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The Effects of Different Sterilization Methods of Fibroin/aloe Wound Dressing: *in Vitro*

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Introduction

Wound dressings are one of the main medical options for the treatment of chronic wounds. The wound dressing containing fibroin blended with *Aloe vera* as a natural origin film for wound healing application of biomaterial in medicine. Generally, the limitations of silk fiber that is used as medical materials is allergies and irritation[1]. That causes adverse effect will be removed. So the fibers can be used to be produced of medical materials. Fibroin from silkworm (*Bombyx mori*) has been widely studied as a natural origin scaffold that can lead to reduction of inflammation and the enhancing of immune response and a high proportion of biocompatibility, biodegradability and cell adhesion property of adherent cell including fibroblast[2,3]. For *Aloe Vera* (aloe gel), it has medicinal properties such as anti-bacterial, anti-inflammatory and wound healing activities[4,5].

The sterilization is very important in medical materials because it can eliminate microbial infection and endotoxin. Both of these can contaminate in the stage of wound dressing preparation. Concurrently, the biomedical devices need not only to be sterile but also must be free from pyrogens. Thus, to defense is to reduce the possibility of bacterial contamination by method sterilization, aseptic techniques and operating in clean room in order to reduce both microbes and pyrogens[6,7]. The wound dressing cannot withstand high temperatures. The different sterilization methods that were used in this study are ultraviolet, ozone and Ethylene oxide. Ultraviolet (UV) radiation is generally used for the disinfection in water and some medical material, are effective for killing only microorganisms, viruses or some fungi but not spores. The UV may not be suitable under all situation[8]. Ozone has been used for years as a drinking water disinfectant. However, it also is a toxic gas. So the operating system has to ensure that is safe level for researchers due to the effects are a growing public health concern to limit human exposure to ozone. Though ozone can be toxic, it is a very efficient sterilizer because it has strong oxidizing property against many contaminants and germs such as bacteria, phage, and fungus[9,10]. Other sterilizations, especially Ethylene oxide (EO), is a well-known sterilizing agent for medical devices. However, EO is a hazardous substance for humans and also is a carcinogen for severe skin, respiratory tract and eye irritation[11-13].

The skin cell such fibroblast cell that related *in vitro* study. The important role of skin cell in wound healing. Fibroblast cell is essential to the extracellular matrix deposition and remodeling. It produces high quantities of collagen and recruited to the site of tissue injury are considered essential for successful wound healing[14,15].

This study has reported that the blended fibroin/aloe gel dressing with sterilization. Due to non-cytotoxic in fibroblast cell. For these reasons, the data of *in vitro* studies lead to the next study in diabetic ulcer in order to approve effectiveness of the blended fibroin/aloe gel dressing**.**

Methods

Preparation and determination of wound dressing

Prepare of blended fibroin/aloe gel wound dressing, a simple casting technique were calculated. The protein content in lyophilized fibroin and lyophilized aloe gel was analyzed by using DC protein assay kit followed by Lowry method protocol. The protein content in an amount of 96.21±3.15 w/w and 4.8 ± 1.0% w/w for silk fibroin extract and aloe gel extract, respectively.

- The wound dressing used the lyophilized fibroin extract 540 mg and then, 15 mg of aloe gel extract was dissolved in lactic acid solution (pH 3.8–4.0) and the final volume was 15 ml.
- The resultant solution was placed into the plastic plate 6x6 cm. (3.7% extract/36 cm^2 mold area)
- Allowed to dry at 47 ± 2 ˚C for 10 hour and placed at room temperature until dryness.
- The wound dressing sterilized by using an ozone treatment for 2 hours.
- A scanning electron microscope (SEM Electron Microscopy) was used to observe the surface morphology of the wound dressing at 5.0 KX magnifications.

