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Honey Hydrogel Patch Assisted by Ultraviolet Exposure: Characterization and Adhesion Analyses

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Abstract:
Wound treatment is one of the challenging issues, faced in healthcare despite the development of various advanced technologies. Hydrogels are known to be used in wound treatment and received a lot of attention in the biomedical field due to its ability to store and absorb a significant amount of water which is advantageous for wound healing. The incorporation of honey within the hydrogel increases the resistance towards bacterial infection and induce advanced cell proliferation. However, the fragile structure of honey hydrogel has limited its placement on wounds. The utilization of ultraviolet (UV) light to harden the physical properties of honey hydrogels able to improve and assist the formation of honey hydrogel patches. Therefore, this study aimed to fabricate honey hydrogel patches through UV exposure and evaluate the physio-chemical properties and mucous adhesion strength of the hydrogel patches. The stingless bee honey was incorporated into the matrix of cellulose hydrogel, followed by the exposure of 365 nm UV light at different distances of 1, 2 and 6 cm. The morphology of the hydrogel patches at 1 cm UV exposure was observed to be flatten and possessed more regular structure compared to the other variation of hydrogel patches. The most hydrophilic surface (26.1 ± 0.8)˚ and the greatest ultimate strength ($0.52 \pm 0.3 \times 10^{-3}$ kPa) were also recorded on the similar sample. Therefore, at a shorter UV light exposure distance, distinct hydrogel patches with greater adhesion capability were fabricated for the use in wound healing management.

Keywords: Honey hydrogel; Ultraviolet; Adhesion; Wound healing

1. INTRODUCTION

Wounds are abnormal breaks, tears, or defects in the skin that are produced by thermal/physical trauma or an underlying medical disease (1). Wounds can be characterized as either acute or chronic based on the healing process (2). Acute wounds are tissue injuries that usually heal in about 8 to 12 weeks and leave little or no scaring behind. Chronic wounds, on the other hand, are more likely to recur and require a longer healing time than 12 weeks. Patients who are plagued with abnormalities in wound healing incur severe physical and psychological distress and morbidity (3). As a result, the wound must be properly cared for quick healing and infection prevention. Honey has been used as a medicine for a long time, including as medical dressings to speed up wound recovery and best described as one of the approaches to prevent and limit bacterial infection, hence lowering the wound's bioburden (4). Furthermore, various kinds of bees are producing various type of honeys with diversity medicinal properties (5). Stingless bee honey, also known as Kelulut honey from Malaysia, has been discovered to exhibit exceptional antibacterial properties that make it suitable for medicinal and therapeutic applications (6,7). Moreover, the stingless bee honey possesses higher content of polyphenols and flavonoids comparable to Australian manuka honey, making it more effective in providing anti-inflammatory, antioxidant and antibacterial properties (8).

For sustainable therapeutic delivery, a matrix medium such as hydrogels or polymers, is required to accommodate honey. Hydrogels are three-dimensional (3D) network structures of polymeric materials that contain hydrophilic polymer chains and have the potential to store and absorb a significant amount of water in their interstitial matrices (9). Furthermore, because water makes up the majority of the human body, hydrogels can absorb vast amounts of water are beneficial for biological applications and control the moisture of wound environment to promote better healing and prevent inflammation (10,11). The flexibility and moldability of hydrogels based on desired application has attracted various innovations in biomedical field (12). However, in general, hydrogels have weak mechanical strength due to its high-water content (13). Nevertheless, the mechanical properties of cellulose-based hydrogels can be altered by physical or chemical means.

