nature portfolio

https://doi.org/10.1038/s41551-021-00740-x

Supplementary information

Inhibition of aberrant tissue remodelling by mesenchymal stromal cells singly coated with soft gels presenting defined chemomechanical cues

In the format provided by the authors and unedited

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Supplementary Text

Modeling MSC-mediated degradation of collagen by interstitial collagenases

We constructed a set of differential equations to model the effect of donor MSCs on collagen levels in lung tissue challenged by bleomycin (**Fig. 5A**). We use a simple equation to describe the kinetics of donor MSCs after *i.t.* delivery as follows:

$$\frac{dMSC}{dt} = (\beta_{MSC} - \alpha_{MSC})MSC \qquad (Eq. 1.1)$$

β_{MSC} is the production (or proliferation) rate, while α_{MSC} is the death rate of donor MSCs. Based on the biodistribution kinetics data from *i.t.* delivery of MSCs (**Fig. S7**), β_{MSC} = 0, and α_{MSC} = 0.937/day, which is equivalent to decay $t_{1/2}$ = 0.74 day or 17.78 hr.

The kinetics of bleomycin after delivery is described as follows:

$$\frac{dBleo}{dt} = -\alpha_{bleo}Bleo \qquad (Eq. 1.2)$$

α_{bleo} is the clearance rate of bleomycin and set to 0.2505/day or $t_{1/2}$ = 2.77 day, starting from 2 day after bleomycin treatment as described[1].

The kinetics of TNFα in the host is described as follows:

$$\frac{dTNF\alpha}{dt} = \beta_{tnf\alpha}Bleo - \alpha_{tnf\alpha}TNF\alpha \qquad (Eq. 1.3)$$

β_{tnfa} is the production rate of TNFα in the host induced by bleomycin and set to 0.135. α_{tnfa} is the decay rate of TNFα and set to 0.08. These values are based on the previously described kinetics of TNFα when a single dose of bleomycin is administered[2].

The kinetics of interstitial collagenases is described as follows:

$$\frac{dMMP}{dt} = \beta_{basal} + \beta_{mmp}\theta_{tnf\alpha}MSC - \alpha_{mmp}MMP \qquad (Eq. 1.4)$$

β_{basal} is the basal production rate of collagenases by the host and set to 300 pg/ml/day, which is within the previously described range in lung tissue[3]. Production of collagenases by donor MSCs depends on two factors: 1. β_{mmp}: the maximum production rate of collagenases from donor MSCs (varied during simulation from 0.4 to 4.0); 2. θ_{tnfa}: the dose response of TNFα to activate TNFα receptors on donor MSCs:

$$\theta_{tnf\alpha} = \frac{1}{1 + \left(\frac{K_{tnf\alpha}}{TNF\alpha}\right)^2} \qquad (Eq. 1.5).$$

, where K_{tnfa} is TNFα concentration to achieve the half-maximum activation of TNFα receptors and set to 1 ng/ml as described[4]. α_{mmp} is the degradation rate of collagenases, for instance, by host TIMPs, and set to 0.693/day or $t_{1/2}$ = 1 day as described[5].

The kinetics of total collagen in lung tissue is described as follows:

$$\frac{dCol}{dt} = \beta_{col}Bleo - \delta(Col) \qquad (Eq. 1.6)$$

β_{col} is the maximum production rate of collagen induced by bleomycin (Bleo) and set to 0.22 μg/mg tissue/day starting 1 week after instillation of bleomycin (**Fig. S8B, iii**) as described[6]. $\delta(Col)$ is the rate of collagen degradation based on the Michaelis-Menten equation:

$$\delta(Col) = V_{max}\frac{Col^2}{Col^2 + {K_{col}}^2} = \frac{\alpha_{col}MMP}{1 + \left(\frac{K_{col}}{Col}\right)^2} \qquad (Eq.\,1.7).$$

α_{col} is the catalytic rate constant of interstitial collagenases and set to 0.02/day or $t_{1/2}$ = 34.67 day as described[7]. K_{col} is the concentration of collagen at the half-maximum rate of degradation and set to 25 µg/mg tissue or ~83 µM collagen (assuming tissue density ~ 1 mg/µl and molar mass of collagen = 300 kDa), which is close to the previously described range for MMP1 and MMP13[8].

