

RSC Advances



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This *Accepted Manuscript* will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

Surface modification of PVDF membrane by cross-linked collagen

Lishun Wu¹, Junfen Sun^{2*}, Faqin Tong²

1. Department of Chemistry and Chemical Engineering, Heze University, Daxue Road 2269, Heze,

Shandong Province, 274015, P.R.China

2. State Key Laboratory for modification of Chemical Fibers and Polymer Materials, College of

Material Science & Engineering, Donghua University, North People Rd. 2999, Songjiang,

Shanghai 201620, P.R.China

Corresponding Author: Junfen Sun, junfensun@dhu.edu.cn

Tel number: 86-18602105973, Fax number: 8621-67792855

Abstract

Polyvinylidene fluoride (PVDF) membrane was modified by low temperature plasma treatment and grafted with cross-linked collagen. The crosslinker of collagen was glutaraldehyde. The effects of glutaraldehyde concentration, crosslinking time and crosslinking temperature on membrane properties and surface structure were investigated. Properties of modified membrane were characterized by contact angle and pure water flux. Surface structure of membrane dyed with acid dye was observed by polarizing microscope. The modified membrane was further characterized concerning permeability and adsorption capacity. The bovine serum albumin (BSA) was used as a model protein. Cell culture ability of membrane was examined by methyl thiazolyl tetrazolium (MTT) method. The amount of grafted collagen on membrane surface reached the maximum when collagen was cross-linked with 0.4 wt% glutaraldehyde for 1 h at 35°C. The BSA adsorption capacity and cell culture ability examination indicated that PVDF membrane grafted with cross-linked collagen had good hydrophilicity and biocompatibility.

Key words: Polyvinylidene fluoride (PVDF); membrane; collagen; glutaraldehyde; cell culture

1. Introduction

Polyvinylidene fluoride (PVDF) is a semicrystalline polymer with repeated unit of $-(\text{CH}_2\text{CF}_2)_n-$. It exhibits excellent mechanical property, corrosion and heat resistance, radiation and chemical stability. Moreover, it shows good flexibility and higher strength [1, 2]. The products prepared with PVDF are widely applied in many fields such as food, biological medicine and water treatment industry [3]. PVDF membrane is widely used in membrane distillation, sewage treatment and wine filtration in the recent years. The requirement for the surface properties of PVDF membrane is different when PVDF membrane is applied in different fields. For example, hydrophilicity modification is used to improve antifouling property of PVDF membrane in the fields of food and water treatment in order to reduce protein adsorption of membrane surface [4]. While hydrophobicity modification is needed in the field of membrane distillation [5]. Moreover biocompatibility of membrane is required in the field of biological medicine [6, 7]. In general, the modification methods about membrane surface include blending and surface treatment [8]. Plasma treatment is a common method to change the surface properties of membrane and is an easy way to treat membrane. Grafting polymerization is further induced and other functional groups are introduced on membrane surface [9-13]. There are two advantages for grafting polymerization modification induced by low temperature plasma treatment. One is that the surface property does not decay for a long time. The other is that the surface property is effectively improved. PVDF membrane has been modified by Akashi [12] and Yang [14] by using plasma treatment to improve hydrophilicity of membrane surface. The studies show that hydroxyl, carboxyl and carbonyl groups are generated on the membrane surface. After PVDF membrane was treated by plasma,

poly(acrylic acid) (PAA), immunoglobulin G (IgG), poly(glycidylmethacrylate) (PGMA) and 2-methacrylic acid-3-(bis-carboxymethylamino)-2-hydroxypropyl ester (GMA-IDA) were introduced on membrane surface by You [9], Paslaru [11], Young [15] and Li [16] respectively to improve hydrophilicity and biocompatibility of membrane. Carbon tetrafluoride (CF₄) was grafted on the surface of PVDF membrane via plasma treatment by Yang [17] and the modified membrane was used for direct membrane distillation.

Collagen protein is a crucial biological material and mainly consists of glycocoll, proline, alanine and so on. Collagen is one of the major constituents of extracellular matrix and is well known for low antigenicity, excellent biocompatibility and biodegradability [18]. It has good biocompatibility in comparison to other synthetic materials. The study on collagen protein is very active nowadays. It is widely applied in the fields of medicine and health care, cosmetics and food now. Moreover researches and applications about collagen increase gradually in the fields of nerve conduits, artificial skin, food packaging and microbiological culture media [19-22]. However the application about collagen is limited due to lower mechanical strength and fast degradation in the body. So crosslinker is used to combine collagen molecules with covalent bonds to improve the stability and mechanical strength of collagen.

Glutaraldehyde is a most widely used crosslinker with two functional groups. It has good water solubility as well as lower price. Two Schiff alkalies will form by the reaction between two aldehyde groups and two amine groups in identical or different molecules, and two collagen molecules are combined by a five carbon bridges [23, 24]. Aldehyde group could react with residue amine, amide and other groups of collagen molecules [25]. As a crosslinker, glutaraldehyde shows a lot of advantages such as high reaction activity with protein, good

crosslinking performance and good stability of crosslinking points between glutaraldehyde and protein. Furthermore, glutaraldehyde combines the linear molecular chains of protein and netty molecular chains of protein form in solution, which keep the fine structure of protein molecular chains in space. The collagen cross-linked by glutaraldehyde on hydroxyapatite [26], galactomannan [27], aminolyzed poly(l-lactic acid) [28] and chitosan [18] was reported. However the research concerning grafting cross-linked collagen on PVDF membrane surface by plasma treatment has not been found yet. To graft cross-linked collagen on PVDF membrane surface by plasma treatment improves the hydrophilicity and biocompatibility of PVDF membrane because collagen contains hydrophilic groups. In this study, the cross-linked collagen was grafted on the surface of PVDF membrane to improve the hydrophilicity and biocompatibility of PVDF membrane. The contact angle, pure water flux, dyeing characterization, protein adsorption capacity and cell culture ability were investigated to study the influence of cross-linked collagen on the hydrophilicity and biocompatibility of modified PVDF membrane.

