Original Research

The impact of quercetin on wound healing relates to changes in αV and β1 integrin expression

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Impact statement

Scar formation during wound healing can be problematic for patients but there are limited therapies available to treat or prevent excess fibrosis at wound sites. This work examines the impact of quercetin, a flavonoid that decreases fibrosis, on wound healing, and relates quercetin's effects to changes in integrin expression on the surface of fibroblast cells. To our knowledge, this is the first report that quercetin alters integrin expression or that this impact may be part of the mechanism by which quercetin prevents fibrosis. This work demonstrates that quercetin can be used to modulate integrin expression and that this effect may in turn reduce fibrosis during wound healing. Furthermore, this paper identifies the modulation of integrin expression as a possible therapeutic target in preventing scars. This information could be used to improve therapeutics to aid in the cosmetic and functional results following wound healing.

Abstract

Overly fibrotic wound healing can lead to excess scar formation, causing functional impairment and undesirable cosmetic results. However, there are few successful treatments available to prevent or remediate scars. This study sought to explore the molecular mechanisms by which quercetin, a naturally-occurring antifibrotic agent, diminishes scar formation. Using both mice and fibroblast cells, we examined quercetin's impact on fibrosis and the wound healing rate, and potential molecular mechanisms underlying the quercetin-mediated reduction of fibrosis. While cultured fibroblasts demonstrated normal growth in response to quercetin, quercetin increased surface αV integrin and decreased β1 integrin. These changes in surface integrin expression may impact factors that contribute to fibrosis including cell migration, proliferation, and extracellular matrix production. In both quercetin-treated and control mice, wounds healed in about 14 days. Masson's trichrome stain revealed diminished fibrosis at the wound site in quercetin-treated animals despite the normal healing rate, indicating the potential for better cosmetic results without delaying healing. An *in vitro* scratch wound model using cells plated on an artificial extracellular matrix demonstrated delayed closure following quercetin treatment. The extracellular matrix also ameliorated quercetin's effect on αV integrin. Thus, αV integrin recruitment in response to quercetin treatment may promote the quercetin-mediated decrease extracellular matrix because cells require less extracellular matrix to migrate into a wound. With added extracellular matrix, β1 integrin remained diminished in response to quercetin, indicating that quercetin's effect on β1 integrin expression is independent of extracellular matrix -mediated signaling and is likely driven by inhibition of the intracellular mechanisms driving β1 expression. These findings suggest that quercetin could alter the cells' interactions with the extracellular matrix through the regulation of integrin expression to promote a decrease in fibrosis. Furthermore, this work demonstrates that this naturally occurring and commercially available supplement could be used to improve wound healing by impacting integrin expression, leading to a lower extracellular matrix requirement to achieve healing.

Keywords: Wound, fibrosis, integrin, quercetin, skin, fibroblast

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Introduction

Cutaneous wounds can range from insignificant cuts that heal relatively quickly to substantial wounds that take a long time to close or fail to heal entirely.[1] These larger, slow to heal wounds can be problematic and can lead to significant sequelae or even death. It is estimated that more than 6.5 million Americans were afflicted with chronic wounds in 2009. On the other end of the healing spectrum, 40–70% of surgical patients experience hypertrophic scarring, or a large cutaneous defect at the surgical site that results from an overgrowth of skin and connective tissue, which can both impair function and be cosmetically undesirable. It is important for the body to heal skin wounds quickly, as these wounds can be painful and may

also become infected if they remain open too long. However, an excessive healing response that leads to fibrosis and scarring is also undesirable. Thus, wounds must heal quickly, but not too robustly. However, it is unclear how best to promote healing without causing excess fibrosis and scar formation.

Wound healing is a complex process involving the orchestration of immune and skin cells with the structural proteins that compose the extracellular matrix (ECM).[2,3] Within days after the initial injury, cells at the wound site, including both skin and immune cells, begin to produce ECM components. In the following weeks, cells at the wound site produce, absorb, and reshape the ECM and these processes impact the strength and quality of the final closure.[4] The ECM also affects the behavior of cells at the wound site, promoting survival, guiding migration, and altering the metabolism of the cells that go on to heal damaged skin. However, the factors that determine whether an ideal amount of ECM will be laid down at a wound site are unclear. Some individuals experience inadequate healing following an injury, while in others, overzealous production of ECM occurs, leaving an excessive scar. Furthermore, it is unclear what factors impact cell growth, such that an ideal cell-to-ECM ratio is achieved at the wound site.

