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The therapeutic potential of cannabinoids for integumentary wound management

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Abstract

The increasing legalization of *Cannabis* for recreational and medicinal purposes in the United States has spurred renewed interest in the therapeutic potential of cannabinoids (CBs) for human disease. The skin has its own endocannabinoid system (eCS) which is a key regulator of various homeostatic processes, including those necessary for normal physiologic wound healing. Data on the use of CBs for wound healing are scarce. Compelling pre-clinical evidence supporting the therapeutic potential of CBs to improve wound healing by modulating key molecular pathways is herein reviewed. These findings merit further exploration in basic science, translational and clinical studies.

KEYWORDS

alternative medicine, endocannabinoid, inflammation, keratinocytes, microenvironment

1 | INTRODUCTION

The medicinal properties of the *Cannabis* plant have been known for millennia. The first known writings referencing the use of *Cannabis* to treat disease date back to Emperor Shen Nung, the father of Chinese medicine, in 2700 BCE.[1] In the modern age, CBs have found utility in the treatment of various medical conditions including glaucoma,[2] epilepsy,[3] cancer,[4] multiple sclerosis[5] and others. The use of CBs to treat skin disorders including psoriasis, atopic dermatitis and hair growth disorders continues to be explored.[6–9] The cutaneous endocannabinoid system (ECS) is well-characterized as a key player in the maintenance of skin homeostasis via regulation of epidermal differentiation, barrier formation and more.[6] Scant literature exists on the potential applications of CBs for the treatment of skin wounds, which remain a significant international threat to public health and the economy. Medicare spending on wounds is estimated conservatively at an annual US $ 30 billion.[10] Meanwhile, chronic or non-healing wounds have associated mortality rates exceeding many common cancers.[11] Additionally, there is a demand to speed the healing of acute wounds (eg surgical wounds) and to achieve more desirable cosmetic outcomes, that is, reduced or eliminated scarring. As such, the need to add to the clinician's arsenal of weapons in the war against wounds cannot be overstated. Herein, we present a review of the literature surrounding the therapeutic potential of CBs for the treatment of human skin wounds. The goal of this article is to summarize relevant pre-clinical data which demonstrates the ability of CBs to modulate the cellular and molecular pathways known to be involved in wound healing, thus highlighting areas for further basic science, translational and clinical research.

2 | THE CUTANEOUS ENDOCANNABINOID SYSTEM

The skin acts as a barrier against the environment and its accompanying insults. Research shows that the skin is characterized by complex innate neuro-immuno-endocrine function, in which the cutaneous ECS plays a key role.[12] Maccarone and team were the first

to demonstrate that the skin has its own ECS, namely the machinery necessary to synthesize, breakdown and respond to cannabinoid compounds.[13]

The ECS is comprised of: endogenous cannabinoids (eCBs) such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG); the "classic" G-protein-coupled CB receptors (CB1R and CB2R); and enzymes for biosynthesis, transport and metabolism.[6] The CB1R is mainly expressed in the central nervous system, where it is responsible for the psychoactive effects of the *Cannabis* plant, and to a lesser extent in many peripheral tissues such as the heart and lung.[14] The CB2R is the principle cannabinoid receptor of the immune system, therein expressed 10–100 fold greater than the CB1R, where its activation leads to suppression of the immune response.[15] Expression of CB1R and CB2R has been noted in a wide variety of skin cells and appendages including keratinocytes, melanocytes, fibroblasts, sebaceous cells and various cell lineages of the hair follicle.[8,16]

Phytocannabinoids (pCBs), the classical cannabis plant-derived compounds, number in the hundreds and also act upon the ECS.[8] The most prominent pCBs include tetrahydrocannabinol (THC), the main psychoactive compound in cannabis, and cannabidiol (CBD), the main non-psychoactive pCB.[8] Other pCBs found abundantly in the *Cannabis* plant include cannabigerol (CBG), cannabichromene (CBC), cannabivarin (CBV) and cannabidivarin (CBDV).[17] Phytocannbinoids are phenolic terpenes which are synthesized in acid form (eg CBDA) in the *Cannabis* plant—their pharmacologic effects are attributed to their neutral, decarboxylated products (eg CBD).[18] Synthetic cannabinoids have also been engineered, such as arachidnoyl-2-chloroethylamide (ACEA), a selective CB1 agonist, and GP1a, a selective CB2 agonist, among many others.[19,20] Synthetic cannabinoids may be phenolic or indolic and can thus have structures quite different from pCBs.[18]

