Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Bi-content micro-collagen chip provides contractility-based biomechanical readout for phenotypic drug screening with expanded and profiled targets

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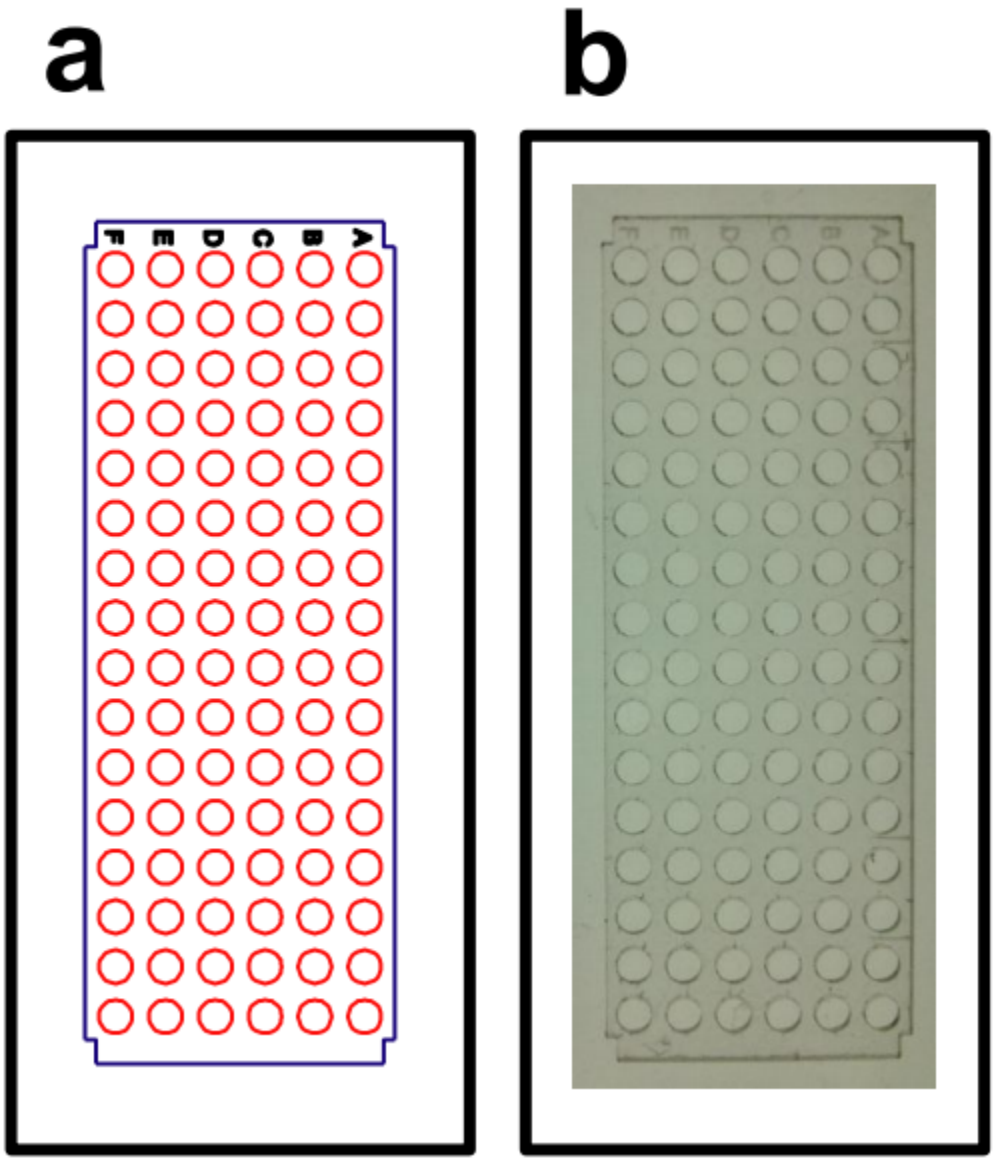
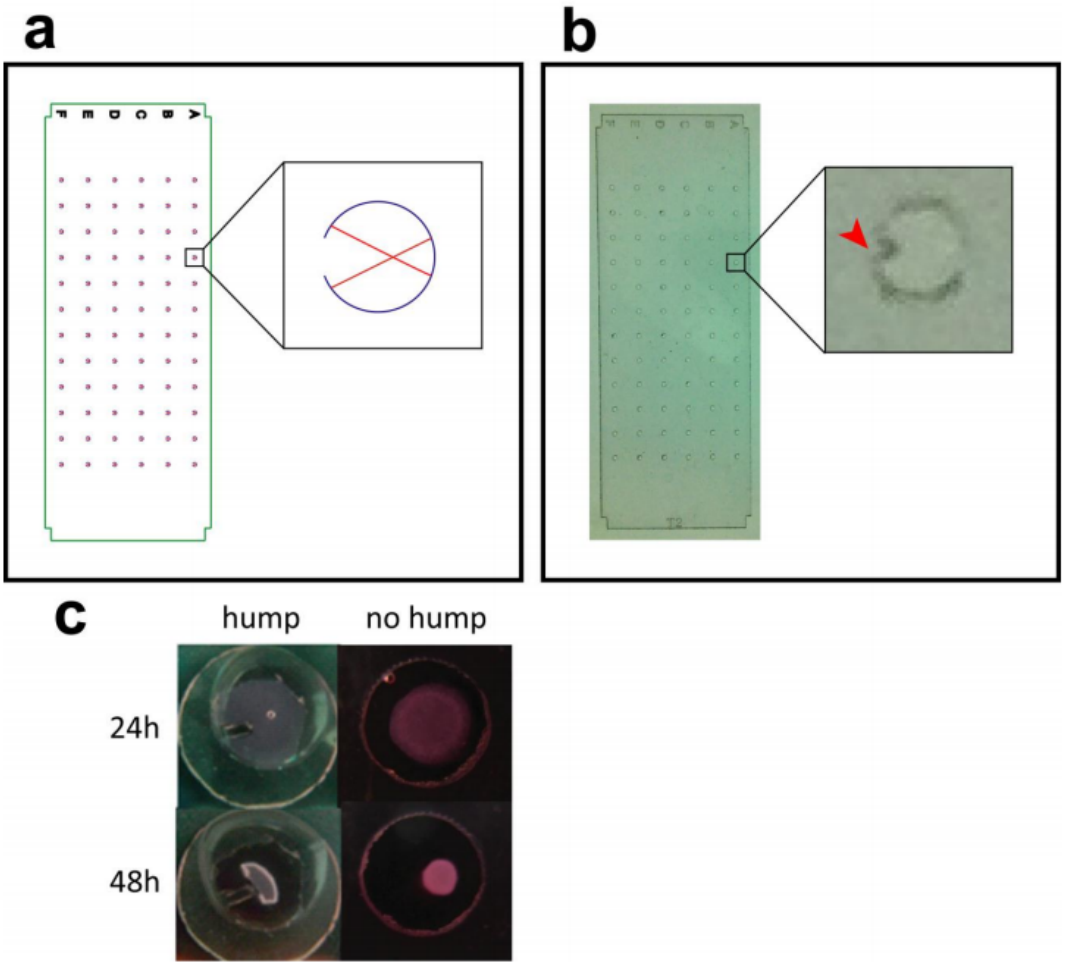
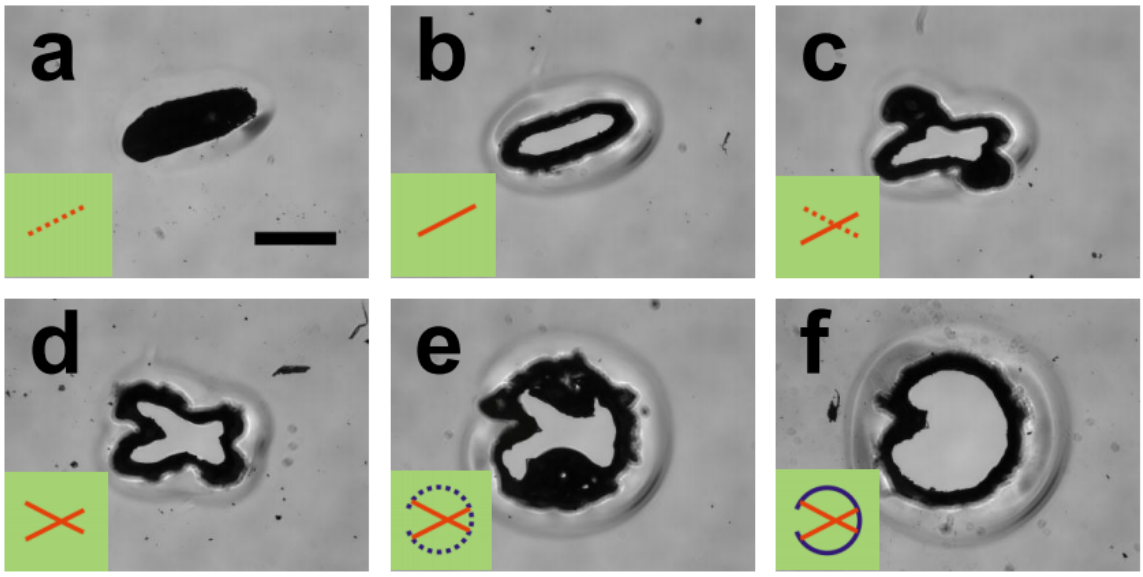