2. Experimental

2.1 materials

Poly(vinylidene fluoride) (PVDF) flat membrane with reported pore size of 0.45 μ m was purchased from the Millipore Co.Ltd (US). Collagen (Mn=3000Da) was purchased from Tianfu garden biological technology Co.Ltd (China). Helium (the purity was 99.99%) was obtained from Lmgas Co.Ltd of Shanghai (China). Acid dye (M-B) was purchased from Yayun Co. Ltd of Shanghai (China). Glutaraldehyde (analysis grade) and bovine serum albumin (BSA, Mw=67000) were bought from Shanghai Chemical Reagent Company (China).

2.2 Membrane modification method

Glutaraldehyde was added into 25 g/l collagen solution. The concentration range of glutaraldehyde was changed from 0 to 0.6 wt%. The crosslinking temperature was monitored from 25°C to 55 °C and the crosslinking time was adjusted from 0 to 2 h.

PVDF membrane was cut into small pieces (5cm×5cm) and arranged in the chamber of low temperature plasma treatment apparatus (HD-1A, ZhongKe ChangTai Plasma Technology Co., Ltd, China). Radio frequency power and inductive coupled electrode were adopted. The treatment condition was selected as argon medium, 20 Pa gas pressure, 50 W power and 90 s processing time.

After PVDF membrane was treated by low temperature plasma treatment, it was rapidly immersed into the 25 g/l cross-linked collagen solution at room temperature to initiate grafting reaction. The membrane was taken out in 60min and washed with distilled water to clean the collagen adsorbed on the membrane surface, and stored in wet state. We found 25°C of grafting temperature and 60 min of grafting time were good for PVDF membrane in previous study. So 25°C of grafting temperature and 60 min of grafting time was used in this work.

2.3 Pure water flux

The membranes were subjected to pure water flux estimation at a trans-membrane pressure of 0.1MPa under cross-flow filtration. The permeability was measured under steady-state flow. Pure water flux was calculated as follows:

$$J_w = \frac{Q}{A\Delta t}$$

Where Q was the quantity of permeate collected (in l), and A was membrane area (m^2), Δt was the sampling time (h), J_w was pure water flux ($\text{l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$).

2.4 Contact angle

Contact angle of modified PVDF membrane was measured at room temperature ($25\pm 1^\circ\text{C}$) by using sessile drop method. A computer-controlled video contact angle meter (OCA40, Dataphysics Company, Germany) was used. Water contact angle measurements (seven measures on different positions per sample, 1 μL drops of distilled water) were carried out and each measurement was considered to have $\pm 3^\circ$ accuracy.

2.5 The dyeing of membrane surface

0.5 g/l acid dye (M-B) solution was prepared with distilled water. Grafted PVDF membrane was clipped into small pieces (1.5 cm \times 2 cm) and put into the dye solution for 2h. After the membrane was dyed completely, it was taken out and washed with distilled water. The membrane was soaked up with filter paper and photographs of the surface of dyed membranes were taken on a Leica DM750P polarizing microscope (Germany).

2.6 BSA static adsorption

The static protein adsorption capacity of membranes was determined with bovine serum albumin (BSA). The membranes were dried at 30°C in a vacuum oven before examination. The samples containing 2 g/l BSA were incubated with an exact amount of membranes in sealed containers under continuous shaking at 25°C . The membrane adsorbed the BSA thereby reducing the BSA concentration in the bulk. The equilibrium BSA concentration after 24 h was monitored in time with a UV-1800 spectrophotometer which was produced by SHIMADZU Company. The BSA depletion was measured at 280nm with 5mm quartz cuvettes.

2.7 Cell culture ability examination

Cell culture ability of PVDF membrane was evaluated by methyl thiazolyl tetrazolium (MTT)

method. The experimental process was described as follow: (1) The PIEC cells (pig artery endothelial cells) in the wall of the culture vessel were digested by trypsin. (2) The cells were counted with the method of blood cell counting plate and were suspended in water to achieve appropriate concentration. (3) A piece of membrane and PIEC cells were put into the holes of a 96 holes plate. The number of PIEC cells was ten thousands per hole. Fresh nutrient solution was added in the holes until the total bulk of each hole was 200 μl . (4) The 96 holes plate was put in incubator for overnight at 37°C. (5) The nutrient solution was changed and the total volume of each hole was kept as 200 μl . (6) The 96 holes plate was cultured in incubator for 24h at 37°C. (7) The liquids in the 96 holes plate were poured out. 1 μl MTT was added in each hole and the plate was cultured in incubator for 4 h at 37°C. (8) 100 μl DMSO was added and was shaken for 20min. (9) The optical density value was test by an enzyme standard instrument (MUTISKAN, Labsystems company, Finland) at 570 nm. The enzyme standard instrument was used to detect samples (less than 250 μl) on a 96 holes plate with colorimetric method.

3. Results and discussions

3.1 Effect of glutaraldehyde concentration on properties and surface structure of modified PVDF membrane

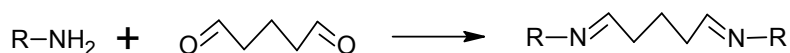
3.1.1 Contact angle and pure water flux of modified PVDF membrane

The effect of glutaraldehyde concentration on the contact angle and pure water flux of modified membrane is shown in Fig. 1. PVDF membrane surface is treated with plasma and the surface is further grafted with collagen cross-linked by glutaraldehyde. As shown in Fig. 1, the contact angle decreases and pure water flux increases with increasing glutaraldehyde concentration when glutaraldehyde concentration is less than 0.4 wt%. The contact angle reaches

the minimum value of 56.7° and pure water flux reaches the maximum value of $245.6 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ when the concentration of glutaraldehyde is 0.4 wt%. While the contact angle increases and pure water flux decreases with further increasing glutaraldehyde concentration when the concentration of glutaraldehyde is above 0.4 wt%.

After PVDF membrane is modified by low temperature plasma treatment, there are a large number of free radicals such as hydroxyl, carbonyl, carboxyl and other polar groups on the membrane surface [12]. Collagen protein molecule possesses terminal amine and carboxyl groups. Generally collagen protein adopts two ways to be grafted on the membrane surface. The first way is that part of the radical groups on membrane surface could transfer to protein to form protein free radicals [29, 30]. The protein free radicals react with radical groups on membrane surface and protein is grafted on membrane surface because of coupling termination. The second way is that the unreacted amine groups on collagen molecules react with the carbonyl groups on membrane surface and collagen molecule is introduced on the surface of membrane [12].

