Graphite/poly (vinyl alcohol) hydrogel composite as porous ringy skirt for artificial cornea

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A B S T R A C T
Graphite/poly (vinyl alcohol) (PVA) hydrogel composites, which were designed as the porous ringy skirt surrounding the transparent core of a novel artificial cornea, were prepared by using the freeze/thawing process and the particle-leaching technique. The properties of the composites, including the water content, the mechanical strength, the porous architecture and the interactions between the graphite and PVA, were investigated. The tissue responses to the composite and pure PVA hydrogel were studied by in vivo implantation in the dorsal muscles of mice. The results showed that chemical interactions were present between the graphite and PVA in the composite, which benefited the combination of the two phases and enhanced the uniform distribution of graphite particles in the PVA matrix. However, the present of graphite in the PVA hydrogels reduced the tensile strength, elongation at break and water content of the composite. Moreover, the porous graphite/PVA hydrogel composite had interconnective pore structure with high porosity and enough mechanical strength. According to the histological analysis of 1 week and 12 weeks post-implantation, the graphite/PVA hydrogel composites showed less inflammatory reactions than the PVA hydrogels at the 1 week post-implantation. Moreover, compared to pure PVA hydrogel, the graphite/PVA hydrogel composite exhibited enhanced migration and infiltration of cells, and more neovascularization and tissue ingrowth. These in vivo characteristics will be beneficial for the long-term biofixation of artificial cornea. Therefore, the porous graphite/PVA hydrogel composite has a potential to be used as novel artificial cornea skirt.

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1. Introduction

Cornea, situated at the front of eyes, is a crucial element of ocular optical system. When cornea is permanently damaged by injuries including acid or alkali burns, traffic accidents or other corneal diseases, the best way to repair cornea and restore the vision is corneal transplantation [1,2]. However, successful human cornea allografts are difficult to obtain due to the lack of donor cornea and the aggressive immunoresponses, especially in underdeveloped countries where an organized eye banking system seems unlikely to become widely available in the foreseeable future [2,3]. Fortunately, artificial cornea studies presented a promising solution for these problems [2–4].

PVA hydrogel resembles organic tissue and has a high elastic modulus even though the water content is very high. PVA hydrogel has been employed in several biomedical applications including drug delivery, tissue engineering scaffold and artificial organs etc. [2,5–9]. Especially, considering its good biocompatibility, high elasticity and desirable optical property, PVA hydrogel has been used for artificial cornea. However, a major problem in cornea substitution is the mechanical or biological fixation of the artificial cornea. As PVA hydrogel itself cannot adhere to natural tissues, long-term biofixation of PVA hydrogel implant is difficult. Recently, research on the fixation of PVA hydrogel artificial corneas has attracted more interests.

Currently, artificial corneas with a porous skirt are more widely studied and more likely to be successful than those without [10]. A porous nano-hydroxyapatite (n-HA)/poly (vinyl alcohol) hydrogel composite was used as an artificial cornea skirt and presented good biocompatibility after implantation in rabbit eyes [11]. The results suggested that the n-HA particles played an important role because of their excellent biocompatibility with natural tissues. However, a potential problem for n-HA/PVA composite scaffold is that the n-HA particles may partially biodegraded after long-term implantation and deduced Ca^2+ ion content increase gradually in the body fluid around the implant [12,13]. As a result, the deposition of apatite may occur on the surface of transparent optical core, which finally leads to the poor transparency of the optical cornea, and causes the failure of the cornea grafting. In addition, over long-term implantation, more newly formed fibrous tissues and even bone tissues may grow on/in the skirt of the artificial cornea due to the osteoconductivity and bioactivity of HA [14,15], which results in tissue hyperplasia and causes patients who have the artificial cornea implantation to undergo discomfort.

