Supplementary file

Materials and methods

2.4. Real Time PCR

For gene expression analyses, cDNA template underwent PCR amplification (40 cycles) using the SYBR Green (PowerUp™ SYBR® green master mix, Applied Biosystems, Foster City, CA, USA) and sequence specific primers (Primer sequences listed in Table S1).

Table S1. Primer sequences

Gene	Sequence
ALP	fw: gtacaacaccaatgcccagg
	rv: cagatttcccagcgtccttg
GAPDH	fw: cgaccactttgtcaagctca
	rv: aggggagattcagtgtggtg
OPN	fw: ggtcactgattttcccacgg
	rv: ctcctcgctttccatgtgtg
Runx2	fw: ctgtggttactgtcatggcg
	rv: aggtagctacttggggagga
SP7	fw: agtggagttggtgaatcggt
	rv: aacgagttctcagcctgtga

2.5.1. Calvarial model

Table S2. Grouping of animals in calvarial model

Group number	implant	lifetime	group size
1	none	3/8 weeks	6/6
2	Collagen gel alone	3/8 weeks	6/6
3	collagen gel + 0.5 µg BSP	3/8 weeks	6/6
4	collagen gel + 5 µg BSP	3/8 weeks	6/6

2.5.2 Femur defect model

Table S3. Grouping of animals in femora model

Group	**implant**	**lifetime**	**group size**
1	Empty defect	4/8 weeks	3/3
2	PLA alone	4/8 weeks	3/3
3	PLA + collagen gel (PLA+coll)	4/8 weeks	5/5
4	PLA + collagen gel + 0.5 µg BSP (PLA+coll+BSPlow)	4/8 weeks	5/5
5	PLA + collagen gel + 5 µg BSP (PLA+coll+BSPhigh)	4/8 weeks	5/5
6	PLA + collagen gel + 2 µg BMP-7 (PLA+coll+BMP-7)	4/8 weeks	5/5

Results

3.1. The first step to characterize the effect of BSP immobilized in collagen type I were in vitro assays. Human osteoblasts (hOB) were seeded in collagen type I gels with and without BSP. Regarding morphology of human osteoblasts no differences were observed whether they were seeded in mono- or in co-culture with HUVECs (**Figure S1**).

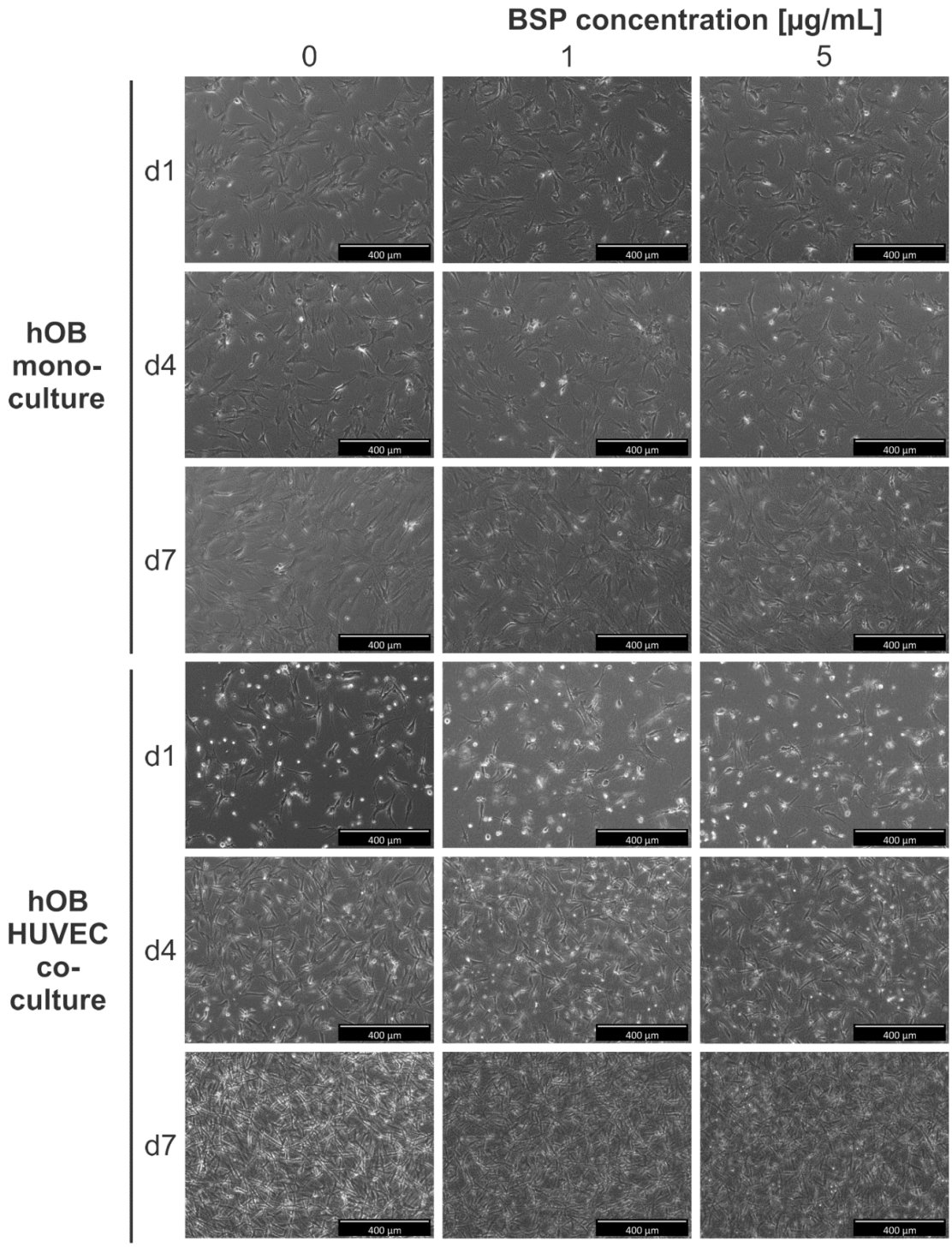

Figure S1. hOB seeded in three dimensional collagen type I gels in mono- and in coculture with endothelial cells (HUVECs).

