

Review

The Rediscovery of Honey for Skin Repair: Recent Advances in Mechanisms for Honey-Mediated Wound Healing and Scaffolded Application Techniques

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Abstract: Honey is a honey-bee product obtained mainly by the enzymatic processing of nectar from a variety of plants, which leads to the wide range of colours and flavours available on the market. These organoleptic and nutritional features are influenced by the chemical composition, which in turn depends on the botanical origin. Bioactive compounds account for honey beneficial activity in medical applications, which explains the extensive use of honey in ethno-pharmacology since antiquity, from cough remedies to dermatological treatments. Wound healing is one of the main therapeutic uses of honey, and various design options in pharmaceutical technology such as smart delivery systems and advanced dressings are currently being developed to potentiate honey's valuable properties for better performance and improved final outcome. In this review, we will focus on the latest research that discloses crucial factors in determining what properties are most beneficial when considering honey as a medicinal product. We will present the most recent updates on the possible mechanisms responsible for the exceptional effects of this ageless therapeutical remedy on skin repair. Furthermore, the state-of-the-art in application techniques (incorporation into scaffolds as an alternative to direct administration) used to enhance honey-mediated wound-healing properties are explored.

Keywords: honey; antioxidant; antibacterial; anti-inflammatory; polyphenolics; skin; healing; hydrogels

1. Introduction

Honey is a sugar-based food product obtained by a broad range of botanical sources (unifloral or multifloral) and different geographical origins. This diversity is reflected by the distinctive pattern of aroma, flavour, colour, and texture of different honey varieties. These organoleptic features, described by the sensory characterisation, are highly interrelated with the peculiar physicochemical composition, including soluble bioactive compounds and volatile organic compounds (VOCs), which constitute the chemical fingerprint of that specific honey variety [1–4]. Reports of using honey and its related bee products (propolis, beeswax, pollen) in traditional medicine stretch back centuries, since the first reports from Sumer, and then in ancient Egypt, Greece, Rome, and Asia [5], due to their appreciated and renowned health benefits. However, despite the many hypotheses on the mechanisms for the numerous beneficial effects, honey's full potential has started to be unlocked only recently, with more comprehensive characterisations of its physicochemical composition (as described in Section 2 of this paper), and the further investigation of the medical activity of natural bioactive compounds also found in honey [6–15]. Honey has been shown to possess beneficial properties with diverse applications in otorhinolaryngology [16,17], respiratory tract diseases [18,19], cardiovascular diseases [20–22], metabolic disorders [23–26], oncology [27,28]. However, the focus of this review is on the use of honey in the management and treatment of skin disease and in particular wounds of various natures, exploring the possible mechanisms by which honey may enhance skin regeneration.

Wound healing is a highly articulated process influenced by various factors, whose disbalance can cause complications, with improper hypertrophic scarring, and impaired tissue repair, resulting in chronic wounds [29–32]. Among the wound complications, infections represent a major concern, given the alarming surge in antibiotic resistance incidence in recent years [33,34]. In this context, the use of topical agents should be regarded as a valuable alternative to systemic antibiotics, when the latter is not deemed essential. Local antimicrobial administration allows high concentration of active compounds in the delimited affected area, with limited or no side effects in the healthy circumjacent skin [35]. Furthermore, the use of natural antimicrobial agents, for which antibacterial resistance has not been reported, is becoming more prominent, as complementary or alternative options to conventional treatments [36]. Honey, with its extensive use in traditional healing and dermatology, represents a valuable candidate to promote wound healing and complete skin regeneration.

Together with a renewed attention for traditional approaches and apitherapy applications in skin medicine [37,38], advanced strategies for wound repair are gaining increasing importance. In particular, functionalised dressings are being designed not only to cover the wound and protect from external contaminations but also to actively enhance and accelerate the healing process [39]. These sophisticated developments in wound care might represent the bridge between the history of ethnopharmacology and phytotherapy, and the needs of future medicine, with the final goal of developing a cost-effective complimentary addition to conventional medications. The main features pursued in an ideal wound dressing are the ability to support healing and shield the wound from further harm and tissue loss whilst incorporating satisfactory fluid control properties. These properties serve to avoid maceration (damage from the over-retention of fluids on the surrounding healthy skin). The ideal dressing should also adhere delicately to the skin avoiding secondary damage upon removal of the dressing from the newly formed skin underneath and prevent excessive scarring [40].

Our review focusses on the latest research demonstrating the promising therapeutical virtues of different honey types, with particular attention to the articles published in the last five years. Starting with the compositional characterisation of honey, in relation to different honey varieties and peculiar compounds, honey antimicrobial, antioxidant, and anti-inflammatory properties as well as the suggested mechanisms are described. Then, the application of honey in the management of different skin diseases and wounds is presented, with case reports and clinical studies, to highlight the latest evidence that demonstrates its healing benefits and also its limitations. Finally, the state-of-the-art of honey incorporation into scaffolds and technological devices for wound healing is illustrated, with a critical analysis of the pivotal parameters that can be used to optimise and further enhancement the intrinsic remarkable properties of honey.

2. Honey Physicochemical Composition

Honey is the product of various modifications, mainly enzymatic and operated by honey bees, of the nectar or other secretions of plants, i.e., nectar honey or honeydew honey, respectively, as defined by the Revised Codex Standard for Honey [41], which meets all the legal requirements for food products, from chemical composition to labelling, in order to guarantee a quality product that conforms to the highest standards. From a chemical point of view, honey is a super-saturated solution of sugars, mainly fructose and glucose, at a concentration of not less than 60 g/100 g. Other sugars (mono-, di-, tri-, and polysaccharides) are also contained, up to 80% of the product total composition, with high variability in the relative ratio depending on the botanical origin, although not specifically linked to it [42]. The sugar mixture itself and water content are both indicators of honey optimal ripeness, and they also represent the primary factors to assess adulteration, while defining the rheological features responsible for honey texture [43]. Honey moisture content should not exceed 20%, although for Heather honey (*Calluna*), a value of 23% is deemed acceptable [41].

Sugar mixture and water content are not the only physicochemical parameters by which honeys can be characterised. A maximum quantity of 0.1 g/100 g water-insoluble solid particles [41] derived from the honey collection, such as pollen, are typically also present and can be used for the melissopalynological analysis as part of the botanical classification [44]. Honey also contains minerals and vitamins; proteins are mainly represented by enzymes such as invertase, diastase, and glucose oxidase. Among the amino acids found in honey, proline is often quantified, with a minimum value of 180 mg/kg required as an indicator of maturation and authenticity [45]. Proline has been found to be the most abundant amino acid in Estonian honeys (257–1328 mg/kg), followed by phenylalanine and glutamine [46]. Another parameter that is strictly monitored is hydroxymethylfurfural (HMF), whose quantity should not exceed 40 mg/kg (80 mg/kg for honey from tropical countries) [41]. It is a by-product of the Maillard reaction between amino acids and sugars and of the acidic degradation of monosaccharides (mainly fructose), and a marker of long storage at high temperatures [47].

3. Honey Phenolic Fraction and Bioactive Compounds

Phenolic compounds are recognised as responsible for the widely investigated antioxidant properties of honey, as indicated by a positive correlation with the water- and lipid-soluble antioxidants capacity [46]. Their content is derived from its floral origin (both if it is single-origin or multifloral) and contributes to honey's colour [48]. A significant correlation ($r = 0.6$, $n = 36$, $p < 0.001$) was confirmed by Kavanagh et al., with Irish dark honeys showing higher total phenolic content (TPC) [49]. Interestingly, the same study has also revealed that phenolic compounds were found to be significantly more abundant in Irish urban honeys than in rural samples. More specifically, in this study, Irish heather honey showed comparable chemical composition to Manuka honey (MH) with regard to its TPC (68.16 ± 2.73 and 62.43 ± 10.03 mg GAE/100 g, respectively, where GAE stands for gallic acid equivalents). A TPC of 88.7 mg GAE/100 g and a lipid-soluble antioxidants capacity of 60.7 mg TE (Trolox equivalents)/100 g represented the highest values among the Estonian honeys analysed by Kivima et al., and they were both registered in heather honey, which was also the darkest sample, with the most red tones [46]. Noticeable levels of total phenolics were also confirmed by Salonen et al. [42] for heather honey from Nordic countries. This highlights the potential of heather honey for biomedical applications, considering that honey antioxidant activity is correlated to the beneficial effects of honey on various health issues [50] and to the antibacterial efficacy [42]. Similar TPC values for Manuka honey to those observed by Kavanagh et al. have been also measured by Nguyen et al. (72.1 ± 2 and 75.4 ± 0.8 mg GAE/100 g), but in this case, they were shown to be significantly higher than other unifloral honeys from traditional Indian medicinal plants such as tulsi plant (*Ocimum tenuiflorum* L.) and alfafa (*Medicago sativa*), respectively 50.6 ± 2.7 and 18.3 ± 0.3 mg GAE/100 g [48]. Moreover, additional factors may act independently or jointly with the honey phenolics, leading to an additive or synergistic (potentiated) effect. As such, further investigations on the medicinal effect of alternative honey varieties to Manuka honey could still disclose a high therapeutic power.

