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ARTICLE

Carbazole-grafted Silicone Hydrogel with a High Refractive Index for Intraocular Lens

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Refractive index (RI) is an important parameter for intraocular lens biomaterials. In this paper, a novel carbazole-grafted methacrylic polysiloxane macromonomer (MA-CZ-PDMS) with a high RI value was synthesized and its structure was confirmed by proton nuclear magnetic resonance (¹H NMR). The polysiloxane macromonomer can copolymerize with monomers of 3-methacryloxypropyl tris(trimethylsiloxy) silane (TRIS) and N,N-dimethylacrylamide (DMA) to prepare transparent high RI silicone hydrogel. Increasing MA-CZ-PDMS macromonomer content in the silicone hydrogels improved its water content, RI value, and mechanical properties, however decreased slightly glass transition temperature (T_g) and transmittance of blue light. The carbazole-grafted silicone hydrogel possessed good cytocompatibility and *in vivo* biocompatibility in rabbit eyes. The results demonstrate that the carbazole-grafted silicone hydrogel has a potential application as high RI ocular refractive correction biomaterial.

1. Introduction

Cataract, one of the most important causes of blindness due to opacification in the natural crystalline lens, can be treated using an artificial intraocular lens (A-IOL) instead of natural lens contents to restore the ocular refractive power [1,2]. Ideal artificial IOL materials should have a low modulus and high RI value [3-5]. Low modulus enables the IOL implant by minimally invasive surgery and change the shape upon action of the ciliary muscle, so change focus for different viewing distances. High RI value makes the lens focus more sensitive to shape change from muscle's adjustment. Moreover, high RI lens materials can be cut thinner, providing a higher refractive power and reducing the friction between lens and iris. This will therefore improve biocompatible of lens in eyes especially for certain patients requiring this increase in add power as a result of their refraction (e.g. high myopia) and corneal curvature values[6].

Silicone hydrogel has been extensively used as intraocular lens [7-9] material due to its attractive properties of good biocompatibility, optical transparency and low modulus. However, conventional silicone hydrogel has a relative low RI value due to the low RI value of polysiloxane backbone and water in it. This may induce significant hyperopia due to RI mismatch [10,11]. The RI

value of polysiloxane can be improved by grafting benzene ring on its side-chain. For example, Xiaojuan Hao et al [12] synthesized a phenyl group-containing polysiloxane via re-equilibration of octamethylcyclotetrasiloxane (D₄) and phenyl-grafted siloxane copolymer. Its RI value reach 1.46 and suitable for injectable *in situ* curable accommodating intraocular lens. Introducing epithio-group on the side-chain is another way to improve RI value. For example, Xiaokang Lan et al [13] synthesized 1,3,5,7-tetra-(3-glycidoxypropyl) tetramethylcyclotetrasiloxane (TGCS) by hydrosilation reaction of D₄H and allyl glycidyl ether at the presence of Pt-catalyst, and then replaced the oxygen atoms with sulfur atoms using potassium thiocyanate to obtain siloxane episulfide. The siloxane episulfide has a higher RI value of 1.51 than that of TGCS.

Carbazole, obtained as a byproduct from the residues of coal-tar distillation, possesses a rigid fused ring structure and its N atom has lone pair electrons forming n-π conjugate with benzene ring. This endows carbazole molecule with specific photoelectric properties. The synthesis of carbazole-grafted siloxane has been the focus of many research groups around the world. However, their aims are mainly focus on developing materials with specific photoelectric properties. For example, Tao Wang et al [14] synthesized carbazole-bound epoxy tetramethylcyclotetrasiloxane (Ep-Cz-Si) and epoxy methylhydrogen siloxane oil (Ep-Cz-SiO), which showed strong self-photosensitizing effects in photopolymerization. The RIs of Ep-Cz-Si and Ep-Cz-SiO improve with increasing carbazole content. Yujing Hua et al [15] synthesized polymeric photosensitizers for photoinitiated cationic polymerization by hydrosilation reaction of D₄H and vinyl carbazole. Kevin D.Belfield et

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al [16] synthesized polysiloxane covalent attachment of charge-transporting carbazole on the side-chain.

To our knowledge, carbazole-grafted polysiloxane have rarely been reported to be used as ocular refractive correction biomaterials in the literature up to now. In this paper, we grafted high RI carbazole molecule on the side-chain of polysiloxane to synthesize polysiloxane macromonomer with high RI value, and then copolymerized with hydrophilic monomer of DMA to prepare ocular refractive correction biomaterial with high RI value. The carbazole-grafted silicone hydrogels were characterized by both physicochemical and biological properties to explore the feasibility as intraocular lens biomaterials.

2. Experimental Part

2.1. Materials

Octamethylcyclotetrasiloxane (D_4), 2,4,6,8-tetramethylcyclotetrasiloxane (D_4H), and 1,1,3,3-tetramethyl disiloxane were purchased from Jiangxi Xinghuo Organic Silicone Plant (Nanchang, China) and distilled before used. 2-Allyloxyethanol (99%), trifluoromethane sulfonic acid (98%) and dibutyltin dilaurate (DBTDL) were purchased from Aladdin Industrial Corporation (Shanghai, China). Chloroplatinic acid was purchased from Shanghai Chemical Regent (Shanghai, China). Carbazole, allyl bromide and 2,2'-azo-bis(isobutyronitrile) (AIBN) were purchased from Energy Chemical (Shanghai, China). Methacrylic acid ethyl isocyanide was purchased from Ji'nan YuEast Technology Development Co., Ltd (Jinan, China). 3-Methacryloxypropyl tris(trimethylsiloxy) silane (TRIS), N,N-dimethylacrylamide (DMA) and chicken egg-white lysozyme were purchased from Sigma-Aldrich. BCA Assay Kit K3000 was purchased from Shanghai Biocolor BioScience & Technology Company (Shanghai, China).

