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Role of Skin Stretch on Local Vascular Permeability in Murine and Cell Culture Models

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Background: Excessive mechanical forces, particularly skin stretch, have been implicated in pathological cutaneous scarring. We hypothesize that this reflects, in part, stretch-induced vessel leakage that provokes prolonged wound/scar inflammation. However, this has never been observed directly. Here, a mouse model was used to examine the effect of skin flap stretching on vascular permeability. An in vitro model with pseudocapillaries grown in a stretchable chamber was also used to determine the effect of stretching on endothelial cell morphology and ion channel activity.
Methods: Murine skin flaps were stretched with a biaxial stretching device, after which FITC-conjugated-dextran was injected and imaged with fluorescence stereomicroscopy. Endothelial cells were induced to form pseudocapillary networks in an elastic chamber. The chamber was stretched and differential interference contrast microscopy was used to assess cell morphology. In other experiments, markers for Ca^{2+} influx and K^{+} efflux were added before a single stretch was conducted. Histamine served as a positive-control in all experiments.
Results: Cyclic stretching (20%) increased the vascular permeability of skin flaps almost as strongly as histamine. Both stimuli also partially disrupted the pseudocapillary networks, induced cell contraction, and created gaps between the cells. Both stimuli caused sustained K^{+} efflux; stretching had a milder effect on Ca^{2+} influx.
Conclusions: Excessive cyclical stretching strongly increased the vascular permeability of skin vessels and in vitro pseudocapillaries. This effect associated with increased K^{+} efflux and some Ca^{2+} influx. Inhibiting such early stretch-induced signaling events may be an effective strategy for treating and preventing hypertrophic scars and keloids. *(Plast Reconstr Surg Glob Open 2022;10:e4084; doi: 10.1097/GOX.0000000000004084; Published online 27 January 2022.)*

INTRODUCTION

The mechanisms that underlie normal and aberrant cutaneous wound healing and scar formation are complex and remain poorly understood. This has hindered the development of therapeutic interventions that improve wound healing and scarring. However, previous studies suggest that various local, genetic, and systemic factors contribute to abnormal wound healing and the resulting formation of pathological scars.[1] Of these, local mechanical force is of particular interest because it may be easier to target therapeutically/prophylactically than genetic or systemic factors.

There are multiple lines of evidence showing that mechanical force drives aberrant wound healing.[2] In particular, it is now well known that skin sites that are highly mobile and are thus constantly or frequently subjected to tension are highly prone to both thickening of the skin and the dysfunctional wound healing that leads to keloidal and hypertrophic scarring.[3,4] Keloids are an especially interesting case in point: despite the fact that systemic and genetic factors play key roles in keloid development, these scars are nonetheless most likely to occur at very specific, highly mobile skin sites, namely, the anterior chest, shoulder, scapula, and lower abdomen.[5] These observations have greatly shaped wound and scar treatment paradigms.

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For example, it is now recommended that wounds and scars should be stabilized with fixable materials such as tapes, bandages, and silicone sheets to limit skin stretching, thereby promoting smooth wound healing and preventing pathological scar formation.[5]

However, further research is needed to elucidate the mechanisms by which mechanical forces on the skin shape abnormal wound healing and scarring.[6,7] One important mechanism relates to inflammation in the wound: although early transient inflammation is an essential part of normal wound healing, there is evidence that this stage is aberrantly prolonged in scars that evince pathogenic growth.[8] Moreover, it has been shown with agent-based model and finite element modeling, in vitro, animal, case, and observational studies that stretching strongly promotes inflammation.[9] However, the mechanisms by which stretching induces/aggravates wound/scar inflammation have not yet been elucidated. We believe that stretch-induced increases in vascular permeability play a key role: specifically, we postulate that mechanical stretch induces endothelial dysfunction that leads to vascular hyperpermeability and the influx into the wound/scar of inflammatory factors, and that this amplifies and aberrantly extends wound/scar inflammation.[5]

In this study, we have performed the real-time analysis of the behavior of blood vessels exposed to skin stretch. We intravitaly observed how excessive cyclic stretch can cause vascular permeability in a skin flap on the dorsal median side of a mouse. An in vitro model with pseudocapillaries grown in a stretchable chamber was also used to determine the effect of stretching on endothelial cell morphology and ion channel activity.[10,11]

Takeaways

Question: How does repetitive/continuous skin tension, which is thought to promote hypertrophic scar and keloid growth, enhance vascular permeability?

Findings: We showed for the first time with intravital microscopy that excessive equibiaxial cyclical stretching markedly increased vascular permeability in the murine skin flap. We succeeded in suggesting that mechanical force exerts the effect on vascular permeability by disrupting the endothelial barrier.

Meaning: β-hydroxybutyrate, which can inhibit stretch-induced Ca^{2+} response, K_{ATP} channel opening, and NLRP3 activation, may be a promising therapeutic candidate for keloid scars.

MATERIALS AND METHODS

Animals

Male 10- to 15-week-old Type ICR mice (30–35 g) were purchased from Sankyo Labo Service Corporation (Tokyo, Japan). All animal experiments were approved by the Animal Experiments Ethical Review Committee of Nippon Medical School (Approval No. 28-011). Mice were fully anesthetized during all experimental procedures. Mouse sample sizes reflected tracer-image variability observed in a pilot study (data not shown).

