

RESEARCH ARTICLE

Therapeutic candidates for keloid scars identified by qualitative review of scratch assay research for wound healing

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Abstract

The scratch assay is an *in vitro* technique used to analyze cell migration, proliferation, and cell-to-cell interaction. In the assay, cells are grown to confluence and then ‘scratched’ with a sterile instrument. For the cells in the leading edge, the resulting polarity induces migration and proliferation in attempt to ‘heal’ the modeled wound. Keloid scars are known to have an accelerated wound closure phenotype in the scratch assay, representing an overactivation of wound healing. We performed a qualitative review of the recent literature searching for inhibitors of scratch assay activity that were already available in topical formulations under the hypothesis that such compounds may offer therapeutic potential in keloid treatment. Although several shortcomings in the scratch assay literature were identified, caffeine and allicin successfully inhibited the scratch assay closure and inflammatory abnormalities in the commercially available keloid fibroblast cell line. Caffeine and allicin also impacted ATP production in keloid cells, most notably with inhibition of non-mitochondrial oxygen consumption. The traditional Chinese medicine, shikonin, was also successful in inhibiting scratch closure but displayed less dramatic impacts on metabolism. Together, our results partially summarize the strengths and limitations of current scratch assay literature and suggest clinical assessment of the therapeutic potential for these identified compounds against keloid scars may be warranted.

Introduction

The scratch assay is an *in vitro* technique used to analyze cell migration, proliferation, and cell-to-cell interaction. In the assay, cells are grown to confluence and then ‘scratched’ with a sterile instrument. For the cells in the leading edge, the resulting polarity induces migration and proliferation to attempt to ‘heal’ the modeled wound [1]. Keloids represent a disordered scar formation marked by an overactivation of proliferation and migration, which may represent overactivation of epithelial-to-mesenchymal transition (EMT) [2–4]. Although EMT is definitionally limited to epithelial derived cells such as keratinocytes (KC), scratch assay closure

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time can be assessed in the scratch assay across varying cell types [5]. Given that the established treatments for keloid scars corticosteroids and 5-fluorouracil both inhibit cellular proliferation and migration [6,7], we hypothesized that systematically reviewing the current literature might identify known inhibitors of scratch assay healing times which present therapeutic potential for patients with keloid scars. We thus performed a qualitative review of the recent literature searching for inhibitors of scratch assay activity that were already available in topical formulations.

Although several shortcomings in the scratch assay literature were identified, caffeine and allicin successfully inhibited the abnormalities of proliferation/migration in the commercially available keloid fibroblast cell line compared to the healthy volunteer cell line control. Allicin also inhibited production of the inflammatory mediator interleukin (IL-) 6. Caffeine and allicin treatment inhibited mitochondrial oxidative phosphorylation (OxPhos), which worsened the inherent defect in keloid cells. However, treatment with the mitochondrial ATP inhibitor rotenone failed to inhibit scratch closure and suggested that caffeine and allicin may exert influence through their effect on non-mitochondrial ATP production. The traditional Chinese medicine shikonin was also successful in inhibiting scratch closure but displayed less dramatic impacts on metabolism. Collectively, our results partially summarize the strengths and limitations of current scratch assay literature and suggest clinical assessment of the therapeutic potential for these identified compounds against keloid scars may be warranted.

Methods

This work was approved by the IRB of the National Institutes of Health.

Qualitative literature assessment

The quoted phrase “scratch Assay” and “scratch wound assays” were searched in PubMed on 5/1/2019. Search results were limited to those published on 1/1/2016 or later. A team of three reviewers (AA, CC, and IM) performed title and abstract level review to eliminate papers focusing on either neoplastic metastasis or those recommending changes to scratch assay methods. Remaining papers were read in detail to assess the use of the scratch assay, the cell types employed, and the impact of the stimulants or challenges tested.

Cell cultures and scratch assay

All cells used in this study were purchased from commercial biobanks (www.atcc.org) or Thermo Fisher Addexbio. Cell lines were not collected for this study, were collected through medically prescribed processes, and were completely de-identified to the researchers before access. Human primary fibroblasts (ATCC PCS-201-012) and primary human keloid fibroblasts (ATCC CRL-1762) were purchased from American Tissue Culture Collection (ATCC; Manassas, VA). HaCaT keratinocytes were purchased from Thermo Fisher Science (Waltham, MA). All cells were cultured and proliferated as previously described [8]. 96 or 24 well plates (Corning; Corning, NY) were coated with 1mg/mL rat tail collagen (Roche; Basel, Switzerland) overnight at 4°C. For 96 well plates, 17,000–25,000 cells and for 24-well plates 100,000–150,000 cells were added and allowed to adhere to the culture plate (cell number was matched across conditions within each given experiment). 12–24 hours later cells were scratched using the Autoscratch (BioTek; Winooski, VT). Cells were placed in the Cytation 5 (BioTek) at 37°C with 5% CO₂; images and enumeration were performed by the Scratch App (BioTek). Chondroitin from shark cartilage, caffeine, and folic acid were purchased from Sigma (St Louis, MO). HaCaT cells were cultured in Defined Keratinocyte-Serum Free Media (Gibco,

ThermoFisher) in T75 flasks. Once cells reached 80% confluence, they were trypsinized and seeded into a 24-well plate at 150,000 cells/well prior to undergoing the scratch assay as above.

Immunofluorescence staining

Cells were fixed with 4% paraformaldehyde (PFA; Cat. No. 15710; Electron Microscopy Sciences, Hatfield, PA) for 20 minutes. After fixation, cells were processed for the immunostaining protocol. Cells were washed with 1X PBS for three times 5 minutes each to get rid of the PFA solution. Cells were permeabilized with 0.5% Triton X-100 (T8787-100ML; Sigma-Aldrich) solution for 15 minutes. Followed by a PBS wash and cells were blocked with 5% normal goat serum (Cat. No. 50062Z; Thermo Fisher Scientific) for 60 minutes. Rabbit Anti-Vimentin antibody (Cat. No. #5741; Cell Signaling Technology, Danvers, MA) solution was prepared in the 1:1 PBS and normal goat serum solution. Primary antibody in 1:500 dilution was incubated for 60 minutes at room temperature. Followed by three PBS wash for 5 minutes each to remove the unbound antibody. Cells were incubated with anti-Rabbit-568 Alexa flour secondary antibody (Cat. No. A-11034; Thermo Fisher Scientific) solution at 1:750 dilution in phosphate buffered saline (PBS) and normal goat serum solution for 30 minutes. Followed by three PBS wash 5 minutes each to remove the unbound secondary antibody. Next cells were stained with DAPI solution (Cat. No. 62248; Thermo Fisher Scientific) 1:2000 dilution in PBS for 30 minutes at room temperature. Followed by cells were washed four time with PBS solution 5 minutes each to remove the unbound DAPI solution. After that cells were imaged with Cytation 5 fluorescence microscope (BioTek). All the images were analyzed and processed with Gen5 software (BioTek).

Multiplex for chemokines and cytokines

Multiplex cytokines and chemokines were performed using the Bio-plex kits per manufacturer instructions (Bio-RAD; Hercules, CA).

Seahorse

Cellular oxidative phosphorylation (OXPHOS) and glycolysis were measured using the Seahorse Bioscience Extracellular Flux Analyzer (XFe96, Seahorse Bioscience Inc., North Billerica, MA, USA) by measuring oxygen consumption reate (OCR; indicative of respiration) and extracellular acidification rate (ECAR; indicative of glycolysis) in real time according to manufacturer's protocol.

Briefly, 10,000 fibroblasts from the healthy volunteer cell line, keloid patients were seeded in 96-well cell culture microplates designed for XFe96 in 200 μ l of appropriate growth media. Fibroblasts were cultured with various stimuli for 2 hours. Prior to measurements, growth media was removed and replaced with 180 μ l pH ready Seahorse Assay Media (Agilent; Catalog #103575–100) and incubated in the absence of CO₂ for 1 hour in the Biotek Cytation1 instrument during which time pre-assay brightfield images were collected. Cells were sequentially treated with oligomycin (2 μ M), carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazine (FCCP) (0.5 μ M), and rotenone + Antimycin A (0.5 μ M). OCR and ECAR were then measured in a standard six-minute cycle of mix (2 min), wait (2 min), and measure (2 min). Basal levels of OCR and ECAR were recorded first, followed by OCR and ECAR levels following injection of compounds that inhibit the respiratory mitochondrial electron transport chain, or ATP synthesis. All OCR and ECAR values were normalized following the Seahorse Normalization protocol. Briefly, after the assay cells were stained with 2 μ g/mL Hoechst 33342 (ThermoFisher Scientific) for 30 minutes while performing post-assay brightfield imaging. Cells were then

imaged and counted using the Biotek Cytation 1. Cell counts were calculated by Cell Imaging software (Agilent) and imported into Wave (Agilent) using the normalization function.

Statistical analysis

To determine statistical significance, analysis of variance (ANOVA) with multiple-comparison corrections were applied using GraphPad Prism 8 software (San Diego, CA). Data are presented as the mean \pm SEM. A *P* value of less than 0.05 was considered significant.

Results

Most scratch assay articles evaluate metastasis rather than wound repair

A PubMed search revealed 1,331 articles contained the phrase “scratch assay” or “scratch wound assay” (Fig 1A). To focus on recent publications, we limited to those published after 2016 and found 859. Our goal was to assess tissue repair and not neoplastic metastasis; thus, title and abstract level assessment was used to eliminate 449 articles. An additional 28 articles were eliminated due to their focus on methods of the scratch assay rather than influencing wound healing pathways. The remaining 382 articles were evaluated to derive the cell type used, the stimuli employed, the impact on inhibition or enhancement of the scratch assay closure time, and the scientific shortcomings (Fig 1A).

Cell types were often tested in isolation

12.8% of publications that used endothelial cells and thus were reflective of endothelial-to-mesenchymal transition (EndoMT) rather than EMT (Fig 1B). Stem cells were used in 9.1% of papers which comment on the important role EMT plays in embryologic development [9]. Of the 207 publications that used epithelial cells, keratinocytes (KC), and fibroblasts (FB) were the most common cell lines used in scratch assay analysis (Table 1; Fig 1C). Among KC, 42 of the 58 (72.4%) studies used HaCaT cells, an immortalized, aneuploid cell line from adult human skin [10]. Primary skin cells were only used in 26.3% of the studies employing KC and none of the publication we evaluated directly compared HaCaT cells to primary cultures.

It is important to note that EMT does not definitionally occur in FB cells, which are already in a mesenchymal phenotype, however FB cells can undergo the inverse process of mesenchymal to epithelial transition (MET). Therefore, scratch assay results that use FB comment on wound closure via cellular migration, proliferation, or both. FB studies however used primary cells in 57 of the 105 studies (54.3%). Tissue sourcing of the primary FB cells studied was varied; 54.4% used FB from skin, 15.8% from gingiva, 10.5% from pulmonary organs, 7% from cardiac, 3.5% from tendons, and 8.8% from other tissues (Fig 1C). 83.3% of cell lines used were from animals (Fig 1C).

Experiments often lacked proper controls, especially for natural products

Natural products were frequently used in scratch assay experiments evaluating potential agents that could enhance wound healing in FB, KC, and epithelia (Table 1). However, control groups were often limited to diluent rather than a similar but comparable challenge. As one singular but demonstrative example, researchers showed enhanced scratch healing in HaCaT cells exposed to crocodile serum [25]. However, while the crocodile serum did demonstrate a dose response curve, no competing serum was used in the studies (such bovine serum). Therefore, it is unclear whether the findings are unique to the specific serum they used or if any serum would have similar effects as suggested by research demonstrating similar impacts of the serum-product lactoferrin [11]. There are several other examples of plant or animal extracts

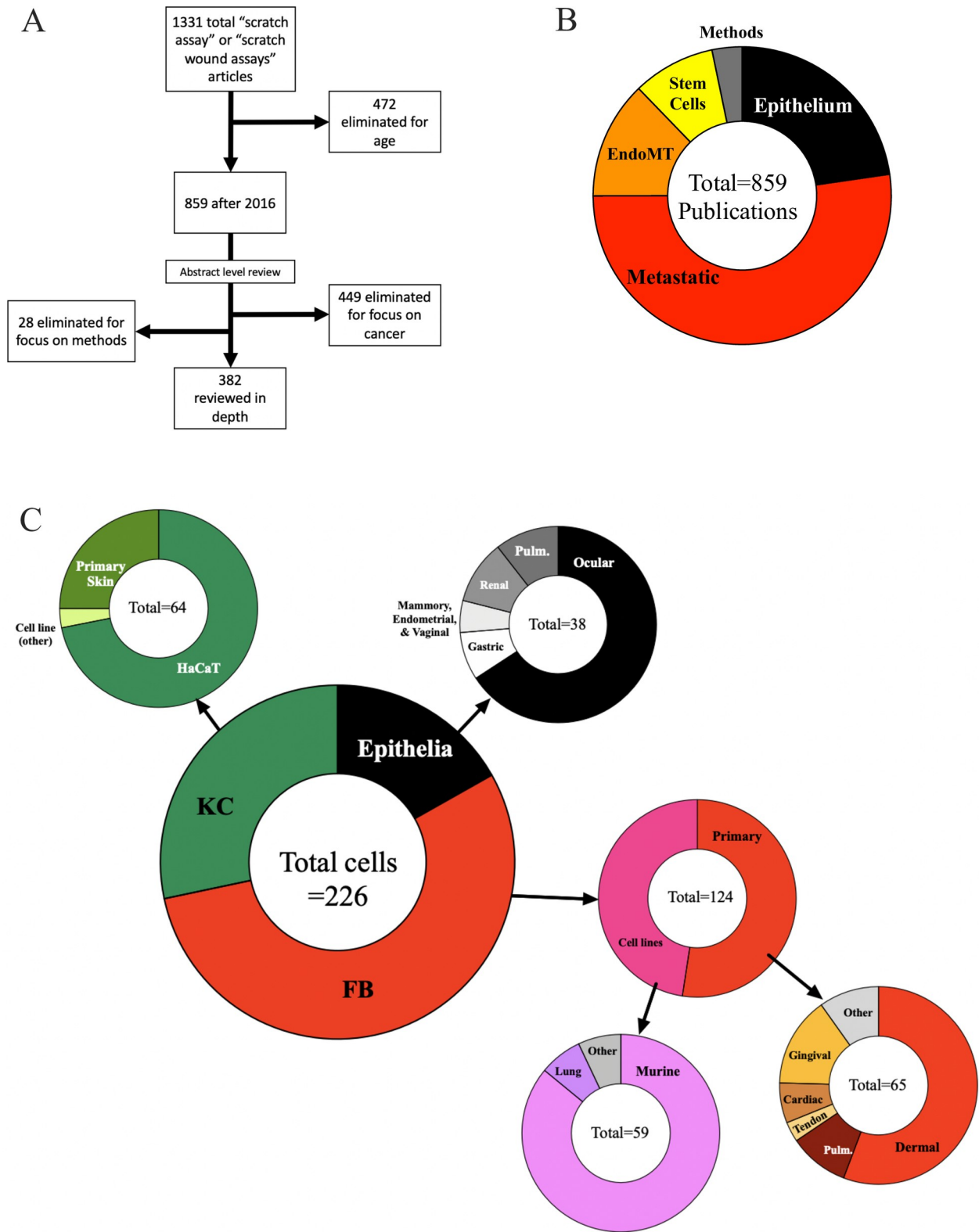


Fig 1. Articles using the scratch assay vary by cell type. (A) Progression and enumeration of articles found during qualitative literature review. (B) Pie chart of the total articles uncovered separated by general wound healing (often EMT) type or EndoMT. (C) Pie chart breakdown of total cell types used in the identified literature. KC = keratinocyte; FB = fibroblasts. EMT does not definitionally occur in FB, which are already of the mesenchymal phenotype.

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Table 1. Effect of different stimuli on scratch assay in keratinocytes, fibroblasts, and epithelial cells. Migration rate of scratch assay in response to cell signalers, natural products, metabolic mediators, immune mediators, drugs, and other stimuli.

HaCaT Keratinocytes		
Increase	Decrease	No Impact
Cell Signalers		
<ul style="list-style-type: none"> •EGF [11,12] •Pep19-2.5 [13] •Hepatocyte Growth Factor (HGF) [14] •micro RNA 21 [15] •ERBB2 [16] •Keratinocyte Growth Factor (KGF) [17] •AES16-2M (ERK activating peptide) [18] •GFP-Smad2 [19] •Lipofectamine and KGF-mRNA [17] •SIS3 (Smad3 phosphorylation specific inhibitor) [19] •Liraglutide, a Glucagon-like peptide-1 analogue (concentration dependent) [20] •Thrombin [21] •AHR siRNA [22] 	<ul style="list-style-type: none"> •EGF receptor inhibitor (EGFRi) [19] •JNK inhibitor (JNKi) [19] •MEK1 inhibitor (MEKi) [19] •Enhanced green fluorescent protein (eGFP) with Lipofectamine [17] •LY 294002 (PI3K inhibitor) [20] •IDR-1018, a synthetic innate defense regulator peptide, in normoxia [23] •EGFR antagonist (AG1478) [21] •ERK1/2 antagonist (UO126) [21] •AHR antagonist, CH223191 [22] •RIPK4 via TGFβ [24] 	<ul style="list-style-type: none"> •ERBB3 [16] •ERK inhibitor (U0126) [18] •IDR-1018 in hypoxic condition [23] •ALK5 (TGF-β receptor I) inhibitor (TGFRi) [19] •Cytochalasin D [22] •PXR siRNA [22] •PXR antagonist, SPA70 [22] •JNK inhibitor (SP600125 and indirubin) [22]
Natural Products		
<ul style="list-style-type: none"> •<i>Crocodylus siamensis</i> serum [25] •<i>Vitex negundo</i>, <i>Emblica officinalis gaertn</i>, and <i>Tridax procumbens</i> mix [26] •Tracheloside [27] •Fish scale derived gelatin nanofibrous scaffolds [28] •Quercetin (plant flavonoid) [29] •Aloe vera extract [30,31] •Chitosan, polyvinyl alcohol S-nitroso-N-acetyl-DL-penicillamine gel (taken from eggs) [32] •<i>Aloe purpurea Mascarene</i> (APM) [31] •<i>Annona crassiflora</i> flavonoid seed extracts [33] •glucan extract of <i>Ziziphus jujuba</i> [34] •Indirubin [22] 	<ul style="list-style-type: none"> •Chondroitin sulfate (ECM polysaccharide) [35] •Caffeine [36] •<i>Rhodomyrtone</i> [37] •<i>Annona crassiflora</i> flavonoid peel extracts [33] 	<ul style="list-style-type: none"> •<i>A. tormentorii</i> [31] •<i>A. lomatosphyloides</i> [31] •<i>A. macra</i> [31] •<i>A. purpurea</i> (Réunion) [31] •Quercetin (dose dependent) [29] •<i>Calendula officinalis</i> n-hexanic, ethanolic or aqueous extracts [14] •single triterpenes (α-amyrine, β-amyrine, lupeol, taraxastene) [14] •β-carotene [14] •Triterpene esters [14] •Tannic Acid (TA) [38]
Metabolic Mediators		
<ul style="list-style-type: none"> •Insulin [39] •Melatonin [40] •Neurotensin [39] •Substance P [39] •Human and bovine lactoferricin [11] 		<ul style="list-style-type: none"> •Allantoin [27] •Lactoferricin in presence of mitomycin C [11]
Immune Mediators		
<ul style="list-style-type: none"> •IL-8 [41] •poly I:C via IL-8 [41] •Neutrophil extracellular traps [42] •TGFβ [18] 	<ul style="list-style-type: none"> •Chloroquine via poly I:C [41] •Anti TGFβ [19] 	<ul style="list-style-type: none"> •anti-IL-8 antibody [41]
Drugs		
<ul style="list-style-type: none"> •Phenytoin [43] •Remifentanil pretreatment (RPC) via H2O2 [44] 	<ul style="list-style-type: none"> •Nanoemulsion [43] •Mitomycin C [20,22] •Rapamycin [45] •H2O2 [44] •3 Methyladenine (3-MA) countering Remifentanil + H2O2 [44] 	<ul style="list-style-type: none"> •Rifampicin [22]
Other		
<ul style="list-style-type: none"> •Amniotic membrane [19] •Tannic acid (TA)-modified Silver nanoparticles (AgNPs) [38] •Ag(salH)2 [46] •AgNO3 [46] •Stem cell media [47] •human stromal vascular fraction gel [48] •gallium/cerium-doped phosphate glass fibers [49] •Mycosporine-like Amino acids: shinorine, porphyra-334, mycosporine-glycine-alanine, or bostrychine [50] •Media with 20% fetal calf serum (FCS) vs 10% FCS [50] 		<ul style="list-style-type: none"> •Silver nanoparticle [51] •AgNO3 (silver nitrate) [46] •ozonated PBS [52]
Keratinocytes Other		
Increase	Decrease	No Impact
Cell Signalers		

