Surface Modified of Silicone rubber by Atmospheric pressure plasma jets and UV Induced-Graft Polymerization of Anti-Bacterial Hydrogels

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Abstract: In this study, the zwitterion hydrogels were grafted onto the treatment of low-temperature atmospheric pressure plasma jet activated silicone rubber surface to improve its anti-biofouling and antibacterial property was demonstrated. Finally, a chemical or natural crosslink agent is used as the crosslinking. Then the zwitterionic hydrogel was synthesized with Hyaluronic acid (HA) by crosslinking the PEG-diamine, which enables the urinary catheter to achieve a low tribological property and dual antibacterial effect.

Keywords: silicon rubber, zwitterion hydrogel, APPJ, hyaluronic acid

1. INTRODUCTION

Silicone rubber is widely used as a natural medicine material because of its excellent softness and elasticity with high hydrophobic properties, such as medical implants for voice prostheses, urinary catheters, and contact lens material. Silicone rubber is highly inert, does not react with most chemicals, and is biocompatible with specific mechanical properties [1-2].

Zwitterionic polymers are essential in various industrial, biological, and medical fields. Moreover, they have many other excellent properties, such as strong hydrophilicity, excellent biocompatibility, and antifouling property. The hydration of zwitterionic polymers plays an essential role in resisting protein adsorption, which depends on the chemical structures, such as polymer density, cation and anion groups and space length, and charge arrangement [4-6].

Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan (GAG). Appeal towards clinical applications of HA is driven by its biocompatibility, biodegradability, processability, and tunable mechanical properties. Various chemical and physical crosslinking methods are available to produce HA-based hydrogels. HA is a fast-degrading polymer in ancrosslinked stete, but crosslinking significantly improves biodegradation [3].

Silicone rubber is commonly used in many fields.In recent years, patients who have been placed in catheters have been exposed to high-risk bacterial infections [1-2]. In order to improve this problem, silicone rubber was chosen as the substrate. In this study, the silicone rubber was modified by APPJ pre-treatment to improve surface activation of the graft polymerization of zwitterion hydrogels and then cross-linked HA onto the substrate. The experimental flow chart of this study is shown in Fig. 1.

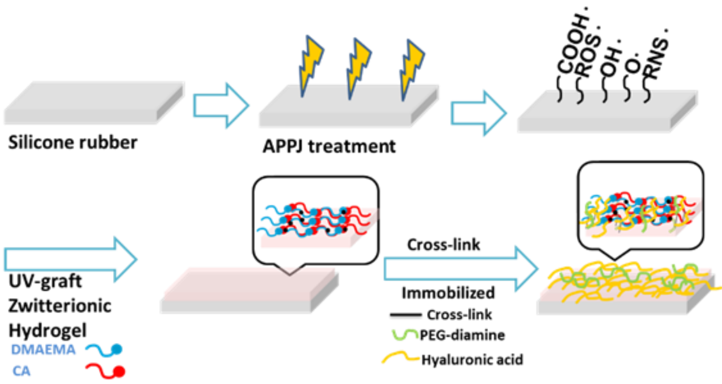

Fig.1 The schematic illustration of the preparation of functionalization surface modification with APPJ pretreatment and then graft polymerization of zwitterion hydrogels onto the silicone rubber and cross-linked HA.

2. EXPERIMENTAL

2.1. Pre-treatment of materials

Silicone rubber was offered from Taiwan GUL CO., LTD, and cut into a suitable size of about 10 × 10 × 10 mm. The silicone rubber was washed with neutral detergent, alcohol, and deionized water ultrasonically for 15 min in each solution and dried in a desiccator to remove contaminants and organic matter on the surface.

2.2. Atmospheric pressure plasma jet Activation Pre-Treatment

In this study, atmospheric pressure plasma (61G20, ae Plasma 41 Co., Ltd Taiwan) was used to treat the surface of the silicone rubber samples. It has a rotating jet head (Φ=35mm) by an AC power system and a moving stage for area scanning. The plasma is generated using pure argon as the working gas, supplied at a constant flow rate of 20 slm. Silicone rubber substrates were subjected to APPJ-treated pretreatment to form peroxide groups and activate groups on the surface. The processing power was at 600 W for a treatment time of the 120s respectively, and the distance between the plasma source and the surface of the samples was 20 mm.