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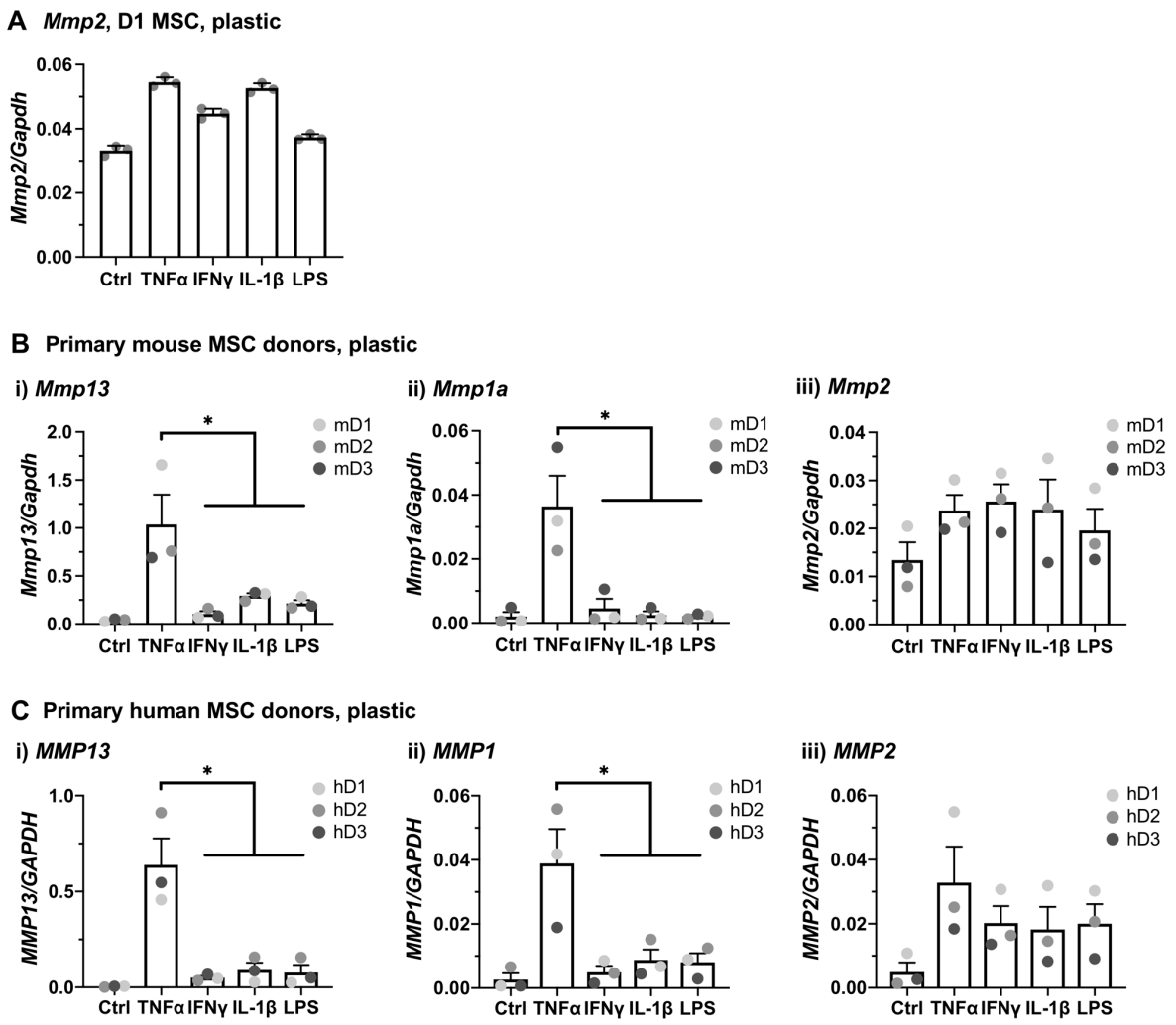

Figure S1. Effects of soluble inflammatory mediators on the expression of MMP isoforms in MSCs on plastic culture. Effects of control (Ctrl; PBS), TNFα, IFNγ, IL1β (100 ng/ml each), and LPS (2000 ng/ml) after treatment for 3 days on plastic culture on expression (normalized to *Gapdh* or *GAPDH*) of **(A)** *Mmp2* in D1 mouse MSCs. $n = 3$ independent experiments, each performed in three replicates. **(B)** (i) *Mmp13*, (ii) *Mmp1a,* and (iii) *Mmp2* in primary mouse bone marrow MSCs. *, (i) $p = (0.6\sim2.6) \times 10^{-2}$, (ii) $p = (2.5\sim4.2) \times 10^{-3}$. **(C)** (i) *MMP13*, (ii) *MMP1,* and (iii) *MMP2* in primary human bone marrow MSCs. *, (i) $p = (0.7\sim1.3) \times 10^{-3}$, (ii) $p = (0.8\sim1.7) \times 10^{-2}$. For (B)-(C), $n = 3$ independent experiments (from 3 different mouse or human donors), each performed in three replicates, and individual *p*-values were derived from one-way ANOVA followed by Tukey's multiple comparisons test. All data are shown as mean ± SEM.