The intramolecular and intermolecular crosslinking points form between glutaraldehyde molecule and collagen molecule after collagen is cross-linked by glutaraldehyde. Two aldehyde groups at both ends of the glutaraldehyde form Schiff bases with amine groups of collagen molecules respectively. The two Schiff bases with five carbon bridges connect collagen molecules so that the molecular weight of collagen becomes bigger and the stability of collagen is strengthened. The chemical equation of the reaction is as follows:



The quantity of collagen grafted on each active centre of membrane surface increases when membrane surface is grafted with cross-linked collagen. The contact angle of membrane decreases

and water flux increases because of the good hydrophilicity of collagen. When glutaraldehyde concentration is 0.4 wt%, collagen protein grafted on membrane surface reaches a maximum value, which leads to a minimum contact angle and a maximum water flux at this point. However the contact angle increases and water flux decreases when the glutaraldehyde concentration further increases. This is attributed to two reasons. Firstly, when glutaraldehyde concentration is over 0.4 wt%, the number of terminal amine groups of collagen protein molecules cross-linked with glutaraldehyde increases with the increasing glutaraldehyde concentration, which results in excessive crosslinking reaction and high crosslinking degree. Moreover the residual quantity of terminal amine groups decreases and the number of reactive centre reacting with the carboxyl groups on membrane surface reduces so that the quantity of collagen introduced onto membrane surface decreases. Secondly, collagen protein molecules may turn into dense cluster structures because of excessive crosslinking reaction when glutaraldehyde concentration is over 0.4 wt%. The remaining reactive terminal amine groups are wrapped up in the cluster and the number of reactive centre of the cross-linked collagen decreases, which makes less amount of collagen be grafted on PVDF membrane surface. So with further increasing glutaraldehyde concentration, the quantity of collagen grafted on membrane surface decreases, the hydrophilicity of PVDF membrane decreases and the contact angle increases.

3.1.2 Characterization of membrane surface structure

In order to observe the collagen grafted on membrane surface, PVDF membrane was dyed by acid dye (M-B) and photographs with different magnification were taken. Photographs of dyeing membrane surface are shown in Fig. 2. The magnification of each membrane is 40 and 100 respectively. There are a lot of polar groups such as hydroxyl, carboxyl and amine groups in the

collagen molecules. These polar groups can be dyed under acid or alkaline condition. It means that the collagen on the surface of PVDF membrane can be dyed by direct dye, acid dye and reactive dye. In this work, acid dye was used to dye PVDF membrane. As shown in the Fig. 2A, the surface of original PVDF membrane is clean. The darker parts on the membrane surface (Fig. 2B) are linear collagens and the darker parts on the membrane surface (Fig. 2C and D) are the cross-linked collagens. Many cross-linked collagens are grafted on the surface of PVDF membrane, as shown in Fig. 2B and C. The size of dark points on the surface of PVDF membrane increases, while the amount of dark points decreases with increasing glutaraldehyde concentration from 0.4 wt% to 0.5 wt%, as shown in Fig. 2C and D. It is suggested that less collagen is grafted on the surface of PVDF membrane when glutaraldehyde concentration is 0.5 wt%.

A large number of active centres such as free radicals and carbonyl groups form on membrane surface after PVDF membrane is modified by low temperature plasma treatment. It is possible that each active centre has a chance to react with one collagen molecule during collagen grafting reaction process. The uncross-linked collagens have low molecular weight. The collagens with low molecular weight are introduced on the active centre of membrane surface when the collagens are not cross-linked by glutaraldehyde. Collagen molecular weight increases several times after crosslinking. The collagens with high molecular weight are introduced on active centres of membrane surface during grafting reaction process when the collagens are cross-linked. However it is difficult to graft excessively cross-linked collagens on membrane surface. The above phenomenon is observed directly after membrane surfaces are dyed.

3.2 Effect of crosslinking time on properties and surface structure of modified PVDF membrane

3.2.1 Contact angle and pure water flux

Fig. 3 shows contact angle and pure water flux of PVDF membrane grafted with collagen in different crosslinking time. As shown in Fig. 3, the contact angle of membrane decreases and pure water flux increases gradually with extending crosslinking time when crosslinking time is less than 1h. The contact angle reaches a minimal value of 56.1° and pure water flux reaches a maximal value of $245.6 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ when crosslinking time is 1h. The contact angle increases and pure water flux decreases rapidly with extending crosslinking time when crosslinking time is over 1h.

The aldehyde groups of the glutaraldehyde react with the terminal amine groups of collagen and schiff bases form during crosslinking reaction process of collagen and glutaraldehyde. Consequently, covalent bonds form among collagen molecular chains, which results in high molecular weight and stability of collagen. However the reaction rate is slow because of low concentrations of collagen and glutaraldehyde in solution.

The crosslinking degree of collagen increases and the molecular weight of cross-linked collagen increases gradually with extending crosslinking time. The amount of collagen introduced on membrane surface during grafting reaction process gradually increases so that the contact angle of membrane decreases and pure water flux increases when crosslinking time is less than 1h. While the crosslinking degree of collagen is too high and the dense clusters form when crosslinking time is over 1h. The number of remaining terminal amine groups and the functional groups participated in grafting reaction decrease, and most of the remaining terminal amine groups of collagen are wrapped up in the clusters. The amine groups embedded in the clusters are difficult to react with the active centres on membrane surface, which results in less amount of collagen grafted on membrane surface. Therefore, the contact angle of membrane increases and pure water

flux decreases.

3.2.2 Characterization of membrane surface structure

Fig. 4 shows the Photographs of Dyed PVDF membrane grafted with cross-linked collagen in different crosslinking time. The crosslinking time of collagen in glutaraldehyde solution is 0, 1h, 2h respectively and the photographs of each dyed membrane are shown in Fig. 4A, B and C. More collagens are introduced on the surface of membrane when crosslinking time is 1 h, as shown in Fig. 4B. Less collagen is introduced on the surface of membrane when crosslinking time is 2 h, as shown in Fig. 4C. It indicates that the collagen is over cross-linked and collagen might form dense cluster structures when crosslinking time is too long. The number of reaction active centre of collagen reduces and most of the active centre is wrapped up in the clusters, which block the grafting reaction and less amount of collagen is introduced on the surface of membrane. Therefore, the efficiency of grafting collagen on PVDF membrane is the highest when collagen is cross-linked with glutaraldehyde for 1h.

