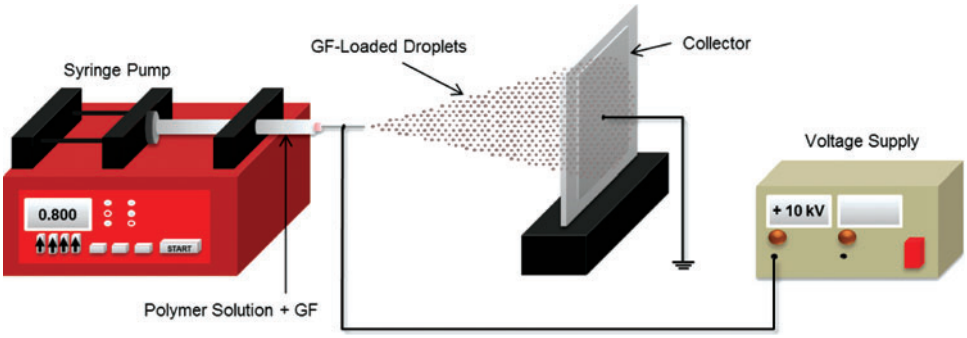
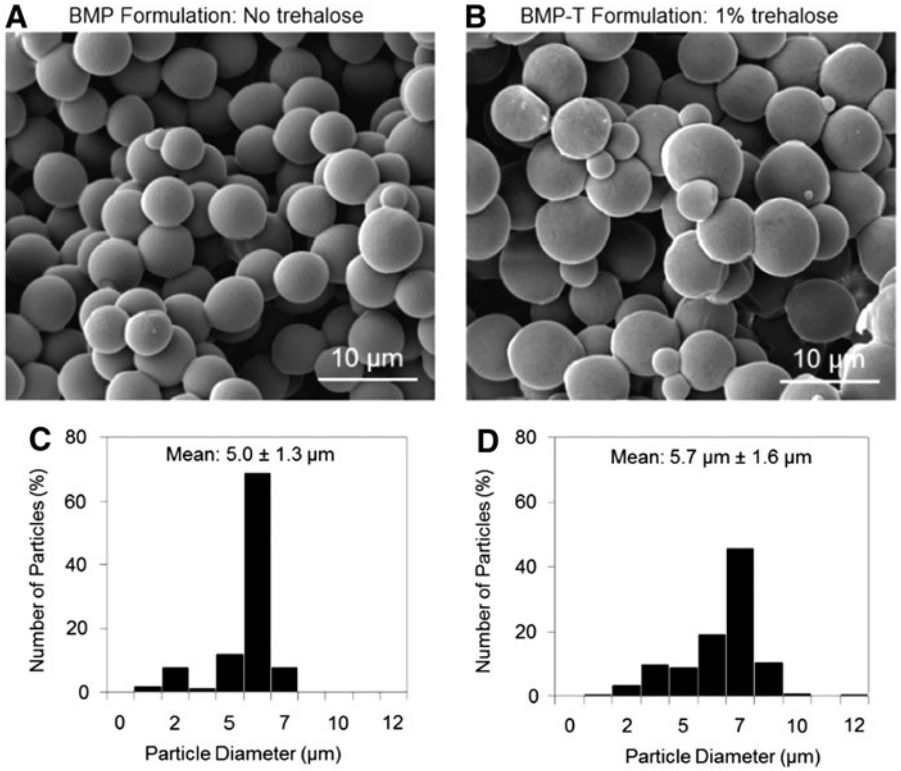
Supplementary Data

Electrospraying Setup and Particle Microstructure



SUPPLEMENTARY FIG. S1. Electrospraying setup.

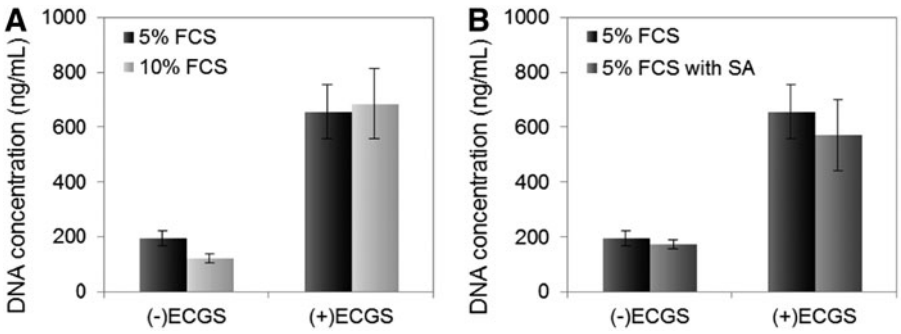


SUPPLEMENTARY FIG. S2. Scanning electron microscopy (SEM) images and particle size distributions of electrosprayed poly(lactic-co-glycolic acid):poly(ethylene glycol) (PLGA:PEG) microparticles loaded with 1% wt serum albumin (SA) without addition of trehalose **(A, C)** and with addition of 1% wt trehalose in the initial polymer solution **(B, D)**. Mean sizes ± standard deviations (SD), $n = 150–200$.

