Vietnam Journal of Biotechnology **20**(1): 89-97, 2022

COMBINING OF SILK FIBROIN AND *ALOE VERA* GEL TO FABRICATE WOUND DRESSINGS

Vu Nguyen Doan[1,2,✉], Ha Le Bao Tran[1,2]

[1]*Laboratory of Tissue Engineering and Biomedical Materials, University of Science, Vietnam National University, 227 Nguyen Van Cu Road, District 5, Ho Chi Minh City, Vietnam*
[2]*Department of Physiology and Animal Biotechnology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University, 227 Nguyen Van Cu Road, District 5, Ho Chi Minh City, Vietnam*

✉To whom correspondence should be addressed. E-mail: dnvu@hcmus.edu.vn

Received: 20.11.2021
Accepted: 05.02.2022

SUMMARY

Currently silk fibroin is used more and more in the biomedical researches, including a potential research direction in creating wound dressing. *Aloe vera* gel has been used as a traditional herbal with many properties suitable for treatment of burns such as anti-inflammatory, anti-bacterial, anti-fungal, especially improvable wound healing. Therefore, the prepared fibroin/ *Aloe vera* gel film (FAV) was an ideal material for wound dressings. In this study, sericin is removed from the silk to obtain fibroin fiber. *Aloe vera* gel is purchased from Traditional Medicine Institute, Ho Chi Minh city, Viet Nam. Fibroin fiber and *Aloe vera* gel are dissolved by formic acid adding calcium chloride ($CaCl_2$). Created FAV are then evaluated in some characteristics such as surface structure, tensile strength, absorbency, dehydration rate, biodegradation ability, preventing bacteria ability and cytotoxicity test. The results showed that FAV possessed good mechanical properties, suitable water vapor transmission rate, effective prevention of bacterial penetration and non-cytotoxic. This study is the first step to creating foundation and orientation for the development of commercial wound dressings.

Keywords: *Aloe vera* gel, burn treatment, silk fibroin, wound dressing, wound healing.

INTRODUCTION

Burns are a global public health problem, accounting for an estimated 265.000 deaths annually. The majority of these occur in low- and middle-income countries and almost half occur in the South-East Asia Region (Moore *et al*., 2014). Burn healing process is often accompanied by the use of wound dressings which should promote a moist environment in the wound and serve as a shield against external factors like dust and bacteria; enhance water and vapor permeation, enables gaseous exchange and promote epithelialization (Nanchahal *et al*., 2002; Heiko *et al*., 2017). In particular, wound dressings should be cost-effective to meet the demand for treatment for low-income people.

Fibroin protein, isolated from silkworm (*Bombyx mori*), is an important polymer that provides a set of material options for biomaterials. Silk fibroin is widely used in biomedical fields such as vascular regeneration, heart valve regeneration, nerve regeneration, bone regeneration, skin regeneration and drug delivery system. Due to its unique properties of high mechanical strength, excellent biocompatibility and biodegradability, silk fibroin has been explored for the development of wound dressings

(Vepari, Kaplan, 2007; Cao, Wang, 2009; Cai *et al*., 2010; Lammel *et al*., 2010; Yu *et al*., 2017; Ke *et al*., 2018; Mehdi *et al*., 2018).

In Vietnam, there are abundance and high efficiency traditional drugs for application of burns treatment. *Aloe vera* gel contains many physiologically active substances include glycoproteins, anthraquinones, polysaccharides, and low molecular weight species that have various biological properties including antiinflammatory, immunomodulatory, antimicrobial, antifungal, hypoglycemic, and gastroprotective. Therefore, *Aloe vera* gel is used in various commercial products to apply on wound, burn, and frost-bite treatment, diabetes treatment, and gastric ulcer treatment. In addition, many researches investigate *Aloe vera* gel which conjugates with synthetic and natural polymers has positive effects on mechanical, biocompatibility and degradation of resulting films (Darokar *et al*., 2003; *Boudreau*, Beland, 2006; Maenthaisong *et al*., 2007).

In this research, we created membranes from silk protein by natural evaporation method that help to control components as well as many features of the membrane as pore size, biodegradation degree..., and created favorable conditions for combining traditional drugs with membranes.

