Supplementary Data

Supplementary Methods

Isolation of patient-derived fibroblasts and cell culture

Fibroblasts were isolated as previously described.[1] Briefly, tissues were incubated overnight at 4°C in dispase (Invitrogen, Carlsbad, CA) to remove the epidermis. The dermal tissue was minced and incubated in collagenase (Invitrogen)/hyaluronidase (Sigma, St. Louis, MO)/DMEM-F12 (Invitrogen) for 1 h. Red blood cell lysis buffer (Sigma) was added for 2 min, and cells were collected by centrifugation. Cultures were routinely checked for mycoplasma.

Flow cytometry

Flow cytometry was conducted to confirm fibroblast identity of all 12 isolated strains as characterized by elevated expression of mesenchymal markers: CD73, CD105, and CD140b and low expression of hematopoietic marker: CD31. Cell suspensions were incubated with primary antibodies (all PE-conjugated; BD Pharmingen, San Jose, CA) for CD markers or an isotype control for 30 min on ice, washed, and resuspended in blocking buffer. Flow cytometry was performed on the 12 strains using an FACSCalibur (Becton-Dickinson, San Jose, CA), and percentage of cells positive for each marker was determined using Summit V4.3 software (Dako, Carpentaria, CA).

Scratch wound assay

To measure cell motility, fibroblasts were grown to confluence on tissue culture plates in fibroblast growth media and scratch wounds were created as previously described.[5] Scratch assays were performed on the 12 fibroblast strains in triplicate or quadruplicate wells, and two to four areas of each scratch were chosen at random and imaged at 0 and 8 h postwounding. The area of the scratch was quantified using ImageJ software and expressed as percent closure ± SD.

Self-assembled extracellular matrix model media

Component	*Company*	*Volume (1 L total)*
DMEM (1 g/L glucose)	Invitrogen	675 mL
F12	Invitrogen	56.25 mL
FetalClone II	Hyclone	50 mL
Adenine (18 mM)	ICN (Aurora, OH)	10 mL
Penicillin/streptomycin	Invitrogen	6.8 mL
HEPES (0.19 g/mL)	Sigma-Aldrich	10 mL
Hydrocortisone	Sigma-Aldrich	2 mL
Cholera toxin	MP Biomedicals (Santa Ana, CA)	1 mL
Epidermal growth factor	Austral Biological (San Ramon, CA)	1 mL
Insulin (5 mg/mL)	EMD Biosciences (San Diego, CA)	1 mL

Hydrogel preparation and migration

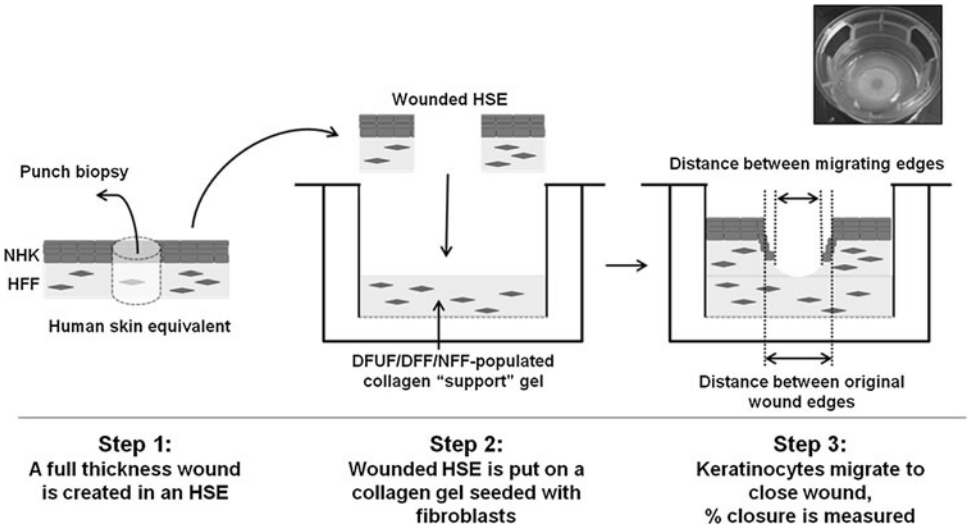
High G-block-containing alginate, MVG (M:G = 40:60; MW ~250 kDa; ProNova Biomedical, Oslo, Norway) was used to also obtain low-molecular alginate (MW ~50 kDa) by gamma irradiation (3 MRad) as previously described (EH&S Lab at MIT, Cambridge, MA).[35] To facilitate degradation and cell release, 1% of the sugar residues in the alginate chains were oxidized using sodium periodate (Sigma-Aldrich) and the alginate was dialyzed and lyophilized as described.[36] Alginate chains were further modified to contain RGD-binding domains (GGGGRGDSH, Peptides 2.0, Virginia; two RGD per HMW chain or per five LMW chain) using carbodiimide chemistry as previously described,[37] dialyzed, and lyophilized.