Integrins and their ligands play a substantial role in the formation, modulation, and remodeling of the ECM associated with wound healing.[5] Integrins consist of two subunits, an α and a β subunit, each of which have many subtypes. These two subunits associate together and bind to a variety of ECM proteins, including collagen, fibronectin, and others. Two specific integrin subunits, αV and β1 appear to play a role in fibroblast behavior at wound sites. It has been shown that an increase in αV integrin may increase fibroblast migration and improve wound healing.[6] Additionally, αV integrin may also play a role in fibroblast proliferation at wound sites.[7] On the other hand, β1 subunit-containing integrins have been associated with both fibroblast migration and the initiation of fibrosis.[8] Both of these integrin subunits are important for fibroblast adhesion to several ECM components, specifically fibronectin and collagen.[5] Thus, it is reasonable to assume that altering the levels of either or both αV and β1 integrin expressed on fibroblasts might alter fibroblast behavior and improve the quality of a healed wound by decreasing fibrosis. However, how best to impact integrin expression and behavior is not known.

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one) is a naturally occurring flavonoid found in tea and berries that may have anti-fibrotic properties.[9] In addition to being present in a variety of foods, quercetin supplements are currently available over-the-counter in the United States. Previous studies have shown a healing benefit when quercetin is applied to a wound.[10,11] One study demonstrated accelerated wound healing in rats and mice when quercetin was applied topically to the wound.[10] In this study, the effect was shown to be associated with an increase in fibroblast proliferation, a decrease in immune cell infiltration, and changes in signaling in fibrosis-associated signaling pathways. Additionally, other studies have indicated that quercetin decreases fibrosis and scar formation in wound healing, both *in vivo* and *in vitro*.[9,12,13] However, none of these studies examined the impact of quercetin treatment on integrin expression.

The purpose of this study is to explore the impact of quercetin on wound healing. Specifically, we explore the quercetin-dependent alterations in integrin levels and the resulting impact on ECM production. These results may help identify possible methods for improving the quality of the wounds during and after the healing process.

Materials and methods

Mice

Wild-type C57Bl/6J mice (Jackson Labs, Bar Harbor, ME) were used as our mouse wound healing model. All procedures were approved by the Institutional Animal Care and Use Committee prior to experimentation.

Procedure

Animals were anesthetized using isofluorane inhalation and shaved. Wounding was performed with an 8 mm biopsy punch (Integra Miltex, Plainsboro, NJ). Mice were treated daily with 100 μL of 10 μM quercetin solution in sterile saline containing 10% dimethylsulfoxide (DMSO) by volume. On post-operative days 0, 1, 3, 7, 10, and 14, mice were anesthetized for photography and measurement of wound sizes.

Measurements of wounds

Wounds were measured using two perpendicular measurements with calipers at the horizontal and vertical centers of the wounds. These numbers were then averaged and represented as the average wound diameter. Areas were calculated from these diameters.

Trichrome stain of wounds

On post-operative day 10 or 14, mice were sacrificed under anesthesia. Wound area was cut out, fixed in formalin, and embedded in paraffin. Masson's trichrome stain of the wound site was performed in accordance with kit instructions (Sigma Aldrich, St. Louis, MO). Photographs were taken using an Olympus BX51 microscope (Olympus, Center Valley, PA) at 4× and 10× magnification.

Cells

L929 fibroblast cells served as the experimental model. Cells were grown in RPMI media supplemented with 10% fetal bovine serum. Where indicated, cells were either plated directly on plates or on plates pre-coated with 0.02% gelatin supplemented with 5 μg/mL fibronectin (Sigma Aldrich, Darmstadt, Germany) for at least half an hour at 37°C.

Scratch assay

Six well plates (Corning, Corning, NY) were coated with a matrix of 0.02% gelatin supplemented with 5 μg/mL fibronectin (Sigma Aldrich, Darmstadt, Germany) for at least

half an hour at 37°C. These plates were plated with 50,000 cells and grown for four days before beginning the experiment. A scratch was created with a 100 μL pipet tip, after which cells treated with 20 μM quercetin in DMSO and monitored for 8 h following the start of the experiment. Cells were photographed using an Olympus IX51 microscope (Olympus, Center Valley, PA). Quantification was performed by tracing wound areas using FIJI.[14]

Cell counts

Cells were treated for 24 h with 20 μM quercetin in DMSO. They were removed from the plate with gentle scraping and stained with trypan blue. Counts were performed using a hemocytometer. Number of cells per plate well were calculated.