MATERIALS AND METHODS

Materials

Silkworm cocoons (*Bombyx mori*), which had similar sized, brightness and roundness, were purchased from August to November at Bao Loc city, Lamdong province, Vietnam**.** *Aloe vera* commercial gel (100%) was purchased from Traditional Medicine Institute, Ho Chi Minh City, Vietnam. All chemicals used in this study were purchased from Sigma Aldrich Chemical Co (USA).

Fabricating films from fibroin combined with *Aloe vera* gel (FAV)

Silkworm cocoons were cut into small pieces and treated with boiling aqueous solution of 0.5% sodium carbonate for 45 minutes with stirring. Then, fibroin fibers were rinsed three times with distilled water, dried at 60°C and stored at room temperature. The fibroin fibers were dissolved in formic acid 88% supplemented calcium chloride (½ of fibroin mass) and *Aloe vera* gel for 30 minutes with stirring. The solution was poured onto glass dishes which were putted in the fume hood for 2 days. FAVs were obtained, washed twice with distilled water and dried at 60°C. Then, FAVs were packed and irradiated at the dose of 25 kGy. FAVs were fabricated with fibroin concentrations 2%, 3%, 4%, 5% (w/v), respectively.

Evaluating quality of FAVs

Surface structure

The FAVs were sent to The Department of Optics - Optoelectronics, Hanoi University of Technology (Vietnam) to observe surface structure by scanning electron microscope (S-3000N, Hitachi, Japan).

Tensile strength

The FAVs tensile strength was measured by Universal Material Testing Machine (A&D, Japan) at The Laboratory of Mechanic- Ho Chi Minh City University of Technology (Vietnam).

Absorbency

The absorbency of FAVs was determined by using BS EN 13726-1:2002 section 3.2 free swell absorptive capacities. For this test, 1cm x 1cm FAVs specimens were prepared. The solution A (2.298g sodium chloride, 0.368g calcium chloride dihydrate are added to 1 L of deionized water) was prepared for the testing. All the prepared FAVs specimens were weighed before tested and placed in petri dishes. The solution A was warmed to 37±1°C and dispensed slowly and gently onto the specimens in the petri dishes. The petri dishes were then placed in an incubator for 30 minutes at 37±1°C (body temperature). After 30 minutes of conditioning the dishes were removed from the incubator and suspended by one corner by using tweezers to allow excessive

solution to drip off for 30 seconds and reweighed for wet mass.

Absorptive capacity (%) = (B-A)100/A;

where, B is the wet mass of specimens, A is the dry mass of specimens

Dehydration rate (Chellamani et al., 2014)

The dehydration rate was determined by measuring the difference between the mass of wet and dry specimens. The mass of wet specimens (1x1 cm) was determined after submerging them in an excess volume of solution A at 37±1°C for 30 minutes. The specimens were taken out from fluid and suspended by a corner for 30 seconds for free drainage. After draining they were put into Petri dishes and kept in an incubator for 24 hours at 37±1°C, they were re-weighed.

Dehydration rate (g/min) = W-D/T

where, W is the wet mass of specimens, D is the dry mass of specimens, T is the test period in hours.

Biodegradability

The specimens (1x1 cm) were dried in an incubator for 24 hours at 37±1°C. The mass of dry specimens was determined before placing on the solution A absorbed gauze. The specimens were taken out and suspended by a corner for 30 seconds for free drainage. After draining they were put into petri dishes and kept in an incubator for 24 hours at 37±1°C. Then they were re-weighed at the periods: 6, 12, 18, 24, 30, 36, 42 and 48 hours.

Bacterial preventing ability

Test specimens were cut into circular discs (15 mm in diameter) and placed on the top of the agar plates which were placed outside the environment for 24 hours. The plates which not covered were used as control group. Then they were incubated at 37°C, 95% RH for 24 hours. The growth of bacteria was observed after removing the specimens.

Cytotoxic test

Toxicity of FAVs were examined by direct contact method (ISO 10993-5). Fibroblasts which were provided by Laboratory of Tissue engineering and Biomedical Materials (TEBM), University of Science, Vietnam National University, Ho Chi Minh City were cultured until confluency (95%) which was formed on surface of cultured discs. Then, test specimens (1/10 of area of cultured discs) were placed in the center of the surface. After incubation at 37±1°C for 24 to 26 hours, cell medium was examined microscopically for the response around the test samples. The reactivity was graded as 0, 1, 2, 3 and 4 based on zone of lysis, vacuolization, detachment and membrane disintegration. As per the ISO 10993–5 the achievement of numerical grade greater than 2 is considered as cytotoxicity.

