Cellular response to substrate rigidity is governed by either stress or strain

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Supporting Material

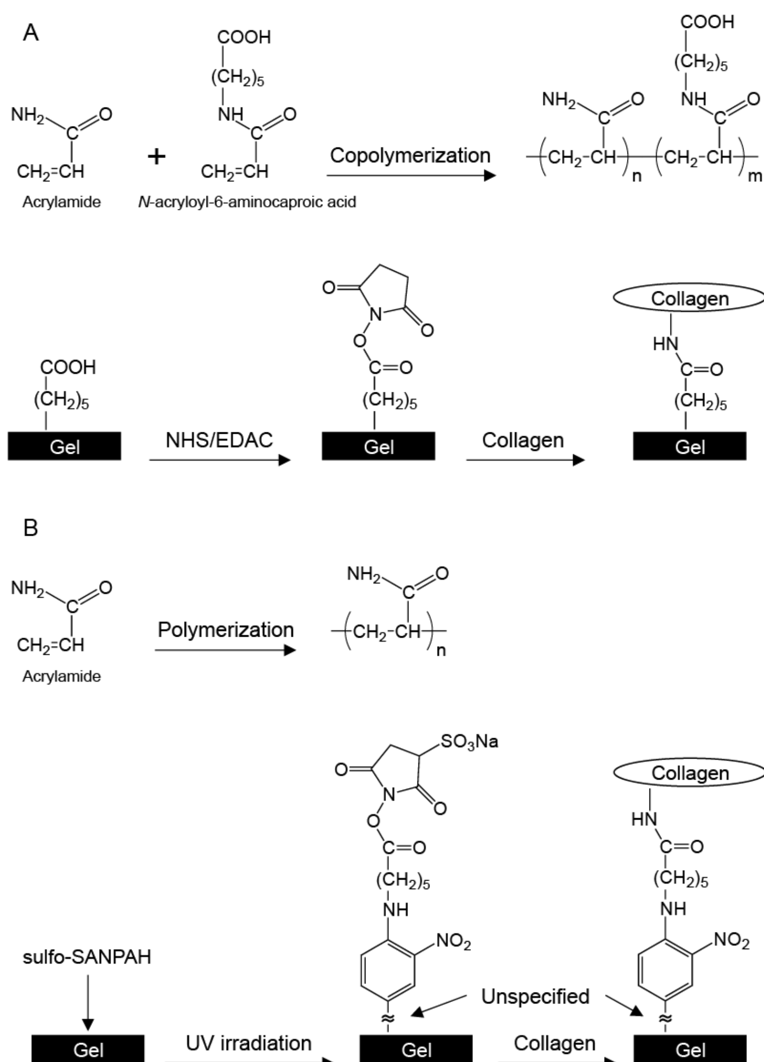

FIGURE S1 Schematic representation of collagen immobilization on ACA (*A*) and sulfo-SANPAH (*B*) gels. (*A*) ACA and AAm monomers were co-polymerized and fully hydrated. After the carboxyl groups of the ACA molecules, which were incorporated into the gel-network, were functionalized with EDAC and NHS, they were allowed to react with the primary amines of collagen molecules through dehydration condensation. (*B*) Sulfo-SANPAH was conjugated to the AAm network, and then the sulfo-NHS groups of sulfo-SANPAH were allowed to react with the primary amines of collagen molecules. Note that the reaction between the sulfo-SANPAH and AAm network, which is mediated by UV-induced nitrene, has neither been specified nor defined.