Cleaning validation

Agar plate culture technique is one of basic bacterial cultivation techniques for cleaning validation. Nutrient agar is a general medium for bacteria growth

- The wound dressing were sterilized using an ultra-violet concentration 51.2 J/cm^2 for 16 hours, an ozone concentration 0.01 ppm/cm^2 for 2 hours and Ethylene oxide sterilized by Naresuen hospital. Then put on nutrient agar medium for contamination test, compared with non-sterile dressing.
- The Nutrient agar culture was incubated at 37 ˚C for 24 hour.
- The result were recorded. If they showed colonies of microbes or bacteria growth, this meant contamination.

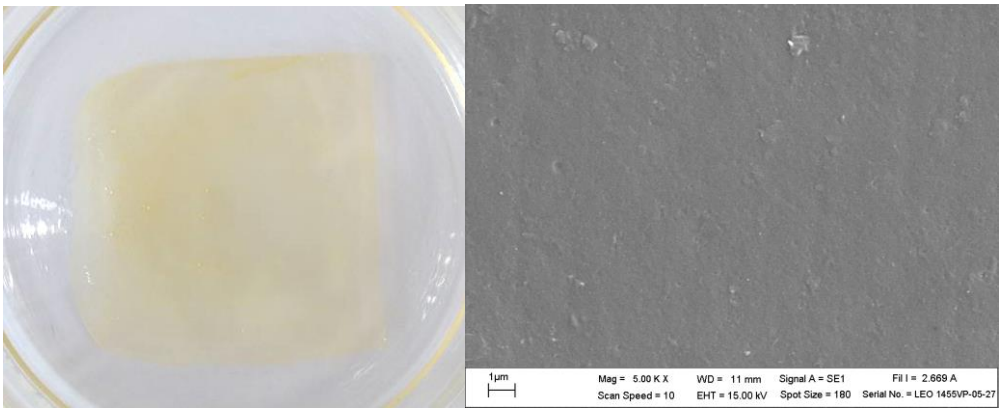
Cytotoxicity assay

The sterilized dressing was soaked with sterilized PBS (pH 7.4). The fibroblasts cell (1x10^5 cell/well) were seeded in 24 well plate then the sterilized dressing were put in trans well cell seeding and incubated at 37˚C in 5% CO2 incubator for 24 hrs. After that, discarded medium and washed with sterilized PBS (pH 7.4) to remove the old medium, two millimeter of DMEM free-serum was added. The cells viability was quantified by XTT assay. The sodium 3'-[-1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonicaid hydrate] (XTT) assay was used to cytotoxic of wound dressing to fibroblasts cell line. The supernatant was collected to 96-well plate to measure absorbance at 490 nm by using spectrophotometer.

Results

Preparation and determination of wound dressing

Figure 1 showed the wound dressing containing 3.7% extract/36 cm^2 mold area and the surface of wound dressing under SEM. The wound dressing containing 2.7% w/v fibroin blended with 1% w/v aloe gel extract in 0.01% lactic acid solution. The wound dressing showed the roughness of surface that is suitable for cell attachment and rapid regeneration. Which morphological characterization of biomaterial features such as surface roughness is important with respect to tissue mechanical properties.



A B

Figure 1 The wound dressing containing 3.7% extract/36 cm^2 mold area (A) and the surface of wound dressing under SEM (B).

Cleaning validation

The wound dressing sterilized by using ultra-violet concentration 51.2 J/cm^2 for 16 hours, an ozone concentration 0.01 ppm/cm^2 for 2 hour and EO sterilized by Naresuen hospital and nutrient agar was cleaning validation. The result showed non-bacterial growth with tree sterilization methods sterilized dressing then non-sterilized showed bacterial growth.

