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Effect Gamma Irradiation on Starch-Based Biomaterials Composites for Wound Healing

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ABSTRACT

This study aims to investigate the properties of wound dressing on human cells in vitro, in terms of moisture content and tensile strength of non-irradiated and irradiated films. The hydrogel films were fabricated via solvent casting method with different compositions, made of sago starch and polyvinyl alcohol solution as base sample. Then, some of films were radiated using gamma irradiation at dosage of 15 kGy for 8 h. Those non-irradiated and irradiated films went through the moisture content test and tensile test. The non-irradiated and irradiated films were sterilized using UV radiation and gamma irradiation, respectively for in-vitro technique. Through radiation, the water content in films improved as the number of breaks in polysaccharide chains of the molecules per energy absorbed decreases with increasing of water content. While, the tensile strength of irradiated films were doubled with the addition of optimum amount of seaweed, due to breaking of double bonds chain that produce strong mechanical strength. The sterilized films had colour changes after 10 h, and gamma irradiation provided better crosslinking which can sustain longer time on wound to increase the healing process compared to UV exposure. The results concluded that the irradiated films improved the properties of hydrogel films compared to non-irradiated films.

INTRODUCTION

Wound healing is a process of replacing missing cellular structures and tissue layers, which categorized under three subsequent actions in certain periods and areas: inflammation phase, proliferation or tissue formation phase, and remodelling phase (Alfarra *et al.*, 2014). The most concerning factor in producing an effective wound healing is to provide solutions that minimized the pain and trauma of the patient. Hence, the innovation of wound dressing innovation depends on its healing mechanism and antimicrobial activity abilities (Kunal & Manjumdar, 2006). Sago starch consists of polysaccharides, acts as the healing agent and it also non-toxic, biocompatible and biodegradable. The sago starch can enhance the function of polymorph nuclear cells as well as fibroblastic proliferation and migration (Alfarra *et al.*, 2014).

Gamma irradiation is a simple process, that has excellent penetration that can readily penetrate dense or complex products, where the exposure time as significant control parameter. This method is harmless as no chemical initiator needed. The application of gamma irradiation is reported to generate free radicals that are capable of inducing molecular changes and fragmentation of starch. It also promote cross-linking between the bonds as the OH- bonds that would be reduced and more bond were formed which can enhanced the mechanical strength, reduced viscosity and increased water solubility of biodegradable film for wound dressing application (Raffi *et* al., 1980; AlKaisey *et Ial., 2003)*

In vitro studies was done to provide extent research in producing effective wound dressing film. In this study, the hydrogel films of wound dressing are applied on the surface of human dermal cell, to know the water absorption ability and identify suitable mechanical properties of hydrogel films. Thus, the aim of this study is to investigate the properties of wound dressing on human cells *in vitro*, in terms of moisture content and tensile strength of non-irradiated and irradiated films.

MATERIALS AND METHOD

Materials

There are five materials involved in this study which are sago starch (S), polyvinyl alcohol (P), distilled water, seaweeds and silver nanoparticles (N). The sago starch solution was prepared by mixing 4 g of sago starch (S) powder with 100 g of distilled water, by stirring using magnetic stirrer at 80 °C until it dissolved homogeneously. The polyvinyl alcohol (P) solution is a combination of 10 g of P with 100 g of distilled water together, where this solution was heat and stirred gently until all the powder completely dissolved. Silver nanoparticles (N) in solution form were purchased with 2000 part per million (ppm) concentrations, where 3 g of silver nanoparticles was used in every mixture, as antimicrobial agent. Brown seaweeds (BSW), were washed with distilled water to get rid of any adhering foreign matter, followed by air drying at room temperature. 12.5 g of seaweed was mixed with 0.325 L distilled water, and was stirred magnetically until all seaweeds completely dissolved. The filter paper was used to filter the seaweeds solution to remove undissolved seaweeds.

The hydrogel film was fabricated via solvent casting method for 24 h at room temperature (25 °C). The first formulation made was 3:7 ratio of sago starch solution and polyvinyl alcohol solution, which was labelled as P.S, as a base sample. The other samples were added with silver nanoparticles and seaweed, as listed in Table 1, and categorized as non-irradiated samples. The irradiated samples were prepared using same compositions as non-irradiated samples, yet these samples were radiated using gamma rays at Malaysian Institute for Nuclear Technology Research (MINT) with dosage of minimum 15.2 kGy and maximum of 15.8 kGy for 8 h. Table 1 summarized the non- irradiated and irradiated films and their compositions.

