Biological and Physical Properties of a Modification Silicone Liner

Huiru Gu1,2#, Huiqin Luan2,3,4#, Zhongjun Mo2,3,4, Yang Song2,3,4 and Yubo Fan1,2*

1 Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, School of Biological Science and Medical Engineering, Beihang University, Beijing 100191, China
2 Key Laboratory of Intelligent Control and Rehabilitation Technology of the Ministry of Civil Affairs, National Research Center for Rehabilitation Technical Aids, Beijing 100176, China
3 Beijing Key Laboratory of Rehabilitation Technical Aids for Old-Age Disability, Beijing 100176, China
4 Key Laboratory of Rehabilitation Technical Aids Analysis and Identification of the Ministry of Civil Affairs, Beijing 100176, China
Email: yubofan@buaa.edu.cn

Abstract. Silicone liners provide comfortability for amputees, meanwhile effectively protecting their skin surface and subcutaneous tissue. Silicone liners used in prosthetics are mainly used for filling, cushioning, and suspending a receiving cavity. At present, biological and mechanical properties of silicone liners in prosthetic cavity remain unclear, and the price is expensive for amputees. We improve the silicone material preparation process to reduce the price as well as to ensure the mechanics and biosafety of the material. Characterization of silicone liner surface, cellular toxicity of silicone liner, bacterial adhesion on the surface of silicone liner, and mechanical properties were evaluated and compared among commercial materials (sample A and B) and our improved silicone liner material (sample C). The water contact, the macroscopic surface and the calculated mechanical parameters have no significant differences among the three types samples. The extract of three types silicone liner have no cytotoxicity reactivity. Samples C markedly reduced the adhesion of E. coli compared with sample A. The improved silicone material preparation process ensured the biological and mechanical properties and reduced the price of these silicone liners, thereby increasing their potential as silicone liners of prosthetics cavity.

Keywords. Silicone liner, cytotoxicity, bacterial adhesion, prosthetics cavity.

1. Introduction

The installation of lower limb prostheses is an effective method for amputees to compensate for missing movement functions, alleviate other activity problems, and return to society. Silicone liners used in prosthetics play a role in an interfacial connection between a prosthetics socket and the residual limb of a patient. Silicone liners provide comfortability for amputees while effectively protecting their muscle tissue, skin surface, and helping to suspend a receiving cavity. A critical interface will be formed during exercise between the silicone liner and the skin of a residual limb. At this interface, movement pressure and the corresponding frictional force will affect the skin and other subcutaneous tissues of the residual limb. The process of repeated mechanical effect between the
silicone liner material and skin of residual limb will result in blisters and ulceration on the skin, thereby causing fever and pain to the patient. Therefore, a patient’s longstanding wearing of a prosthetic silicone liner frequently exhibits symptoms, including sweat accumulation, irritation, discomfort, bacteria breeding, and infection in the residual limb [1, 2], thus seriously affecting the quality of life of prosthetic wearers. Studies have shown that sores/chafing and skin irritation from prosthetic sockets prevent people from walking in fields and limit rapid walking [3].

Silicone liners used in prosthetics are typically polymeric inserts made of silicone as the main raw material. Repeated mechanical forces of the residual and prosthetic materials may cause wear, aging, and failure of the prosthetic polymeric material [4, 5]. The health of the residual limb skin in lower limb amputees is crucial, but the socket of the prosthesis creates a warm and humid microenvironment that encourages bacterial growth and skin breakdown. Improved mechanical and biological properties of prosthetic silicone liners can protect the physiological functions of the skin and subcutaneous tissue. Based on the abovementioned factors, silicone liners must demonstrate improved biocompatibility and mechanical properties. Studies have reported that skin is more comfortable in touching soft and smooth objects than hard, rough objects [6-9]. From the feel, the toughness and elasticity of silicone liners are excellent; these materials are not easily deformed given external forces and feel smooth. The prosthesis liner must generally satisfy the requirements of durability, fit, safety, and suspension [10, 11]. The comfort, preventing rashes and sores were very important when patient use prosthesis [12]. In particular, using silicone liners for amputees can reduce physical energy consumption, increase wearing time of artificial limbs, extend walking distance and level and scope of activities, and improve the quality of life. To the best of our knowledge, the biological and mechanical characteristics of prosthetic silicone liners have not been sufficiently investigated.