Cannabinoids, particularly pCBs, often exert their effects through "non-classic" cannabinoid receptors such as the vanilloid-type transient receptor potential channels(TRPVs). Along these lines, CBG has been demonstrated to activate α-2 adrenoceptors and moderately antagonise serotonin receptor $5HT_{1A}$. Interestingly, CBs have the capacity to bind to intracellular receptors such as the nuclear receptor transcription factor protein peroxisome proliferator-activated receptor gamma (PPARγ).[8] This highlights the potential for epigenetic mechanisms of action for CBs; indeed, CBs are known to affect keratin gene expression via DNA methylation.[21] In fact, epigenetic regulatory mechanisms make at least some contribution to most of the major effects of CBs[22] and such mechanisms have in recent years gained much

TABLE 1 Select cannabinoids and their major targets

Class	Compound	Receptor activity
Endogenous cannabinoids (eCBs)	Anandamide (AEA)	CB1R (Partial)[84] TRPV1/4[84,85] GPR55[85]
	2-Arachidonoylglycerol (2-AG)	CB1R[84] CB2R[84] GPR55[85]
Phytocannabinoids (pCBs)	Tetrahydrocannabinol (THC)	CB1R[85] CB2R[85] GPR55[85] PPARγ[85]
	Cannabidiol (CBD)	CB1/2R: Non-specific antagonist (Varied, context-dependent)[7]
	Cannabigerol (CBG)	CB1/2R[7] TRPV1[7] TRPA1[7]
	Cannabinol (CBN)	CB1R (Weak)[7] CB2R (Partial)[7]
	Cannabichromene (CBC)	TRPA1[7] AEA Reuptake Inhibitor[7]
	Tetrahydrocannabivarin (THCV)	Antagonizes THC (low doses) [7] CB1R (high doses)[7]
Synthetic cannabinoids	Arachidonyl-2'-chloroethylamide (ACEA)	CB1R (Antagonist)[33]
	GP1a	CB2R[32]
	JWH133	CB2R[39]
	AM630	CB2R (Antagonist)[20]
	JWH105	CB2R[62] CB1R (Weak)[62]

Note: Receptor activities of cannabinoids discussed in this review. Many other cannabinoids exist. Receptor activities are agonism unless otherwise specified. Major receptor binding activities are highlighted; others exist.

attention as significant drivers of the wound healing process.[23] The CBs discussed herein and their major ligands are outlined in Table 1.

Endocannabinoid signalling is markedly complex. Some studies demonstrate conflicting receptor activities for certain CBs; for example, one study found CBG to have low affinity for CB1 and CB2 receptors,[17] while another found CBG to be a partial agonist of CB2 as well as active at CB1, the latter due to an unknown underlying molecular mechanism.[18] CBs may act indirectly on ECS tone, such as in the case of CBG and CBC which both inhibit cellular AEA uptake. CBs can even exert opposite effects on the same receptor.[9] For example, CBD alone is not particularly active at the classic CB receptors, however may antagonize CB1 in the presence of CB1 agonists (eg THC), or may context-dependently activate CB1 through the inhibition of breakdown enzymes and resulting passive increase in CB1 tone.[7,9,24] CB receptors have also been noted to exhibit biased agonism, whereby their choice of intracellular effector pathway differs from agonist to agonist.[9] CB1 and CB2 have also been observed to form heteromers, which affects their ligand affinity.[18] The ECS is further modulated by intricate biochemical phenomena including signalling bias, agonist-induced receptor downregulation and the regulatory effects of the membrane lipid microenvironment.[9] Thus, it is difficult to precisely determine the cellular effects of CBs and their receptors, and caution is necessary when extrapolating results from *in vitro* and animal studies.[9] Nonetheless, pre-clinical data suggest that cannabinoids may have therapeutic potential in the treatment of patients with skin wounds, both chronic and acute.

3 | CHRONIC WOUNDS

Normal physiologic wound healing occurs in a series of distinct yet overlapping phases: inflammation, proliferation and maturation.[25] Although wound closure is often used colloquially to refer to wound healing, they are two separate processes; wound closure is achieved when re-epithelialization is complete, either by primary (eg as with sutures) or secondary (via keratinocyte migration) intention, but wound healing is not technically complete until maturation and remodelling have occurred. A chronic wound is defined as any wound which fails to proceed in an orderly manner through the regular physiologic stages of healing.[25] Venous leg ulcers (VLUs), diabetic foot ulcers (DFUs) and pressure ulcers (PUs) are the most commonly considered, but any type of wound may be thus characterized (eg surgical wounds, burns). Such wounds suffer from a lack of consistent standards for diagnosis and prognostication,[26] and they are frequently recalcitrant to standard-of-care therapeutic strategies; data from the US Wound Registry suggest that healing rates exceeding 40% are not likely to be possible for chronic wounds today.[27] As such the need to expand the clinician's arsenal of tools to combat wounds is tremendous, and research suggests that CBs may have utility in this domain through modulation of keratinocyte phenotype and various components of inflammatory microenvironment.