(Continued)

Table 1. (Continued)

<ul style="list-style-type: none"> •JWH015 (cannabinoid receptor type 2 agonist) [53] •PHD-2—Protein Hydroxylase Domain Containing Protein 2 [54] •IOX2 (PHD-2 inhibitor) in hypoxia [54] •Epidermal growth factor (EGF) [55] •CD163 overexpressing macrophages [56] •Indirubin [22] 	<ul style="list-style-type: none"> •AM281 (cannabinoid receptor type 1 antagonist) [53] •AM360 (cannabinoid receptor type 2 antagonist) [53] •Cldn-1 knockdown [57] •ZO-1 knockdown [57] 	<ul style="list-style-type: none"> •Ocln knockdown [57]
Natural Products		
<ul style="list-style-type: none"> •Spirulina extract [58] •Cholinergic acid (C) [59] •Quercetin [55] •Hydroxysafflor Yellow A, a derivative of safflower [60] •<i>H. perforatum</i> oil extract [61] 		<ul style="list-style-type: none"> •Hidradenitis suppurativa (HS)[62] •Myricetin-3-O-rhamnoside (M) [59] •<i>P. perca</i> extract [59]
Drugs		
	<ul style="list-style-type: none"> •Ingenol mebutate [63] •Sulfur Mustard in hypoxic condition [54] 	
Other		
<ul style="list-style-type: none"> •Adipose derived stem cells in hypoxic conditions [64] •Conditioned medium dermal stromal cells (cmDSCs) [65] •Light Emitting Diode (LED) (significance not indicated) [66] •DermaLife media [57] 	<ul style="list-style-type: none"> •Chronic wound [62] •Orofacial clefts (OFC) [67] •Rodent keratinocytes versus mouse keratinocytes [68] 	<ul style="list-style-type: none"> •conditioned medium adipose stromal cells (cmASCs) [65] •EpiLife [57]
Human Dermal Fibroblasts		
Increase	Decrease	No Impact
Cell Signalers		
<ul style="list-style-type: none"> •EGF [69,70] •Fli1 siRNA [71] •siKI RNA [72] •bFGF (basic fibroblast growth factor) [70] •Apelin and ML-233 (apelin receptor activator) [73] •Klotho (KI), gene that encodes αKI protein [72] •Mono-epoxy-tocotrienol-α (MeT3α) [74] •JHW015 (a cannabinoid receptor type 2 agonist) [53] •PHD-2—Protein Hydroxylase Domain Containing Protein 2 [54] •IOX2 (PHD-2 inhibitor) in hypoxia [54] •Cartilage acidic protein 1 [75] 	<ul style="list-style-type: none"> •AQP1 siRNA [71] •AM281 (cannabinoid receptor type 1 antagonist) [53] •AM360 (cannabinoid receptor type 2 antagonist) [53] •Compound 21 [76] •Cryptotanshinone [77] 	<ul style="list-style-type: none"> •scrRNA [72]
Natural Products		
<ul style="list-style-type: none"> •Exopolysaccharides (EPS) from Nitratireductor spp PRIM-31 [78] •Spirulina platensis (algae) [58] •<i>Moringa oleifera</i> [79] •Myricetin-3-O-β-rhamnoside (M) [59] •Cholinergic acid (C) [59] •Saffron [80] •<i>Triticum vulgare</i> extract [81] •Cupuassu butter [82] •<i>Hypermongone C</i> extracted [83] •<i>Moringa oleifera</i> fraction [79] •<i>C. Papaya</i> extract [84] 	<ul style="list-style-type: none"> •Myricetin-3-O-β-rhamnoside [59] •<i>P. perca</i> extract [59] •Allicin [85] 	<ul style="list-style-type: none"> •<i>Moringa oleifera</i> ethyl acetate fraction [79]
Metabolic Mediators		
<ul style="list-style-type: none"> •Angiotensin 2 [76] •Vitamin combinations: B9 and B12; B3, B5, B6, and B10; and B3, B5, and B7 [86] 		<ul style="list-style-type: none"> •Vitamin Combination: B3, B5, B6, B9, B10, and B12 [86] •Vitamin C [86]
Drugs		
<ul style="list-style-type: none"> •Estradiol [87] •Pimecrolimus [88] 	<ul style="list-style-type: none"> •Arsenic [87,89] •Sulfur mustard in hypoxia [54] •Mitomycin C [77] 	
Immune Mediators		
<ul style="list-style-type: none"> •IL-6 [90] •CXCL-8 [90] •Histatins variants (Hst1, Hst2, cyclic Hst1) [90] •TGFβ [71] 		
Other		

(Continued)

Table 1. (Continued)

<ul style="list-style-type: none"> •Scleroderma disease [71] •Conditioned medium dermal stromal cells (cmDSCs) [65] •Conditioned medium adipose stromal cells (cmASCs) [65] •Fibrocytes [91] •Platelet-rich plasma [92] •Human Adipose Derived Stem Cell (HADSC) Extracellular Vesicles (EV) [93] •5% and 10% cerium chloride (CeCl3) [94] •Human stromal vascular fraction gel [48] •Silver nanoparticles [95] •Synthetic Quanzizoline Compound [96] •Dermal skin cells versus adipose skin cells [65] •curcumin-silica nano-particle [97] 	<ul style="list-style-type: none"> •Chlorogenic acid [59] •Depleted uranium [92] 	<ul style="list-style-type: none"> •Serum free medium and BSA [93]
Human Primary Fibroblasts, Other		
Increase	Decrease	No Impact
Cell Signalers		
<ul style="list-style-type: none"> •TRAM 34 (K+ channel 3.1 inhibitor) [98] •Fibrocytes [91] •JWH015 (cannabinoid rec agonist) [53] •Irisin [99] •FKBP10 KD via TGFβ1 [100] •PDRN (polydeoxyribonucleotide) [101] •Enamel matrix proteins (EMPs) [102] •miRNA-34a inhibitor [103] •MiRNA-34a and delta-like protein 1 (DLL1) siRNA [103] 	<ul style="list-style-type: none"> •Human amniotic epithelial cells [104] •TRAM 34 (calcium/calmodulin activated K+ channel 3.1 inhibitor) via TGFβ [98] •FK506-binding protein 10 (FKBP10) knockdown [100] •ERK inhibitor via IL-25 [105] •SB (p38 inhibitor) via IL-25 [105] •SP (JNK inhibitor) via IL-25 [105] •Bay (NFκB inhibitor) via IL-25 [105] •Ullrich congenital muscular dystrophy [106] •Anti-collagen VI 3C4 antibody (3C4-PA) [106] •SB203580 (p38 MAPK inhibitor) [107] •NS398 (COX-2-specific inhibitor) [107] •Heat-shock protein 27 (Hsp27) siRNA [107] •MicroRNA 34 (miR-34a) mimic [103] 	<ul style="list-style-type: none"> •Enhanced green fluorescent protein (eGFP) [17]
Natural Products		
<ul style="list-style-type: none"> •Polydeoxyribonucleotides-a fragmented DNA from (Oncorhynchus mykiss) sperm [69] •Maltodextrin/ascorbic acid [108] •Coumestrol/hydroxypropyl-β-cyclodextrin [109] •Hypromellose (HMPC) [109] •Platelet-rich plasma (PRP) [110] •Platelet-rich plasma (PRP) and fibrin [111] •Indirubin [112] •Ozone [113] 	<ul style="list-style-type: none"> •Eonurine extract [114] •H. italicum [115] 	
Immune Mediators		
<ul style="list-style-type: none"> •IL-25 [105] •TNF-α [107] 	<ul style="list-style-type: none"> •IL-37 [116] 	<ul style="list-style-type: none"> •IL-1β [101] •TNF-α in presence of HIF [117]
Metabolic Mediators		
<ul style="list-style-type: none"> •Bradykinin [118] •Insulin w/o adipocytes [119] 	<ul style="list-style-type: none"> •2DG in cells from patients with rheumatoid arthritis [120] •3-bromopyruvate in cells from patients with rheumatoid arthritis [120] 	
Drugs		
	<ul style="list-style-type: none"> •Pirfenidone [121] •anti-IL 6 [112] •anti-IL 8 [112] 	
Other		
<ul style="list-style-type: none"> •Adipocytes from non-diabetics (ND) [119] •Biodentine [122] 	<ul style="list-style-type: none"> •All trans retinoic acid [123] •Zoledronic acid [111] •Ullrich congenital muscular dystrophy (UCMD) [106] •anti-collagen VI 3C4 antibody (3C4-PA) [106] •Oxygenating therapeutic (Ox66TM) [124] 	<ul style="list-style-type: none"> •1% FBS vs 10% FBS in DMEM [108] •Cobalt chloride (CoCl2) [102] •TheraCal [122] •Xeno III [122]
Animal Fibroblasts		
Increase	Decrease	No Impact
Cell Signalers		

(Continued)

Table 1. (Continued)

<ul style="list-style-type: none"> •Apelin and ML-233 (apelin receptor activator) [73] •High affinity small peptide ligand, H1 [125] •Endodontic paste [126] •PDGF [127–130] •Protease-activated receptor-4 activating peptide (PAR-4AP) [131] •Thrombin [131] •Bioinspired hydrogels with basic fibroblast growth factor [132] •ATP [133] •microRNA 103 mimic [134] •synthetic peptide SVVYGLR [135] •PDGF [136] •cGAMP [137] 	<ul style="list-style-type: none"> •Apln (apelin peptide—a G protein couple receptor) siRNA [73] •apelin receptor (aplnr) siRNA [73] •knockdown of tRNA selenocysteine 1 associated protein 1 (Trnau1ap) [138] •NS398 (COX-2 inhibitor) [139] •DKK-1 (Wnt/β-catenin antagonist) [139] •JNK inhibitor (SP600125) [140] •SP600125 and Bay [140] •PI3K inhibitor (LY294002) [140] •DALBK (Bradykinin rec 1 antagonists) [141] •HOE (Bradykinin rec 2 antagonists) [141] •PDTC (NFkB receptor inhibitor) [141] •PK (Bradykinin rec 2 antagonists) [130] •miRNA-103 inhibitor [134] •SPHK1 [134] •Platelet derived growth factor [142] 	<ul style="list-style-type: none"> •SF in presence of Bay 11–7082, NF-B inhibitor [143]
Natural Products		
<ul style="list-style-type: none"> •<i>Thunnus obesus</i> (big eye tuna) extract [144] •3-epimasticadienolic acid (pistachio hull extract) [145] •<i>Pistacia vera L.</i> hull extract (select fractions) [145] •n-butanol [145] •<i>Talaromyces purpureogenus</i> (fungus) silver nanoparticles [146] (significance not indicated) •Sea bass extract [147] •<i>Achemilla vulgaris</i> extract [148] •polyherbal formulation of <i>Vitex negundo</i>, <i>Emblica officinalis</i> Gaertn, <i>Tridax procumbens</i> [26] •Cipladine (iodine cream) [149] •<i>A. Sacata</i> leaf extract (significance not indicated) [149] •terpinolene, α-phellandrene (monoterpenes) [127] •grape seed extract [150] •<i>Eugenia dysenterica</i> (Myrtaceae) oil (significance not indicated) [151] •Prangos ferulacea roots extract [152] •<i>Terminalia sericea</i> extracts [128] •S. nux-vomica-ZnO nanocomposite [153] •<i>Lafoensia pacari</i> leaf [129] •Vegetable oil blend [130] •Flavonoid extract oil palm leaf [154] •<i>Allophylus spicatus</i> [155] •<i>Ocimum gratissimum</i> [155] •<i>Jasminum dichotomum</i> [155] •<i>Phioliota nameko</i> [156] •<i>H. perforatum</i> oil extract [61] •Punica granatum and polymeric film [157] •Triticum aestivum extract with chitosan [158] •Sorocea guilleminina Gaudich extract [159] 	<ul style="list-style-type: none"> •Ethyl Acetate Fractions of <i>Allophylus spicatus</i> [142] •Ethyl Acetate Fractions of <i>Ocimum gratissimum</i> [142] •Ethyl Acetate Fractions of <i>Jasminum dichotomum</i> [142] 	<ul style="list-style-type: none"> •<i>Struthanthus vulgaris</i> extract [136] •<i>Pistacia vera L.</i> hull extract (select fractions) [145] •Ozonated PBS [52] •Ozone therapy for wound healing [52] •<i>Philenoptera cyanescens</i> folium cum fructus extract [155] •<i>Melanthera scandens</i> herba extract [155] •<i>Annona senegalensis</i> extract from folium [155] •<i>Cissua quadrangularis L</i> extract from herba [155] •<i>Gymnanthemum coloratum</i> extract from radix and folium [155] •<i>Indigofera pulchra</i> extract from herba [155] •<i>Leonotis nepetifolia</i> var. africana extract from herba [155] •<i>Millettia thonningii</i> extract from cortex [155] •<i>Rourea coccinea</i> extract from radix and folium [155] •<i>Thomningia sanguinea</i> extract from herba [155] •<i>Trichilia monadelpha</i> extract from cortex [155] •<i>Triumfetta rhomboidea</i> extract from radix [155] •<i>Uvaria ovata</i> extract from radix, cortex, and folium [155] •<i>Ocimum gratissimum</i> extract from herba [155] •<i>Jasminum dichotomum</i> extract [155]
Metabolic Mediators		
<ul style="list-style-type: none"> •Allantoin [145] •Bradykinin [141] 		<ul style="list-style-type: none"> •Insulin [119] •Subcutaneous adipocytes [119]
Immune Mediators		
<ul style="list-style-type: none"> •IL-6 [160] •Lipopolysaccharide [139] •TGFβ1 [161] •TNF [141] •Bay 11–7082 (NFkB inhibitor) [140] 	<ul style="list-style-type: none"> •NALP3KO via ATP [133] •High lung macrophage MHCII expression [162] •low MHCII + PDGF-AA blocking antibody [162] •Lipopolysaccharide [140] •TNF antibody [141] •Bay 11–7082 (NFkB inhibitor) [143] 	
Drugs		
<ul style="list-style-type: none"> •Chloroform [145] 	<ul style="list-style-type: none"> •NaOCl sodium hypochlorite [163] 	
Other		

(Continued)

Table 1. (Continued)

<ul style="list-style-type: none"> •Silk fibroin [143] •Chitosan, polyvinyl alcohol S-nitroso-N-acetyl-DL-penicillamine gel (taken from eggs) [32] •Silver nanoparticles [51,164] •Cipladine (iodine cream) [149] •Induced pluripotent stem cell-derived exosomes [165] •Self-assembled Graphene Quantum Dots (sGQDs) [150] •Electrical stimulation [166] •Poly(2-hydroxyethyl methacrylate)/polypyrrole hydrogel [166] •Sponges w/carboxymethyl chitosan and collagen peptides [167] •Gold nanoparticles (AuNP) [168] •Stromal vascular fraction (SVF) [169] •Quinone-based chromenopyrazole (QCP) antioxidant-laden silk fibroin electrospun nanofiber scaffold [170] •Pulmonary fibrosis associated RNA overexpression [171] •Titanium dioxide nanoparticle biofilm [172] •Neonatal cardiac fibroblasts infected with ETV2 [173] •exosomes platelet rich plasma [174] •tonsil derived Stem cell media [175] •gallium/cerium-doped phosphate glass fibers [49] 	<ul style="list-style-type: none"> •iodoform-based paste [126] •Tp-AgNPs [146] •SB203580 (p38 MAPK inhibitor) [160] •Ferrous nanoparticles [176] •Elaidic and linoleic (fatty acids) [119] •Light exposure [177] •Primary rat alveolar macrophages (AMO)-derived monocyte chemotactic protein-induced protein 1 (MCP1) knockdown [178] •Oxymatrine and Notch signaling pathway inhibitor (DAPT) via TGF-β1 [161] 	<ul style="list-style-type: none"> •Ca(OCl₂) (calcium hypochlorite) [163] •Stromal vascular fraction in normoglycemia [169] •VEGF factor E (VEGF-E) [179]
Epithelial Cells		
Increase	Decrease	No Impact
<i>Cell Signalers</i>		
<ul style="list-style-type: none"> •Epidermal Growth Factor [12] •tBHQ (Nrf2 inducer) [180] •Erythroid E2-related factor 2 (Nrf2) [180] •siRNA-knockdown of epiplakin [181] •IWR-1 (Wnt inhibitor) [182] •Platelet-derived growth factor isoform BB (PDGFBB) [183] •Corneal Mesenchymal stromal cells exomes [184] •VEGF [185] •ATF 2 and ATF 7 [186] •Chitinase-like protein YKL-40 [187] 	<ul style="list-style-type: none"> •Tankyrase inhibitor XAV939 via TGF-β [188] •Placental growth factor via hypoxia [189] •Lamin A/C (LMNA) knockdown [190] •neonatal Fc receptor [191] 	<ul style="list-style-type: none"> •Myocardin-related transcription factor A (MRTF-A) signaling inhibitor CCG-1423 [192] •miR-363 [182] •PGF [189]
<i>Natural Products</i>		
<ul style="list-style-type: none"> •Centell asiatica extracts [193] •Crocin (antioxidant carotenoid in saffron) via PDGFBB [183] •Pentacyclic triterpene-rich Centella extract [193] •Asiaticoside Centella extract [193] •Madecassoside Centella extract [193] •Lactobacillus crispatus [185] •L. crispatus supernatant [185] 	<ul style="list-style-type: none"> •Fucus distichus subspecies evanescens extract [194] •Casein hydrolysates (concentration and fraction dependent) [195] 	<ul style="list-style-type: none"> •non silencing siRNA [196] •Heat killed L. crispatus [185] •L.acidophilus [185]
<i>Metabolic Mediators</i>		
<ul style="list-style-type: none"> •Aqueous lysophosphatidic acid [197] •Hypokalemia [198] 	<ul style="list-style-type: none"> •Estradiol [199] 	<ul style="list-style-type: none"> •Pepsin [200] •1,25-dihydroxy vitamin D3 [201] •Substance P [202] •All-trans retinoic acid receptor agonist [203] •BMS493 (retinoic acid antagonist) [203]
<i>Immune Mediators</i>		
<ul style="list-style-type: none"> •NLRP siRNA [204] •IL1-β [205] •NLRP3 siRNA [204] •TGFβ [205] 	<ul style="list-style-type: none"> •S. aureus [206] 	<ul style="list-style-type: none"> •mTOR-siRNA [196] •Hla mutant S. aureus [206]
<i>Drugs</i>		
	<ul style="list-style-type: none"> •Bevacizumab [207] •Canakinumab via TGFβ and IL1β [205] •Aflibercept/Ranibizumab [208] 	<ul style="list-style-type: none"> •Axitinib [209]
<i>Other</i>		
<ul style="list-style-type: none"> •Stromal Fibroblasts Conditioned Mediums (SFCM) [202] •Low shear stress induced to HCEC before scratching [210] •Differentiation in bronchial cells [211] 	<ul style="list-style-type: none"> •Plumbagin [212] •Biomass fuel smoke extract [204] •Cigarette smoke extract [204] •Cigarette smoke condensate [213] •Crocin via PDGFBB [183] 	<ul style="list-style-type: none"> •Intermittent Hypoxia [214] •Hypoxia [189] •Amphiphilic block polymer polyethylene glycol-polycaprolactone [209] •Low-intensity pulsed ultrasound [215] •Particulate Material (PM2.5) (significance not indicated) [216] •Sunitinib malate loaded with biocompatible poly (lactic-co-glycolic acid) nanoparticles [217]

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being tested against diluent alone rather than a competing challenge of similar, but distinct, molecular complexity. Overall, such a limitation may not have any practical implications given that a product that induces wound healing may be beneficial regardless of the mechanism. However, it does present a limitation on mechanistic insights since, for example, the effects of crocodile serum may be due the added nutrient density of the culture media. Mechanistic interpretations are easier for studies where the only variable is the addition of one cytokine or molecule than the studies where a highly complex stimulant is compared to water or saline.