Silicone is used as a prosthetic liner considering its remarkable comfort and biocompatibility. Most silicone liners’ price is costly despite their comfortability, which is approximately 290-1800 dollars. Moreover, the service life of silicone is relatively short, that is, lasting for only 2-3 years of use. At present, prosthetic silicone liners are expensive for amputees. On the basis of non-cytotoxicity and comfortability requirements, we improve the silicone material preparation process in this study to ensure the mechanics and biosafety of the material and try to reduce the price. In addition, we compare the mechanical and biological properties of other manufacturers and our improved prosthetic silicone liner materials. This study will be beneficial to researching prosthetic socket issues, providing insight into the design mechanism of prosthetic silicone liners, and helping improve the fitting for patient prosthesis.

2. Material Preparation
Table 1 list the formulation of a high-temperature vulcanization (HTV) silicone. In the studied formulation, which was represented by X (X = 0, 5, 10, 20, and 30 phr), only the fumed silica content varied, whereas all the other ingredients were constant. Using internal mixer (Haake, Rheocord90) at room temperature to 100 RPM speed ratio will blend the ingredients for 10 minutes. The mixture was then passed through a two-roll mill at a speed ratio of 1:1:1. The silicone sample was obtained by molding a mixture of an iron frame having a thickness of 2 mm and 10 mm at 170 °C with pressure of 6 MPa to achieve an optimization curing time.

| Table 1. The formulation of HTV silicone. |
|----------------------------------------|
| Composition    | Proportion |
| Silicone rubber | 100        |
| Curing agent   | 1.5        |
| Fumed silica   | X          |
2.1. Characterization of a Silicone Liner Surface

Surface morphology was evaluated by using a stereomicroscope (Olympus SZ61, Japan). Contact angle goniometer was used to measure the surface wettability of each silicone (Dataphysics OCA25, Germany).

2.2. Detection the Cellular Toxicity of Silicone Liner

Samples A and B were purchased from American and German manufacturers, respectively. Sample C was prepared by our research group. The preparation of the sample extract is presented as follows [13]: a 1 ml extract was added to a silicone liner per 0.4 g sample, set at 37 °C, and extracted after 24 hours. The experiment groups were categorized as follows: blank control, negative control, positive control, and silicone liner groups A, B, and C.

The cytotoxicity of the silicone samples was detected by L929 mouse fibroblasts (ATCC CCL1, NCTC clone 929, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences). Cells were incubated in essential medium (Hyclone, USA) supplemented with 10% fetal bovine serum (Hyclone, USA) and 1% penicillin-streptomycin (Invitrogen, USA) in a humidified atmosphere of 5% CO2 at 37 °C. These cells were subsequently used in the cell viability assay.

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was used to measure cell viability (Sigma, USA). The density of cells was 1 × 10^4 cells/mL and the cells were seeded in 96-well culture plates. The cell cultured with 0.4 g/mL concentrations of extractions in 5% CO2 at 37 °C for 72 h. The MTT solution was used to wash and treat the cell at 37 °C for 1 h after treatment. The microplate reader was used to detect the absorbency at 490 nm (TECAN, Switzerland).

2.3. Detection the Bacterial Adhesion on the Surface of Silicone Liner Samples

An Escherichia coli strain (Trans10 chemically competent cell transfected with pUC19) was used to conduct bacterial adhesion assessment. The E. coli strain was sub-cultured in LB Nutrient Agar cultured from frozen stock before each assay (Thermo Scientific 700, America), at 37 °C about 24 h, and until the mid logarithmic growth. 1.5 cm × 1.5 cm of the silicone liner samples (A, B, and C) were prepared. Then the culture was added to a six-well culture plate, which contained a silicone-lined sample and incubated for 48 h at 37 °C.