The variety of phenolic and structurally related compounds observed in honey is determined mainly by the floral origin and the geographical collocation. Some examples are listed here and shown in Table 1 merely to offer a brief (but by no means exhaustive) illustration of the diverse assortment of phenolics measured in the latest published characterisation of different honey varieties. The honey phenolic fraction can include benzoic acids such as syringic acid, its aldehyde syringaldehyde, and its ester methylsyringate. The latter has been detected in Manuka honey [51,52], Iranian unifloral honeys such as Persian rose, hawthorn, and thyme [51], and also identified as a biomarker of asphodel honey [53,54]. Gallic acid was the most concentrated phenolic acid quantified in strawberry tree honey, accounting for 54.44% of the phenolic acid content, which was followed by 4-hydroxybenzoic acid and caffeic acid [55]. Homogentisic acid has been identified in peculiarly high concentrations in strawberry tree honey [54], of which it represents,

together with abscisic acid derivatives, a specific marker of botanical origin [56,57]. Ellagic acid, typically found in raspberry honey, was also detected in lingonberry honey by Salonen et al. [42]. Phenyllactic acid and p-HBA (para-hydroxybenzoic acid) were the benzoic acids found in the highest concentration in honeys from Iran [51]. Cinnamic acid has been detected in chestnut honey [58] and is typically found in high concentrations in heather honey, together with myricetin and abscisic acid derivatives [46]. Coumaric acid is the most represented cinnamic acid derivative in buckwheat honey [42]. Among the flavonoids, pinobanksin has been found to be abundant across various Iranian honey samples of different botanical origin [51]. It was also the most abundant flavonoid, together with pinocembrin (36% and 23% of total flavonoid content, respectively), identified in Manuka honey [52]. The flavonol kaempferol was the most represented flavonoid (41.2% of the total) in strawberry tree honey, with quercetin and luteolin being also highly abundant [55]. Chrysin has been found in various unifloral honeys, such as astragal, chicory, white clover, and hawthorn honey [51].

Phenolic compounds have been extensively researched, and the rationale for their use as ethno-medicinal products has been confirmed by numerous studies [59,60]. These natural anti-inflammatory and antioxidant agents are widely present in various plants and natural products of traditional use in wound care and folk medicine for various skin conditions [61]. Polyphenol-rich crude extracts from Alaskan wild berries showed promising potential in vitro for tissue regeneration and wound closure, in particular due to the proanthocyanidin fractions playing a significant role in the expression of extracellular matrix constituents, such as integrins and collagen, and stimulating mitochondrial vital processes [62]. *Bletilla striata*'s phenols-rich extract has been shown to effectively promote healing in a mice model of burn wounds, with notable wound reduction by the fifth day of treatment compared to control. The components of the beneficial extract were identified as protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, p-hydroxybenzaldehyde, 3-hydroxycinnamic acid, and ferulic acid [63]. These bioactive compounds and structurally related compounds have been also identified in honeys of various botanical sources [42,46,58,64,65], suggesting that honey might share the same virtues and represent a valuable agent in wound medicine.

Royal jelly (RJ) has been highly regarded in traditional medicine and widely investigated for its distinctive antimicrobial properties [66]. RJ's peculiar compound, 10-hydroxy-2-decenoic acid (10-HDA), also known as queen bee acid, has shown promising results in vitro on human colorectal cancer (CRC) by tackling critical pathways in the pathogenesis of carcinomas, markedly reducing the levels of cytokines involved in pro-inflammatory signaling and exerting bactericidal activity against pathogens responsible for infections of the gastrointestinal tract [67]. A significant downregulation of melanin production and inhibited expression of melanogenesis-related peptides were observed in melanoma cells upon treatment with 10-HDA, showing the potential to treat hyper-pigmentation skin conditions [68]. In light of their noteworthy beneficial properties, identification of RJ compounds in honey could be of significant interest for the applications of honey in skin treatment. 10-HDA was found in high concentrations in the aliphatic acids fraction analysed in pine herb honey and multifloral honey by Isidorow et al. [69]. It was also identified by Levya-Jimenez in Iranian honeys of different botanical origin, although dihydroxy-decenoic acid was the most abundant RJ-derived compound in these samples. However, this was not found to be present at significant concentrations even in the most actively antimicrobial samples [51], suggesting it might not significantly contribute to this activity. RJ-derived fatty diacids (decanedioic and decenedioic acids) have been detected in Scottish honeys, together with their glycosides with preliminary data finding them more concentrated in antimicrobially active samples [65].

Table 1. Chemical structure of some of the most frequently researched phenolic compounds detected in honey and examples of their occurrence in honeys of different botanical origin.

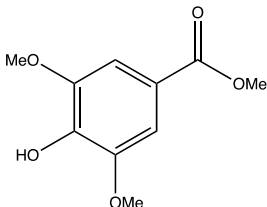
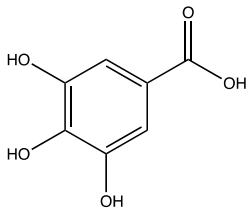
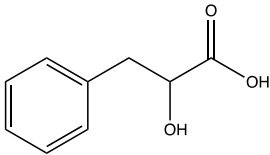
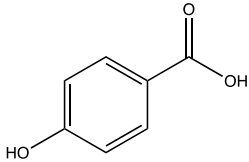
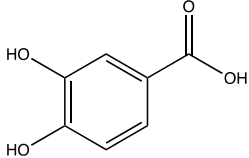
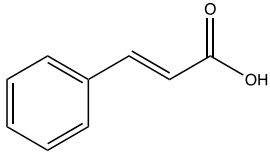
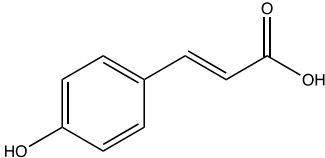
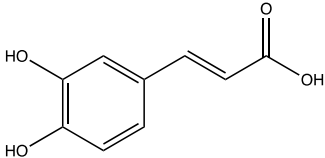
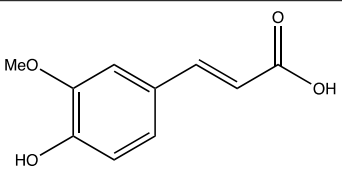
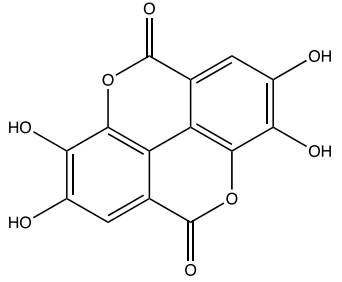
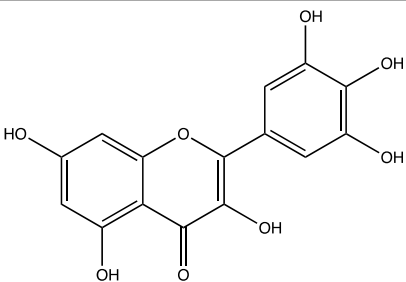
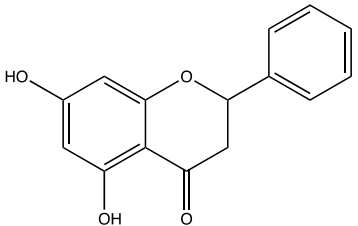
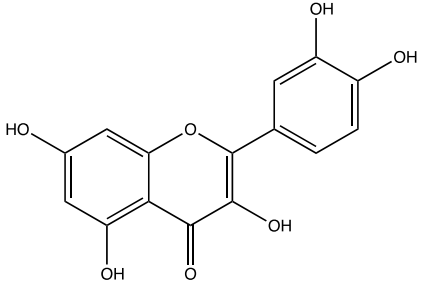
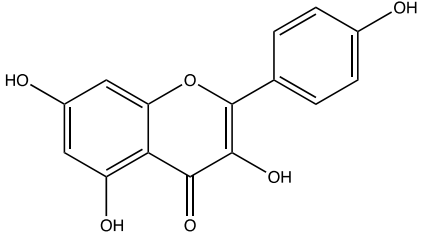
Chemical Structure	Compound Name	Honey Varieties
	Methylsyningate	Persian rose, Hawthorn, Thyme [51], Asphodel [53,54], Agastache [70], Manuka [52,65]
	Gallic acid	Strawberry tree [55], Chestnut [58], Mint, Raspberry [71]
	Phenyllactic acid	Agastache, Jarrah [70], Manuka [65]
	para-Hydroxybenzoic acid	Strawberry tree [55], Chestnut [58], Raspberry, Sunflower, Mint [71], Buckwheat [72]
	Protocatechuic acid	Chestnut [58], Raspberry, Mint, Thyme, Honeydew [46,71], Buckwheat [72]
	Cinnamic acid	Chestnut [58], Heather [46]
	Coumaric acid	Raspberry, Sunflower, Thyme [71], Buckwheat [42,72], Juazeiro [64], Tilia, Honeydew, Sunflower [73]
	Caffeic acid	Strawberry tree [55], Chestnut [58]

Table 1. Cont.

