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Development of high refractive ZnS/PVP/PDMAA hydrogel nanocomposites for artificial cornea implants

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ABSTRACT

A series of high refractive index (RI) ZnS/PVP/PDMAA hydrogel nanocomposites containing ZnS nanoparticles (NPs) were successfully synthesized via a simple ultraviolet-light-initiated free radical co-polymerization method. The average diameter of the ZnS NPs is ~3 nm and the NPs are well dispersed and stabilized in the PVP/PDMAA hydrogel matrix up to a high content of 60 wt.% in the hydrogel nanocomposites. The equilibrium water content of ZnS/PVP/PDMAA hydrogel nanocomposites varied from 82.0 to 66.8 wt.%, while the content of mercaptoethanol-capped ZnS NPs correspondingly varied from 30 to 60 wt.%. The resulting nanocomposites are clear and transparent and their RIs were measured to be as high as 1.58–1.70 and 1.38–1.46 in the dry and hydrated states, respectively, which can be tuned by varying the ZnS NPs content. In vitro cytotoxicity assays suggested that the introduction of ZnS NPs added little cytotoxicity to the PVP/PDMAA hydrogel and all the hydrogel nanocomposites exhibited minimal cytotoxicity towards common cells. The hydrogel nanocomposites implanted in rabbit eyes can be well tolerated over 3 weeks. Hence, the high RI ZnS/PVP/PDMAA hydrogel nanocomposites with adjustable RIs developed in this work might potentially be a candidate material for artificial corneal implants.

1. Introduction

The human cornea, the main refractive element of the eye and a prerequisite for good vision, is a clear and transparent tissue which functions like a window that controls the entry of light into the eye. It consists of a highly organized group of cells and proteins which are arranged in three main cellular layers for different functions: an outermost multilayered protective epithelium, a central stroma with keratocytes consisting primarily of water (78%) and collagen (16%) [1], and an innermost single-layer endothelium that governs fluid and solute transport and maintains water balance for optical transparency. Myopia and other forms of cornea-related refractive errors represent the commonest cause of visual disability today. It has been reported that corneal disease and damage are the primary reasons for vision loss caused in ~10 million people worldwide [2]. To date, the most common medical treatment for vision correction is laser-assisted in situ keratomileusis (LASIK) surgery, which is the irreversible reshaping of the cornea by laser energy. Furthermore, corneal function is related to its transparency and any corneal disease or damage to the stroma or endothelium may reduce the transparency, leading to the loss of vision or blindness. The widely adopted treatment for most forms of corneal blindness is corneal transplantation with human donor tissue. However, due to the fact that the demand far exceeds the supply in many parts of the world, corneal transplantation with donor corneas is not always possible [3]. The critical shortage of human corneal donor tissue has resulted in various efforts to develop corneal substitutes as an urgent need [4]. An artificial cornea (keratoprosthesis), which was first developed over a century ago, has great potential application in treating cornea-related diseases and vision errors [5,6]. Moreover, the use of a refractive implant made of biocompatible materials is relatively obvious as the preferred method for creating a successful long-term refractive change of the cornea, either for myopia or hyperopia. The ability to specify an accurate refractive change would be enhanced, and most importantly the procedure is additive and reversible, not removing tissue [7,8].

In recent years, many research groups have attempted to develop various materials as corneal replacements [9–17]. Hydrogels were the first type of biomaterials developed for use in the human body and have wide applications in biomedical areas such as drug delivery, soft contact lenses, tissue engineering scaffolds, biosensors and soft tissue replacement [18–22]. Hydrogels with a cross-linked network structure can absorb large amounts of water while maintaining their network structures to form water-swollen polymeric materials. Hydrogel biomaterials resemble hydrodynamic properties of cells and tissues due to their soft tissue-like physical

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properties and can also improve the biocompatibility due to their surface hydrophilicity and high intrinsic mobility of the polymer chains [23,24]. However, due to the low refractive index (RI) of water at 1.33, hydrogel biomaterials with high water contents generally have low RIs. The incorporation of high RI inorganic building blocks such as TiO_2, ZnO and ZnS into organic matrices is an effective way of increasing the RIs of the polymeric materials [25–27].

In a preceding paper, we developed a high RI organic–inorganic interpenetrating network (IPN) hydrogel nanocomposite incorporating ZnS nanoparticles (NPs) (ZnS/PHEMA/PAA). The nanocomposite exhibited a high RI of 1.49 in the hydrated state with an equilibrium water content of 60.2% [28]. In this work, we further developed a series of high RI organic–inorganic hybrid ZnS/PVP/PDMAA hydrogel nanocomposites with tunable ZnS NPs and water contents. Nanometer-sized ZnS particles were incorporated into a PVP/PDMAA hydrogel matrix. Different from the previous PHEMA/PAA system, the RIs of the ZnS/PVP/PDMAA nanocomposites are easily adjustable by varying the contents of ZnS NPs. The physical and biological properties of the resultant hydrogel nanocomposites were extensively characterized and their suitability as potential artificial corneal implants was also investigated.