Statistical methods

The data obtained was processed by the Excel program, and the Least Significant Differentiation (LSD) was calculated at a probability of p = 95% by means of differential analysis (Analysis of Variance - ANOVA) under the Statgraphic Program 7.0, 1997 of the University of Michigan (USA).

RESULTS AND DISCUSSION

FAVs fabricating

The fibroin fibers were dissolved in $CaCl_2$ solution, and binding between Ca2+ and tyrosine of fibroin caused disruption of the van der Waals forces and hydrogen bonds. This result caused fibroin to swell and dissolve (Mehdi *et al*., 2018). After formic acid evaporated, remaining ingredients would dry out quickly and form film (Fig 1). FAVs with 2%, 3%, 4%, 5% (w/v) fibroin concentrations were linked.

Surface structure of FAVs

According to Figure 2, the films with 2%, 3% and 4% fibroin concentration had structure of fibers intertwined to form holes on surface (FAV2: 67.96 - 196.12 µm, FAV3: 33.98 – 171.84 µm, FAV4: 32.98 – 120.39 µm). While the surface of films with 5% fibroin concentration were homogeneous and didn't observed holes.

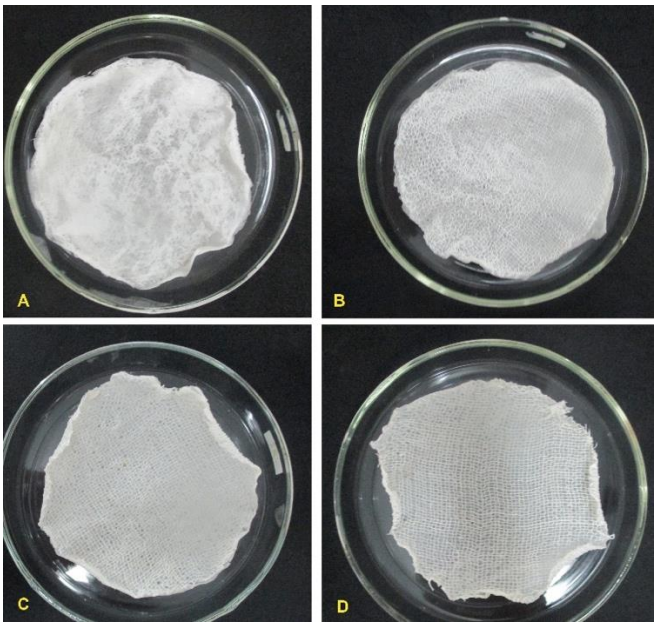

Figure 1. FAVs with different fibroin concentrations. A. FAV2 (FAVs with 2% fibroin). B. FAV3 (FAVs with 3% fibroin). C. FAV4 (FAVs with 4% fibroin). D. FAV5 (FAVs with 5% fibroin).