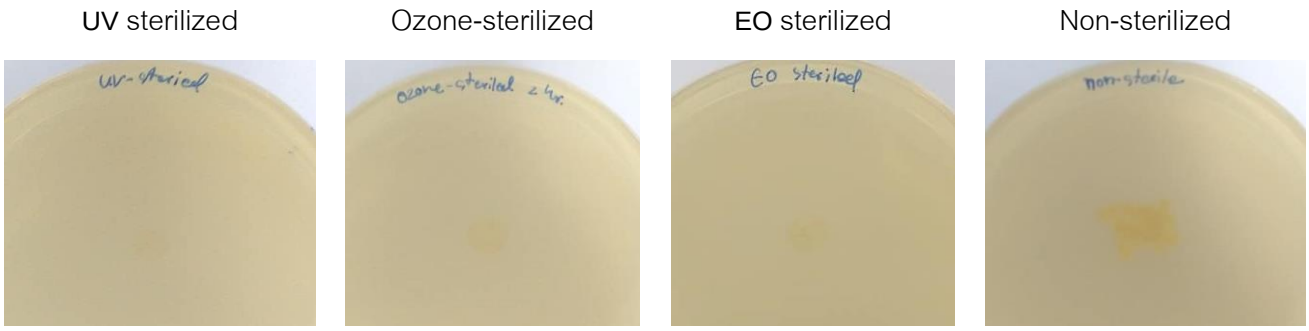


Figure 2 The cleaning validation of dressing sterilized by different sterilization methods

Cytotoxicity assay

For a quantitative assessment of cell viability, XTT assay was used to cytotoxic of wound dressing to fibroblasts cell. The obtained results are expressed as absorbance value at 490 nm for 24 hour. Figure 3 show percentage of cell viability. The results showed that the wound dressing when compared to the positive control, After 24 hours of culture on the wound dressing then UV sterilized, Ozone sterilized as well as EO sterilized cell viability increased compared to the positive control. The present of wound dressing that stimulated fibroblast cell generation.

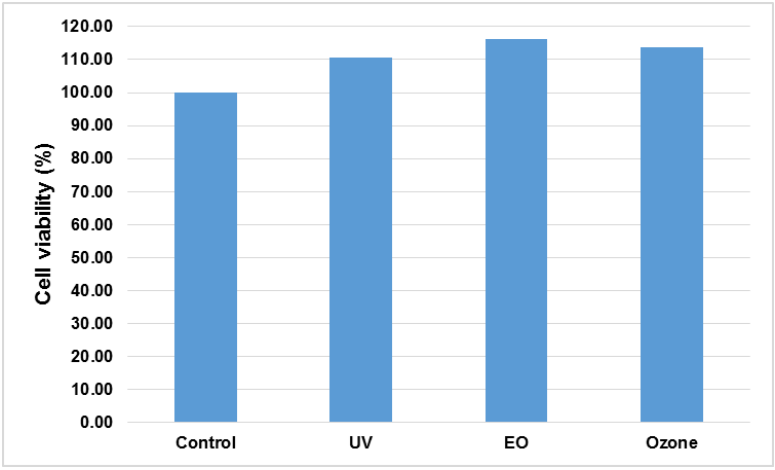


Figure 3 Percentage of cell viability by XTT in fibroblast cells (passage 5). UV sterilized, EO sterilized as well as Ozone sterilized. Data are present as absorbance value at 490 nm (mean ± SD, N=3)

Discussion

The wound dressing containing 2.7% w/v fibroin blended with 1% w/v aloe gel extract as a natural origin non-cytotoxic with fibroblast cells. The cytotoxicity demonstrated that the wound dressing is non-cytotoxic revealed that wound dressing present higher rates of cellular viability than the control. The present cell viability read to cell generation by active ingredient of wound dressing. Although, sterilization by EO was present higher rates of UV satirized and Ozone sterilized but do not indicate statistically significant differences ($p < 0.05$). UV light as a disinfectant is that the radiation is not very penetrating, so the wound dressing to be sterilize must be directly exposed to the rays. Then EO sterilized was fragile with this dressing. Ozone sterilized is a more reasonable option because it can be done not effected in stability of dressing. However, three sterilized methods it may not the effective against all bacterial such pyrogen. Another method such heat sterilization was effectiveness with protein may use filtration membrane for pyrogen to prepared the wound dressing and clean zone.

The sterilization under sterile conditions control of extraction protein or the wound dressing can be sterilized after optimize are prepared. The proceed of optimize should be prepared in the clean room in order to reduce both microbes and pyrogens.

Conclusion

The wound dressing developed from blending between the silk fibroin and aloe gel extracts was non-cytotoxicity with fibroblast cell.

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