Findings were limited in mechanistic validity

One of the limitations in scratch assay publications included the failure to use objective statistical methods to evaluate results. For example, some papers would rely on photographs of healed scratches but not offer measurements of impact beyond visual comparisons [112]. In addition, some publications failed to experimentally block the pathway claimed as mechanistic. As one example, researchers suggested nicotine induced scratch closure through modulation of α SMA (alpha smooth muscle actin; a potential EMT modulator) but did not experimentally block or neutralize α SMA to validate the mechanistic claims [218].

Commercially available inhibitors of the scratch assay were identified

Despite the limitations in the literature, we aimed to identify potential candidates for topical products that could inhibit scratch closure. Our criteria were to identify treatments that were: (a) already through the drug development pipeline; (b) available in a topical formulation; and (c) present a reasonable side effect profile. Based on these criteria we identified chondroitin [35], caffeine [36], and allicin [85] as potential scratch assay closure inhibitors.

Chondroitin failed to inhibit scratch assay results

In direct contrast with the prior report [35] chondroitin enhanced scratch closure in healthy volunteer (HV) primary fibroblast line cells (FB; Fig 2A) and HaCaT keratinocytes (Fig 2B and 2C). Of note, the prior report using chondroitin extracted it directly from pig trachea [35] whereas our assay used pharmaceutical-grade chondroitin from shark cartilage. Given the failure to inhibit scratch closure, chondroitin was not evaluated further.

Caffeine inhibited scratch closure in healthy and keloid cell line fibroblasts

In contrast to chondroitin, caffeine recapitulated the literature through inhibition of the scratch assay in a dose dependent manner (Fig 2D). While inhibition of wound healing pathways would be most often viewed negatively, such inhibition may be beneficial in patients with keloid fibroblasts which display pathologic overactivity of wound healing [2–4]. Similar to the prior research [5,219], we found that keloid fibroblasts demonstrated increased closure over time in the scratch assay (Fig 2E and 2F; area under the curve HV-FB = 654, 95%CI 612.5–690.3; area under the curve KEL-FB = 829, 95%CI 778.5–879.5). At lower concentrations, caffeine inhibited scratch repair in HV-FB more than for keloid FB (Fig 2G), however at concentrations above 1mg/mL equivalent inhibition was seen (Fig 2H).

Consistent with prior reports in KC [220], keloid FB had significantly more immunofluorescent staining per cell for the mesenchymal phenotype marker vimentin. Vimentin cellular expression was inhibited by caffeine (Fig 3A and 3B). To elucidate how caffeine may be influencing mesenchymal phenotypes in these cells we first evaluated the impact on cytokines previously associated with keloid scars [221]. Supernatant from keloid FB accumulated significantly more TGF β 2, but not TGF β 1 or TGF β 3 (Fig 3C–3E). However, caffeine did not

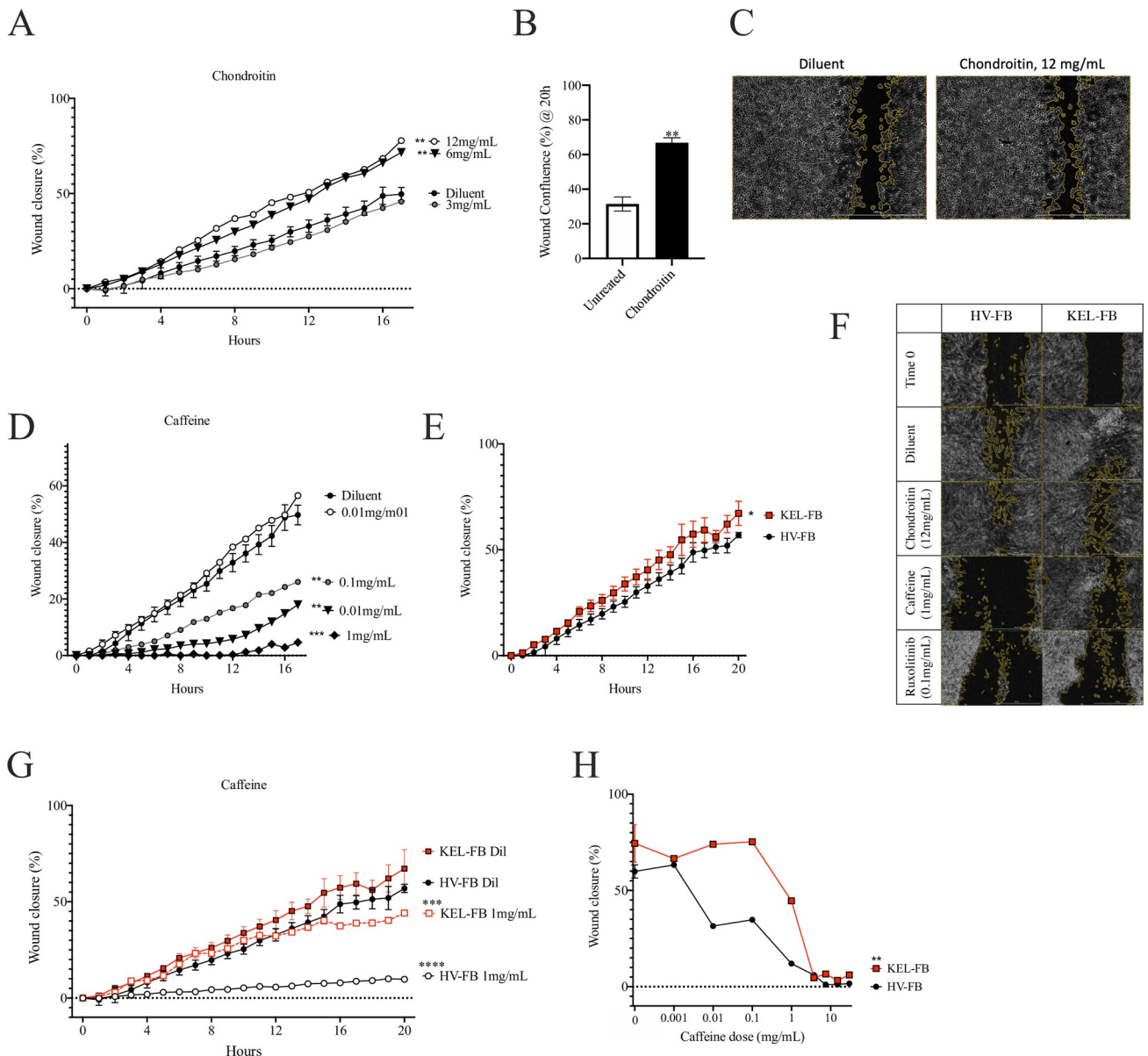


Fig 2. Caffeine, but not chondroitin, inhibit scratch repair in keloid and healthy volunteer fibroblasts. (A) Wound closure over 18 hours for healthy volunteer fibroblast line (HV-FB) treated with indicated concentrations of chondroitin. (B-C) Wound closure (B) and representative image (C) for HaCaT keratinocytes at 20hours with treatment of 12mg/mL of chondroitin at 18 hours. (D) Wound closure over 18 hours for HV-FB treated with indicated concentrations of caffeine. (E-F) Wound closure over 22 hours and representative images at 22 hours (F) for HV-FB or a commercially available fibroblasts cell line from a patient with keloid scarring (KEL-FB). (G) Wound closure over 22 hours for HV-FB and KEL-FB treated with 1mg/mL caffeine. (H) Wound closure at 14 hours for HV-FB and KEL-FB treated with indicated concentrations of caffeine. Results are representative of three independent experiments and displayed as mean + SEM for triplicate wells. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; for statistical comparison of area under the curve versus HV-FB under diluent stimulation conditions as determined by ANOVA with Sidak adjustment. Masking on representative images of scratch assay performed by Scratch App (BioTek).

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significantly influence production of any TGF β isotype in keloid FB (Fig 3C–3E). Keloid fibroblasts also displayed an increased supernatant accumulation of interleukin (IL-) 6 (Fig 3F) and a reduced production of IL-8 (Fig 3G). However, caffeine did not correct these abnormalities, nor did it significantly influence production of RANTES (Fig 3H), CXCL1 (Fig 3I), hepatocyte growth factor (HGF; S1A Fig), CCL2 (S1B Fig), vascular endothelial growth factor (VEGF; S1C Fig), or platelet derived growth factor (PDGF; S1D Fig).

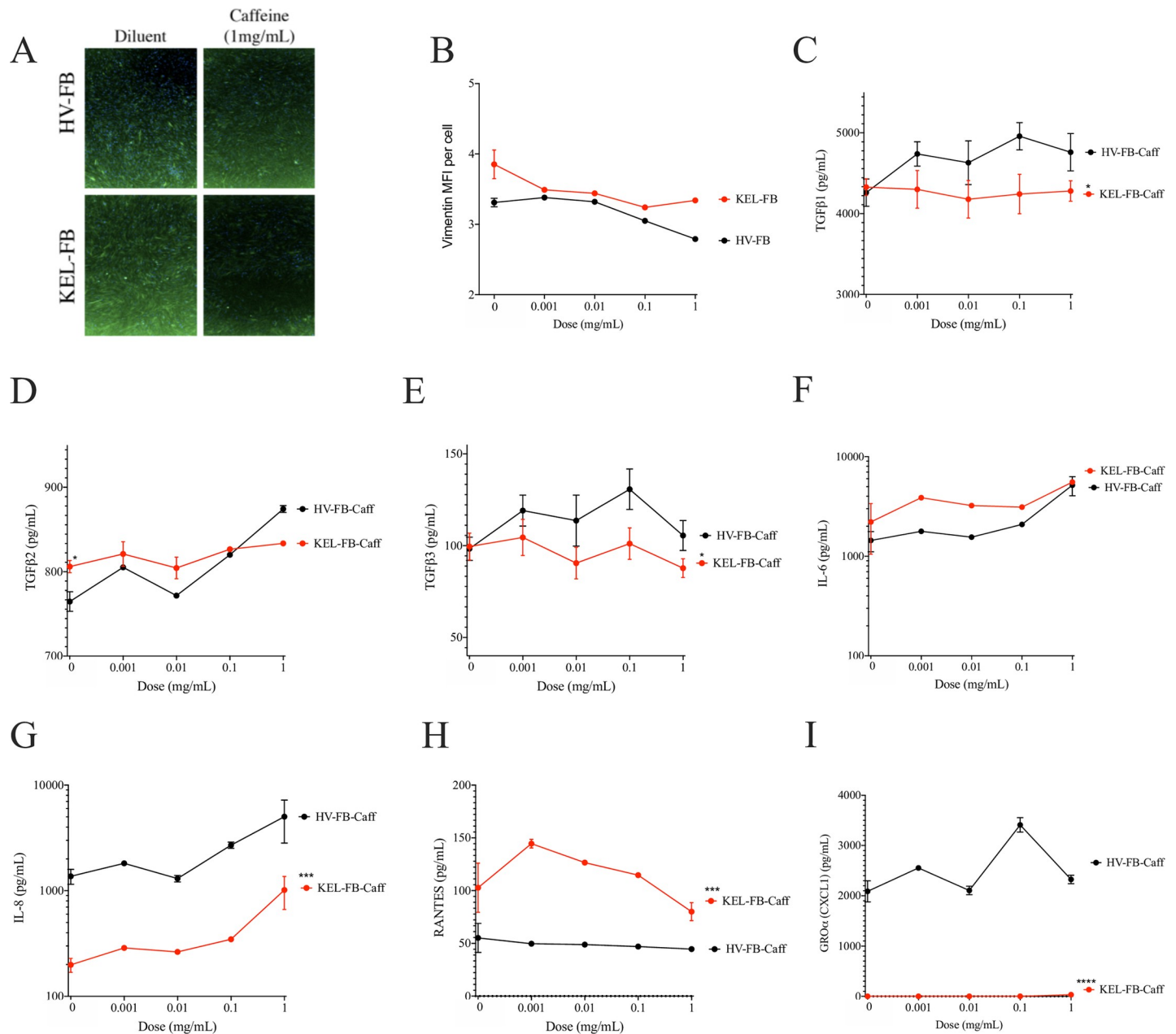


Fig 3. Impact of caffeine on vimentin expression and cytokine production. (A) Representative images from vimentin stain in healthy volunteer (HV-) or keloid-derived (KEL-) fibroblast cell lines (FB). (B) Mean fluorescence intensity (MFI) per cell for vimentin for HV-FB and KEL-FB treated with indicated doses of caffeine. (C-I) Supernatant accumulation of cytokines and chemokines for cells treated with caffeine. Transforming growth factor beta 1 (TGFβ1; C), TGFβ2 (D), TGFβ3 (E), interleukin (IL-) 6 (F), IL-8 (G), RANTES (H), and CXCL1/GROα (I) are shown. Results are representative of two independent experiments and displayed as mean + SEM for triplicate wells. * = $p < 0.05$; *** = $p < 0.001$; for statistical comparison of area under the curve versus HV-FB under diluent stimulation conditions as determined by ANOVA with Sidak adjustment.

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Caffeine altered metabolic activity in keloid fibroblasts

Cells from keloid scars are known to display Warburg metabolism—a form of metabolic disruption associated with cancer cells in which over expression of STAT3, in conjunction with JAK2, drives a tendency for rapidly proliferating cells to generate ATP via glycolysis rather than OxPhos, even with available oxygen [222,223]. Consistent with these reports, keloid

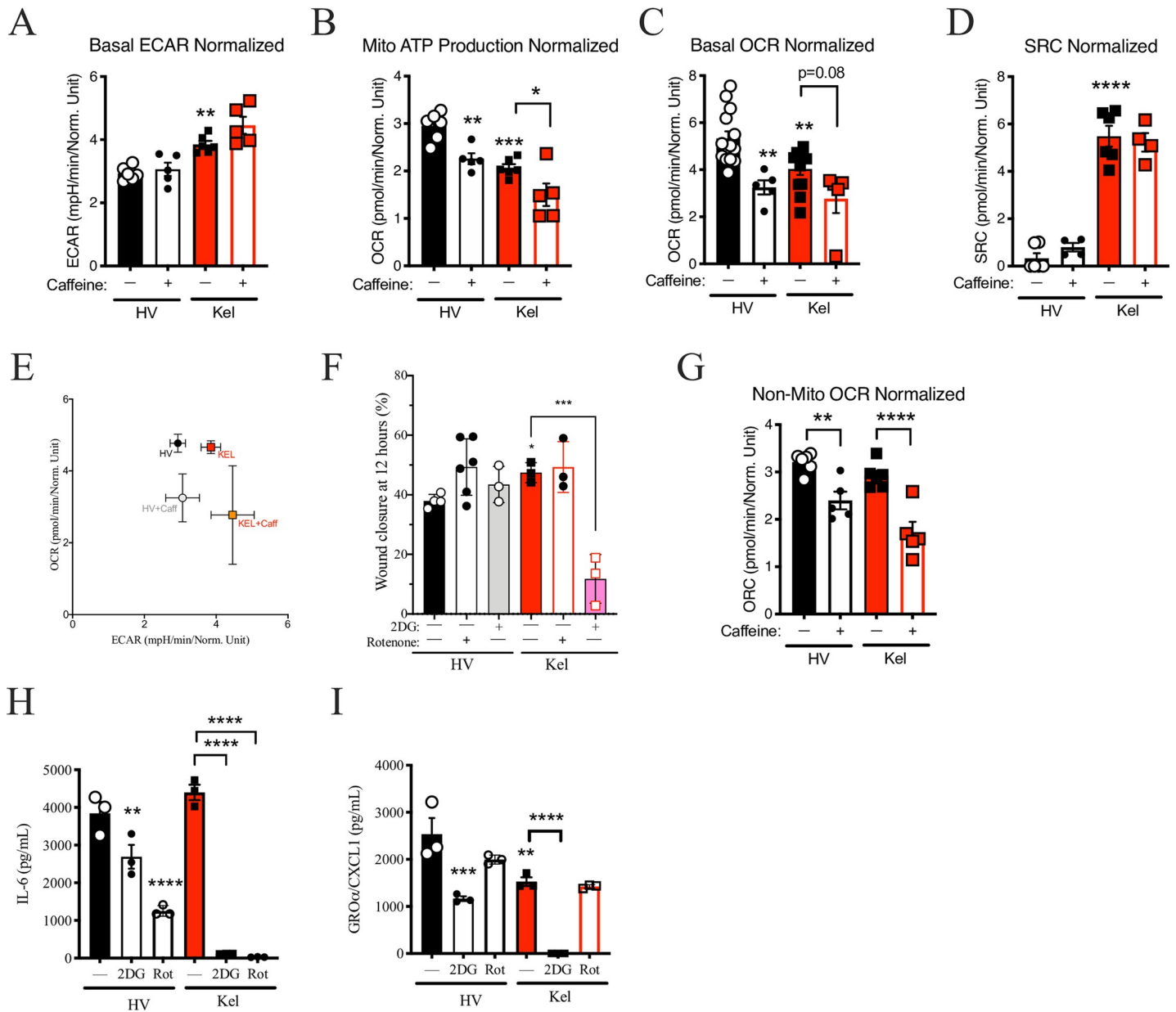


Fig 4. Caffeine impacts metabolic function. Seahorse assay was performed on fibroblast cell lines (FB) from healthy volunteer (HV) or keloid scars (Kel). Results for extracellular acidification rate (ECAR; a measure of glycolysis; A), mitochondrial (mito) ATP production (B), basal oxygen consumption rate (OCR; C), spare respiratory capacity (SRC; D), and ratio of basal ECAR to OCR (E) are shown. (F) Wound closure at 12 hours for keloid FB or healthy FB with treatment with diluent, the glycolysis inhibitor 2DG, or the mitochondrial OxPhos inhibitor rotenone. (G) Seahorse results for non-mitochondrial OCR for cells treated with diluent or caffeine. (H-I) Supernatant accumulation of interleukin (IL-) 6 (H) and CXCL1 (I). Results are representative of two independent experiments and displayed as mean + SEM with dots indicating replicate wells. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$ as determined by ANOVA with Sidak adjustment.

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fibroblasts demonstrated: more glycolytic activity as measured by extracellular acidification rate (ECAR) in the Seahorse assay (Fig 4A); a significant reduction in mitochondrial ATP production (Fig 4B); a significant reduction in basal oxidative phosphorylation as measured by the oxygen consumption rate (OCR; Fig 4C); and a higher spare respiratory capacity (SRC; Fig 4D). Rather than impact glycolysis, caffeine further inhibited OCR and mitochondrial ATP production in keloid FB (Fig 4A–4E).