2.2. Synthesis of Carbazole-grafted Methacrylic Polysiloxane Macromonomer (MA-CZ-PDMS)

N-allyl Carbazole(ACZ) was synthesized by the conventional method as described in the literature[17]. Briefly, KOH (14 g) was dispersed in 80 mL of N,N-dimethyl formamide (DMF), in which 6.6 g of carbazole was added and stirred for 40 min at room temperature. Allyl bromide (7.26 g) was dissolved in 10 mL of DMF, and then added in the DMF solution of carbazole by drop-wise over 30 min. The mixture was stirred overnight at room temperature, and then poured into 1 L of water to precipitate followed by filtration to obtain white crude product of N-allyl carbazole (ACZ). The crude ACZ was purified three-times by recrystallization in ethanol, and then dried *in vacuo* at 40°C.

Carbazole-grafted methacrylic polysiloxane macromonomer (MA-CZ-PDMS) was synthesized by a four-step reaction. Briefly, 1.65 g (12.28 mmol) of end-capper of 1,1,3,3-tetramethyl disiloxane, 10 g (33.71 mmol) of D_4 and 20 g (83.16 mmol) of D_4H were dispersed in 30 mL of dried toluene in a 100-mL three-neck boiling flask. After the temperature was raised to 40°C,

trifluoromethane sulfonic acid (200 μ L) was added, stirred for 24 h under nitrogen atmosphere, and then washed repeatedly with purified water until the pH of the mixture became neutral. After water was separated, the residual liquid was dried with anhydrous magnesium sulfate and then toluene was distilled off under reduced pressure. The residual liquid was dissolved in 20 mL of acetone and then reprecipitated in 40 mL of methanol. This was followed by the removal of volatile components *in vacuo* to give a transparent liquid of polymethylhydrosiloxane (PMHS).

PMHS (2 g) and ACZ (2.5 g) were dispersed in 25 mL of dried toluene and heated to 80°C under N_2 , in which 5 mL of dried toluene containing 0.0012 g chloroplatinic acid was added by drop-wise. The reaction mixture was maintained for 48 h to synthesize carbazole-grafted hydrosiloxane copolymer (CZ-PDMS), and then the temperature was set to 60°C and 2 g of 2-allyloxyethanol and 0.0006 g of chloroplatinic acid were added. The reaction was allowed to stand for another 12 h, and the solvent of toluene was removed under reduced pressure. The product was further purified using a chromatography column to obtain a transparent and pale yellow viscous liquid of hydroxyl- and carbazole-grafted polysiloxane copolymer (HO-CZ-PDMS).

HO-CZ-PDMS (2.83 g) was dissolved in 30 mL of acetone containing 0.0020 g of DBTDL, in which 1 mL of acetone containing 0.098 g of methacrylic acid ethyl isocyanide was added by drop-wise. The reactive was allowed to stand for 6 h at room temperature. Finally, acetone was distilled off under reduced pressure to obtain a transparent pale yellow viscous liquid of carbazole-grafted methacrylic polysiloxane macromonomer (MA-CZ-PDMS).

2.3. Preparation of Silicone Hydrogel with High Refractive Index

MA-CZ-PDMS, TRIS, DMA, initiator of AIBN and diluent of 1-hexanol were mixed with the formulations as described in Table 1. The mixture was injected into the cavity of polypropylene plate mold separated by polypropylene frame with a thickness of 0.2 mm, and then cured at 80°C for 24 h. The silicone hydrogel membranes were stripped out of the mold and the diluent of n-hexyl alcohol was removed *in vacuo* at 80°C, and then immersed into anhydrous alcohol/acetone (5:1, v/v) for 30 min to remove uncured monomers. Finally, the hydrogel membranes were swollen to equilibrium in distilled water.

Table 1. Formulations of carbazole-grafted silicone hydrogel membranes with a high refractive index

Samples	MA-CZ-PDMS (g) ¹	TRIS (g)	DMA (g)	Hexanol (g)	AIBN (g)
1	0.1770 (61 wt%)	0.0502	0.0662	0.06	0.0016
2	0.1937 (66 wt%)	0.0335	0.0662	0.06	0.0016
3	0.2105 (72 wt%)	0.0167	0.0662	0.06	0.0016
4	0.2823 (81 wt%)	0	0.0662	0.07	0.0018

Note: The data in brackets is weight percent of MA-CZ-PDMS macromonomer

versus total monomer weight of MA-CZ-PDMS, TRIS and DMA.

2.4. Methods and Measurements

2.4.1. NMR Spectra

¹H-NMR spectra were recorded on a 400-MHz instrument (Bruker AC200) with CDCl₃ as a solvent. H content of Si-H in PMHS copolymer, the average number of dimethyl siloxane and Si-H unit of PMHS copolymer were also determined by ¹H-NMR spectra by adding 0.029 g of dioxane as internal standard in 0.063 g of PMHS.

2.4.2. Gel Permeation Chromatography (GPC)

The molecular mass of PMHS was determined by GPC (Waters, E2695). The inject volume of samples was 80 μL and THF was used as mobile phase at a flow rate of 1.0 mL/min. Calibration was done with polyoxytetramethylene standards with molecular weight from 800 to 90000 Daltons.