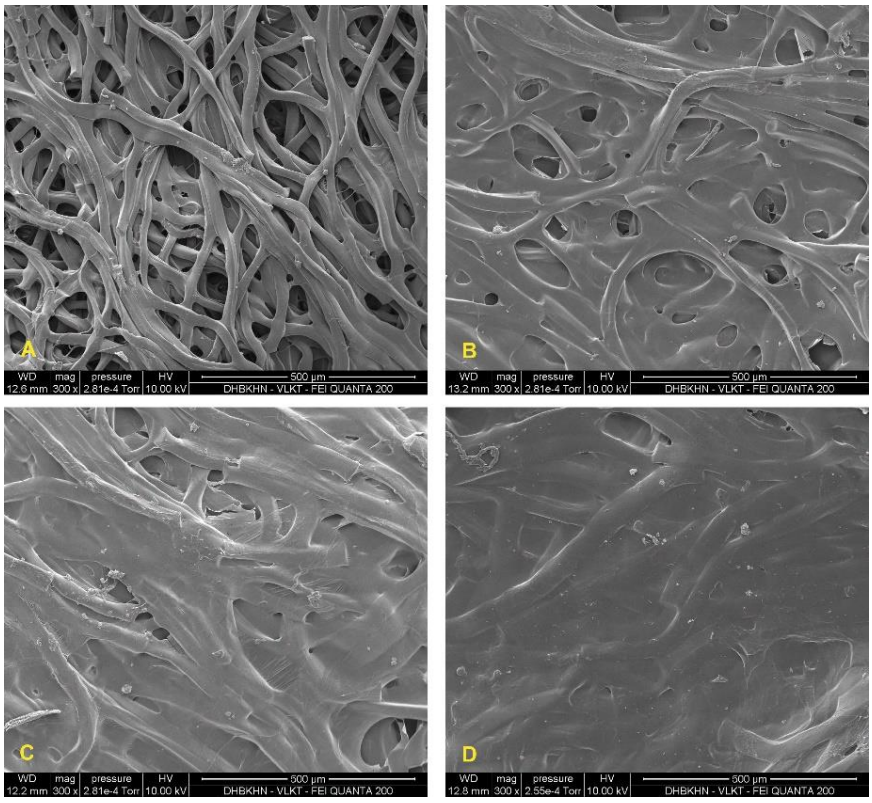

Figure 2. SEM image of the FAVs with X300 magnification. A. FAV2. B. FAV3. C. FAV4. D. FAV5.

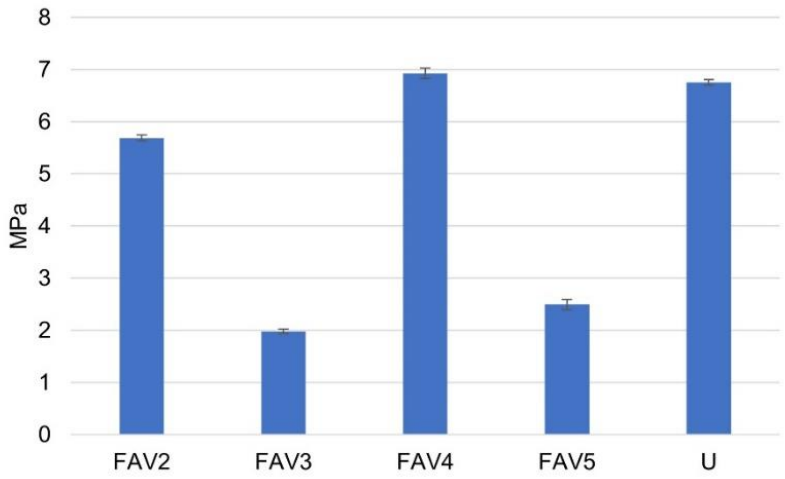

Figure 3. Tensile strength (MPa) of FAVs.

Tensile strength of FAVs

The wound dressing must have mechanical strength equivalent to human skin (≥1.8 MPa) (Vanessa *et al*., 2006; Mehdi *et al*., 2018). According to Figure 3, tensile strength of all formed FAVs and commercial film (U) were greater than 1.8 MPa. In natural state, silk fibroin was organized in β-sheet crystals alternated by amorphous regions, which provided strength and resilience to the protein. FAVs with 2% and 4% fibroin concentrations could reconstruct higher β-sheet content so they had higher tensile strength.

Absorbency of FAVs

According to Figure 4, the absorbance of film was inversely proportional to the fibroin concentration (2% > 3% > 4% > 5%), the highest absorbency of films with 2% fibroin concentration was about 795.78%. FAVs with 2% and 3% fibroin concentrations had appropriate absorbency for wound dressing (≥ 400%) due to the surface had many holes to help the film absorbs quickly (Muhammet *et al*., 2013; Yu *et al*., 2017). The films with 5% fibroin concentration didn't observe hole on the surface so that they slowly absorbed.