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Aside from the anti-inflammatory and antioxidant properties, moderate UV exposure is good for wound treatment and re-establishing skin homeostasis (14,15). There are a number of factors that influence the efficacy of UV light in creating biological changes (16). On the other hand, UV light is also found to participate in increasing the entanglement of polymeric matrix which hardens its physical properties (17). The entanglement is achieved through chemical modifications of crosslinking between cellulose and ethylene glycol domains. The UV exposure would induce partly cross-linked covalent bonds to undergo expansion-contraction transition which enhances the hydrogel's mechanical properties (18). Besides, UV irradiation prevents the use of physical or chemical crosslinking agents which leaves toxic reaction remnants or generate waste during the process (18). Hence, the projection of UV exposure at specific parameters on hydrogels able to solidify a part of the honey hydrogels to attain more distinct structure and to control hydrogel's dissolvability. Therefore, UV light at different parameters was exposed on honey hydrogels in this study for the solidification purpose in developing honey hydrogel patches, while expecting the delivery of UV therapeutic values on wound lesion during future application. The effects of UV light on honey hydrogel patches were evaluated in terms of chemical composition, morphology, and mucosal adhesion.

2. MATERIALS AND METHODS

2.1 Materials Preparation

Sodium carboxymethyl cellulose (SCMC, Mw = 90,000 g/mol), hydroxypropyl methyl cellulose (HPMC, Mw = 10,000 g/mol), polyethylene glycol (PEG) 400 and analytical grade ethanol (70%) were purchased from Sigma Aldrich (Missouri, USA). Stingless bee, honey at 22% concentration was purchased from Bahtera Yubalam Enterprise, Malaysia. The cellulose hydrogel was prepared referring to Lo et al., 2021 (19). In short, 10% (w/w) HPMC and 15% (w/w) SCMC were dissolved slowly in ethanol at 60°C to avoid agglomeration. Then 5% w/w PEG was added followed by 40% (w/w) distilled water. Finally, after the ethanol was evaporated, 30% (w/w) stingless bee honey was pipetted into the cellulose mixture and stirred for 10 minutes. The hydrogel mixture was transferred onto a petri dish and dried in an oven (UNB 300, Memmert, Germany) at 40°C for 2 days. Afterwards, it was transferred into a refrigerator for 1 day at 4°C prior to the analysis and labelled as HH (honey hydrogel).

2.2 Ultraviolet Exposure

The hydrogels were exposed to UV light (365 nm) (UVGL-25 Compact UV Lamp, UVP, Cambridge, United Kingdom) by varying the exposure distances at 6, 2 and 1 cm. The selected distances were based on the preliminary trials to examine the influences of UV intensity on the hydrogel surface properties. These distances represent high (1 cm), medium (2 cm) and low (6 cm) irradiance levels to systemically evaluate the effect of UV energy gradient on crosslinking and surface morphology. The hydrogels were exposed for 30 minutes to allow the top surface of the hydrogels to be hardened. These hydrogels were labelled HHP-d (honey hydrogel patches, d = UV irradiation distance).

2.3 Morphology Visualization

The morphology of the hydrogels after the UV exposure was visualized under scanning electron microscope (SEM) (Tabletop Microscope TM300, Hitachi, Japan) at magnifications of 100× and 1000×.

2.4 Wettability Analysis

The VCA Optima equipment (Optima, AST Product, Inc, USA) was used to measure the water contact angle of the hydrogels. 2 μL of distilled water droplet was dripped over the sample surfaces at a rate of 1 μL/s. The angle of the water droplet was measured (n=3) and recorded to determine the surface hydrophilicity. Statistical significance was calculated by one-way ANOVA with Tukey's test.

2.5 Tensile Adhesion Test

Texture analyzer (CT3 Texture Analyzer, AMETEK Brookfield, USA) was used to measure and plot the adhesion tensile strength of the hydrogels towards chicken breast (Figure 1). The breast of the chicken was purchased from the butcher shop and directly used to maintain the presence of mucous membrane on the surface. The chicken breast mucosal tissue was chosen due to its ready availability, ease of handling, and similar mucous membrane characteristics relevant for adhesion testing. This mucosal model is biologically relevant substrate to assess the adhesion under moist conditions and allows comparative analysis of adhesive strength changes after UV treatment (20). The hydrogels were cut into 30 × 15 mm size and the bottom surface of the hydrogel was attached on the chicken tissue while the UV irradiated surface facing outwards. The loading force was standardized at 0.05 N at a speed of 1 mm/s (n=3).