Chemical Structure	Compound Name	Honey Varieties
	Ferulic acid	Buckwheat [72], Juazeiro [64]
	Ellagic acid	Raspberry, Lingonberry [42], Juazeiro [64]
	Myricetin	Strawberry tree [55], Thyme, Rape, Mint, Raspberry, Sunflower [71], Malicia [64], Heather [46]
	Pinocembrin	White clover, Hawthorn, Black cumin [51], Manuka [52], Tilia, Acacia, Honeydew, Sunflower [73]
	Quercetin	Strawberry tree [55], Juazeiro, Malicia [64], Brassica [46], Honeydew, Acacia [73]
	Kaempferol	Strawberry tree [55], Juazeiro, Malicia [64], Brassica [46]

4. Antimicrobial Properties of Honey

Phenolic compounds critically modulate the antimicrobial properties of honey against common wound infections-causing strains, both Gram-positive, such as *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*), and Gram-negative bacteria as *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). When tested using a disc diffusion method, phenolic acid extracts were found to inhibit bacteria at a lower minimal inhibitory concentration (MIC) than the correspondent whole honey sample extract. This shows the prominent contribution of phenolic constituents, working synergistically, to the overall honey antibacterial activity [51]. Such phenolics-mediated anti-bacterial action seems to rest on a sophisticated balance between radical scavenging and pro-oxidants functions: depending on the concurring factors (i.e., hydrogen peroxide-rich honeys and the presence of transition metals), these renowned antioxidant agents can in fact auto-generate reactive oxygen species (ROS) and activate hydroxyl radicals. This triggers propagation of the Fenton reaction [74,75], resulting in the observed antibacterial effect by bacterial damage. The direction that this equilibrium tends to also depends on the pH value: pro-oxidant action has been observed at fairly neutral pH, such as in diluted honeys [76]. The total phenolic content of honeys from Nordic countries has been shown to correlate with their inhibitory capacity against *S. aureus* at 15% dilution ($r = 0.57$), with buckwheat, heather, sweet clover, and polyfloral honeys having the most pronounced antibacterial activity [42]. However, the total phenolic content and antioxidant activity of Scottish honeys of different botanical origins have not been found to be correlated with antibacterial activity [65]. The investigation of Bueno-Costa et al. on Brazilian honeys confirmed these findings, but a significant correlation ($r = 0.4424$) was instead observed between total flavonoids levels and antibacterial activity against *Bacillus cereus* (*B. cereus*) [77].

Nonetheless, the determinant factor for honey antimicrobial activity is yet to be unequivocally agreed on, which complicates the comparison between honey varieties described in healing applications. A conventional simplification leads to a distinction between peroxide and non-peroxide antibacterial activity: the first being hydrogen peroxide-mediated and the latter often identified with Manuka honey but not limited to it. However, the hypothesis of a multifactorial system seems more verisimilar, and it is generally accepted [78].

The osmotic pressure generated upon honey application on a wound site is regarded to be a primary contributor to the pro-healing features of honey of all botanical origins. In fact, the flux of fluids along the concentration gradient due to honey's high sugar content effectively washes the wound area from contaminants, debris, and bacteria, and it transports nutrients while regulating the moisture balance on the exudative wound [79]. Fyfe et al. reported that a negative sugar control (consisting of a saturated solution of 38.5% fructose, 33.3% glucose, 6.2% maltose, and 7.3% sucrose in distilled water) inhibited the bacteria tested although not as effectively as the compared honey samples [65]. A similar observation was made in a study by Salonen et al., with artificial honey prepared with analogous sugar composition to Finnish honey and acidified to pH 3.5 with HCl [42]. However, a 75% sucrose (*w/w*) artificial honey did not show antibacterial activity against any of the bacteria tested by Matzen et al. [80]. Therefore, it is suggested that the high osmolality is not decisive to the medicinal properties of honey by itself, although it could contribute to them.

Honey presents an intrinsic acidic pH, typically ranging between 3 and 4.7, due to its organic acids content [48,49,81]. This inherent acidity is deemed to be one of the primary contributors, for the antimicrobial activity of honey as it creates an unfavourable environment for bacterial growth [82]. However, no correlation between pH and antibacterial activity was found in Stingless bee honeys [83] nor in Polish honeys against *Staphylococci* [84]. The acidity is also believed to play a role in the capacity of honey to restore the skin barrier properties that can be affected by various medical conditions. Inflammatory diseases (atopic prone skin, eczema, seborrheic dermatitis), skin microbiome alteration, topical infections (candidiasis), and wounds can in fact compromise the epidermal acidic milieu [85]. The use of topical agents with slightly acidic pH, such as honey, can help

establish and maintain skin physiological pH (in the range between 4.5 and 6.0) and the re-deposition of the “acid mantle” which is important for optimal skin barrier function in the stratum corneum (the outermost epidermal layer) [86]. Topical treatment with honey can also enhance wound healing by creating an unfavourable environment for proteolytic enzymes, such as elastase or matrix metalloproteinases (MMPs). These are otherwise found to be hyperactive in the alkaline environment typical of chronic and infected wounds (up to 7.5–8.9), and as a consequence of this, the skin repair is impaired by the continuous degradation of recently deposited tissue [85].

Another suggested mechanism for the widely clinically observed capacity of honey to fight bacterial infections and promote skin regeneration is due to the production of hydrogen peroxide (H_2O_2) during the glucose oxidase (GOx)-mediated conversion of glucose to its correspondent acid. Furthermore, honey also upregulates AQP3 aquaporin's expression, with consequent improved diffusion of H_2O_2 , which results in increased calcium Ca^{2+} cytoplasmatic levels by Ca^{2+} channels activation. Different Ca^{2+} release intensities and kinetics have been linked to different honey varieties. Augmented intracellular Ca^{2+} thereupon governs numerous biochemical chains of events that ultimately boost wound repair [87]. However, conflicting results are reported in the literature about the role played by H_2O_2 in the antibiotic efficacy of honey, and this might also due to the time-sensitive variations of H_2O_2 levels after honey production. H_2O_2 content in Western Australian honeys did not show a correlation with the antiseptic performance, although H_2O_2 reduction operated by the enzyme catalase did affect the samples' antimicrobial power [88]. Comparable results to these reported by Roshan et al. were also described by Bucekova and co-workers about honeydew honeys with no significantly different results in the antibacterial in vitro evaluations, despite divergent H_2O_2 concentrations across the samples [76]. On the other hand, opposite observations were collected by the same author in an attempt to elucidate the contribution of H_2O_2 to the antimicrobial effect of different blossom honeys—namely rapeseed, acacia and wildflower, which were ordered here by increasing antibacterial activity. In fact, Bucekova et al. reported a statistically significant correlation between antibacterial effect and both total phenolic and H_2O_2 . However, interestingly, no statistical correlation was registered between the measured H_2O_2 -producing enzyme GOX and the levels of H_2O_2 . Furthermore, while again dramatically inhibited by treatment with catalase, such antimicrobial activity was instead not affected when GOx was proteolytically digested upon the addition of proteinase K [44]. These results suggest that another system, other than the protein-controlled one, must be co-responsible for the honey peroxide-mediated activity. Therefore, these observations could confirm the hypothesis of a phytochemical-dependent production of H_2O_2 in honey as an alternative to the widely accepted GOx biochemical pathway [76]. A synergism between H_2O_2 and phenolics has been also linked to the antiseptic activity exerted by Corsican honeys through irreversible plasmidic DNA damage on *P. aeruginosa* cultures [74].

Manuka honey (MH) is currently regarded as the gold standard for medical applications, and a specific unit, the Unique Manuka Factor (UMFTM), has been adopted to indicate its authenticity based on the content of specific markers, such as methylglyoxal (MGO), dihydroxyacetone (DHA), and leptosperin at the time of pre-packing food quality testing [89]. MGO originates from the precursor DHA found in the nectar, and its content depends on the presence of endogenous co-factors, such as phenolic content (mainly phenyllactic acid and methoxyphenyllactic acid) and aminoacidic composition (in particular proline) that could trigger Maillard-like side reactions, and environmental parameters such as storage temperature and length of time [90,91]. Given the vast attention granted to Manuka honey as the only medical-grade honey recognised so far, its fingerprint compound MGO has recently attracted increasing interest, with research focusing on its identification in other honey varieties. The MGO content in honeys of different floral origins than Manuka is highly variable. Terio et al. conducted a quantification of MGO in Italian honeys, showing a MGO wide range (0.4–24.01 mg/kg) with cherry (18.62 ± 3.69 mg/kg) and almond honey (17.88 ± 4.18 mg/kg) having the highest concentrations among the analysed varieties,

but still significantly lower than the levels measured in Manuka honey. However, this study did not include an evaluation of the antibacterial activity of the analysed honey samples, so further investigations would be needed to assess whether MGO content is relevant to the antibacterial mechanism of action of these Italian honey varieties [91]. MGO was found in high quantities (up to $166 \pm \text{mg/kg}$) in Nordic honey samples (mire and polyfloral), but it was totally absent in heather honey and in some samples of other varieties such as caraway, sweet clover, and dandelion. However, MGO content was not found to correlate with the inhibitory capacity of Nordic honeys against *P. aeruginosa* or *S. aureus* at 15% honey dilution [42]. Eleven honey samples of varieties produced from Danish flora showed a significantly lower MGO content (less than $5 \mu\text{g/mL}$) than commercial Manuka honey ($54.33 \mu\text{g/mL}$). Nonetheless, Water mint, Linden, and mixed organic flora honey were shown to cause the greatest degree of bacterial growth inhibition of all the tested honey varieties, even on the Gram-negative bacteria (*P. aeruginosa* and *E. coli*) against which Manuka was instead ineffective. Such activity was significantly affected by treatment with catalase, suggesting that it is ascribable mainly to the peroxide-mediated mechanism [80].