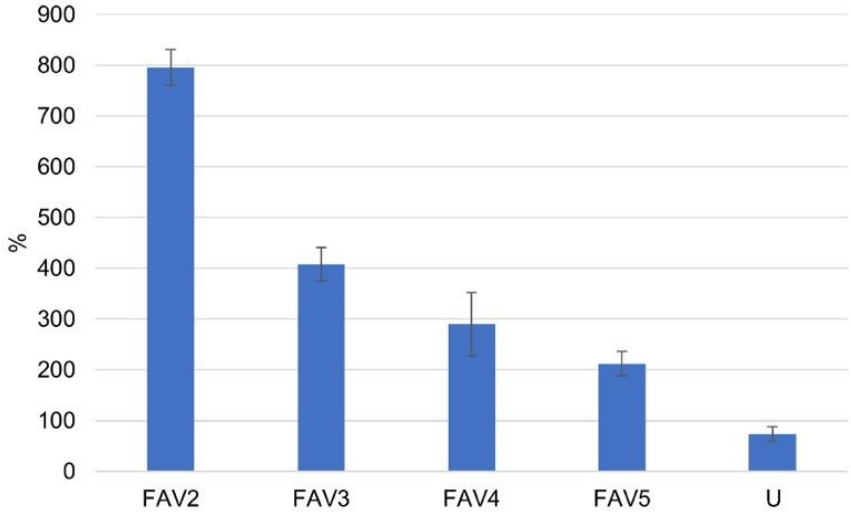

Figure 4. Absorbency of FAVs.

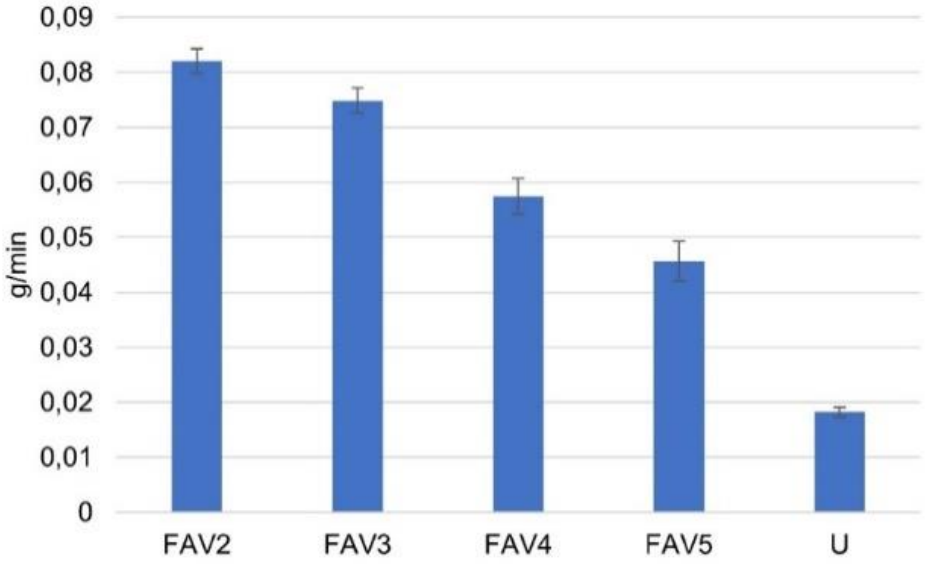

Figure 5. Dehydration rate of FAVs.

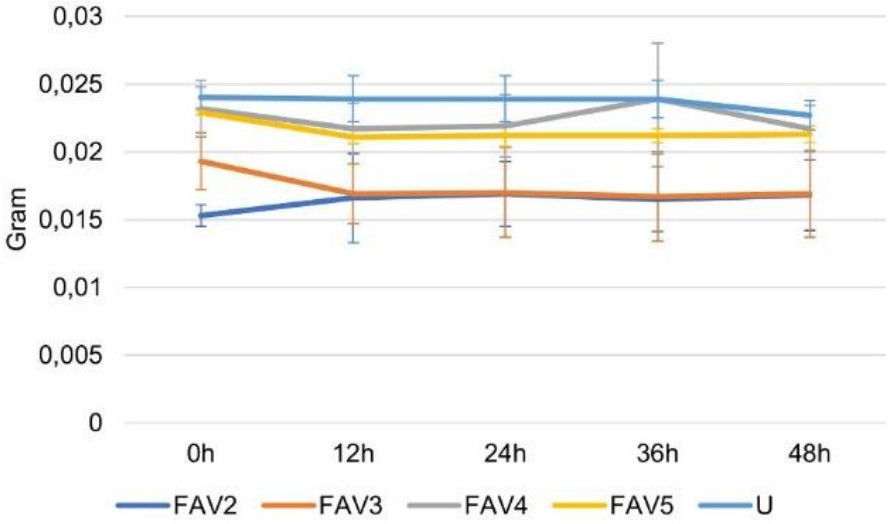

Figure 6. Weight of FAVs through the timeline.