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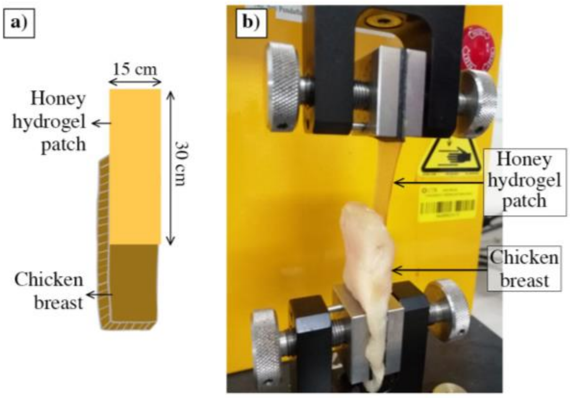

Figure 1. a) Adhesion of bottom layer of honey hydrogel patch on chicken breast tissue and b) vertical tensile adhesion test.

3. RESULT AND DISCUSSION

3.1 Ultraviolet Exposure

Earlier, all HH were exposed to two types of UV wavelength of 255 nm and 365 nm. The 365 nm UV was chosen due to its ability to harden the hydrogel surface from the preliminary study. Moreover, 365 nm UV wavelength falls within UVA range, which has relatively low energy compared to shorter wavelengths (255 nm, UVA) (21). UVA is considered safer for biological materials and has better penetration through materials and tissue than UVC, minimizing the risk of excessive damage to the honey's bioactive compounds such as polyphenols and enzymes (22). Hence, exposing the 365 nm UV for 30 minutes was able to harden the hydrogels on the top surface by forming a distinct and intact hydrogel structure.

3.2 Morphology Visualization

Figure 2 shows the surface morphology of the honey hydrogel at different UV irradiation distances. The structure irregularities were reduced where the surfaces became flatter as the distances of UV exposure decreased. The HH without UV exposure have uneven and irregular surfaces compared to the HHP-*d*.