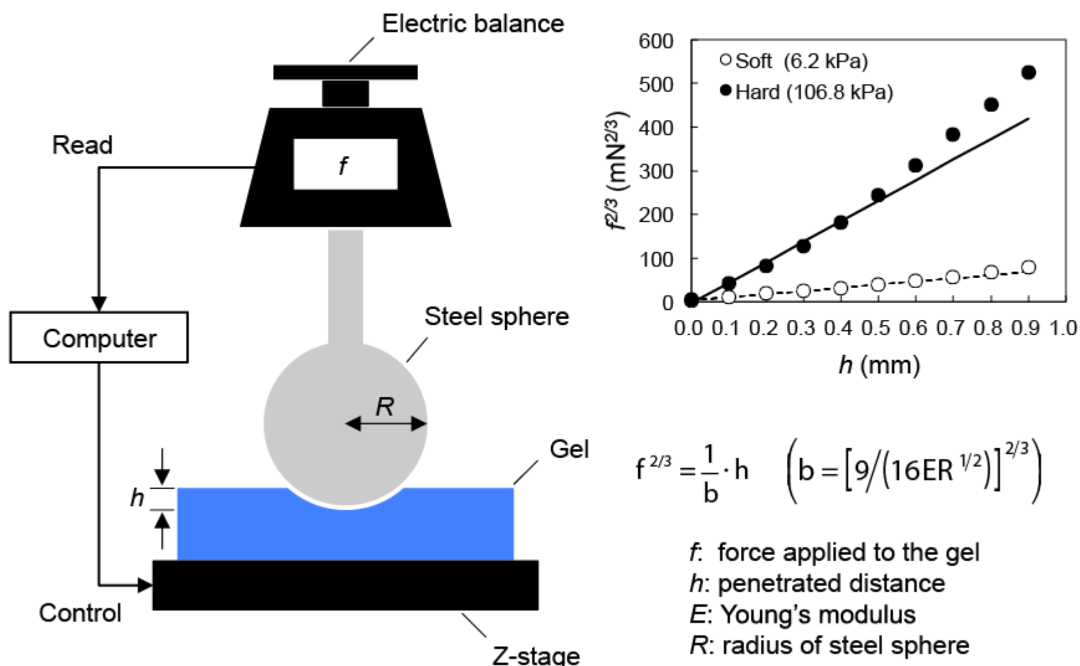

FIGURE S2 Schematic illustration of the penetration method to measure the gel elasticity. A gel placed on a z-axis stage (resolution of step; 0.1 mm) was compressed with a steel sphere (6 mm in diameter), which was directly connected to an electric balance. The penetrated distance (h) was controlled and the force applied to the gel (f) was measured. The Hertz model was applied to fit the first linear section in the plot $f^{2/3}$ against the indentation depth to ensure that the estimation was consistent with the linear approximation. The plots from a soft (6.2 kPa) gel (*open circles*) and a stiff (106.8 kPa) gel (*filled circles*) are exemplified.