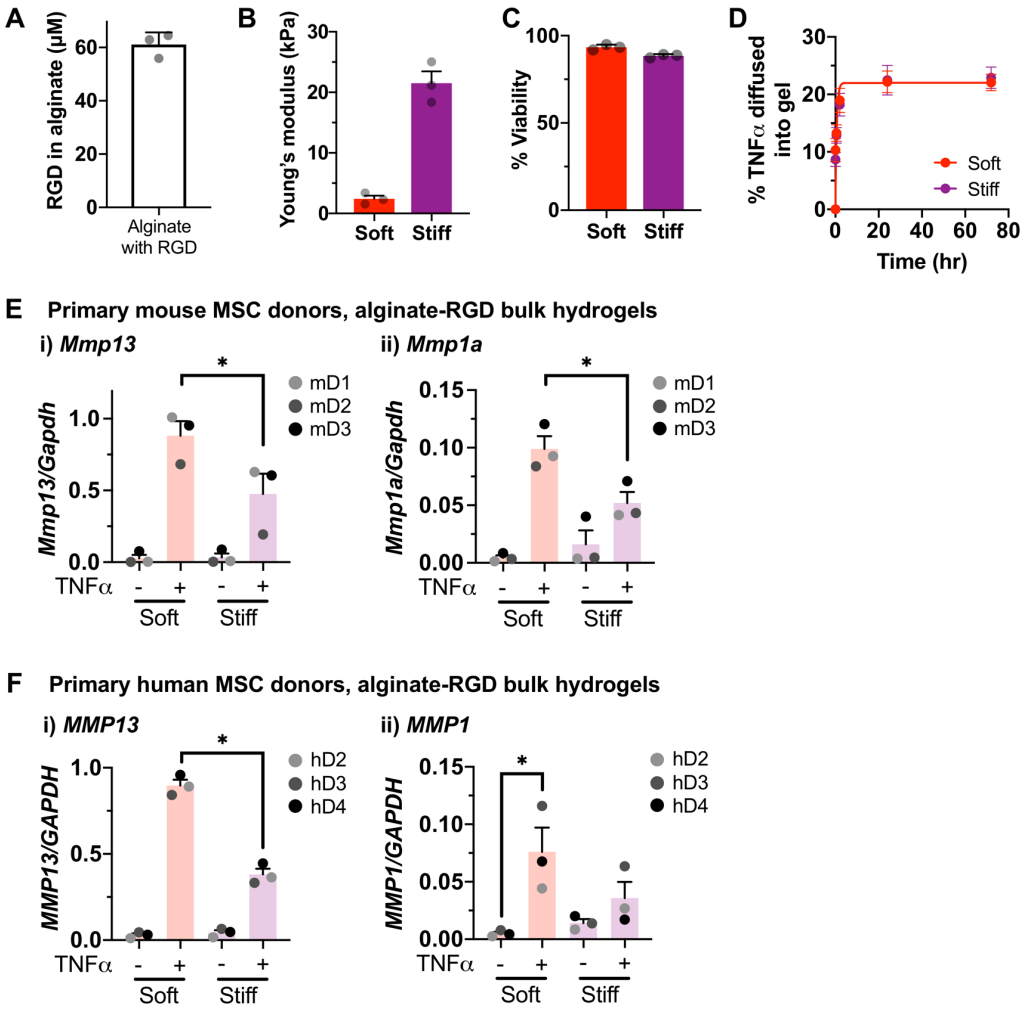

Figure S2. Effects of hydrogel elasticity on TNFα-induced expression of collagenases in primary MSC donors. (A) The concentration of RGD conjugated to alginate (LF200, ~240 kDa, 1% w/v) measured by LavaPep assay. $n = 3$ batches. **(B)** Young's modulus of soft and stiff bulk alginate-RGD gels measured by atomic force microscopy. $n = 3$ independent experiments, each averaged from six indentations of random regions. **(C)** Percentage viable (calcein$^+$) cells after encapsulation of D1 mouse MSCs in soft or stiff bulk gels and culturing for 3 days measured by flow cytometry. $n = 3$ independent experiments, each performed in two replicates. **(D)** *In vitro* diffusion kinetics of exogenously added murine recombinant TNFα (100 ng/ml) into soft or stiff bulk gels determined by ELISA. One-phase association kinetics fit, $t_{1/2} = \sim 0.43$hr, plateau = ~22% for both soft and stiff gels. $n = 3$ independent experiments, each performed in two replicates, mean ± SEM. **(E)** Effects of TNFα (100 ng/ml) on expression of (i) *Mmp13* and (ii) *Mmp1a* in primary mouse bone marrow MSCs in bulk alginate-RGD gels after 3-day treatment in culture. *, (i) $p = 4.8 \times 10^{-2}$, (ii) $p = 3.3 \times 10^{-2}$. **(F)** Effects of TNFα (100 ng/ml) on expression of (i) *MMP13* and (ii) *MMP1* in primary human bone marrow MSCs in bulk alginate-RGD gels after 3-day treatment in culture. *, (i) $p = 2.6 \times 10^{-6}$, (ii) $p = 1.9 \times 10^{-2}$. For (E)-(F), $n = 3$ independent experiments (from 3 different mouse or human donors), each performed in three replicates, and individual p-values were derived from one-way ANOVA followed by Tukey's multiple comparisons test. All data are shown as mean ± SEM.