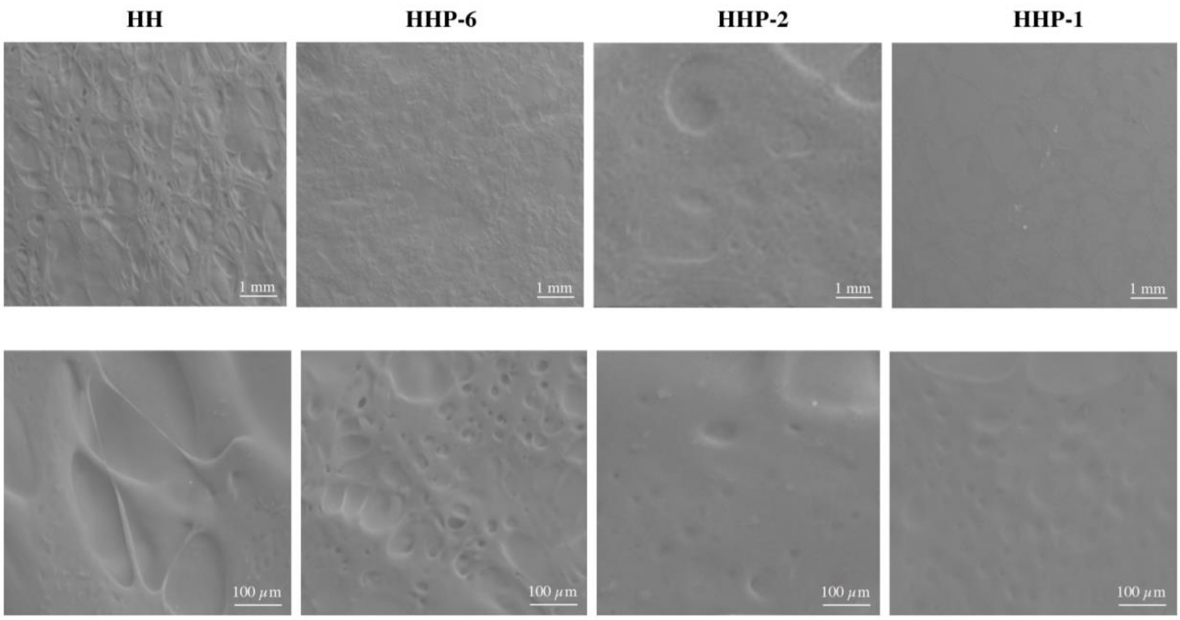

Figure 2. Morphology of HH and HHP-*d* after at 100× (top) and 1000× (bottom) magnifications.

The surface regularity was visualized due to the initiation of radical polymerization upon the UV exposure which produced stable covalent C–C bonds hence, a stable hydrogel (23). Radical polymerization is a type of polymerization process in which a polymer is formed from the reaction between monomers and free radicals. The free radicals are generated by chemical or physical initiator, such as UV radiation (24). Covalent bonds are chemical bonds that are formed by the sharing of electrons between two atoms and are considered to be very strong (24). In this study, the UV exposure may assist in the formation of covalent bonds, thus providing the hydrogel network with stability, mechanical strength and maintaining the physical structure over time.

In the previous study, non-UV irradiated (control) sample has shown irregular structure due to alternate high and low temperature storages during the drying and cooling processes (25,26). The process of drying and cooling are commonly used for the preservation and storage of hydrogels, and during these processes, the hydrogel is exposed to high and low temperatures, which can collapse the physical crosslinks, weaken the hydrogel structure and cause the formation of irregular surface (25,26). Thus, it is proven in this study that the closer UV distance able to reduce the surface irregularity to form a flatter and stable hydrogel surface.

3.3 Wettability Analysis

The wettability properties of the honey hydrogels were evaluated to determine the effect of UV exposure towards the hydrophilicity of the honey hydrogels. Figure 3 shows the water contact angle data of the honey hydrogel surfaces. Although all honey hydrogels recorded lower water contact angle (<90˚), the hydrophilic properties were found to be enhanced on the HHP-*d* surfaces, especially on the HHP-2 and HHP-1. The HHP-*d* show great hydrophilicity as the UV irradiation distance decreased and indicates that the honey hydrogel patches favored water and liquids. The surfaces with regular and flat structures (HHP-*d*) have smaller water contact angle than the irregular and uneven surface (HH), as the water tends to spread out on flat surface (27). UV irradiation increases the surface energy of the hydrogel undergoing mild photo-oxidation (28). The presence of several functional groups on the surface of the HH, especially hydroxyl or carbonyl group (19), upon UV crosslinking alters the morphology and enhances the hydrophilicity. UV irradiation induces the formation of highly reactive hydroxyl and hydroperoxide groups on the cellulose surface through photo-oxidation of peroxyl radicals, increasing the abundance of –OH, C–O, C–H and C–OH bonds, which enhances the surface hydrophilicity by elevating the polar functional groups (18).

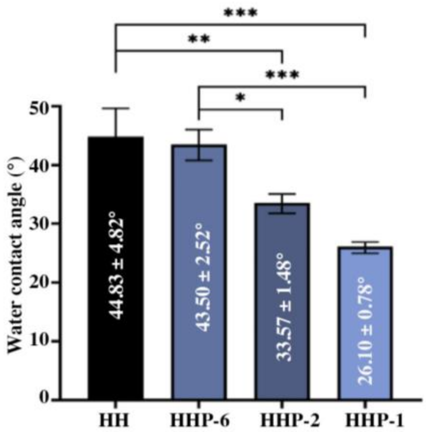

Figure 3. Graph of water contact angle measurements of honey hydrogel (control) and honey hydrogel patches after UV irradiation. (*p<0.05, **p<0.01, ***p<0.001)