In Vivo Skin-stretching Study

The MCB1 skin-stretching device was purchased from CellScale (Ontario, Canada) (Fig. 1A). Three murine groups were prepared: a cyclic-stretched group (n = 16), a nonstretched negative-control group (n = 4), and a histamine-injected positive-control group (n = 3). After shaving, the skin flap was elevated by dissecting the skin from the attached fascia. The vessels arising from the brachial artery to the iliac artery and the epigastric artery and vein were kept intact (Fig. 1B). The mice were placed in the left lateral tilt position and the inner side of the island flap with left lateral thoracic artery was attached tautly face-up at its superior and inferior edges to the skin-stretching device and either subjected to 45 minutes of equibiaxial (superior-inferior) cyclic stretching (20%, 1 Hz), left unstretched for 45 minutes, or left unstretched for 44 minutes and then injected intravenously with 0.5 ml of 10 mg histamine dihydrochloride (Sigma-Aldrich, St. Louis, Mo.) dissolved in 5 ml of PBS.[12,13] At the 45-minute time point, the tail veins of all mice were injected with 0.2 ml FITC-conjugated 70 kDa dextran tracer (Sigma-Aldrich) with a 27-gauge disposable needle (Terumo Corp., Tokyo, Japan). The 70 kDa dextran was selected because normal cutaneous blood vessels do not allow its passage into the interstitial space.[14] The FITC-dextran solution was prepared by dissolving 3 mg FITC-dextran in 0.6 ml PBS (Wako, Tokyo, Japan). Leakage of the tracer into the surrounding tissue was imaged by positioning the mice on the stage of a fluorescence stereomicroscope (M165FC, Leica Microsystems, Wetzlar, Germany) and taking images with a Thorlabs Quantalux sCMOS camera (Thorlabs, Newton, N.J.).

Stretch-induced Cytomorphology of In Vitro Capillary Networks

A stretchable chamber was molded by using Silpot 184 W/C silicone elastomers (Dow Corning Toray, Tokyo, Japan), after which it was coated with collagen (Cellmatrix type I-A, Nitta Gelatin, Osaka, Japan) and overlaid with Matrigel (Corning Life Sciences, Tewksbury, Mass.). Human dermal microvascular endothelial cells (HMEC-1) purchased from American Type Culture Collection (CRL-3242, ATTC, Manassas, Va.) were grown in MCDB-131 (Gibco, Grand Island, N.Y.) supplemented with 10% FBS at 37°C in a humidified atmosphere of 5% CO_2 and then seeded on the stretchable chamber. Cells were incubated for 1 day to allow them to form pseudocapillary networks. The chamber was then attached to a pulse-motor-driven stretching machine (ST-600W, STREX, Osaka, Japan)[14] mounted on the stage of an inverted microscope (IX73, Olympus, Tokyo, Japan). Cells were either left alone for 50 minutes (negative-control), subjected to uniaxial cyclic stretch for 5 minutes and then left alone for 45 minutes, or treated with 10 μM histamine for 50 minutes (positive-control). The 5-minute stretching session consisted of cyclic 1 second-long 20% stretches that were repeated every 5 seconds (n = 6).

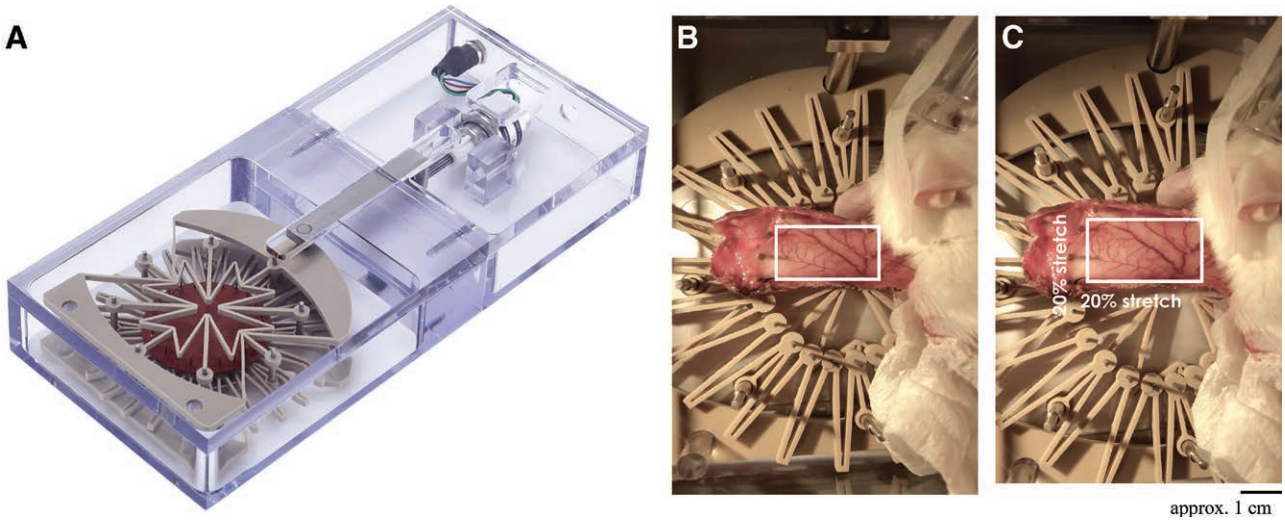

Fig. 1. The stretching device and the murine skin flap before and during skin stretch. A, The stretching device (MCB1, CellScale, Canada) bears 24 mounting pins that are organized in a radial pattern on a ring with a deformable interface. B, The surgically generated rectangular abdominal skin flap is puncture-mounted to the ring at its superior and inferior edges. The main blood vessels in the flap that rise from the brachial artery to the iliac artery are kept intact, as are the epigastric artery and vein. C, The skin flap is exposed to biaxial stretch stimulation with a maximum magnitude of 20% and a cycle frequency of 1 Hz for 5 minutes (see Video 1 [online]). The white boxes in (B) and (C) indicate the region of interest in the center and the extent of stretch.