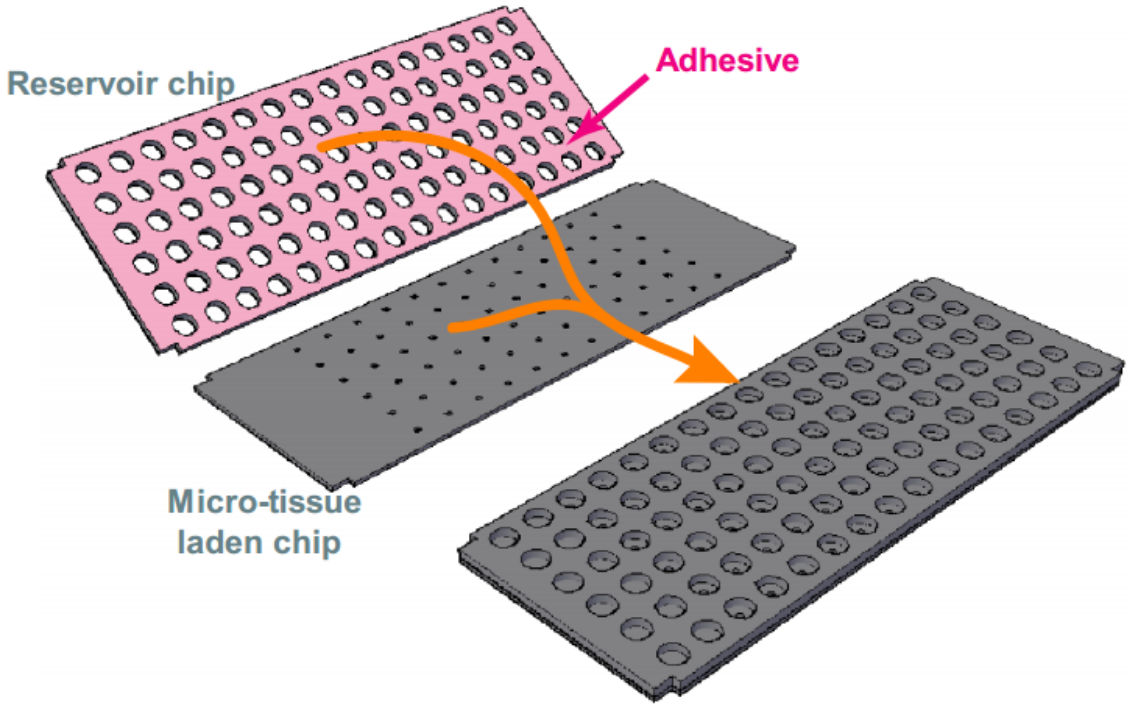
Supplementary Figure 1. Fabrication of reservoir chips. (a) Drawing of reservoir chips by *CorelDRAW* software. Size of a chip is similar to that of a glass slide (78 mm × 26 mm). Lines of different colors indicate the correspondent engraving order and laser energy. **(b)** Photograph of a reservoir chip fabricated by laser engraving



