Supplementary Methods and Figures

Supplement. 1 Processes of the free flow confluence (FFC) method used to make the PDMS substrate with stiffness variations.

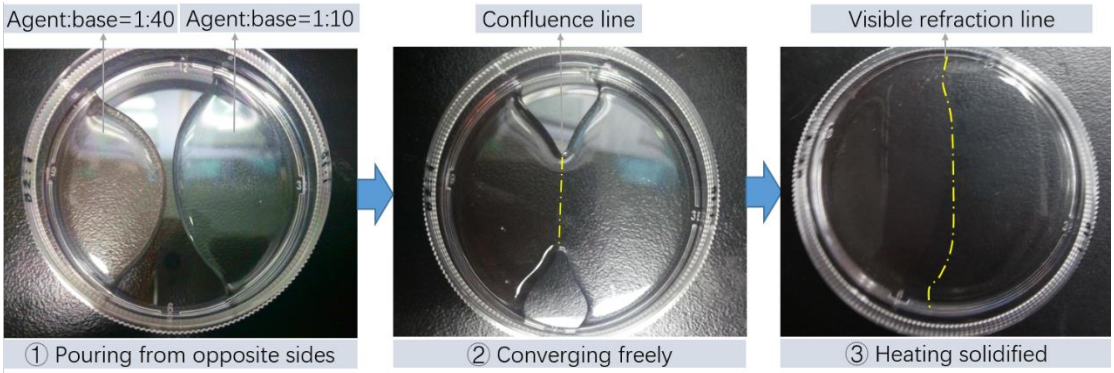

Figure S1. Processes of the free flow confluence (FFC) method to produce the stiffness gradient PDMS substrate.

Firstly: Pour different mass ratios of mixed liquid PDMS into the opposite sides of the cell culture dish. In this process, the higher mass ratio liquid has a relatively higher fluidity, which would flow faster than the lower one and should be poured a few minutes later than the lower mass ratio liquid PDMS.

Secondly: Let the poured liquid PDMS flow freely, and make sure the two different mass ratios of PDMS liquid converge in the middle of the dish.

Thirdly: Keep the dish steady and horizontal, and heat the fluid PDMS to a solid substrate in the oven at 80°C for 6 hours. Sterilize and modify the solid PDMS substrate under ultraviolet irradiation for more than 4 hours.

Supplement. 2 Mast cells in the overall skin and the measurement of distance from mast cell to specific tissue.

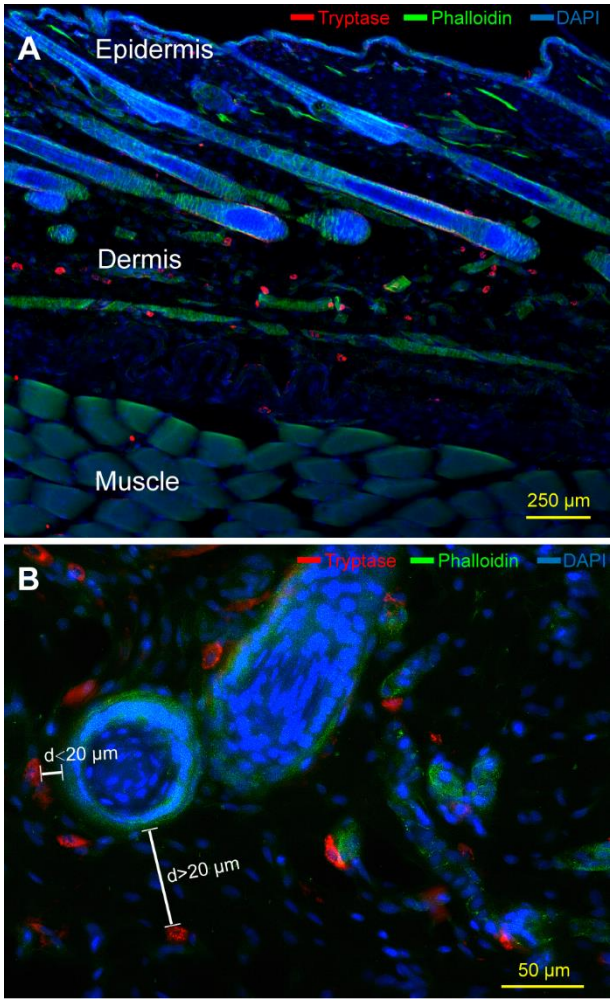

Figure S2. Mast cells in the overall skin and the measurement of distance from mast cell to specific tissue. (A) Mast cells in the overall scale of the skin. (B) The distance measurement of mast cell to hair follicle tissue. 'd' represents the vertical distance from one cell to specific tissue. Immunofluorescence histochemical staining was used to co-localization tryptase (red), phalloidin (green), and DAPI (blue).