Quantitatively evaluation the bacterial adhesion to the silicone liner samples was using static microplate assay in previous study [14]. After bacterial adhered for 48 h, each silicone liner sample was removed from the media and washed four times by PBS. Then the 0.1% crystal violet solution (Jiancheng Bioengineering Institute, Nanjing, China) was used to stain for 15 min. Then each sample was washed three times by distilled water and was air-dried about 40 min. After 2 mL 95% ethanol dissolved crystal violet-stained biofilms, the 96-well plate containing 20 μL of the solubilized crystal violet was measured the OD (optical density) through the microplate reader. Similar treatments were performed on silicone-lined samples without bacterial adhesion, and the OD values of these samples were used as negative controls. The sample was then dehydrated with a series of fractionated ethanol, dried at a critical point, and sputtered with gold. Finally, the samples were detected by a scanning electron microscope (SEM, Helios, FEI, Netherland).

2.4. Mechanical Characterization

A mechanical test of the three types of sample was measured on a material testing system (AG-IS MO, Shimadzu, Japan).

3. Statistical Analysis

All values were expressed by mean ± standard deviation (SD). One-way analysis of variance of SPSS 15.0 was used to perform statistical analysis. The statistical significance level was set at P < 0.05.
4. Results

4.1. Surface Characteristic
The surface morphology of sample was detected by a stereomicroscope. The image revealed that the macroscopic surface lack significant differences among the three groups (figures 1a-1c). The surface characterization of the samples was evaluated by hydrophilization, and the hydrophilization was assessed by the water contact angles. The water contact of samples B and C was larger than sample A. No evident difference was observed among the three groups (figures 2a-2c).

![Figure 1](image1.png)

**Figure 1.** Microscope images showing the surface morphology of samples. (a): Silicone liner A, (b): Silicone liner B, (c): Silicone liner C. Magnification: 45×, Scale bar: 100 µm (down right).

![Figure 2](image2.png)

**Figure 2.** Water contact angles of the sample surface. Samples A, B, and C represent the silicone liners.

4.2. Cytotoxicity Assessment
Table 2 displays that the extracts of the three types of silicone liner samples have no cytotoxicity reactivity.

|                   | OD      | RGR%    |
|-------------------|---------|---------|
| Silicone liner A  | 0.9804±0.0492 | 111.3015% |
| Silicone liner B  | 0.8949±0.0383  | 101.5985% |
| Silicone liner C  | 1.0071±0.0095  | 114.3207% |
| Blank control     | 0.8885±0.0388  | /       |
| Negative control  | 0.9773±0.0925  | 110.9496% |
| Positive control  | 0.0613±0.0032  | 6.9592%  |

Note: Values are mean ± SD.

4.3. Evaluation of Bacterial Adhesion
Microplate assay was used to quantify the adherent bacteria, as illustrated in figure 3. Samples B and C markedly reduced the adhesion of E. coli compared with sample A. There is no significant difference between sample B and C. Figures 4a-4c depict the representative SEM.
Figure 3. The results from sample A, B and C after incubation with Escherichia coli using static microplate assay. *p < 0.05.

Figure 4. The images of the bacteria adhered to surface of silicon liner were obtained by SEM. A silicone liner A, B silicone liner B, C silicone liner C.
The pictures of the bacteria adhered to the three sample surfaces. These pictures demonstrated that adhesion bacterial is obviously decreased on the sample C surface. This SEM result is match to the evaluation using the static microplate.

4.4. Mechanical Characterization
None of the calculated parameters showed any considerable differences among silicone liner samples A, B, and C (table 3).

| Table 3. Mechanical parameters of the silicone liner. | Maximum load (N) | Hardness (HA) |
|----------------------------------------------------|-----------------|--------------|
| Silicone liner A                                    | 261.13±2.35     | 22.31±1.14   |
| Silicone liner B                                    | 271.04±7.28     | 21.81±1.37   |
| Silicone liner C                                    | 253.45±10.67    | 24.23±1.56   |

Note: Values are mean ± SD.