Scratch closure in keloid fibroblasts was dependent on glycolysis

A targeted inhibitor of glycolysis, 2DG, inhibited scratch results in keloid, but not HV cell line fibroblasts (Fig 4F and 4G). While the chemical inhibitor of mitochondrial oxidative phosphorylation (OxPhos), rotenone, did not impact the scratch assay results in either cell type (Fig 4F). This suggests that while mitochondrial ATP production was diminished in keloid line FB, the impact on scratch closure was independent of its further inhibition. However, non-mitochondrial OCR was preserved in keloid FB and inhibited by caffeine (Fig 4G). 2DG and rotenone inhibited the production of IL-6 in both keloid and healthy fibroblasts (Fig 4H). However, only 2DG inhibited CXCL1 (Fig 4I) indicating a potential role of metabolism in IL-6 mediated inflammation and neutrophil recruitment beyond the reported connection between IL-6 and glycolysis [224].

Alliin altered metabolism and scratch closure

In our literature review, Alliin was also identified as an inhibitor of wound healing [85] and subsequently revealed modulation of metabolism via JAK2/STAT3 [225]. Alliin did inhibit scratch closure in both HV and keloid fibroblasts in a dose dependent fashion (Fig 5A and 5B). At higher doses, alliin caused a greater degree of cellular detachment from the plate (Figs 5A and S2A). Alliin did not significantly impact ECAR (Fig 6C), SRC (Fig 6D), or mitochondrial ATP production (Fig 5E). However, alliin selectively inhibited basal OCR (Fig 6F) and non-mitochondrial OCR (Fig 6G) while shifting the ECAR-OCR ratio (Fig 5H) in keloid fibroblasts. Vimentin staining was similarly reduced in dose-response fashion (Fig 5I–5J). At moderate doses, the staining pattern and cell morphology became disordered in both HV and keloid cells (Fig 5J). Although inhibition of IL-6 occurred to a greater degree in keloid cells than HV (Fig 5K), no rescue of CXCL1 production was seen (Fig 5L).

Shikonin inhibited keloid fibroblasts without impacting metabolism

Given the potential that metabolic alterations could inhibit keloid fibroblasts as seen with caffeine and alliin, we revisited our original 1,331 articles to search for pharmacologically available products that inhibited the scratch assay with known impacts on metabolism. As previously described, Shikonin inhibited EMT in cancer cell lines [226], regulated Warburg physiology in keloid fibroblasts, and improved outcomes in murine burn models [227]. However, in our analysis shikonin did not alter basal ECAR (Fig 6A), basal OCR (Fig 6B), or SRC (Fig 6C). Shikonin inhibited mitochondrial ATP production but not non-mitochondrial OCR in keloid cells (Fig 6D and 6E). Shikonin also partially normalized the ECAR-to-OCR ratio (Fig 6F). Despite the paucity of metabolic impacts, shikonin inhibited the scratch assay results in both HV and keloid FB (Fig 6G) and suppressed vimentin staining in HV, but not keloid, cells (Fig 6H). Although shikonin inhibited the overproduction of IL-6 in keloid cells (Fig 6I), it did not normalize the production of CXCL1 (Fig 6J). Table 2 summarizes the impacts of each treatment on the measured outcomes.

Discussion

The scratch assay is a widely used in vitro tool for assessing EndoMT, all three types of EMT, and overall wound healing [9]. The potential difference in results between commercial grade and extracted chondroitin are representative of a limitation of scratch assay research using molecularly complex stimuli. While such distinctions may not limit therapeutic benefit of the product tested, it is often unclear if the results are specific to the exact stimuli used or if the effects would be shared within the stimuli's general category. Therefore, given that many

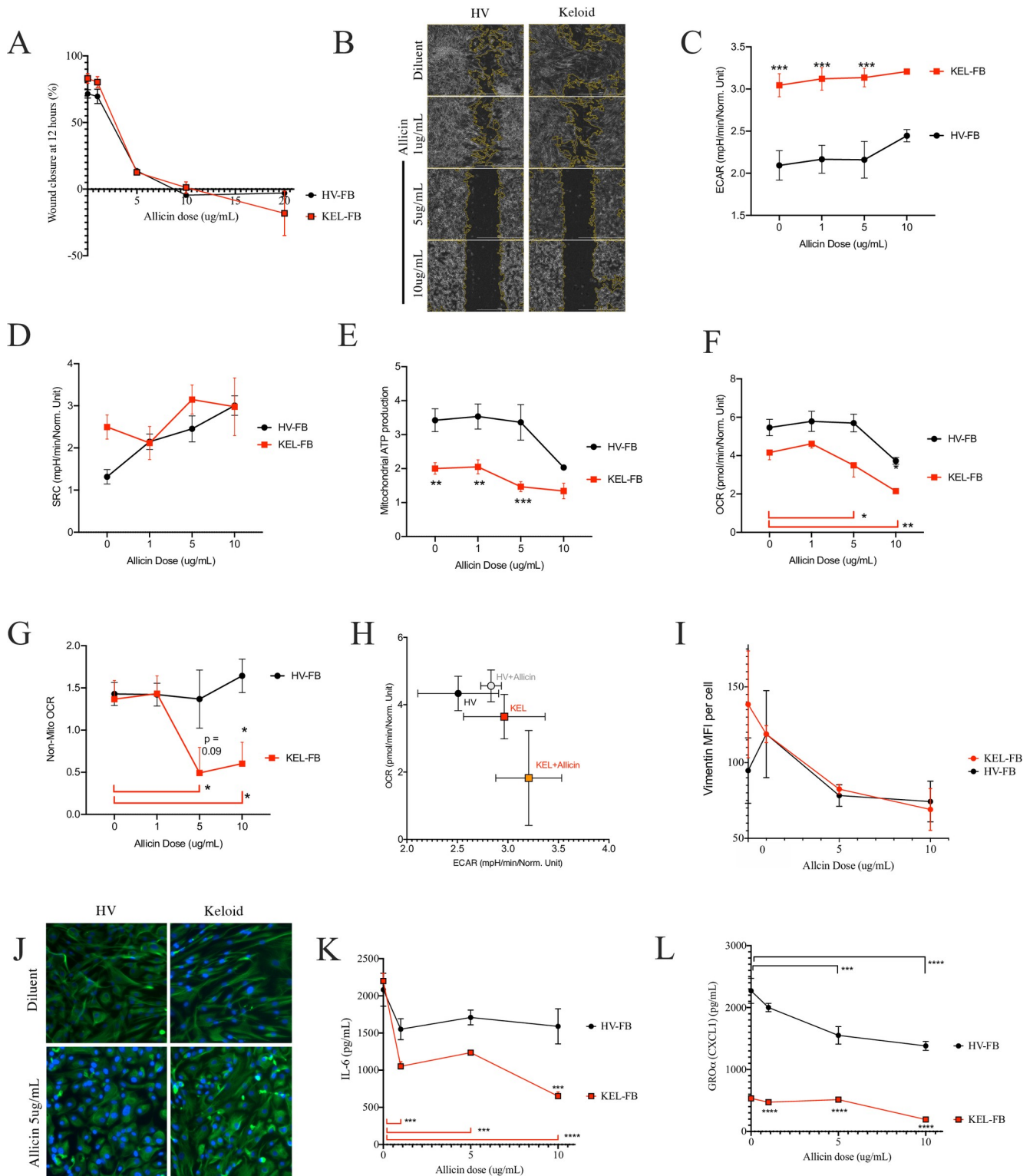


Fig 5. Allicin impacts scratch outcomes and metabolic function. (A-B) Wound closure as 12 hours (A) and representative image (B) for keloid (KEL-) or health volunteer (HV-) fibroblast cell lines (FB) after treatment with indicated doses of allicin. (C-F) Seahorse assay results for extracellular acidification rate (ECAR; C), spare respiratory capacity (SRC; D), mitochondrial (mito) ATP production (E), basal oxygen consumption rate (OCR; F), non-mitochondrial OCR (G), and ratio of basal ECAR to OCR (H) are shown. (I-H) Mean fluorescence intensity (MFI) per cell (I) and representative images (H) for vimentin for HV-FB and KEL-FB treated with indicated doses of allicin. (K-L) Supernatant accumulation of interleukin (IL-) 6 (K) and CXCL1 (L). Results are

representative of two independent experiments and displayed as mean + SEM (A-G, I-L) or SD (H) for triplicate wells. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$ as determined by ANOVA with Sidak adjustment compared with HV in under similar conditions unless indicated; red brackets indicate statistical assessment for keloid FB while black brackets represent statistical assessment for HC-FB. Masking on representative images of scratch assay performed by Scratch App (BioTek). Negative values on scratch healing indicate inhibition of scratch repair (healing times that were slower than diluent control).

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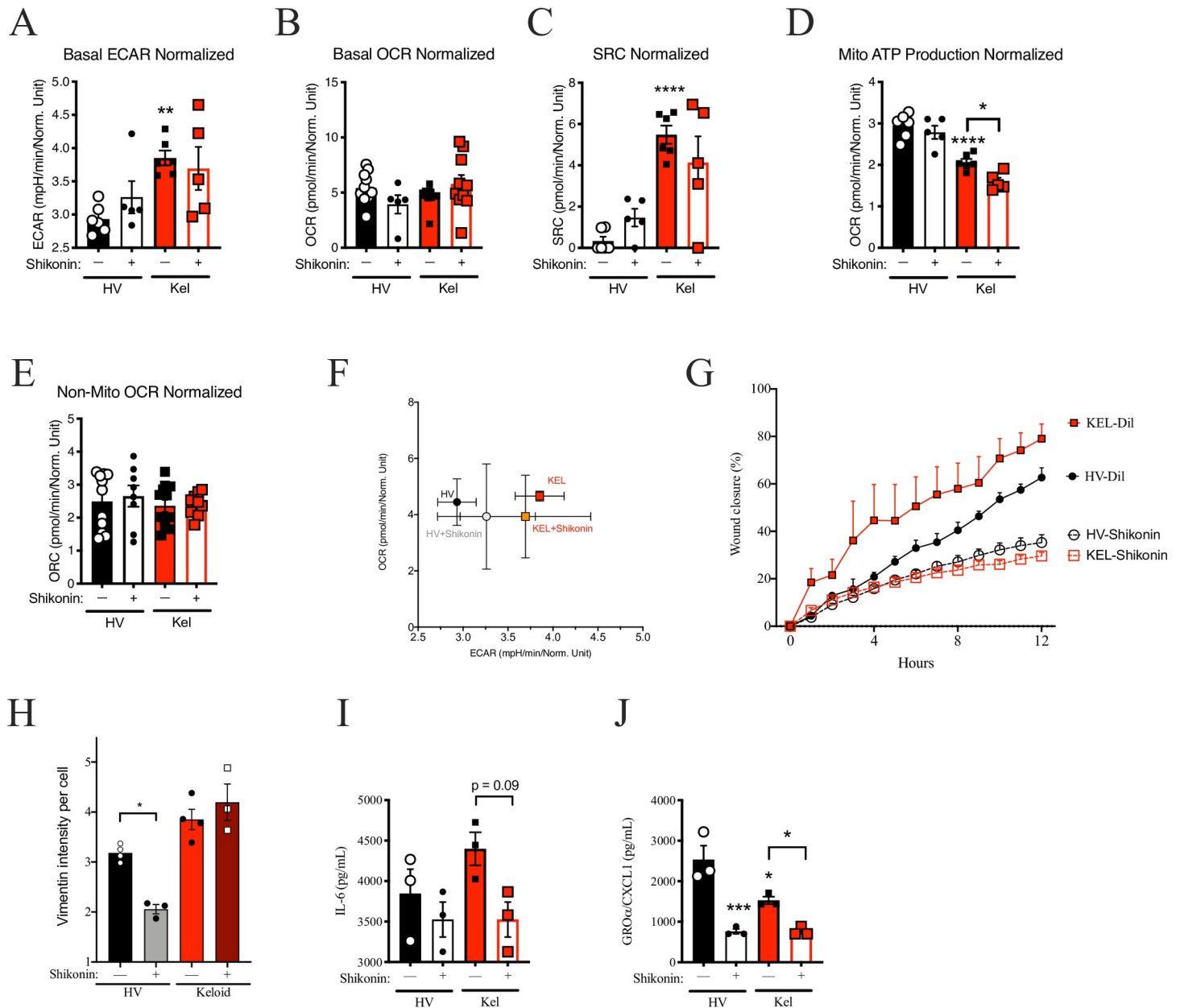


Fig 6. Shikonin impacts scratch outcomes without influencing metabolic function. (A-F) Seahorse assay results for keloid (KEL-) or health volunteer (HV-) fibroblasts (FB) for extracellular acidification rate (ECAR; A), basal oxygen consumption rate (OCR; B), spare respiratory capacity (SRC; C), mitochondrial (mito) ATP production (D), non-mitochondrial ATP production (E), and ratio of basal ECAR to OCR (F) are shown. (G) Wound closure as 12 hours for HV and KEL-FB after treatment with shikonin (10µM). (H) Mean fluorescence intensity (MFI) per cell for vimentin for HV-FB and KEL-FB treated with shikonin (10µM). (I-J) Supernatant accumulation of interleukin (IL-) 6 (I) and CXCL1 (J). Results are representative of three independent experiments and displayed as mean + SEM (A-E, G-J) or SD (F) for triplicate wells. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$ as determined by ANOVA with Sidak adjustment compared with HV in under similar conditions unless indicated.

<https://doi.org/10.1371/journal.pone.0253669.g006>

Table 2. Summary of impacts of compounds on healthy volunteer and keloid fibroblasts. Summary of impacts of the glycolysis inhibitor (2DG), the mitochondrial ATP inhibitor (rotenone), caffeine, allicin, and shikonin on scratch assay healing time, extracellular acidification rate (ECAR), mitochondrial ATP production (Mito-ATP), non-Mito-ATP, the proinflammatory interleukin (IL-6), and the neutrophil chemokine CXCL1.

Stimulant	EMT	ECAR	Mito-ATP	Non-Mito-ATP	IL-6	CXCL1
Healthy volunteer fibroblast cell line						
2DG	—	↓	—	↓	↓	↓
Rotenone	—	—	↓	—	↓	—
Caffeine	↓	—	↓	↓	—	—
Allicin	↓	—	↓	↓	↓	↓
Shikonin	↓	—	—	—	—	↓
Keloid fibroblast cell line						
Keloid derived (vs HV-FB)	↑	↑	↓	—	↑	↓
2DG	↓	↓	—	↓	↓	↓
Rotenone	—	—	↓	—	↓	—
Caffeine	↓	—	↓	↓	—	—
Allicin	↓	—	↓	↓	↓	↓
Shikonin	↓	—	↓	—	↓	↓

<https://doi.org/10.1371/journal.pone.0253669.t002>

papers also failed to compare their findings against more than one cell type, researchers may have difficulty extrapolating findings beyond the exact parameters of the presented experiment. However, such research may occur prior to 2016 or have been performed using the transwell assay rather than scratch assay [221].

Our findings are also limited in the sole use of monolayer cultures of fibroblasts as the primary focus of the complex pathology of keloid scars. In addition, although the use of a commercial cell line allows other researchers greater opportunity for testing the reproducibility of our findings, our results are nonetheless limited to singular cell lines and thus cannot comment on the impact of body site or individual patient variation in healthy donors or patients with keloids. Furthermore, many scratch assay methods employ anti-proliferative agents to limit the interpretation of scratch closure results to migration. By avoiding use of these agents our results better reflect *in vivo* wound closure, which relies on both cell proliferation and migration; however, failure to use anti-proliferative treatments in our methods precludes us from commenting on whether the results seen on scratch closure were due to impacts on proliferation, migration, or both.

Despite limitations of the literature and our assay, we identified three over-the-counter treatments that improved modeled outcomes in the commercially available keloid fibroblast cell line: caffeine, allicin, and subsequently shikonin. Caffeine is available over the counter in creams marketed for reducing “cellulite” and diminishing “bags under the eyes”. However, our results suggest that caffeine may be less ideal due to an increased potency inhibiting wound healing in healthy cell line FB than those from keloids (Fig 2E) and failed to impact the hyperinflammatory state of keloid line FB (Fig 3F). Allicin, a sulfur containing metabolite extracted from garlic, has also been used to mitigate murine models of fibrotic disorders like keloid scars and pulmonary fibrosis [85,225,228]. Allicin may present the most promising candidate for clinical trials given it appeared more potent against keloid FB than healthy cells in its OxPhos inhibition (Fig 5F), cell toxicity (Figs 5A and S2A), and IL-6 inhibition (Fig 5K). Shikonin has long been a traditional Chinese medicine with anti-scarring claims [227]. While shikonin inhibited scratch closure (Fig 6G) and IL-6 production (Fig 6I) in keloid line cells, the mechanism of action is unclear given shikonin failed to impact vimentin expression (Fig 6H) and had less pronounced impacts on metabolism (Fig 6A–6E).

However, the impacts of allicin and caffeine on OxPhos worsened, rather than reverse, the inherent mitochondrial ATP defect in keloid cells (Figs 4B, 4C, 5E and 5F). The reduction in non-mitochondrial OCR seen with caffeine and allicin treatment may indicate a role for reactive oxygen species and/or NOX mediated metabolism in the pathogenesis of keloids [229]. Thus, elucidation of the maladaptive impact of Warburg metabolism in keloid cells is essential to discover a treatment that reverses the underlying metabolic disorder in keloid derived cells. Furthermore, given that all of our identified treatments also inhibited wound closure in healthy FB and could theoretically prevent normal wound healing, each should likely be avoided in the immediate aftermath of an injury or surgery.

While our evaluations were successful in identifying commonly available drugs with therapeutic potential in cell models of keloid scars, we could not uncover a unifying mechanism for their actions. Caffeine and allicin may work through reducing mitochondrial ATP production and non-mitochondrial OCR beyond what the cell can tolerate. Meanwhile, 2DG blocks the glycolytic pathway that keloid cells are programmed to prefer. However, shikonin also inhibited scratch closure without similar impacts on metabolism. Adding further complexity, reductions in IL-6 did not correlate with any of the identified metabolic alterations. Furthermore, while most keloid histology does not indicate a strong role for neutrophils, the stark reduction in neutrophil chemokines like CXCL1 may indicate a role for neutrophils early in the tissue repair pathology such as seen in other disorders [230,231]. Overall, our results suggest that the scratch assay is a valuable research tool but the current literature limits extrapolation between research groups' findings. Despite these limitations, our results support the consideration of clinical trials investigating use of available wound healing inhibitors, most reasonably allicin, in the treatment and/or prevention of keloid scars.