3.1 | Keratinocyte phenotype modulation

Keratins are intermediate filament proteins found in epithelial cells which are instrumental for differentiation, migration and proliferation.[28] Keratin expression varies based on keratinocyte phenotype; for example, basal cells of the epidermis express K5 and K14 while corneocytes express differentiation-specific keratins 1 and 10.[28] In acute wounding, keratinocytes which normally differentiate to form rigid structures must adopt a flexible morphology conducive to migration and wound closure.[29] Accordingly, upon injury, keratinocytes at the wound edge downregulate K1/K10 and instead express keratins 6, 16 and 17—so-called "inducible" (aka activated or proliferative) keratins.[28] These activated keratinocytes migrate to form a monolayer over the exposed dermis, after which they revert back to their basal (K5/K14) or suprabasal (K1/K10) phenotypes.[30] Overexpression of inducible keratins K16 and K17 has been correlated with hyperproliferative skin diseases such as psoriasis and squamous cell carcinoma.[28] Conversely, K17 knockout mice exhibit markedly delayed wound closure while K17-deficient keratinocytes exhibit impaired protein synthesis and cell growth.[28]

Keratinocytes at the non-healing edge of chronic wounds demonstrate a sustained proliferative phenotype which precludes wound closure.[30] For example, the wound edge of venous leg ulcers is characterized by a hyperproliferative epidermis with hyperkeratosis and parakeratosis, with keratinocytes exhibiting persistent upregulation of proliferative markers (K6/16/17) and downregulation of differentiation markers (K1/10, filaggrin).[31] These cells fail to properly execute either the differentiation or activation pathways, do not migrate across the wound bed and thus wound closure cannot be achieved.[30,31] Similar observations have been made in diabetic foot ulcers.[30]

The observation of these dysregulated skin cells at the wound edge lends additional credence to the use of sharp debridement as a foundational aspect of wound care, in this case by removing the cells with functional impairment in molecular pathways.[31] However, many wounds fail to close despite combining debridement with other standards of care and even advanced therapies. Pre-clinical data demonstrate that the endocannabinoid system plays a role in modulating keratinocyte phenotype and keratin expression which may be beneficial in the treatment of chronic wounds.

Although there is evidence that cannabinoid receptors, both classic and non-classic, have a role in keratinocyte proliferation, some findings are contradictory. When Macaronne and team first characterized the endocannabinoid system of the skin, they noted that AEA downregulated the expression of K1 and K10 in HaCaT cells (a spontaneously transformed aneuploid immortal keratinocyte cell

line) via a CB1R-dependent mechanism.[13] These findings were contradicted by later studies demonstrating a pro-differentiative effect of CB1R agonism; HaCaT cells treated with the CB1-specific agonist arachidonylcyclopropamide (APA) were found to exhibit an increase in K1 and K10, and CB1R-knockout mice exhibited decreased markers of epidermal differentiation.[32] This may be explained by the fact that AEA is also known to bind to several non-classic CB receptors (TRPV1, PPARγ, GPR55).[32]

The aforementioned CB1R-knockout mice exhibited, in addition to decreased markers of epidermal differentiation, impaired epidermal barrier recovery and increased keratinocyte proliferation. These findings were reversed in CB2R-knockout mice, and can be extrapolated to suggest that CB1R activation inhibits proliferation and promotes differentiation, while CB2R activation does the opposite.[32] To further support an anti-proliferative effect of CB1 agonism, treatment of organ-cultured human skin with the specific CB1 agonist arachidonoyl-chloro-ethanolamide (ACEA) inhibited keratinocyte proliferation and decreased the expression of proliferative keratins K6 and K16.[33] Thus whether CB1R agonism results in increased or decreased keratinocyte differentiation has yet to be fully explored, but the evidence appears to favour a pro-differentiative and anti-proliferative effect profile. Later studies on wounded mice confirmed an anti-differentiative effect of selective CB2 agonism and demonstrated decreased proliferation due to selective CB2 antagonism, although no effect of CB2 agonism on proliferation was noted.[20]

Phytocannabinoids may have utility in modulating keratinocyte phenotype. Wilkinson and team found that proliferation of HPV16 e6/e7 transformed hyperproliferative keratinocytes was inhibited by the pCBs THC, CBD, CBN and CBG.[24] Cannabidiol and CBG exhibited the greatest overall activity, with CBD boasting the highest potency.[24] Furthermore, the anti-proliferative effects of these compounds were not abrogated by the addition of CB receptor-specific antagonists, suggesting a non-classic CB receptor-based mechanism.[24] Neither CBD nor CBG is very active at CB receptors which further supports this hypothesis.[24] On the other hand, topical CBD was observed to induce keratinocyte proliferation and increase levels of K16/K17 in the regular skin of healthy mice.[34] These seemingly contradictory findings highlight the complex and contextual mode of action of CBs as discussed previously.