Flow cytometry

Cells were grown on 12-well plates and were treated with 20 uM quercetin for 24 h. Cells were gently scraped off the plate and counted with an automatic cellometer using acridine orange and propidium iodide (AO/PI) (Nexcelom Bioscience, Lawrence, MA). One million cells were stained with Aqua (ThermoFisher Scientific, Waltham, MA) to exclude dead cells and antibodies to αV (Phycoerythrin (PE) rat anti-mouse CD51) and β1 integrin (Pacific Blue Armenian hamster anti-mouse CD29) (Biolegend, San Diego, CA). Cells were analyzed using a BD FACS Canto II (BD Biosciences, San Jose, CA) and FlowJo (FlowJo, Ashland, CA). Mean fluorescence intensity was calculated with isotype-matched antibodies as the controls for specific stains (PE rat IgG 1ak (BD Pharmingen)) for αV and Pacific Blue Armenian hamster IgG for β1 (Biolegend, San Diego, CA)).

Statistical analysis

Statistical analysis was performed with GraphPad (Prism, La Jolla, CA). Graphs demonstrate means with error bars representing standard deviations. Student's *t*-tests were used for comparisons of two groups and ANOVA was used for comparisons of more than two groups. Wound healing rate was analyzed using a linear regression model and ANOVA. Statistical significance was indicated by a $P < 0.05$.

Results

Fibroblast reaction to quercetin

Because quercetin is known to be anti-fibrotic, we hypothesized that quercetin treatment might impact the expression of integrins on fibroblasts. We specifically chose to examine the response of αV integrin and β1 integrin because of their association with cell migration, skin cell proliferation, and fibrosis.[7,8,15] We established an *in vitro* system of fibroblast culture and treated the cells with quercetin at a concentration of 20 μm for 24 h. After 24 h of quercetin treatment, we observed changes in the level of surface expression of both integrin subunits examined, as measured by flow cytometry. Fibroblasts demonstrated decreased surface β1 integrin with quercetin treatment (Figure 1(a)). Given that this