Intracellular Ca^{2+} Response and K^+ Channel Activity of In Vitro Capillary Networks after a Single Stretch

Capillary networks were either subjected to a single 20% uniaxial stretch for 1 second, left unstretched, or treated with 10 μM histamine. Before this, the networks were loaded with either 1 mM Fluo-8 AM (AAT Bioquest, Sunnyvale, Calif.)[15,16] supplemented with 0.2% Cremophor EL (Sigma-Aldrich) or 0.4 μM Thallos-AM dye (TEFlabs, Austin, Tex.)[17] for 30–45 minutes. Fluorescence imaging was conducted with a fluorescence microscope (IX73, Olympus). Imaging data were processed by using ImageJ software (v1.52, National Institutes of Health, Bethesda, Md.). All experiments were performed by a number of four to six.

STATISTICAL ANALYSES

All quantitative fluorescent intensity values were expressed as mean ± standard error of the mean (SEM). Differences between two groups in the in vivo and in vitro experiments were assessed by Student's *t* test. Statistical analyses were carried out using R 4.1.0 (R Core Team, 2021). A *P* value less than 0.05 was considered statistically significant.

RESULTS

Equibiaxial Strain Promotes Vascular Permeability in Skin Flaps

To test the effect of excessive stretch on the vascular permeability in skin flaps, we initially used the ST-600W device to stretch murine skin flaps uniaxially (20% stretch). However, this type of stretching caused the flaps to contract laterally. Therefore, we selected the MCB1 device (Fig. 1A), which can stretch skin flaps with physiologically plausible equibiaxial (superior-inferior) loading. (**See Video 1 [online],** which shows live images during excessive equibiaxial skin flap stretching.) Thus, surgically created rectangular abdominal skin flaps in mice were attached in a taut state at their superior and inferior edges to MCB1 (Fig. 1B). In the study group, the skin flap was then subjected to 45 minutes of 20% equibiaxial stretching with a frequency of 1 Hz (Fig. 1C). By contrast, in negative- and positive-control mice, the skin flap was left tautly attached to the stretching device for 45 minutes (Fig. 1B) but was never stretched. The positive-control mice were injected intravenously with histamine at the 44-minute mark. FITC-dextran was then injected within 1 minute and the leakage of fluorescence into the interstitial space of the skin flap was monitored for 50 minutes with fluorescence stereomicroscopy. In the nonstretched negative-control group, fluorescent dextran was observed in the blood vessels; major FITC-dextran extravasation in the central part of the flap was not observed over 50 minutes after the injection (nonstretch/negative-control group) as shown in Supplemental Digital Content 1. **(See figure, Supplemental Digital Content 1**, which displays the effect of excessive stretching and histamine treatment on vascular permeability in the center of the murine skin flaps, as determined by intravenous injection of FITC-conjugated 70 kDa dextran tracer followed by fluorescence stereomicroscopy. A, Permeability of the skin flaps of the negative-control unstretched [control], stretched [cyclic stretch], and positive-control unstretched histamine-injected (histamine) mice. The skin flap was attached to the stretching device and left unstretched for 45 minutes, after which the mouse was injected with tracer and monitored for 50 minutes [control]. In cyclic stretch, the flap was subjected to 20% biaxial [superior-inferior] stretching with a frequency of 1 Hz for 45 minutes, after

which the tracer was injected and monitored for 50 minutes. In histamine, the flap was attached to the stretching device and left unstretched for 44 minutes, after which the mouse was injected with histamine. One minute later, the mouse was injected with the tracer. Scale bar = approximately 1 cm. Respective zoom-in images of the areas in A are bound by white dashed lines **[Video 2]**. B, The fluorescent intensity of leakage was measured. The intensity data were normalized to the peak responses 5 min after the tracer infusion. The mean ± SEM intensities of one of six representative experiments are shown. There were five mice each in the negative-control, stretched, and positive-control groups in each experiment. *$P < 0.001$, as determined by Student's t test. **http://links.lww.com/PRSGO/B904**.) By contrast, 5 minutes after the injection, the stretched skin flap exhibited a mottled pattern of visible leakage from the microvessels throughout the whole flap (**Supplemental Digital Content 1, http://links.lww.com/PRSGO/B904**). Subsequently, the FITC-dextran spread widely across the interstitial area. The fluorescence intensity peaked at 25 minutes and was much greater than the fluorescence in the negative-control mice at both 25 and 50 minutes. Histamine had a very similar effect as skin stretching (**Supplemental Digital Content 1, http://links.lww.com/PRSGO/B904**).[12,13]

Given that there was some surgical damage-induced leakage of the tracer at the flap edges of the negative-control mice, we quantified the vascular permeability in a rectangular central flap area,[6] which is marked by dashed white lines in Supplemental Digital Content 1 (**http://links.lww.com/PRSGO/B904**). Thus, we zoomed in on these rectangles and scored the fluorescence intensity according to the vascular permeability scale. (**See Video 2 [online],** which shows zoom-in fluorescent images of the areas in **Supplemental Digital Content 1** that are bound by white dashed lines during excessive equibiaxial skin flap stretching.) All intensity data were normalized to the responses 5 min after dextran infusion. In the negative-control mice, fluorescent intensity gradually decreased over time (n = 3). By contrast, the stretched mice showed conspicuous dextran extravasation at 25 minutes [193% higher compared with control, $P < 0.001$ (n = 16)]. Histamine injection had a similar effect (n = 4), although it was 10% smaller than the effect of stretching (**See Supplemental Digital Content 1, http://links.lww.com/PRSGO/B904**).