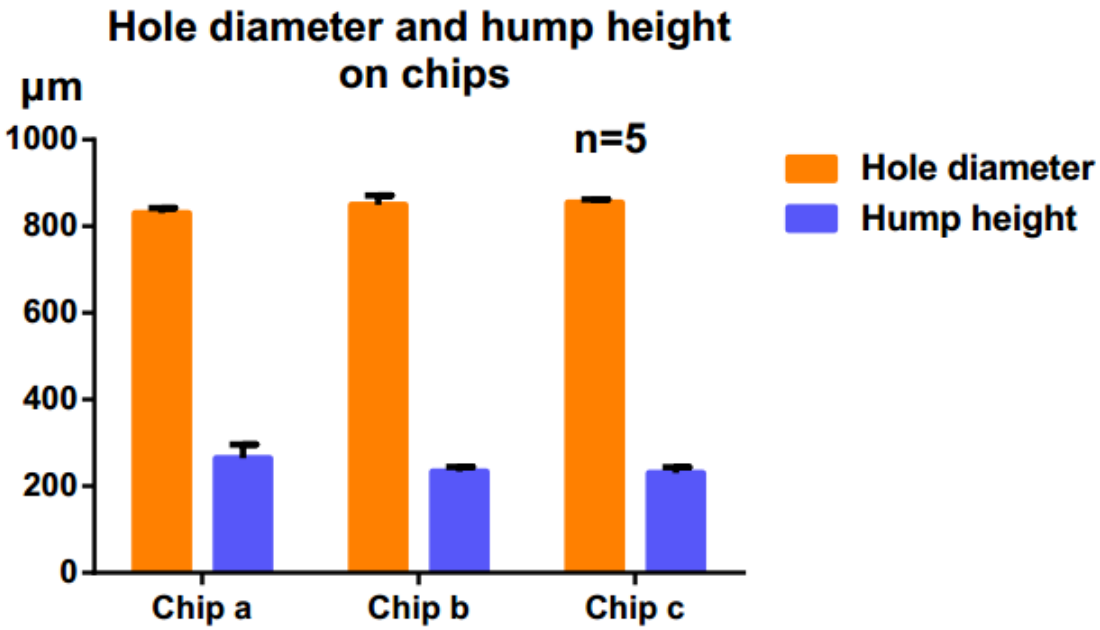
Supplementary Figure 2. Fabrication of micro-tissue laden chips and feasibility test of positioning effect of humps. (a) Drawing of micro-tissue laden chips by *CorelDRAW* software (also see Supplementary Figure 3). Size of a chip is similar to that of a glass slide (78 mm × 26 mm). Lines of different colors indicate the correspondent engraving order and laser energy. Specially, a small hump was designed in each hole to serve as a holder for gel body. **(b)** Photograph of a micro-tissue laden chip. The red arrow head indicates the hump. **(c)** Experiment using bulky collagen gels in wells with or without hump indicates a hump in a well is efficient to hold gel body.