2.3. Surface UV-light grafting of zwitterion hydrogel on **APPJ treated silicone rubber**

The APPJ-treated silicone rubber specimen was soaked in an aqueous solution with mixed 2-carboxyethyl acrylate (CA), and (2-dimethyl amino) ethyl methacrylate (DMAEMA) monomer , and the volume ratio of the monomer solution is 1:1, 1:2, 2:1 respectively. Graft polymerization was performed under UV light (power of 1000W and wavelength of 365 nm) exposure for 15 min. After graft polymerization, the grafted specimens were washed with distilled water overnight to remove the homopolymer aqueous solution.

2.4. HA immobilization on silicone rubber

After the graft polymerization reaction, each substrate was immersed separately and cross-linked with two crosslinking agent EDC/NHS and genipin by mixing PEG-diamine and HA with substrates at 4 °C. The cross-linking time was 1, 2, 12, or 24 hours. After the immobilization process, the substrates were mildly rinsed with distilled water for several cycles to remove the residual cross-linking agents and dry for 24 h at room temperature.

2.5 Characterization analysis

Result analyses of water contact angle(WCA), SEM, FTIR, swelling rate, and antibacterial properties. And the influence of the coefficient of friction at room temperature and in artificial body fluids. Cytotoxicity test is used to culture fibroblasts, the influence of growth on the surface of the substrate, and the attachment situation. In order to evaluate the antibacterial effect, Escherichia coli (E. coli) will be selected for antibacterial experiments.

3. RESULTS AND DISCUSSION

3.1 Wettability of the Surface-Modified silicone rubber sample

The measured water contact angle of the un-modified silicone rubber is $88.5^{\circ} \pm 3.4$.Treatment of samples via APPJ treatment and UV-induced polymerization of zwitterion hydrogels reduces the water contact angle due to the increased surface hydrophilicity cross-linked HA. From the results in the Table I, the surface of substrates treated by APPJ treatment was more hydrophilic than when it was untreated; accordingly, the water contact angle was reduced after APPJ treatment, indicating that the surface becomes hydrophilic and easier for zwitterion hydrogels to graft on it. The increase of surface hydrophilicity may be due to the polar groups produced by APPJ treatment.

After UV grafting and polymerization of different proportions of zwitterion hydrogels on the surface of silica gel, it also belongs to a hydrophilic surface type. Zwitterion-like (CA: DMAEMA ratio is 2:1), and then chemically covalently bonded to the substrate with two cross-linking agents EDC-NHS/PEG-diamine/HA and genipin (GP)/PEG-diamine/HA on the surface, the reaction time is 1 hour, 2 hours, 4 hours and 24 hours respectively. The surface wettability of zwitterion hydrogels will mostly stay the same due to the cross-linking agent immobilizing hyaluronic acid. They are all hydrophilic surfaces. However, after hyaluronic acid is cross-linked with chemical or natural cross-linking agents on the surface of silicone, it can be observed that the longer the cross-linking time, the more hydrophilic the surface becomes.

Table. I Wettability of various substrates after different treatments.

Sample	Untreatment	APPJ treatment (D:2cm/T:2min)	UV-p(CA-co-DMAEMA) 1:1	UV-p(CA-co-DMAEMA) 1:2	UV-p(CA-co-DMAEMA) 2:1
WCA(°)	88.5°±3.4	27.3°±2.6	45.3°±3.1	38.1°±3.8	34.6°±3
					
		EDC-NHS/PEG-diamine/HA 1hr	EDC-NHS/PEG-diamine/HA 2hr	EDC-NHS/PEG-diamine/HA 12hr	EDCNHS/PEG-diamine/HA 24hr
		43°±4.1	30.5°±2.7	27°±3.3	21.3°±2.1
					
		GP/PEG-diamine/HA 1hr	GP/PEG-diamine/HA 2hr	GP/PEG-diamine/HA 12hr	GP/PEG-diamine/HA 24hr
		38.5°±4.5	26.6°±2.4	25°±4.9	18.5°±2.3
					