Excessive Stretching Disrupts Capillary Networks In Vitro

To our knowledge, the effect of excessive stretching or histamine on the cytomorphological characteristics of microvascular networks cultured on Matrigel has not been reported previously. Thus, we induced endothelial cells to form two-dimensional pseudocapillary networks by culturing them on a Matrigel-coated elastic chamber for 1 day. We then started the experiment. Thus, we either left the cells alone for 50 minutes (negative-control), cyclically uniaxially stretched the chamber by 25% with a frequency of 1 Hz once every 5 seconds for 5 minutes and then left the cells alone for another 45 minutes, or added 10 μM histamine to the chamber and left the cells alone for 50 minutes (positive-control). DIC microscopy continuously imaged the cells after starting the experiment. The negative-control cells showed no change over time in terms of their morphology and adhesion to each other (Fig. 2, control). By contrast, in the stretched cultures, imaging at 25 minutes showed that the stretching had caused the cells to contract away from each other and the intercellular adhesions had become frail (Fig. 2, cyclic stretch). This was even more pronounced by 50 minutes. Similarly, histamine treatment caused the endothelial cells to contract rapidly and display weakening of the intercellular substance (Fig. 2, histamine). (**See Video 3 [online],** which shows some of the live imaging during cyclic stretch stimulation.)

Excessive Stretch Induces Intercellular Ca^{2+} Responses and K^+ Efflux

In separate experiments, capillary networks were loaded with Fluo-8-AM[15,16] or Thallos-AM[17] subjected to a single 25% stretch for 1 second or 10 μM histamine (n = 4–6). Fluorescence microscopy was conducted from 30 seconds before stretch/treatment to 150 seconds later (Fig. 3). (**See Video 4 [online],** which shows real-time Ca^{2+} imaging during [A] stretch or [B] histamine stimulation.) The single stretch-induced small intracellular calcium influx that nonetheless rose over time (Fig. 3B and C, $P < 0.001$). By contrast, histamine immediately induced a large transient large calcium influx followed by typical long-lasting Ca^{2+} oscillation (Fig. 3B and C).[18,19] Stretching had a much larger effect on K^+ channel activity (Fig. 4A). (**See Video 5 [online],** which shows real-time Tl^+ responses during [A] stretch or [B] histamine stimulation.) Sustained K^+ release was observed after the single stretch and this response exceeded the K^+ release induced by histamine (Fig. 4B and C).

DISCUSSION

We have previously reported that cyclic skin stretch increases the expression of neuropeptides such as substance P (SP) and calcitonin gene-related peptide in the dermis and epidermis.[20] It has been recognized that calcitonin gene-related peptide coadministrated with SP potentiates microvascular permeability and promotes plasma extravasation in mouse dorsal skin.[21] However, local skin stretch-induced vascular permeability and morphological study on the cell-based model still remained extremely limited.[22] Furthermore, it has been less well described to associate the time-dependent changes in vascular permeability in vivo with those in vitro. Because microenvironmental properties of endothelial cell activities in vivo may not be entirely reproduced in vitro. Here, we designed experiments to determine the contribution of skin stretch stimulation to microvascular responses using in vivo and in vitro assays, including time course. Cell culture models were also used for the preclinical screening for the determination of the drug efficacy. This is the first time that equibiaxial cyclic stretch-induced vascular permeability has been intravitally evaluated in the murine skin flap. Indeed, the effect of quibiaxial cyclic stretching (20%, 1 Hz, 45 min) on vascular relaxation was almost as pronounced as the effect of intravenously injecting 10 μM