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Table 1 Summary of non-irradiated and irradiated samples code and their compositions

Sample Code	SG (ml)	PVA (ml)	SNP (ml)	SW (ml)
P.S	30	70	0	0
P.S.N.	30	70	3	0
P.S.N.SW5	30	70	3	5
P.S.N.SW15	30	70	3	15
P.S.N.SW25	30	70	3	25
P.S (IR)	30	70	0	0
P.S.N. (IR)	30	70	3	0
P.S.N.SW5 (IR)	30	70	3	5
P.S.N.SW15 (IR)	30	70	3	15
P.S.N.SW25 (IR)	30	70	3	25

Note: *P – Poly (vinyl alcohol), S – Sago Starch, N – silver nanoparticles, SW – Seaweeds, IR – irradiation*

Characterization of films

Five samples were cut into 2 cm X 2 cm, and each specimen was measured for its initial moist weight (w_i). The samples were placed in desicator for 24 h with 40 % humidity before placed in the oven at 80 °C for another 24 h. The final dry weight (w_f) of each specimen were weight and data were recorded, where the percentage of moisture content were calculate using Eq 1.

$$\text{Moisture Content (\%)} = [(w_i)-(w_f)] / (w_f) \times 100 \qquad (1)$$

The tensile test was conducted in accordance with MS ISO 527-3 using Shimadzu AGS-X 5kN, at a crosshead speed of 5 mm/min for tensile strength (MPa). The test was conducted under standard laboratory conditions (temperature = 23 ± 2 °C; relative humidity = 50 ± 5 %). The samples were prepared in strip dimension of 10 mm wide and 150 mm long; then, were carefully clamped in between grips that had rubber grip faces. These rubber grip faces are commonly used for thin test specimens to prevent unnecessary failure, through which grips and specimens were safely clamped throughout the test. The break load was measured and the tensile strength was calculated.

Films Sterilization and Media Preparation

The hydrogel films were put in petri dishes and directly sterilized with ultraviolet (UV) radiation for first 6 h, followed by 8 and 10 h subsequently. The cells were cultured and seeding for three weeks, to increase the percentage of live MCF-7 cells per cubic volume This is prepared for application of *in vitro* technique of hydrogel films on the monolayer of epithelium cell.

RESULTS AND DISCUSSION

The moisture content and tensile strength were observed to evaluate the suitability of conducting in vitro studies of hydrogel films on the monolayer MCF-7 cells.

Moisture Content

The significant of moisture content in the wound dressing are to provide barrier to microorganisms, prevent the infection occurred and promote wound healing (Field & Kerstein, 1994). Fig.1 illustrated the effects of non-irradiated and irradiated films with different seaweed content on moisture content. The irradiated films by gamma radiation have improved water content in hydrogel films, as the number of breaks in polysaccharide chains of the molecules per energy absorbed decreases with increasing of water content. It is assumed that in materials low water content excitation with chemical changes with higher efficiency than in material content more water (Hashim *et al.*, 2001). The other effects of radiation on the hydrogel film structure is the polymer molecules can be cross-linked, which increases the mechanical strength and oxidative degradation (Field & Kerstein, 1994).

The moisture contents of non-irradiated films are 30.82%, 22.77%, 31.42%, 26.58%, and 26.29%, respectively. It shows that the percentage of moisture content is decreased when the silver nanoparticle is added to the hydrogel film (P.S.N), as the silver nanoparticle has occupied the pore of the hydrogel film gel. When 5g of seaweed is added to P.S.N., the percentage of moisture content is increased again, due to lower concentration of polysaccharide with high film volume caused increasing in porosity, resulted from high availability of free water particle involved in polymerization (Field & Kerstein, 1994). After that, the higher amount of seaweed had reduced the moisture content of hydrogel films. This is due to high interactions between hydrogel films and hydroxyl groups of seaweed, which leads to the increasing in hydrogen bonding with more seaweed contents. Seaweed is a hydrophilic and water soluble, but due to the interaction, the number of hydrophilic sites available for water molecules is reduced; hence decrease in the capability of water to penetrate the films at ambient temperature (Chung & Liu, 2009).

Meanwhile, the percentages of moisture content for irradiated films are 39.28%, 51.12%, 53.11%, 69.57%, and 79.92%, respectively. The moisture content demonstrated proportional increment trend as more amount of seaweed added to the film. It is resulted from better water retention by polysaccharides from the seaweed.