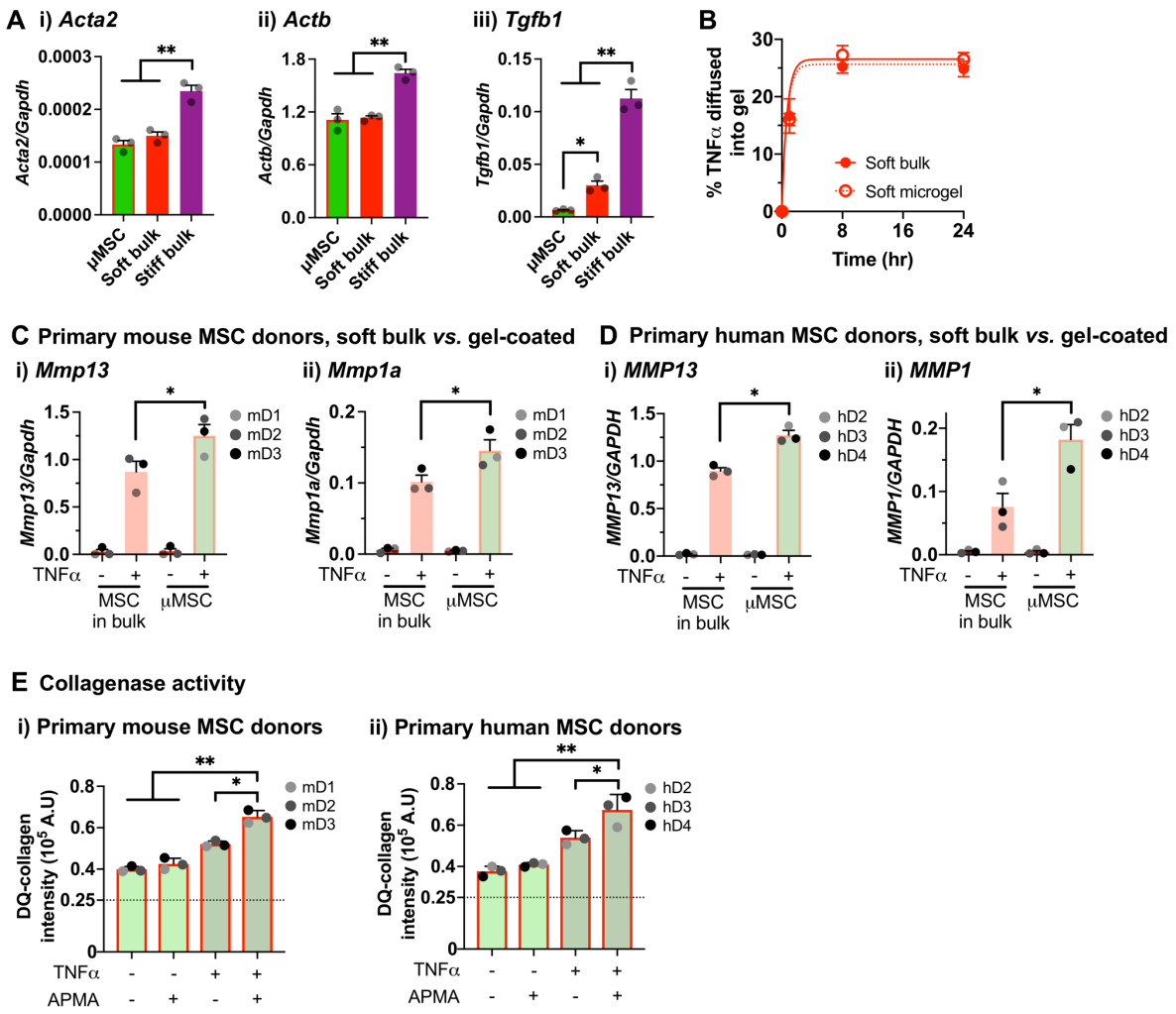

Figure S3. Effects of engineered gel coating on myofibroblast gene expression and TNFα-induced collagenase expression in MSCs. (A) D1 mouse MSCs were encapsulated in gel coating (μMSC), soft or stiff bulk alginate-RGD gels and cultured for 3 days prior to gene expression analysis of (i) *acta2*, (ii) *actb*, and (iii) *tgfb1*. *, (iii) $p = 4.6 \times 10^{-2}$; **, (i) $p = (0.4\sim1.1) \times 10^{-3}$, (ii) $p = (5.8\sim7.6) \times 10^{-4}$, (iii) $p = (2.1\sim9.0) \times 10^{-5}$ via one-way ANOVA followed by Tukey's multiple comparisons test. $n = 3$ independent experiments, each performed in three replicates. **(B)** *In vitro* diffusion kinetics of recombinant murine TNFα into soft bulk gel or soft microgel (~20 μm diameter) quantified by ELISA. One-phase association kinetics fit, $t_{1/2} = \sim0.46$hr, plateau = ~26% for both soft bulk and microgels. $n = 3$ independent experiments, each performed in two replicates. **(C)** Effects of TNFα (100 ng/ml) on expression of i) *Mmp13* and ii) *Mmp1a* in primary mouse bone marrow MSCs in soft bulk gel or gel coating after 3-day treatment in culture. *, (i) $p = 4.7 \times 10^{-2}$, (ii) $p = 3.5 \times 10^{-2}$. **(D)** Effects of TNFα (100 ng/ml) on expression of i) *MMP13* and ii) *MMP1* in primary human bone marrow MSCs in soft bulk gel or gel coating after 3-day treatment in culture. *, (i) $p = 9.1 \times 10^{-5}$, (ii) $p = 6.5 \times 10^{-3}$. **(E)** Collagenase activity in the conditioned media from (i) gel-coated primary mouse MSCs or (ii) gel-coated primary human MSCs. *, (i) $p = 5.0 \times 10^{-4}$, (ii) $p = 2.1 \times 10^{-2}$; **, (i) $p = (4.0\sim9.1) \times 10^{-6}$ (ii) $p = (1.4\sim3.2) \times 10^{-4}$. For (C)-(E), $n = 3$ independent experiments (from 3 different mouse or human donors), each performed in three replicates, and individual *p*-values were derived from one-way ANOVA followed by Tukey's multiple comparisons test. All data are shown as mean ± SEM.