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As we know, graphite has good properties such as surface-adsorption and wearing-resistance. C. Eriksson’s study showed that the surface of graphite could adsorb plasma proteins and cells in blood [16]. The results from in vitro cell culture suggested that graphite had good biocompatibility [17]. Furthermore, various graphite materials have been used as implant materials in biomedical engineering, such as joints, heart valves, and electrodes of biosensors [18–21]. So, graphite may be an ideal additive for skirt to solve the problem of biofixation of artificial cornea because it may effectively enhance the tissue adsorption on the surface of the skirt. Moreover, considering the dark color of iris which is closed to the pupil [22], a black color of the skirt formed by the graphite addition is fit for oriental eyes aesthetically. A draft of the designed artificial cornea described the porous graphite/PVA ring which would be used as a skirt of the transparent core in Fig. 1.

This research developed a possible material for the porous skirt of artificial cornea and will be of interest to the general biomedical engineering audience.

2. Materials and methods

2.1. Raw materials

Poly(vinyl alcohol) (PVA, mean degree of polymerization 1700 ± 50, hydrolysis degree 99%, residual content of acetate groups 0.13%, content of PVA more than 90.5 wt.%) was from Chongqing Beipei Chemical Co. Ltd., China. The contents of carbon and hydrogen of PVA determined by Element Analyze (Carlo Erba 1106, Italy) were 52.20 ± 0.36 wt. % and 9.75 ± 0.12 wt. %, respectively. Micron-grade graphite (the content of carbon is more than 99.85%, the content of combustion residues of graphite is less than 0.15 wt. %) was from Chengdu Chemical Agent Co. Ltd., China. The molar ratio of C:O on the surface of graphite detected by Kratos Xsam800 X-ray photoelectron spectroscopy (XPS, U.K.) was 97.98:2.02. Dimethylsulfoxide (A. R., DMSO) was purchased form Chengdu Chemical Agent Co. Ltd., China. Other reagents used in this work were of analytical grade.

2.2. Preparation of graphite/PVA hydrogel composites with or without pores

Previous research suggested that nanoparticles may have potential risk to human health because of their penetration into cells [23,24]. So, in this paper, micron-grade graphite particles with the size of 10–20 μm (Fig. 2) were selected to prepare graphite/PVA hydrogel composites.

20 g PVA was dissolved into 80 ml 80% DMSO aqueous solution at 90 °C for 3 h to form the 20% PVA solution. NaCl particles, which were used as place holders to control the pore size of finished PVA hydrogels or composites, were milled and sieved to the particle size of 106–150 μm. Graphite and graphite/NaCl were then added into the PVA solution separately with vigorously stirring for 1 h at 90 °C to obtain two kinds of mixed slurries. The slurries were cast into moulds and subjected to one freeze–thawing cycle by keeping at −28 °C for 8 h to freeze and at room temperature for 4 h to thaw. The freeze–thawing cycle was repeated for seven times to form PVA crosslinks. Thereafter, the graphite/PVA hydrogel composites with or without NaCl particles were immersed into deionized water for 3 days to remove DMSO and/or NaCl. Finally, the graphite/PVA hydrogel composites with different porosity were prepared by changing the addition ratio of NaCl particles.

2.3. Characterization tests of graphite/PVA hydrogel composites

The composition and structure of graphite particles, PVA hydrogel and their composites, as well as the interaction between these two phases were determined by X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FT-IR).

Before examination, the samples were pretreated by freeze drying. The fracture surface of each sample was coated with gold and observed by scanning electron microscope (SEM) (JSM-5900LV, Japan) at 20 kV.

Water absorption of the graphite/PVA composites was determined by gravimetric procedure. The water content of the composite was calculated by using the formula:

$$W_2 = \frac{W_k - W_d}{W_k} \times 100\%,$$

where $W_k$ and $W_d$ are the weight of hydrated composite and dried composite, respectively.

The elongation at break and tensile strength of the composite samples with the size of 30 mm × 6 mm × 3 mm were tested using a mechanical testing machine (Reger-3050, China). Five samples with the same graphite content were used in each test group. The tests were carried out at a condition of room temperature and 76% RH. Samples were strained to failure at a rate of 50 mm/min.