Supporting information

S1 Fig. (A-D) Supernatant accumulation of HGF (A), CCL2 (B), VEGF (C) and PDGF (D) for HV-FB and KEL-FB stimulated with indicated doses of caffeine. Results are representative of three independent experiments and displayed as mean + SEM for triplicate wells. **** = $p < 0.0001$; for statistical comparison of area under the curve versus HV-FB under same stimulation conditions as determined by ANOVA with Sidak adjustment.
(PDF)

S2 Fig. (A) Representative image for HV and KEL-FB treated with 20mg/mL allicin for 12 hours. (B) Representative images for HV and KEL-FB cells treated with indicated doses of allicin and stained for vimentin (green) and DAPI (blue). Results are representative of two independent experiments.
(PDF)

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References

1. Kalluri R, Weinberg RA: The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009 Jun; 119:1420–1428. <https://doi.org/10.1172/JCI39104> PMID: 19487818
2. Yuan F-L, Sun Z-L, Feng Y, Liu S-Y, Du Y, Yu S, et al.: Epithelial-mesenchymal transition in the formation of hypertrophic scars and keloids. *J Cell Physiol* 2019 May 20; 234:21662–21669. <https://doi.org/10.1002/jcp.28830> PMID: 31106425
3. Lee Y-S, Liang Y-C, Wu P, Kulber DA, Tanabe K, Chuong C-M, et al.: STAT3 signalling pathway is implicated in keloid pathogenesis by preliminary transcriptome and open chromatin analyses. *Exp Dermatol* 2019; 28:480–484. <https://doi.org/10.1111/exd.13923> PMID: 30916811
4. Kuwahara H, Tosa M, Egawa S, Murakami M, Mohammad G, Ogawa R: Examination of epithelial mesenchymal transition in keloid tissues and possibility of keloid therapy target. *Plast Reconstr Surg Glob Open* 2016 Nov 28; 4:e1138. <https://doi.org/10.1097/GOX.0000000000001138> PMID: 27975033
5. Lim CP, Phan TT, Lim IJ, Cao X: Stat3 contributes to keloid pathogenesis via promoting collagen production, cell proliferation and migration. *Oncogene* 2006 Aug 31; 25:5416–5425. <https://doi.org/10.1038/sj.onc.1209531> PMID: 16619044
6. Gu W-J, Liu H-L: Induction of pancreatic cancer cell apoptosis, invasion, migration, and enhancement of chemotherapy sensitivity of gemcitabine, 5-FU, and oxaliplatin by hnRNP A2/B1 siRNA. *Anticancer Drugs* 2013 Jul; 24:566–576. <https://doi.org/10.1097/CAD.0b013e3283608bc5> PMID: 23525071
7. Yao Y, Yao Q-Y, Xue J-S, Tian X-Y, An Q-M, Cui L-X, et al.: Dexamethasone inhibits pancreatic tumor growth in preclinical models: Involvement of activating glucocorticoid receptor. *Toxicol Appl Pharmacol* 2020 Aug 15; 401:115118. <https://doi.org/10.1016/j.taap.2020.115118> PMID: 32619553
8. Myles IA, Anderson ED, Earland NJ, Zarembler KA, Sastalla I, Williams KW, et al.: TNF overproduction impairs epithelial staphylococcal response in hyper IgE syndrome. *J Clin Invest* 2018 Aug 1; 128:3595–3604. <https://doi.org/10.1172/JCI121486> PMID: 30035749
9. Nieto MA, Huang RY-J, Jackson RA, Thiery JP: EMT: 2016. *Cell* 2016 Jun 30; 166:21–45. <https://doi.org/10.1016/j.cell.2016.06.028> PMID: 27368099
10. Fusenig NE, Boukamp P: Multiple stages and genetic alterations in immortalization, malignant transformation, and tumor progression of human skin keratinocytes. *Mol Carcinog* 1998 Nov; 23:144–158. [https://doi.org/10.1002/\(sici\)1098-2744\(199811\)23:3<144::aid-mc3>3.0.co;2-u](https://doi.org/10.1002/(sici)1098-2744(199811)23:3<144::aid-mc3>3.0.co;2-u) PMID: 9833775
11. Vang Mouritzen M, Jenssen H: Optimized Scratch Assay for In Vitro Testing of Cell Migration with an Automated Optical Camera. *J Vis Exp* 2018 Aug 8; <https://doi.org/10.3791/57691> PMID: 30148500
12. Liarte S, Bernabé-García Á, Armero-Barranco D, Nicolás FJ: Microscopy based methods for the assessment of epithelial cell migration during in vitro wound healing. *J Vis Exp* 2018 Jan 2; <https://doi.org/10.3791/56799> PMID: 29364245
13. Pfalzgraff A, Bárcena-Varela S, Heinbockel L, Gutschmann T, Brandenburg K, Martínez-de-Tejada G, et al.: Antimicrobial endotoxin-neutralizing peptides promote keratinocyte migration via P2X7 receptor activation and accelerate wound healing in vivo. *Br J Pharmacol* 2018 Jul 20; 175:3581–3593. <https://doi.org/10.1111/bph.14425> PMID: 29947028

14. Nicolaus C, Junghanns S, Hartmann A, Murillo R, Ganzera M, Merfort I: In vitro studies to evaluate the wound healing properties of *Calendula officinalis* extracts. *J Ethnopharmacol* 2017 Jan 20; 196:94–103. <https://doi.org/10.1016/j.jep.2016.12.006> PMID: 27956358
15. Wang J, Qiu Y, Shi NW, Zhao JN, Wang YC, Jiang H, et al.: microRNA-21 mediates the TGF- β 1-induced migration of keratinocytes via targeting PTEN. *Eur Rev Med Pharmacol Sci* 2016; 20:3748–3759. PMID: 27735045
16. Dahlhoff M, Gaborit N, Bultmann S, Leonhardt H, Yarden Y, Schneider MR: CRISPR-assisted receptor deletion reveals distinct roles for ERBB2 and ERBB3 in skin keratinocytes. *FEBS J* 2017 Sep 14; 284:3339–3349. <https://doi.org/10.1111/febs.14196> PMID: 28805349
17. Denzinger M, Link A, Kurz J, Krauss S, Thoma R, Schlensak C, et al.: Keratinocyte growth factor modified messenger RNA accelerating cell proliferation and migration of keratinocytes. *Nucleic Acid Ther* 2018 Oct 30; 28:335–347. <https://doi.org/10.1089/nat.2018.0737> PMID: 30376406
18. Lee S, Kim MS, Jung S-J, Kim D, Park HJ, Cho D: ERK activating peptide, AES16-2M promotes wound healing through accelerating migration of keratinocytes. *Sci Rep* 2018 Sep 26; 8:14398. <https://doi.org/10.1038/s41598-018-32851-y> PMID: 30258088
19. Ruiz-Cañada C, Bernabé-García Á, Liarte S, Insausti CL, Angosto D, Moraleda JM, et al.: Amniotic membrane stimulates cell migration by modulating transforming growth factor- β signalling. *J Tissue Eng Regen Med* 2018; 12:808–820. <https://doi.org/10.1002/term.2501> PMID: 28621502
20. Nagae K, Uchi H, Morino-Koga S, Tanaka Y, Oda M, Furue M: Glucagon-like peptide-1 analogue liraglutide facilitates wound healing by activating PI3K/Akt pathway in keratinocytes. *Diabetes Res Clin Pract* 2018 Dec; 146:155–161. <https://doi.org/10.1016/j.diabres.2018.10.013> PMID: 30367901
21. Talati N, Kamato D, Piva TJ, Little PJ, Osman N: Thrombin promotes PAI-1 expression and migration in keratinocytes via ERK dependent Smad linker region phosphorylation. *Cell Signal* 2018 Mar 22; 47:37–43. <https://doi.org/10.1016/j.cellsig.2018.03.009> PMID: 29577978
22. Tanaka Y, Uchi H, Ito T, Furue M: Indirubin-pregnane X receptor-JNK axis accelerates skin wound healing. *Sci Rep* 2019 Dec 3; 9:18174. <https://doi.org/10.1038/s41598-019-54754-2> PMID: 31796845
23. Marin-Luevano P, Trujillo V, Rodríguez-Carlos A, González-Curiel I, Enciso-Moreno JA, Hancock REW, et al.: Induction by innate defence regulator peptide 1018 of pro-angiogenic molecules and endothelial cell migration in a high glucose environment. *Peptides* 2018 Jan 17; 101:135–144. <https://doi.org/10.1016/j.peptides.2018.01.010> PMID: 29353019
24. Dinçer T, Boz Er AB, Er İ, Toraman B, Yildiz G, Kalay E: RIPK4 suppresses the TGF- β 1 signaling pathway in HaCaT cells. *Cell Biol Int* 2020 Mar; 44:848–860. <https://doi.org/10.1002/cbin.11282> PMID: 31825120
25. Jangpromma N, Preecharram S, Srilert T, Maijaroen S, Mahakunakorn P, Nualkaew N, et al.: In Vitro and in Vivo Wound Healing Properties of Plasma and Serum from *Crocodylus siamensis* Blood. *J Microbiol Biotechnol* 2016 Jun 28; 26:1140–1147. <https://doi.org/10.4014/jmb.1601.01054> PMID: 26975771
26. Talekar YP, Apte KG, Paygude SV, Tondare PR, Parab PB: Studies on wound healing potential of polyherbal formulation using in vitro and in vivo assays. *J Ayurveda Integr Med* 2017 Jun 7; 8:73–81. <https://doi.org/10.1016/j.jaim.2016.11.007> PMID: 28601354
27. Kim J, Shin Y-K, Kim K-Y: Promotion of Keratinocyte Proliferation by Tracheloside through ERK1/2 Stimulation. *Evid Based Complement Alternat Med* 2018 Jul 26; 2018:4580627. <https://doi.org/10.1155/2018/4580627> PMID: 30147732
28. Beishenaliev A, Lim SS, Tshai KY, Khiew PS, Moh'd Sghayyar HN, Loh H-S: Fabrication and preliminary in vitro evaluation of ultraviolet-crosslinked electrospun fish scale gelatin nanofibrous scaffolds. *J Mater Sci Mater Med* 2019 May 24; 30:62. <https://doi.org/10.1007/s10856-019-6264-4> PMID: 31127374
29. Yin G, Wang Z, Wang Z, Wang X: Topical application of quercetin improves wound healing in pressure ulcer lesions. *Exp Dermatol* 2018; 27:779–786. <https://doi.org/10.1111/exd.13679> PMID: 29733461
30. Rahman MS, Islam R, Rana MM, Spitzhorn L-S, Rahman MS, Adjaye J, et al.: Characterization of burn wound healing gel prepared from human amniotic membrane and Aloe vera extract. *BMC Complement Altern Med* 2019 Jun 3; 19:115. <https://doi.org/10.1186/s12906-019-2525-5> PMID: 31159783
31. Lobine D, Cummins I, Govinden-Soulange J, Ranghoo-Sanmukhiya M, Lindsey K, Chazot PL, et al.: Medicinal Mascarene Aloes: An audit of their phytotherapeutic potential. *Fitoterapia* 2018 Jan; 124:120–126. <https://doi.org/10.1016/j.fitote.2017.10.010> PMID: 29066297
32. Zahid AA, Ahmed R, Raza Ur Rehman S, Augustine R, Tariq M, Hasan A: Nitric oxide releasing chitosan-poly (vinyl alcohol) hydrogel promotes angiogenesis in chick embryo model. *Int J Biol Macromol* 2019 Sep 1; 136:901–910. <https://doi.org/10.1016/j.ijbiomac.2019.06.136> PMID: 31229545

33. Prado LG, Arruda HS, Peixoto Araujo NM, de Oliveira Braga LE, Banzato TP, Pereira GA, et al.: Antioxidant, antiproliferative and healing properties of araticum (*Annona crassiflora* Mart.) peel and seed. *Food Res Int* 2020 Jul; 133:109168. <https://doi.org/10.1016/j.foodres.2020.109168> PMID: 32466931
34. Fazio A, La Torre C, Caroleo MC, Caputo P, Plastina P, Cione E: Isolation and Purification of Glucans from an Italian Cultivar of *Ziziphus jujuba* Mill. and In Vitro Effect on Skin Repair. *Molecules* 2020 Feb 21;25. <https://doi.org/10.3390/molecules25040968> PMID: 32098024
35. Corsuto L, Rother S, Koehler L, Bedini E, Moeller S, Schnabelrauch M, et al.: Sulfation degree not origin of chondroitin sulfate derivatives modulates keratinocyte response. *Carbohydr Polym* 2018 Jul 1; 191:53–64. <https://doi.org/10.1016/j.carbpol.2018.02.072> PMID: 29661321
36. Ojeh N, Stojadinovic O, Pastar I, Sawaya A, Yin N, Tomic-Canic M: The effects of caffeine on wound healing. *Int Wound J* 2016 Oct; 13:605–613. <https://doi.org/10.1111/iwj.12327> PMID: 25041108
37. Chorachoo J, Saeloh D, Srichana T, Amnuaitik T, Musthafa KS, Sretrirutchai S, et al.: Rhodomystone as a potential anti-proliferative and apoptosis inducing agent in HaCaT keratinocyte cells. *Eur J Pharmacol* 2016 Feb 5; 772:144–151. <https://doi.org/10.1016/j.ejphar.2015.12.005> PMID: 26687635
38. Orłowski P, Zmigradzka M, Tomaszewska E, Ransozek-Soliwoda K, Czupryn M, Antos-Bielska M, et al.: Tannic acid-modified silver nanoparticles for wound healing: the importance of size. *Int J Nanomedicine* 2018 Feb 16; 13:991–1007. <https://doi.org/10.2147/IJN.S154797> PMID: 29497293
39. Mouritzen MV, Abourayale S, Ejaz R, Ardon CB, Carvalho E, Dalgaard LT, et al.: Neurotensin, substance P, and insulin enhance cell migration. *J Pept Sci* 2018 Jul; 24:e3093. <https://doi.org/10.1002/psc.3093> PMID: 29938867
40. Blažević F, Milekić T, Romić MD, Juretić M, Pepić I, Filipović-Grčić J, et al.: Nanoparticle-mediated interplay of chitosan and melatonin for improved wound epithelialisation. *Carbohydr Polym* 2016 Aug 1; 146:445–454. <https://doi.org/10.1016/j.carbpol.2016.03.074> PMID: 27112895
41. Takada K, Komine-Aizawa S, Hirohata N, Trinh QD, Nishina A, Kimura H, et al.: Poly I:C induces collective migration of HaCaT keratinocytes via IL-8. *BMC Immunol* 2017 Apr 24; 18:19. <https://doi.org/10.1186/s12865-017-0202-3> PMID: 28438134
42. Tonello S, Rizzi M, Migliario M, Rocchetti V, Renò F: Low concentrations of neutrophil extracellular traps induce proliferation in human keratinocytes via NF-κB activation. *J Dermatol Sci* 2017 Oct; 88:110–116. <https://doi.org/10.1016/j.jdermsci.2017.05.010> PMID: 28576417
43. Teo SY, Yew MY, Lee SY, Rathbone MJ, Gan SN, Coombes AGA: In Vitro Evaluation of Novel Phenylethylamine-Loaded Alkyd Nanoemulsions Designed for Application in Topical Wound Healing. *J Pharm Sci* 2017; 106:377–384. <https://doi.org/10.1016/j.xphs.2016.06.028> PMID: 27522920
44. Kim CH, Jeong SS, Yoon JY, Yoon JU, Yu SB, Kim EJ: Remifentanyl reduced the effects of hydrogen peroxide-induced oxidative stress in human keratinocytes via autophagy. *Connect Tissue Res* 2017 Nov; 58:597–605. <https://doi.org/10.1080/03008207.2017.1285915> PMID: 28165802
45. Zhang JH, Zhang DX, Zhao LP, Yan TT, Zhang Q, Jia JZ, et al.: [Effect of rapamycin on the migration of human epidermal cell line HaCaT and its possible molecular mechanism]. *Zhonghua Shao Shang Za Zhi* 2016 Jan; 32:40–45. <https://doi.org/10.3760/cma.j.issn.1009-2587.2016.01.011> PMID: 27426069
46. Stathopoulou M-EK, Banti CN, Kourkoumelis N, Hatzidimitriou AG, Kalampounias AG, Hadjikakou SK: Silver complex of salicylic acid and its hydrogel-cream in wound healing chemotherapy. *J Inorg Biochem* 2018 Feb 3; 181:41–55. <https://doi.org/10.1016/j.jinorgbio.2018.01.004> PMID: 29407907
47. Kosol W, Kumar S, Marrero-Berríos I, Berthiaume F: Medium conditioned by human mesenchymal stromal cells reverses low serum and hypoxia-induced inhibition of wound closure. *Biochem Biophys Res Commun* 2020 Feb 5; 522:335–341. <https://doi.org/10.1016/j.bbrc.2019.11.071> PMID: 31761327
48. Ou LD, Zhang AJ, Li A, Tao SJ, Xu MM, Li Q, et al.: [Effect of human stromal vascular fraction gel on the treatment of patients with skin depressed scar and its mechanism]. *Zhonghua Shao Shang Za Zhi* 2019 Dec 20; 35:859–865.
49. Łapa A, Cresswell M, Campbell I, Jackson P, Goldmann WH, Detsch R, et al.: Ga and Ce ion-doped phosphate glass fibres with antibacterial properties and their composite for wound healing applications. *J Mater Chem B, Mater Biol Med* 2019 Nov 28; 7:6981–6993.
50. Orfanoudaki M, Hartmann A, Alilou M, Gelbrich T, Planchenault P, Derbré S, et al.: Absolute Configuration of Mycosporine-Like Amino Acids, Their Wound Healing Properties and In Vitro Anti-Aging Effects. *Mar Drugs* 2019 Dec 31;18. <https://doi.org/10.3390/md18010035> PMID: 31906052
51. You C, Li Q, Wang X, Wu P, Ho JK, Jin R, et al.: Silver nanoparticle loaded collagen/chitosan scaffolds promote wound healing via regulating fibroblast migration and macrophage activation. *Sci Rep* 2017 Sep 5; 7:10489. <https://doi.org/10.1038/s41598-017-10481-0> PMID: 28874692

52. Borges GÁ, Elias ST, da Silva SMM, Magalhães PO, Macedo SB, Ribeiro APD, et al.: In vitro evaluation of wound healing and antimicrobial potential of ozone therapy. *J Craniomaxillofac Surg* 2017 Mar; 45:364–370. <https://doi.org/10.1016/j.jcms.2017.01.005> PMID: 28169044
53. Bort A, Alvarado-Vazquez PA, Moracho-Vilrriales C, Virga KG, Gumina G, Romero-Sandoval A, et al.: Effects of JWH015 in cytokine secretion in primary human keratinocytes and fibroblasts and its suitability for topical/transdermal delivery. *Mol Pain* 2017; 13:1744806916688220. <https://doi.org/10.1177/1744806916688220> PMID: 28326930
54. Deppe J, Popp T, Egea V, Steinritz D, Schmidt A, Thiermann H, et al.: Impairment of hypoxia-induced HIF-1 α signaling in keratinocytes and fibroblasts by sulfur mustard is counteracted by a selective PHD-2 inhibitor. *Arch Toxicol* 2016 May; 90:1141–1150. <https://doi.org/10.1007/s00204-015-1549-y> PMID: 26082309
55. Hujiahemaiti M, Sun X, Zhou J, Lv H, Li X, Qi M, et al.: Effects of quercetin on human oral keratinocytes during re-epithelialization: An in vitro study. *Arch Oral Biol* 2018 Nov; 95:187–194. <https://doi.org/10.1016/j.archoralbio.2018.08.004> PMID: 30130672
56. Ferreira DW, Ulecia-Morón C, Alvarado-Vázquez PA, Cunnane K, Moracho-Vilrriales C, Grosick RL, et al.: CD163 overexpression using a macrophage-directed gene therapy approach improves wound healing in ex vivo and in vivo human skin models. *Immunobiology* 2020; 225:151862. <https://doi.org/10.1016/j.imbio.2019.10.011> PMID: 31711674
57. Zorn-Kruppa M, Volksdorf T, Ueck C, Zöller E, Reinshagen K, Ridderbusch I, et al.: Major cell biological parameters of keratinocytes are predetermined by culture medium and donor source. *Exp Dermatol* 2016 Mar; 25:242–244. <https://doi.org/10.1111/exd.12922> PMID: 26662204
58. Gunes S, Tamburaci S, Dalay MC, Deliloglu Gurhan I: In vitro evaluation of Spirulina platensis extract incorporated skin cream with its wound healing and antioxidant activities. *Pharm Biol* 2017 Dec; 55:1824–1832. <https://doi.org/10.1080/13880209.2017.1331249> PMID: 28552036
59. Moghadam SE, Ebrahimi SN, Salehi P, Moridi Farimani M, Hamburger M, Jabbarzadeh E: Wound Healing Potential of Chlorogenic Acid and Myricetin-3-O- β -Rhamnoside Isolated from Parrotia persica. *Molecules* 2017 Sep 8;22. <https://doi.org/10.3390/molecules22091501> PMID: 28885580
60. Gao S-Q, Chang C, Niu X-Q, Li L-J, Zhang Y, Gao J-Q: Topical application of Hydroxysafflor Yellow A accelerates the wound healing in streptozotocin induced T1DM rats. *Eur J Pharmacol* 2018 Mar 15; 823:72–78. <https://doi.org/10.1016/j.ejphar.2018.01.018> PMID: 29408092
61. Gunes S, Tamburaci S, Tihminlioglu F: A novel bilayer zein/MMT nanocomposite incorporated with H. perforatum oil for wound healing. *J Mater Sci Mater Med* 2019 Dec 14; 31:7. <https://doi.org/10.1007/s10856-019-6332-9> PMID: 31838599
62. Jones D, Banerjee A, Berger PZ, Gross A, McNish S, Amdur R, et al.: Inherent differences in keratinocyte function in hidradenitis suppurativa: Evidence for the role of IL-22 in disease pathogenesis. *Immunol Invest* 2018 Jan; 47:57–70. <https://doi.org/10.1080/08820139.2017.1377227> PMID: 28972431
63. Braun SA, Baran J, Schrumph H, Buhren BA, Bölke E, Homey B, et al.: Ingenol mebutate induces a tumor cell-directed inflammatory response and antimicrobial peptides thereby promoting rapid tumor destruction and wound healing. *Eur J Med Res* 2018 Sep 28; 23:45. <https://doi.org/10.1186/s40001-018-0343-8> PMID: 30266096
64. Riis S, Newman R, Ipek H, Andersen JI, Kuninger D, Boucher S, et al.: Hypoxia enhances the wound-healing potential of adipose-derived stem cells in a novel human primary keratinocyte-based scratch assay. *Int J Mol Med* 2017 Mar; 39:587–594. <https://doi.org/10.3892/ijmm.2017.2886> PMID: 28204820
65. Zomer HD, Varela GKDS, Delben PB, Heck D, Jeremias T da S, Trentin AG: In vitro comparative study of human mesenchymal stromal cells from dermis and adipose tissue for application in skin wound healing. *J Tissue Eng Regen Med* 2019 Mar 21; 13:729–741. <https://doi.org/10.1002/term.2820> PMID: 30773827
66. Huang C, Qian SL, Sun LY, Cheng B: Light-Emitting Diode Irradiation (640 nm) Regulates Keratinocyte Migration and Cytoskeletal Reorganization Via Hypoxia-Inducible Factor-1 α . *Photomed Laser Surg* 2016 Aug; 34:313–320. <https://doi.org/10.1089/pho.2015.4077> PMID: 27244052
67. Mammadova A, Carels CEL, Zhou J, Gilissen C, Helmich MPAC, Bian Z, et al.: Deregulated adhesion program in palatal keratinocytes of orofacial cleft patients. *Genes (Basel)* 2019 Oct 23;10. <https://doi.org/10.3390/genes10110836> PMID: 31652793
68. Stewart DC, Serrano PN, Rubiano A, Yokosawa R, Sandler J, Mukhtar M, et al.: Unique behavior of dermal cells from regenerative mammal, the African Spiny Mouse, in response to substrate stiffness. *J Biomech* 2018 Nov 16; 81:149–154. <https://doi.org/10.1016/j.jbiomech.2018.10.005> PMID: 30361050
69. Hwang K-H, Kim J-H, Park EY, Cha S-K: An effective range of polydeoxyribonucleotides is critical for wound healing quality. *Mol Med Rep* 2018 Dec; 18:5166–5172. <https://doi.org/10.3892/mmr.2018.9539> PMID: 30320361