3.3 Effect of crosslinking temperature on properties and surface structure of modified PVDF membrane

3.3.1 Contact angle and pure water flux

Fig. 5 shows the contact angle and pure water flux of PVDF membranes grafted with cross-linked collagen at different crosslinking temperature. As shown in Fig. 5, the contact angle of PVDF membrane decreases and pure water flux increases slightly with rising crosslinking temperature from 25°C to 35°C. While the contact angle of PVDF membrane increases slowly and pure water flux decreases sharply when the temperature exceeds 35°C. The contact angle of PVDF membrane reaches the minimal value of 55.6° and pure water flux reaches the maximal value of

$271.9 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ when the crosslinking temperature is 35°C . In the range of studied temperature, the contact angle of PVDF membrane varies in a little range and pure water flux changes a lot.

When collagen is cross-linked with glutaraldehyde at 35°C which is close to the temperature of human body (37°C), the molecular chains of collagen elongate and do not shrink in solution. The crosslinking density of molecular chains of collagen was improved and the elongation state of molecular chains of collagen was solidified in solution by using glutaraldehyde to react with collagen. Moreover further grafting reaction of residual active centre is not affected and good graft ratio is kept. Therefore the contact angle of modified PVDF membrane reaches the minimum value and pure water flux reaches the maximum value when crosslinking temperature is 35°C . When the crosslinking temperature rises to 45°C and 55°C , the rate of crosslinking reaction is accelerated. More terminal amine groups participate in crosslinking reaction during reaction process, which leads to excessive crosslinking reaction and a dense cluster structure of collagen. That the residual terminal amine groups are wrapped up in the cluster and block further grafting reaction successfully reduces the ratio of grafting reaction and the amount of collagen on membrane surface. Meanwhile, it is even possible that the denaturation of collagen protein happens at higher temperature and the hydrophilicity of collagen protein becomes worse. Accordingly, the contact angle of PVDF membrane increases and pure water flux decreases rapidly with further rising crosslinking temperature.

3.3.2 Characterization of membrane surface structure

Fig. 6 shows the Photographs of Dyed PVDF membrane grafted with cross-linked collagen at different crosslinking temperature. As shown in Fig. 6, more collagen is introduced on the membrane surface when crosslinking temperature rises from 25°C to 35°C . It indicates that the

ratio of grafting reaction is higher when crosslinking temperature is 35°C. The main reason is that the collagen molecular chains in solution extend better at 35°C. Good crosslinking matrix need possess suitable crosslinking density and a good extension state in space, which do not interfere the next grafting reaction generated by reactive centre. More collagen is introduced on the membrane surface when crosslinking temperature rises from 25°C to 35°C, which makes the contact angle of membrane decrease and pure water flux increase. When the crosslinking temperature further rises to 45°C, the amount of collagen introduced on membrane surface decreases (Fig.6 C). Possible reason is that the rate of crosslinking reaction is accelerated with rising temperature. Excessive crosslinking makes the collagen molecules form dense cluster structures. Reactive centre is wrapped in the clusters, which blocks the next grafting reaction and results in less amount of collagens which are introduced on membrane surface. Meanwhile collagen might be denaturated with further rising crosslinking temperature and part of grafting reaction could not carry out, which makes the contact angle increase and pure water flux decrease sharply.

3.4 BSA static adsorption

Table 1 shows the BSA adsorption capacity of different membranes at pH 6. PVDF is the original PVDF membrane, G-PVDF is the membrane grafted with uncross-linked collagen after plasma treatment, and C-PVDF is the membrane grafted with cross-linked collagen after plasma treatment. The difference between G-PVDF and C-PVDF is that the collagens on the surface of PVDF membrane are cross-linked with glutaraldehyde for different time. The crosslinking time of G-PVDF and C-PVDF is 0 and 1h respectively. As shown in Table 1, for BSA adsorption capacity, C-PVDF < G-PVDF < PVDF. It means that for hydrophilicity, C-PVDF > G-PVDF > PVDF. The

low BSA adsorption capacity contributes to high biocompatibility of C-PVDF membrane.

3.5 Cell culture ability examination

MTT method is adopted to examine the ability of membranes on cell culturing. The OD values of different PVDF membranes are shown in Fig.7. It can be seen from Fig. 7 that the quantity of cells on three different membranes increases with extending culturing time. The growth rate of cells is different for different PVDF membranes. In the first 3 days, the growth rate and quantity of cells on three kinds of membrane are almost the same. In five days, the optical density value (OD value) of different membranes is much different. The OD values of original PVDF membrane, PVDF membrane grafted with uncross-linked collagen (G-PVDF) and PVDF membrane grafted with cross-linked collagen (C-PVDF) are 0.309, 0.489 and 0.562 respectively. It indicates that the growth rate and quantity of cells cultured on C-PVDF membrane have the maximum value among three membranes. High OD value means good cells activity and strong cells culture ability of PVDF membrane. It is suggested that C-PVDF possesses the strongest ability for cell culturing and the best biocompatibility among three different PVDF membranes.

4. Conclusions

PVDF membrane modified by low temperature plasma treatment is used to graft collagen cross-linked by glutaraldehyde. Contact angle reaches the minimum value of 55.6° and pure water flux reaches the maximum value of $271.9 \text{ l}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ when collagen is cross-linked with 0.4 wt% glutaraldehyde concentration for 1 h at 35°C . At this point, the amount of collagen grafted on membrane surface is the highest. For BSA static adsorption capacity, $\text{C-PVDF} < \text{G-PVDF} < \text{PVDF}$. For hydrophilicity and biocompatibility of membranes, $\text{C-PVDF} > \text{G-PVDF} > \text{PVDF}$. C-PVDF membrane possesses the strongest ability for cell culturing and the best biocompatibility compared

to original PVDF membrane and G-PVDF membrane.

Acknowledgements

The authors thank National Natural Science Foundation of China (51203020); Scientific Research Starting Foundation for Returned Overseas Chinese Scholars, Ministry of Education of China; Donghua University central university scientific research special fund (2232012D3-32); Shandong Nature Science Foundation (BS2013HZ027).