2.4.3. Gel Fraction (G)

The carbazole-grafted silicone hydrogels were dried to a constant weight *in vacuo* and weighed before and after extraction in a Soxhlet extractor with 30 mL of acetone for 24 h. G was calculated gravimetrically with the following formula (1):

$$G(\%) = \frac{W_g}{W_0} \times 100\% \quad (1)$$

Where, W_0 and W_g are the weights of the dried hydrogel samples before and after extraction respectively.

2.4.4. Dehydration Kinetics and Equilibrium Swelling Ratio (ESR)

Carbazole-grafted silicone hydrogel samples, swollen to equilibrium in 0.9% saline solution, were weighed (W_w) after carefully absorbing the moisture off the surface with filter paper and place in an oven at 40°C. At certain time intervals, the samples were taken out and weighed (W_t). After 30 min dehydration, the temperature of the oven was raised to 105°C and maintain for 24 h to determine the final equilibrium swelling ratio (ESR) of the silicone hydrogel samples. The dehydration mass (DH) of the samples were gravimetrically calculated with the following formula (2) and final DH after 24 h dehydration at 105 °C was defined as ESR.

$$DH(\%) = \left(\frac{W_w - W_t}{W_w} \right) \times 100\% \quad (2)$$

2.4.5. Light Transmittance

The silicone hydrogel samples, swollen to equilibrium in distilled water, were cut into 10 × 40 mm² strips and attached to the inner surface of a quartz colorimetric cuvette that was full of distilled water. Transmittance of the hydrogel membranes was recorded at a wavelength between 200 and 800 nm with an ultraviolet-visible spectrophotometer (Helios, Thermo Electron). Distilled water was used as a reference solution.

2.4.6. Refractive Index

The refractive index of silicone hydrogels, swollen to equilibrium in distilled water, was determined with an abbe's refractometer (WAY, Shanghai Ineas Instrument) at room temperature.

2.4.7. Mechanical Properties

Carbazole-grafted silicone hydrogel membranes, swollen to equilibrium in distilled water, were cut into 10 × 40 mm² strips. The mechanical properties were determined in three triplicates with a Series IX Automated Materials Testing System (Instron Corporation), with a crosshead speed of 10 mm/min at room temperature and a relative humidity of 50%.

2.4.8. Lysozyme Deposition

Lysozyme deposition of the silicone hydrogels was determined by bicinchoninic acid assay [18]. The hydrated silicone hydrogels were cut into disc with a diameter of 14 mm and immersed respectively in 5 mL of lysozyme (120 μg/mL) phosphate-buffered saline solution (PBS, pH 7.4) at 37°C. After 24 h immersion the silicone hydrogel discs were removed from the lysozyme solution and immersed into 10 mL of 0.9% NaCl solution for 5 s to remove loosely bound lysozyme. The silicone hydrogels deposited lysozyme were blotted on a filter paper and placed in 4 mL of lysozyme extraction solution containing 0.1% trifluoroacetic acid and 50% acetonitrile for 16 h at room temperature. The concentration of lysozyme in extraction solution was determined at 560 nm by a Microplate Reader (Bio-Rad 680, USA) according to specification of BCA Assay Kit. The amount of adsorbed lysozyme on the silicone hydrogel membrane surface was calculated by the concentration of lysozyme in the extraction solution. Every sample was measured in three triplicates.

2.4.9. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) experiments were carried out using a Netzsch DSC 204 F1 Phoenix equipment with a refrigerated cooling accessory. Nitrogen was used as purge gas at a flow rate of 50 mL/min. The calorimeter was calibrated for baseline using no pans. To determine the glass transition temperature (T_g) dried discs (about 10 mg) pieces were accurately weighed in aluminium pans and program-heated from 25°C to 200°C, then cooled to -50°C, and finally heated again up to 200°C; always at 5°C/min.

2.4.10. In Vitro Cytotoxicity

Carbazole-grafted silicone hydrogel discs with a diameter of 14 mm were extracted by immersing in 10 mL of sterile serum-free culture medium (SFM), composed of Gibco Minimum Essential Medium containing 1% non-essential amino acids and penicillin/streptomycin antibiotics at 60 mg/mL and 200 mg/mL for 48 h at 37°C, and then the extract medium was transfer to a sterile vial for the cytotoxicity assay.

L929 cells were seeded at 6×10³ cells/well into individual wells of 96-well culture trays and allowed to attach and spread for 4 h at 37°C in culture medium composed of SFM supplemented with 10% (v/v) fetal bovine serum (FBS). After 4 h incubation, the medium was removed and replaced with extraction test samples. SFM, and 1% (v/v) dimethyl sulfoxide (DMSO) in SFM were used as negative and positive controls, respectively. The cells were then incubated

for a further 24 h in the presence of the test samples and respective controls. Cell viability was measured by incubating the cells with 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, NSW, Australia), 0.5 mg mL^{-1} , for the final 4 h of the culture period and the absorbance was measured at 595 nm. Cell viability was expressed as a percentage of that measured for cells exposed to SFM only.

