

Thermoresponsive antimicrobial wound dressings via simultaneous thiol-ene polymerization and in situ generation of silver nanoparticles

Zahra Abdali^a, Hamid Yeganeh^{b*}, Atefeh Solouk^a, Reza Gharibi^b, Marziyeh Sorayya^b

a) Biomedical Engineering Faculty, Amirkabir University of Technology, Tehran, Iran

b) Iran Polymer and Petrochemical Institute, P.O. Box: 14945/115, Tehran, Iran

Received (in XXX, XXX) XthXXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXXXX 20XX

DOI: DOI: 10.1039/x0xx00000x

S1. Evaluation of wound exudates management and oxygen permeability of dressings

The moisture permeability of the membranes was determined by the measurement of water vapour transmission rate (WVTR) across the material as expressed by ASTM E96/E96M. The WVTR Where expressed in g m⁻² day⁻¹ was calculated using the following equation:

$$WVTR = \frac{W_i - W_t}{A \times t} = \left(\frac{P}{L}\right) \cdot \Delta p$$

, where W_i and W_t are the weight of water containing cup before and after placing in the oven, A is the area of cup mouth (m²), and t is time. In addition, P represents the water vapour permeability (WVP) (the amount of water through unit membrane thickness per unit surface area per unit time by unit vapour pressure difference), L is the membrane thickness, and Δp is the water vapour pressure difference across the membrane. All measurements were carried out at 35 °C and 35 % humidity that corresponded to the average surface temperature of injured skin.¹ Wound dressings often differ significantly in thickness and their evaluations are reported under different experimental conditions of temperature and humidity, thus posing difficulty in comparison and standardization. In this regard, P/L that known as water vapour permeance and is normalized to thickness, often preferred over permeability to describe and compare different dressing materials.²

Further, the value of diffusion coefficient (D) was obtained from the plots of the degree of saturation (W/W_{eq}) against $t^{1/2}/L$, which W_{eq} is equilibrium water absorption, L is the initial thickness of the membrane and t is the swelling time. According to Fickian diffusion mechanism³, the diffusion coefficient (D) of water in the membranes can be determined from the slope of the plot by using following equation:

$$\frac{W}{W_{eq}} = \left(\frac{Dt}{16 \pi L^2}\right)^{0.5}$$

Oxygen penetration through the wound dressings measured using gas permeability analyser at 35±0.1°C and expresses in ml Pa⁻¹ s⁻¹cm⁻².

S2. Thermoresponsivity study

The thermoresponsive behaviour of samples were studied by using differential scanning calorimetry (DSC, 200 F3 Maia®, NETZSCH). A certain amount of deionized water swollen sample at room temperature was placed inside a hermetic aluminium pan. The thermal transitions were recorded in the range of 25 to 45 °C, with a heating rate of 2 °Cmin⁻¹ under a

dry nitrogen atmosphere at a flow rate of 80 mlmin⁻¹. Water was used as the reference in the DSC measurement. The onset temperatures of endothermic peaks were referred to the LCST. To have a better insight about thermoresponsive behaviour of the membranes, they were examined through determination of their surface and bulk hydrophilicity. Surface wettability of prepared films was determined by measurement of static water contact angle at lower and higher temperatures than LCST (25 and 37 °C). Six different water droplets were placed on the surface of membrane at different positions. The contact angle was determined via running Image-Pro® Plus software on the digital pictures taken from interfaces of films and droplets. The values reported were an average of six measurements. Bulk hydrophilicity was evaluated via measurement of equilibrium water absorption (EWA). The films were completely dried at 60 °C, accurately weighed, and immersed in 10.0 ml of water at temperature range from 5 to 45 °C until the equilibrium swelling was attained (after 48 h). The swollen samples were wiped with soft paper tissue before being weighed. EWA% was determined using the following equation:

$$EWA (\%) = \left[\frac{W_s - W_d}{W_d} \right] \times 100$$

, where W_d and W_s are the weights of dry and swelled membrane, respectively. The reproducible results for all swelling studies were obtained with triplicate measurements.

S3. Quantification of the amount of stratum corneum removed

Sterilized membranes were applied to the inner hairless area of the forearm. After 24 h, each dressing was removed from the forearm at a rate of approximately 1 cmsecond⁻¹ by the same operator once at room temperature and another after cooling with an ice pack. Then, a transparent adhesive tape was applied to the adherent surface of each wound dressing following its removal in order to print the stratum corneum to the adhesive tape. Thereafter, the printed stratum corneum on the adhesive tape was stained with Gentian violet (1% solution in water) for 45 seconds and washed off. Then, the stratum corneum printed on the adhesive tape was mounted on a glass slide. The tissue samples attached on a glass slide were examined under an optical microscope.⁴