Dehydration rate of FAVs

According to Figure 5, all formed FAVs were capable of evaporating. Many studies showed that the evaporation rate of nonwoven dressings ranged from 0.027 g/min to 0.036 g/min, for foam and hydroactive dressings it ranged from 0.025 g/min to 0.080 g/min (Muhammet *et al*., 2013; Mehdi *et al*., 2018). Therefore, formed FAVs had suitable evaporation for wound dressing (0.045 g/min to 0,082 g/min).

Biodegradability of FAVs

According to Figure 6 and statistically analyzing, all FAVs with 2%, 3%, 4%, 5%

fibroin concentrations and commercial film (U) had unchanged mass over time (no statistically significant difference). The films undegraded in 48 hours, but the evaluation of the decomposition of films should to be carried out over a longer period of time, leading to prolonged use. Wound dressings for long-term use leaded to reduce treatment cost and avoid pain to patients (Hasatsri *et al*., 2015; Mehdi *et al*., 2018).

Bacterial preventing ability

According to Figure 7, after incubation period, the agar surface of wells which were covered with FAVs and commercial film (U) had no formation of bacterial colony. However, the control wells (not covered with FAVs) appeared bacterial colonies on the surface of agar. In an infected wound, bacteria were present not only on the surface but also within the granulation tissue. The presence of bacteria inside the granulation tissue was one of the reasons for inability to control infection. Therefore, the bacterial preventing ability was one of the criteria to consider on selecting wound dressings (Hasatsri *et al*., 2015).

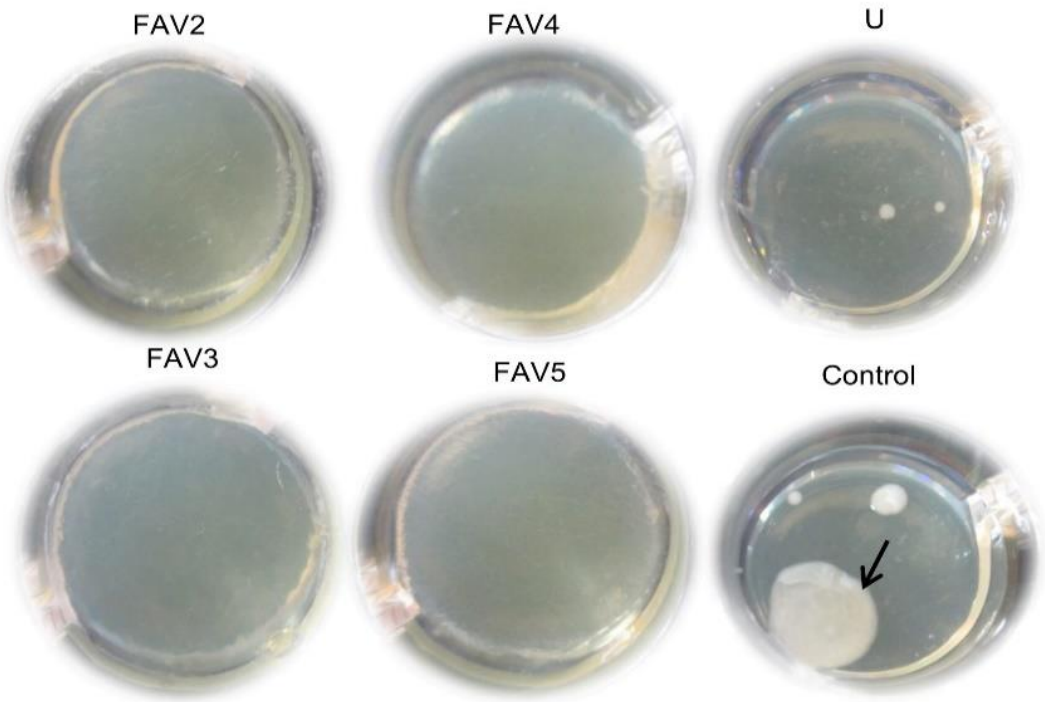

Figure 7. Resistance of FAVs to penetration by outdoor.