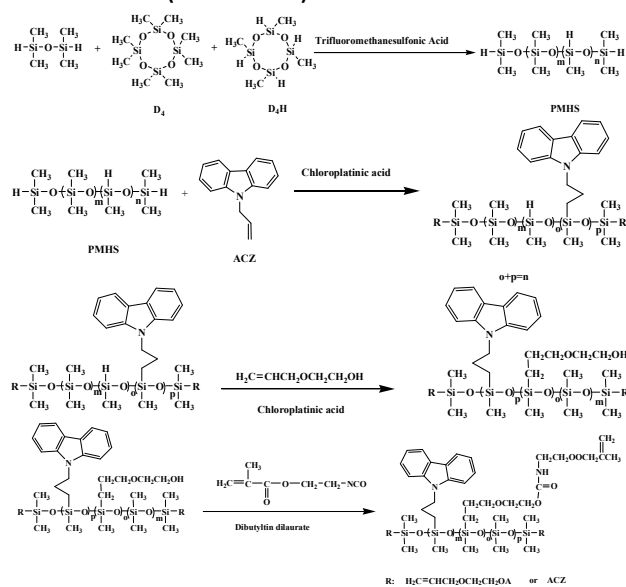
2.4.11. *In Vivo* Biocompatibility and Histology Study

Rabbits weighing 2.0 kg were supplied by the Animal Center of Qilu University of Technology, China (License No. QLU 2014-0012). The corneas of rabbits were carefully observed with a slit lamp after dyeing with fluor-strips (Tianjin Jingming New Technological Development Co.), and rabbits with corneal damage were ruled out. The rabbits were individually housed in a light controlled room at 20°C and a relative humidity of 50%, with no restriction of food and water intake. The Local Ethics Committees for animal experimentation approved all the experiments.

The rabbits were anaesthetized with 10% chloral hydrate by intraperitoneal injection at a dose of 5 mL/kg. Carbazole-grafted silicone hydrogel of sample 3 was inserted into left eye between the cornea and iris through a 3 mm incision along the edge of the cornea. After 30 days of implantation the rabbits were killed by air embolism at eye vein and eyeball implanted carbazole-grafted silicone hydrogel was taken out and immersed in 10 wt% formaldehyde aqueous solutions. Then the pathological changes of involved material and tissues were observed using HE staining with microscope (Olympus BX 41, Japan).

3. Results and Discussions

3.1. Synthesis of Carbazole-grafted Methacrylic Polysiloxane Macromonomer (MA-CZ-PDMS)



Scheme 1. Synthesis route of carbazole-grafting polysiloxane macromonomer (CZ-MA-PDMS)

The synthesis route of MA-CZ-PDMS macromonomer was

shown in Scheme 1. Firstly, the polymethylhydrosiloxane copolymer (PMHS) was synthesized by a ring-opening polymerization of D_4 and D_4H with 1,1,3,3-tetramethyl disiloxane as end-capper by the acid-catalyzed equilibration. Molecular weight of PMHS determined by GPC was about 2457 Daltons. This is consistent with the theoretical molecular weight of PMHS (2576 Daltons), calculated from the ratio of the mass of D_4 , D_4H and end-capper to the molar amount of end-capper. ^1H NMR spectrum of PMHS without and with internal standard (dioxane) was shown in Figure 1(a) and (b), respectively. H content of Si-H in PMHS copolymer was 1.0 wt% calculated as Equation (3), and the average number (m) of dimethyl siloxane unit in the PMHS copolymer was about 12 and the average number (n) of Si-H unit was about 21 calculated as Equations (4) and (5), respectively.

$$H\% = \frac{A_{\text{Si-H}} \times W_{\text{Dioxane}} \times 8}{A_{\text{Dioxane}} \times W_{\text{PMHS}} \times 88} \times 100\% \quad (3)$$

$$\frac{A_{\text{Si-H}}}{A_{\text{Si-CH}_3}} = \frac{n+2}{3n+6m+12} \quad (4)$$

$$\frac{A_{\text{Dioxane}}}{A_{\text{Si-H}}} = \frac{W_{\text{Dioxane}} \times 8 / 88}{W_{\text{PMHS}} \times (n+2) / 60n+74m+134} \quad (5)$$

Where, $A_{\text{Si-H}}$, A_{Dioxane} and $A_{\text{Si-CH}_3}$ was the proton peak area of Si-H peak, dioxane and the methyl group peaks connecting to siloxane, respectively. W_{Dioxane} and W_{PMHS} were the weight of internal standard (dioxane) and sample of PMHS, respectively. m and n were the average number of dimethyl siloxane and Si-H unit in PMHS copolymer.

Carbazole was grafted on the side-chain of PMHS by hydrosilation at the presence of Pt-catalyst. CZ-PDMS was unstable and crosslink occurred when removing solvent of toluene under reduced pressure. This may be ascribed to the instability of the Si-H at the presence of chloroplatinic acid. Moreover, the product is immiscible with usual hydrophilic methacrylic monomer of DMA. Therefore, hydrophilic composition of 2-allyloxyethanol was also grafted on the side-chain of PMHS by hydrosilation to give an amphiphilic polysiloxane copolymer grafting hydroxyl and carbazole on the side chain (HO-CZ-PDMS). ^1H NMR spectrum of HO-CZ-PDMS was shown in Fig 1(c). It can be seen that the resonance peak at 4.7 ppm corresponding to Si-H wasn't observed. This indicated that all the Si-H bonds have involved into the reaction of hydrosilation. ^1H NMR spectra of final MA-CZ-PDMS macromonomer was shown in Fig 1(d), in which obvious carbon-carbon double bond peak, locating at $\delta=6.11$ and 5.57 ppm, was observed. This indicated that carbazole-grafted polysiloxane macromonomer was successfully synthesized. The ratio of Si-H bonds grafted carbazole (S_1), methacrylic acid ethyl isocyanide (S_2) and allyloxyethanol (S_3) was respectively about 53.88 mol%, 7.54 mol% and 38.58 mol% calculated by the equation (6), (7) and (8):

$$S_1 = \frac{f_1/2}{f_2} \times 100\% \quad (6)$$

$$S_2 = f_3 \times S_1 \quad (7)$$

$$S_3 = (1 - S_1 - S_2) \times 100\% \quad (8)$$

where f_1 is the area of the methyl group peak connecting to silicone (6.95) with the area of carbazole peak at 8.04 ppm (2H, ArH) as reference [Fig 1 (c)] and f_2 is the area of the methyl group peak connecting to silicone (6.45) with the area of Si-H peak at 4.70 ppm as reference in ^1H NMR spectra [Fig 1 (a)]. f_3 is the area of the carbon-carbon double bond peak locating at $\delta=6.11$ and 5.57 ppm (0.14) as shown in Fig 1 (d).

