Supporting Information

Shah et al. 10.1073/pnas.0906501107

SI Text

Materials and Methods. ***Phage display to determine binding sequence to TGF-β1.*** Phage display was performed with the Ph.D. 7 kit (New England Biosystems). The M13-phage library (containing 2×10^9 phages and all possible 7-mer sequences) was added to 96-well microtiter plates, which had been exposed to 50 µL 10 µg/mL growth factor solution for 18 h at 0 °C. After incubation at room temperature for 60 min, the unbound phage was removed by rinsing with 0.1% v/v Tween-20. Bound phage was subsequently eluted by incubating with growth factor solution for 60 min. The presence of binding phage was determined by serial dilution of the phages and subsequent plating with *E. Coli* on agar plates. The number of plaques formed can be related to the number of phages in the original mixture. The phages were subsequently amplified for 4 h in *E. Coli* at 37 °C. In subsequent panning rounds, the concentration of Tween-20 was raised to 0.5% and the binding and elution times were decreased and increased, respectively, to increase the stringency of the selection process. After three rounds of panning, the recovered phage mixture is diluted and plated with bacteria on agar. Subsequently, plaques are isolated containing a single DNA sequence. After purification, ten clones were sequenced. Next, ELISA screening was used to eliminate false positives and determine relative binding strengths of the selected clones. Each clone was incubated for 1 h on growth factor coated microtiter plates, the plates were rinsed with TBS/Tween-20 and subsequently HRP-conjugated anti-M13 antibody (Amersham Biosciences) was incubated for 1 h. After addition of ABTS, the absorbance at 450 nm was measured after 30 min to determine the relative binding strengths of the isolated clones. The strongest binder was subsequently selected and incorporated within the PA structure.

Peptide Amphiphile Synthesis. The following reagents, or equivalents, were used as received: HBTU (2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), piperidine, DIEA (n, n,-diisopropylethlamine), DMF (n, n-dimethylformamide), DCM (dichloromethane), TFA (trifluoroacetic acid), TIS (triisopropylsilane). All water was purified by reverse osmosis and filtered using a Millipore™ system to a resistivity of 18.2 Mohm-cm. 9- Fluorenylmethoxycarbonyl (Fmoc) protected amino acids and orthogonally protected Fmoc- Lys(Mtt)-OH were purchased from EMD Biosciences (La Jolla, CA). For the TGF-binding PA, the peptides were synthesized on Rink amide resin via solid-phase methodology on an automated peptide synthesizer (CS Bio Co. model 136XT). The resin was first swelled in DCM and DMF, and then Fmoc deprotection was performed with 30 vol % piperidine in DMF solution for 10 min, repeated twice. Amino acid couplings were done with 4.0 equivalents of the Fmoc- protected amino acid (0.5 M in DMF), 3.8 equivalents HBTU (0.475 M in DMF) and 6.0 equivalents of DIEA (0.75 M in DMF) for 1 h per coupling. Each solution was combined and pre-activated by bubbling with high purity nitrogen gas for 3 min prior to being added to the resin-containing reaction vessel. Each coupling was performed once. For a 1 mmole reaction scale, 30 mL of solution was used for each deprotection and washing step. All reagents were stored and reactions performed under high purity nitrogen gas. Multiple DCM and DMF washing steps were done between each reaction step. The first amino acid coupled to the Rink amide resin was an orthogonally protected Fmoc-Lys(Mtt)-OH. After coupling, the Mtt protecting group was selectively removed with 1% TFA in DCM. The fatty acid lipophilic component of the PA was coupled to the epsilon amine of the Lys as described above, except 2.0 equivalents of the dodecanoic acid was dissolved in a 50/50 mixture of DMF/DCM and combined with 1.9 equivalents of HBTU and 3.0 equivalents of DIEA in DMF. This coupling was repeated three times, after which the product was checked for free amines by the ninhydrin reaction (also known as the 'Kaiser test') and the reaction repeated if necessary to obtain a negative result for free amines. After the fatty acid was attached, the remaining peptide sequence was synthesized as described, progressing from C to N terminus, and leaving the N terminus of the peptide as a free amine.

The filler PA was synthesized as described above, except a preloaded glutamic acid Wang resin was used. Additional amino acids were coupled proceeding from C to N terminus. After coupling of the N-terminal valine, the peptide was capped with a palmitoyl moiety, which was accomplished as described above but substituting palmitic acid for dodecanoic acid.

Cleavage and deprotection of PAs from the resin was carried out in a mixture of TFA:TIS:water in ratio of 95.0:2.5:2.5 for 3 h. TFA was removed by rotary evaporation and was halted prior to complete dryness. The remaining viscous peptide solution was triturated with cold (-20 °C) diethyl ether. The solution was agitated to ensure good mixing then re-cooled to -20 °C overnight to allow complete precipitation. The resulting precipitated PA was collected, washed three times with cold ether, and dried under vacuum. PAs were then dissolved in an aqueous solution with sufficient ammoniumhydroxide to obtain a pH of 9 and purified using an Agilent, Inc. model 1100 preparative HPLC. An elution gradient of water and acetonitrile (each containing 0.1 vol % ammonium hydroxide buffer) was used. UV-absorption was monitored at 220 nm wavelength, and the eluent of the primary peak collected. To remove the water and acetonitrile following preparative HPLC, PA solutions were lyophilized for at least 48 h. The resulting PAs were characterized using mass spectroscopy, high performance liquid chromatography (HPLC), and amino acid analysis.