The UMF indicator is described as linked to the antibacterial power by comparison with a reference compound, e.g., 5 UMF is assigned to a honey inducing a microbial inhibition equal to that obtained by a 5% phenol positive control [92]. However, the MGO content in MH can increase during honey storage due to DHA conversion [90], generating incongruence with the claims reported on the labelling. Interestingly, Hixon et al. recorded the performance in the bacterial clearance test to be significantly greater in samples with lower UMF compared to MH of up to 20 UMF [92]. Similar results were also observed by Girma et al. [93], which compared MIC in vitro against different bacteria for MH of various UMF. These results highlight the urgency for a univocal criterion applicable to all honey varieties, which would be crucial in allowing a standardised comparison of antibacterial potency of honey across different botanical species.

The Manuka honey antibacterial effect seems to be incompatible with peroxide-mediated activity, as MGO supplementation has been shown to impede GOx activity in non-Manuka honeys by morphological damage and the formation of a glycated derivative structure. As a result, GOx-modulated H_2O_2 production was significantly compromised in a dose-proportional fashion [94]. However, an interaction between peroxide and non-peroxide systems has been described by Henatsch et al., who studied the antibacterial activity of honey in relation not only to its content of MGO and analogous α -dicarbonyl compounds but also to their conversion into different free radicals in the presence of either hydrogen peroxide or amino acids (specifically arginine and lysine) [95].

Majtan et al. also reported how MGO structurally inactivates other peptides deemed responsible for the therapeutical applications of honey in dermatology, such as the bee-peptide defensin-1, with possible impaired antibacterial activity [96]. Studies conducted on non-Manuka honey suggest that defensin-1 might be a crucial element to the antibacterial activity of honey of some geographical and botanical origin while being irrelevant in other samples. Defensin-1 levels in honeydew honey do not seem to influence the antibacterial effect on cultured *S. aureus*, which is responsible for many nosocomial infections. This was confirmed by the uncompromised antibacterial activity despite protein inactivation by peptidase [76]. In contrast, proteinase K-treated Greek honeys had their microbiological properties negatively impacted, as demonstrated by higher honey concentrations needed to achieve the same bacterial inhibition (i.e., higher MIC) [97]. Nevertheless, besides the irregular antibacterial function reported in the discordant literature, this peptide could give a pivotal contribution to the skin regenerative effect traditionally shown by honey and royal jelly. The defensin-1-induced wound-healing advancement is achieved in vitro by significantly increased matrix metalloproteinase-9 (MMP-9) release and consequently enhanced keratinocytes chemotaxis and neo-vascularisation in vivo [98]. This restorative mechanical process is fundamental to the re-establishment of an effective skin barrier following epithelial cell migration after an injury; thus, such encouraging results offer a further explanation for the use of bee products in wound medicine.

In the struggle against hospital-acquired infections, biofilm eradication from chronic wounds represents a substantial challenge [99]. Heather honey showed analogous effectiveness to Manuka honey against the biofilm formation from different species, namely *Acinetobacter baumannii* (*A. baumannii*), *E. coli*, *Salmonella enteritidis* (*S. enteritidis*), and *P. aeruginosa*. A shared key element in the inhibition of the *P. aeruginosa* biofilm for both the honey varieties could be benzoic acid, which has been predicted by molecular docking to efficiently bind the bacterial enzyme PaDsbA1. In doing so, it would alter the protein structural arrangement, hence compromising the biological functionality of fimbriae, flagellae, and adhesion factors, which are crucial for biofilm establishment. However, heather honey promoted *E. faecalis* and *Klebsiella pneumoniae* (*K. pneumoniae*) biofilms, while Manuka honey was ineffective on *S. aureus* [100]. This different susceptibility of the bacterial species to honey of different botanical origin could be considered when evaluating which honey variety to use to treat a given infected wound. Portuguese heather honey effectively inhibited *Candida tropicalis* (*C. tropicalis*) planktonic population at a lower concentration (MIC 12.5% (*w/v*), minimum fungicidal concentration 50% (*w/v*)) than Manuka honey (MIC 25% (*w/v*), MFC 50% (*>w/v*)). The two honey varieties were also compared on their antimicrobial action on single and multi-species biofilms of *C. tropicalis* with *P. aeruginosa*. A significant reduction in the cell viability of *C. tropicalis* was observed for both heather and Manuka honey at a concentration of 50% (*w/v*). In regard to *P. aeruginosa*, Manuka honey was more effective in decreasing the cell count already at a concentration of 25% (*w/v*) and up to 4 log (CFU/cm²) reduction at 50% (*w/v*) in the single biofilm. Nonetheless, a significant cell inhibition compared to control was obtained with heather honey at 50% (*w/v*). When assessed in association with conventional antifungal treatment, the supplementation with honey allowed the administration of a 50% lower dose of fluconazole, but higher inhibition was still achieved with honey monotherapy at 50% (*w/v*) [101]. Different honey varieties have been shown to be remarkably effective against various bacteria, and more broadly against pathogens, including fungi, which is responsible for infections non-responsive to conventional drugs. This indicates that honey represents a potential contributor to the new frontier of natural antimicrobial products emerging as promising agents in antibiotic resistance.

Ultimately, *Apis mellifera*, commonly known as honey bee, is not the only insect responsible for honey production. The species of nectar-foraging insects seems to determine the phytochemicals pattern in the ripened honey, thus leading to a different effectiveness against bacterial infections. Malaysian honeydew honey has been reported to have a higher bactericidal effect against *S. aureus* and *E. coli* in vitro when produced by the stingless bee species *Heterotrigona itama* rather than *Apis cerana* and *Geniotrigona thoracica* [78]. Of the eight stingless bee honey samples analysed by Rosli et al., honey produced by *Homotrigona fimbriata* has been found to possess the highest inhibitory capacity against five bacteria: *Serratia marcescens* (*S. marcescens*), *E. coli*, *Bacillus subtilis* (*B. subtilis*), *Alcaligenes faecalis* (*A. faecalis*), and *S. aureus*. The least active honey was produced by *H. erythrogastra*, and it has been shown to be inactive against the tested bacteria at all the evaluated concentrations [83]. In contrast, *H. erythrogastra* honey from Borneo was shown to be broadly active against all the bacteria strains tested by Tuksitha et al. Honey produced by *G. thoracica* has been shown to be significantly more effective than the honey samples from other stingless bees against both Gram-positive (*Staphylococcus xylosus* (*S. xylosus*)) and Gram-negative (*P. aeruginosa* and *Vibrio parahaemolyticus* (*V. parahaemolyticus*)). Interestingly, *H. itama* honey was both the least active antimicrobial agent against Gram-negative bacteria and the richest in phenolics, while significant Gram-negative bacteria inhibition was obtained with the honey samples with the highest level of flavonoids (*G. thoracica*). This has been hypothesised to be due to flavonoids-mediated disruption of the outer bacterial membrane integrity or impaired DNA synthesis [102].

5. Anti-Inflammatory Properties of Honey

Honey has been long appreciated for its exceptional ability to de-escalate *phlogosis*, hence the traditional use as a medicament on chronic inflammatory skin conditions and in the management of persistent symptoms such as unremitting discomfort, itchiness often

associated with skin abrasion, and laceration due to scratching, all severely affecting the quality of patients' everyday life. Along with the conventional treatments prescribed to soothe the affected skin (predominantly topical corticosteroids), growing interest has been expressed for natural remedies in the management of disorders with a main inflammatory component such as eczematous lesions and psoriasis [103,104]. Alangari et al. researched the performance of Manuka honey on atopic dermatitis (AD) patients and tried to elucidate the responsible biochemical mechanisms by AD-related in vitro models. Both evaluations confirmed a significant ameliorative effect by direct MedihoneyTM (medical-grade MH-based commercial ointment) and MH extracts application. Inflammation was reduced as confirmed by lower IL-4 stimulation on the chemokine ligand CCL26 (a chemotactic involved in pro-inflammatory and allergic response). In addition, the suppression of histamine release from mast cells was obtained in a concentration-related fashion [105].

ROS-mediated oxidative damage has been linked to chronic inflammation and compromise of wound healing; hence, targeting and modulating the systems responsible for aberrant inflammation is emerging as a promising strategy to promote skin repair [106,107]. Unlocking the mechanisms of the antioxidant and radical scavenging activity of honey could possibly allow the full potential for honey application to be leveraged in wound medicine. Alvarez-Suarez reported a statistically improved wound closure rate by Manuka honey pre-treatment through enhanced human dermal fibroblast viability and migration. MH also showed a significant protective effect on an oxidative stress model and reduced ROS generation than control and artificial honey. This might be due to augmented 5'-AMP-activated protein kinase (AMPK) phosphorylation [52]. The activation of AMPK ultimately results in the upregulated expression of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, and glutathione s-transferase, as also observed by Gasparrini and co-workers [108]. When assessed in a macrophagic LPS-induced inflammation model, Manuka honey has shown to modulate a series of crucial peptides (caspase 3, p38, pErk1/2, AMPK, SIRT1, and PGC1 α). As a result, dose-dependent downregulation of apoptosis and augmented mitochondrial metabolism were observed, with cellular proliferation and migration [109], confirming again Manuka honey's ability to promote wound closure. Again, however, it should be noted that the basis for this observed effect was not explored in this study.