5. Discussion
Silicone liners used in prosthetics are mainly used for filling, cushioning, comfort, and other functions. However, most prosthetic silicone liners are made of silicone, which is highly hydrophobic. Traditionally, the tendency of a liquid to diffuse on a solid surface is described using the wetting [15, 16] to evaluate a hydrophobic or hydrophilic material. The hydrophilization character of the silicones was evaluated by the water contact angles. The contact angle of the three types of sample was all greater than 90°. A large contact angle indicated that the sample surface is not wetted by the liquid, and the wettability and spread of the liquid are minimal.

Long-time silicone liner wearers may cause infection because their external environment is suitable for the attachment and colonization of bacteria. Therefore, multiple microorganisms often appear in the prosthesis of wearers [14, 17], thereby possibly leading to increased inflammation [18]. Surface hydrophobicity of some materials can affect bacterial adhesion, which have been research by some studies [19-21]. It is an important physical factor during the adhesion process that the surface the bacteria itself is hydrophobicity [22-24]. Bacteria with hydrophilic characteristics can voluntarily adhere to the hydrophilic surfaces, while those with the hydrophobic properties generally adhere freely to the hydrophobic surfaces [14, 24]. Silicone liner surface is hydrophobic. Therefore, in the present study, we used the highly hydrophobic E. coli. Moreover, E. coli was used to assess the bacterial adhesion to the surfaces of silicone liner. These results indicated that the ability of sample C surface resistant to bacterial adhesion is more than sample A surface. Moreover, these findings implied that the effect of inhibitory E. coli adhesion on Sample A is less than the effect adhesion on sample C. In addition, some substances which leach from silicone liner might cause skin damage, such as blister, redness, and ulceration, thereby resulting in fever. So cell viability test can evaluate the cytotoxicity of solution, which can leach from sample. Our results showed that the three types of silicone liner samples do not induce cytotoxicity.

The macroscopic surface morphology revealed that the three types of silicone surfaces display minor changes in the surface morphology. The micro-surface morphologies of the silicone surfaces are demonstrated by SEM. The surfaces were rougher in samples A and C than in sample B. These results indicated that sample C has caused small increases on the roughness surface in the nanometer level without obvious change in the micrometer level. In addition, the mechanical properties of silicone liner affect its durability and comfort. No obvious change is observed in the silicone liner, thereby indicating that the preparation method can be used to improve products, which can be used by amputees.

We prepared the silicone liner with minor improvement the surface morphology. In addition, a hydrophobic surface can inhibit bacteria from sticking to the surface without causing cytotoxicity. Our findings suggested that the silicone liner (sample C) can be used to relieve discomfort, improve its
biocompatibility and reduce prosthesis-related infections among silicone liner wearers. In addition, the prepared sample C might be cheaper than the ones (samples A and B).

6. Conclusions
The main goal of silicone liner preparation is to ensure biological and mechanical properties and reduce the price of these materials. The sample prepared in this study is consistent with the requirements. Moreover, the improved silicone liner can inhibit the bacterial sticking to the surface of liner without causing cytotoxicity. These results imply that the improved silicone liner materials can be used to enhance the biocompatibility of prosthetic silicone liners to relieve discomfort or infections of prosthetic silicone liner wearers. In addition, we conclude that the present silicone liner is unrelated to moisture permeability, and developing new prosthetic socket which can allow heat release and sweat drainage is necessary.

Conflicts of Interest
The authors declare that they have no conflict of interest.

Acknowledgements
This work was funded by National Key R&D Program of China (No. 2018YFB1107000), Sichuan Science and Technology Program (No. 2018SZ0036) and Supported by the National Natural Science Foundation of China (No. 11902089). *These authors contributed equally to this work and should be considered co-first authors.