Given the body of evidence, we suggest that topical CBD may ameliorate keratinocyte dysregulation at the edge of non-healing wounds by inhibiting overactive keratinocyte proliferation. The effects of CB1R agonism on differentiation are unclear, but it appears to lend an anti-proliferative effect which would also be useful to treat the hyperproliferative wound edge. CB2R antagonism may also be anti-proliferative. It is worth considering that, since wound edge keratinocytes are trapped in a mixed phenotypic "limbo," tipping them over the edge into either committed proliferation or differentiation would be therapeutically valuable. Thus, the fact that cannabinoids have demonstrated their role in modulating this process merits their continued exploration as agents to improve wound healing, preferably in human skin.

3.2 | Inflammatory dysregulation

Some degree of inflammation is necessary for regular wound healing. However, chronic wounds are characterized by a prolonged, inappropriate, self-perpetuating inflammatory state which impairs healing.[35] In such wounds, the complex and intricate balance between cytokines, chemokines, proteases and their inhibitors has fallen into disequilibrium.[35] CBs generally exert anti-inflammatory effects although the associated underlying mechanisms are not well-characterized.[36] Nonetheless, CBs have demonstrated the ability to modulate several pathways involved in the deleterious inflammatory microenvironment of chronic wounds, including pro-inflammatory cytokine balance, macrophage tone and matrix metalloprotease (MMP) activity.

In chronic wounds there exists a relative abundance of pro-inflammatory cytokines, chiefly TNFα and IL-1β, and a resulting persistence of inflammatory cells (chiefly macrophages and neutrophils).[37] In a murine wound model, dermal injection of GP1a (a selective CB2R agonist) resulted in significantly decreased infiltration by neutrophils and macrophages, as well as decreased levels of IL-1, IL-6, TNFα and TGFβ.[20] Similarly, treatment of HACAT cells with *cannabis sativa* extract (CSE) led to a decrease in TNFα-mediated release of IL-8.[36] Chronic wounds also demonstrate increased M1 (inflammatory) macrophage tone with decreased M2 (anti-inflammatory) macrophage activity.[38] In a murine wound model, intraperitoneal injection of selective CB2R agonists led to a significant decreased in M1 tone with only a slight increase in M2 tone.[39] Systemic administration of GP1a resulted in similar findings in a mouse model of traumatic brain injury.[40] Thus it seems that selective CB2 agonism has the potential to ameliorate multiple dysregulated inflammatory pathways in chronic wounds, and this potential merits further exploration in studies solidifying this relationship and establishing a link to healing outcomes.

Matrix metalloproteases are a family of zinc-dependent endopeptidases necessary for various components of repair including extracellular matrix (ECM) remodelling, cellular migration and angiogenesis.[41] An imbalance between MMPs and their inhibitors (TIMPs; tissue inhibitors of metalloproteases) is a well-known contributor to the pathophysiology of chronic wounds; an excess of MMPs and/or deficiency of TIMPs results in tissue breakdown in excess of tissue production, as well as destruction of endogenous growth factors, and subsequently impaired healing.[42] Overexpression of many MMPs, most commonly MMP-2 and MMP-9, has been observed in chronic wounds.[41–43] To this end, HaCaT cells treated with either CSE or CBD exhibited decreased production of MMP-9.[36] Intraperitoneal injection of ACEA (selective CB1R-agonist) in a murine spinal cord injury model produced significantly reduced levels of spinal MMP-2 and MMP-9.[19] Furthermore, THC has been observed to inhibit cancer cell invasion in mice by inhibiting MMP-2,[44] and *in vitro* through upregulation of TIMP-1.[45] Finally, CB2 agonism decreased expression of MMP-1 and MMP-3 in ocular human Tenon's fibroblasts (HTFs).[46] The effects of CBs on MMP profiles specifically in wounds, and

the subsequent relationship to wound healing outcomes, merit further exploration.

The effects of CBs on MMPs may be beneficial to improve healing outcomes in patients with diabetic foot ulcers (DFUs) undergoing dermal grafting with synthetic skin substitutes. In a study of 35 patients with DFUs, elevated protease levels (EPA) in wound effluent prior to the procedure were found to be the only significant negative predictor of graft integration.[47] Thus, the MMP-modulating capabilities of CBs may be able to improve graft integration in this setting. Additionally, THC has been observed to attenuate the allograft response and delay skin graft rejection via CB1-dependent activation of myeloid suppressor cells in mice.[48] Furthermore, CB2 activation inhibits the *in vitro* mixed lymphocyte reaction, a surrogate assay for graft rejection.[49]