As with the antimicrobial properties, a greater degree of attention is reserved for the Manuka variety rather than other honeys in regard to their antioxidant benefits. Nonetheless, Sardinian strawberry tree (*Arbutus unedo*) honey (STH), also described as "bitter honey", is also appreciated for its exceptional phenolic content and antioxidant properties [110], and it was shown to exert targeted cytotoxic performance at lower doses than Manuka honey (MH) on colon cancerous and metastatic cells, with enhanced ROS production [50]. For both STH and MH, a high significant correlation was observed between polyphenols and flavonoids contents, and there was also a strong relationship between these values and the total antioxidant capacity of honey, suggesting how the virtuous health properties can be ascribed to the notable phytochemical composition. In particular, STH from the Berchidda area had the highest value of total polyphenols of all the samples (1.00 ± 0.02 g GAE/Kg), including MH (0.89 ± 0.01 g GAE/Kg), and its total flavonoids content was also significantly higher (108.20 ± 2.69 g CAE/Kg) than the MH value (71.90 ± 0.03 g CAE/Kg). These results are in line with the values reported by Di Petrillo et al. (969.7 ± 8.5 mg GAE/kg) [54]. The same bitter honey was further analysed for its anti-proliferative effect, and the biological mechanisms inducing its protective and antineoplastic features were thoroughly disclosed by Afrin et al. [55,111]. While a full discussion on the findings of such an in-depth investigation is beyond the scope of this review, whose focus is on the honey benefits on skin, it is worth attention that the expression of honey's health-giving properties is modulated on the biological target. Cell migration was in fact significantly suppressed by STH pre-treatment in cancerous cells, as confirmed by the lowered expression of invasion factors such as MMP-2 and MMP-9, where it indicates the honey capacity to inhibit the invasive potential of metastatic cells. Conversely, STH promoted the migration of non-neoplastic control cells in a wound-healing model, which is

a promising result for skin repair applications. Likewise, oxidative stress would provoke biological aberration in healthy cells, and it is regulated in physiological conditions by compounds acting as free-radical scavengers. However, ROS expression is significantly augmented by STH in a dose-proportional fashion, and the activity of antioxidant cellular enzymes is down-regulated in colorectal cancer cells: whilst this might seem paradoxical for a well-known antioxidant product, the pro-oxidative environment is intended to induce apoptosis and reduce malignant cells proliferation [111]. This duality explains the broad applicability of honey for health issues characterised by distinctive pathogenesis.

When discussing and researching the positive attributes of a medical use agent, as for any other bioactive compounds, it is imperative to keep in mind the aphorism *Sola dosis facit venenum*; i.e., the dose makes the poison. This means that different concentrations of the therapeutic agent need to be evaluated to define the cut-off dose above which the side effects are observed. Neutrophils play a pivotal role in regulating the inflammatory response to an injury by the modulation of signal cascades in fibrosis and wound healing [112]. Therefore, it is of interest to quantify the effect of honey culturing and specifically the cytotoxic dose (quantified as above 3–5%) on these cells when honey is used in wound care [79]. In the same *in vitro* study, despite the initial promotion of the inflammatory response, a significant reduction in superoxide release was observed at non-cytotoxic doses after 24 h, together with inhibited chemotaxis of neutrophils and I κ B α phosphorylation, indicating a significant overall dose-proportional anti-inflammatory outcome. It is rightfully suggested by Minden-Birkenmaier et al. that a thorough evaluation of the possible release kinetics should be conducted, and that a prolonged release, able to maintain a steady concentration of the active compounds in the area over an extended time frame, might be more advantageous than high concentrations administered with a burst delivery.

6. Latest Advances of Honey Applications in Wound Care

Honey and other bee products have been known for centuries for their beneficial properties on several diseases and have been extensively used in traditional medicine, alone or in association with other therapeutic regimens [5]. The treatment of burn lesions is amongst the numerous honey ethno-pharmacological applications, and they are worthy of mention for their relevance to the aim of this paper. The rationale for this usage has been illustrated in a 2015 Cochrane review that highlighted that the healing time for partial thickness burns medicated with honey bandages is 4 to 5 days shorter than with conventional dressings medication (high-quality evidence). Similarly, an expedited outcome is observed on post-surgical infected wounds when treated with honey rather than with antiseptic rinses plus gauze application, with fewer side effects (moderate-quality evidence) [113]. A 2017 Cochrane review comparing different antiseptics for the treatment of burns stated that burn injuries tend to heal faster with honey treatments than if treated with topical antibiotics (moderate certainty) or non-antibacterial unconventional medicaments (high certainty) [114]. Most of the studies summarised in these review papers were conducted using Manuka honey, but since this review, other honey varieties and bee products have also been investigated and their beneficial effects disclosed. For example, the association of chestnut honey and RJ into an ophthalmic formulation promoted corneal healing of a chemical burn model on rats. This was also confirmed by the significantly increased levels of α 4 β 1-integrin on histological sections after two weeks of treatment [115]. A synergistic effect with natural products of different origin has also been reported for honey promotion of burn healing. For example, mixtures of Euphorbia honey and *A. sativum* L. in different proportions were found to be more effective compared to conventional treatment such as silver sulfadiazine and betadine, functionally reducing the time needed to achieve epithelialisation and burn wound contraction, with no side effects such as allergic reactions [116]. A synergistic effect, as increased inhibition zone *in vitro*, has also been observed for honey combined with antimicrobial drugs, suggesting a possible use of honey as a complementary aid to conventional antibiotics in wound treatment [78]. Furthermore, supplementation of honey with propolis extract has been shown to improve honeys antimicrobial activity

with synergistic effect [117]. An additive effect was instead observed in the scratch assay with a more significant wound closure compared to control. It has been hypothesised that these increased beneficial effects of honey might be explained by the improved antioxidant and anti-inflammatory activity upon propolis extract addition [118]. Another mechanism responsible for the ability to promote wound healing could be the propolis-mediated increased expression of AQP3 and consequent augmented Ca^{2+} , a mechanism also observed in honey [87], as described earlier in this review. These encouraging results show that honey and bee products of different botanical origins can be employed to promote more rapid healing and to effectively treat infections that colonise burns complicating skin recovery. Therefore, it can be concluded from the latest studies that honey application represents a valuable natural approach in the treatment of burns, both alone or in association with alternative or conventional medicaments.

In the field of wound medicine, Diabetic Foot Ulcers (DFU) are currently a critical challenge with an alarming estimated incidence in 15% of diabetic patients, resulting in a dramatic compromise of their quality of life and high mortality [119,120]. Microbial infections represent the main complication, particularly given the insurgence of antibiotic-resistant strains together with impaired wound healing, and the aggravation of symptoms can often result in limb amputation [121]. Due to the severity of the potential consequences, urgent progress in the management of diabetic ulcerations is required, with early intervention and preventative plans of action as cardinal elements in the therapeutical strategy. Clinical use of honey as an alternative option in the treatment of DFUs is described in the medical literature, with the main limitations being the small number of patients involved in the studies and the difficulty of quantifying the dose employed in case of direct honey application, as the quantity is usually determined simply by the need to cover the wound and surrounding skin area [122–127]. Astrada et al. illustrated the case study of a large leg ulceration on a female diabetic patient with metatarsal exposure and concurrent systemic infection, requiring surgical debridement of necrotic and devitalised tissue. After two months of daily propolis-enriched Trigona honey applications, full re-epithelialisation could be observed on the wound site, which on clinical observation was observed to display reduced signs of inflammation [128]. Six patients diagnosed as at risk of limb amputation due to infected DFUs non-respondent to conventional therapies were successfully treated with Manuka honey-containing commercial formulations. The infections, due to bacteria resistant to antibiotics, were managed in 2.6 weeks (average time), and the honey treatments also reduced malodour after just a few days of application. The use of these cost-effective medical grade honey products, enriched with vitamin C and E, induced autolytic debridement and replacement with granulation tissue within 3.5 weeks, thus reducing the healing time [129]. In all the examined cases, honey application induced positive clinical outcome with improved tissue regeneration and successful wound contraction, with no allergic reactions nor maceration of the periwound area. Further studies, possibly randomised clinical trials with significantly more patients recruited, is now needed to corroborate these promising results and support an evidence-based use of honey in the treatment of DFUs.

Honey destined for consumption as a food product contains inactive endospores and other innocuous contaminants, and so it needs to be filtered to remove the impurities [130] or decontaminated by gamma-ray exposure prior to the application on wounds in order to meet the standards for medical use [131]. Likewise, gamma irradiation is employed to sterilise hydrogels designed to treat skin injuries [132,133]. Furthermore, gamma irradiation is an essential step in the gelation of some polymer materials, as it initiates the process of crosslinking [134–136]. However, despite neutralising the total bacterial count on samples, this process of sterilisation does not compromise honey's efficacy against bacteria and biofilms, as demonstrated on fir honeydew honey at 10, 20, and 30 kGy [137]. Nonetheless, this treatment induced a significant dose-related reduction in small peptides such as defensin-1 at radiation doses over 10 kGy, which was probably by conformational alteration and aggregation, whereas higher molecular weight proteins such as GOx were not affected. Despite this report of a reduction in defensin-1, considering that the honey antimicrobial activity is ascribed to a multifactorial mechanism and that the other factors do not seem

to be affected by the sterilisation, honey can be considered a safe and effective alternative option for the management of infected wounds.