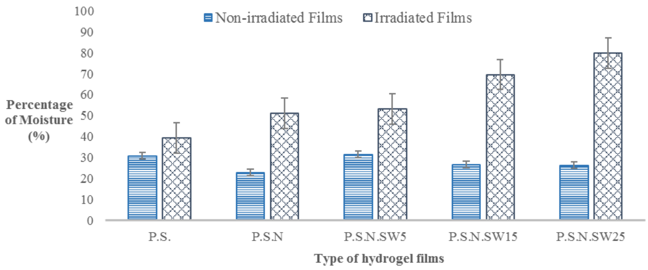

Fig. 1 Percentage of moisture content in the hydrogel films

Tensile Strength

Tensile strength is important to study the strength of wound dressing. The performance and long term stability of wound dressing are determined through mechanical strength. Fig. 2 exemplifies the effects of non-irradiated and irradiated films with different seaweed content on tensile strength.

The tensile strength of non-irradiated films are 5.55 MPa, 8.55 MPa, 6.46 MPa, 6.96 MPa, and 7.71 MPa, respectively. The tensile strength increased when the silver nanoparticle was added to the hydrogel films. Silver nanoparticle is a metallic component which has attracted attention ability (Cota *et al.*, 2008). There is decrement in tensile strength when 5 g of seaweed was added and increment in tensile strength when 15 g and 25 g of seaweed were added to P.S.N hydrogel film. This is due to increment in calcium ions inside seaweed which enlarged tensile strength of the mixture (Mandal *et al.*, 2014).

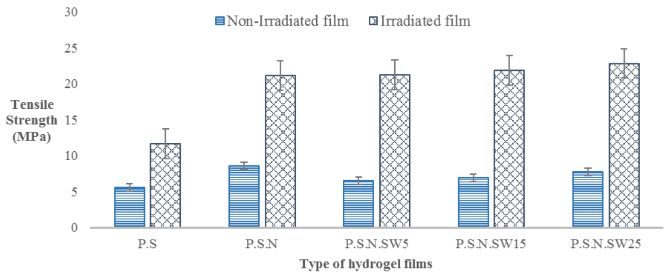

Fig. 2 Graph of tensile strength in the hydrogel films

The tensile strengths of irradiated films are 11.65 MPa, 21.13 MPa, 21.28 MPa, 21.89 MPa, and 22.83 MPa, respectively. From the

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graphs, the tensile strength rises proportionally to the increment amount of seaweed added to the films. This is due to chemical structure of the seaweed that contains blocks of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers that caused maximization of mechanical properties of the films (Chung & Liu, 2009). In fact, the network structure by the existence of these blocks become stronger after the process of crosslinking. The existence of sago and seaweed contributed to variation in degree of crosslinking and network structure (Bhat & Karim, 2009), where gamma ray irradiation did improve tensile strength with the addition of optimum amount of seaweed. This is due to breaking of main chains to produce (C=O) and vinyl (C=C) from the irradiation effects, where these double bonds chain could sustain a strong mechanical strength (Chung & Liu, 2009).

Effect of sterilization on film mechanical strength and cell viability

The in vitro experiments in this study starts with films sterilization and applied the films onto upper layer of the cells after the cells is confluent with enough live cells cultured inside petri dish medium. The films sterilization serves for antibacterial purpose for safely used to avoid cells death or contamination. The irradiated hydrogel films were sterilized using gamma ray irradiation of 15 kGy for 8 h, as this type of radiation is very penetrating, pure and stable state of process then exposed to UV lights. While the control was only exposed to UV lights without irradiation (Bhat & Karim, 2009). UV lights also enhanced the crosslinking of the films in providing low hydrophilic properties and increased of tensile strength. The effects of UV lights exposure to the films, yet is much lower compared to gamma radiation in terms of purification of method and penetration capability.