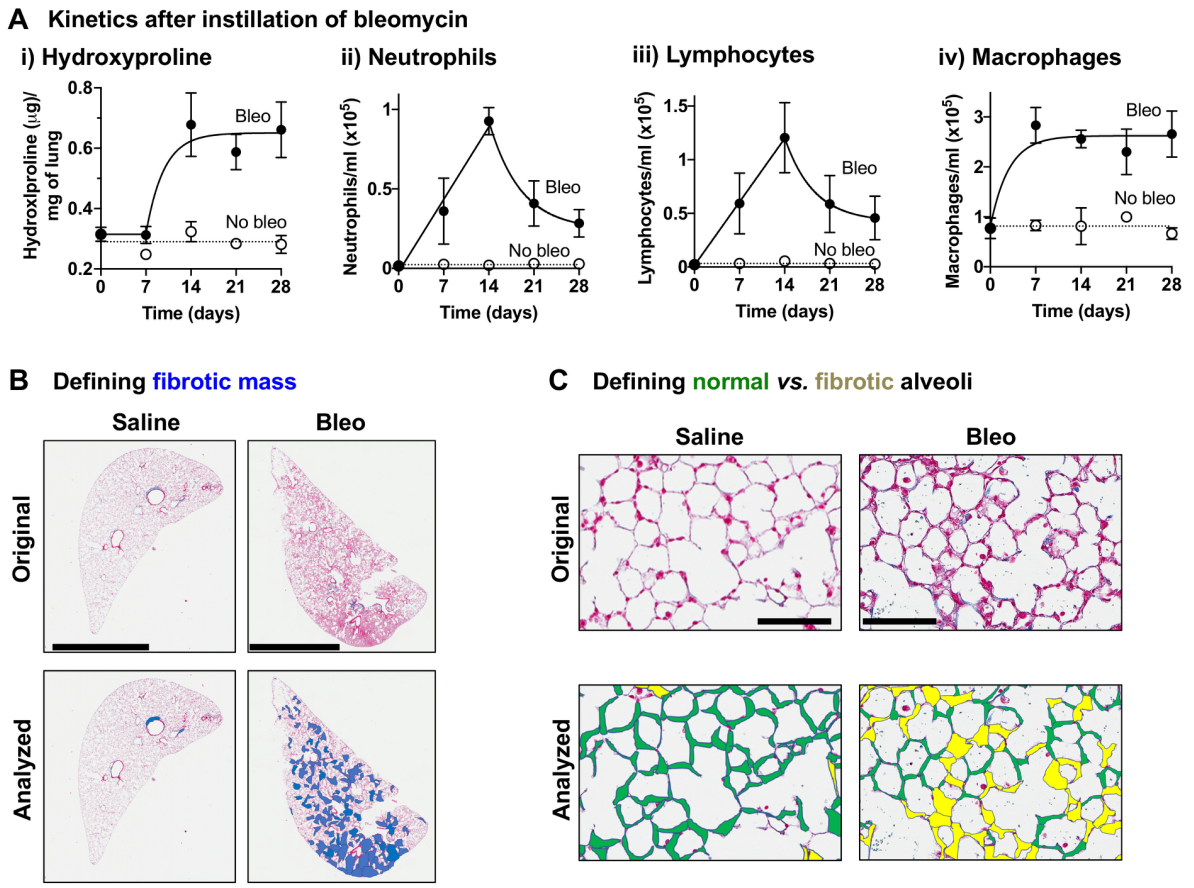

Figure S4. Characterization of fibrotic lung injury induced by bleomycin. (A) Kinetics of (i) collagen deposition, and infiltration of (ii) neutrophils, (iii) lymphocytes, and (iv) macrophages in bronchoalveolar lavage fluid in the first 28 days (4 weeks) after a single dose of bleomycin (bleo) treatment (0.015U per 20 g mouse). Data points from the no bleo group are fitted to a straight line with slope = 0. For (i) and (iv), data points from the bleo group are fitted to a one-phase association kinetics curve with $t_{1/2} \sim 2$ days from 7-day and initial time points, respectively. For both (ii) and (iii), data points from the bleo group are fitted to a straight line between 0-14 days, and an exponential decay curve between 14-28 days with $t_{1/2} \sim 3$ days. $n = 3$ animals. Data are shown as mean ± SD. **(B)** Representative images selected from 9 sections showing automatic selection of fibrotic mass (blue) from histology sections with Masson's trichrome staining at low magnification (scale bar = 3 mm). **(C)** Representative images out of 9 tissue sections showing automatic selection of normal (green) and fibrotic (yellow) alveoli from Masson's trichrome stained histology sections at high magnification (scale bar = 200 µm).