3.3 | Nitric oxide

Nitric oxide (NO) plays a key role in various processes necessary for normal tissue repair, including angiogenesis, granulation tissue formation, keratinocyte migration, collagen production and the activation and upregulation of growth factors.[50] NO also exhibits antibacterial and antibiofilm activity via generation of peroxynitrite ($^{-}OONO$) through the superoxide (O^{2-}) pathway.[51,52] Nitric oxide synthase is responsible for the synthesis of NO from L-arginine and exists in multiple isoforms: endothelial (eNOS) in vascular endothelial cells, neuronal (nNOS) in neurons and inducible (iNOS) in many cell types of the skin and immune system (keratinocytes, fibroblasts, vascular endothelial cells, macrophages).[50] iNOS is upregulated by pathogens, IFNα and TNF in acute inflammatory processes such as wounding (inflammatory phase) and infection.[50] This produces a high concentration of NO which is pro-inflammatory, upregulating MMPs and modulating leucocyte recruitment.[52] On the other hand, eNOS and nNOS are constitutively expressed, generating low levels of NO which are associated with an anti-inflammatory effect crucial for the proliferation and maturation phases of normal wound healing.[52]

Abnormal NO production is a known contributor to impaired healing in chronic wounds, especially diabetic wounds. Macrophages, keratinocytes and fibroblasts in diabetic wounds exhibit decreased iNOS expression, resulting in lower numbers of macrophages and a subsequent decrease in pathogen clearance. Furthermore, impaired NO production inhibits keratinocyte proliferation and differentiation, thus hindering re-epithelization. Finally, fibroblasts in chronic wounds express lower levels of iNOS and eNOS with resulting senescence and impaired ECM formation. Indeed, diabetic wound fluid has been observed to carry lower levels of NO than healthy wounds.[50] This relationship has been further demonstrated in murine studies of NOS deficiency [53] and NOS-inhibiting drugs.[50] A study of 16 patients with chronic leg ulcers found significantly greater healing rates in wounds with higher iNOS expression.[54]

Cannabinoids have the ability to modulate nitrergic signalling. The effects of CBs on such can be stimulatory or inhibitory depending on the type of cell or tissue involved.[55] Phytocannabinoids in general inhibit iNOS-dependent NO formation, in line with their well-known anti-inflammatory effects. For example, THCV inhibited LPS-induced iNOS-dependent NO production in murine peritoneal macrophages via CB2 agonism.[56] Cannabichromene, which is not particularly active at the classic CB receptors, and THC exerted similar effects via unclear mechanisms with the former being possibly related to TRPA1 activation.[57] CBG also reduced iNOS expression and NO production in murine gut macrophages via CB2 agonism in a mouse model of inflammatory bowel disease (IBD).[58]

These results would appear to suggest a deleterious effect for pCBs on healing of chronic wounds, at least as far as nitregic signalling is confirmed, as we have thus far discussed that iNOS deficiency is associated with impaired wound healing while pCBs reduce iNOS expression in inflammatory cells. On the other hand, excessive NO is associated with overexuberant inflammation and resulting tissue damage; for example, excess NO from iNOS overexpression may contribute to excess collagen formation in keloids.[50,52] Thus NO can be a beneficial messenger or a toxic intermediate,[55] and a deficiency or excess of NO can be deleterious depending on the cellular context.[55] It would certainly be logical to hypothesize that since iNOS is associated with a high-inflammation state, as are the beds of chronic wounds, that inhibiting iNOS would help improve chronic wound healing. All this is to say that further studies examining the effects of CBs on nitrergic signalling and the subsequent link to wound healing outcomes are necessary to truly coalesce the nature of this relationship.

4 | ACUTE WOUNDS

There is a theory that wound healing in mammals evolved for rapid healing under dirty conditions, and that the trade-off for this is scarring.[20] CBs have been observed to regulate various parameters involved in acute wound healing, including fibrosis and re-epithelialization. This has the potential to improve the healing speed of acute wounds and, as others have pointed out, may be beneficial in the quest to achieve scar-less wound healing.[9]

Although deposition of collagen other ECM by fibroblasts is necessary for healing, this phenomenon also leads to fibrosis and scarring[59] The anti-fibrotic effects of CB2R activation are well-documented *in vitro* and in mouse models. Treatment with CB2-selective agonist JWH133 resulted in decreased fibrosis in a mouse model of bleomycin-induced lung fibrosis, characterized by decreased fibroblast proliferation, migration, and expression of type I collagen and alpha-smooth muscle actin (α-SMA).[60] Dermal injection of GP1a (a selective CB2R agonist) has been observed to decrease fibroblast infiltration, collagen deposition and TGF-β in murine excisional wound models.[20,61] Treatment with AM630, a selective CB2R antagonist, reversed these effects.[61] CB2 agonism has also been found to inhibit TGF-β1-induced ECM deposition in HTFs *in vitro* and was thus posited to have a potential protective effect on corneal scarring.[46]