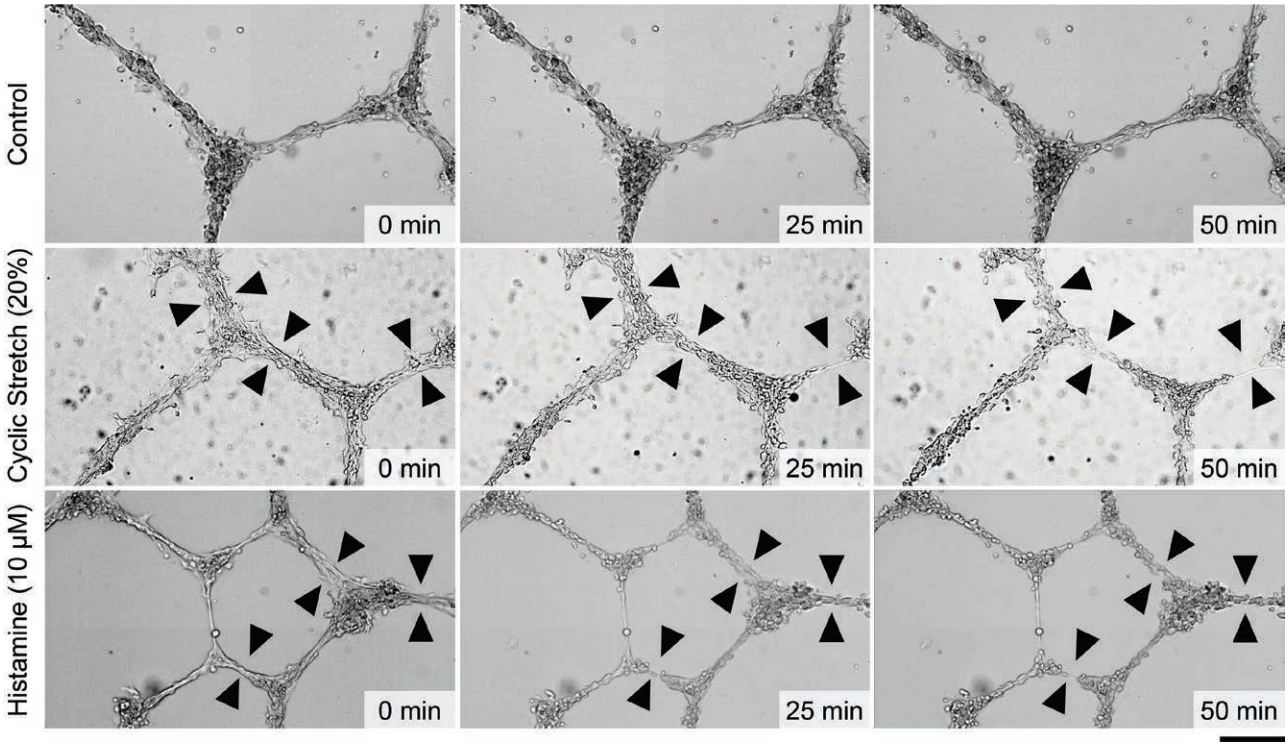

Fig. 2. Effect of excessive stretching and histamine treatment on the cytomorphology of pseudocapillary networks that were generated by endothelial cells in vitro. Representative data on the endothelial pseudocapillary networks cultured on Matrigel in a silicone stretch chamber were either left alone for 50 minutes as a negative-control (control), subjected to 20% uniaxial stretching for 1 second every 5 seconds for 5 minutes (nominal 60% extension) and then left alone for 45 minutes (cyclic stretch), or treated with 1 µm histamine for 50 minutes as a positive-control (histamine). Representative differential interference contrast microcopy images are shown. Intercellular areas of interest are indicated by pairs of opposing black or white arrowheads. The scale bar is approximately 200 µm.

histamine (1-min pretreatment). Thus, mechanical force can induce the vascular hyperpermeability, which associates with strong inflammation.

We also identified that mechanical force triggers the contraction of the endothelial cells and impairs the endothelial networks.[23] Thus, excessive stretch on capillaries may cause the endothelial junctions to weaken, thereby abruptly inducing acute barrier breakdown. This notion is supported by a recent study by Gawlak et al[24] that explored why the excessive tissue distension caused by suboptimal ventilator support or regional ventilation heterogeneity associates with life-threatening conditions such as brain and pulmonary edema. They asked whether cyclic stretch of pulmonary endothelial cells provoked vascular leakage. Indeed, they found that 18% cyclic stretch doubled the permeability of human pulmonary artery endothelial cell monolayers, as shown by increased binding of FITC-labeled avidin to the biotinylated collagen-coated plate. This change also associated with changes in the Rho GTPase signaling pathway, which is triggered in focal adhesions between endothelial cells and is involved in cellular mechanotransduction. Significantly, the stretched pulmonary endothelium monolayer expressed inflammatory markers.[24] Mechanical skin stretch-induced tension at the focal adhesions seemingly cause acute endothelial barrier dysfunction as well as histamine stimulation.[12,13]

We further used our pseudocapillary network model to determine whether stretch activates cytoskeletal contraction by increasing intracellular Ca^{2+} influx and/or K^+ efflux since we noted that stretching did not disrupt the networks when Ca^{2+} was absent or the K^+ channel inhibitor glibenclamide was present (Fig. 5). (**See Video 6 [online],** which shows stretch-induced [A] cytomorphology of pseudocapillary networks and the intercellular [B] Ca^{2+} responses/[C] K^+ efflux in the presence of Ca^{2+}-free or glibenclamide.) (**See Video 7 [online],** which shows real-time Tl+ responses during stretch or histamine stimulation.) Multiple studies suggest that Ca^{2+} and K^+ channels participate in stretch-induced vascular hyperpermeability and inflammatory responses.[25–31] Moreover, recent studies suggest that mechanical stretch of lung endothelial cells leads to NLRP3 inflammasome activation,[32] which is triggered by K^+ efflux.[28–31] This stretch triggers cytosolic Ca^{2+} and K^+ concentration changes and induces the cells to contract, which in turn causes gaps to appear between the cells. These gaps increase the permeability of the blood vessels, thus aiding the extravasation of inflammatory cells and factors into the perivascular area.[33] The ultimate effect is increased local inflammation that does not subside as long as tension remains present. This prevents the wound healing process from progressing from the early inflammatory stage to the proliferative and remodeling phases; as a result, wound/scar fibroblasts are constantly