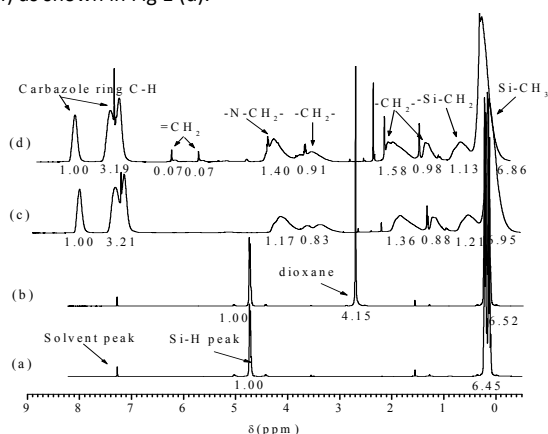


Figure 1. ^1H NMR spectra of (a) PMHS, (b) PMHS with internal standard dioxane, (c) hydroxyl- and carbazole-grafted polysiloxane copolymer (HO-CZ-PDMS) and (d) carbazole-grafted methacrylic polysiloxane macromonomer (MA-CZ-PDMS).

3.2. Preparation of Carbazole-grafted Silicone Hydrogels with a High Refractive Index

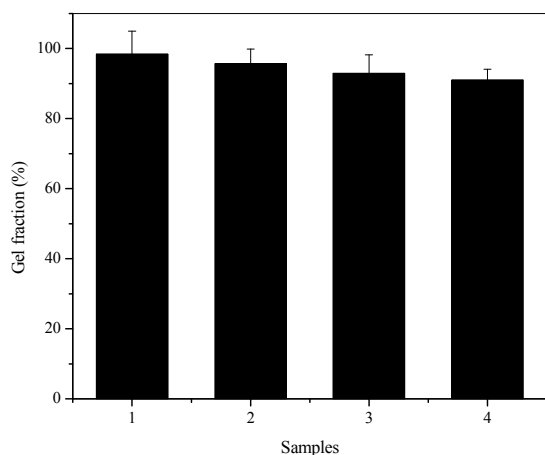


Figure 2. Gel fraction of samples 1-4 with MA-CZ-PDMS content of 61, 66, 72 and 81 wt%, respectively.

DMA was chosen as hydrophilic monomer to prepare silicone hydrogel, in which the hydrophilic compositions enable it absorb water to govern a flexible property [19]. Small molecular siloxane monomer TRIS was also copolymerized in it to reduce the modulus of the final polymer through reduction of the crosslinking density

[20]. To obtain high RI silicone hydrogel, high content of carbazole-grafted polysiloxane macromonomer (MA-CZ-PDMS) was added. After 24 h curing, transparent and pale yellow silicone hydrogels were obtained. Si-O bond was high stable and silicone hydrogel has been extensively used as biomaterials such as contact lens or intraocular lens. Moreover there is no degradable bond in comonomers (TRIS and DMA) and carbazole molecule grafting on the side-chain of polysiloxane. Therefore, the carbazole-grafted silicone hydrogel was nondegradable. MA-CZ-PDMS was soluble in acetone. Therefore, the silicone hydrogel was extracted with acetone in a Soxhlet extractor. The G values corresponding to samples 1-4 were obtained and shown in Figure 2. Sample 1 with free MA-CZ-PDMS of 61 wt% has a highest G value of 98%, and then it decreased to 91% with the free content of MA-CZ-PDMS increase to 81 wt%. This indicated that MA-CZ-PDMS macromonomer can well copolymerize with methacrylic monomers of TRIS and DMA.

Figure 3 reveals that the dehydration occurs mainly at the initial period of 5 min, in which the dehydration ratio is 48.89 wt%, 59.08 wt%, 66.21 wt% and 65.61 wt% of total ESR corresponding to samples 1-4 respectively. ESR of the carbazole-grafted silicone hydrogel exhibits dependence on the composition as shown in Figure 3, and increasing the ratio of MA-CZ-PDMS to TRIS, fixing hydrophilic monomer of DMA, will increase its ESR. This is mainly ascribed to the hydrophilic molecule grafting on the side-chain of MA-CZ-PDMS, making the macromonomer has a higher hydrophilicity than TRIS. According to peppas [21], the percolation of water through hydrogels starts when the water content reaches ca. 20%. This provides wettability and comfort. As shown in Figure 3, samples 1-4 have ESR of 24.26%, 27.54%, 28.91%, and 30.56 % respectively, and all the silicone hydrogels have sufficient value of ESR. Intraocular lens made of these gels would keep percolation of aqueous humor in eye.