Honey incorporation within scaffolds with different designs has been explored as a strategy to potentiate its medical effects on skin injuries, providing a favourable environment to facilitate complete healing across all the skin strata. Typical design considerations include an easy-to-handle structure that is suitable for commercialisation and while also safe for home medication. Pectin, a natural polyuronate, has been used to produce Manuka honey-loaded hydrogels (PHH) to treat excisional wounds in rats, with significant acceleration of wound contraction compared with direct liquid honey application or control (no treatment). It is interesting to note that at the end of the observation time, new hair follicles and organised fibrous tissue with reduced inflammation could be observed not only in the PHH group but also on the pectin blank hydrogel group [132]. This suggests that the polymer itself played an important role in achieving complete healing and indicates the paramount importance of selecting the most suitable material for the designated medical application. The inclusion of stingless bee honey and curcumin within composite nanofibrous membranes improved their therapeutical potential in terms of the radical scavenging ability, compared to direct application of these healthful natural agents on the wound. The polymeric structures were shown to be highly cytocompatible, although in this study by Samraj et al., the honey-loaded membrane did not significantly reduce the wound closure time compared to the conventional treatment (povidone iodine). However, over 95% inhibition of all the bacterial strains tested was obtained upon honey addition to the gelatin membrane, while a maximum of 25% was achieved when only curcumin was loaded into the membrane [138]. Iranian honeys from three different provinces were compared by Mirzaei et al. on a rat burn model, with the thymol-rich Damavand sample showing the greatest antibacterial effect with early onset of signs of wound recovery. Honey incorporation in an alginate scaffold further shortened the healing time (from 16 to 14 days) with remission from infection. Interestingly, in the same study, the analysis of the honey sample from the Ardabil province showed a higher level of sucrose (right below the maximum concentration allowed, above which it is indicative of adulteration) and reduced diastase activity denoting possible excessive heating as compared to the other samples. Ardabil honey's ability to promote wound healing was lower compared to the other samples, suggesting that honey authenticity and quality is a cardinal parameter to be considered for all the therapeutically effective honey varieties to ensure remarkable medical properties [139].

7. Commercially Available Honey-Based Products for Skin Repair

Various honey-based products are available on the market and have been approved for medical use with different indications, such as Actilite[®], Activon Tulle[®], Activon Tube[®], L-Mesitran[™], TheraHoney[®], MediHoney[®], Revamil[®], Principelle IF[™], examples of which are shown in Figure 1. Honey and honey-containing commercial products are currently used in clinical practice for the treatment of wounds of different aetiology. Zeleníková et al. described a statistically significant amelioration in patients aged over 65 with refractory wounds of various nature, with reduced wound size and improved pain relief (measured with the Visual Analog Scale) reported in the intervention group. This group was treated with the commercial honey dressings Actilite[®] (containing a mixture of Manuka honey and Manuka oil, respectively 99% and 1%, as shown in Figure 1) [140] for 90 days, compared to the control group [141]. A commercial ointment with 48% Medical Grade Honey (MGH-L-Mesitran[™] ointment) [142] has been used in monotherapy to treat wounds of different origin (post-surgical and not) in pediatric patients. No discomfort was reported upon application, and minimal scarring was observed [143,144]. However, the antioxidant vitamins C and E, and the other components (calendula officinalis, aloe vera, essential oils, lanolin) also contained in the formulation might have played a role in this positive outcome, given their intrinsic advantageous properties for skin health [145–148]. L-Mesitran[™] Soft (gel containing 40% MGH supplemented with vitamin C and E) has been reported to be effective against clinical isolates of vaginal *Candida albicans* (*C. albicans*), with a minimal

inhibitory concentration (MIC) of 25–50% and a minimal fungicidal effect observed at 50% (MFC). Interestingly, when raw Mexican Yucatan MGH (declared as the same honey used in the commercial product) was directly applied, no fungistatic or fungicidal effect was observed at 40% dilution (maximum concentration tested) [149]. Similar results were also reported against clinical isolates of multi-resistant *C. auris* and other *Candida* species responsible for nosocomial infections. A dose-dependent inhibition was achieved with L-Mesitran™ Soft on all fungal species investigated, while equal honey concentrations (Brasilian blossom honey) not only were shown to be significantly less effective in reducing fungal proliferation but even stimulated the growth of *C. albicans* and *Candida glabrata* (*C. glabrata*). The antifungal effect for the raw medical grade honey was observed instead at a minimum concentration of 40% [150]. These findings suggest again that the other components of the formulations are critical to the successful clinical outcome, possibly potentiating the beneficial properties of honey [151–154]. TheraHoney® impregnated dressings [155] were compared to sustained-release ionic silver hydrophilic dressings in a prospective, double-blind, randomised clinical trial to evaluate the capacity to enhance the healing of neuropathic diabetic foot ulcers and eradicate concurring infections, thus reducing hospitalisation. No statistically significant difference was observed between the two groups, indicating that both treatments are comparably effective and represent valuable options for the management of DFUs [156]. TheraHoney® gel (with medical-grade Manuka honey) has been used in association with conventional antibiotic drugs to treat wounds following cochlear implants in three pediatric patients, with the remarkable promotion of surgical site repair on previously non-healing ulcerations [157]. MediHoney® paste (with Active Leptospermum Manuka Honey) [158] was successfully used as an alternative to conventional mouth rinses to topically treat oral mucositis following chemotherapy treatments in ten pediatric patients, showing enhanced healing observed after 3 days as well as alleviated pain and reduced bleeding within 5 days [159]. Local application of MediHoney® on surgical wounds following the implantation of bone-anchored hearing devices significantly improved the clinical outcome, with reduction of the time necessary to achieve complete healing [160]. These examples of the use of honey-based products in clinical settings, despite the limited number of patients involved and the absence of a control group in the case reports, provide an indication of the increasing acknowledgment of honey-based medical devices as a valuable option to conventional treatments.



Figure 1. Examples of commercially available honey-based dressings for wound healing. (a) Actilite® non-adherent viscose net dressing coated with 99% Manuka honey and 1% Manuka oil. © Image courtesy of Brightwake Ltd. (trading as Advancis Medical); (b) Principelle IF® Honey based bio-active wound care dressing containing medical grade dark buckwheat honey with no synthetic components. © Image courtesy of Principelle B.V.

8. Functional Aspects of Honey-Loaded Scaffolds for Wound Healing

Every health condition to be treated and every specific application of a therapeutic device requires specific features in order to obtain the best possible mitigation of the symptoms or achieve a full recovery. In the design process of a loaded scaffold, an accurate evaluation of the most suitable polymer to be employed depends on the required functions, e.g., the tensile strength for scaffolds destined to areas subjected to intense mechanical stress, the ability to improve cell proliferation for hydrogels for wound healing, and antimicrobial properties for infected wounds. For example, wider inhibition zones on a disc diffusion test were measured for chitosan-based honey hydrogels than for those fabricated with carbopol, indicating a higher antimicrobial activity *in vitro*, when equal honey concentrations were compared. Furthermore, the 75% honey–chitosan hydrogel showed better antibacterial performance than pure honey when evaluated using the disc diffusion method against four common burn-infecting bacteria, and it also induced the fastest burn wound closure among the tested preparations [161]. These results could be attributed to the intrinsic potential of chitosan for biomedical applications [162,163] which seemingly potentiated and improved honey's virtues.

These results could be attributed to the intrinsic potential of chitosan for biomedical applications [162,163], which seemingly potentiated and improved honey's virtues. Honey incorporation into a scaffold and its penetration into the tridimensional polymer network has also been shown to alter the mechanical performance by inducing structural modifications. This might potentially improve some functional attributes and negatively affect others; therefore, the concentration of honey has to be carefully evaluated in order to accurately tailor the scaffold's behaviour based on the specifics of the tissue to be treated. Chestnut honey incorporation on carboxymethyl cellulose (CMC) hydrogels affected the structural strength with reduced capacity to withstand compression load compared to plain CMC gels [134]. This should be kept in mind as it might assume greater importance depending on the location of the wound to be treated to ensure that the hydrogel maintains structural integrity throughout the application time. Interestingly, in a study by Bonifacio et al., Manuka honey was exploited as molecular spacer in gellan gum hydrogels for cartilage implants, with improved compressive moduli and flexibility. Furthermore, these cytocompatible hydrogels induced chondrogenic differentiation with the deposition of cartilage integral constituents such as collagen II, glycosaminoglycans (GAGs), and proteoglycans, which resulted in effective cartilage reconstruction. When tested against MDR *Staphylococci* pathogens typically isolated in post-arthroscopy infected joints, the scaffolds have been shown to significantly reduce the biofilm viability in comparison with controls at each time point [164]. Honey addition (up to 2%) to alginate bioink reduced its viscosity in a dose-dependent way without appreciably affecting the overall rheological features such as extrusion and printability. However, honey significantly enhanced fibroblast viability, proliferation, and adhesion when compared with the plain alginate membrane, with promising applicability to bioprint compatible bioengineered human skin tissues substitutes [165]. These contrasting results indicate that the impact of honey incorporation on the scaffold functionality cannot be assumed and should be evaluated as a matter of course, as the biomedical outcome cannot prescind from an optimised structural and mechanical performance.