2.4. In vivo experiment

The porous graphite/PVA hydrogel composites and the porous PVA hydrogels were prepared in a cylindrical shape with a diameter of 10 mm and a length of 2 mm. Before surgery, the cylindrical samples were sterilized with 70% ethanol for 3 h and washed three times with physiological saline. Then the samples were implanted in the dorsal muscle of adult mice. At 1 week and 12 weeks post-implantation, the
samples together with surrounding tissues were explanted, fixed in a 10% formalin solution (pH=7.4) and dehydrated in gradient ethanol solution (from 70% to 100% (v/v)), then embedded in paraffin. The paraffin samples were sectioned into 5 μm in thickness and stained with hematoxylin and eosin (H&E), and observed by optical microscope (Olympus, GX51, Japan). The tissue response was independently rated by at least two persons according to the following scoring system: − = no infiltration till +++ += severe infiltration of e.g. macrophages/giant cells and lymphocytes. Furthermore, sections were examined for the presence of fibrin, the induction of vascularization and the formation of fibrous capsules around the implants.

3. Results and discussion

3.1. IR analysis

The FT-IR spectra in Fig. 3 show the characteristic absorptions of PVA hydrogel, graphite and the graphite/PVA hydrogel composite. In the composite, the following absorption peaks of PVA and graphite are observed without shift, including 3600–3000 cm⁻¹ (O–H), 3000–2800 cm⁻¹ (CH, CH₂), 1450–1350 cm⁻¹ (CH₂), and 1161 and 1118 cm⁻¹ (C–O–C, C–C) as reported elsewhere [25–28]. However, some significant changes of the peaks of the PVA were observed in the composite. The C–C stretching vibration peak presented at 918 cm⁻¹ and 854 cm⁻¹[27] in the only PVA hydrogel, the former shifted to 899 cm⁻¹ and the latter disappeared in the composites. The C–H wagging of PVA at 1238 cm⁻¹ and the C=O or C=C stretching of PVA at 1710 cm⁻¹ also disappear in composite[25,26,29]. The presence of OH, CH, CH₂ and C–O–C in pure graphite arose from graphite production process, which was similar to those of C=O, C=C and C–O–C in pure PVA [25]. In addition, it was reported in the literature that the unsaturated boundary effect of the graphite particle was avoided by the presence of OH, COO⁻ and CH groups on the surface of the flake crystal graphite particles [30]. That caused the presence of OH peak of graphite at 3600–3000 cm⁻¹ in IR spectra in Fig. 3c.

The disappearance and shift of the characteristic absorption peaks might result from the hydrogen bond interaction of different groups between graphite and PVA, such as bindings between OH of graphite and OH of PVA, and even between OH of graphite and COO⁻ of PVA. The interaction between graphite and PVA should have positive effect on the interface behavior and mechanical properties of the composite.
3.2 XRD analysis

Fig. 4 shows relevant XRD patterns of PVA hydrogel, graphite and the graphite/PVA hydrogel composite. It is known that PVA is a semicrystalline polymer. There are strong interactions between the polymer chains of PVA, which result from the formation of hydrogen bondings between the hydroxyl groups. PVA had an obvious diffraction peak at $2\theta = 20^\circ$ and was characteristic for an orthorhombic lattice. In graphite/PVA hydrogel composite, however, the crystallinity of the PVA phase decreased as shown in Fig. 4b, indicating that the crystal structure of PVA was changed after forming the composite. This can be explained that the preferential formation of H-bonds between the graphite particles and the polymer inhibit the formation of H-bonds between PVA chains which contributed to PVA crystallinity [30].

3.3 Mechanical property

Mechanical properties such as tensile strength and elongation at break are important for cornea biomaterials. After graphite/PVA hydrogel composites sufficiently swelled in deionized water at 37 °C for 5 days, the effect of graphite content on the tensile strength and elongation at break of the composite is shown in Fig. 5. Unsurprisingly, the elongation at break and the tensile strength decreased with the increase of graphite content. However, the mechanical properties did not decrease excessively compared to the PVA only hydrogels, even when the graphite content was 10 wt.% the composite still exhibited a tensile strength of more than 3 MPa with the elongation at break of 350%.