3.2 Surface morphology of surface-modified silicone rubber samples

A scanning electron microscope (SEM) was used to observe the surface morphology and structural changes of the silicone rubber substrate after treatment, as shown in Fig. 2 . In Fig. 2; there are tiny pores on the surface of the untreated silicone rubber substrate. However, after APPJ treatment, it becomes less noticeable, and different proportions of zwitterion hydrogel are grafted. It can be seen from the SEM image that when When the ratios of CA and DMAEMA are 1:1 and 1:2, needles appear on the surface. When the ratio of CA and DMAEMA is 2:1, a layer of hydrogel can be observed on the surface of the silicone rubber substrate. Fig.3 shows that the silicone rubber substrate was fixed on the surface after EDC-NHS/PEG-diamine/HA and GP/PEG-diamine/HA, respectively, and the treatment time was different. The treatment with NHS/PEG-diamine/HA lasted 1, 2 ,12 hours. As time goes by, it can be found that there are more and more particles of different sizes on the surface. A uniform film coating can be observed after 24 hours, with GP/PEG-diamine/HA in 2 and 12 hours. It can be seen from the SEM image that it is coated with a thin film, and needles appeared on the surface after 24 hours and were crosslinked for 1 hour.

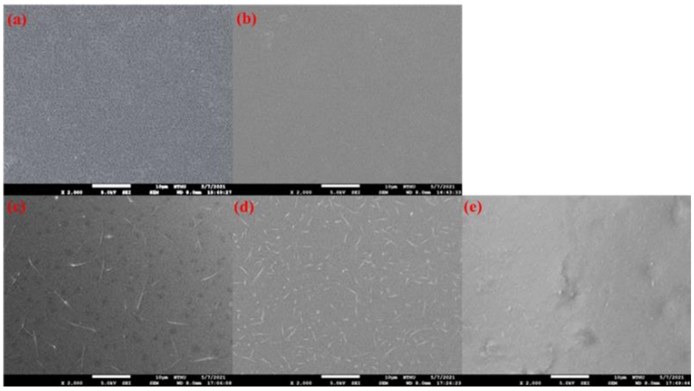

Fig.2 Surface morphologies of (a)Untreatment、(b)APPJ treatment (D:2cm/T:2min) 、(c) APPJ treatment/UV-p(CA-co-DMAEMA)1:1、(d)APPJ treatment/UV-p(CA-co-DMAEMA)1:2 、(e)APPJ treatment+UV-p(CA-co-DMAEMA) 2:1

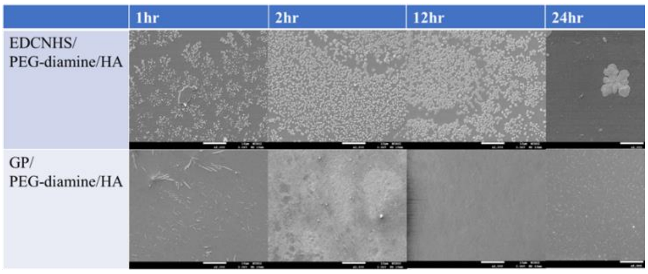

Fig.3 Surface morphologies of silicone rubber substrate after different cross-linking treatment time

3.3 Cytocompatibility assay of surface-modified silicone rubber samples

In order to understand whether the silicone rubber substrate will produce toxicity to the cells after different surface treatments, a cytotoxicity test was carried out. The commercially available mouse fibroblasts (NIH-3T3) used were added to the substrate using cell culture technology for cell culture and tested for their cytotoxicity.Fig.4 shows the effect of the surface modification of the silicone rubber substrate on cell growth. It can be seen from the figure that the O.D value of the untreated silicone rubber substrate is more significant than 0.7, indicating that the silicone rubber substrate itself is non-toxic. There is no cytotoxicity after grafting different proportions of zwitterionic hydrocolloids after atmospheric plasma treatment.

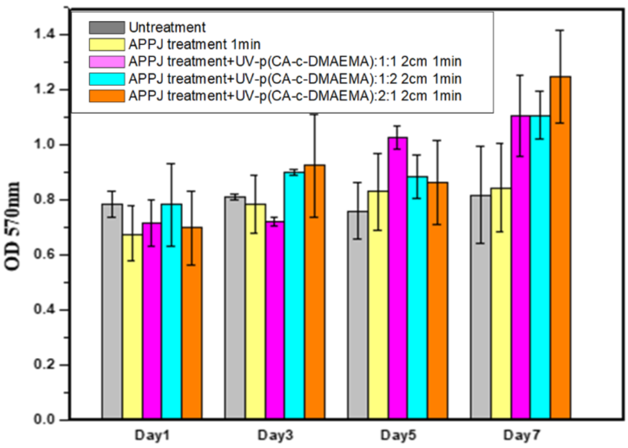

Fig.4 In vitro cytocompatibility of various substrates after different treatments over a period of 1 to 7 days.