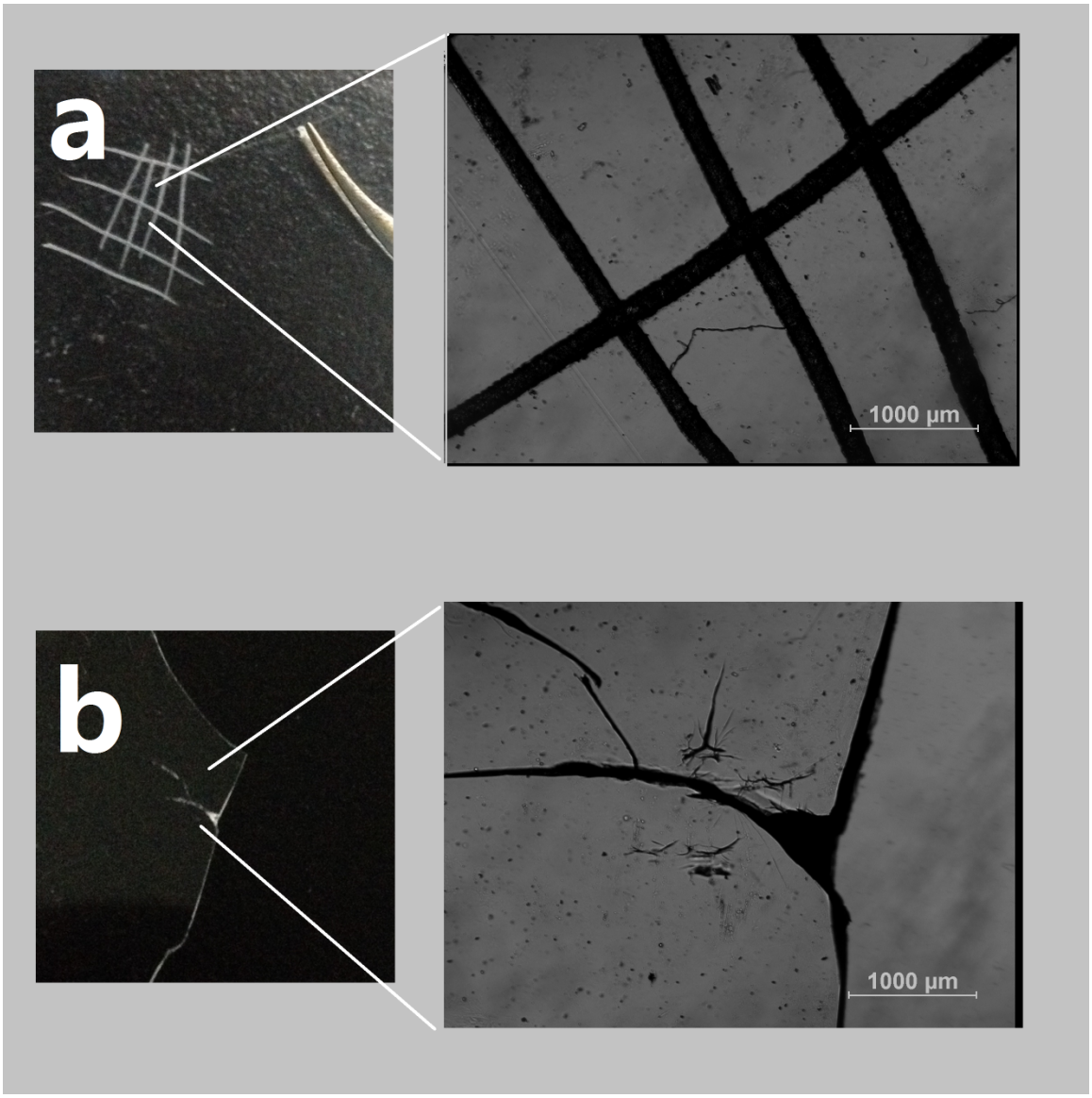
Supplementary Figure 3. Fabrication process of a hump by laser engraving. Accuracy has remained as a common problem for microfabrication using lasers. To obtain inerratic and uniform humps in micro-tissue laden chips, laser engraving process was attentively optimized. Dash lines indicate that the line has been engraved once, while full lines indicate that the line has been engraved twice. Lines are engraved twice at low energy to avoid deformation of humps by high temperature.