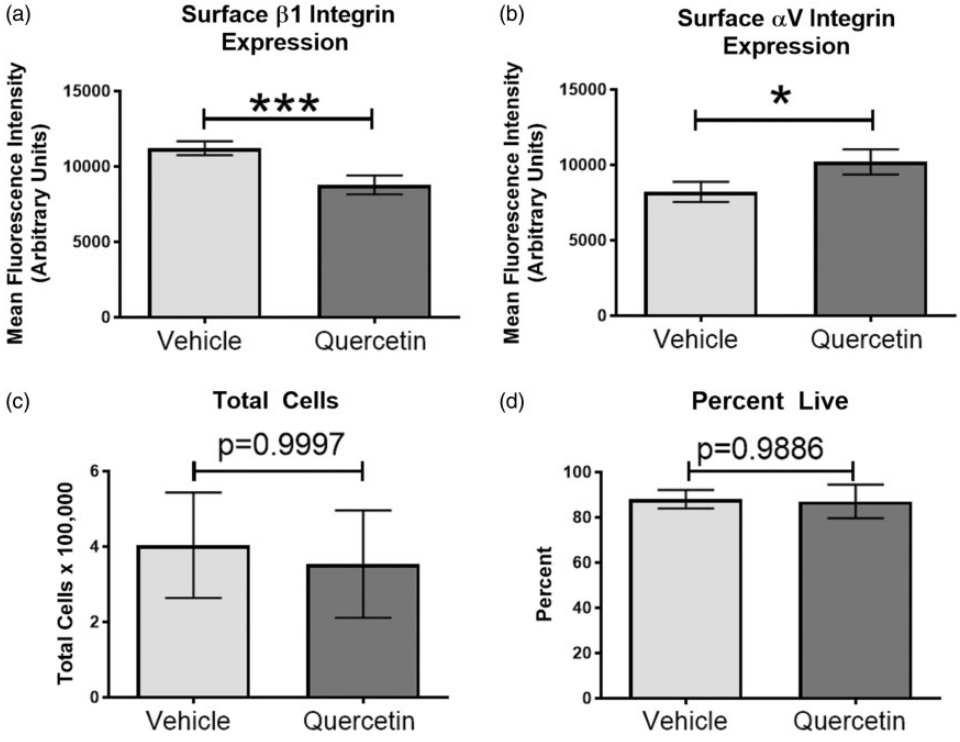

Figure 1 Fibroblast Response to Quercetin. (a) Surface β1 integrin expression on L929 fibroblasts is decreased compared with control. (b) Surface αV integrin expression on quercetin treated fibroblasts is higher than control. (c, d). Quercetin treatment had no effect on total cell number (c) or cell survival (d). Graph values indicate mean ± standard deviation. *$P < 0.05$; ***$P < 0.001$

integrin subunit is associated with focal adhesions, we suspected that this finding may indicate a change in cell adherence in response to quercetin. Additionally, quercetin treatment increased surface αV integrin (Figure 1(b)). Because αV integrin has been associated with promoting cell migration, this finding may mean that quercetin makes cells more capable of entering a wound site, even in the absence of substantial ECM deposition. The changes in αV and β1 integrin therefore may be indicative of a pro-migration phenotype that requires less ECM to facilitate wound closure. Thus, the alteration of integrin components on the surface of skin cells may be part of the mechanism by which quercetin diminishes fibrosis and may improve the quality of healed wounds *in vivo*.

Integrin expression has been shown to impact fibroblast growth which, if impaired, could worsen wound healing.[5,16,17] Following the finding that quercetin alters integrin expression, we sought to demonstrate that quercetin would not impact cell proliferation or survival. We measured both total cell number and percent live cells in the plated fibroblasts treated with 20 uM quercetin for 24 h. Total cell counts (Figure 1(c)) and percent live cells (Figure 1(d)) were similar in the quercetin-treated and control groups. This indicates that quercetin and its effects on integrin expression are non-toxic at the tested concentration and that this treatment does not impair or improve cell proliferation or survival.

Quercetin does not impair wound healing in mice

Based upon our experience *in vitro*, in which quercetin did not impair cell growth or survival, we sought to determine whether quercetin would impact wound healing *in vivo*. To this end, we employed a mouse model of wound healing using C57Bl/6J mice. Mice were wounded with an 8 mm biopsy punch and wounds were allowed to heal by second intention. Mice were treated daily with intraperitoneal injections of quercetin. Following the biopsy punch, we observed that wounds in wild-type mice heal in roughly 14 days. We measured the wounds with calipers to find the average diameter and used these diameters to calculate approximate wound areas. Daily quercetin treatment had no impact on the wound healing rate over the 14-day healing period, based upon these calculated areas ($P = 0.55$) (Figure 2(a)).

Images of typical wounds from the experiments are shown in Figure 2(b). The wounds of quercetin-treated mice look grossly normal. Immediately following wounding, which is superficial and does not extend into the underlying adipose layer, the wounds are relatively bloodless and look very similar in both groups. By day 1 post-wounding, wounds stretch to be larger and clots begin to form. On day 3 post wounding, clots are fully formed and contraction appears to be beginning at the edges of the wounds. By day 7, the unhealed area is substantially smaller and the clot matures in preparation to fall off. On day 10, the wounded area is substantially smaller than its peak size, returning to roughly the size of the original injury. Additionally, wounds have typically unroofed by day 10. Finally, on day 14, the wound area has continued to contract