3.4 Tensile Adhesion Analysis

The tensile adhesion test was conducted to evaluate the adhesiveness of the honey hydrogels on the chicken mucous tissues. The stress-strain curves obtained were plotted and presented in Figure 4. The ultimate strength of each hydrogel was calculated from the curve and the average ultimate strength were 0.03 ± 0.05 kPa, 0.30 ± 0.03 kPa, 0.37 ± 0.04 kPa and 0.52 ± 0.03 kPa for the HH, HHP-6, HHP-3 and HHP-1, respectively. The exposure of UV at different distances greatly impacted the adhesion of honey hydrogels onto mucous surfaces. Hydrogels with greater high crosslinking density is more rigid and have a stronger mechanical structure due to the interconnected network of polymer chains, which provides stronger physical bond and increases its adhesion to a surface (29). Therefore, from the results, the HHP-*d* have better ultimate strength than the HH.

The basic principle of adhesion is through the formation of junctions between the cellulose base of honey hydrogel and the mucus membrane surfaces (30). The tissue adhesive property of the adhesive cellulose (SCMC, HPMC) used in the hydrogel fabrication aided in the junction formation during the contact with the mucous membrane of the chicken breast. The formation of these junctions can also be achieved through various other methods, such as the use of specific polymers or surface modification techniques, that can increase the hydrogel's ability to interact with the mucus membrane and promote the formation of junctions. The surface energy of a hydrogel is an important factor that can affect its adhesion to other surfaces (31). Hydrogels that have a high surface energy, such as high hydrophilicity, tend to interact well with water molecules, which allows them to adhere to wet surfaces more effectively. Therefore, the HHP-*d* have better adhesion on the mucous surfaces compared to the HH. UV irradiation enhances the stability and hydration retention of cellulose-based hydrogels by inducing crosslinking which creates a denser polymer network that improves mechanical strength. Crosslinked structure also reduces degradation and prevents excessive water loss while preserving flexibility and biocompatibility, making the hydrogel more durable and effective during storage and use (18).

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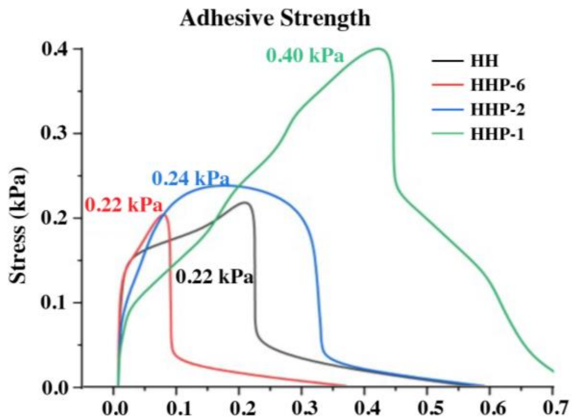

Figure 4. Stress-strain curves from tensile adhesion analysis of HH and HHP-*d* onto mucous tissues.

4. CONCLUSION

In conclusion, the effects of UV irradiation at varying distances were investigated on the honey hydrogels for the purpose of hardening one side of the hydrogel. The surface characterization of the HH showed that smoother and flatter surface and significant increase in hydrophilicity were achieved after the UV exposure. Stronger adhesion between the honey hydrogels and the mucous layer was recorded on the honey hydrogels that been exposed with UV at the lowest distance (HHP-1). Therefore, UV is seen as one of the techniques to form distinct structure of honey hydrogels that possess strong adhesion on the mucous tissues for wound treatment.

AUTHORSHIP CONTRIBUTION STATEMENT

Belal Yahya Hussein Al-Tam: methodology, experimental work, formal analysis, investigation, data collection, writing – original draft. Rathosivan Gopal: methodology, investigation, validation. Ling Le Ee: visualization; writing – review & editing. Syafiqah Saidin: conceptualization, funding, supervision, resources, writing – review & editing.

DATA AVAILABILITY

Data are available within the article or its supplementary materials

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

DECLARATION OF GENERATIVE AI

The authors declare that they have not used any type of generative AI for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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