In Vitro cell differentiation studies. Primer pair sequences used for RT-PCR were as follows: Aggrecan–TGTCCACAAAGTCTTCACCTGTGTAG; GTGAGGACCGTCTACGTGCAT and Collagen II Alpha I–TGGTGAAAGAGGACGGACTGG; ACCAGCAGGACCGACAGGAC.

SEM fixation and imaging. For processing cell encapsulated PA scaffolds for SEM analysis, samples were dehydrated with a gradient of water-ethanol mixtures until the gel was in 100% ethanol. The samples were then critically point dried in a Polaron E3000 Critical Point Drying Apparatus and sputter coated with 3nm of a gold palladium alloy in a Cressington 208 HR Sputter Coater. Samples were imaged on a Hitachi S-4800 II SEM (Hitachi, Pleasanton, CA).

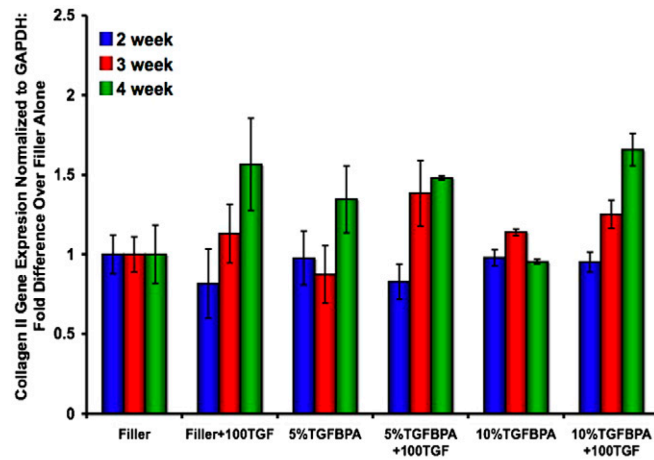

Fig. S1. Type II collagen gene expression from hMSCs cultures in PA gels at 2, 3, and 4 weeks. Filler PA = Filler; 100 ng/mL of TGF = 100TGF; 5 mol% TGFBPA = 5% TGFBPA; 10 mol% TGFBPA = 10%TGFBPA.

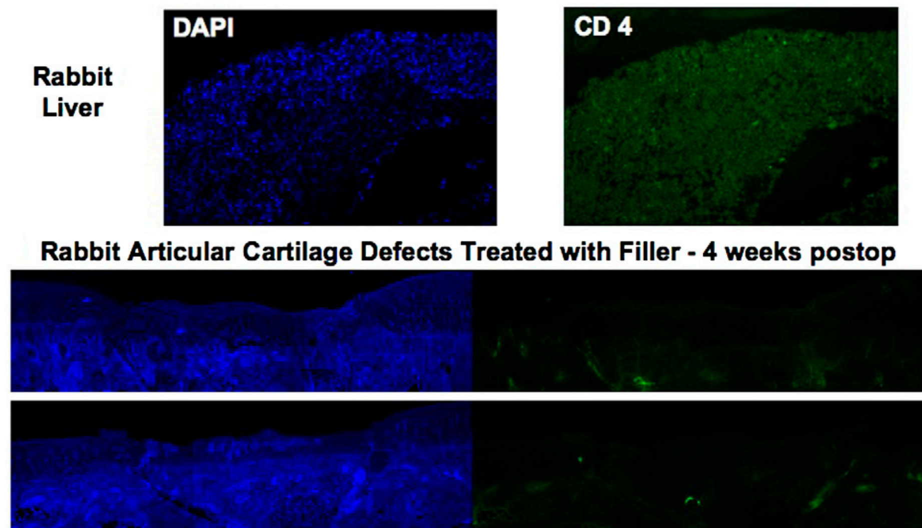

Fig. S2. Biocompatibility of PAs within articular cartilage defects. DAPI and CD4 immunohistochemical stained sections of rabbit liver (*Upper*) and articular cartilage defects treated with filler PA + rhTGF-β1 4 weeks postop (*Bottom*) showing no apparent immune response resulting from PA treatment.

Table S1. Description of scoring for histological evaluation

O'Driscoll category	Score
Nature of the predominant tissue	
Cellular morphology	
Hyaline articular cartilage	4
Incompletely differentiated mesenchyme	2
Fibrous tissue or bone	0
Safranin-O staining of the matrix	
Normal or nearly normal	3
Moderate	2
Slight	1
None	0
Structural characteristics	
Surface regularity	
Smooth and intact	3
Superficial horizontal lamination	2
Fissures: 25–100% of thickness	1
Severe disruption, including fibrillation	0
Structural integrity	
Normal	2
Slight disruption, including cysts	1
Severe disintegration	0
Thickness	
100% of normal adjacent cartilage	2
50–100% Of normal cartilage	1
0–50% of normal cartilage	0
Bonding to the adjacent cartilage	
Bonded at both ends of the graft	2
Bonded at one end, or partially at both ends	1
Not bonded	0
Freedom from cellular changes of degeneration	
Hypocellularity	
Normal cellularity	3
Slight hypocellularity	2
Moderate hpocellularity	1
Severe hypocellularity	0
Chondrocyte clustering	
No clusters	2
< 25% of the cells	1
25–100% of the cells	0
Adjacent cartilage degenerative joint disease	
Normal, no clusters, normal staining	3
Normal cell, mild clusters, mod staining	2
Mild/moderate hypocellularity, slight staining	1
Severe hypocellularity	0