The final concentration of alginate gels was 2% w/v (HMW:LMW = 25:75) in serum-free DMEM. Gels contained $1e^6$ cells per 60 μL and were ionically cross-linked with 4% v/v 1.22 M calcium sulfate solution. For cell release studies, 60 μL gel was ejected per well ($n = 4$) and fed with fibroblast growth media. Gels were transferred daily to fresh wells, and cells that had attached overnight were trypsinized and counted. The number of cells released is expressed as average number of cells per well ± SD. Identical gels were constructed for cell delivery in the *in vivo* mouse wound-healing model.

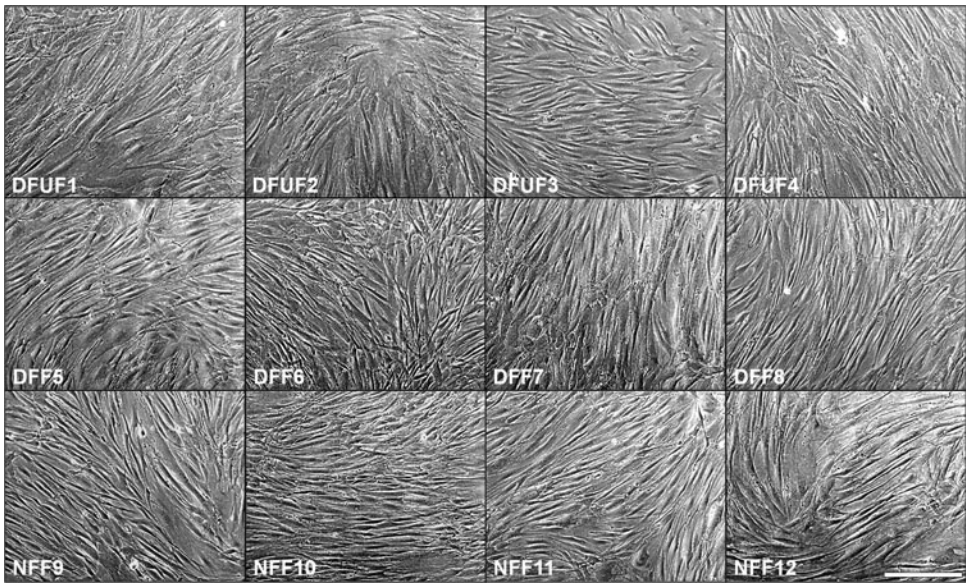
Supplementary Table S1. Patient-Derived Cell Strains Express Fibroblast Lineage Markers, But Not a Hematopoietic Marker by Flow Cytometry

Cell Line	CD31	CD73	CD105	CD140b
HFF	2	95	95	99
DFUF1	0	99	98	98
DFUF2	0	99	95	98
DFUF3	0	99	99	92
DFUF4	0	99	99	99
DFF5	0	99	99	99
DFF6	0	99	98	98
DFF7	0	99	99	98
DFF8	0	99	99	99
NFF9	0	99	99	99
NFF10	0	99	98	96
NFF11	3	99	99	99
NFF12	0	99	99	99