70. Monsuur HN, Boink MA, Weijers EM, Roffel S, Breetveld M, Gefen A, et al.: Methods to study differences in cell mobility during skin wound healing in vitro. *J Biomech* 2016 May 24; 49:1381–1387. <https://doi.org/10.1016/j.jbiomech.2016.01.040> PMID: 26903411
71. Yamashita T, Asano Y, Saigusa R, Taniguchi T, Nakamura K, Miura S, et al.: Increased expression of aquaporin-1 in dermal fibroblasts and dermal microvascular endothelial cells possibly contributes to skin fibrosis and edema in patients with systemic sclerosis. *J Dermatol Sci* 2019 Jan; 93:24–32. <https://doi.org/10.1016/j.jdermsci.2018.09.007> PMID: 30270117
72. Markiewicz M, Panneerselvam K, Marks N: Role of Klotho in migration and proliferation of human dermal microvascular endothelial cells. *Microvasc Res* 2016 May 31; 107:76–82. <https://doi.org/10.1016/j.mvr.2016.05.005> PMID: 27260080
73. Doğan A: Apelin receptor (Aplnr) signaling promotes fibroblast migration. *Tissue Cell* 2019 Feb; 56:98–106. <https://doi.org/10.1016/j.tice.2019.01.003> PMID: 30736911
74. Xu C, Bentinger M, Savu O, Moshfegh A, Sunkari V, Dallner G, et al.: Mono-epoxy-tocotrienol- α enhances wound healing in diabetic mice and stimulates in vitro angiogenesis and cell migration. *J Diabetes Complicat* 2017; 31:4–12. <https://doi.org/10.1016/j.jdiacomp.2016.10.010> PMID: 27839658
75. Letsiou S, Félix RC, Cardoso JCR, Anjos L, Mestre AL, Gomes HL, et al.: Cartilage acidic protein 1 promotes increased cell viability, cell proliferation and energy metabolism in primary human dermal fibroblasts. *Biochimie* 2020 Feb 18; 171–172:72–78. <https://doi.org/10.1016/j.biochi.2020.02.008> PMID: 32084494
76. Chisholm J, Gareau AJ, Byun S, Paletz JL, Tang D, Williams J, et al.: Effect of compound 21, a selective angiotensin II type 2 receptor agonist, in a murine xenograft model of Dupuytren disease. *Plast Reconstr Surg* 2017 Nov; 140:686e–696e. <https://doi.org/10.1097/PRS.0000000000003800> PMID: 29068929
77. Li Y, Shi S, Gao J, Han S, Wu X, Jia Y, et al.: Cryptotanshinone downregulates the profibrotic activities of hypertrophic scar fibroblasts and accelerates wound healing: A potential therapy for the reduction of skin scarring. *Biomed Pharmacother* 2016 May; 80:80–86. <https://doi.org/10.1016/j.biopha.2016.03.006> PMID: 27133042
78. Priyanka P, Arun AB, Ashwini P, Rekha PD: Functional and cell proliferative properties of an exopolysaccharide produced by *Nitratireductor* sp. PRIM-31. *Int J Biol Macromol* 2016 Apr; 85:400–404. <https://doi.org/10.1016/j.ijbiomac.2015.12.091> PMID: 26772917
79. Gothai S, Arulselvan P, Tan WS, Fakurazi S: Wound healing properties of ethyl acetate fraction of *Moringa oleifera* in normal human dermal fibroblasts. *J Intercolt Ethnopharmacol* 2016 Feb 8; 5:1–6. <https://doi.org/10.5455/jice.20160201055629> PMID: 27069722
80. Alemzadeh E, Oryan A: Effectiveness of a *Crocus sativus* Extract on Burn Wounds in Rats. *Planta Med* 2018 Nov; 84:1191–1200. <https://doi.org/10.1055/a-0631-3620> PMID: 29791931
81. Tito A, Minale M, Riccio S, Grieco F, Colucci MG, Apone F: A *Triticum vulgare* Extract Exhibits Regenerating Activity During the Wound Healing Process. *Clin Cosmet Investig Dermatol* 2020 Jan 14; 13:21–30. <https://doi.org/10.2147/CCID.S216391> PMID: 32021367
82. Sano K, Kawanobe H, Someya T: Effect of cupuassu butter on human skin cells. *Data Brief* 2018 Dec; 21:516–521. <https://doi.org/10.1016/j.dib.2018.10.026> PMID: 30370321
83. Moghadam SE, Moridi Farimani M, Soroury S, Ebrahimi SN, Jabbarzadeh E: Hypermongone C Accelerates Wound Healing through the Modulation of Inflammatory Factors and Promotion of Fibroblast Migration. *Molecules* 2019 May 27;24. <https://doi.org/10.3390/molecules24102022> PMID: 31137844
84. Soib HH, Ismail HF, Husin F, Abu Bakar MH, Yaakob H, Sarmidi MR: Bioassay-Guided Different Extraction Techniques of *Carica papaya* (Linn.) Leaves on In Vitro Wound-Healing Activities. *Molecules* 2020 Jan 24;25. <https://doi.org/10.3390/molecules25030517> PMID: 31991676
85. Sun H-H, Wang J-C, Feng X-M, Zhu S-L, Cai J: Allicin inhibits proliferation and promotes apoptosis of human epidural scar fibroblasts. *World Neurosurg* 2020 Apr; 136:e460–e468. <https://doi.org/10.1016/j.wneu.2020.01.031> PMID: 31953094
86. Rembe J-D, Fromm-Dornieden C, Stuermer EK: Effects of vitamin B complex and vitamin C on human skin cells: is the perceived effect measurable? *Adv Skin Wound Care* 2018 May; 31:225–233. <https://doi.org/10.1097/01.ASW.0000531351.85866.d9> PMID: 29672394
87. Pinto BI, Lujan OR, Ramos SA, Propper CR, Kellar RS: Estrogen mitigates the negative effects of arsenic contamination in an in vitro wound model. *Appl In Vitro Toxicol* 2018 Mar 1; 4:24–29.
88. Xu P, Chen J, Tan C, Lai R-S, Min Z-S: Pimecrolimus increases the melanogenesis and migration of melanocytes in vitro. *Korean J Physiol Pharmacol* 2017 May; 21:287–292. <https://doi.org/10.4196/kjpp.2017.21.3.287> PMID: 28461770
89. Pinto BI, Cruz ND, Lujan OR, Propper CR, Kellar RS: In vitro scratch assay to demonstrate effects of arsenic on skin cell migration. *J Vis Exp* 2019 Feb 23; <https://doi.org/10.3791/58838> PMID: 30855562

90. Boink MA, Roffel S, Nazmi K, van Montfrans C, Bolscher JGM, Gefen A, et al.: The influence of chronic wound extracts on inflammatory cytokine and histatin stability. *PLoS One* 2016 Mar 28; 11:e0152613. <https://doi.org/10.1371/journal.pone.0152613> PMID: 27018788
91. Ling C, Nishimoto K, Rolfs Z, Smith LM, Frey BL, Welham NV: Differentiated fibrocytes assume a functional mesenchymal phenotype with regenerative potential. *Sci Adv* 2019 May 8; 5:eaav7384. <https://doi.org/10.1126/sciadv.aav7384> PMID: 31086819
92. Pinto BI, Tabor AJ, Stearns DM, Diller RB, Kellar RS: A Bench-Top In Vitro Wound Assay to Demonstrate the Effects of Platelet-Rich Plasma and Depleted Uranium on Dermal Fibroblast Migration. *Appl In Vitro Toxicol* 2016 Sep 1; 2:151–156. <https://doi.org/10.1089/aivt.2016.0001> PMID: 28971114
93. Choi JS, Cho WL, Choi YJ, Kim JD, Park H-A, Kim SY, et al.: Functional recovery in photo-damaged human dermal fibroblasts by human adipose-derived stem cell extracellular vesicles. *J Extracell Vesicles* 2019 Jan 20; 8:1565885. <https://doi.org/10.1080/20013078.2019.1565885> PMID: 30719241
94. Ramenzoni LL, Weber FE, Attin T, Schmidlin PR: Cerium chloride application promotes wound healing and cell proliferation in human foreskin fibroblasts. *Materials (Basel)* 2017 May 24; 10. <https://doi.org/10.3390/ma10060573> PMID: 28772932
95. Chinnasamy G, Chandrasekharan S, Bhatnagar S: Biosynthesis of Silver Nanoparticles from *Melia azedarach*: Enhancement of Antibacterial, Wound Healing, Antidiabetic and Antioxidant Activities. *Int J Nanomedicine* 2019 Dec 11; 14:9823–9836. <https://doi.org/10.2147/IJN.S231340> PMID: 31849471
96. Bagheri E, Saremi K, Hajiaghaalipour F, Faraj FL, Ali HM, Abdulla MA, et al.: Synthesis of Novel Derivatives of Quinazoline Schiff base Compound Promotes Epithelial Wound Healing. *Curr Pharm Des* 2018; 24:1395–1404. <https://doi.org/10.2174/1381612824666180130124308> PMID: 29384057
97. Mirzahosseini M, Khorsandi K, Hosseinzadeh R, Ghazaeian M, Shahidi FK: Antimicrobial photodynamic and wound healing activity of curcumin encapsulated in silica nanoparticles. *Photodiagnosis Photodyn Ther* 2020 Mar; 29:101639. <https://doi.org/10.1016/j.pdpdt.2019.101639> PMID: 31899378
98. Anumanthan G, Gupta S, Fink MK, Hesemann NP, Bowles DK, McDaniel LM, et al.: KCa3.1 ion channel: A novel therapeutic target for corneal fibrosis. *PLoS One* 2018 Mar 19; 13:e0192145. <https://doi.org/10.1371/journal.pone.0192145> PMID: 29554088
99. Pullisaar H, Colaianni G, Lian A-M, Vandevska-Radunovic V, Grano M, Reseland JE: Irisin promotes growth, migration and matrix formation in human periodontal ligament cells. *Arch Oral Biol* 2020 Mar; 111:104635. <https://doi.org/10.1016/j.archoralbio.2019.104635> PMID: 31869727
100. Knüppel L, Heinzelmann K, Lindner M, Hatz R, Behr J, Eickelberg O, et al.: FKBP10 regulates lung fibroblast migration via collagen VI synthesis. *Respir Res* 2018 Apr 19; 19:67. <https://doi.org/10.1186/s12931-018-0768-1> PMID: 29673351
101. Baek A, Kim Y, Lee JW, Lee SC, Cho S-R: Effect of polydeoxyribonucleotide on angiogenesis and wound healing in an in vitro model of osteoarthritis. *Cell Transplant* 2018 Oct 12; 27:963689718804130.
102. Song ZC, Li S, Dong JC, Sun MJ, Zhang XL, Shu R: Enamel matrix proteins regulate hypoxia-induced cellular biobehavior and osteogenic differentiation in human periodontal ligament cells. *Biotech Biochem* 2017 Dec 5; 92:606–618. <https://doi.org/10.1080/10520295.2017.1370131> PMID: 29205072
103. Zhang W, Hsu P, Zhong B, Guo S, Zhang C, Wang Y, et al.: MiR-34a Enhances Chondrocyte Apoptosis, Senescence and Facilitates Development of Osteoarthritis by Targeting DLL1 and Regulating PI3K/AKT Pathway. *Cell Physiol Biochem* 2018 Jul 26; 48:1304–1316. <https://doi.org/10.1159/000492090> PMID: 30048987
104. Gottipamula S, Sundarrajan S, Chokalingam K, Sridhar KN: The effect of human amniotic epithelial cells on urethral stricture fibroblasts. *J Clin Transl Res* 2019 Sep 8; 5:44–49. PMID: 31579841
105. Park S-K, Jin Y-D, Park Y-K, Yeon S-H, Xu J, Han R-N, et al.: IL-25-induced activation of nasal fibroblast and its association with the remodeling of chronic rhinosinusitis with nasal polyposis. *PLoS One* 2017 Aug 3; 12:e0181806. <https://doi.org/10.1371/journal.pone.0181806> PMID: 28771607
106. Sardone F, Santi S, Tagliavini F, Traina F, Merlini L, Squarzone S, et al.: Collagen VI-NG2 axis in human tendon fibroblasts under conditions mimicking injury response. *Matrix Biol* 2016 Mar 2; 55:90–105. <https://doi.org/10.1016/j.matbio.2016.02.012> PMID: 26944560
107. Saini S, Liu T, Yoo J: TNF- α stimulates colonic myofibroblast migration via COX-2 and Hsp27. *J Surg Res* 2016 Apr 25; 204:145–152. <https://doi.org/10.1016/j.jss.2016.04.034> PMID: 27451881
108. Salgado RM, Cruz-Castañeda O, Elizondo-Vázquez F, Pat L, De la Garza A, Cano-Colín S, et al.: Maltodextrin/ascorbic acid stimulates wound closure by increasing collagen turnover and TGF- β 1 expression in vitro and changing the stage of inflammation from chronic to acute in vivo. *J Tissue Viability* 2017 May; 26:131–137. <https://doi.org/10.1016/j.jtv.2017.01.004> PMID: 28162862
109. Bianchi SE, Machado BEK, da Silva MGC, da Silva MMA, Bosco LD, Marques MS, et al.: Coumestrol/hydroxypropyl- β -cyclodextrin association incorporated in hydroxypropyl methylcellulose hydrogel

- exhibits wound healing effect: in vitro and in vivo study. *Eur J Pharm Sci* 2018 Jul 1; 119:179–188. <https://doi.org/10.1016/j.ejps.2018.04.019> PMID: 29665401
110. Nguyen PA, Pham TAV: Effects of platelet-rich plasma on human gingival fibroblast proliferation and migration in vitro. *J Appl Oral Sci* 2018 Jul 10; 26:e20180077. <https://doi.org/10.1590/1678-7757-2018-0077> PMID: 29995149
 111. Steller D, Herbst N, Pries R, Juhl D, Hakim SG: Positive impact of Platelet-rich plasma and Platelet-rich fibrin on viability, migration and proliferation of osteoblasts and fibroblasts treated with zoledronic acid. *Sci Rep* 2019 Jun 5; 9:8310. <https://doi.org/10.1038/s41598-019-43798-z> PMID: 31165745
 112. Huang M, Wang L, Zeng S, Qiu Q, Zou Y, Shi M, et al.: Indirubin inhibits the migration, invasion, and activation of fibroblast-like synoviocytes from rheumatoid arthritis patients. *Inflamm Res* 2017 May; 66:433–440. <https://doi.org/10.1007/s00011-017-1027-5> PMID: 28265680
 113. Akdeniz SS, Beyler E, Korkmaz Y, Yurtcu E, Ates U, Araz K, et al.: The effects of ozone application on genotoxic damage and wound healing in bisphosphonate-applied human gingival fibroblast cells. *Clin Oral Invest* 2018 Mar; 22:867–873. <https://doi.org/10.1007/s00784-017-2163-6> PMID: 28699091
 114. Li N, Xu Q, Liu Q, Pan D, Jiang Y, Liu M, et al.: Leonurine attenuates fibroblast-like synoviocyte-mediated synovial inflammation and joint destruction in rheumatoid arthritis. *Rheumatology* 2017 Aug 1; 56:1417–1427. <https://doi.org/10.1093/rheumatology/kex142> PMID: 28431044
 115. Addis R, Cruciani S, Santaniello S, Bellu E, Sarais G, Ventura C, et al.: Fibroblast proliferation and migration in wound healing by phytochemicals: evidence for a novel synergic outcome. *Int J Med Sci* 2020 Apr 7; 17:1030–1042. <https://doi.org/10.7150/ijms.43986> PMID: 32410832
 116. Liu Y, Gao W: [Interleukin-37 inhibits proliferation, migration and induces apoptosis of rheumatoid arthritis fibroblast-like synoviocytes (RAFLS) by inhibiting STAT3]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2020 Mar; 36:236–241. PMID: 32389171
 117. Petito V, Lopetuso LR, Arena V, Stigliano E, Boninsegna A, Bibbò S, et al.: Direct effect of infliximab on intestinal mucosa sustains mucosal healing: exploring new mechanisms of action. *Dig Liver Dis* 2016 Apr; 48:391–398. <https://doi.org/10.1016/j.dld.2015.12.008> PMID: 26804809
 118. Chu E, Saini S, Liu T, Yoo J: Bradykinin stimulates protein kinase D-mediated colonic myofibroblast migration via cyclooxygenase-2 and heat shock protein 27. *J Surg Res* 2017; 209:191–198. <https://doi.org/10.1016/j.jss.2016.10.014> PMID: 28032559
 119. Dittfeld C, Bienger K, Andres J, Plötze K, Jannasch A, Waldow T, et al.: Characterization of thoracic fat depots—expression of adipokines and remodeling factors and impact of adipocyte conditioned media in fibroblast scratch assays. *Clin Hemorheol Microcirc* 2018; 70:267–280. <https://doi.org/10.3233/CH-170341> PMID: 30507567
 120. Garcia-Carbonell R, Divakaruni AS, Lodi A, Vicente-Suarez I, Saha A, Cheroutre H, et al.: Critical Role of Glucose Metabolism in Rheumatoid Arthritis Fibroblast-like Synoviocytes. *Arthritis Rheumatol* 2016; 68:1614–1626. <https://doi.org/10.1002/art.39608> PMID: 26815411
 121. Tao Y, Chen Q, Zhao C, Yang X, Cun Q, Yang W, et al.: The in vitro anti-fibrotic effect of Pirfenidone on human pterygium fibroblasts is associated with down-regulation of autocrine TGF- β and MMP-1. *Int J Med Sci* 2020 Mar 5; 17:734–744. <https://doi.org/10.7150/ijms.43238> PMID: 32218695
 122. Giraud T, Jeanneau C, Bergmann M, Laurent P, About I: Tricalcium silicate capping materials modulate pulp healing and inflammatory activity in vitro. *J Endod* 2018 Nov; 44:1686–1691. <https://doi.org/10.1016/j.joen.2018.06.009> PMID: 30217466
 123. Ng-Blichfeldt J-P, Alçada J, Montero MA, Dean CH, Griesenbach U, Griffiths MJ, et al.: Deficient retinoid-driven angiogenesis may contribute to failure of adult human lung regeneration in emphysema. *Thorax* 2017 Jan 13; 72:510–521. <https://doi.org/10.1136/thoraxjnl-2016-208846> PMID: 28087752
 124. Gueldner J, Zhang F, Zechmann B, Bruce ED: Evaluating a novel oxygenating therapeutic for its potential use in the advancement of wound healing. *Toxicol In Vitro* 2017 Sep; 43:62–68. <https://doi.org/10.1016/j.tiv.2017.06.005> PMID: 28599845
 125. Zhao Y, Wang Q, Jin Y, Li Y, Nie C, Huang P, et al.: Discovery and Characterization of a High-Affinity Small Peptide Ligand, H1, Targeting FGFR2IIIc for Skin Wound Healing. *Cell Physiol Biochem* 2018 Sep 7; 49:1033–1048. <https://doi.org/10.1159/000493287> PMID: 30196288
 126. Cuppini M, Zatta KC, Mestieri LB, Grecca FS, Leitune VCB, Guterres SS, et al.: Antimicrobial and anti-inflammatory drug-delivery systems at endodontic reparative material: Synthesis and characterization. *Dent Mater* 2019 Jan 11; 35:457–467. <https://doi.org/10.1016/j.dental.2019.01.002> PMID: 30642636
 127. de Christo Scherer MM, Marques FM, Figueira MM, Peisino MCO, Schmitt EFP, Kondratyuk TP, et al.: Wound healing activity of terpinolene and α -phellandrene by attenuating inflammation and oxidative stress in vitro. *J Tissue Viability* 2019 May; 28:94–99. <https://doi.org/10.1016/j.jtv.2019.02.003> PMID: 30792116