Supplement. 3 RBL-2H3 cells prefer the area with stiffness variations and distribute along stiffness changing curved boundaries.

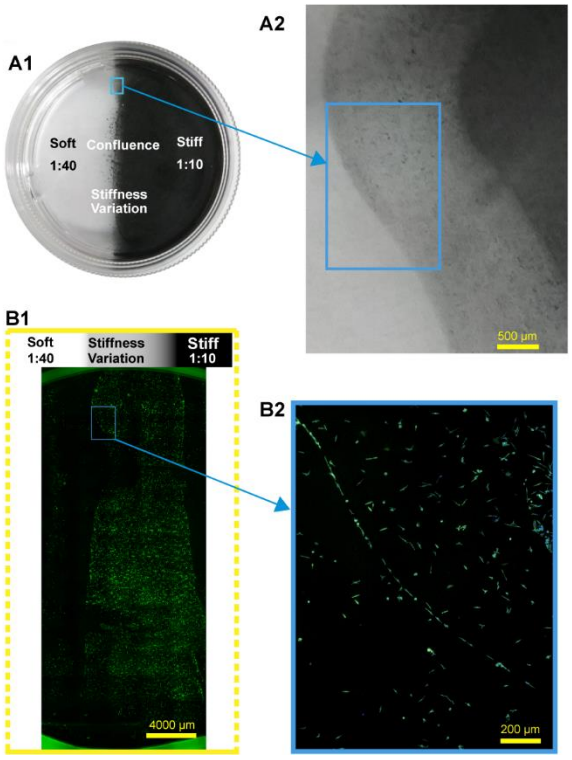

Figure S3. RBL-2H3 cells prefer the area with stiffness variations and distribute along stiffness changing curved boundaries. (A1-A2) The PDMS confluence belt bends at the edge of the culture dish. (B1-B2) RBL-2H3 cells are distributed along the stiffness gradient's curved boundaries. Adherent RBL-2H3 cells was dyed green by phalloidin. The blue rectangular boxes in (A1), (A2), (B1), and (B2) represent a similar position in the culture dishes.

Supplement. 4 Traditional manual acupuncture might modify the stiffness of local tissues through the lifting and twisting motion of the needles.

Mechanical stimulation induces the accumulation and degranulation of mast cells in the stress-changing region.

In clinical treatment, the lifting and twisting motion of manual acupuncture can twine collagen fibers and result in changes in the stiffness of local tissue [1]. To test and verify whether mast cells accumulate in the stress-changing region (or in the area with stiffness variations), we applied acupuncture to the rat ST36 and examined the number and degranulation ratio of mast cells in the paraffin section of the skin tissue (Fig. S4C1-C3). The statistical results show that the lifting and twisting motion of acupuncture has significantly increased the cell number and degranulation ratio (Fig. S4A-B).