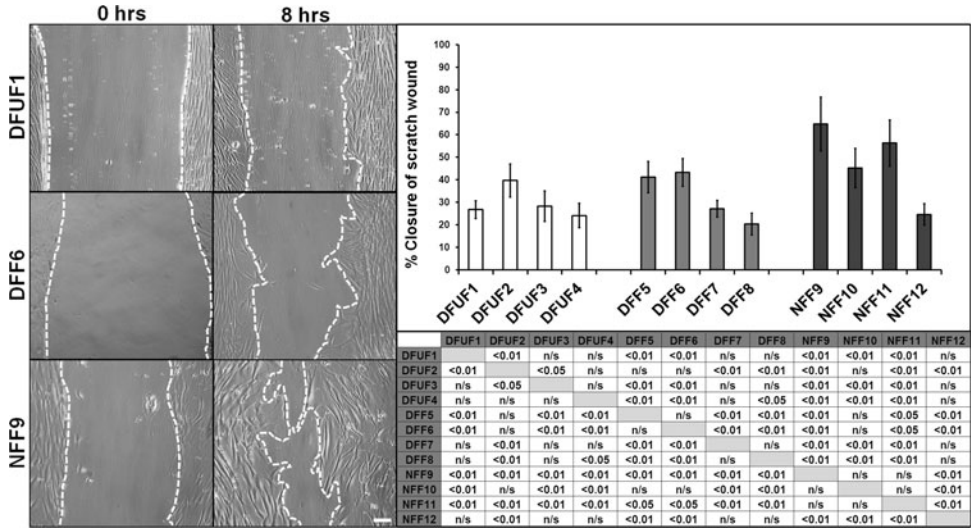
Values expressed as percentage of cells positive for each marker.



SUPPLEMENTARY FIG. S1. Schematic of 3D re-epithelialization model. A healthy human skin equivalent tissue is constructed using keratinocytes and fibroblasts derived from human foreskin; normal human keratinocytes (NHK), and human foreskin fibroblasts (HFF). Then, a 5 mm punch biopsy is used to create an excisional ''wound'' in the center of the tissue. This wounded tissue is then placed on a collagen ''support'' gel populated with the fibroblast strain of interest. Keratinocytes can then migrate over the support gel to close the wound. After 72 h, the mean percent wound closure of several tissue sections is determined by measuring the distances between the original wound edges and the migrated epithelial tongues. Insert shows a photograph of the re-epithelialization model from above after step 2. 3D, three dimensional. HSE, human skin equivalent.

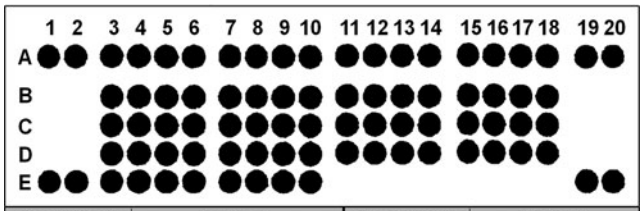


SUPPLEMENTARY FIG. S2. Twelve primary patient-derived cell strains exhibit fibroblast morphology. Representative bright-field images of 12 patient-derived fibroblast strains are shown. All cells exhibited a spindle-like morphology, typical of fibroblasts. Scale bar, 20 μm.