128. Parkar H, Aiyegoro OA, Steenkamp P, Steenkamp V: Extracts of *Terminalia sericea* Enhance Cell Migratory Activity of Endothelial Hybrid and Fibroblast Cells In Vitro. *Planta Med* 2017 Dec; 83:1397–1404. <https://doi.org/10.1055/s-0043-113324> PMID: 28770552
129. Pereira LOM, Vilegas W, Tangerina MMP, Arunachalam K, Balogun SO, Orlandi-Mattos PE, et al.: Lafoensia pacari A. St.-Hil.: Wound healing activity and mechanism of action of standardized hydro-ethanolic leaves extract. *J Ethnopharmacol* 2018 Jun 12; 219:337–350. <https://doi.org/10.1016/j.jep.2018.02.038> PMID: 29501673
130. Guidoni M, de Christo Scherer MM, Figueira MM, Schmitt EFP, de Almeida LC, Scherer R, et al.: Fatty acid composition of vegetable oil blend and in vitro effects of pharmacotherapeutical skin care applications. *Braz J Med Biol Res* 2019 Feb 14; 52:e8209. <https://doi.org/10.1590/1414-431X20188209> PMID: 30785481
131. Kleeschulte S, Jerrentrup J, Gorski D, Schmitt J, Fender AC: Evidence for functional PAR-4 thrombin receptor expression in cardiac fibroblasts and its regulation by high glucose: PAR-4 in cardiac fibroblasts. *Int J Cardiol* 2018 Feb 1; 252:163–166. <https://doi.org/10.1016/j.ijcard.2017.10.019> PMID: 29249425
132. Zhang X, Kang X, Jin L, Bai J, Liu W, Wang Z: Stimulation of wound healing using bioinspired hydrogels with basic fibroblast growth factor (bFGF). *Int J Nanomedicine* 2018 Jul 4; 13:3897–3906. <https://doi.org/10.2147/IJN.S168998> PMID: 30013343
133. Ito H, Kanbe A, Sakai H, Seishima M: Activation of NLRP3 signalling accelerates skin wound healing. *Exp Dermatol* 2018; 27:80–86. <https://doi.org/10.1111/exd.13441> PMID: 28887870
134. Li F, Yao J, Hao Q, Duan Z: miRNA-103 promotes chondrocyte apoptosis by down-regulation of Sphingosine kinase-1 and ameliorates PI3K/AKT pathway in osteoarthritis. *Biosci Rep* 2019 Oct 30;39. <https://doi.org/10.1042/BSR20191255> PMID: 31652455
135. Uchinaka A, Kawaguchi N, Ban T, Hamada Y, Mori S, Maeno Y, et al.: Evaluation of dermal wound healing activity of synthetic peptide SVVYGLR. *Biochem Biophys Res Commun* 2017 Sep 23; 491:714–720. <https://doi.org/10.1016/j.bbrc.2017.07.124> PMID: 28751213
136. Vittorazzi C, Endringer DC, Andrade TU de, Scherer R, Fronza M: Antioxidant, antimicrobial and wound healing properties of *Struthanthus vulgaris*. *Pharm Biol* 2016; 54:331–337. <https://doi.org/10.3109/13880209.2015.1040515> PMID: 25915104
137. Mizutani Y, Kanbe A, Ito H, Seishima M: Activation of STING signaling accelerates skin wound healing. *J Dermatol Sci* 2020 Jan; 97:21–29. <https://doi.org/10.1016/j.jdermsci.2019.11.008> PMID: 31813660
138. Hu X, Luo J, Lai H, Li M, Zheng X, Nie T, et al.: Knockdown of Trnau1ap inhibits the proliferation and migration of NIH3T3, JEG-3 and Bewo cells via the PI3K/Akt signaling pathway. *Biochem Biophys Res Commun* 2018 Sep 5; 503:521–527. <https://doi.org/10.1016/j.bbrc.2018.05.065> PMID: 29758194
139. Li X-J, Huang F-Z, Wan Y, Li Y-S, Zhang WK, Xi Y, et al.: Lipopolysaccharide Stimulated the Migration of NIH3T3 Cells Through a Positive Feedback Between β -Catenin and COX-2. *Front Pharmacol* 2018 Dec 19; 9:1487. <https://doi.org/10.3389/fphar.2018.01487> PMID: 30618773
140. Xuan Y, Chi L, Tian H, Cai W, Sun C, Wang T, et al.: The activation of the NF- κ B-JNK pathway is independent of the PI3K-Rac1-JNK pathway involved in the bFGF-regulated human fibroblast cell migration. *J Dermatol Sci* 2016 Apr; 82:28–37. <https://doi.org/10.1016/j.jdermsci.2016.01.003> PMID: 26829882
141. França AJVB du V, De Faveri R, Nunes R, Steimbach VMB, Santin JR, Quintão NLM: The role of kinins in the proliferation of fibroblast primed with TNF in scratch wound assay: Kinins and cell proliferation. *Int Immunopharmacol* 2018 Dec; 65:23–28. <https://doi.org/10.1016/j.intimp.2018.09.036> PMID: 30268800
142. Bayrami Z, Hajiaghaee R, Khalighi-Sigaroodi F, Rahimi R, Farzaei MH, Hodjat M, et al.: Bio-guided fractionation and isolation of active component from *Tragopogon graminifolius* based on its wound healing property. *J Ethnopharmacol* 2018 Nov 15; 226:48–55. <https://doi.org/10.1016/j.jep.2018.08.002> PMID: 30096362
143. Park YR, Sultan MT, Park HJ, Lee JM, Ju HW, Lee OJ, et al.: NF- κ B signaling is key in the wound healing processes of silk fibroin. *Acta Biomater* 2018; 67:183–195. <https://doi.org/10.1016/j.actbio.2017.12.006> PMID: 29242162
144. Lin X, Chen Y, Jin H, Zhao Q, Liu C, Li R, et al.: Collagen Extracted from Bigeye Tuna (*Thunnus obesus*) Skin by Isoelectric Precipitation: Physicochemical Properties, Proliferation, and Migration Activities. *Mar Drugs* 2019 May 1;17. <https://doi.org/10.3390/md17050261> PMID: 31052462
145. Sarkhail P, Navidpour L, Rahimifard M, Hosseini NM, Souri E: Bioassay-guided fractionation and identification of wound healing active compound from *Pistacia vera* L. hull extract. *J Ethnopharmacol* 2020 Feb 10; 248:112335. <https://doi.org/10.1016/j.jep.2019.112335> PMID: 31654800

146. Hu X, Saravanakumar K, Jin T, Wang M-H: Mycosynthesis, characterization, anticancer and antibacterial activity of silver nanoparticles from endophytic fungus *Talaromyces purpureogenus*. *Int J Nanomedicine* 2019 May 9; 14:3427–3438. <https://doi.org/10.2147/IJN.S200817> PMID: 31190801
147. Chen J, Jayachandran M, Xu B, Yu Z: Sea bass (*Lateolabrax maculatus*) accelerates wound healing: A transition from inflammation to proliferation. *J Ethnopharmacol* 2019 May 23; 236:263–276. <https://doi.org/10.1016/j.jep.2019.03.012> PMID: 30862523
148. Tasić-Kostov M, Arsić I, Pavlović D, Stojanović S, Najman S, Naumović S, et al.: Towards a modern approach to traditional use: in vitro and in vivo evaluation of *Alchemilla vulgaris* L. gel wound healing potential. *J Ethnopharmacol* 2019 Jun 28; 238:111789. <https://doi.org/10.1016/j.jep.2019.03.016> PMID: 30904703
149. Bolla SR, Mohammed Al-Subaie A, Yousuf Al-Jindan R, Papayya Balakrishna J, Kanchi Ravi P, Veer-araghavan VP, et al.: In vitro wound healing potency of methanolic leaf extract of *Aristolochia saccata* is possibly mediated by its stimulatory effect on collagen-1 expression. *Heliyon* 2019 May 20; 5:e01648. <https://doi.org/10.1016/j.heliyon.2019.e01648> PMID: 31193473
150. Kumawat MK, Thakur M, Gurung RB, Srivastava R: Graphene quantum dots for cell proliferation, nucleus imaging, and photoluminescent sensing applications. *Sci Rep* 2017 Nov 20; 7:15858. <https://doi.org/10.1038/s41598-017-16025-w> PMID: 29158566
151. Mazutti da Silva SM, Rezende Costa CR, Martins Gelfuso G, Silva Guerra EN, de Medeiros Nóbrega YK, Gomes SM, et al.: Wound Healing Effect of Essential Oil Extracted from *Eugenia dysenterica* DC (Myrtaceae) Leaves. *Molecules* 2018 Dec 20;24. <https://doi.org/10.3390/molecules24010002> PMID: 30577426
152. Yousefi K, Hamedeyazdan S, Hodaei D, Lotfipour F, Baradaran B, Orangi M, et al.: An in vitro ethnopharmacological study on *Prangos ferulacea*: a wound healing agent. *Bioimpacts* 2017 Apr 26; 7:75–82. <https://doi.org/10.15171/bi.2017.10> PMID: 28752071
153. Steffy K, Shanthi G, Maroky AS, Selvakumar S: Potential bactericidal activity of *S. nux-vomica*-ZnO nanocomposite against multidrug-resistant bacterial pathogens and wound-healing properties. *J Trace Elem Med Biol* 2018 Dec; 50:229–239. <https://doi.org/10.1016/j.jtemb.2018.07.009> PMID: 30262284
154. Che Zain MS, Lee SY, Sarian MN, Fakurazi S, Shaari K: In Vitro Wound Healing Potential of Flavonoid C-Glycosides from Oil Palm (*Elaeis guineensis* Jacq.) Leaves on 3T3 Fibroblast Cells. *Antioxidants (Basel)* 2020 Apr 17;9. <https://doi.org/10.3390/antiox9040326> PMID: 32316665
155. Freiesleben SH, Soelberg J, Nyberg NT, Jäger AK: Determination of the wound healing potentials of medicinal plants historically used in Ghana. *Evid Based Complement Alternat Med* 2017 Feb 23; 2017:9480791. <https://doi.org/10.1155/2017/9480791> PMID: 28326125
156. Sung T-J, Wang Y-Y, Liu K-L, Chou C-H, Lai P-S, Hsieh C-W: *Pholiota nameko* Polysaccharides Promotes Cell Proliferation and Migration and Reduces ROS Content in H₂O₂-Induced L929 Cells. *Antioxidants (Basel)* 2020 Jan 10;9. <https://doi.org/10.3390/antiox9010065> PMID: 31936888
157. Costa NN, de Faria Lopes L, Ferreira DF, de Prado EML, Severi JA, Resende JA, et al.: Polymeric films containing pomegranate peel extract based on PVA/starch/PAA blends for use as wound dressing: In vitro analysis and physicochemical evaluation. *Mater Sci Eng C Mater Biol Appl* 2020 Apr; 109:110643. <https://doi.org/10.1016/j.msec.2020.110643> PMID: 32229007
158. Sandhya J, Veeralakshmi S, Kalaiselvam S: Tripolyphosphate crosslinked *Triticum aestivum* (wheat-grass) functionalized antimicrobial chitosan: Ameliorating effect on physicochemical, mechanical, in vitro cytocompatibility and cell migration properties. *J Biomol Struct Dyn* 2020 Mar 12;1–10. <https://doi.org/10.1080/07391102.2020.1736160> PMID: 32107986
159. Figueiredo F de F, Cechinel Filho V, Damazo AS, Arunachalam K, Colodel EM, Ribeiro M, et al.: *Sorocea guilleminiana* Gaudich.: Wound healing activity, action mechanisms, and chemical characterization of the leaf infusion. *J Ethnopharmacol* 2020 Feb 10; 248:112307. <https://doi.org/10.1016/j.jep.2019.112307> PMID: 31629026
160. Nishikai-Yan Shen T, Kanazawa S, Kado M, Okada K, Luo L, Hayashi A, et al.: Interleukin-6 stimulates Akt and p38 MAPK phosphorylation and fibroblast migration in non-diabetic but not diabetic mice. *PLoS One* 2017 May 23; 12:e0178232. <https://doi.org/10.1371/journal.pone.0178232> PMID: 28542434
161. Zhao L, Xu Y, Tao L, Yang Y, Shen X, Li L, et al.: Oxymatrine Inhibits Transforming Growth Factor β 1 (TGF- β 1)-Induced Cardiac Fibroblast-to-Myofibroblast Transformation (FMT) by Mediating the Notch Signaling Pathway In Vitro. *Med Sci Monit* 2018 Sep 9; 24:6280–6288. <https://doi.org/10.12659/MSM.910142> PMID: 30196308
162. Looney AP, Bhattacharya M: Fibroblast Gap-closure Assay-Microscopy-based in vitro Assay Measuring the Migration of Murine Fibroblasts. *Bio Protoc* 2019 Aug 20;9. <https://doi.org/10.21769/BioProtoc.3333> PMID: 31531389

163. Blattes GBF, Mestieri LB, Böttcher DE, Fossati ACM, Montagner F, Grecca FS: Cell migration, viability and tissue reaction of calcium hypochlorite based-solutions irrigants: An in vitro and in vivo study. *Arch Oral Biol* 2017 Jan; 73:34–39. <https://doi.org/10.1016/j.archoralbio.2016.08.037> PMID: 27658125
164. Giri VP, Pandey S, Kumari M, Paswan SK, Tripathi A, Srivastava M, et al.: Biogenic silver nanoparticles as a more efficient contrivance for wound healing acceleration than common antiseptic medicine. *FEMS Microbiol Lett* 2019 Aug 1;366. <https://doi.org/10.1093/femsle/fnz201> PMID: 31580434
165. Kobayashi H, Ebisawa K, Kambe M, Kasai T, Suga H, Nakamura K, et al.: <Editors' Choice> Effects of exosomes derived from the induced pluripotent stem cells on skin wound healing. *Nagoya J Med Sci* 2018 May; 80:141–153. <https://doi.org/10.18999/nagjms.80.2.141> PMID: 29915432
166. Lu Y, Wang Y, Zhang J, Hu X, Yang Z, Guo Y, et al.: In-situ doping of a conductive hydrogel with low protein absorption and bacterial adhesion for electrical stimulation of chronic wounds. *Acta Biomater* 2019 Apr 15; 89:217–226. <https://doi.org/10.1016/j.actbio.2019.03.018> PMID: 30862548
167. Cheng Y, Hu Z, Zhao Y, Zou Z, Lu S, Zhang B, et al.: Sponges of Carboxymethyl Chitosan Grafted with Collagen Peptides for Wound Healing. *Int J Mol Sci* 2019 Aug 9;20. <https://doi.org/10.3390/ijms20163890> PMID: 31404991
168. Tan G, Onur MA: Cellular localization and biological effects of 20nm-gold nanoparticles. *J Biomed Mater Res A* 2018 Mar 10; 106:1708–1721. <https://doi.org/10.1002/jbm.a.36373> PMID: 29468810
169. Tan SS, Yeo XY, Liang ZC, Sethi SK, Tay SSW: Stromal vascular fraction promotes fibroblast migration and cellular viability in a hyperglycemic microenvironment through up-regulation of wound healing cytokines. *Exp Mol Pathol* 2018 Apr 3; 104:250–255. <https://doi.org/10.1016/j.yexmp.2018.03.007> PMID: 29621477
170. Kandhasamy S, Arthi N, Arun RP, Verma RS: Synthesis and fabrication of novel quinone-based chromenopyrazole antioxidant-laden silk fibroin nanofibers scaffold for tissue engineering applications. *Mater Sci Eng C Mater Biol Appl* 2019 Sep; 102:773–787. <https://doi.org/10.1016/j.msec.2019.04.076> PMID: 31147050
171. Sun J, Su W, Zhao X, Shan T, Jin T, Guo Y, et al.: LncRNA PFAR contributes to fibrogenesis in lung fibroblasts through competitively binding to miR-15a. *Biosci Rep* 2019 Jul 31;39. <https://doi.org/10.1042/BSR20190280> PMID: 31273058
172. Ismail NA, Amin KAM, Majid FAA, Razali MH: Gellan gum incorporating titanium dioxide nanoparticles biofilm as wound dressing: Physicochemical, mechanical, antibacterial properties and wound healing studies. *Mater Sci Eng C Mater Biol Appl* 2019 Oct; 103:109770. <https://doi.org/10.1016/j.msec.2019.109770> PMID: 31349525
173. Lee S, Lee DH, Park B-W, Kim R, Hoang AD, Woo S-K, et al.: In vivo transduction of ETV2 improves cardiac function and induces vascular regeneration following myocardial infarction. *Exp Mol Med* 2019 Feb 12; 51:1–14.
174. Liu X, Wang L, Ma C, Wang G, Zhang Y, Sun S: Exosomes derived from platelet-rich plasma present a novel potential in alleviating knee osteoarthritis by promoting proliferation and inhibiting apoptosis of chondrocyte via Wnt/ β -catenin signaling pathway. *J Orthop Surg Res* 2019 Dec 30; 14:470. <https://doi.org/10.1186/s13018-019-1529-7> PMID: 31888697
175. Jung H, Kim HS, Lee JH, Lee JJ, Park HS: Wound Healing Promoting Activity of Tonsil-Derived Stem Cells on 5-Fluorouracil-Induced Oral Mucositis Model. *Tissue Eng Regen Med* 2020; 17:105–119. <https://doi.org/10.1007/s13770-019-00226-7> PMID: 32002842
176. Domac BH, AlKhatib S, Zirhli O, Akdogan NG, Öçal Dirican ŞC, Bulut G, et al.: Effects of PEGylated Fe-Fe₃O₄ core-shell nanoparticles on NIH3T3 and A549 cell lines. *Heliyon* 2020 Jan; 6:e03124. <https://doi.org/10.1016/j.heliyon.2019.e03124> PMID: 31909281
177. Ipek T, Hanga MP, Hartwig A, Wolffsohn J, O'Donnell C: Dry eye following cataract surgery: The effect of light exposure using an in-vitro model. *Cont Lens Anterior Eye* 2018; 41:128–131. <https://doi.org/10.1016/j.clae.2017.11.003> PMID: 29223650
178. Wang X, Zhang Y, Zhang W, Liu H, Zhou Z, Dai X, et al.: MCP1P1 Regulates Alveolar Macrophage Apoptosis and Pulmonary Fibroblast Activation After in vitro Exposure to Silica. *Toxicol Sci* 2016 Feb 10; 151:126–138. <https://doi.org/10.1093/toxsci/kfw029> PMID: 26865670
179. Wise LM, Bodaan CJ, Mercer AA, Riley CB, Theoret CL: Orf virus interleukin-10 and vascular endothelial growth factor-E modulate gene expression in cultured equine dermal fibroblasts. *Vet Dermatol* 2016 Oct; 27:434–e114. <https://doi.org/10.1111/vde.12370> PMID: 27550846
180. Chen N, Du B, Zhou H, Shen F, Li J, Xie Z: Abnormal expression of Nrf2 may play an important role in the pathogenesis and development of adenomyosis. *PLoS One* 2017 Aug 17; 12:e0182773. <https://doi.org/10.1371/journal.pone.0182773> PMID: 28817677
181. Kokado M, Okada Y, Miyamoto T, Yamanaka O, Saika S: Effects of epiplakin-knockdown in cultured corneal epithelial cells. *BMC Res Notes* 2016 May 20; 9:278. <https://doi.org/10.1186/s13104-016-2082-7> PMID: 27206504