References

- [1] S. Rajabzadeh, C. Liang, Y. Ohmukai, T. Maruyama, H. Matsuyama, *J. Membr. Sci.* 423-424 (2012), 189.
- [2] Z. L. Cui, N. T. Hassankiadeh, S. Y. Lee, K. T. Woo, J. M. Lee, A. Sanguineti, V. Arcella, Y. M. Lee, E. Drioli, *J. Membr. Sci.* 473(2015)128.
- [3] C.Y. Feng, K.C.Khulbe, T. Matsuura, A. F. Ismail, *Sep. Purif. Technol.* 111 (2013)43.
- [4] C. Q. Zhao, X. C. Xu, J. Chen, F. L. Yang, *J. Environ. Che. Eng.* 1 (2013)349.
- [5] Z. Y. Wang, L. Q. Sun, Q. Wang, B. A. Li, S. C. Wang, *Eur. Polym. J.* 60 (2014)262.
- [6] J. Yuan, J. Q. Meng, Y. L. Kang, Q.Y. Du, Y. F. Zhang, *Appl. Surf. Sci.* 258 (2012)2856.
- [7] Y. Chang, Y. J. Shih, R. Ruaan, A. Higuchi, W. Chen, J. Lai, *J. Membr. Sci.* 309(2008) 165.
- [8] F. Liu, N. A. Hashim, Y. T. Liu, M.R. Moghareh Abed, K. Li, *J. Membr. Sci.* 375 (2011) 1.
- [9] S. You, G. U. Semblante, S. Lu, R. A. Damodar, T. Wei, *J. Hazard. Mater.* 237-238(2012)10.
- [10] C. Yao, X. S. Li, K.G. Neoh, Z. L. Shi, E.T. Kang, *Appl. Surf. Sci.* 255(2009) 3854.
- [11] E. Paslaru, M. C. Baican, E. G. Hitruc, M. T. Nistor, F. Poncin-Epaillard, C. Vasile. *Colloids Surf. B.* 115(2014)139.
- [12] N. Akashi, S. Kuroda, *Polymer* 55(2014)2780.
- [13] Y. Chang, C. Y. Ko, Y. Shih, D. Quemener, A. Deratani, T. Wei, D. Wang, J. Lai, *J. Membr. Sci.* 345(2009)160.
- [14] X. Yang, R. Wang, L. Shi, A. G. Fane, M. Debowski, *J. Membr. Sci.* 369(2011)437.
- [15] T. Young, H. Chang, D. Lin, L. Cheng, *J. Membr. Sci.* 350(2010)32.
- [16] S. Li, C. Wang, C. Chen, *J. Membr. Sci.* 318(2008) 429.
- [17] C. Yang, X. Li, J. Gilron, D. Kong, Y. Yin, Y. Oren, C. Linder, T. He, *J. Membr. Sci.*

456(2014)155.

[18] Y. Liu, L. Ma, C. Gao, *Mater. Sci. Engin. C.* 32(2012)2361.

[19] A. Lionel, A. Michael, R. Tomas, *Biomaterials.* 32(2011)1543.

[20] T. Takahashi, T. Kondo, K. Tanaka, S. Hattori, S. Irie, S. Kudoh, S. Imura, H. Kanda, *Polym. Degrad. Stabil.* 97(2012)1002.

[21] C. Huang, Y. Chien, T. Ling, H. Cho, J. Yu, Y. Chan, *Biomaterials* 31(2010)8271.

[22] R. R. Rao, A. Jiao, D. H. Kohn, J. P. Stegemann, *Acta Biomater.* 8(2012) 1560.

[23] A. K. Dutta, A. Nayak, G. Belfort, *J Colloid Interf. Sci.* 324(2008)55.

[24] S. B. Adeloju, A. T. Lawal, *Anal. Chim. Acta.* 691(2011)89.

[25] S.Farris, K. M. Schaich, L. Liu, P. H. Cooke, L. Piergiovanni, K. L. Yam, *Food Hydrocolloid.* 25(2011) 61.

[26] M. Kikuchi, H. N. Matsumoto, T. Yamada, Y. Koyama, K. Takakuda, J. Tanaka, *Biomaterials* 25(2004)63.

[27] S.D Figueiro, J. C. Goes, R.A Moreira, A.S.B Sombra, *Carbohydr. Polym.* 56(2004) 313.

[28] Y. Zhu, M. B. Chan-Park, *Anal. Biochem.* 363(2007)119.

[29] A. S. Domazou, V. Zelenay, W. H. Koppenol, J. M. Gebicki, *Free Radical Bio. Med.* 53(2012)1565.

[30] Z. Yu, Xi. Li, X. Wang, X. Ma, X. Li, K. Cao, *J. Chem. Sci.* 124(2012)537.

Table 1 BSA adsorption capacity of different membranes

Membrane	PVDF	G-PVDF	C-PVDF
Adsorption capacity (mg/g membrane)	3.43	2.83	1.81

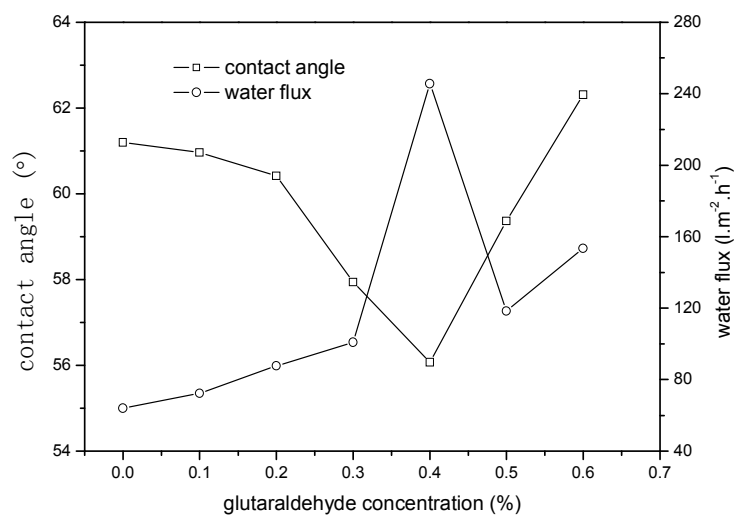


Fig. 1 Contact angle and pure water flux of PVDF membrane grafted with collagen cross-linked by different glutaraldehyde concentration

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, crosslinking time is 1h and crosslinking temperature is 25°C.)