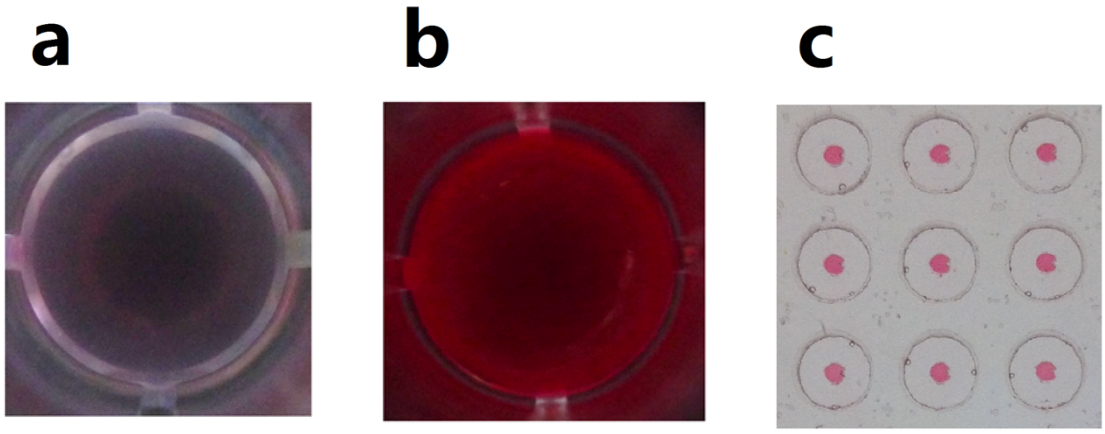
Supplementary Figure 4. Assembly of 3CChip by adhesive. Reservoir chip and micro-tissue laden chip are aligned and then assembled in-between a double-sided adhesive tape (Pink).



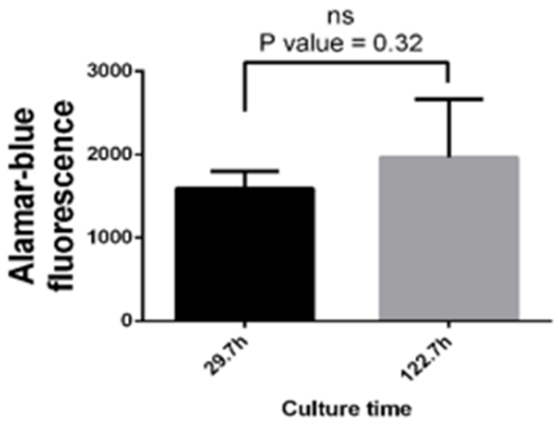
Supplementary Figure 5. Uniformity of hole diameter and hump height on chips. Five holes were randomly selected from 3 chips (Chip a, b and c), and hole diameter and hump height were measured. Good uniformity is shown in every chip and between chips. Error bars indicate standard deviations (n=5).



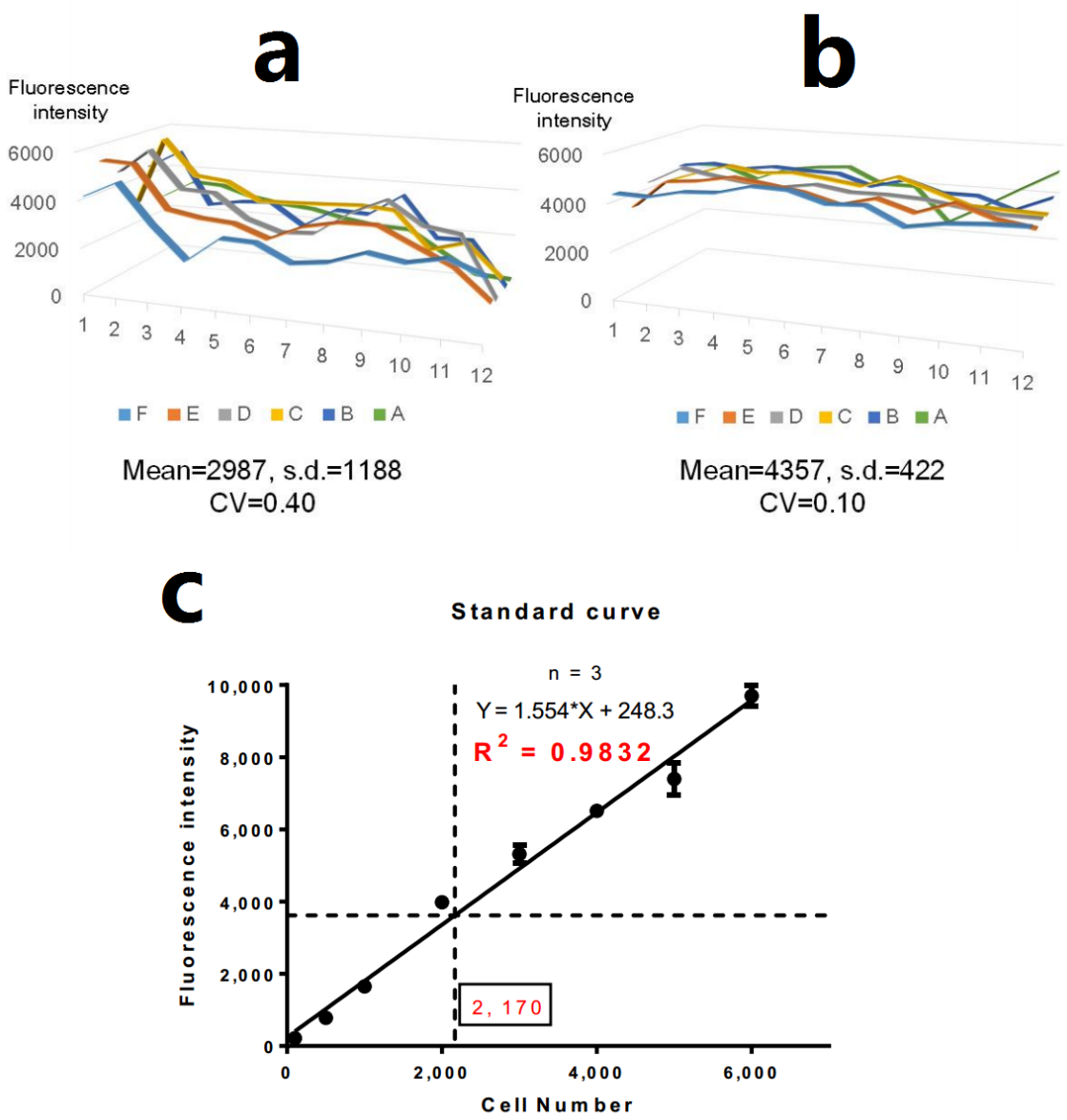
Supplementary Figure 6. Black rims of micro-wells are not necessarily burning residual. In Figure 1d, thick and black rims of wells can be observed. This is not necessarily caused by burning residual, but more likely phantom under microscope, which can be proved by that **(a)** scratches by tweezers or **(b)** physical cracks of PMMA also result in black rims under microscope.