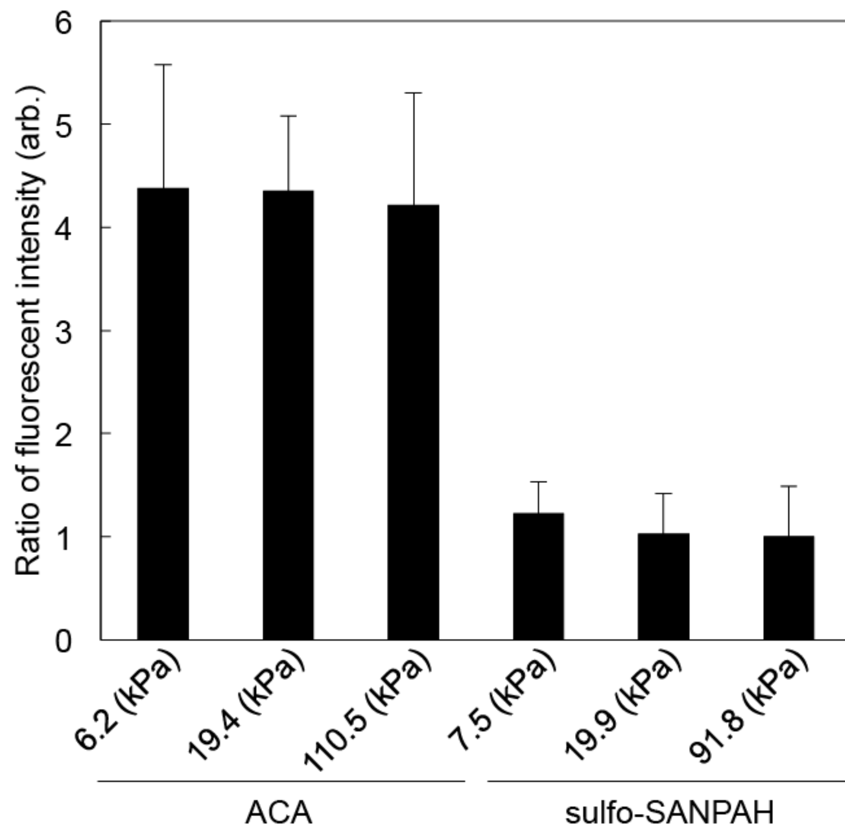

FIGURE S3 Quantitation of collagen immobilized on gels. Bovine collagen was immobilized onto ACA and sulfo-SANPAH gels, and subjected to anti-collagen immunofluorescence staining as in Fig. 1 (see Materials and Methods). The fluorescence images were acquired by a confocal microscope under equal settings; including laser power, pinhole size and gain of photonmutiplier tube. Images were analyzed at 8-bit gray scale using ImageJ software. The fluorescence intensity was quantified after background subtraction using the images from staining of ACA and sulfo-SANPAH gels that were not coated with collagen. Five separate areas were analyzed for each sample. The quantities of the fluorescence intensity were averaged from two independent experiments and scaled with the mean value of 91.8 kPa sulfo-SANPAH gels set at one.