CB2 activation may also accelerate wound closure, as GP1a increases re-epithelialization speed in wounded mice.[20] Treatment

TABLE 2 Wound healing molecular pathways modulated by cannabinoids

Molecular pathway of interest	Receptor action	Effect	Ligand	Model	Potential utility for wounds
Keratinocyte phenotype	CB1 Agonism	Anti-proliferative	None	CB1R KO mice [32]	Chronic wound edge
			ACEA	Ex vivo human skin[33]	
		Anti-differentiative	AEA	HaCaT[13]	~
		Pro-differentiative	APA	HaCaT[32]	
			None	CB1R KO Mice[32]	
	CB2 Agonism	Pro-proliferative	None	CB2R KO Mice[32]	~
		Anti-differentiative	None	CB2R KO Mice[32]	
		Anti-differentiative	GP1a	Murine excisional wound[20]	
	CB2 Antagonism	Anti-proliferative	AM630	Murine excisional wound[20]	Chronic wound edge
	Varied	Anti-proliferative	CBD/CBG/CBN/ THC	HPV transformed keratinocytes[24]	Chronic wound edge
		Pro-proliferative	CBD	Murine healthy skin[34]	
MMPs	CB1 Agonism	Decreased MMP-9	ACEA	Murine spinal cord injury[19]	Chronic wound bed
	CB2 Agonism	Decreased MMP-1/3	HU308 JWH133	Human tenon's fibroblasts *in vitro*[46]	Chronic wound bed
	CB1/CB2 Agonism	Decreased MMP-2	THC	Murine glioma[44]	Chronic wound bed
		Increased TIMP-1	THC	*In vitro* human cervical cancer cells[45]	
	Varied	Decreased TNFα-mediated MMP-9	CSE	HaCaT[36]	Chronic wound bed
		Decreased MMP-9	CBD	HaCaT[36]	
Inflammatory cytokines	CB2 Agonism	Decreased IL-1, IL-6, TNFα, TGFβ	GP1a	Murine excisional wound[20]	Chronic wound bed
	Varied	Decreased IL-8	CSE	HaCaT[36]	Chronic wound bed
Macrophage phenotype	CB2 Agonism	Decreased M1 Increased M2	GP1a	Murine traumatic brain injury[40]	Chronic wound bed
		Decreased M1 Slight increase M2	GP1a JWH133	Murine wounds[39]	
Nitric oxide signalling	CB2 Agonism	Reduced iNOS expression Inhibited NO production	CBG	Murine peritoneal macrophages[58]	Chronic wound bed (Conflicting evidence)
			THCV	Murine peritoneal macrophages[56]	
	Uncertain – Possibly TRPA1 Agonism		CBC	Murine peritoneal macrophages[57]	
	Uncertain		THC	Murine peritoneal macrophages	

(Continues)

TABLE 2 (Continued)

Molecular pathway of interest	Receptor action	Effect	Ligand	Model	Potential utility for wounds
Fibrosis	CB2 Agonism	Decreased fibroblast proliferation and migration Decreased Collagen 1 and αSMA	JWH133	Murine bleomycin-induced lung fibrosis [60]	Acute wounds
		Decreased fibroblasts Decreased TGFβ	GP1a	Murine excisional wound[20]	
		Decreased collagen deposition Decreased TGFβ	GP1a	Murine excisional wound[61]	
		Decreased TGFβ-induced ECM deposition	HU308 JWH133	Human tenon's fibroblasts *in vitro*[46]	
	CB1/CB2 Agonism	Increased TGFβ	JWH015	Murine excisional wound	~
Melanocytes/ Pigmentation	CB1 Agonism	Increased melanin content, tyrosinase activity	CBD	Cultured human epidermal melanocytes	Scar dyspigmentation
		Increased melanogenesis	AEA	Cultured human epidermal melanocytes	Scar dyspigmentation
Re-epithelialization	CB2 Agonism	Accelerated re-epithelialization Promoted migration	GP1a	Murine excisional wounds[20]	Acute wounds
	CB1/CB2 Agonism	Faster scratch gap closure	JWH015	Primary cultured human keratinocytes/ Fibroblasts[62]	Acute wounds
Stem cells	CB2 Agonism	Activation of cellular pathways for survival, metabolism, protein synthesis	JWH133	Cultured human bMSCs[72]	Any wound
		Enhanced anti-inflammatory activity	THC	Cultured human bMSCs[73]	Any wound
		Increased number of viable cells	JWH133	Cultured human aMSCs[74]	Any wound
	Varied	Increased number of viable cells	CBD, CBDA, CBGA, THCV	Cultured mouse bMSCs[75]	Any wound