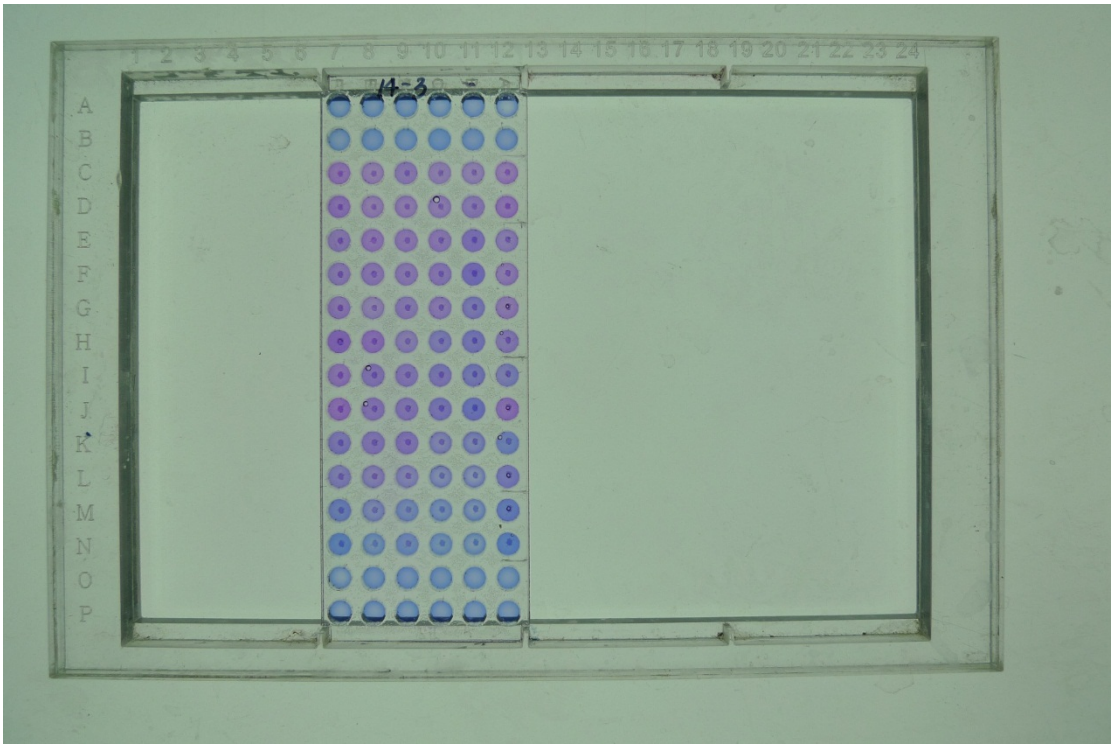
Supplementary Figure 7. Collagen gel without cells does not automatically contract neither in 96-well plate nor on the chip. (a) After gelation, collagen gel (2 mg/ml) was incubated with complete medium in a well of 96-well plate at 37 °C and no contraction can be observed after 3 days. **(b)** To make the result in (a) more evident, the collagen gel was fixed with 2.5 % glutaraldehyde and dyed with ponceau S. Contraction can still not be observed. **(c)** After gelation, collagen gel (2 mg/ml) was incubated with complete medium on 3CChip at 37°C for 3 days. The chip was then fixed with 2.5 % glutaraldehyde and dyed with ponceau S. No contraction can be observed.