3.4 Water content

Water content is another crucial character for hydrogel used for artificial cornea material, because it will influence the biocompatibility of hydrogel material. Fig. 6 displays the water content of the composite with different graphite content. It can be seen that the water content decreases slowly with the increase of graphite content within 10 wt.%. But when the graphite content exceeded 10 wt.%, the water content dropped apparently. It seems that the interactions between molecules of PVA and water might be blocked by the graphite due to the spatial effect, the surface-adsorption of graphite, and the interactions between graphite and PVA in the composite. Additionally, as shown in Fig. 6, a water content of more than 75% is desirable to the composite with the graphite content of 10 wt.%, compared to the PVA only hydrogel.

When the graphite content exceeded 10 wt.%, the composites were unfit to prepare artificial cornea skirt because of their decreasing fluidity and the graphite falling partially off the composites. Therefore, the graphite contents of 10 wt.% and 5% was fit to prepare further porous graphite/PVA hydrogel composites, and the content of 10 wt.% was selected to use in this paper considering the adsorption capability of the graphite.

Fig. 8. Light micrographs of graphite/PVA hydrogel composite (a, b) and PVA hydrogel (c, d) at 1 week after implantation. Neovascularization (arrow) was found in the pores of the composite. Original magnification ×400. HC, PH and M present graphite/PVA hydrogel composite, PVA hydrogel and muscle tissue, respectively.
3.5. Porous graphite/PVA hydrogel composite

As the skirt for artificial cornea, the materials are expected to have an interconnective porous network, and the mechanical properties and water content similar to nature donor cornea. Porous inorganic/organic composites with different porosity were made using a particle-leaching method by controlling the addition amount of water-soluble particles. The porous graphite/PVA hydrogel composite with a porosity of about 60% (cursorily evaluated by using the volume of the water in the pores of the composite and the volume of the composite) and a water content of more than 90 wt.% was obtained by leaching out 55 wt.% NaCl particles, as shown in Fig. 7. Due to the freeze-drying dehydration process of the hydrogel samples which caused volume shrinkage of the samples, the pore size and porosity could be reduced by this process. So, the actual pore size and porosity should be higher than the Fig. 7 shown. As a skirt for artificial cornea needs to support intraocular pressure of human eyes, and provide enough strength to facilitate surgery suture. The composite had an interconnective porous network with a tensile strength of 0.32±0.07 MPa, which was much higher than normal intraocular pressure of human eyes (about 3 KPa) [31], and suitable for suturing in cornea operation. Therefore, the porous graphite/PVA hydrogel composites could fulfill the mechanical requirements of the cornea skirt.

3.6. In vivo biocompatibility

The histological micrographs of porous graphite/PVA hydrogel composites and porous PVA hydrogels after implantation for 1 week and 12 weeks are shown in the Figs. 8 and 9, respectively. It can be observed that new tissues formed surrounding the brims (Fig. 8a and c, Fig. 9a and c) and grew into the pores of the implanted materials (Fig. 8b and d, Fig. 9b and d). Furthermore, we used a semi-quantitative histomorphometric analysis to score tissue reaction (Table 1), and evaluated the number/presence of fibrin, induction of vascularization, infiltration of polymorph nuclear cells, lymphocytes, macrophages etc.

At 1-week postoperatively (Fig. 8), early inflammatory response was presented in both graphite/PVA hydrogel composite and PVA hydrogel, which was predominantly characterized by the presents of macrophages and lymphocytes. However, there were less inflammatory cells in the pores and in the edges of the composites than those of the PVA hydrogels (Table 1). At this time point, the infiltration or ingrowth of tissue cells was observed in the pores of all implanted materials (Fig. 8). More fibroblasts, fibrin and even new blood vessels can be found in the pores of the composites than those of PVA hydrogels (Table 1). In addition, at this moment, all implants were encapsulated by the capsules primarily consisted of active fibroblasts.