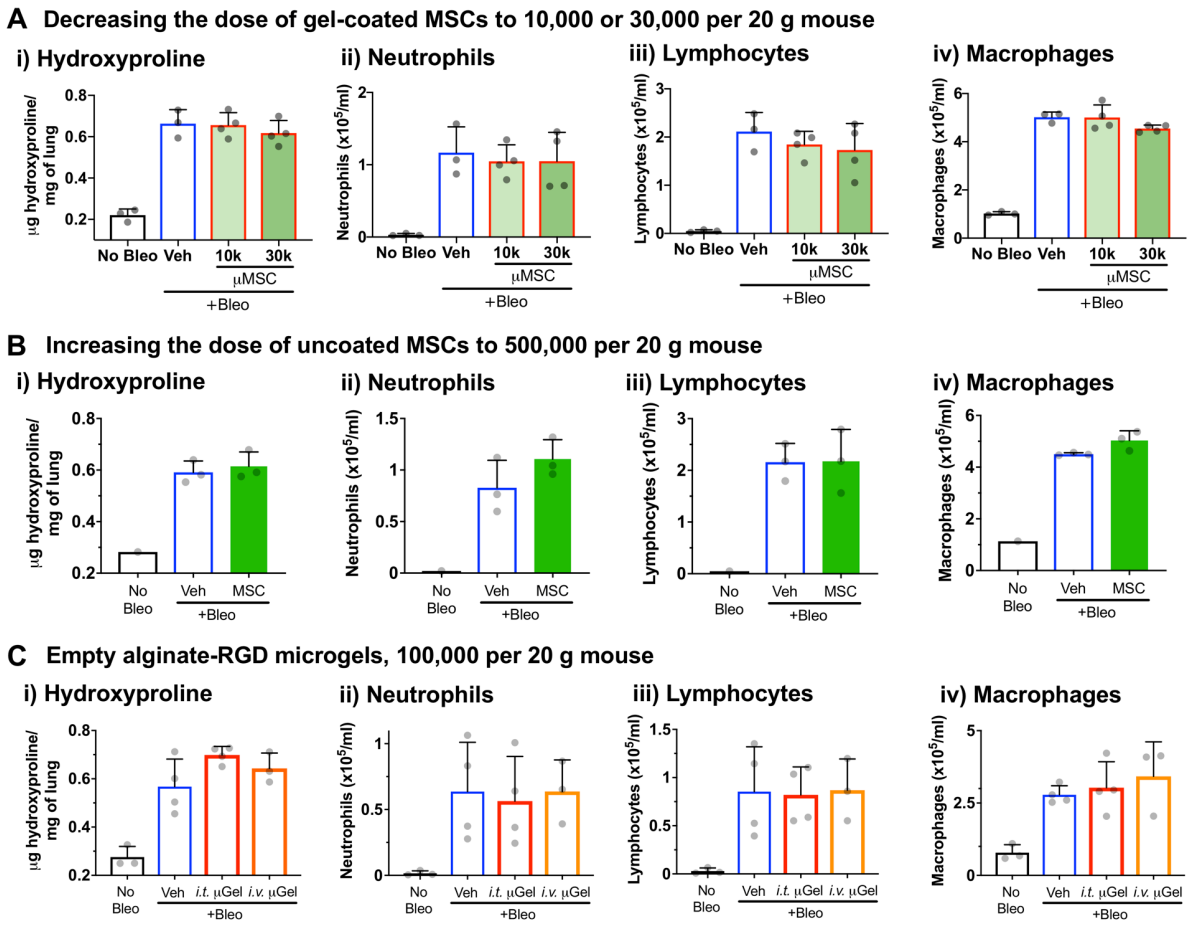

Figure S5. Characterization of cell dose and microgels in fibrotic lung injury. The experimental design is described in **Fig. 3A**. **(A)** Effects of gel-coated D1 mouse MSCs (µMSC) with lower doses (10,000 and 30,000 per 20 g mouse) on (i) hydroxyproline levels, and infiltration of (ii) neutrophils, (iii) lymphocytes, and (iv) macrophages in bronchoalveolar lavage fluid. $n = 3$ animals for no bleo or bleo with vehicle (veh), $n = 4$ for bleo with each tested dose of gel-coated MSCs. **(B)** Effects of uncoated D1 mouse MSCs with increased cell dose (500,000 per 20 g mouse) on (i) hydroxyproline levels, and infiltration of (ii) neutrophils, (iii) lymphocytes, and (iv) macrophages in bronchoalveolar lavage fluid. $n = 1$ animal for no bleo, $n = 3$ for bleo with either vehicle or MSCs. **(C)** Effects of alginate-RGD microgels (µGel, ~20 µm in diameter) without cell encapsulation (100,000 per 20 g mouse) delivered via intratracheal (*i.t.*) or intravenous (*i.v.*) route on (i) hydroxyproline levels, and infiltration of (ii) neutrophils, (iii) lymphocytes, and (iv) macrophages in bronchoalveolar lavage fluid. $n = 3$ animals for no bleo and bleo + *i.v.* µGel groups, $n = 4$ for other groups. All data are shown as mean ± SD.

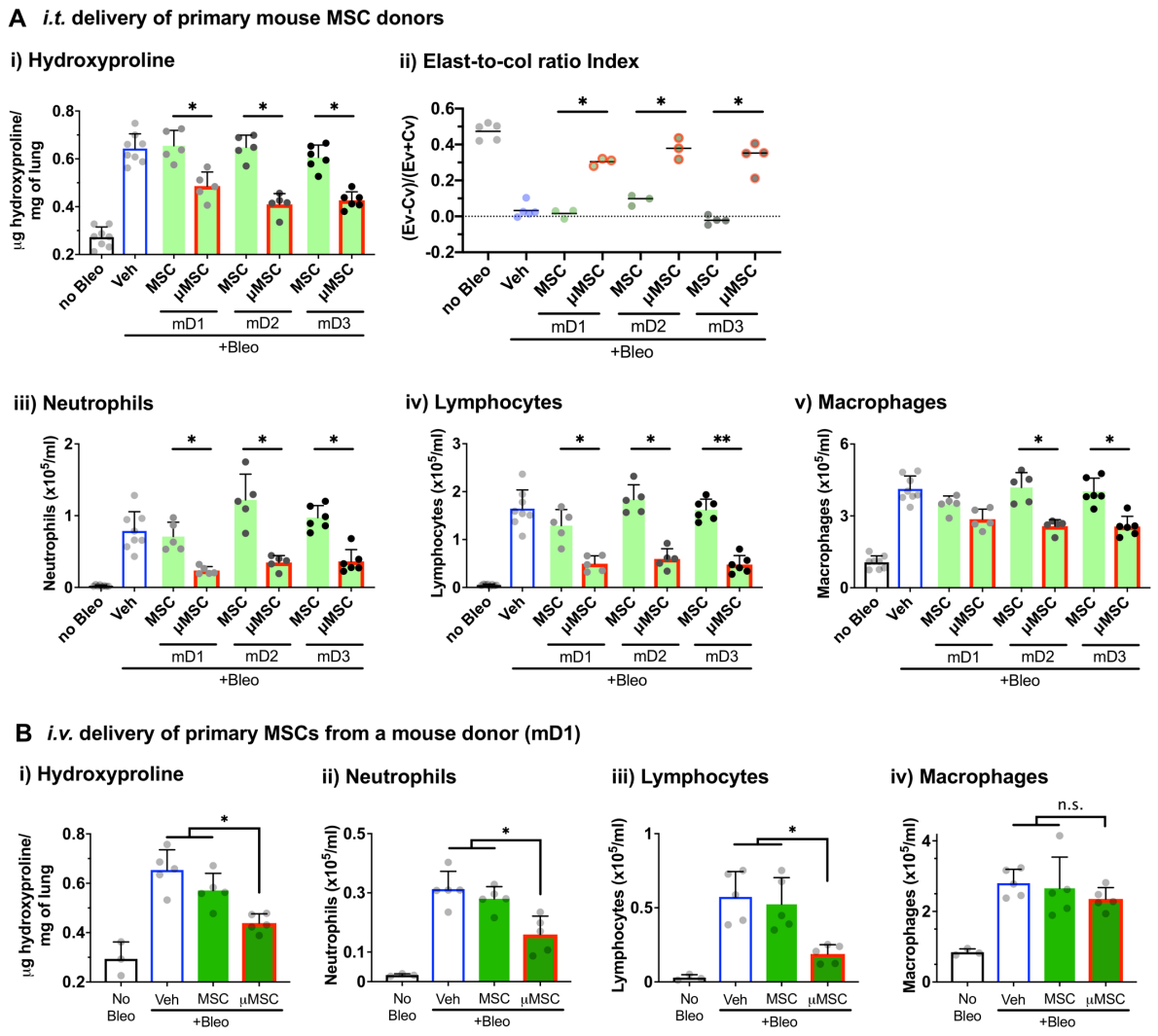

Figure S6. Effects of primary mouse MSCs on fibrotic lung injury. The experimental design is described in **Fig. 3A**. Treatment 1 week after bleomycin (bleo) – vehicle (veh), uncoated MSCs or gel-coated MSCs (µMSC) with the dose 100,000 per 20 g mouse. **(A)** Intratracheal (*i.t.*) delivery of primary MSCs from 3 different mouse donors. (i) Hydroxyproline levels [*, $p = (0.1\sim4.5) \times 10^{-2}$]. (ii) Parenchymal elastin (E_v)-to-collagen (C_v) volume ratio index $(E_v - C_v)/(E_v + C_v)$ [*, $p = (0.1\sim4.1) \times 10^{-2}$]. Quantification of (iii) neutrophils [*, $p = (0.2\sim4.9) \times 10^{-2}$], (iv) lymphocytes [*, $p = (0.3\sim4.6) \times 10^{-2}$; **, $p = 5.0 \times 10^{-5}$], and (v) macrophages [*, $p = (1.3\sim2.7) \times 10^{-2}$] in bronchoalveolar lavage fluid. For (i) and (iii)-(v), $n = 8$ animals for each of no bleo and bleo only; $n = 5$ for each of mD1 and mD2 MSC donor; $n = 6$ for mD3 MSC donor. For (ii), $n = 5$ animals for each of no bleo and bleo only; $n = 3$ for each of mD1 and mD2 MSC donor; $n = 4$ for mD3 MSC donor, each data point averaged from three fields of view from a lung of each animal. **(B)** Intravenous (*i.v.*) delivery of primary MSCs from a mouse donor (mD1). (i) Hydroxyproline levels [*, $p = (0.9\sim4.6) \times 10^{-2}$]. Quantification of (ii) neutrophils [*, $p = (2.1\sim4.2) \times 10^{-2}$], (iii) lymphocytes [*, $p = (2.4\sim4.9) \times 10^{-2}$], and (iv) macrophages [n.s., $p > 0.05$] in bronchoalveolar lavage fluid. $n = 3$ animals for no bleo, $n = 5$ for other groups. Individual p-values were derived from one-way Welch ANOVA followed by Dunnett T3 multiple comparisons test. All data are shown as mean ± SD.

