Electronic Supplementary Material (ESI) for Integrative Biology

Supporting Information

Supplementary Figures

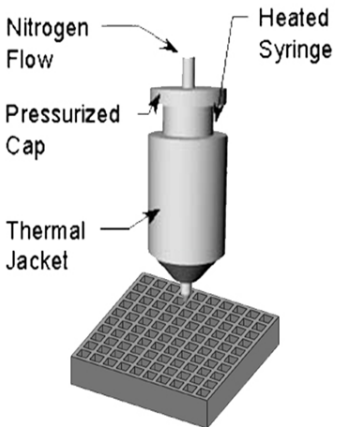


Fig. S1 Well system fabrication. Free form fabrication with 3D-plotters. Material extrusion occurred by nitrogen pressure. Wells are rectangular, with closed bottom and sides, and opened top. The strand distance is defined by the symmetrical space between one well wall and one well (empty space) and repeated throughout the device.

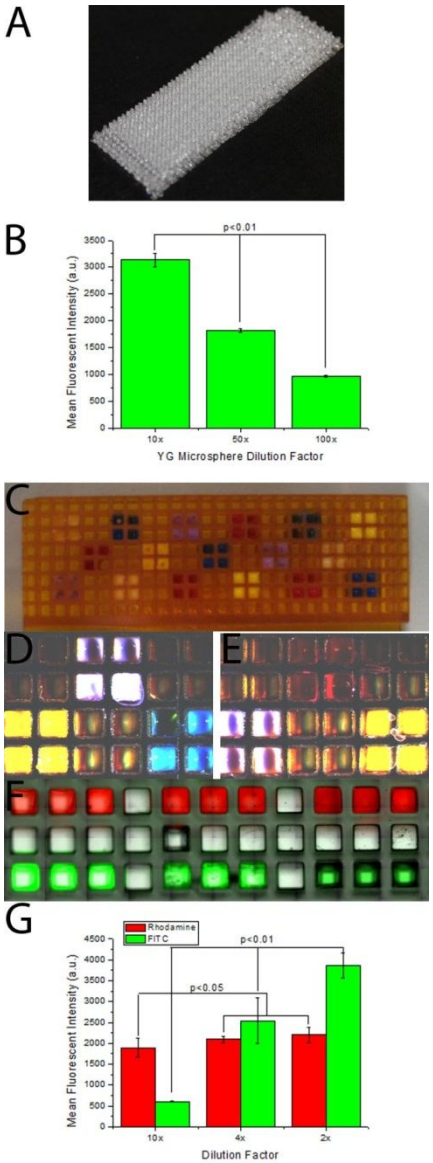


Fig. S2 Well robustness tested with chemicals and fluorescent markers. Screening devices of two types were customized to fit an automatic confocal light microscopy: one made of poly(L-lactic acid) (PLLA) manufactured via fused deposition modeling (A), and the other made of an acrylic photopolymerizable resin and manufactured via stereolithography (C). Diluted fluorescent microspheres (YG: λ = 441-486 nm) were quantified and assessed (B), indicating that well autonomy was maintained with microspheres and wells were suitable for sample randomization (Color dyes: red, blue, yellow, purple, white) (D,E). By seeding Rhodamine red and FITC in opposite directions and measuring fluorescence after 30 min., potential arbitrary biases were considered (F, G). One-way ANOVA followed by Tukey's post-hoc test was used to evaluate statistical significance, where bars and error bars represent the mean fluorescence (n=12 wells) and their standard deviation, respectively ($p < 0.05$).

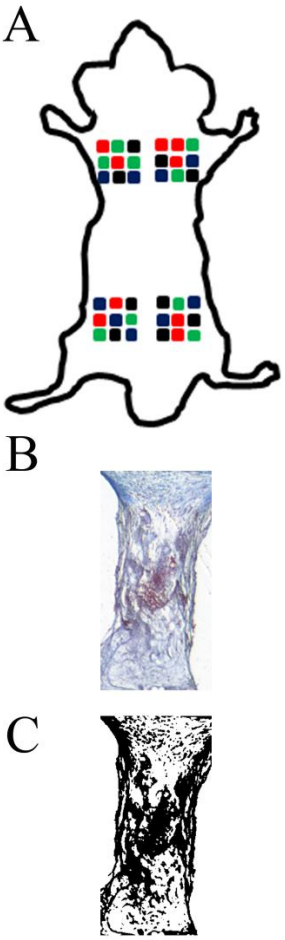
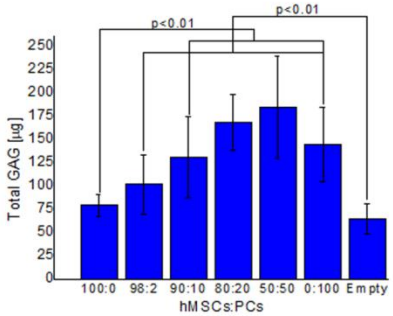


Fig. S3 Four 9-well devices were implanted subcutaneously per animal (A), where each mouse contains 36 randomized conditions. Red represents hMSCs cell numbers; blue represents bPCs cell numbers; green represents hMSCs:bPCs co-culture ratios; and black represents wells without cells. Representative image used for analysis with imageJ (B), which shows the well area cut from a slide stained with Masson's trichrome. Images of wells were converted into binary images (C), which quantified tissue pixels (black) for estimation of the percent area of tissue (tissue area/total area*100%) in a well.