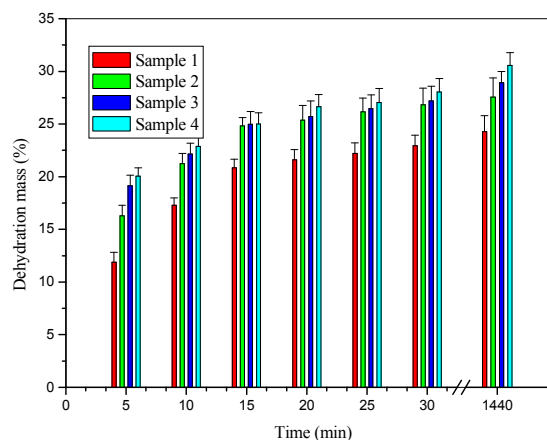


Figure 3. Dehydration kinetics (dehydration mass vs time) of carbazole-grafted silicone hydrogels with a high RI value

The transmittance (T%) of the carbazole-grafted silicone hydrogel, measured on an UV-Vis spectrophotometer, is shown in

Figure 4. it can be seen that the transmittance of sample 1, 2, 3 and 4 is greater than 90% at the wavelength more than 530 nm, 524 nm, 550 nm and 580 nm, respectively. This indicated that T% slightly decreased with increasing the content of carbazole-grafted siloxane composition in the silicone hydrogels although which increased the water content in the silicone hydrogels. The most sensitive wavelength for human eye is about 555 nm corresponding to yellow and green light. Therefore, the carbazole-grafted silicone hydrogels especially for samples 1, 2, and 3 were suitable for biomaterial used as intraocular lens. Moreover, the incorporation of carbazole-grafted siloxane in the silicone hydrogels decreased the transmittance during the wavelength between 400 nm and 500 nm. The more the content of carbazole-grafted siloxane in the hydrogels, the lower the transmittance is. This may mean that the silicone hydrogel can partly block high energy purple and blue light, with a wavelength from 400 nm to 500 nm, which can induce retinal damage [22].

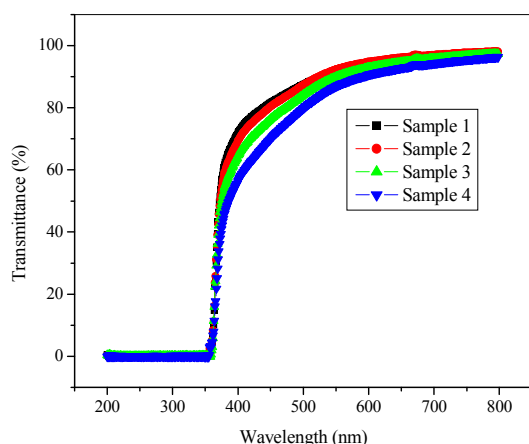


Figure 4. Transmittance of carbazole-grafted silicone hydrogels

Refractive index is an important parameter for intraocular lens. Lens materials with high refractive index can be cut thinner. This will greatly improve the biocompatible in eye especially for the refractive correction of high myopia. For swollen hydrogel material it is difficult to improve its RI value because of the low RI value of hydrogel backbone and water in the hydrogel. According to the classical electromagnetic theory, RI is inversely proportional to the molecular volume and proportional to the degree of molar refraction proportional to the dielectric polarization rate. Therefore, the material's RI is associated with molecular polarizability per volume of material. Carbazole molecules have rigid fused ring, and nitrogen atom in the ring has a lone pair electrons that can form $n-\pi$ conjugate with benzene ring, making carbazole molecule can be polarized under the action of electric field section of the light. This makes carbazole molecule possessing high refractive index. From Figure 5, It can be seen that RI value increase with the increase of high RI carbazole-grafted polysiloxane composition in the hydrogels although it increase the ESR of the hydrogels. This indicated that

the high RI value is mainly ascribed to the carbazole molecule grafted on the side-chain of MA-CZ-PDMS macromonomer. For ophthalmic refractive correction instrument, there is following relation, as illustrated in Equation (9), between diopter and refractive index of the lens. Therefore, it can be calculated that the lens' diopter will increase to -6.125D from -5.00D corresponding to RI value increase to 1.49 (carbazole-grafted silicone hydrogel, sample 3) from 1.40 (conventional silicone hydrogel) if we use a similar model for lens with -5.00 D under hypothesized two kind of hydrogel has similar swelling ratio.

$$D = (n - 1) \times \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad (9)$$

Where, D is diopter of lens; n is the refractive index of the lens; R_1 and R_2 are curvature radius of lens' inner and outer sphere respectively.

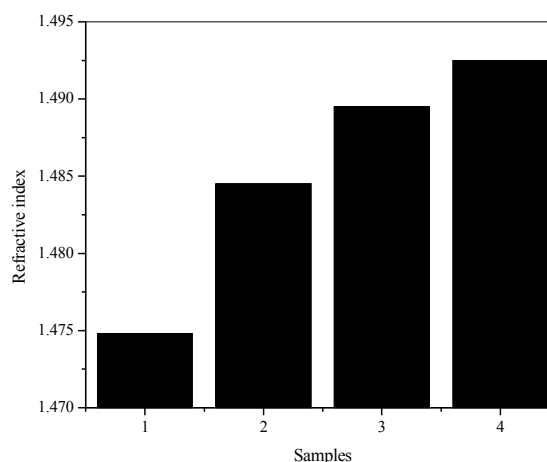


Figure 5. Refractive index of carbazole-grafted silicone hydrogel of samples 1-4

The mechanical properties of the carbazole-grafted silicone hydrogels are measured using a tensile tester and the results are shown in Table 2. The hydrogels are high flexible. The elongation at break of samples 1-3 is more than 100% and that of sample 4 is 86%. This ensure the intraocular lens from carbazole-grafted silicone hydrogel can be inserted by minimally invasive surgery. Moreover, the increase of MA-CZ-PDMS content in the hydrogels increases their tensile strength and Young's modulus. It may be attributed to the rigidity and big volume of carbazole molecule that form interchain junctions and thus, reduce the elasticity of the polymer chain.