Once honey has been incorporated within the scaffold, an in-depth characterisation of the developed formulation should be conducted in order to evaluate some critical parameters to ensure that the treatment effectiveness is optimised, as summarised in Figure 2.

The release rate of the medically active agent is one of these crucial aspects, as it determines important features such as the honey concentration at the wound and periwound area. It can be modulated to prevent the inconvenience of honey leaking from the scaffold, as shown in Figure 3, and consequently reduce the dressing change frequency, with important implications on the patients' adherence to the therapy, as the manipulation may cause discomfort [166]. Honey release is usually determined by an indirect quantification

of either sugars or markers (such as methylglyoxal for Manuka honey) liberated in the medium. The maximum absorbance at specific wavelengths can be measured on a UV-Vis spectrophotometer and plotted against time [138,161]; alternatively, HPLC quantification can be employed to determine the cumulative release [164]. For highly hydrophilic and biodegradable polymeric structures, drug diffusion and scaffold degradation are considered the driving forces of the release process [138,161]. On the other hand, this process of structural degradation can be counterbalanced by an increased degree of crosslinking i.e., chain entanglement, with improved rigidity and structural integrity of the network, hence improved honey retention [167]. For example, a significant reduction in methylglyoxal was observed from Ca^{2+} and Mg^{2+} -crosslinked gellan gum composite hydrogels when compared with the non-crosslinked equivalents [164]. Different experimental protocols are described in the literature for the evaluation of drug release *in vitro*, with the immersion volume being variable across the papers. For honey-alginate bioprinted scaffolds, a volume of 10 mL of phosphate-buffered saline (PBS) was chosen [165]. Samraj et al. carried out the *in vitro* release test by directly submerging the membrane in PBS buffer at a controlled temperature of 37 °C, but the volume of the medium was not specified [138]. Alternatively, for amorphous honey hydrogels, the sample can be inserted into a dialysis bag, as described by El-Kased et al. [161]. A burst release is conventionally detected at the beginning of the observation time, which is followed by a plateau, in hydrogels where the drug loading was performed by the direct mixing method [161,166]. However, a sustained release was observed from gelatine-based electrospun nanofibrous membranes, with $99 \pm 0.5\%$ cumulative honey release only achieved after 24 h of immersion [138]. This shows that different release kinetics can be established by employing alternative tissue engineering strategies, thus allowing for a controlled extended release.

The capacity of a wound dressing to swell and uptake a considerable volume when immersed in an extracellular fluid-resembling medium is indicative of its ability to absorb wound exudate and to maintain a curative moist environment on the affected skin area [168]. As with the evaluation of the drug release kinetics, the volume of the medium employed to assess the water absorption features of the scaffold is highly variable across the published literature. Samraj et al. conducted the swelling study by soaking a 1×1 cm sample in 50 mL of PBS at 37 °C, and a maximum swelling of 500% of the original dry weight was recorded. However, no comparative evaluation was reported between the blank membrane and the drug-loaded ones, so a consideration could not be drawn on the contribution of honey to the swelling capacity of the unloaded gelatin nanofibrous membrane [138]. Azam et al. carried out the swelling test in analogous experimental conditions (PBS, 37 ± 0.5 °C) but did not specify the immersion volume. Increasing honey concentrations affected hydrogel's absorption capacity from $512 \pm 21\%$ for the blank film down to $197 \pm 9\%$ for the 10% (*w/v*) honey-loaded sample [169]. This is in line with the results by Sarhan, which described a fivefold reduction of the percentage of swelling (from 520% to 100%) when honey concentration was increased from 10% to 30% (at a fixed crosslinking level) in honey (H)/polyvinyl alcohol (P)/chitosan (CS) electrospun nanofibers. Honey addition also impacted on the structural features of the fibres, with an increase of almost 100 nm in the mean diameter for any 10% increase in honey concentration [167]. It has been hypothesised that the frequently observed reduction in swelling ability of hydrogels upon honey addition might be ascribed to honey high water solubility, causing a higher rate of degradation when the scaffold is immersed in water [167]. Another explanation might be that honey occupies the polymer's sites for hydrogen bonding, which are otherwise available for interactions with water molecules [169]. Conversely, honey addition to double cross-linked alginate hydrogels led to a significant improvement in fluid uptake capacity up to 700% (measured in 10 mL deionised water), with an optimum observed with the 4% honey structure, which showed pronounced water absorption for 40 h followed by a plateau. This is in line with the controlled degradation kinetics, which involved non-crosslinked residuals first [170]. Analogously, the swelling ratio of honey–silk fibroin scaffolds significantly increased with higher honey concentrations, which was possibly due to honey hygroscopicity generating

an osmotic pressure that directed the medium influx. However, the degradation rate also increased in the fibrous mats with higher honey loading, together with a reduction in compressive strength. These effects on the mechanical features could be explained by the enhanced pore size and porosity of the tridimensional structures upon the incorporation of increasing honey quantities [171]. Similarly, when incorporated as an additive during the electrospinning process of silk fibroin (SF) solutions, Manuka honey has been found to function as an effective hydrophilicity-enhancer, significantly increasing the water retention capacity of the control (pristine silk fibroin scaffolds) at both the honey concentrations tested (1% and 5%), up to a maximum swelling ratio of almost 400% of the initial dry weight [172].

Although a dose-dependency of honey antibacterial and antioxidant properties has been described in the literature [79,109], when it is incorporated into a scaffold, simply increasing its concentration does not necessarily guarantee that the beneficial effects are maximised. For example, among the formulations developed by Rajput et al. for silk fibroin-based skin substitutes (loaded with multifloral honey up to 10% of the final concentration), as imaged in Figure 4, the best pro-healing performance with reduced scar formation was obtained with the 4% honey scaffold.

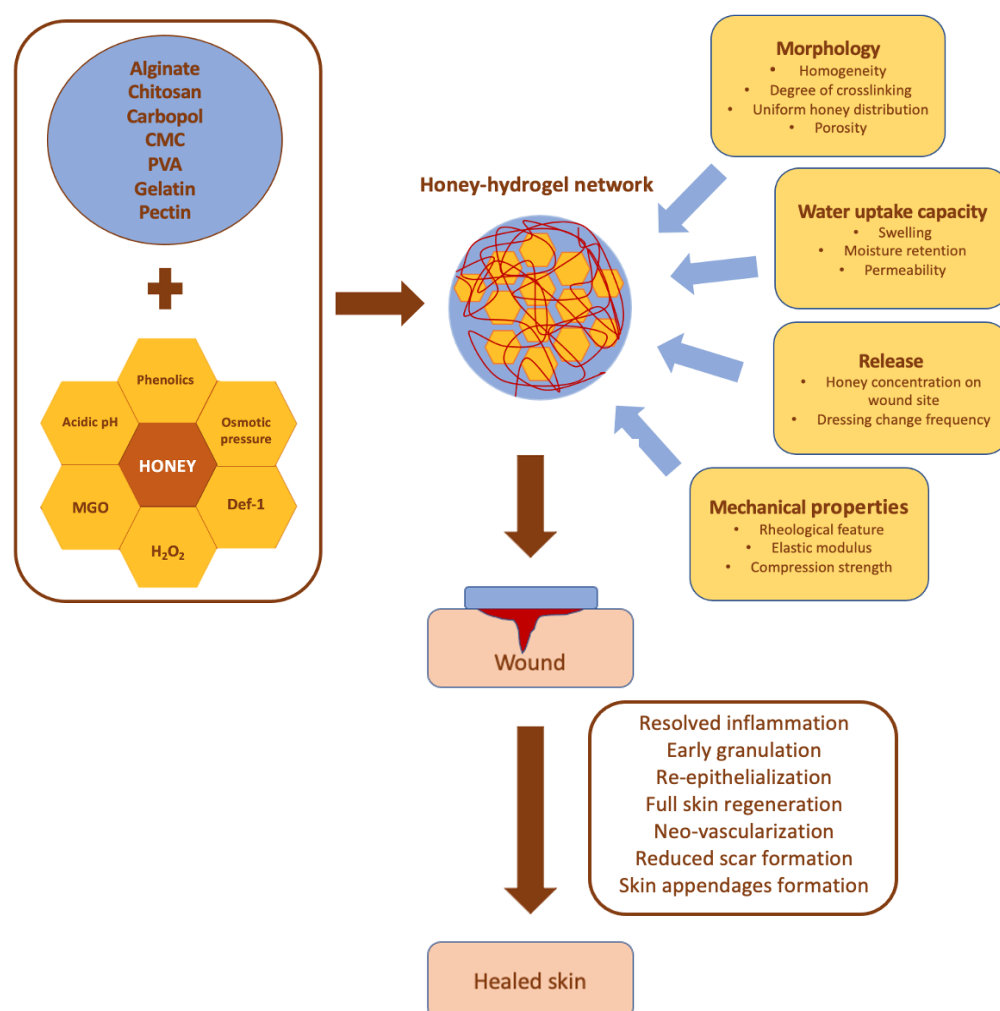


Figure 2. Schematic representation illustrating critical aspects for the optimisation and characterisation of a honey-loaded scaffold for wound repair, with examples of polymers successfully demonstrated in published literature, as discussed in Section 8.