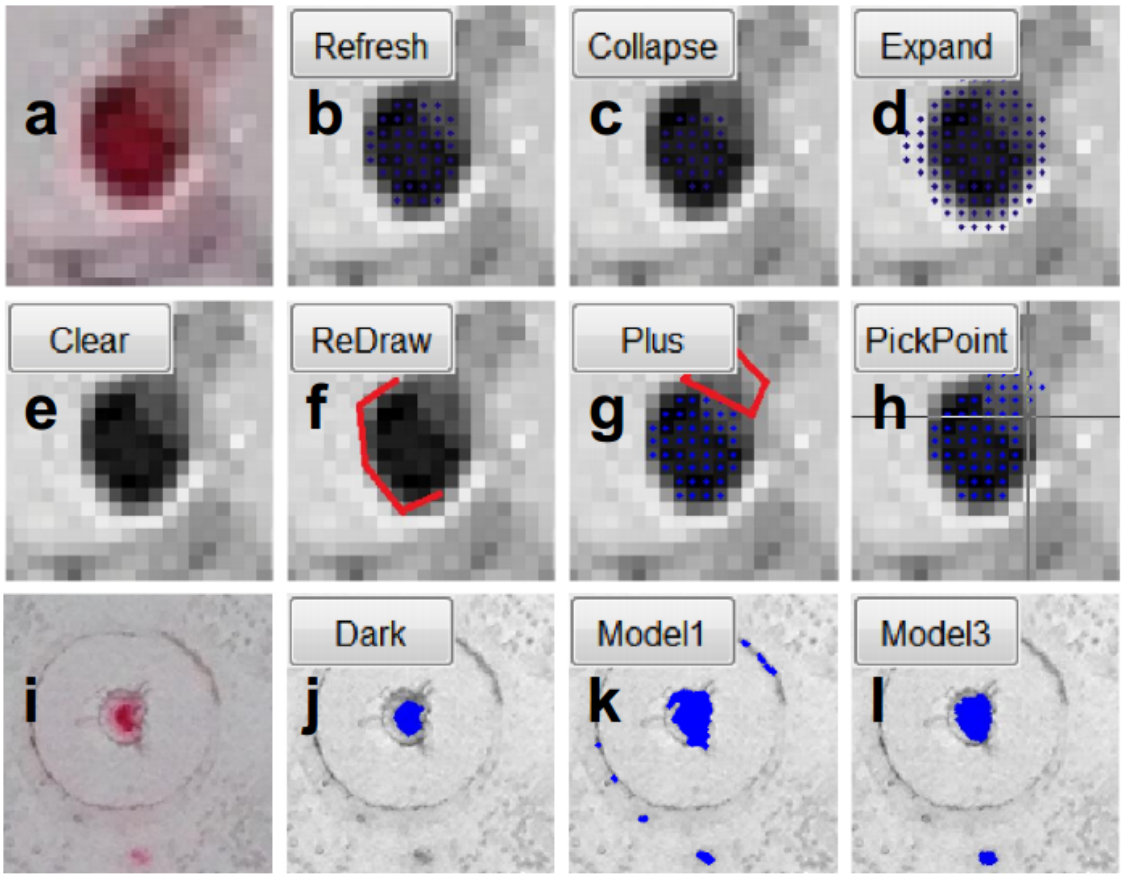
Supplementary Figure 8. Long term cell cultivation and drug response. (a) Cardiac fibroblasts on 3CChip could survive at least for 5 days and showed weak proliferation on chip after 5 days (no significance, p value = 0.32). **(b and c)** Cardiac fibroblasts could still show drug (simvastatin) response trend after 5 days drug exposure, but the ICV_{50} and ICC_{50} value were both smaller than the result tested using cells cultured for 30 hours. ICV_{50} is smaller than ICC_{50} at 5 days can be explained by that collagen gel contracted at low drug concentration at the beginning and remained the same extent of contraction during long time culture, while cells at low drug concentration were constantly killed during long time exposure to drug.



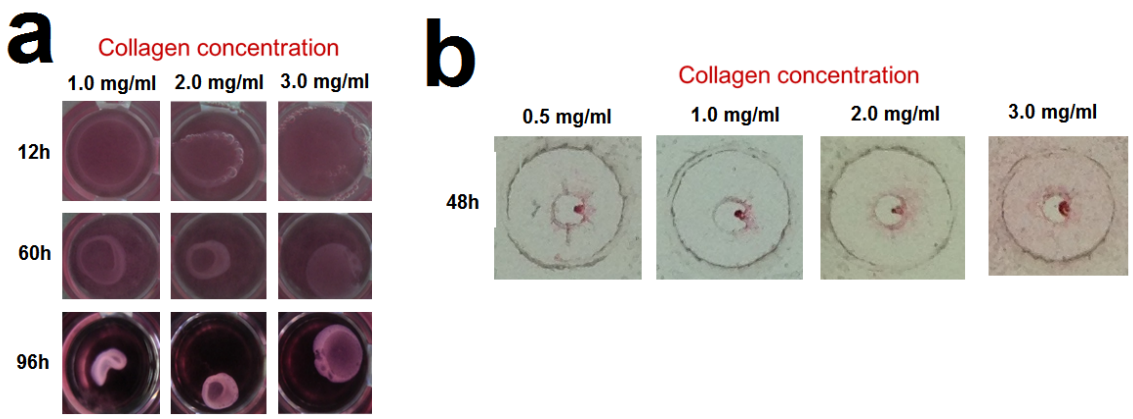
Supplementary Figure 9. Comparison of different scraping approaches to load cell-collagen mix and actually loaded cell number by scraping with a bent glass rod. Cell-collagen mix of HT1080 was scraped with **(a)** a glass slide or **(b)** a bent glass rod. Chips were incubated with Alamar Blue and the number of loaded cells in each well was reflected by fluorescence intensity obtained using on-chip Alamar Blue assay. Cell-collagen mix scraped with a bent glass rod showed less variation than that with a glass slide, as shown by reduced CV value (Coefficient of variation, calculated as the ratio of SD to mean). Background signals of Alamar Blue measured in non-cell control were subtracted form original readout values. **(c)** Standard curve of cell number to fluorescence intensity. The standard curve was obtained by HT-1080 cells seeded in collagen hydrogel via Alamar Blue assay.