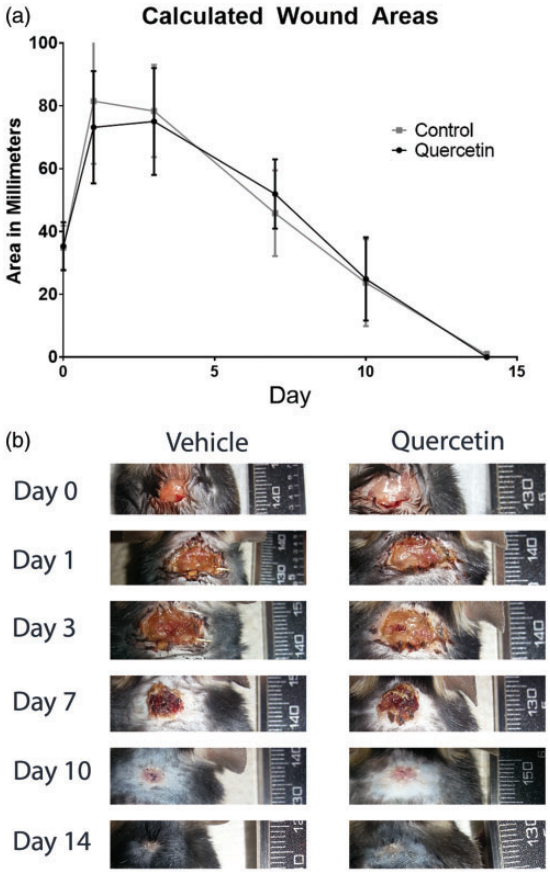

Figure 2 (a, b) Wild-type mice treated with quercetin heal at the same rate as controls, based on a linear regression analysis of the calculated areas of their wounds that remained open over the course of 14 days ($P = 0.55$). Graph values indicate mean ± standard deviation. *In vivo* wound healing. Gross wound healing for vehicle-treated mice (left column) and quercetin-treated mice (right column) were similar over the course of the 14-day experiment. (A color version of this figure is available in the online journal.)

and the remaining scar appears to be formed. Thus, in the quercetin-treated group, wound healing appears to proceed normally, closing the wound at the same rate and with the same visual quality as in the control mice. Thus, quercetin appears not to impair wound healing in wild-type C57Bl/6J mice.

Quercetin diminishes fibrosis in healing wounds

To understand whether quercetin has an impact on the quality of healed wounds, we sought to characterize the extent of fibrosis using Masson's trichrome stain. We chose the day 10 and day 14 time points to examine the degree of fibrosis in the wounds late in healing. Sections shown are characteristic sections from both time points. When compared to controls, we found that quercetin-treated mice have a decrease in fibrosis as revealed using Masson's Trichrome Stain, shown at both 4× and 10× magnifications. The frequency and density of ECM fibers, in blue, are substantially decreased following quercetin treatment compared with controls (Figure 3). These findings demonstrate that, while the wounds in both groups are

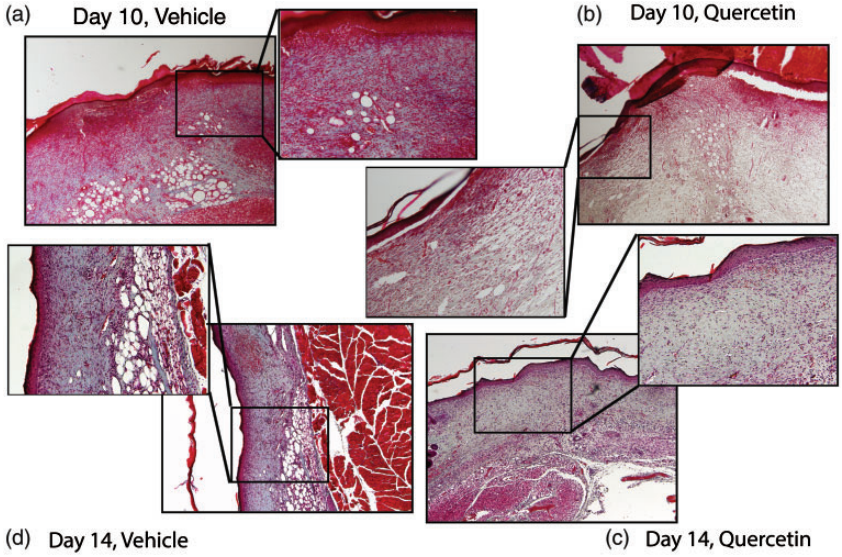

Figure 3 Evidence of quercetin-related wound healing advantages *in vivo*. Masson's Trichrome stain of wound sites. On both day 10 and day 14 post-wounding, sections of wounds from vehicle-treated mice (a, c, respectively) had more fibrosis present, characterized by a blue color, than wounds from quercetin-treated mice (b, d, respectively). Characteristic sections are shown. (A color version of this figure is available in the online journal.)

closed, less ECM was required to achieve the closure in the mice treated with quercetin.