3.4 Antibacterial test of surface-modified silicone rubber samples

In this experiment, Gram-negative Escherichia coli were selected for testing to confirm that the silicone rubber substrate has an antibacterial effect after modification.The antibacterial effect is determined by the size of the inhibition zone after antibacterial and compared with the untreated silicone rubber substrate. Antibacterial test of different proportions of zwitterion hydrogel grafted with APPJ treatment. It can be observed that there is no antibacterial zone on untreated and APPJ-treated silica gels. Grafting of different proportions of zwitterion hydrogel can be observed. No matter what the ratio is, there is an apparent inhibition zone. Table. 1 shows the diameter of the antibacterial zone and the corresponding grade after the APPJ treatment grafted with different proportions of zwitterion hydrogel and treated at different crosslinking times. It can be seen from the table that the diameter of the inhibition zone does not change much whether the silicone rubber substrate is treated with different crosslinking times or whether chemical or natural crosslinking agents are used.

Table I. Zone of inhibition in agar diffusion tested against the surface-modified silicone rubber sample.

	Untreatment	1hr	2hr	12hr	24hr
EDCNHS/ PEG-diamine/HA	A	B	C	D	E
	0	17	17mm	19mm	16.5mm
	–	+++	+++	+++	+++
GP/ PEG-diamine/HA	A	B	C	D	E
	0	18	17mm	19mm	20mm
	–	+++	+++	+++	+++

+Inhibitory zone diameter ≤10 mm low sensitive,++10 mm < Inhibitory zone diameter <15 mm is highly sensitive, +++ Inhibitory zone diameter ≥15 mm is significantly sensitive,−show no bacteria grow.

3.6 Friction test analysis of surface-modified silicone rubber samples

The modified silicone rubber tube was used as the experimental group, and the untreated silicone rubber tube was used as the control group for comparison, referring to the specification of ASTM D1894. The test method uses SHIMADZU AGS-X equipment to conduct dynamic and static friction tests with unique measuring fixtures. According to the experimental results in Fig. 5, it can be known that the surface friction coefficient of the untreated silicone rubber tube in room temperature drying is 0.973μ, while the improved coefficient of friction after massaging is 1.302μ, while it is 1.261μ and 0.461μ in artificial urine at 37°C, which proves that this method can effectively increase the lubricity.

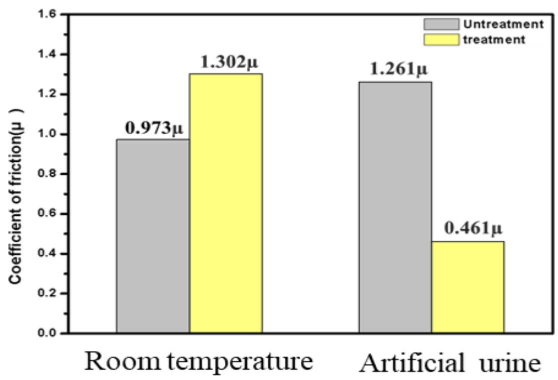

Fig.5 Silicone rubber tube friction test results in different environments

4. CONCLUSIONS

In this study, the zwitterion hydrogels were grafted onto the direct treatment of atmospheric pressure plasma jet (APPJ) -activated surface. Graft polymerization of zwitterion hydrogels, 2-carboxyethyl acrylate (CA), and (2-dimethyl amino) ethyl methacrylate (DMAEMA), then generating a carboxylic acid group and tertiary amine groups at the surface after via APPJ pretreatment. The possibility of plasma treatment for surface offers free radical and covalent grafting of zwitterionic hydrogel with –COOH and –NH_3 group to improve its anti-biofouling property antibacterial property was demonstrated. Finally, a chemical or natural crosslink agent is used as the intermediate crosslinking. Then the zwitterionic hydrogel was synthesized with Hyaluronic acid (HA) by crosslinking the polyethylene glycol diamine (PEG-diamine), which enables the sample to achieve a low tribological property and dual antibacterial effect. By combining multiple surface treatment methods, two different materials of silicone rubber are further enhanced. The silicone rubber sample function is expected to give the surface multifunctional properties (anti-protein adhesion, bacterial adhesion) through surface modification and evaluate the potential for clinical applications.

5. REFERENCES

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