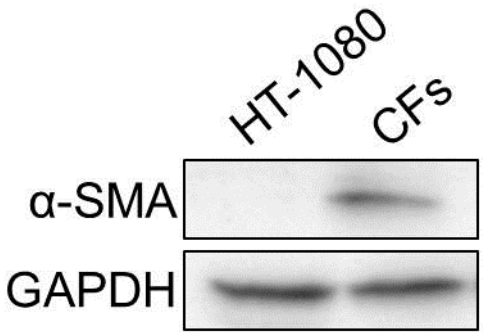
Supplementary Figure 10. Home-made adaptor to micro-plate reader for fluorescence measurement. Size of the adaptor is the same as a standard plate. The protuberances of the adaptor can fit four pieces of the3CChip. The position of holes on 3CChip is consistent with the hole distribution of a 384-well plate allowing convenient fluorescence measurement by a common microplate reader under 384-well plate mode.



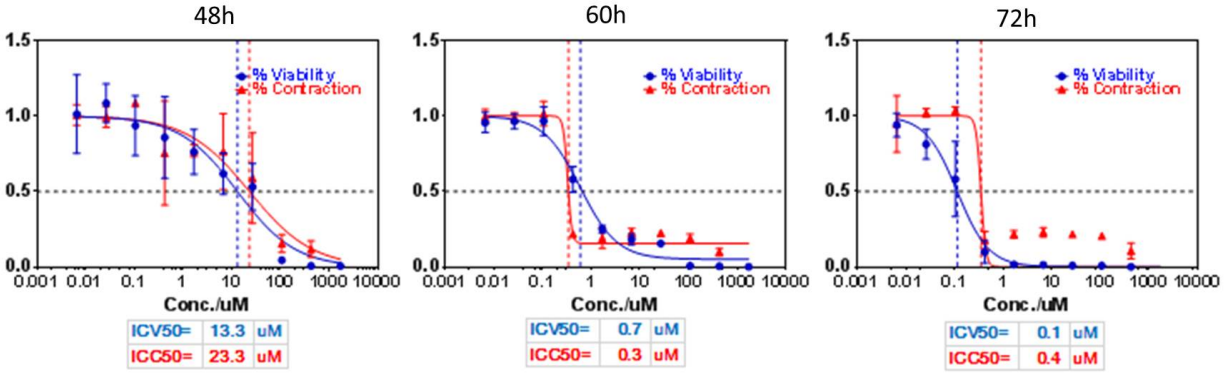
Supplementary Figure 11. Demonstration of using *'CheckCircle.m'* software to measure numbers of pixels of colored lumps dyed with ponceau S. (a) A magnified picture of a colored lump of a unit before treatment. **(b)** Click 'Refresh' to show the recognizing result. **(c)** Click 'Collapse' to make the recognized pixels collapse to a circle of pixels smaller than previous image (result of 3 times of clicking is shown here). **(d)** Click 'Expand' to make the recognized pixels expand to a circle of pixels bigger than previous image (result of 3 times of clicking is shown here). **(e)** Click 'Clear' to clear all the pixels recognized. **(f)** Click 'Redraw' to re-draw a region that should be recognized. The previously recognized pixels will be cleared. **(g)** Click 'Plus' to add appointed pixels. **(h)** Click 'PickPoint' to adjust the pixels recognized accurately. Left click will change the status of a pixel of recognition. Right click will exit this function. **(i)** Picture of a unit before changing of analyzing model. **(j)** Result of analysis by Dark model, which is suitable for most cases. **(k)** Result of analysis by Model1. This model suits the conditions where color lumps are dyed slightly and there are not enough noisy points. **(l)** Result of analysis by Model3. This model is suitable for conditions where gels are slightly contracted.



Supplementary Figure 12. Effects of collagen concentration on contraction rate. (a) HT-1080 cells in the same density were embedded in collagen matrix of different concentrations. Experiment was performed in 24-well plate. **(b)** On-chip collagen contraction assay was performed at the same cell density of HT-1080 and collagen concentration varied as 0.5, 1.0, 2.0 and 3.0 mg/ml. Collagen gel contracted at all concentrations on chip after 48 h cultivation.



Supplementary Figure 13. Western blot analysis on α-SMA expression in CFs and HT-1080. α-SMA was nearly undetectable in HT-1080 cell line. Primary antibody for α-SMA was from Abcam (ab5694).



Supplementary Figure 14. Time-dependent ICC and ICV curves in Dox treated HT-1080. Three sets of 3CChip (Dox against HT-1080) were set up in parallel and drug response was analyzed at different time points (48 h, 60 h and 72 h). Change of curve patterns were clearly observed, indicating observation time as a critical factor to be considered for drug testing on 3CChip.