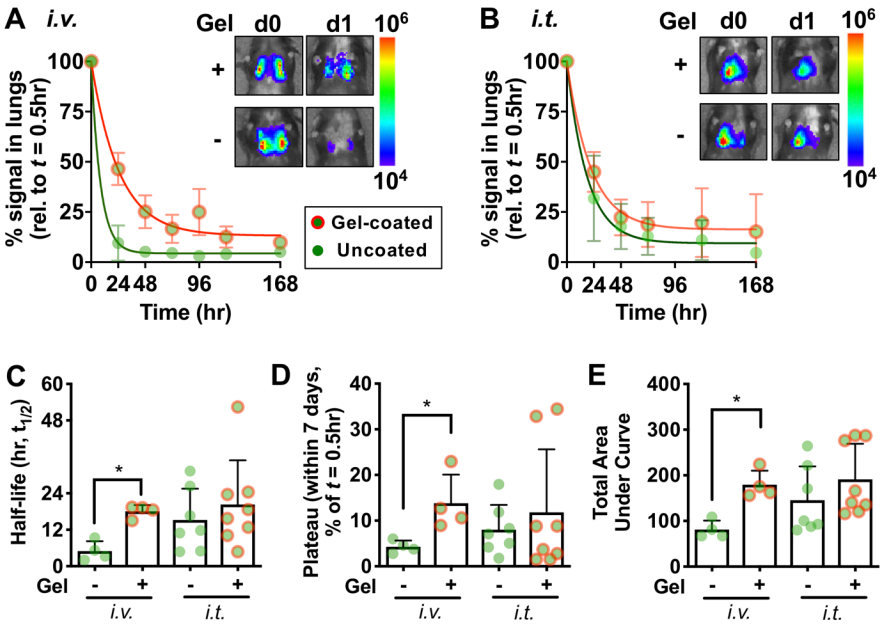

Figure S7. Effects of gel coating on *in vivo* residence of firefly luciferase-expressing MSCs in the lungs. Kinetics of MSCs after **(A)** intravenous (*i.v.*) and **(B)** intratracheal (*i.t.*) delivery, based on average radiance (photons/sec/cm^2/sr) in the lung region. Inlet: representative bioluminescence images. Data are fitted to an exponential decay curve. Quantification of each kinetics data set in terms of **(C)** half-life [*, $p = 4.9 \times 10^{-4}$], **(D)** plateau [*, $p = 2.5 \times 10^{-2}$], and **(E)** total area under curve [*, $p = 1.7 \times 10^{-3}$]. *p*-values are derived from two-tailed unpaired T-test with equal SD. $n = 4$ animals for *i.v.* groups, $n = 7$ for *i.t.* uncoated MSCs, $n = 8$ for *i.t.* gel-coated MSCs. All data are shown as mean ± SD.

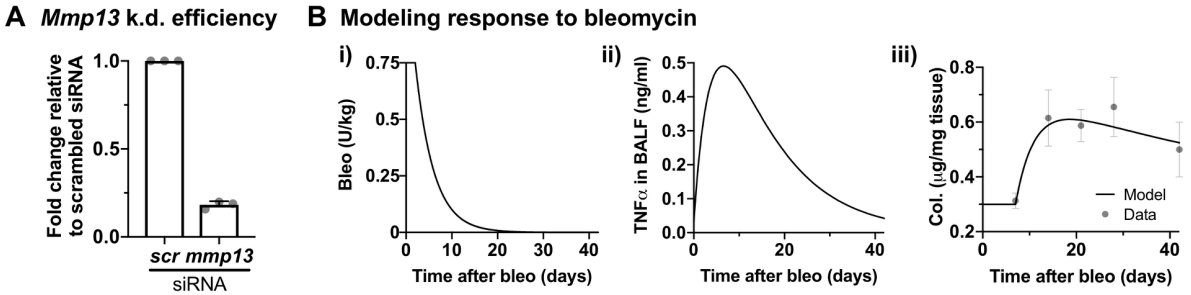

Figure S8. Understanding mechanisms behind therapeutic efficacy of gel-coated MSCs in fibrotic lung injury. (A) siRNA knockdown efficiency of *Mmp13* in D1 MSCs relative to scrambled (*scr*) siRNA. $n = 3$ independent experiments, each performed in three replicates. **(B)** Simulation results showing kinetics of (i) bleomycin (U/kg) after administration, (ii) TNFα in bronchoalveolar lavage fluid (BALF, ng/ml), and (iii) total collagen (μg/mg tissue). For (iii), experimental data from 3 animals are also shown. Mean ± SD.