Note: Summary of the effects of cannabinoids on molecular and cellular processes relevant to wound healing.

of cultured human primary keratinocytes with JWH015, a CB2-receptor agonist with weak CB1 activity, induced faster gap closure in a scratch gap assay.[62] Interestingly, JWH015 induced an increased in TGF-β which appears contradictory to the above-mentioned findings of TGF- β reduction via CB2 agonism, though this can likely be explained by the CB1 activity. Indeed, both the pro-migratory effect and upregulation of TGF- β by JWH015 were abrogated by administration of either CB1- or CB2-selective antagonists.[62] Studies from other organ systems also support the potential of CBs to improve acute wound closure. For example, blockade or knockout of CB2R in mice produces delayed corneal wound closure.[63] Furthermore, cannabinoids improved healing speeds in a scratch assay of murine colonic epithelial cells (treatment with CBD).[64]

Hypopigmentation and hyperpigmentation are a common complication of wounding, especially in the case of surgical excisions.[65] This may be a source of cosmetic and psychosocial concern for patients.[65] There is currently a lack of effective treatment options for scar dyspigmentation, and literature surrounding the mechanisms of scar repigmentation is scarce.[66] In cultured human epidermal melanocytes, CBD increased melanin content and tyrosinase activity, while AEA stimulated melanogenesis, both in CB1-dependent manners.[67,68] However, care must be taken as resultant hyperpigmentation may be an undesirable side effect, though this relationship is

made even more complex as CBDs anti-inflammatory effects may reduce pigmentation as well. This highlights the need for further research characterizing the effects of CBs on melanocytes, melanocyte stem cells and post-wound pigment abnormalities.

5 | STEM/PROGENITOR CELLS

Stem cells are instrumental for all phases of normal wound healing, and chronic wounds are known to be characterized by dysfunction thereof.[69] Indeed, various case reports and RCTs have demonstrated the efficacy of exogenous cultured stem cells in treating chronic wounds.[69] Such treatments remain challenging to apply given their high cost and difficulty in culturing and standardizing large numbers of cells.[69] The modulation of various endogenous stem cells populations that contribute to wound healing may be an attractive, alternative therapeutic strategy for wounds.

Stem cells from the basal layer of the interfollicular epithelium (IFE) and the bulge of the hair follicle (HF) are involved in the re-epithelialization of wounds.[70] The effects of cannabinoids on IFE stem cells are evident in the modulation of keratinocyte proliferation and differentiation as discussed previously. Lineage-tracing experiments have revealed that HF bulge stem cells transiently contribute to re-epithelialization but are not present in the wound bed several weeks later; indeed, mice lacking hair follicles demonstrate a delay in early re-epithelialization but do not exhibit complete abrogation of this process.[70] Thus, bulge stem cells contribute to wound healing but are not necessary for it.[70] Various HF cell populations have been observed to express cannabinoid receptors[8]; indeed, CB1R expression is upregulated in HF epithelium during catagen, while CB1 agonism has been found to induce HF catagen in mice.[8] The CB receptor expression and effects of CBs on HF bulge stem cells during wounding have not yet, to our knowledge, been robustly explored, representing a direction for future research.

Mesenchymal stromal cells (MSCs), progenitor cells of mesodermal origin,[71] from various tissue compartments contribute to wound healing and exhibit modulation by CBs. There is some debate surrounding their classification as stem cells.[71] Nonetheless, MSCs promote cellular migration, angiogenesis, epithelialization and granulation, demonstrate immunosuppressive properties, and inhibit fibrosis.[71,72] Upon wounding, MSCs from the HF dermal sheath, interfollicular dermis, adipose tissue (aMSCs) and bone marrow (bMSCs) all contribute to the regeneration of damaged or lost tissue[69,71]; bone marrow MSCs alone may contribute up to 20% of wound fibroblast populations.[69]

Cultured MSCs express all the components of the eCS.[72] CB2 agonism of cultured human bMSCs resulted in the activation of pathways promoting cell survival, protein synthesis and increased metabolism, while 2-AG was a chemoattractant for bMSCs in a CB2R-dependent fashion.[72] THC-mediated CB2R signalling also enhanced the immunomodulatory effects of bMSCs, enhancing IL-10 production and inhibiting pro-inflammatory cytokine release from LPS-stimulated microglia.[73] Furthermore, CB2 agonism increased the number of aMSCs *in vitro* while CB1 agonism resulted in decreased metabolic activity and cell number.[74] Various pCBs including CBD and cannabigerolic acid (CBGA) increased the number of viable mouse bMSCs cultured *in vitro*.[75] Thus, it appears that CB2 agonism has generally favourable effects on MSC proliferation/survival and enhances their anti-inflammatory activity, which may prove useful for acute or chronic wounds of various aetiologies.