Cytotoxic test of FAVs

Cells around and beneath the FAVs were still normal morphology and proliferation (Fig 8. C, D, E, F). On the contrary, in culture plate containing latex, all of fibroblasts were deformed, round, formed clusters and completely detached from the culture surface (Fig 8. B). According to ISO 10993–5, all FAVs with fibroin concentrations of 2%, 3%, 4%, 5% had cytotoxic levels at 0. Therefore, they were nontoxic to human fibroblasts (Ke *et al*., 2018).

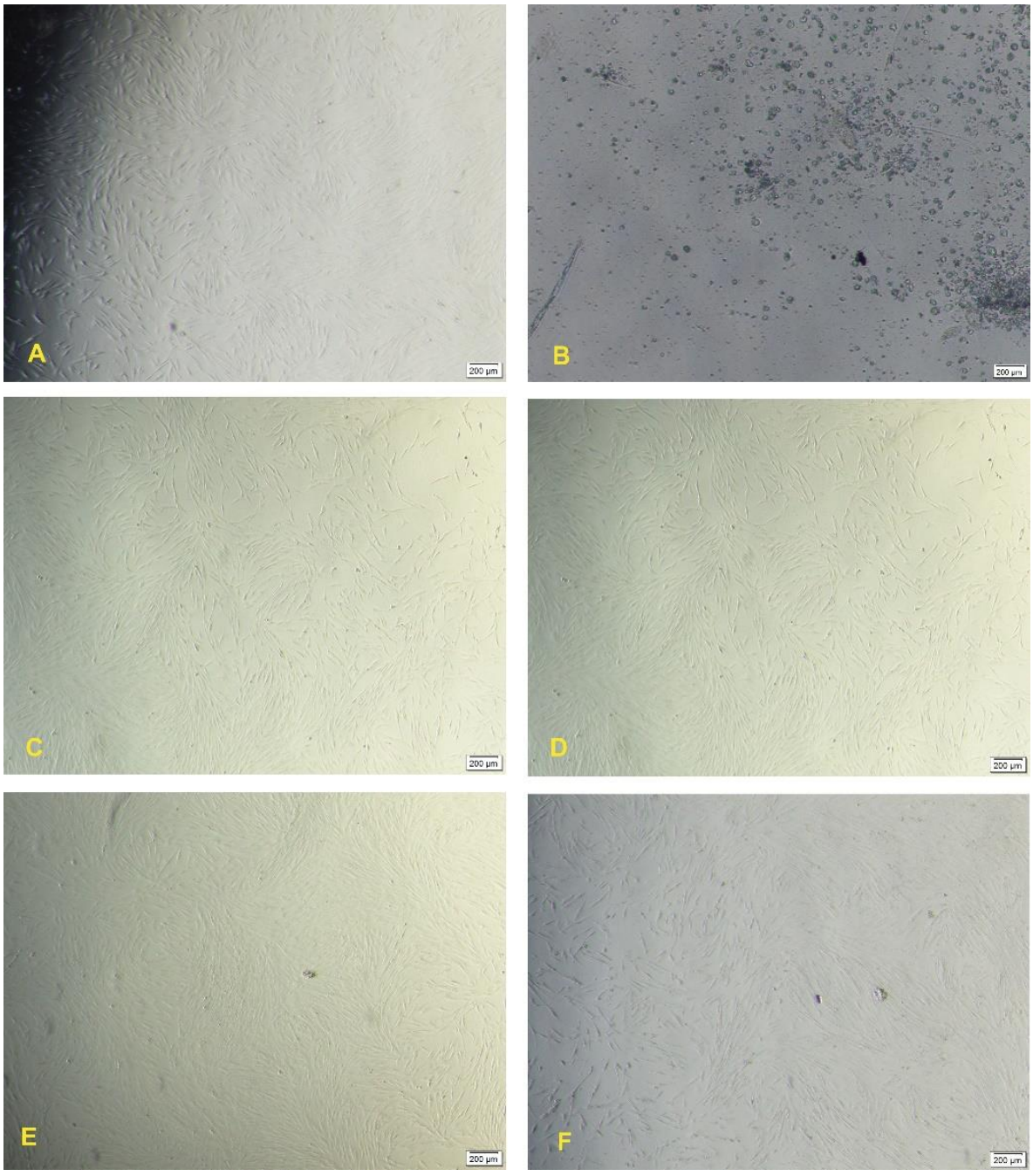

Figure 8. The cells below FAVs with X40 magnification. A. Control; B. Latex; C. FAV2; D. FAV3; E. FAV4; F. FAV5.