Table 2. Mechanical properties of the carbazole-grafted silicone hydrogels

Samples	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)
1	1.08±0.11	117±38	0.83±0.09
2	1.12±0.24	125±46	0.98±0.19
3	1.24±0.22	106±26	1.16±0.32
4	1.25±0.35	86±24	1.21±0.41

Figure 6 presents DSC thermographs of carbazole-grafted silicone hydrogels. The T_g values of carbazole-grafted silicone hydrogel samples 1-4 are 56, 42, 35 and 33°C, respectively. In the hydrogel, T_g value can be effected by pendent group of carbazole and the content of Si-O component. Generally, T_g decreased with the increase of Si-O component in silicone hydrogel. However, there are two main sides about the influence of pendent groups on T_g of a polymer [23]. One is that pendent groups can reduce the interaction between polymer backbones and this leads to a low T_g ; the other is that steric effect of pendent groups could decrease flexibility of polymer chains, and make the T_g of polymer increasing. The T_g value of carbazole-grafted silicone hydrogel was far more than that of silicone hydrogel (usually lower than 0°C). Therefore, the steric effect of carbazole molecule is a dominant aspect due to its rigidity and big volume, and as a result, it makes flexibility of polymer chains decreasing markedly. This result is accordance with its mechanical properties described above. Moreover, the T_g value of the silicone hydrogel decreased with the addition of carbazole-grafted polysiloxane. This may be ascribed to the increase of soft Si-O component in the hydrogel.

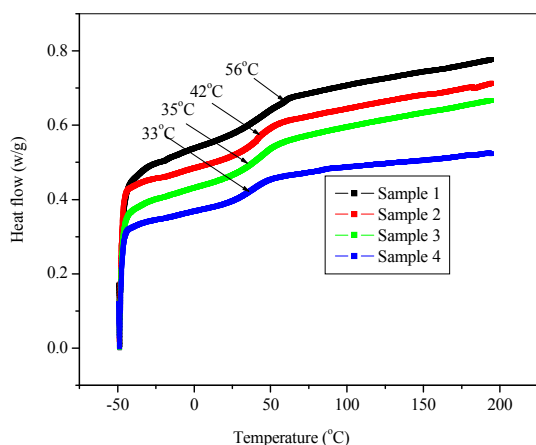


Figure 6. DSC thermograms for carbazole-grafted silicone hydrogel samples 1-4

Protein components in normal tears, such as lysozyme, lactoferrin, immunoglobulins, lipocalin, vitronectin, may enhance contact lens wettability, but their overall accretion could be detrimental to ocular health. Proteins deposited on a lens may become denatured with their resultant allergenicity contributing to adverse ocular syndromes [24]. Lysozyme is usually used to evaluate protein deposition on lens. Senchyna et al [25] reported that significantly lower levels of lysozyme adsorb on silicone hydrogel lenses versus conventional PHEMA-based hydrogel lens. Lysozyme deposition on the carbazole-grafted silicone hydrogels was determined and the results were shown in Figure 7. The amount of lysozyme deposited on the silicone hydrogel is about 6.37 μg per silicone hydrogel disc when the content of free carbazole-grafted polysiloxane macromonomer is at 61 wt%, and decreases to 0.32 μg as the addition content of carbazole-grafted polysiloxane

macromonomer was increased to 81 wt% in the polymerization solution with fixed hydrophilic component of DMA. This may be the reason that increase carbazole-grafted polysiloxane composition in the silicone hydrogel increased material water content and changed surface charge.

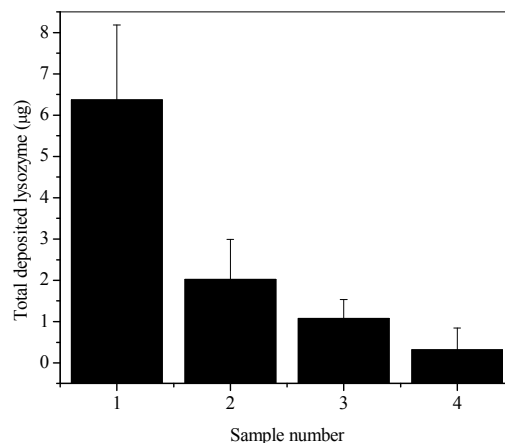


Figure 7. The amount of total lysozyme deposited on per carbazole-grafted silicone hydrogel disc with a diameter of 14 mm

3.3. *In Vitro* Cytotoxicity and *In Vivo* Biocompatibility of Carbazole-grafted Silicone Hydrogel

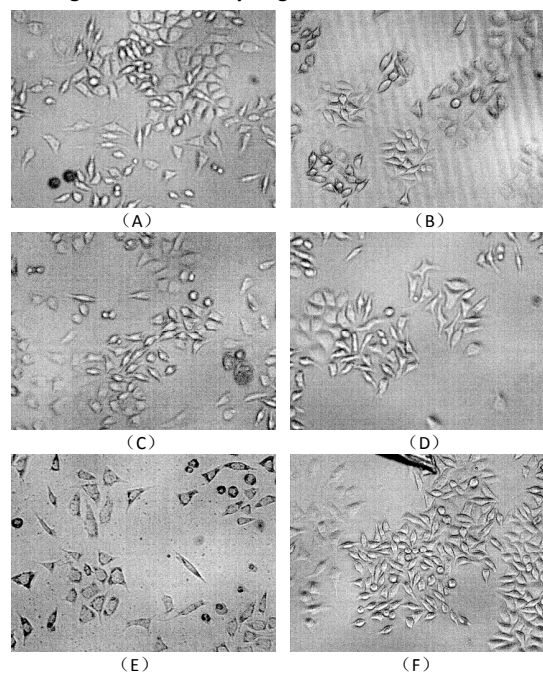


Figure 8. Representative morphology of L929 cells exposed to extract from carbazole-grafted silicone hydrogel of sample 1 (A), sample 2 (B), sample 3 (C) and sample 4 (D) compared to controls DMSO (E) and controls SFM (F).