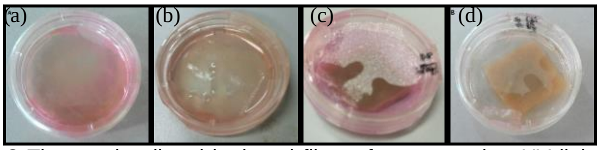

Fig. 3 The non-irradiated hydrogel films after exposed to UV light. (a) P.S film exposed to 6 h UV light,(b) P.S film exposed to 10 h UV light, (c) P.S.N.SW5 film exposed to 6 h UV light, (d) P.S.N.SW5 PVA/SG/SNP/SW5 film exposed to 10 h UV light

Fig. 3 displays the non-irradiated hydrogel films of P.S and P.S.N.SW5, exposure to UV light for 6 h and 10 h in suitable temperature range and concentration of photosensitize along with the absence of oxygen. These films were next immersed in media for 24 h. Fig. 3(a) demonstrates that the P.S film was separated into small pieces, due to low UV exposure time which minimizes the cross-linking, although it has tendency to increase the surface modification of the films. Fig. 3(b) shows the film ability to have better water absorption and tensile strength, as the UV exposure time increased from 6 h to 10 h. This deduces that UV lights succeed to enhance crosslinking of the films and surface tension between bonding of the films. It increases tensile strength of the films, to sustain longer period in moisture surrounding. Fig. 3(c) and Fig. 3(d) show the same trend of P.S.N.SW5 films, as the films without any seaweed.

Fig. 4 shows the observation of media preparation with confluence cells for each samples of non-irradiated films (exposed to UV lights) for 24 h and 48 h, respectively. It is observed that the color changes from pink yellowish to brown yellowish. The films were easily disintegrated, where P.S films indicates bursting of the films after 48 h. The films with seaweed content though, show the ability to sustain their mechanical properties without indication of films disintegration. This is due to sufficient tensile strength and water absorption ability from the seaweed to have longer time on the wounded area.

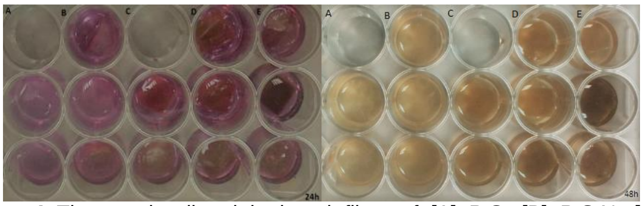

Fig. 4 The non-irradiated hydrogel films of [A] P.S, [B] P.S.N, [C] P.S.N.SW5, [D] P.S.N.SW15, [E] P.S.N.SW25 after 24 h (on left) and 48 h (on right).

While Fig. 5 demonstrates the irradiated films after 24 h and 48 h, respectively. These films also show the colour changes from pink yellowish to brown yellowish, resulted from the normal cellular respiration called glycolysis which leads to acidification of the media, suggesting that the cells have normal growth. The cells stipulate the requirement of more media and nutrient to support the respiration process and before the acidification become a problem to the cell viability (Ng *et al.*, 2004). Therefore, the changing of colour shown that the cells able to live with the presence of the hydrogel films.

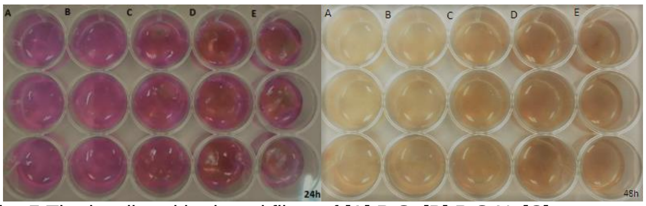

Fig. 5 The irradiated hydrogel films of [A] P.S, [B] P.S.N, [C] P.S.N.SW5, [D] P.S.N.SW15, [E] P.S.N.SW25 after 24 h (on left) and 48 h (on right).

The irradiated films (Fig. 5) show more pale color of brown changes compared to non-irradiated films, indicate that irradiated films had provided better crosslinking to support more nutrient from seaweed and starch for the cells to grow and able to live in longer h. Thus, these results illustrate that the films could sustain longer time on wound to increase the healing process. The films that are not sterilized, however, can contaminate with bacteria which over the chances of cell to live. The existing of bacteria on the film diminished the cells or the risk of having competition with the cells to gain much nutrient available in the media.

CONCLUSION

The hydrogel films of non-irradiated and irradiated films were successfully fabricated and were compared through moisture content and tensile strength of the films. The properties of hydrogel films improved with gamma irradiation proportionally increased with the addition of seaweed. Films sterilization were done through UV lights and gamma ray. The films sterilized under UV lights were easily disintegrated, yet the addition of seaweed helps to sustain their mechanical properties. Meanwhile, the films sterilized under gamma irradiation had virtuous crosslinking compared to the other films. Thus, gamma irradiation helped in improving properties of hydrogel films that suitable for in vitro studies.

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