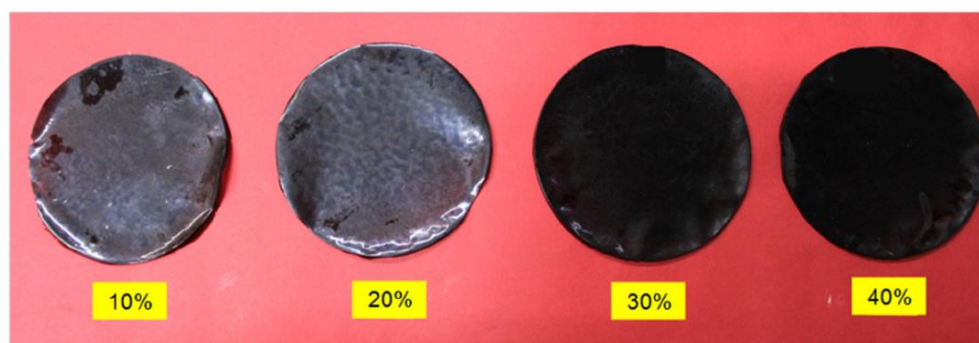


Figure 3. Pictomicrographs of DNG/Ch/MH (dextran/nanosoy/glycerol/chitosan with Manuka honey) dressings at different drug concentrations (10–40%). Reproduced from International Journal of Biological Macromolecules 120 (2018) 1581–1590; Singh et al., Scar-free healing mediated by the release of aloe vera and Manuka honey from dextran bionanocomposite wound dressings (reproduced with permission from [166], Elsevier, 2018).

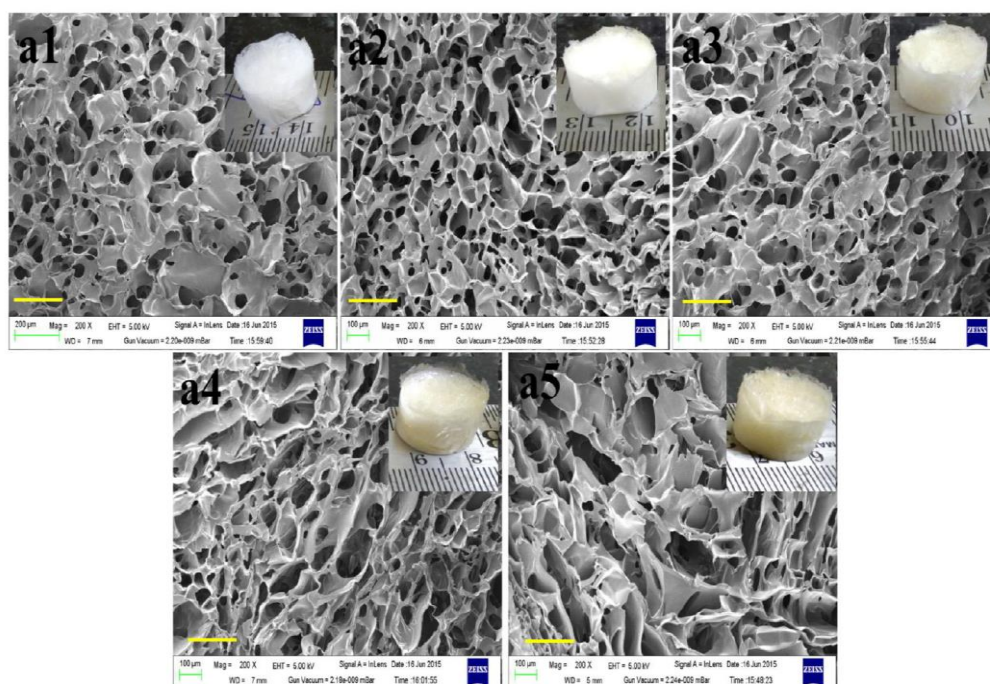


Figure 4. Microphotographs by SEM showing surface morphology of crosslinked silk fibroin (SF) and honey silk fibroin scaffolds (a1) SF, (a2) HSF1 with 1% honey concentration, (a3) HSF2 with 2% honey concentration, (a4) HSF4 with 4% honey concentration, and (a5) HSF6 with 6% honey concentration. Reproduced from Materials, 12 (2020), 100703; Rajput et al.; Honey-loaded silk fibroin 3D porous scaffold facilitates homeostatic full-thickness wound healing (reproduced with permission from [171], Elsevier, 2020).

This sample had an optimal microstructure, which allowed a flux of nutritious substances to the wound site, with enhanced fibroblast proliferation and migration. In particular, a full skin regeneration occurred both at the epidermal and dermal level, with the prevalence of collagen I over collagen III, angiogenesis, and formation of skin appendages such as hair follicles and sebaceous glands after 2 weeks of treatment [171]. Similarly, dual crosslinked alginate hydrogels presented a disarranged crystalline structure for high honey concentrations, while honey incorporation up to 4% ensured a morphology favourable for cellular adhesion, a significantly better bactericidal activity compared to the other honey dilutions, and ultimately skin re-epithelialisation with characteristics indicative of healthy uninjured skin and minimal scar thickness [170]. The successful loading of Manuka

honey into dextran-based dressings was achieved for concentrations up to 20% with a uniform distribution of honey. However, when the honey concentration was above 20%, the dressings were found to be fragile, sticky, and stiff. In addition to these structural disadvantages, a functional drawback was also observed, as the antibacterial activity of the highly concentrated samples was also compromised, in particular against the Gram-negative *E.coli*, as demonstrated by a halved inhibition zone diameter at concentrations beyond 20% [166]. The outcome achieved with a treatment can be affected not only by the intrinsic properties of that given honey variety but also by the protocol design. A factor to take into consideration could be the number of honey applications during the observation time as well as the frequency of the dressing replacements. For example, two daily applications of thyme honey on a rat wound model induced faster healing with a significantly higher fibroblast count and collagen deposition, and early formation of granulation tissue with angiogenesis compared to the control and the single daily application [130].

Lastly, particular attention should be given to the possible interactions between the compound of interest and the hydrogel components, other active agents, and excipients, and how these can affect the pharmacokinetic aspects of the active principle, for example by modulation of its release. For instance, it is worth mentioning that the incorporation of polyphenols within a scaffold or delivery system might restrict their antioxidant potential, as shown by a diminished responsiveness to radical scavenging tests such as ABTS and DPPH of different quercetin–hydrogel formulations compared to the free flavanol [173]. In addition, no statistical difference in antibacterial potency was observed between Manuka honey samples with different UMF when these were loaded into scaffolds for medical engineering, despite a significantly different bacterial inhibition being obtained with direct application of the same honeys [92].

9. Conclusions

Honey has been shown to be much more than a simple food product, but rather a valuable medical product with multiple mechanisms and beneficial virtues. The varying antimicrobial, antioxidant, and anti-inflammatory properties of honey are responsible for the diverse and broad range of varieties of applications of honey being investigated as powerful topical treatments for healing and wound repair. The phenolic compounds intrinsic to honey's composition are recognised as significant contributors to its widely investigated antioxidant properties. Critically however, whilst these phenolic compounds have been shown to modulate the antimicrobial properties of honey against common wound infections, the full breadth of determinant factors for honey antimicrobial activity is yet to be unequivocally agreed on, with peroxide-mediated mechanisms also suggested as a plausible mechanism. This complexity prompts the need for further research to explore the likely multifaceted, potentially synergistic components of honey's medicinal properties. In addition to its antibacterial properties, the anti-inflammatory properties of honey were also reviewed, and again, multiple potential mechanisms to account for this activity have been proposed. However, regardless of the ambiguities surrounding the underlying mechanical mechanisms, the evidence for the net positive impacts of honey as a component within topical wound treatments is becoming more broadly established in the literature. Whilst Manuka honey is the most famous honey to be utilised in healing, as explored in this review, a number of other honey varieties are also being shown as effective in this regard. In particular, the use of honeys in the treatment of burn wounds have been robustly demonstrated to improve treatment outcomes in terms of speed of healing, skin regeneration, and reduction of scarring.

One of the most exciting aspects currently emerging in the field of honey-enhanced wound healing involves the incorporation of honey within an increasing variety of scaffolds to potentiate its medical effects on wound healing. In this second part of this review, the design considerations of effective honey-loaded scaffolds were explored. Here, too, it is important to recognise that the impact of honey incorporation on the scaffold functionality cannot be assumed, with differing configurations resulting in contrasting results. Partic-

ularly exciting is the potential for the controlled release of honey by optimising scaffold designs. Therefore, it can be concluded that advanced honey wound dressings obtained by the incorporation of honey into polymeric or fibrous structures represent a promising novel option for skin regeneration and enhanced wound healing. From the evidence collected and described here, it can be seen that the advantageous properties of honey-loaded devices on skin medicine depend on the careful optimisation of numerous factors in order to offer a natural product with up-to-standard performance, if not improved, when compared with the conventional products.

In addition to research to elucidate the underlying mechanisms driving honey's medicinal properties, and the exploration of advanced design scaffolds for honey wound dressings, systematic studies with greater numbers of patients are now needed to substantiate the current evidence and further investigate the beneficial effects of honey in skin and wound healing. Bringing these key aspects together could enable medicine to fully harness the significant potential of honey to improve therapeutic wound outcomes, so that sufferers of complex wounds, including chronic wounds, infected wounds, and burn wounds can realise significantly more positive outcomes in the future.

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