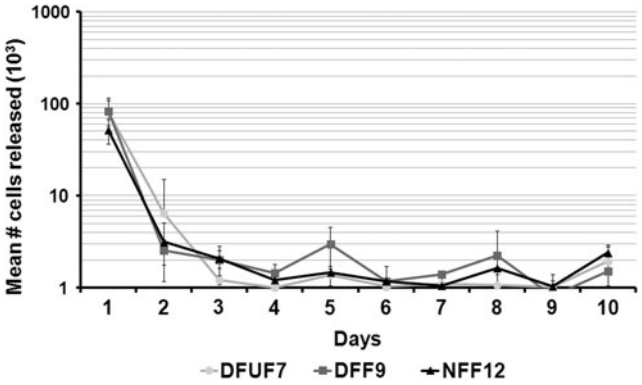
	DFUF1	DFUF2	DFUF3	DFUF4	DFF5	DFF6	DFF7	DFF8	NFF9	NFF10	NFF11	NFF12
DFUF1		<0.01	n/s	n/s	<0.01	<0.01	n/s	n/s	<0.01	<0.01	<0.01	n/s
DFUF2	<0.01		<0.05	n/s	n/s	n/s	<0.01	<0.01	<0.01	n/s	<0.01	<0.01
DFUF3	n/s	<0.05		n/s	<0.01	<0.01	n/s	n/s	<0.01	<0.01	<0.01	n/s
DFUF4	n/s	n/s	n/s		<0.01	<0.01	n/s	<0.05	<0.01	<0.01	<0.01	n/s
DFF5	<0.01	n/s	<0.01	<0.01		n/s	<0.01	<0.01	<0.01	n/s	<0.05	<0.01
DFF6	<0.01	n/s	<0.01	<0.01	n/s		<0.01	<0.01	<0.01	n/s	<0.05	<0.01
DFF7	n/s	<0.01	n/s	n/s	<0.01	<0.01		n/s	<0.01	<0.01	<0.01	n/s
DFF8	n/s	<0.01	n/s	<0.05	<0.01	<0.01	n/s		<0.01	<0.01	<0.01	n/s
NFF9	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		n/s	n/s	<0.01
NFF10	<0.01	n/s	<0.01	<0.01	n/s	n/s	<0.01	<0.01	n/s		n/s	<0.01
NFF11	<0.01	<0.01	<0.01	<0.01	<0.05	<0.05	<0.01	<0.01	n/s	n/s		<0.01
NFF12	n/s	<0.01	n/s	n/s	<0.01	<0.01	n/s	n/s	<0.01	<0.01	<0.01	

SUPPLEMENTARY FIG. S3. DFUFs and DFFs exhibit impaired motility compared with NFFs. Representative bright-field images of scratch wounds of one fibroblast strain per type (DFUF1, DFF6, and NFF9) at 0 and 8 h postwounding. *Dotted line* demarcates migrating edge. Scale bar, 10 μm. Quantification of motility 8 h postwounding is expressed as mean percent scratch closure ± SD ($n = 4$). DFUFs and DFFs tended to migrate slower compared with NFFs. Significance was determined by one-way ANOVA and Tukey's HSD *post hoc* tests as indicated in the table. DFFs, type II diabetic, nonulcerated foot-derived fibroblasts; DFUFs, type II diabetic foot ulcer-derived fibroblasts; NFFs, nonulcerated foot-derived fibroblasts; n/s = not significant.



Coordinate	Target	Coordinate	Target
A1,A2	Reference Spot	C7,C8	IL-13
A3,A4	C5/C5a Complement	C9,C10	IL-16
A5,A6	CD40 Ligand*	C11,C12	IL-17
A7,A8	G-CSF	C13,C14	IL-17E
A9,A10	GM-CSF	C15,C16	IL-23*
A11,A12	CXCL1*	C17,C18	IL-27
A13,A14	I-309	D3,D4	IL-32α
A15,A16	sICAM-1	D5,D6	IP-10
A17,A18	IFN-γ*	D7,D8	I-TAC
A19,A20	Reference Spot	D9,D10	MCP-1*
B3,B4	IL-1α	D11,D12	MIF*
B5,B6	IL-1β	D13,D14	MIP-1α
B7,B8	IL-1ra	D15,D16	MIP-1β
B9,B10	IL-2	D17,D18	PAI-1*
B11,B12	IL-4	E1,E2	Reference Spot
B13,B14	IL-5	E3,E4	RANTES
B15,B16	IL-6*	E5,E6	SDF-1*
B17,B18	IL-8*	E7,E8	TNF-α
C3,C4	IL-10	E9,E10	sTREM-1
C5,C6	IL-12	E19,E20	Negative Control

SUPPLEMENTARY FIG. S4. Cytokine array spot locations. Coordinates of antibody spot locations are shown for a panel of cytokines. Cytokines detected in DFUF1, DFF6, and NFF10 conditioned media are marked with an asterisk.



SUPPLEMENTARY FIG. S5. DFUFs, DFFs, and NFFs migrate out of a hydrogel vehicle at similar rates. Quantification of the mean number of fibroblasts that migrated out of hydrogel in culture daily ± SD ($n = 4$). These data showed that DFUF1, DFF6, and NFF10 survived in the hydrogel vehicle and were able to migrate out over 10 days, indicating that hydrogel is a suitable vehicle for *in vivo* transplantation.