Table 1

<table>
<thead>
<tr>
<th>Implant</th>
<th>Time (weeks)</th>
<th>Fibrin</th>
<th>Fibroblasts</th>
<th>PMN b</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Blood vessels</th>
<th>Capsule c</th>
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<tr>
<td>PVA</td>
<td>1</td>
<td>+</td>
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<td>±</td>
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<tr>
<td>Graphite/PVA</td>
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</table>

a Tissue reactions were rated from sp, ± till ++++: sporadic till extensive; -: not present.
b Polymorph nuclear cells i.e. granulocytes.
c Active fibroblasts and newly formed collagen fibers were identified as structural components of the capsules surrounding the PVA hydrogels or the hydrogel composites, not including in the pores of them.
and newly formed collagen fibers, while the capsules around the composites were thinner than those around the PVA hydrogels (Table 1). However, Fig. 7a and c showed the interface between implanted materials and muscle tissues, suggesting that the interface connection of the composites with muscle tissues seemed tighter than those of the PVA hydrogels.

At 12-week postoperatively (Fig. 9), inflammatory cells significantly decreased in and around all implants. Less lymphocytes, but little more macrophages were observed in and around the composites than those in and around the PVA only hydrogels. At this time point, more fibroblasts, collagen fibers and neonatal blood vessels were observed in the pores of all implants than the weeks before (Fig. 9, Table 1), and they were significantly more observed in the pores of the composites than in the PVA hydrogels. The thickness of the capsules around all implants became decreasing, and could hardly observe around the composites at this moment.

During the 12 weeks implantation period, especially at 1 week, the graphite/PVA hydrogel composite showed slighter inflammatory reactions than the PVA hydrogel, which was evaluated by the number of inflammatory cells, such as macrophages and lymphocytes. However, the macrophages in the pores of the composites were more than in the PVA hydrogel. It possibly resulted from a foreign-body response to the graphite particles in the composites, which may elicit the degradation of the graphite particles in the composites, though the proof was not sufficient.

Previous studies suggested that the tissue reactions to the surgical intervention and subsequent intramuscular implantation were generally similar to the reaction scheme typical of the wound healing processes and foreign-body invasion [32]. Wound healing was suggested to be a complex process involving the interplay of different cell types in the wounded tissues, including inflammatory cells and fibroblasts [33]. In this paper, numerous fibroblasts, collagen fibers, reticular cells and neonatal blood vessels can be observed in the pores of the graphite/PVA hydrogel composites at 1 week and 12 weeks post-implantation, especially at 12 weeks. The infiltration and proliferation of these cells, and the formation of new blood vessels can be thought as tissues ingrowth or wound healing. The surface-adsorption of the graphite might promote the infiltration and migration of cells, thereby enhance the formation of new tissues in the pores of the composites. On the other hand, the foreign-body response to the graphite particles might stimulate macrophages migration or proliferation. Moreover, macrophages have been known to promote the formation of the fibrocytes and vascular endothelial cells [34]. However, the mechanism of the formation of more vascularization in graphite/PVA composite still needs further research.

Consequently, compared to pure PVA hydrogel, the graphite/PVA hydrogel composite exhibited enhanced migration and infiltration of cells, and more neovascularization and tissue ingrowth, which is crucial for the implantation of artificial cornea scaffold because they are helpful to long-term anchoring of the artificial cornea. In this study, the implantation of the porous graphite/PVA hydrogel composites in the rat dorsal muscle provides a preliminary evaluation of tissue reaction to the artificial cornea scaffold material. Further research on this scaffold material is intensely needed due to the substantial difference of physiology environment between eye and muscle.

4. Conclusion

Graphite/PVA hydrogel composites used as the porous ringy skirt of a novel artificial cornea were investigated in this paper. The porosity and pore size could be easily adjusted by leaching out of the soluble NaCl particles. FT-IR and XRD analyses showed the presence of intermolecular interactions between graphite and PVA in the composite. According to the histological analysis, the graphite/PVA hydrogel composite showed slighter inflammatory reactions than the PVA hydrogel at the 1 week post-implantation. Moreover, compared to pure PVA hydrogel, the graphite/PVA hydrogel composite exhibited enhanced migration and infiltration of cells, and more neovascularization and tissue ingrowth, which will be beneficial for the long-term fixation of artificial cornea. Furthermore, the porous graphite/PVA hydrogel composites had enough mechanical strength and high porosity, and have a potential to be studied as novel artificial cornea skirt. Further study will be carried out on the preparation and animal experiments of the artificial core-skirt cornea, that is, a dense PVA transparent core with the skirt of porous graphite/PVA hydrogel composite.

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