2. Materials and methods

2.1. Materials

Zinc acetate dihydrate ($Zn(Ac)_2{\cdot}2H_2O$, 98%, Alfa-Aesar), mercaptoethanol (ME, 98%, Alfa-Aesar), thiourea (99%, Alfa-Aesar), N-vinyl-2-pyrrolidone (NVP, 99%, Acros Organics), N,N-dimethylacrylamide (DMAA, 99.5%, Alfa-Aesar), 2-hydroxy-2-methylpropiophenone (Darocur 1173, 97%, Sigma–Aldrich), triethylene glycol dimethacrylate (TEGDMA, 97%, Sigma–Aldrich), bovine serum albumin (BSA, Sigma–Aldrich), bicinchoninic acid solution (Sigma–Aldrich), copper (II) sulfate solution (Sigma–Aldrich, 4% (w/v) prepared from copper (II) sulfate pentahydrate) and BSA (Quick Start™, standard set, BIO-RAD) were purchased and used without further purification. N,N-dimethylformamide (DMF, Fisher Scientific) was of high-performance liquid chromatography grade and purified by vacuum distillation prior to use. All other solvents were of analytical grade and were used as-received.

Cell culture dishes and flasks, centrifuge tubes and serological pipettes were purchased from Becton Dickinson (Franklin Lakes, NJ). Dulbecco modified Eagle's medium (DMEM), Ham's F-12, HEPES, penicillin and streptomycin, L-glutamine, 0.05% trypsin-0.02% ethylenediaminetetraacetic acid (EDTA) solution were acquired from Invitrogen-GIBCO BRL (Grand Island, NY). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT). Mouse NIH 3T3 fibroblasts (ATCC CCL 92) were acquired from American Type Culture Collection (ATCC, Rockville, MD). Dispase II was purchased from Roche (Mannheim, Germany). Mitomycin-C, bovine insulin, human transferrin, hydrocortisone, human epidermal growth factor (EGF), cholera toxin and other reagents were from Sigma–Aldrich (St Louis, MO). CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (MTS) was obtained from Promega (Madison, USA).

2.2. Synthesis of ME capped ZnS NPs

The synthesis procedure of ME capped ZnS NPs was the same as that in our previous work [28], which was adopted from the literature [29]. A three-necked round-bottom flask 500 ml in volume, equipped with a magnetic stirrer, a condenser and nitrogen purging, was charged with $Zn(Ac)_2{\cdot}2H_2O$ (22.0 g, 0.1 mol), ME (11.6 g, 0.148 mol), thiourea (5.5 g, 0.072 mol) and DMF (300 ml). The solution was refluxed at 160 °C for 10 h under continuous stirring and nitrogen purging. The resultant mixture was concentrated to 80 ml by rotary evaporation, after which the solid was precipitated using excess ethanol. The solid precipitate was collected and washed thoroughly with methanol before being dried in vacuum.

2.3. Synthesis of ZnS/PVP/PDMAA hydrogel nanocomposites

The powder of ME capped ZnS NPs with desired weight ratio (30, 40, 50 and 60 wt.% in the final composites at dry state) was dispersed in the mixture consisting of DMF and DMAA. After stirring for 0.5 h at room temperature, the monomer NVP was added into the mixture. The weight ratio of DMAA:NVP:DMF was 2:2:1. The resultant mixture was stirred for another 0.5 h. After adding the photoinitiator (Darocur 1173, 1 vol.%, with respect to the monomer) and the cross-linking agent (TEGDMA, 1 vol.%, with respect to the monomer), the mixture was ultrasonicated for ~30 s and then the transparent precursor solution was transferred into a Teflon spacer (250 mm in thickness and 20 mm in inner diameter) positioned on a glass plate (1.0 mm thick). After a second glass plate was put on top of the spacer, the solution was exposed to an ultraviolet (UV) light source (200–2500 nm) for 10 min, during which free-radical-induced gelation occurred and a transparent hydrogel was formed which is insoluble in DMF. The resultant ZnS/PVP/PDMAA hydrogel nanocomposites were extensively washed with water to exchange the solvent in the hydrogel and then immersed in deionized water for at least 3 days to eliminate any unreacted components, as well as to attain equilibrium water content in the swelling state.

2.4. Materials characterization

Fourier transform infrared (FTIR) spectra were recorded on a Bio-Rad digilab FTS 3100 spectrometer. The FTIR pellets were made from ~2 mg of the sample and 100 mg of KBr. Thermogravimetric analysis (TGA) was carried out in a Perkin Elmer Diamond TG/DTA instrument at a heating rate of 10 °C min^{-1} from ambient temperature to 700 °C under a flow of nitrogen at 200 ml^{-1} min. The RIs of the IPN hydrogel nanocomposites at a wavelength of 589 nm were measured on a NAR-4T and NAR-1T solid abbe refractometer at 20 °C, using methylene iodide containing sulfur solution (for dry state) and monobromonaphthalene (for hydrated state) as contact liquids. The sample was cut to 20–30 mm in length, ~8 mm in width and 3–10 mm in height, followed by washing and surface polishing for RI measurement. The RIs of three duplicates cut from the same sample were measured for consistency checking.

2.5. Swelling studies

The equilibrium water content of the ZnS/PVP/PDMAA hydrogels was estimated by comparing the dry and the swollen weights. The swollen gels soaked in deionized water were taken out and patted dry and the weight was measured regularly until the equilibrium was reached. The equilibrium water percentage was calculated using Eq. (1):

$$W\% = (W_s - W_d)/W_s \times 100\% \quad (1)$$

where W_s and W_d are the weights of swollen and dry nanocomposites, respectively.

2.6. Viability/cytotoxicity tests

The in vitro viability/cytotoxicity of the ZnS/PVP/PDMAA hydrogel nanocomposites was studied using WST-1 {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate} assay and LIVE/DEAD® assay. The WST-1 test was carried out using a Transwell cell culture system, which is an indirect