While appearing grossly normal with fewer ECM fibers, the presence and cellular appearance of both the dermal and epidermal layers appear unchanged. Thus, quercetin appears to specifically impact ECM deposition, without impairing the ability of skin cells to replicate or migrate. Given that skin cells migrate into the wound through the adhesion of integrins to the ECM during the wound healing process, this result is unexpected.[18] However, this may be due to altered integrin expression, allowing cells to migrate more efficiently in spite of diminished ECM. Furthermore, the cells' ability to migrate adequately may downregulate further ECM production, thus proceeding in a manner that leads to adequate closure of the wound with less ECM.

Fibroblast growth is impaired when quercetin is combined with an artificial extracellular matrix

Following the finding that quercetin did not impact cellular growth *in vitro* or alter wound closure rate *in vivo*, but did diminish ECM production *in vivo*, we sought to test the reaction of quercetin-treated cells to an ECM that is already present. We hypothesized that the addition of ECM to the system might impair quercetin-mediated integrin alterations and thereby impact wound healing. To test this hypothesis, fibroblast cells were grown on an ECM made of 0.02% bovine gelatin supplemented with 5 μM fibronectin. Fibroblast cells grow and survive on this modeled ECM. However, when treated with quercetin in the context of added ECM, cells grew less and were less likely to survive (Figure 4(a) and (b)). Furthermore, fibroblasts were less able to migrate and close an *in vitro* model wound than in the control group (Figure 4(c) and (d)). Thus, the advantage of quercetin in diminishing the levels of fibrosis appears to occur prior to the appearance of the ECM. A similar experiment was performed without the artificial ECM to compare the migration across the plate. However, in this experiment, the fibroblasts in the control group, but not the quercetin-treated group, did not remain adherent to the plate, but rather peeled off the plate in two sheets (data not shown). These findings further hint that quercetin makes cells more capable of adhering to and migrating in their environment and that, following quercetin treatment, cells may require less ECM to migrate.

We sought to determine whether αV or β1 integrin changes induced by quercetin were impacted by the addition of ECM to the plate. When treated with the artificial ECM, fibroblast cells demonstrated the same decrease in β1 integrin expression when treated with quercetin as they had when no ECM was added (Figure 4(e)). However, the increase in αV Integrin levels was attenuated with this treatment (Figure 4(f)). Thus, when the ECM was already present, quercetin dampened the increase in αV integrin expression without changing the quercetin-mediated reduction of β1 integrin levels. This result may suggest that the cells are less able to expand and migrate or adhere to their current surroundings. These changes may have made the cells less likely to survive as well given that integrins are associated with survival and proliferation.[19] These results further support the interpretation that quercetin's anti-fibrotic effects may be attenuated by the presence of a pre-constructed ECM, such as that present in certain engineered tissue structures. Additionally, these findings highlight a potential role for αV integrin-mediated adherence and migration in the mechanism by which quercetin reduces fibrosis. Finally, the fact that the artificial ECM does not prevent the decrease in surface β1 integrin expression in response to quercetin indicates that this reaction may be more dependent on "inside-out" signaling rather than "outside-in" integrin signaling. Inside-out signaling occurs when integrin behavior responds to intracellular