Potential harmful constituents released from implanted biomaterials, including raw material monomer, low molecular polymer, initiator and solvent, can cause inflammation and tissue response. Therefore, four carbazole-grafted silicone hydrogel samples prepared with different content of carbazole-grafted

polysiloxane macromonomer (Table 2) was selected to evaluate the cytotoxic effect using both *in vitro* cytotoxicity and *in vivo* biocompatibility. Extracts from the sample with different percent of MA-CZ-PDMS were screened for the cytotoxic effect of leachables, and representative morphology of L929 cells exposed to it is shown in Figure 8. The morphology of the cells exposed to the carbazole-grafted silicone hydrogel extracts was similar to that observed for L929 cells in the presence of the SFM control medium, and cell density besides sample 4 is not visibly reduced. Cell viability was determined by MTT assay and the results were shown in Figure 9. The cell viability for sample 1-3 was more than 100% and sample 4 was 98%. This indicated that the introduction of carbazole molecule into the silicone hydrogel did not adversely affect the *in vitro* cellular response compared to the SFM medium.

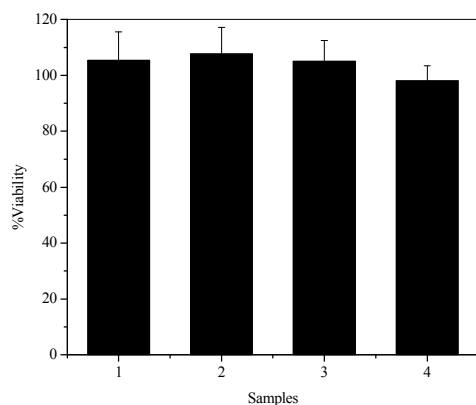


Figure 9. Cytotoxicity assay for high RI carbazole-grafted silicone hydrogels

Carbazole-grafted silicone hydrogel with a thickness of 0.2 mm was inserted into rabbit eyes, as shown in Figure 10, to evaluate *in vivo* biocompatibility of the hydrogel. During the experiment of 30 days, the rabbit has a normal growth and no obvious tear flush and eye irritation was observed. The cornea and iris in contact with carbazole-grafted silicone hydrogel were observed after HE staining and shown in Figure 11. It can be seen that corneal endothelial layer cells were normal and one or two cell layers were observed on the surface of implanted material. Although the type of cells has not been identified, the fibroblast is a possible source of the cells considering its fibroblast-like shape [Fig.11 (a)]. The attached growth of cell on the surface of implanted material indicated that carbazole-grafted silicone hydrogel has an excellent biocompatibility. Moreover, cell growth on material surface may lead to the decrease of transmittance. Therefore, this prompted that carbazole-grafted silicone hydrogel may need surface treatment, e.g. by irradiation with ions, plasma or UV light, when it was used as ocular implanted material such as intraocular lens. Besides, a slight proliferation of pigment cells was observed on the surface of iris which is in contact with the implanted material; however, no significant invasion of inflammatory cells was observed herein [Figure 11(b)]. This further indicated that there is no cytotoxic substance released from the

implanted biomaterials, and the cell proliferation is mainly ascribed to surface characterization of carbazole-grafted silicone hydrogel [26].



Figure 10. Rabbit eye after 30-days implantation of carbazole-grafted silicone hydrogel strip (sample 4)

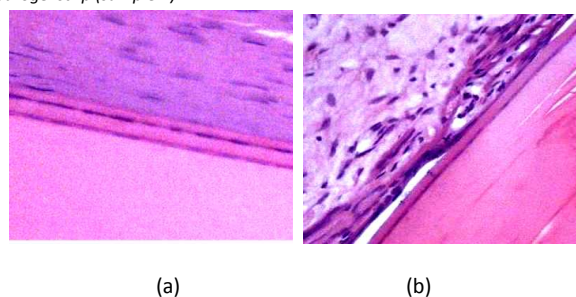


Figure 11. The cornea (a) and iris (b) in contact with implanted carbazole-grafted silicone hydrogel of sample 3 with a high refractive index ($\times 40$)

Conclusions

Carbazole can be grafted on the side-chain of polysiloxane by hydrosilylation reaction to obtain liquid polysiloxane macromonomer with a high refractive index, which can copolymerize with the methacrylic monomer of TRIS and DMA to prepare transparent high RI silicone hydrogels. The carbazole-grafted silicone hydrogel has good cytocompatibility and *in vivo* biocompatibility in rabbit eyes. The RI value of the silicone hydrogel is tunable and can be controlled by the incorporation ratio of carbazole-grafted polysiloxane macromonomer in the silicone hydrogels. The silicone hydrogel shows a low lysozyme deposition and possesses blocking characteristics to blue light. Targeted properties such as swelling degree, light transmittance and mechanical properties show promise as an intraocular lens.

Acknowledgements

The projects were supported by National Nature Science Foundation of China (Nos. 51163007 and 21276149); Nature Science Foundation of Jiangxi Province (No. 20114BAB206025), Nature Science Foundation of Shandong Province (No. ZR2013BQ014), Science and Technology Development Program of Shandong

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Province (No.2014GSF118009), Nature Science Foundation of Jiangxi Agricultural University (No. 09003332), and Program for Scientific Research Innovation Team in Colleges and Universities of Shandong Province.

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