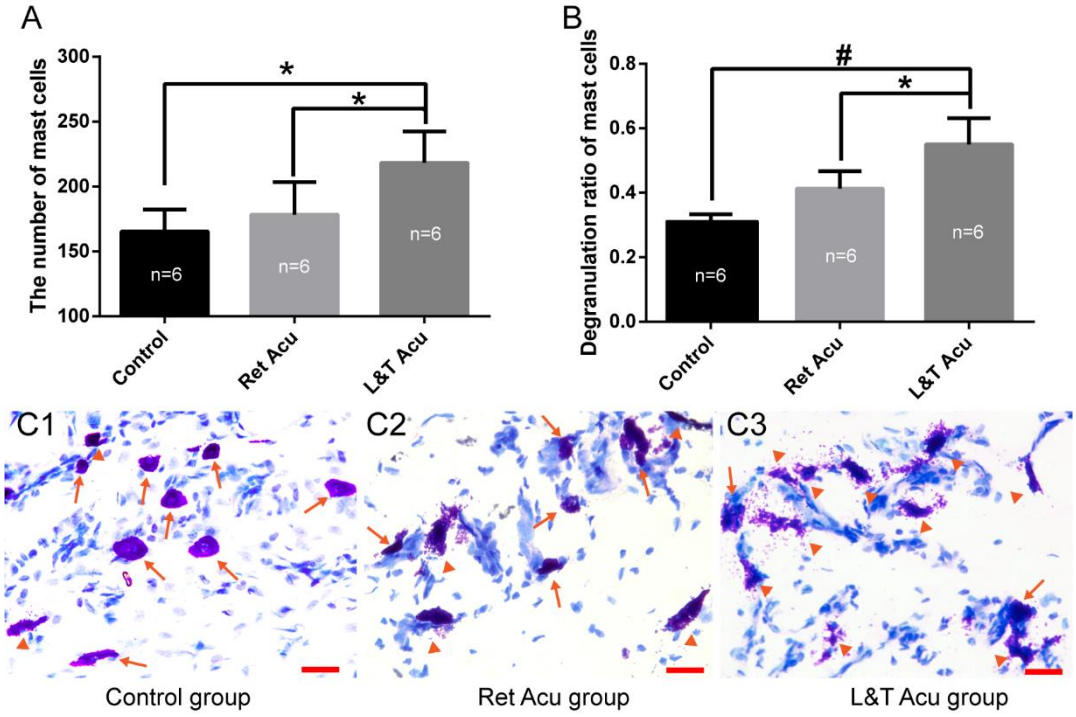

Figure S4. Lifting and twisting motion of acupuncture induces the accumulation and degranulation of mast cells. (A-B) The number and degranulation ratio of mast cells in different motion treatments in the rat ST36. The subjects were divided into three groups: the 'control' group, without acupuncture treatment; the 'Ret Acu' group, with the needle inserted into the tissue but no lifting and twisting motion; and the 'L&T Acu' group, with the needle inserted into the tissue and with the lifting and twisting motion every 1 minute. The total treatment time is 30 minutes in all groups. *$p<0.05$ versus 'L&T Acu' group, #$p<0.01$ versus 'L&T Acu' group. Values are given as the means±SE with N=6. (C1-C3) The examination of paraffin sections of the skin tissue with 'control', 'Ret Acu' and 'L&T Acu' treatment, respectively. The brick red arrows point to the mast cells without degranulation, and the triangle points to the mast cells with degranulation in each group. Scale bar=20 μm, Abbrevation: Ret Acu: retention acupuncture; L&T Acu: lifting and twisting acupuncture.

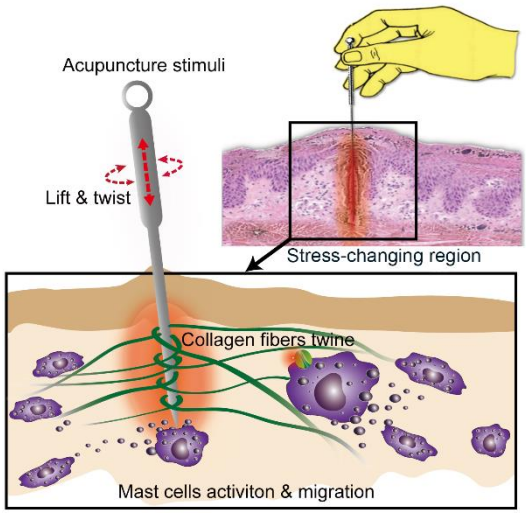

Figure S5. The intermittent motion of lifting and twisting of an acupuncture needle forms a stress-changing region and induces the accumulation and degranulation of mast cells in the skin. Acupuncture needles in the skin can twine collagen fibers and other matrix tissues to form a continuously changing stress region with the manual motion of lifting and twisting. We deem that the changing rigidity of the tissue is a matrix mechanical signal that induces mast cells to accumulate in the treated area. This mechanical stimulation or injury can also induce the degranulation of mast cells.

Despite mast cell distribution along the areas with stiffness variations of different tissues in physiological conditions, the changing of local tissue stiffness under pathological or therapeutic conditions will affect the concentration and status of mast cells. Our results show that the lifting and twisting motion of needles in the skin could induce the accumulation and degranulation of mast cells. This process is presented in a diagrammatic sketch (Fig. S5). Langevin and her colleagues have performed a thorough study on the connective tissues in the skin in order to investigate the mechanisms of acupuncture [2]. During the motion of the needle, connective tissue winds and tightens around the needle [1], and the local tissue forms a high stress-changing region [2]. Wu ML [3] and Luo MF [4] found that the number of mast cells significantly increased after acupuncture relative to a nonstimulated control. Zhang D [5] and Huang M [6] found that the degranulation ratio of mast cells increased after manual acupuncture. Based on previous findings and our experimental results, we assume that the lifting and twisting motion of the needle could induce changes in the local tissue stiffness with the movement of the needle. The changing stiffness could be a mechanotransduction signal to mechanically sensitive receptors and channels on mast cells, such as TRPV2 [7]. Morphologic changes in mast cells and others induced by acupuncture needling play a crucial role in cytoskeletal remodeling, which may stimulate significant downstream cascades.

Supplemental References

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