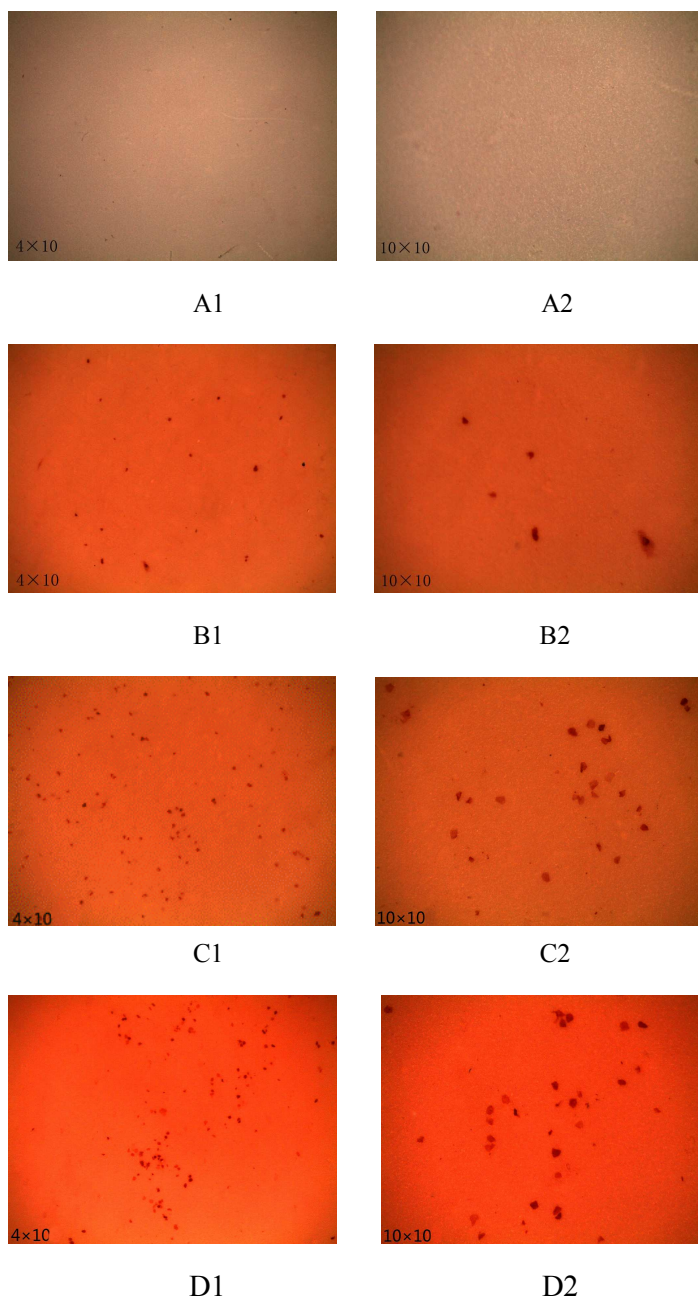


Fig.2 Photographs of dyeing PVDF membrane grafted with collagen cross-linked by different glutaraldehyde concentration.

A is original PVDF membrane. The glutaraldehyde concentration of B, C and D is 0, 0.4 wt% and 0.5 wt% respectively

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, crosslinking time is 1h and crosslinking temperature is 25°C.)

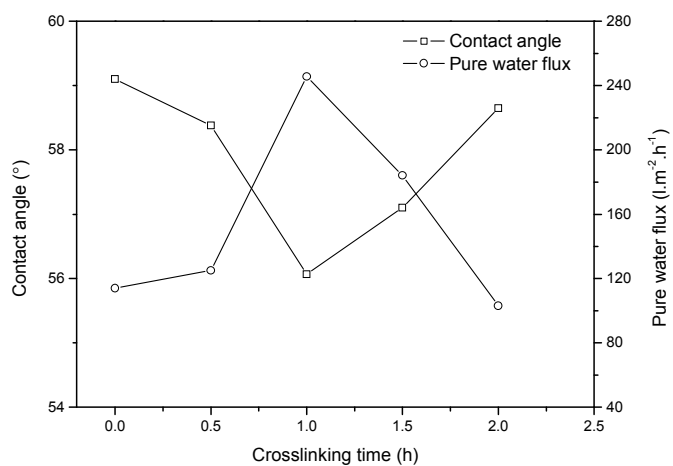


Fig. 3 Contact angle and pure water flux of PVDF membrane grafted with collagen in different crosslinking time

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking temperature is 25°C.)