References
[1] Karaca S, Kulac M and Cetinkaya Z J 2008 Etiology of foot intertrigo in the District of Afyonkarahisar, Turkey: a bacteriologic and mycolologic study J Am Podiatr Med Assoc. 98 42-4
[2] Gray M, Black J M and Baharestani M M J 2011 Moisture-associated skin damage: overview and pathophysiology J Wound Ostomy Cont. 38 233-41
[3] Hagberg K and Branemark R J 2001 Consequences of non-vascular trans-femoral amputation: a survey of quality of life, prosthetic use and problems Prosthet Orthot Int 25 186-94
[4] Wong A S W and Li Y J 2004 Relationship between thermophy silogoical response and psychological thermal perception during exercise wearing aerobic wear J Therm Biol. 29 791-6
[5] Li W, Qu S X and Zhou Z R J 2006 Reciprocating sliding behaviour of human skin in vivo at lower number of cycles Tribol. Lett. 2 165-170
[6] Essick G K J 2010 Quantitative assessment of pleasant touch Neurosci Biobehav R. 34 192-203
[7] Rochelle A, Ida C and Henric W J 2014 Touch perceptions across skin sites: differences between sensitivity, direction discrimination and pleasantness Front Behav Neurosci. 8 54
[8] Kelley C T 2016 Fabric-covered polymeric prosthetic liner 39-42
[9] Guest S and Essick G K M 2016 Psychophysical Assessment of the Sensory and Affective Components of Touch (Springer New York)
[10] Li W, Pang Q and Jiang Y J 2012 Study of Physiological Parameters and Comfort Sensations During Friction Contacts of the Human Skin Tribol. Lett. 48 293-304
[11] Tan X J and Zhang X Y J 2002 The problems of prosthetic sockets Chin J Clinical Rehabil. 20 2987-88
[12] Legro M W J 1999 Issues of importance reported by persons with lower limb amputations and prostheses J. Rehabil. Res. Dev. 36 155
[13] General Administration of Quality Supervision, I.A.Q.O. Biological evaluation of medical devices--Part 5: Test for in vitro cytotoxicity, N.C.O.S. Devices, N.C.O.S. Devices^Editors. 2003, China Standards Press: Beijing p. 12
[14] JaeSang K and Kanghee C J 2017 Adherence of coagulase-negative staphylococci to plastic
tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices; Hydrophilic surface modification of poly(methyl methacrylate)-based, Colloids and Surfaces B: Biointerfaces. J. Clin. Microbiol. 158 287-294
[15] Churaev N V and Sobolev V D J 2007 Wetting of low-energy surfaces Adv. Colloid. Interface Sci. 134-135 15-23
[16] Cohen C J 2010 Wetting and dewetting transition: an efficient toolbox for characterizing low-energy surfaces Langmuir. 26 15345-9
[17] Vasquez R J and Linberg J V J 1989 The anophthalmic socket and the prosthetic eye. A clinical and bacteriologic study Ophthal. Plast. Reconstr. Surg. 5 77-80
[18] Goldfarb H J and A I Turtz J 1966 A detergent-lubricant solution for artificial eyes Am J Ophthalmol. 61 1502-5
[19] Braga P C and Reggio S J 1995 Correlation between reduction of surface hydrophobicity of S. aureus and the decrease in its adhesiveness induced by subinhibitory concentrations of brodimprom Pharmacol Res. 32 315-9
[20] Kang S and Choi H J 2005 Effect of surface hydrophobicity on the adhesion of S. cerevisiae onto modified surfaces by poly(styrene-ran-sulfonic acid) random copolymers Colloids Surf. B 46 70-7
[21] Li B and Logan B E J 2004 Bacterial adhesion to glass and metal-oxide surfaces Colloids Surf. B. 36 81-90
[22] Xu L C and Siedlecki C A J 2014 Staphylococcus epidermidis adhesion on hydrophobic and hydrophilic textured biomaterial surfaces, Biomed Mater 9 035003
[23] Salerno B and Brian M J 2003 A macro- and micro-scale analysis of the molecular details of bacterial adhesion to hydrophilic and hydrophobic surfaces Ukr J. Phys Opt. 4 107-109
[24] An Y H and Friedman R J J 1998 Concise review of mechanisms of bacterial adhesion to biomaterial surfaces J. Biomed. Part A. 43 338-348