CONCLUSION

In this study, a FAV film was successfully prepared by using silk fibroin as the substrate and *Aloe vera* gel as the active element. However, FAV4 and FAV5 failed property of absorbency, while FAV2 and FAV3 achieved all property of wound dressing. The results showed that the FAV films had suitable absorbency (400%-800%) and mechanical properties (≥1.8 MPa)

with many holes on the surface, could provide wound areas with a suitably moist environment and acted as an effective protective barrier to inhibit the penetration of bacteria. In addition, cytotoxic test results demonstrated that the prepared FAV films were biocompatible to human fibroblasts. So the film with 2% and 3% fibroin/*Aloe vera* gel could be chosen to target the wound dressing applications. This section provided an in-depth understanding of relationship between the fibroin concentrations and their wound dressing properties. The inferences drawn from this section were crucial in order to design and develop more effective and novel modern wound dressings made of fibroin and *Aloe vera* gel.

REFERENCES

Boudreau MD, Beland FA (2006) An Evaluation of the Biological and Toxicological Properties of Aloe Barbadensis (miller), Aloe Vera. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 24: 103-154.

Cai Z, Mo X, Zhang K, Fan L, Yin A, He C, Wang H (2010) Fabrication of Chitosan/Silk Fibroin Composite Nanofibers for Wound-dressing Applications. *Int J Mol Sci* 11: 3529-3539.

Cao Y, Wang B (2009) Biodegradation of Silk Biomaterials. *Int J Mol Sci* 10: 1514-1524.

Chellamani K, Vignesh BR, Veerasubramanian D (2014) Quality evaluation methods for textile substrates based wound dressings. *IJETAE* 4: 811-817.

Darokar MP, Rai R, Gupta AK, Shasany AK, Rajkumar S, Sunderasan V, Khanuja SPS (2003) Molecular assessment of germplasm diversity in *Aloe* spp. using RAPD and AFLP analysis. *J Med Arom Plant Sci* 25: 354-361.

Hasatsri S, Yamdech R, Chanvorachote P, Aramwit P (2015) Physical and biological assessments of the innovative bilayered wound dressing made of silk and gelatin for clinical applications. *J Biomater Appl* 29: 1304-1313.

Heiko S, Daniel JT, Stephan H, Jörg H, Ursula M (2017) Skin Wound Healing: An Update on the Current Knowledge and Concepts. *Eur Surg Res* 58: 81-94.

Ke W, Qian M, Xiao Q, Shu DW (2018) Silk Fibroin and its Application in Tissue Engineering. *Curr Trends Fashion Technol Textile Eng* 4: 1-3.

Lammel AS, Hu X, Park SH, Kaplan DL, Scheibel TR (2010) Controlling silk fibroin particle features for drug delivery. *Biomaterials* 31: 4583-4591.

Maenthaisong R, Chaiyakunapruk N, Niruntraporn S, Kongkaew C (2007) The efficacy of *Aloe vera* used for burn wound healing: A systematic review. *Burns* 33: 713-718.

Mehdi F, Fatemeh M, Yousef F, Ali K, David LK (2018) Overview of Silk Fibroin Use in Wound Dressings. *Trends Biotechnol* 36: 907-922.

Moore Z, Butcher G, Corbett L, McGuinness W, Snyder R, Van Acker K (2014) Managing wounds as a team–exploring the concept of a team approach to wound care. *J Wound Care* 23: 1-38.

Muhammet U, Subhash CA, Tahir AS (2013) *In vitro* characterisation and evaluation of different types of wound dressing materials. *J Med Eng Technol* 1: 1-17.

Nanchahal J, Dover R, Otto WR (2002) Allogeneic skin substitutes applied to burns patients. *Burns* 28: 254-257.

Vanessa J, Joseph EG, Keith GH (2006) Wound dressings. *BMJ* 332: 777-780.

Vepari C, Kaplan DL (2007) Silk as a Biomaterial. *Prog Polym Sci* 32: 991-1007.

Yu Q, Hui W, Kai W, Ya Y, Ru-Yue Z, Ick SK, Ke-Qin Z (2017) A Review of Structure Construction of Silk Fibroin Biomaterials from Single Structures to Multi-Level Structures. *Int J Mol Sci* 18: 237-258.