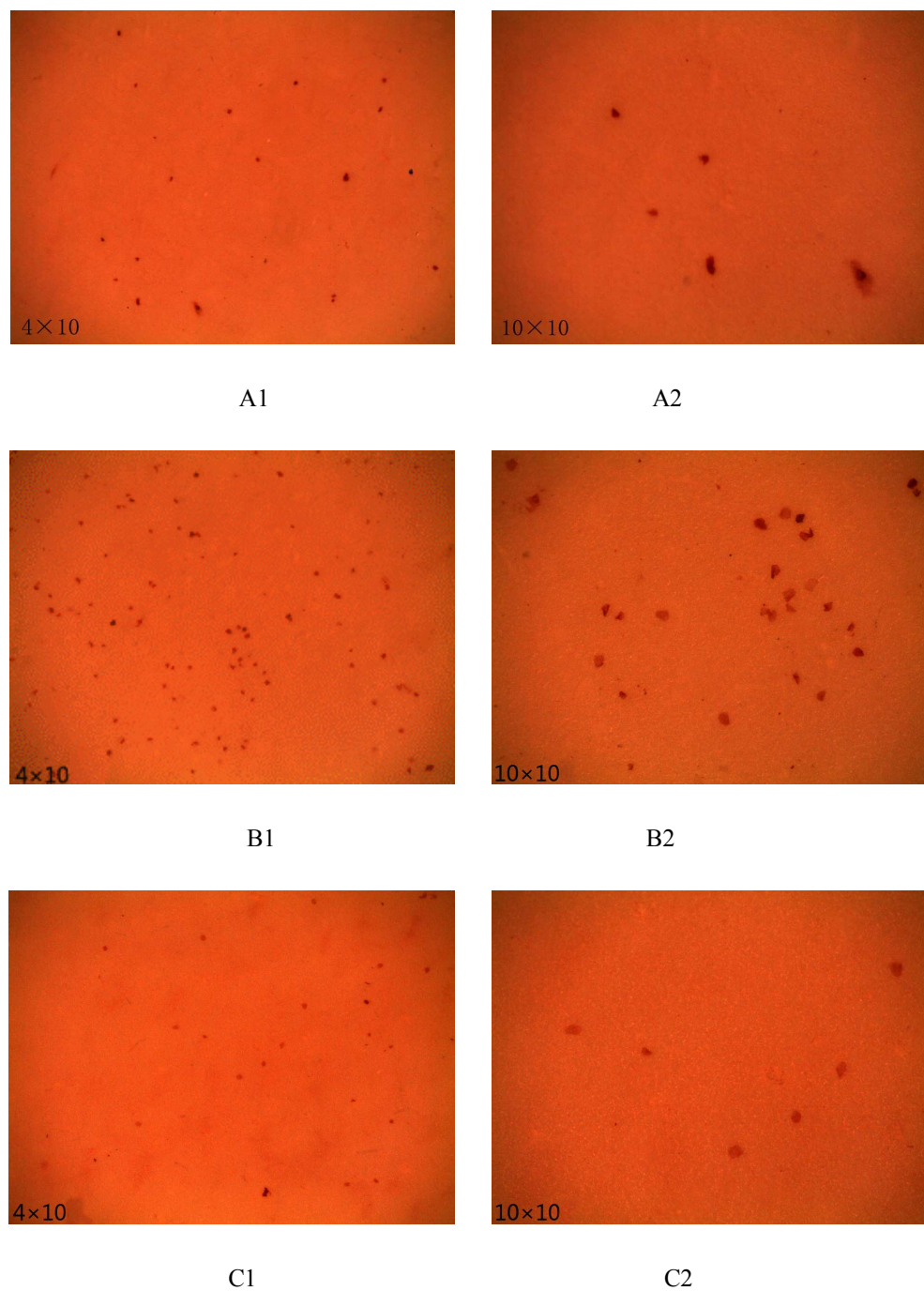


Fig. 4 Photographs of Dyeing PVDF membrane grafted with cross-linked collagen in different crosslinking time.

For A, B and C, the crosslinking time is 0h, 1h and 2h respectively

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking temperature is 25°C.)

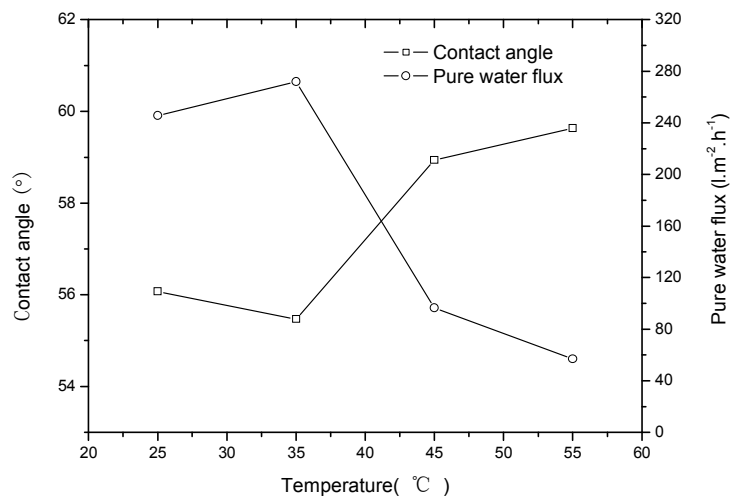


Fig. 5 Contact angle and pure water flux of PVDF membranes grafted with cross-linked collagen at different temperature

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking time is 1h.)