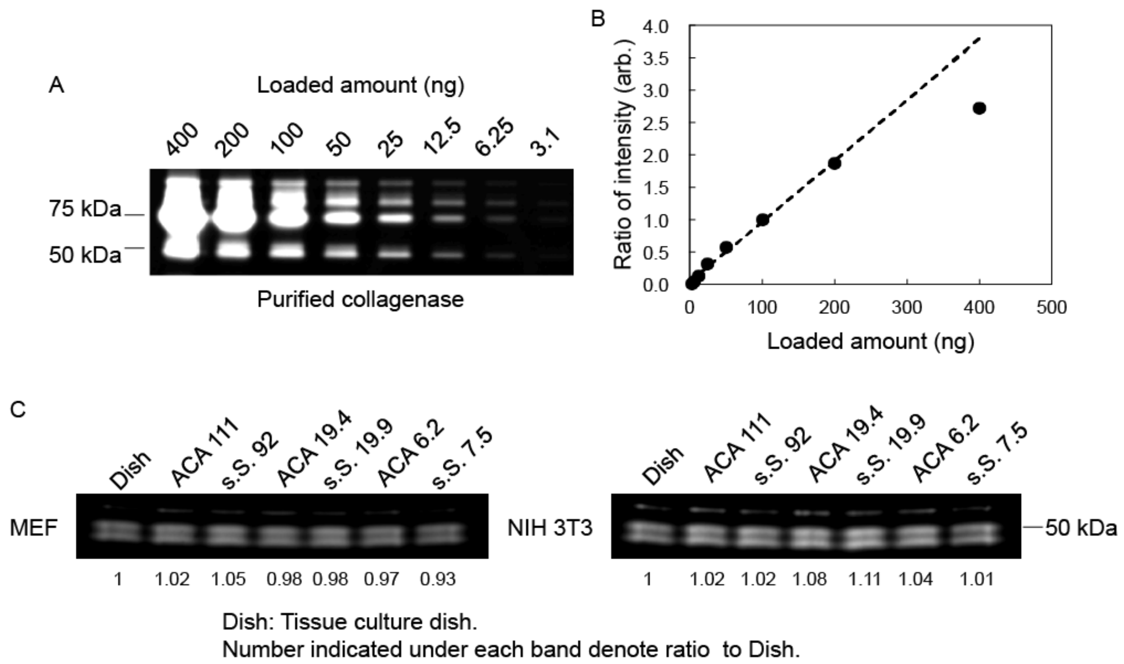

FIGURE S4 The activity of MMP released from cells does not change, depending on the stiffness of ACA and sulfo-SANPAH gels. MMP activity in the medium was quantified by zymography using gelatin as a substrate. (*A*) Defined amounts of purified collagenase were loaded. (*B*) 6.25 to 200 ng of collagenase was linearly detected. (*C*) MMP activity in the medium from MEFs and NIH3T3 cells culture on ACA and sulfo-SANPAH gels with indicated elasticities. ‘Dish’ denotes MEFs or NIH3T3 cells on standard plastic culture dishes. The quantities of MMP activity were scaled with the value from Dish set at one, and noted below the gel pictures.

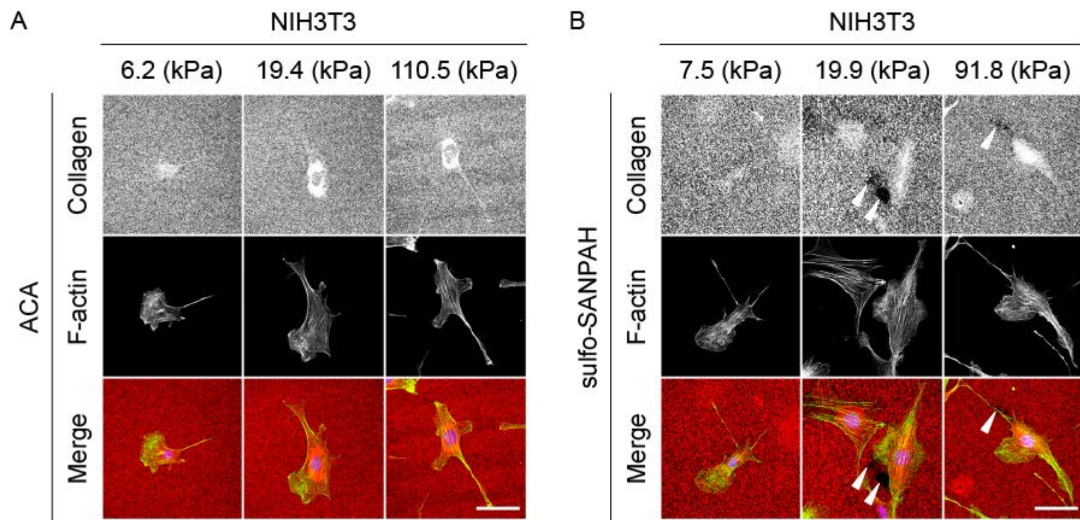

FIGURE S5 Immunofluorescence staining of collagen attached to ACA (*A*) and sulfo-SANPAH (*B*) substrates on which NIH3T3 cells were plated. Staining was conducted after 12 h of cell culture as in Fig. 1. Arrowheads point to areas devoid of collagen. Scale bars represent 50 µm.

Supporting Materials and Methods

Gelatin zymography assay

The gelatin zymography was performed to determine the activity of MMP. Culture medium was collected and mixed with 2× loading buffer (100mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 1% bromophenol blue). Proteins in the medium were separated by SDS-PAGE using a 7.5% polyacrylamide gel containing 1 mg/ml gelatin. After the electrophoresis, the gel was incubated in 2.5% Triton X-100 in deionized water with gentle agitation for 30 minutes at room temperature for protein renaturation. The gel was then incubated in developing buffer (50 mM Tris, 0.2 M NaCl, 5 mM $CaCl_2$, 0.02% Brij35, pH7.5) for 12 hours at 37 ˚C, and subjected to Coomassie Brilliant Blue staining. The proteolytic activity was visualized as clear bands in contrast to the blue background from the stained gelatin, and quantified using a gel imager (Gel Doc^{TM} EZ; Bio-Rad, CA).