A



B

hMSCs:PCs	total DNA	GAG/DNA	GAG/initial % PC
(%:%)	µg	µg/µg	($\cdot 10^{-5}$ g)
100:0	12.6 ± 4.8	7.3 ± 3.5	-
98:2	13.8 ± 4.1	6.1 ± 2.4	5.08
90:10	11.4 ± 7.4	9.3 ± 1.5	1.31
80:20	14.3 ± 3.0	9.4 ± 2.4	0.84
50 :50	19.2 ± 7.9	10.3 ± 3.9	0.37
0:100	20.2 ± 5.8	13.2 ± 3.2	0.14
empty scaffold	6.8 ± 1.8	10.5 ± 1.1	-

C

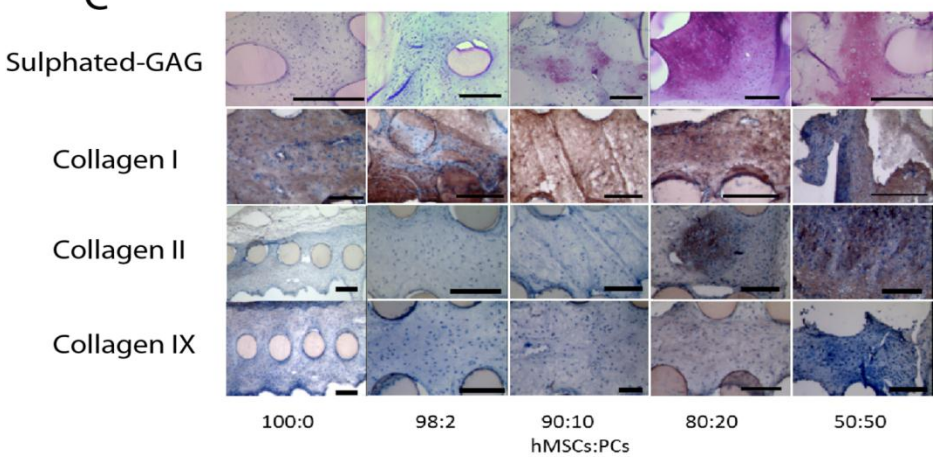


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Fig. S4 Conventional in vivo method to evaluate co-culture ratios of bPCs and hMSCs. 3D scaffolds of 300PEOT55PBT45 are seeded with cocultures ratios in nude mice (8 weeks). Increasing ratios of bPCs, increase the total glucosaminoglycans (GAG)/construct (A). Means were evaluated for significance with two-way Anova followed by Tukey's ($p < 0.01$). 10-20 % bPCs showed comparable efficiency in GAG production (B). 10-20 % bPCs is thus sufficient to induce sulphated proteoglycans (SAF-O (red)) and to promote production of collagens (C) type I, II and IX (Brown). Counterstained to observe the nuclei (dark blue), cytoplasm (light blue) and scaffold (white). Scale bars: 200 µm.

Supplementary Tables

Table S1. Several materials can be used to manufacture implantable well systems according to their processing parameters.

Material	Cartridge Temperature [°C]	1st layer Strand Distance [mm]	2nd layer and higher strand distance [mm]	Layer thickness[mm]	Inner Needle diameter [mm]
PEOT/PBT 300/55/45	200	0.3	1.5	0.2	0.4
PEOT/PBT 1000/70/30	190	0.3	1.5	0.2	0.25
Polylactic Acid	220	0.3	1.5	0.2	0.4
Alginate	25	0.5	3	0.2	0.5

Table S2. hMSCs conditions. The percentage (%) of mice of the total population (10 mice/week) from weeks 2 (light grey) and week 4 (dark grey) showing significantly ($p<0.05$) higher amounts of tissue. One-way ANOVA analysis of the means (n=3 slides) of tissue percent area from each condition were compared between each other for each statistically independent mouse.

Cells #s	25,000	12,000	6,000	Control
25,000		0%	33%	25%
12,000	13%		11%	25%
6,000	0%	13%		50%
Control	50%	50%	38%	

Table S3. Primary chondrocytes conditions. The % of mice of the total population (10 mice/week) from weeks 2 (light grey) and 4 (dark grey) showing significantly ($p<0.05$) higher amounts of tissue. One-way Anova analysis of the means (n=3 slides) of tissue percent area from each condition were compared between each other for each statistically independent mouse.

Cells #s	25,000	12,000	6,000	Control
25,000		25%	25%	38%
12,000	50%		50%	50%
6,000	25%	0%		38%
Control	25%	25%	25%	

Table S4. Co-culture conditions. The percentage (%) of mice of the total population (10 mice/week) from weeks 2 (Light grey) and week 4 (Dark grey) showing significantly ($p<0.05$) higher amounts of tissue. One-way ANOVA analysis of the means (n=3 slides) of tissue percent area from each condition were compared between each other for each statistically independent mouse.

hMSCs:chondrocytes	80/20	50/50	20/80	Control
80/20		11%	22%	44%
50/50	33%		33%	44%
20/80	33%	11%		11%
Control	56%	11%	33%	