [4] can be used in the future to prepare hydrogel nanoparticles of the same composition for use in BNCT. However, hydrogels that are monolithic films are more convenient for material characterization, as needed in the current study. These hydrogels have been shown to contain about 90% water at physiological pH and ionic strength [7,8], and hence about 10,000 ppm by wt. of boron, based on the nominal monomer composition.

2.2. Exposure of smart hydrogels to thermal neutrons and/or gamma rays

Hydrogels synthesized by both thermal and UV curing methods were placed in separate glass vials containing PBS buffer and were irradiated at room temperature with gamma rays emitted from a J.L. Shephard Mark I ^{137}Cs irradiator in the Department of Pathology at the University of Utah School of Medicine. Gamma doses ranged from 70 Gy to 25.7 kGy, as calculated using a conversion factor of 10 mGy/R in water. Note that for each dose value, a separate hydrogel sample (from the same synthesis batch) was employed. In order to simulate BNCT conditions, hydrogels synthesized by both thermal and UV curing methods in separate polypropylene microcentrifugation tubes containing deionized water were placed for 30 min in the University of Utah TRIGA Reactor (UUTR) operating at 90 kW. Different vials were placed within the thermal neutron irradiation (TI) port, and within the fast neutron (FNIF) irradiation port. Table 1 shows the doses of both neutrons and gamma rays in the TRIGA reactor, as calculated by the Monte Carlo simulation code MCNPX [18].

2.3. Measurement of hydrogel glucose response

Hydrogels with the compositions described above are already known to be glucose-responsive hydrogels that shrink with increase in the environmental glucose concentration at fixed pH and ionic strength [7,8]. This apparently occurs because an increase in the environmental glucose concentration increases the fraction of negatively charged boronic acid moieties within the hydrogel, thereby changing the structuring of water molecules within the hydration sphere surrounding the hydrogel [8]. As in Lin et al. [6], the variation in hydrogel osmotic swelling pressure with change in glucose concentration was measured by placing the hydrogel film in a "macrosensor". The macrosensor consists of an off-the-shelf piezoresistive pressure transducer (Endevco model 8510B-2, San Juan Capistrano, CA, USA, full-scale pressure 2 psig) with a cylindrical stainless steel sensing area (diameter 3.2 mm) that is completely covered with the hydrogel film (thickness ≈ 400 μm). The hydrogel is held in place with a cap having a top surface that consists of a rigid porous membrane through which mass transfer occurs. Glucose response tests were performed by placing the macrosensor in a well-stirred temperature-controlled continuous-flow test platform that allows us to vary the glucose concentration of the aqueous environment without handling the sensor. The environmental glucose concentration was varied at room temperature between zero and 5.0 mM (the normal physiological value) at a fixed pH of 7.4 and at a fixed ionic strength of 150 mM. The change in hydrogel osmotic swelling pressure with change in environmental glucose concentration was detected as a change in the mechanical pressure measured by the pressure transducer. Measurements were performed on hydrogels from the same synthesis batch both before and after exposure to radiation.

3. Results

3.1. Effect of gamma irradiation

Fig. 1 shows the time dependence of the glucose response for a UV-cured hydrogel, both before and after exposure to gamma rays

Table 1
Hydrogel doses in TRIGA reactor.

Irradiation port	Neutron dose (kGy)	Gamma dose (kGy)	Total dose (kGy)
TI	66.8	1.92	68.7
FNIF	844	2.16	846