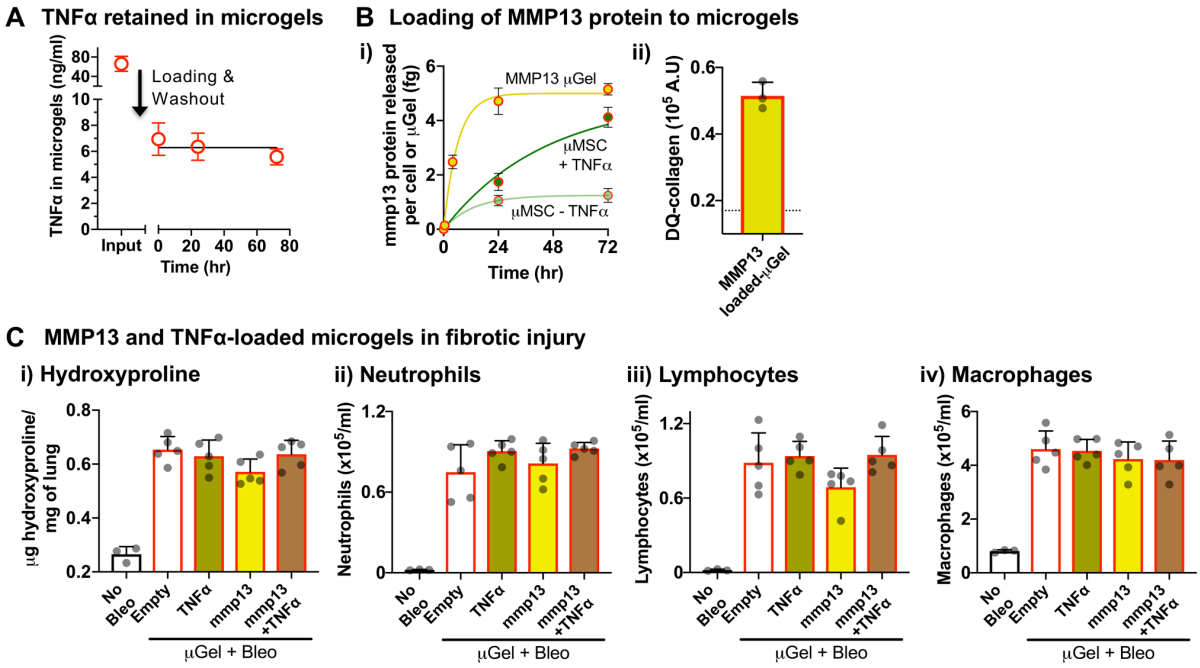

Figure S9. Effects of alginate microgels loaded with recombinant proteins on fibrotic lung injury. (A) Quantification of recombinant TNFα retained in alginate-RGD microgels (~20 μm diameter) after incubation of TNFα (~65 ng/ml) for 1 day, followed by washout and incubation in culture for 3 days. *n* = 3 independent experiments, each performed in two replicates, mean ± SEM. **(B)** Characterization of alginate-RGD microgels loaded with catalytically-active mouse MMP13 proteins. (i) *In vitro* mouse MMP13 protein release kinetics determined by ELISA. One-phase association kinetics fit, ($t_{1/2}$, plateau) values for MMP13-loaded microgels (μGel): (4.3 hr, 5.0 fg/gel), gel-coated MSCs with TNFα (μMSC + TNFα): (30.7 hr, 4.8 fg/cell), gel-coated MSCs without TNFα (μMSC - TNFα): (8.9 hr, 1.25 fg/cell). (ii) Collagenase activity of MMP13-loaded microgels. The media were collected from MMP13-loaded microgels (50,000 gels per 200 μl) for 1 day, followed by mixing with fluorogenic collagen-I substrate (DQ-collagen, 100 μg/ml) and incubating for 1 day before readout. The dotted line indicated a background level from a complete DMEM. *n* = 3 independent experiments, each performed in two replicates, mean ± SEM. **(C)** Effects of alginate-RGD microgels loaded with TNFα or MMP13 proteins (100,000 per 20 g mouse) on (i) hydroxyproline levels, and infiltration of (ii) neutrophils, (iii) lymphocytes, and (iv) macrophages in bronchoalveolar lavage fluid. Microgels were *i.t.* delivered 3 weeks after bleomycin treatment, followed by readout after 1 week of microgel treatment. *n* = 3 animals for no bleo, *n* = 5 for bleo with each treatment group, mean ± SD.

Table S1. Primer sequences for quantitative PCR

Target	Sequence
Mouse *Gapdh* (NM_008084)	F: CTTTGTCAAGCTCATTTCCTGG R: TCTTGCTCAGTGTCCTTGC
Mouse *Mmp1a* (NM_032006)	F: CCAGTTAAACTTGACGCTGC R: GAAACTGTGGATGTCTCTGGG
Mouse *Mmp2* (NM_008610)	F: ACCAAGAACTTCCGATTATCCC R: CAGTACCAGTGTCAGTATCAGC
Mouse *Mmp13* (NM_008607)	F: TTGATGCCATTACCAGTCTCC R: ACATGGTTGGGAAGTTCTGG
Mouse *Acta2* (NM_007392)	F: GTGAAGAGGAAGACAGCACAG R: GCCCATTCCAACCATTACTCC
Mouse *Actb* (NM_007393.5)	F: ACCTTCTACAATGAGCTGCG R: CTGGATGGCTACGTACATGG
Mouse *Tgfb1* (NM_011577.2)	F: CCTGAGTGGCTGTCTTTTGA R: CGTGGAGTTTGTTATCTTTGCTG
Human *GAPDH* (NM_002046)	F: ACATCGCTCAGACACCATG R: TGTAGTTGAGGTCAATGAAGGG
Human *MMP1* (NM_002421)	F: GCACAAATCCCTTCTACCCG R: TGAACAGCCCAGTACTTATTCC
Human *MMP2* (NM_001127891)	F: ACCCATTTACACCTACACCAAG R: TGTTTGCAGATCTCAGGAGTG
Human *MMP13* (NM_002427)	F: GATGACGATGTACAAGGGATCC R: ACTGGTAATGGCATCAAGGG

Supplementary Code. Source code for mathematical modeling. The MATLAB code consists of two files and were used to solve a set of ordinary differentiation equations for modeling of MSC-mediated collagen degradation.