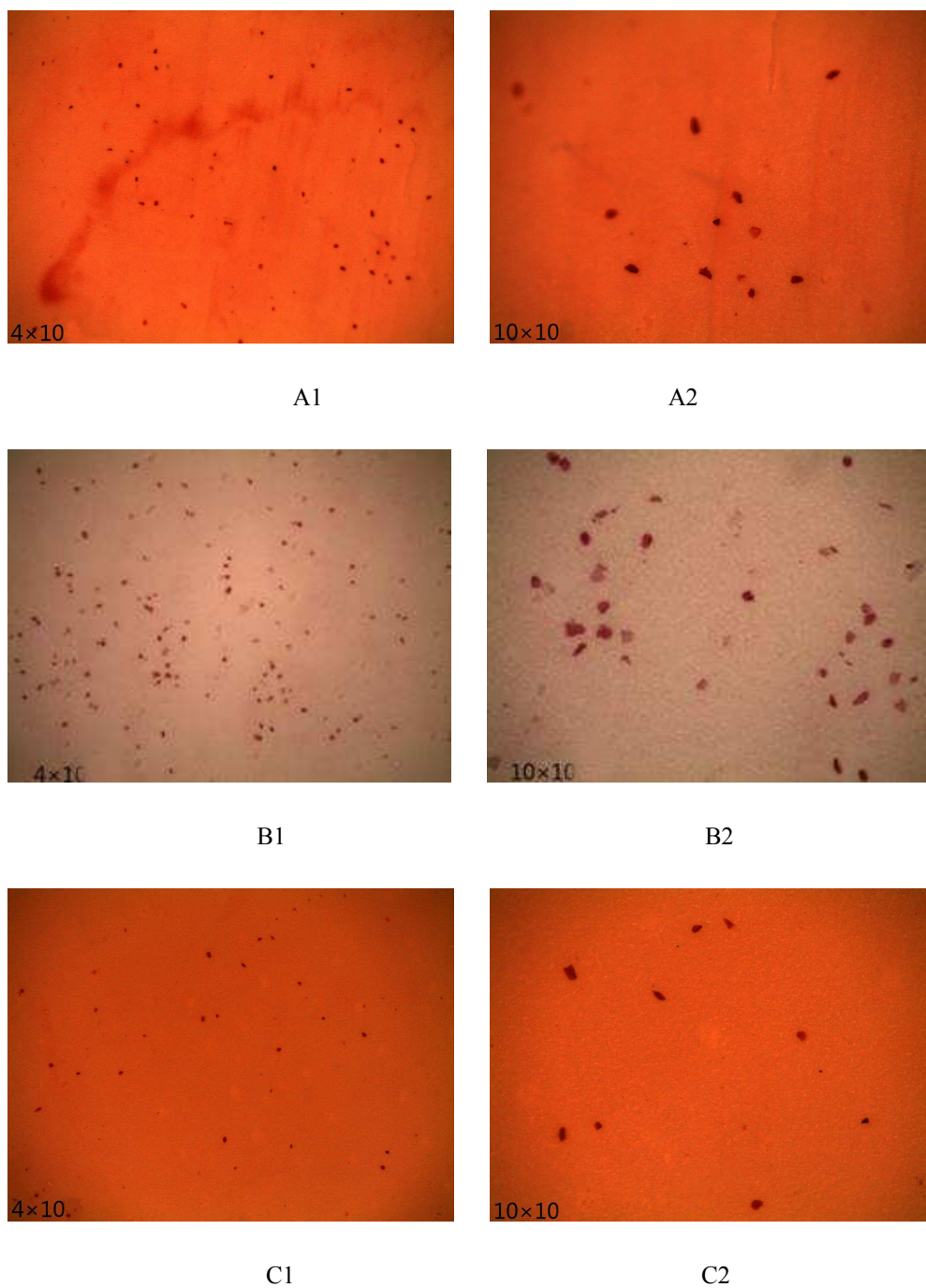


Fig. 6 Photographs of Dyeing PVDF membrane grafted with cross-linked collagen at different temperature.

The crosslinking temperature of A, B and C is 25°C, 35°C and 45°C respectively.

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking time is 1h.)

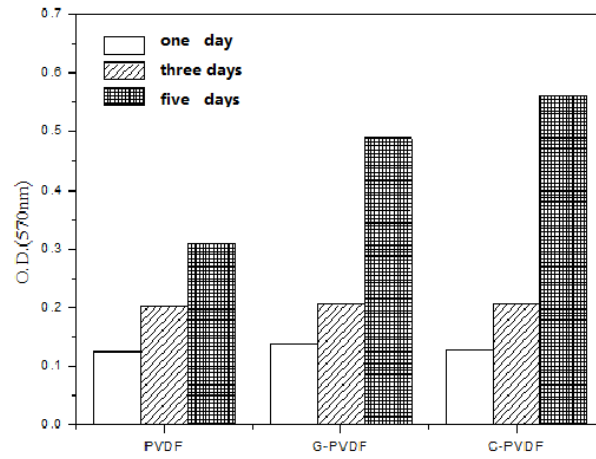


Fig. 7 OD values of different PVDF membranes

Caption list

1. Table 1 BSA adsorption capacity of different membranes
2. Fig. 1 Contact angle and pure water flux of PVDF membrane grafted with collagen cross-linked by different glutaraldehyde concentration

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, crosslinking time is 1h and crosslinking temperature is 25°C.)

3. Fig.2 Photographs of dyeing PVDF membrane grafted with collagen cross-linked by different glutaraldehyde concentration.

A is original PVDF membrane. The glutaraldehyde concentration of B, C and D is 0, 0.4 wt% and 0.5 wt% respectively

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, crosslinking time is 1h and crosslinking temperature is 25°C.)

4. Fig. 3 Contact angle and pure water flux of PVDF membrane grafted with collagen in different crosslinking time

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking temperature is 25°C.)

5. Fig. 4 Photographs of Dyeing PVDF membrane grafted with cross-linked collagen in different crosslinking time.

For A, B and C, the crosslinking time is 0h, 1h and 2h respectively

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking temperature is 25°C.)

6. Fig. 5 Contact angle and pure water flux of PVDF membranes grafted with cross-linked collagen at different temperature

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking time is 1h.)

7. Fig. 6 Photographs of Dyeing PVDF membrane grafted with cross-linked collagen at different temperature.

The crosslinking temperature of A, B and C is 25°C, 35°C and 45°C respectively.

(The plasma discharge power is 50W, working pressure is 20 Pa, medium is argon, processing time is 90s. Collagen concentration is 25 g/l, glutaraldehyde concentration is 0.4 wt%, and crosslinking time is 1h.)

8. Fig. 7 OD values of different PVDF membranes

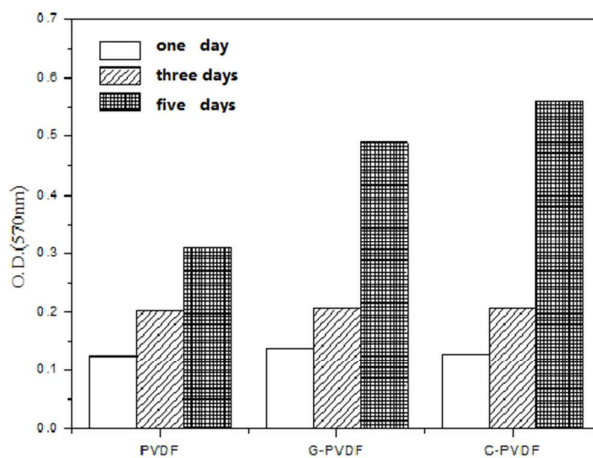
Surface modification of PVDF membrane by cross-linked collagen

Lishun Wu¹, Junfen Sun^{2*}, Faqin Tong²

1. Department of Chemistry and Chemical Engineering, Heze University, Daxue Road 2269, Heze, Shandong Province, 274015, P.R.China

2. State Key Laboratory for modification of Chemical Fibers and Polymer Materials, College of Material Science & Engineering, Donghua University, North People Rd. 2999, Songjiang, Shanghai 201620, P.R.China

Polyvinylidene fluoride (PVDF) membrane was modified by grafting collagen which was cross-linked by glutaraldehyde after plasma treatment. Surface structure of membrane was observed by polarizing microscope after dyed and cell culture ability of membrane was examined by methyl thiazolyl tetrazolium (MTT) method. The modified PVDF membrane has high OD value compared to original PVDF membrane. The BSA adsorption capacity and cell culture ability examination indicate that PVDF membrane grafted with cross-linked collagen has good hydrophilicity and biocompatibility.



OD values of different PVDF membranes