Proliferation was analyzed using the alamarBlue assay (Figure S2). Figure S2 demonstrates that BSP applied in low concentrations enhanced proliferation of primary human osteoblasts in mono- and in co-culture with endothelial cells. The most significant effect was observed after 4 days.

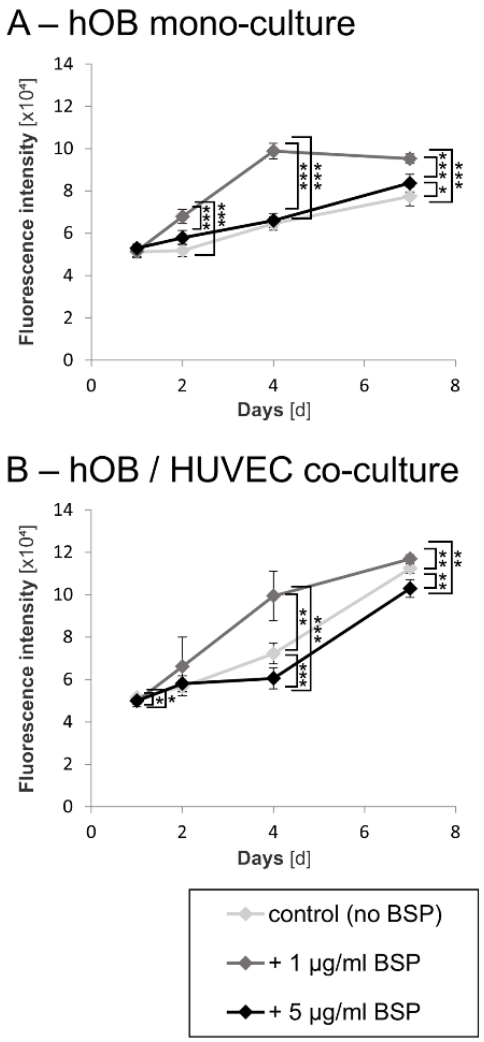

Figure S2. Cell viability of hOBs in monoculture (A) and in co-culture with HUVECs (B) in collagen gels with immobilized BSP. Results are expressed as mean ± SD (n = 9). Games-Howell or Tukey-HSD posthoc tests (dependent on Levene's test) revealed significant differences (*p<0.05, **p<0.01, ***p<0.001).

3.5. BSP release was measured as described in materials and methods and is shown in Fig. S3.

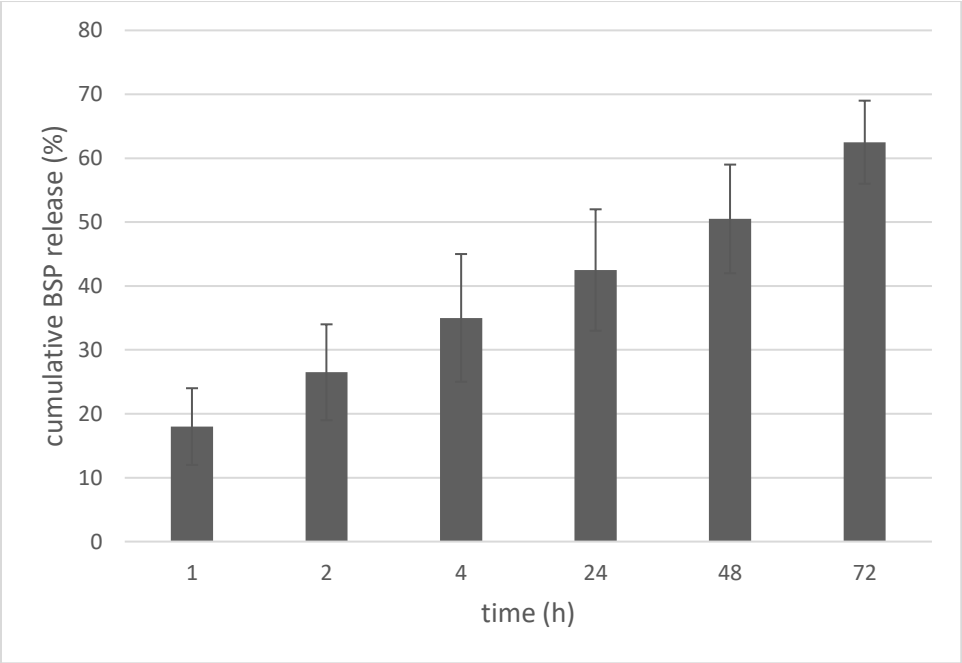

Figure S3. Cumulative BSP release from the PLA-collagen scaffold over 72 hours. Two different concentrations were used for the release assays (BSP-low: 100 ng and BSP-high: 500ng). The diagram shows the BSP release averaged and presented as cumulative percentage (n=3).