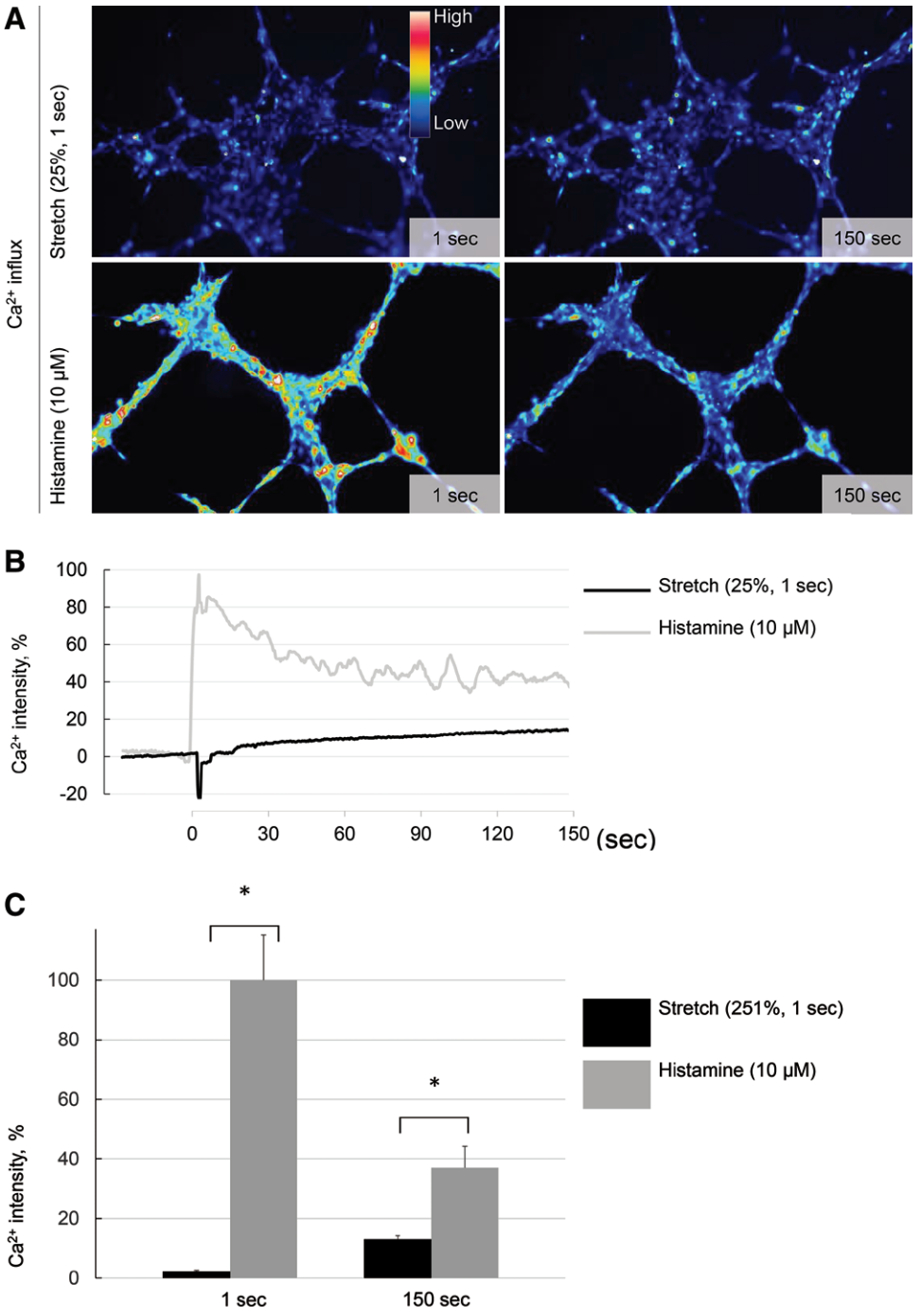

Fig. 3. Effect of excessive stretching and histamine treatment on the Ca^{2+} dynamics in pseudocapillary networks. Capillary networks were cultured on Matrigel in a silicone stretch chamber with a Ca^{2+} indicator dye (Fluo-8AM) for 30–45 minutes and then subjected to a single 25% uniaxial stretch for 1 second or treated with 1 μM histamine. The cultures were imaged continuously from 30 seconds before stretch/treatment to 150 seconds later. A, Representative images of the stretched (top) and histamine-treated (bottom) networks. Scale bar is approximately 200 μm. B, C, Quantification of the Ca^{2+} responses to the stimuli. The fluorescence intensity traces of 50 cells in each culture dish were averaged and normalized to the peak value obtained with ionomycin treatment. B, The change in intensity over time. C, The average values of the normalized traces at 1 and 150 seconds. The data are from four separate experiments. All quantitative data show the mean ± SEM. Three groups were prepared: a stretched group (n = 6), a nonstretched negative-control group (n = 4), and a histamine-added positive-control group (n = 6). *$P < 0.001$, as determined by Student's *t* test.