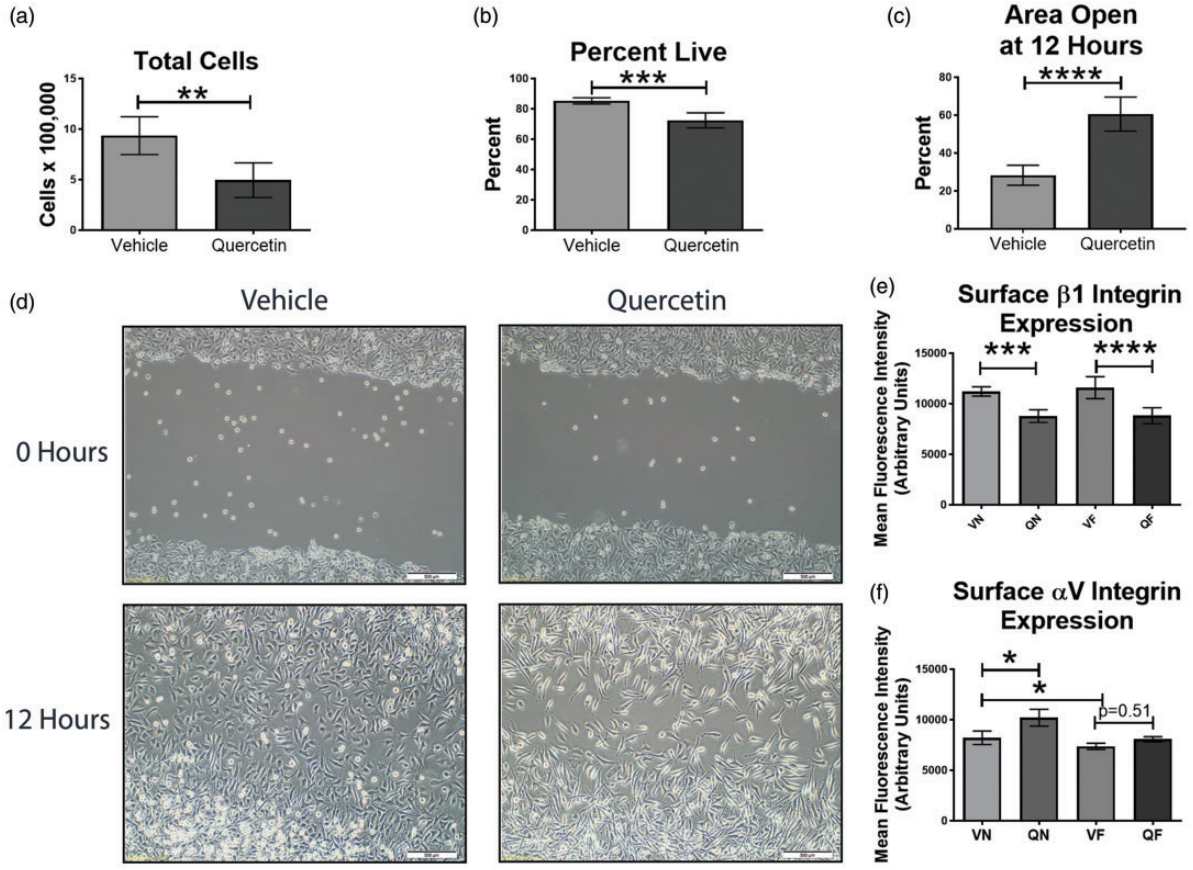

Figure 4 Artificial extracellular matrix alters response to quercetin. (a) Cell proliferation is diminished when cells grown on an artificial ECM made of gelatin and fibronectin. (b) Cell survival is also diminished on the same ECM. (c, d) When grown on gelatin and fibronectin, quercetin diminishes migration into an *in vitro* model of a wound. (e) Cells grown on gelatin and fibronectin retain their decreased β1 integrin expression following quercetin treatment. (f) When grown on gelatin and fibronectin, cells do not increase their αV levels, unlike the cells grown in the absence of the ECM components. Graph values indicate mean ± standard deviation. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$

changes, while outside-in signaling occurs when integrin behavior is determined by the content of the ECM.[20] Changes in αV integrin following quercetin treatment may be more responsive to the outside-in mechanism of integrin signaling. These possible differences in regulation may explain their different response to the application of an artificial ECM.

Discussion

Given the prevalence of excessive undesirable scarring following wounding, manipulating the formation of, and skin cell reaction to, ECM is an attractive therapeutic strategy. Treatments that decrease the formation of ECM without substantially impairing cell growth are ideal for improving the appearance of healed skin without delaying closure. The purpose of this study was to explore the use of a flavonoid, quercetin, which is currently available over the counter, to improve wound healing. This work demonstrates that quercetin can be used to reduce fibrosis at a wound site without delaying the closure *in vivo*, ideal characteristics for a therapeutic intervention. Additionally, quercetin treatment does not alter the *in vitro* growth of fibroblasts in the absence of ECM. The mechanism by which quercetin impacts wound healing appears to involve altered cell surface expression of αV and β1 integrin subunits, a finding that, to our knowledge, has not previously been reported.

The fact that quercetin treatment increases αV integrin and decreases β1 integrin on the surface of skin cells may reflect an anti-fibrotic and pro-adhesion phenotype in which cells require less ECM for their migration and proliferation.[6–8] The fact that αV integrin's increase in response to quercetin is abolished in the presence of ECM may indicate that with ECM already present, high αV is no longer needed to augment migration. The loss of quercetin-mediated increased expression of αV integrin is associated with decreased fibroblast proliferation, decreased survival, and delayed *in vitro* wound closure. This may mean that αV provides important survival signaling in the context of quercetin treatment and that without this increase, cell growth is impaired when quercetin is present. On the other hand, β1 integrin is decreased by quercetin treatment regardless of whether ECM is present or absent. This may indicate that the expression β1 subunit-containing integrins is reduced as a result of quercetin's impact on internal cellular signals rather than altered interaction with ECM.