6 | MAJOR OPEN QUESTIONS

Further research is required to confirm the specific effects of CBs on the parameters outlined in this article and their subsequent relationship to wound healing (for example, is CB1 agonism pro- or anti-differentiative for keratinocytes?). Existing studies have limitations; for example, HaCaT cells are known not to express TRVP1 of which CBD is a well-characterized ligand.[21] Some studies discussed herein were not in the context of wounding.[32] Additionally, most of these studies were *in vitro* which highlights the need for more *in vivo* and clinical studies as *in vitro* results do not always translate reliably into these settings. We suggest that the effects of cannabinoids continue be explored in murine *in vivo* wound models or in ex vivo wound models such as a cultured bilayer living cellular construct[28] or cultured human skin wound models such as the one described by Glinos et al[76] Such studies would set the stage for clinical studies to truly elucidate the effects of endocannabinoid system modulation on wound healing in humans. Finally, the pharmacology of the major pCB CBV, and that of many less abundant minor pCBS (eg cannabielsion [CBE], cannabicyclol [CBL]), has yet to be studied and as such their therapeutic potential for not only wound healing but all of medicine remains untapped.[17]

7 | CONCLUSIONS AND PERSPECTIVES

The legalization of *Cannabis* for medical and recreational purposes has become increasingly prevalent in the United States. Accordingly, interest in therapeutic applications for cannabinoids (CBs) has skyrocketed, with 55% of dermatologists reporting a patient-initiated conversation about cannabinoids in the past year.[77] The medical cannabis market in the USA is expected to grow to US $ 12.5 billion by 2025.[78] Thus the iron is hot to strike with impactful research on the therapeutic applications of CBs for human skin disease. Herein, we summarized existing evidence supporting the therapeutic benefits of CBs for patients with skin wounds.

We suggest that the application of topical cannabinoids would be beneficial for wound healing. Table 2 contains a summary of the evidence for the ability of CBs to modulate key molecular pathways of wound healing. Notably, CB1R agonism at the non-healing edge of chronic wounds may improve healing via inhibition of the characteristically hyperproliferative keratinocytes found therein. This could be combined with CB2R agonism in the wound bed to ameliorate the dysregulated inflammatory microenvironment, namely through

reduction of pro-inflammatory cytokines, regulation of MMPs, and shifting from M1 (pro-inflammatory) to M2 (anti-inflammatory) macrophage tone. CBD also has potential to treat chronic wounds, as it exerts generally anti-inflammatory properties, appears to inhibit the proliferation of hyperproliferative keratinocytes, and has been observed to decrease expression of MMP-9. Furthermore, CB2R agonism may be of benefit in acute wound healing by speeding wound closure and reducing fibrosis to potentially lessen or eliminate scarring. Finally, CB2 agonism promotes the survival, proliferation and anti-inflammatory activity of MSCs which may prove beneficial for acute or chronic wounds.

The use of topical CBs for wounds minimizes the possibility of negative side effects; indeed, most topical cannabinoid formulations have negligible systemic absorption within commonly used carriers (eg lotions, creams, oils) which should assuage concerns about systemic side effects.[7] CBD in particular is attractive due to its lack of psychotropic effects and added ability to modulate pain.[7] Topical CBD has already been used safely in two small case series of orphaned diseases (Epidermolysis Bullosa [EB] and Pyoderma Gangrenosum) wherein patients reported decreased pain and, for the EB group, subjective decreases in healing time.[79,80] A topical CB formulation containing CBD, THC and various terpenes and flavonoids has also been successfully used in a small cohort of two patients with biopsy proven-calciphylaxis; clinically significant analgesia and closure were achieved in 0.6 and 2.5 months, respectively.[81] These few studies suggest that CBs hold great promise for the treatment of classically difficult or recalcitrant wound subtypes.

Translating CBs into the clinical setting may be limited by various factors including physician knowledge and comfort as well as perceived stigma. A recent survey of 249 physicians across 39 states revealed significant gaps in knowledge about CBs and discomfort in discussing CBs and their use with patients.[82] Furthermore, 48% of dermatologists reported concern about a negative stigma if they were to propose treatment with a topical CB.[77] This concern may be tempered with the knowledge that, according to Pew research, over 90% of adults in the US support the use of *Cannabis* for medicinal purposes.[83] It is important for clinicians to differentiate between the *Cannabis* plant, hemp oil, THC, CBD and the multitude of other various *Cannabis* derivatives as this is often an area of confusion for clinicians and patients alike. Robust clinician education will be instrumental as the therapeutic potential of CBs continues to mature.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

All authors made significant contributions to the conception, writing and reviewing of the manuscript, revision of intellectual and technical content, and gave final approval of the version to be published. All authors assume responsibility and accountability for the information contained herein.

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