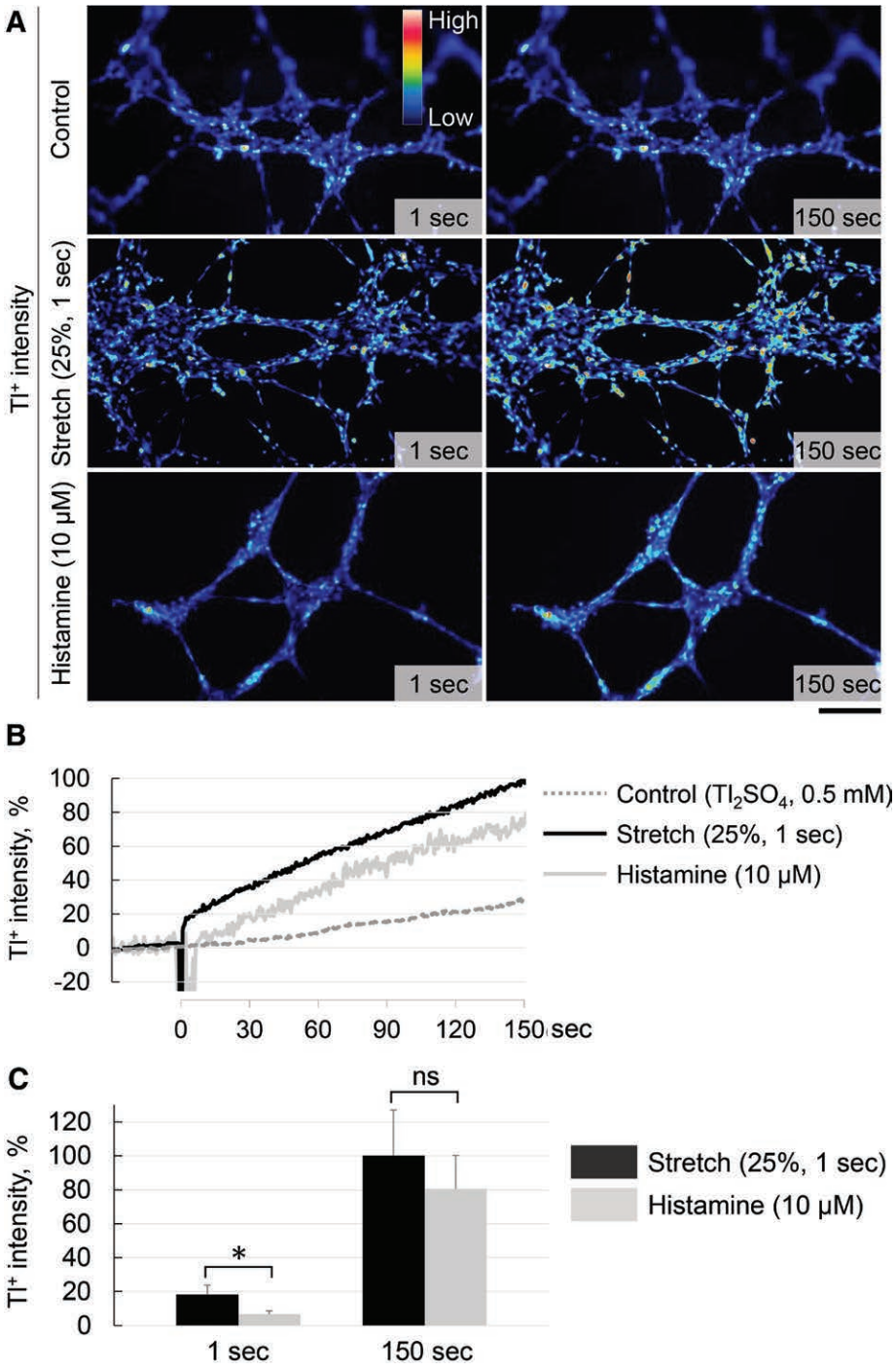

Fig. 4. Effect of excessive stretching and histamine treatment on K^+ channel activity influx in pseudocapillary networks. Capillary networks were cultured on Matrigel in a silicone stretch chamber with a dye indicating K^+ channel activity (Thallos-AM) for 30–45 minutes and then subjected to a single 25% uniaxial stretch for 1 second or treated with 1 μM histamine. The cultures were imaged continuously from 30 seconds before stretch/treatment to 150 seconds later. A, Representative images of the unstretched untreated (top), stretched (middle), and unstretched histamine-treated (bottom) networks. Scale bar is approximately 200 μm. B, C, Quantification of the K^+ channel activity caused by the stimuli. The fluorescence intensity traces of 50 cells in each culture dish were averaged and normalized to the peak value obtained with ionomycin treatment. B, The change in intensity over time. C, The average values of the normalized traces at 1 and 150 seconds. The data are from four separate experiments. All quantitative data show the mean ± SEM. Three groups were prepared: a stretched group (n = 6), a nonstretched negative-control group (n = 4), and a histamine-added positive-control group (n = 4). *P < 0.01. ns, not significant, as determined by Student's t test.