Additional factors that mediate the effects of quercetin demonstrated in this work remain to be elucidated.

However, integrin signaling appears involved in the decrease in fibrosis in response to quercetin. Integrins must be activated to participate in ECM adhesion and their behavior is mediated by both ''inside-out'' and ''outside-in'' activation.[20] Inside-out activation occurs when integrin behavior responds to intracellular signaling changes while outside-in activation occurs when integrin behavior is determined by the content of the ECM. Unfortunately, this study does not determine whether the integrins on the surface of the fibroblasts were activated or not. More work is required to demonstrate whether quercetin impacts the activation of integrins on the surface of skin cells and, if so, how this impacts the proliferation and migration of these cells. However, it is clear that quercetin modulates expression of the integrin subunits evaluated in this study. Furthermore, it is known that integrins impact ECM formation at wound sites. Integrins, specifically αV-containing integrins, but also some β1-containing integrins are known to cleave transforming growth factor-β in the ECM, a behavior that is often, but not always, pro-fibrotic.[5] By altering the integrin content on the cell surface, quercetin may diminish signaling via pro-fibrotic pathways while maintaining cell adhesion and the ability of the cells to migrate. These results hint that more exploration of quercetin's impact on fibrosis and, specifically on integrin expression and activation, may yield promising treatment strategies. The results also demonstrate that quercetin's mechanism of action may involve modulation of integrin expression and behavior. However, more work is needed to determine which integrin subunit combinations are most important for quercetin's role wound healing. Additional experimentation should also be performed to examine the impact of quercetin on fibroblast growth and migration in the context of other ECM components. One ECM component that has the potential to either synergize or antagonize quercetin in impacting fibroblast growth and migration is Fibulin-5, which as Furie *et al*.[21] demonstrate reduces the growth potential of keloid-derived fibroblasts. These studies also demonstrate that, in the presence of an anti-integrin β1 antibody in combination with a coating of fibulin-5 on a culture plate, fibroblasts were significantly less able to adhere. Thus, quercetin, which reduced β1 integrin expression in the present study, might also synergize with fibulin-5 to decrease fibroblast adhesion potential and might promote cell migration. On the other hand, a combination of fibulin-5 and quercetin might also decrease cell proliferation sufficiently to impair wound healing, in a manner similar to that seen with the artificial ECM composed of gelatin and fibronectin. Another avenue of further experimentation would be to explore quercetin's effects on other cell types that participate in wound healing, including skin cells such as melanocytes or keratinocytes, immune participants in wound healing such as neutrophils, macrophages, lymphocytes or histiocytes, or cells that mediate neovascularization such as smooth muscle cells or vascular endothelial cells.[1] A more complete examination of the impact of quercetin on all the cells present at the wound site would not only improve our understanding of how quercetin could prevent the formation of ECM fibers in wounds and thereby decrease scarring but might also help identify other therapeutic targets by which wound healing could be improved. Thus, more work is needed to better understand the mechanism by which quercetin improves wound healing to fully utilize its therapeutic potential.

Other studies have employed quercetin treatment and have demonstrated improvements in wound healing.[10,11,22,23] Additionally, there is substantial interest in incorporating quercetin into ECM-like scaffolds as part of a bioengineering strategy to improve wound healing.[23] While this is an attractive strategy, our work suggests that quercetin may be more effective without the use of artificial ECM. Furthermore, our results add to the evidence that quercetin is anti-fibrotic and may be an effective therapy for patients in whom excess scarring is likely to be a problem, such as patients with hypertrophic scarring or keloids, or patients in whom skin injuries cross a joint and may impair function if excessive scar forms. This work also highlights the role that integrins may play in the effects of quercetin on wound healing, which to our knowledge has not previously been documented. Thus, more work should be done to improve our understanding of the mechanism by which quercetin improves fibrosis and alters integrin expression in order to employ this medication, which is currently available in the United States, as a therapeutic.

Authors' contributions: Both authors contributed to the data analysis, figure creation, literature review, and writing of this manuscript.

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DECLARATION OF CONFLICTING INTERESTS

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