S4. In vitro cytocompatibility assays

Biocompatibility of prepared membranes was evaluated against human dermal fibroblast at low passage number (between 1 and 5), by either microscopic investigation of the fibroblast cells morphology after direct contact with samples or tetrazolium dye-based colorimetric assay (MTT assay). For this purpose, the cells were exposed to the disc shaped samples punched under sterile conditions and incubated at 37 °C for 48 h. A well containing cells and growth medium with no sample was set up as negative control. After incubation time, the cells were washed 3 times with PBS to remove excess medium, and the amounts of survived cells were evaluated by MTT staining assay, which measures the metabolic reduction of 3-(4,5-di-methylthiazol-2-yl)-2,5, diphenyl tetrazolium bromide to a coloured formazan by viable cells.^{5,6}

The possible toxicity of leachates extracted from disc samples was also evaluated. For this purpose, the test samples (1 cm²) were immersed in 1.0 ml culture medium at 37 °C for 24 and 48 h. The cells were exposed to the each extraction and incubated at 37 °C for 48 h. The percentage of relative cell viability was calculated according to following equation:

$$\text{Cell viability} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{positive control}}}{\text{OD}_{\text{negative control}}} \times 100$$

, where OD designates the optical density. In addition, the growth characteristics of fibroblasts and their ability to attach and proliferate onto samples were evaluated by seeding

12,000 cells well⁻¹ directly onto semi-IPN samples. After 48 h of culture, the presence of cells on the semi-IPNs surface and their morphological changes was evaluated by a TMS inverted optical microscope.

S5. Cell attachment and detachment

Human dermal fibroblasts were seeded onto the sample (TRPU-50/Ag- 2.5 as representative example) at a density of 10⁵ cells well⁻¹ in the culture medium and incubated at 37 °C with 5% CO₂. A non-thermoreponsive sample (PU) was used for comparison. After 48 h incubation, cell culture medium removed and sample were rinsed by 37°C PBS to washout the non-adhered cells. Then, cells attached to the samples surface were fixated with 0.5% glutaraldehyde for 1 minute, more permeabilized with 0.5% Triton X-100, stained with 2 µM nucleic acid staining reagent, Hoechst 33342, and incubated for 30 minutes. The same procedure was repeated for other set of the same samples. After incubation for 48 h, the cell culture plates containing samples were transferred to an incubator at 20 °C and incubation was continued for 20 min. Then the samples were washed with PBS at 20°C, fixated, and stained. All stained samples viewed by a fluorescence microscope with an appropriate filter.

S6. Antibacterial study

Antimicrobial activity of prepared membranes was evaluated against *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 15449), and *C. albicans* (ATCC 10231) using “colony forming count” method based on procedure reported in ASTM E 2180-07. Bacteria at inoculated concentration of 2 × 10⁸ CFU ml⁻¹, and membranes with the dimensions of 1 cm × 1 cm were used throughout the tests. Bacterial cultures grew for 18 h at a specified temperature in tryptic soy broth. 1.0 ml of inoculated agar slurry was pipetted onto the test and control samples and allowed to gel. 24 h after contact; surviving microorganisms are recovered via elution of the agar slurry inoculums from the test substrate into neutralizing broth and extracted via vortexing that provides complete removal of the inoculum from the test polymers. Serial dilutions were made, and then spread plates were made of each dilution and incubated at 37 °C for 48 h. Colony numbers were counted and recorded for each dilution plate. The geometric mean of the number of organisms that recovered and percent reduction were determined by the following equations:

$$\text{Geometric mean} = \frac{(\log_{10} X_1 + \log_{10} X_2 + \log_{10} X_3)}{3}$$

$$\% \text{ Reduction} = \frac{(a - b) \times 100}{a}$$

Where X is referred to number of organisms recovered from the incubation period, a is the antilog of the geometric mean of organisms recovered from the incubation period control samples, and b is the antilog of the geometric mean of the number of organisms recovered from the incubation period treated samples.

References

1. L.-O. Lamke, G. E. Nilsson, and H. L. Reithner, *Burns*, 1977, **3**, 159–165.
2. L. Ruiz-Cardona, Y. D. Sanzgiri, L. M. Benedetti, V. J. Stella, and E. M. Topp, *Biomaterials*, 1996, **17**, 1639–1643.
3. J. M. Yang, H. T. Lin, and W. C. Lai, *J. Memb. Sci.*, 2002, **208**, 105–117.

4. H. Matsumura, N. Ahmatjan, Y. Ida, R. Imai, and K. Wanatabe, *Int. Wound J.*, 2013, **10**, 291–294.
5. M. V Berridge, A. S. Tan, K. D. McCoy, and R. Wang, *Biochemica*, 1996, **4**, 15–19.
6. H. Bakhshi, H. Yeganeh, S. Mehdipour-Ataei, M. A. Shokrgozar, A. Yari, and S. N. Saeedi-Eslami, *Mater. Sci. Eng. C*, 2013, **33**, 153–164.