182. Morizane R, Fujii S, Monkawa T, Hiratsuka K, Yamaguchi S, Homma K, et al.: miR-363 induces trans-differentiation of human kidney tubular cells to mesenchymal phenotype. *Clin Exp Nephrol* 2016 Jun; 20:394–401. <https://doi.org/10.1007/s10157-015-1167-2> PMID: 26373846
183. Zhang H, Shang Q, An J, Wang C, Ma J: Crocetin inhibits PDGF-BB-induced proliferation and migration of retinal pigment epithelial cells. *Eur J Pharmacol* 2019 Jan 5; 842:329–337. <https://doi.org/10.1016/j.ejphar.2018.11.001> PMID: 30395849
184. Samaeekia R, Rabiee B, Putra I, Shen X, Park YJ, Hematti P, et al.: Effect of Human Corneal Mesenchymal Stromal Cell-derived Exosomes on Corneal Epithelial Wound Healing. *Invest Ophthalmol Vis Sci* 2018 Oct 1; 59:5194–5200. <https://doi.org/10.1167/iovs.18-24803> PMID: 30372747
185. Takada K, Komine-Aizawa S, Kuramochi T, Ito S, Trinh QD, Pham NTK, et al.: *Lactobacillus crispatus* accelerates re-epithelialization in vaginal epithelial cell line MS74. *Am J Reprod Immunol* 2018 Aug 24; 80:e13027. <https://doi.org/10.1111/aji.13027> PMID: 30144195
186. Meijer BJ, Giugliano FP, Baan B, van der Meer JHM, Meisner S, van Roest M, et al.: ATF2 and ATF7 are critical mediators of intestinal epithelial repair. *Cell Mol Gastroenterol Hepatol* 2020 Jan 17; 10:23–42. <https://doi.org/10.1016/j.jcmgh.2020.01.005> PMID: 31958521
187. Guan R, Lin R, Jin R, Lu L, Liu X, Hu S, et al.: Chitinase-like protein YKL-40 regulates human bronchial epithelial cells proliferation, apoptosis, and migration through TGF- β 1/Smads pathway. *Hum Exp Toxicol* 2020 Apr; 39:451–463. <https://doi.org/10.1177/0960327119891218> PMID: 31797699
188. Schacke M, Kumar J, Colwell N, Hermanson K, Folle GA, Nechaev S, et al.: PARP-1/2 Inhibitor Olaparib Prevents or Partially Reverts EMT Induced by TGF- β in NMuMG Cells. *Int J Mol Sci* 2019 Jan 26;20. <https://doi.org/10.3390/ijms20030518> PMID: 30691122
189. Zhang Y, Zhao L, Wang L, Yang X, Zhou A, Wang J: Placental growth factor promotes epithelial-mesenchymal transition-like changes in ARPE-19 cells under hypoxia. *Mol Vis* 2018 Apr 26; 24:340–352. PMID: 29769799
190. Pongsakul N, Vinaiphath A, Chanchaem P, Fong-Ngern K, Thongboonkerd V: Lamin A/C in renal tubular cells is important for tissue repair, cell proliferation, and calcium oxalate crystal adhesion, and is associated with potential crystal receptors. *FASEB J* 2016 Jun 29; 30:3368–3377. <https://doi.org/10.1096/fj.201600426R> PMID: 27358390
191. Tonsawan P, Dylewski J, Lewis L, Blaine J: Knockout of the neonatal Fc receptor in cultured podocytes alters IL-6 signaling and the actin cytoskeleton. *Am J Physiol Cell Physiol* 2019 Nov 1; 317: C1048–C1060. <https://doi.org/10.1152/ajpcell.00235.2019> PMID: 31553647
192. Kobayashi M, Tokuda K, Kobayashi Y, Yamashiro C, Uchi S-H, Hatano M, et al.: Suppression of Epithelial-Mesenchymal Transition in Retinal Pigment Epithelial Cells by an MRTF-A Inhibitor. *Invest Ophthalmol Vis Sci* 2019 Feb 1; 60:528–537. <https://doi.org/10.1167/iovs.18-25678> PMID: 30707754
193. Wannasarit S, Puttarak P, Kaewkroek K, Wiwattanapatapee R: Strategies for Improving Healing of the Gastric Epithelium Using Oral Solid Dispersions Loaded with Pentacyclic Triterpene-Rich Centella Extract. *AAPS PharmSciTech* 2019 Aug 8; 20:277. <https://doi.org/10.1208/s12249-019-1488-7> PMID: 31396788
194. Rohwer K, Neupane S, Bittkau KS, Galarza Pérez M, Dörschmann P, Roeder J, et al.: Effects of Crude *Fucus distichus* Subspecies *evanescens* Fucoïdan Extract on Retinal Pigment Epithelium Cells—Implications for Use in Age-Related Macular Degeneration. *Mar Drugs* 2019 Sep 16;17. <https://doi.org/10.3390/md17090538> PMID: 31527536
195. Nielsen SD, Purup S, Larsen LB: Effect of Casein Hydrolysates on Intestinal Cell Migration and Their Peptide Profiles by LC-ESI/MS/MS. *Foods* 2019 Mar 6;8. <https://doi.org/10.3390/foods8030091> PMID: 30845637
196. Zhang C, Liu J, Jin N, Zhang G, Xi Y, Liu H: siRNA Targeting mTOR Effectively Prevents the Proliferation and Migration of Human Lens Epithelial Cells. *PLoS One* 2016 Dec 2; 11:e0167349. <https://doi.org/10.1371/journal.pone.0167349> PMID: 27911920
197. Nelson DL, Zhao Y, Fabiilli ML, Cook KE: In vitro evaluation of lysophosphatidic acid delivery via reverse perfluorocarbon emulsions to enhance alveolar epithelial repair. *Colloids Surf B, Biointerfaces* 2018 Sep 1; 169:411–417. <https://doi.org/10.1016/j.colsurfb.2018.05.037> PMID: 29807339
198. Kapincharanon C, Thongboonkerd V: K⁺ deficiency caused defects in renal tubular cell proliferation, oxidative stress response, tissue repair and tight junction integrity, but enhanced energy production, proteasome function and cellular K⁺ uptake. *Cell Adh Migr* 2018 May 4; 12:247–258. <https://doi.org/10.1080/19336918.2017.1356554> PMID: 28820294
199. Kumar N, Srivastava S, Burek M, Förster CY, Roy P: Assessment of estradiol-induced gene regulation and proliferation in an immortalized mouse immature Sertoli cell line. *Life Sci* 2016 Mar 1; 148:268–278. <https://doi.org/10.1016/j.lfs.2016.01.027> PMID: 26784849

200. McCann AJ, Samuels TL, Blumin JH, Johnston N: The role of pepsin in epithelia-mesenchymal transition in idiopathic subglottic stenosis. *Laryngoscope* 2020; 130:154–158. <https://doi.org/10.1002/lary.27879> PMID: 30776094
201. Sel S, Trau S, Paulsen F, Kalinski T, Stangl GI, Nass N: 1,25-dihydroxyvitamin D3 inhibits corneal wound healing in an ex-vivo mouse model. *Graefes Arch Clin Exp Ophthalmol* 2016 Apr; 254:717–724. <https://doi.org/10.1007/s00417-016-3267-4> PMID: 26794222
202. Kowtharapu BS, Murin R, Jünemann AGM, Stachs O: Role of Corneal Stromal Cells on Epithelial Cell Function during Wound Healing. *Int J Mol Sci* 2018 Feb 4;19. <https://doi.org/10.3390/ijms19020464> PMID: 29401709
203. Kim S-J, Park J-H, Lee S-A, Lee J-G, Shin J-M, Lee H-M: All-trans retinoic acid regulates TGF- β 1-induced extracellular matrix production via p38, JNK, and NF- κ B-signaling pathways in nasal polyp-derived fibroblasts. *Int Forum Allergy Rhinol* 2020 Feb 27; 10:636–645. <https://doi.org/10.1002/alr.22525> PMID: 32104972
204. Li C, Zhihong H, Wenlong L, Xiaoyan L, Qing C, Wenzhi L, et al.: The Nucleotide-Binding Oligomerization Domain-Like Receptor Family Pyrin Domain-Containing 3 Inflammasome Regulates Bronchial Epithelial Cell Injury and Proapoptosis after Exposure to Biomass Fuel Smoke. *Am J Respir Cell Mol Biol* 2016; 55:815–824. <https://doi.org/10.1165/rcmb.2016-0051OC> PMID: 27447246
205. Masola V, Carraro A, Granata S, Signorini L, Bellin G, Violi P, et al.: In vitro effects of interleukin (IL)-1 beta inhibition on the epithelial-to-mesenchymal transition (EMT) of renal tubular and hepatic stellate cells. *J Transl Med* 2019 Jan 7; 17:12. <https://doi.org/10.1186/s12967-019-1770-1> PMID: 30616602
206. Putra I, Rabiee B, Anwar KN, Gidfar S, Shen X, Babalooee M, et al.: Staphylococcus aureus alpha-hemolysin impairs corneal epithelial wound healing and promotes intracellular bacterial invasion. *Exp Eye Res* 2019 Feb 27; 181:263–270. <https://doi.org/10.1016/j.exer.2019.02.019> PMID: 30822400
207. Jun JH, Sohn W-J, Lee Y, Kim J-Y: Effects of anti-vascular endothelial growth factor monoclonal antibody (bevacizumab) on lens epithelial cells. *Clin Ophthalmol* 2016 Jun 27; 10:1167–1174. <https://doi.org/10.2147/OPTH.S103443> PMID: 27418802
208. De Cillà S, Farruggio S, Cocomazzi G, Mary D, Alkabes M, Rossetti L, et al.: Aflibercept and Ranibizumab Modulate Retinal Pigment Epithelial Cells Function by Acting on Their Cross Talk with Vascular Endothelial Cells. *Cell Physiol Biochem* 2020 Feb 12; 54:161–179. <https://doi.org/10.33594/000000212> PMID: 32045141
209. Shi S, Peng F, Zheng Q, Zeng L, Chen H, Li X, et al.: Micelle-solubilized axitinib for ocular administration in anti-neovascularization. *Int J Pharm* 2019 Apr 5; 560:19–26. <https://doi.org/10.1016/j.ijpharm.2019.01.051> PMID: 30710659
210. Molladavoodi S, Robichaud M, Wulff D, Gorbet M: Corneal epithelial cells exposed to shear stress show altered cytoskeleton and migratory behaviour. *PLoS One* 2017 Jun 29; 12:e0178981. <https://doi.org/10.1371/journal.pone.0178981> PMID: 28662184
211. Schwartz CM, Dorn BA, Habtemariam S, Hill CL, Chiang T, Reynolds SD: The wound healing capacity of undifferentiated and differentiated airway epithelial cells in vitro. *Int J Pediatr Otorhinolaryngol* 2018 Sep; 112:163–168. <https://doi.org/10.1016/j.ijporl.2018.07.006> PMID: 30055727
212. Chen H, Wang H, An J, Shang Q, Ma J: Inhibitory Effects of Plumbagin on Retinal Pigment Epithelial Cell Epithelial-Mesenchymal Transition In Vitro and In Vivo. *Med Sci Monit* 2018 Mar 13; 24:1502–1510. <https://doi.org/10.12659/msm.906265> PMID: 29532788
213. Rouzair M, Comptour A, Belville C, Bouvier D, Sapin V, Gallot D, et al.: Cigarette smoke condensate affects the retinoid pathway in human amnion. *Placenta* 2017 Oct; 58:98–104. <https://doi.org/10.1016/j.placenta.2017.08.076> PMID: 28962704
214. Hamada S, Sato A, Hara-Chikuma M, Satooka H, Hasegawa K, Tanimura K, et al.: Role of mitochondrial hydrogen peroxide induced by intermittent hypoxia in airway epithelial wound repair in vitro. *Exp Cell Res* 2016 May 15; 344:143–151. <https://doi.org/10.1016/j.yexcr.2016.04.006> PMID: 27093911
215. Iwanabe Y, Masaki C, Tamura A, Tsuka S, Mukaibo T, Kondo Y, et al.: The effect of low-intensity pulsed ultrasound on wound healing using scratch assay in epithelial cells. *J Prosthodont Res* 2016 Oct; 60:308–314. <https://doi.org/10.1016/j.jpor.2016.03.002> PMID: 27026212
216. Cui Y-H, Hu Z-X, Gao Z-X, Song X-L, Feng Q-Y, Yang G, et al.: Airborne particulate matter impairs corneal epithelial cells migration via disturbing FAK/RhoA signaling pathway and cytoskeleton organization. *Nanotoxicology* 2018 Feb 20; 12:312–324. <https://doi.org/10.1080/17435390.2018.1440651> PMID: 29463199
217. Bhatt P, Narvekar P, Lalani R, Chougule MB, Pathak Y, Sutariya V: An in vitro Assessment of Thermo-Reversible Gel Formulation Containing Sunitinib Nanoparticles for Neovascular Age-Related Macular Degeneration. *AAPS PharmSciTech* 2019 Aug 9; 20:281. <https://doi.org/10.1208/s12249-019-1474-0> PMID: 31399890

218. Wang Z, Liu B, Zhu J, Wang D, Wang Y: Nicotine-mediated autophagy of vascular smooth muscle cell accelerates atherosclerosis via nAChRs/ROS/NF- κ B signaling pathway. *Atherosclerosis* 2019 May; 284:1–10. <https://doi.org/10.1016/j.atherosclerosis.2019.02.008> PMID: 30856513
219. Ashcroft KJ, Syed F, Bayat A: Site-specific keloid fibroblasts alter the behaviour of normal skin and normal scar fibroblasts through paracrine signalling. *PLoS One* 2013 Dec 9; 8:e75600. <https://doi.org/10.1371/journal.pone.0075600> PMID: 24348987
220. Hahn JM, McFarland KL, Combs KA, Supp DM: Partial epithelial-mesenchymal transition in keloid scars: regulation of keloid keratinocyte gene expression by transforming growth factor- β 1. *Burns Trauma* 2016 Aug 23; 4:30. <https://doi.org/10.1186/s41038-016-0055-7> PMID: 27574697
221. Limandjaja GC, Niessen FB, Scheper RJ, Gibbs S: The keloid disorder: heterogeneity, histopathology, mechanisms and models. *Front Cell Dev Biol* 2020 May 26; 8:360. <https://doi.org/10.3389/fcell.2020.00360> PMID: 32528951
222. Tan S, Khumalo N, Bayat A: Understanding Keloid Pathobiology From a Quasi-Neoplastic Perspective: Less of a Scar and More of a Chronic Inflammatory Disease With Cancer-Like Tendencies. *Front Immunol* 2019 Aug 7; 10:1810. <https://doi.org/10.3389/fimmu.2019.01810> PMID: 31440236
223. Anderson ED, Sastalla I, Earland NJ, Mahnaz M, Moore IN, Otaizo-Carrasquero F, et al.: Prolonging culture of primary human keratinocytes isolated from suction blisters with the Rho kinase inhibitor Y-27632. *PLoS One* 2018 Sep 12; 13:e0198862. <https://doi.org/10.1371/journal.pone.0198862> PMID: 30208113
224. Kumari N, Das A, Bhatt AN: Interleukin-6 confers radio-resistance by inducing Akt-mediated glycolysis and reducing mitochondrial damage in cells. *J Biochem* 2020 Mar 1; 167:303–314. <https://doi.org/10.1093/jb/mvz091> PMID: 31670806
225. Sun H-H, Feng X-M, Wang J-C, Cai J: Allicin can suppress the activity of vascular endothelial cells probably by regulating JAK2/STAT3 pathway. *Mol Cell Biochem* 2020 Sep 25; <https://doi.org/10.1007/s11010-020-03919-z> PMID: 32975696
226. Matias D, Balça-Silva J, Dubois LG, Pontes B, Ferrer VP, Rosário L, et al.: Dual treatment with shikonin and temozolomide reduces glioblastoma tumor growth, migration and glial-to-mesenchymal transition. *Cell Oncol (Dordr)* 2017 Jun; 40:247–261. <https://doi.org/10.1007/s13402-017-0320-1> PMID: 28401486
227. Vinaik R, Barayan D, Auger C, Abdullahi A, Jeschke MG: Regulation of glycolysis and the Warburg effect in wound healing. *JCI Insight* 2020 Sep 3;5. <https://doi.org/10.1172/jci.insight.138949> PMID: 32750036
228. Krumm P, Giraldez T, Alvarez de la Rosa D, Clauss WG, Fronius M, Althaus M: Thiol-reactive compounds from garlic inhibit the epithelial sodium channel (ENaC). *Bioorg Med Chem* 2012 Jul 1; 20:3979–3984. <https://doi.org/10.1016/j.bmc.2012.05.021> PMID: 22668601
229. Wagner BA, Venkataraman S, Buettner GR: The rate of oxygen utilization by cells. *Free Radic Biol Med* 2011 Aug 1; 51:700–712. <https://doi.org/10.1016/j.freeradbiomed.2011.05.024> PMID: 21664270
230. Das J, Sharma A, Jindal A, Aggarwal V, Rawat A: Leukocyte adhesion defect: Where do we stand circa 2019? *Genes Dis* 2020 Mar; 7:107–114. <https://doi.org/10.1016/j.gendis.2019.07.012> PMID: 32181281
231. Wolk K, Join-Lambert O, Sabat R: Aetiology and pathogenesis of hidradenitis suppurativa. *Br J Dermatol* 2020 Oct 13; <https://doi.org/10.1111/bjd.19556> PMID: 33048349