Supplemental Data

Table S1. Sequences of primers used in quantitative PCR.

Gene (GenBank accession number)	Primer	Sequence
18S rRNA (X01117)	Forward	5'-CCCAGTAAGTGCGGGTCATAA-3'
	Reverse	5'-GATCCGAGGGCCTCACTAAAC-3'
β-actin (NM_031144)	Forward	5'-GAGATTACTGCCCTGGCTCCT-3'
	Reverse	5'-CTTGCTGATCCACATCTGCTG-3'
α-SMA (X06801)	Forward	5'-TGTGCTGGACTCTGGAGATG-3'
	Reverse	5'-GAAGGAATAGCCACGCTCAG-3'
PDGF-Rβ (NM_031525)	Forward	5'-ATGCAGAAATGCTGGGAAGAA-3'
	Reverse	5'-AAACTCCTCATCCACCTGCTG-3'
PPARγ (NM_013124)	Forward	5'-TGACCAGGGAGTTCCTCAAAA-3'
	Reverse	5'-GGCGGTCTCCACTGAGAATAA-3'

Supplemental Methods

TUNEL Assay. The TUNEL assay was performed using the In Situ Cell Death Detection kit with fluorescein labeling (Roche). HSC cultured on polyacrylamide gels coated with type I collagen (0.1 mg/ml) were grown for 3 or 7 days. They were fixed with 4% paraformaldehyde in PBS, pH 7.4 for 1 h at room temperature and then permeabilized

with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Positive control cells were treated with 300 U/ml DNaseI (New England Biolabs) for 10 min at room temperature. Cells were then labeled by incubation with the TUNEL reaction mixture (containing nucleotide mixture and terminal deoxynucleotidyl transferase) for 60 min at 37C, while negative control cells were incubated with only the nucleotide mixture (without TdT enzyme). After washing, cells were analyzed using fluorescence microscopy for detection of fluorescein labeled cells.

Supplemental Figure Legends

Fig. S1: There is minimal deposition of neomatrix onto polyacrylamide gels. HSC were cultured for 7 days on 2.5 kPa polyacrylamide gels coated with either type I collagen or plasma fibronectin. Gels were immunostained with antibodies against α-SMA (red) and either type I collagen or fibronectin (green). Staining demonstrated that gels were uniformly coated with matrix, and manifested minimal deposition of additional matrix over the 7 days of culture, although there is evidence of some collagenase activity (upper left panel). Photos are representative of two independent experiments. Bar, 50 μM.

Fig. S2. HSC on polyacrylamide supports do not demonstrate increased apoptosis. Primary rat HSC were cultured on 0.4 kPa, 2.5 kPa, and 12 kPa polyacryamide supports coated with type I collagen for 3 (A) and 7 (B) days after isolation, after which a TUNEL assay was carried out. Representative areas of the gels are shown. No apoptotic HSC were seen on polyacrylamide substrates of any stiffness. DNAseI treatment of the same cells (positive control; 12 kPa) demonstrates the efficacy of the assay. The left image of

each pair shows TUNEL staining; the right is the merge between TUNEL and bright field with Nomarski optics.