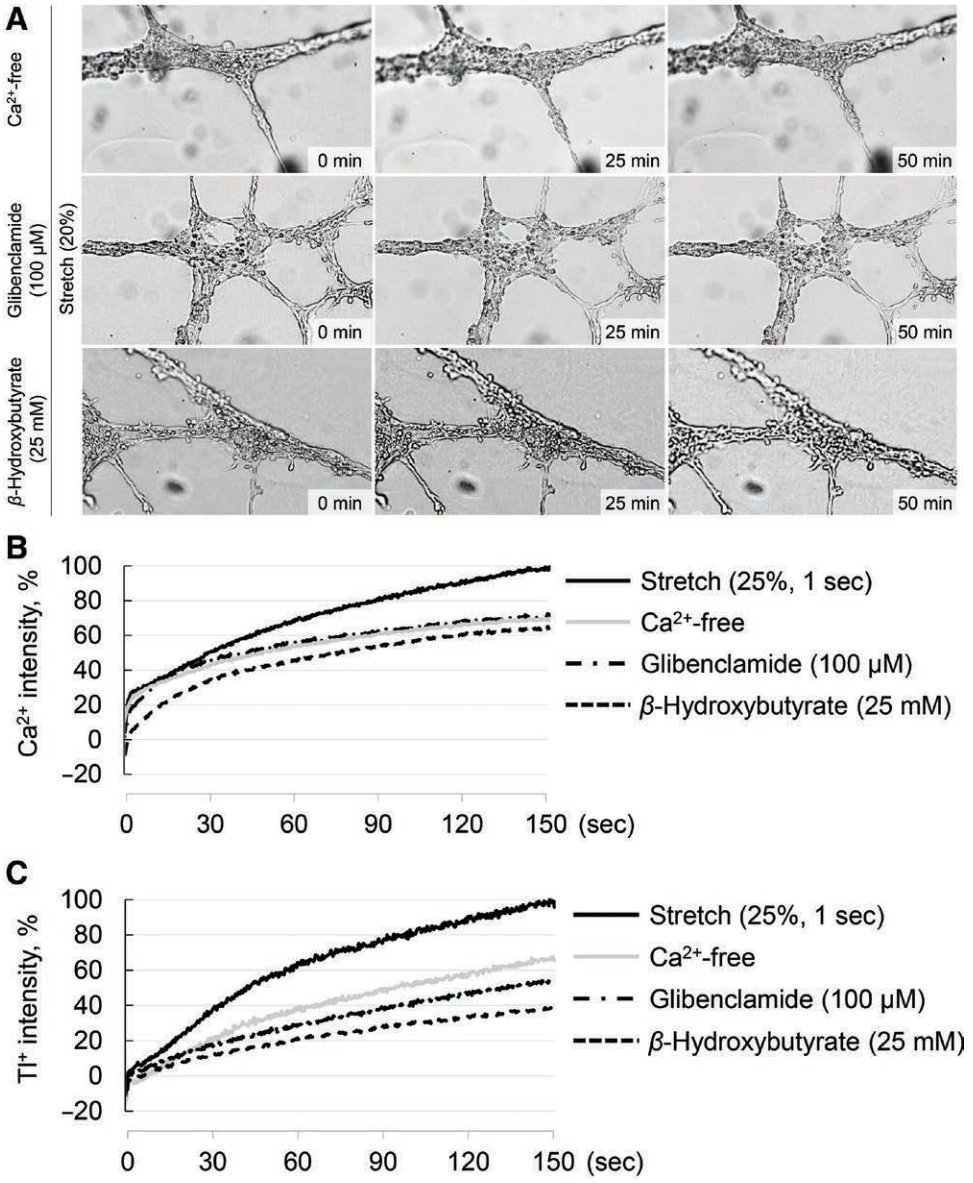

Fig. 5. Effect of excessive stretching treatment on the cytomorphology, Ca^{2+} dynamics, and K^+ channel activity in pseudocapillary networks. A, Stretch-induced change of cytomorphology. B, The intercellular Ca^{2+} responses and (C) K^+ efflux (Tl^+ intensities) under the condition of Ca^{2+}-free, glibenclamide (100 μM) or β-hydroxybutyrate (25 mM) of pseudocapillary networks.

provoked to lay down extracellular matrix, which causes abnormal growth of the scar.[8,34] Vinaik et al[35] revealed that NLRP3-mediated inflammation is present in keloids compared with burn and normal skin. Lee et al[36] indicated a mechanism linking autophagy to inflammation and myofibroblastic differentiation in keloid fibroblasts induced by the Notch/NLRP3 signaling pathway. Indeed, stretch- or histamine-induced endothelial cell contraction was suppressed by β-hydroxybutyrate, which can prevent the influx of Ca^{2+}, the decline of intracellular K^+, and the successive NLRP3 inflammasome[37,39] **(Video 6)**. These preliminary findings indicate that glibenclamide and β-hydroxybutyrate of the therapeutic candidate for NLRP3-related diseases[40] may be a new therapeutic approach toward stretch-dependent inflammation such as keloid.

Although we did not address it in our present study, it is likely that mechanical force such as shear stress also contributes to pathological scarring. When endothelial cells are subjected to shear stress in vitro, their ability to support cytokine-induced leukocyte adhesion and migration increases markedly.[41] Orsenigo et al[42] reported that homodynamic shear stress triggers vascular endothelial cadherin phosphorylation and internalization, resulting in the disruption of endothelial junctions. Albarrán-Juárez et al[43] showed that laminar and disturbed flow activate mechanosensitive cation channel Piezo1, the downstream purinergic P2Y2 receptor, and Gq/G11-mediated signaling pathway. Thus, local tissue deformation induced by skin stretching may increase endothelial cell-mediated leukocyte recruitment and

local inflammation. We are currently assessing the upstream mechanism on stretch-induced immune/inflammatory response of endothelial cells in our in vivo and in vitro skin stretch models.

CONCLUSIONS

Our findings support the notion that repetitive/continuous skin tension exerts mechanical force on blood vessels and that this increases their permeability, thereby promoting inflammatory cell/factor influx into the wound bed and prolonging the inflammatory phase of wound healing and promoting the growth of hypertrophic scars and keloids.[44,45] We speculate that inhibiting the early stretch-induced signaling events in endothelial cells may be an effective strategy for treating and preventing hypertrophic scars and keloids.

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