Culture Conditions for Growth Factor Bioactivity Assessment

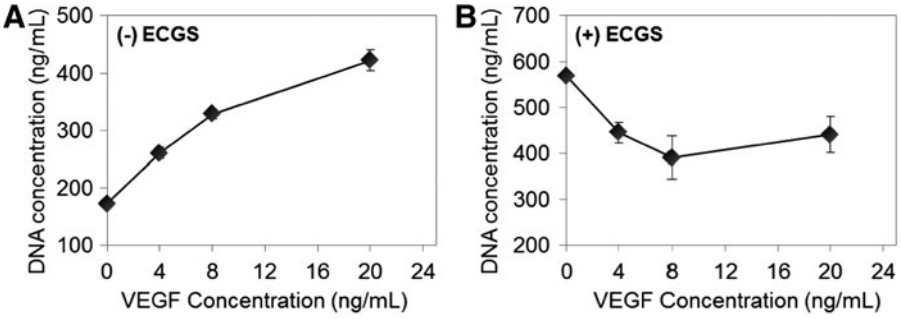
Vascular endothelial growth factor

The effect of endothelial growth supplement (ECGS) and fetal calf serum (FCS) on the proliferation of human umbilical vein endothelial cells (HUVECs) was assessed using a starting seeding density of 3000 cells/well in a 96-well plate and after 3 days of incubation. Results are presented in Supplementary Figure S3A.



SUPPLEMENTARY FIG. S3. **(A, B)** The Effect of fetal calf serum (FCS) amount, serum albumin (SA), and endothelial growth supplement (ECGS) on proliferation of human umbilical vein endothelial cells (HUVECs). Mean ± SE, $n = 5$.

There was a significant impact of ECGS on HUVEC proliferation ($p < 0.001$), where the addition of the supplement increased cell proliferation in a fourfold manner, for any amount of FCS (5% or 10%). Without ECGS, the addition of 10% FCS instead of 5% had a slight, but significant ($p = 0.021$), negative effect on cells. It could be concluded that the ECGS supplement was the most critical factor to proliferation of HUVECs. Since serum albumin (SA) was used as an excipient in electrosprayed microparticles, its effect on cell proliferation was also verified (Supplementary Fig. S3B). It was observed that the presence of SA slightly decreased cell proliferation, but not significantly ($p = 0.4$). Supplementary Figure S4 shows the proliferation results of HUVECs for increasing doses of fresh VEGF. When ECGS was present in the medium, the addition of extra VEGF led to a decrease in cell proliferation (Supplementary Fig. S4B), representative of an excessive GF amount in solution. In the absence of ECGS, the exogenous VEGF delivery significantly increased cell numbers at all concentrations, 4, 8, and 20 ng/mL, in a linear dose-dependent manner (Supplementary Fig. S4A).



SUPPLEMENTARY FIG. S4. **(A, B)** The effect of vascular endothelial growth factor (VEGF) dose on proliferation of HUVECs. Mean ± SE, $n = 5$.

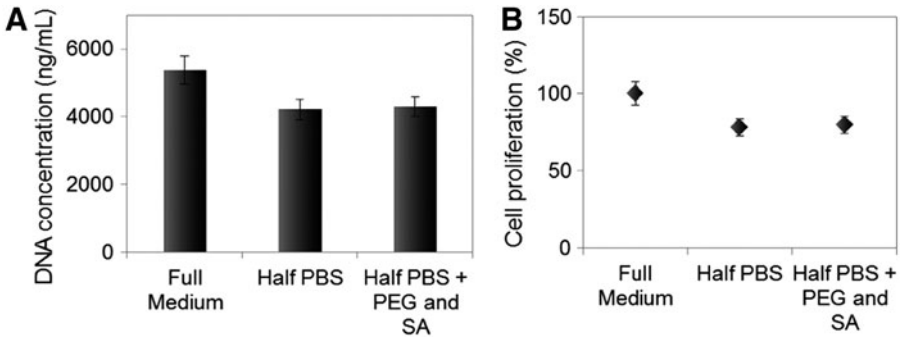
In conclusion, the best conditions for performing an HUVEC proliferation assay to assess VEGF bioactivity were as follows:

- to use VEGF concentrations between 4 and 20 ng/mL,
- in a medium containing 5% FCS and no ECGS,
- for a minimum of 3000 cells/well seeding density since the use of 1000 cells/well showed to be initially insufficient to provide a notable cell response (data not shown),
- for 3 days of incubation before DNA content analysis.

Bone morphogenetic protein-7

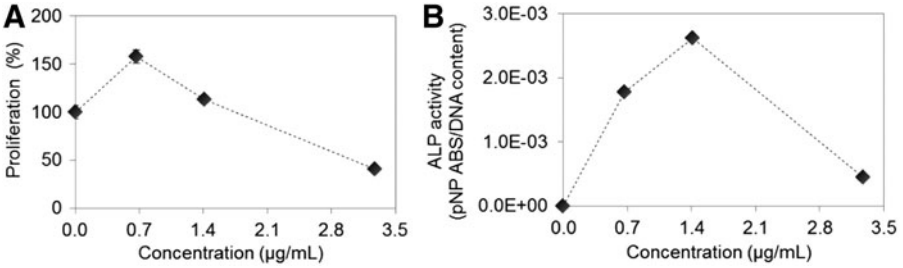
First, the effect of medium on C2C12 proliferation was assessed (Supplementary Fig. S5). A C2C12 seeding density of 5000 cells/well in a 48-well plate was initially used.

Supplementary Figure S5 shows that the presence of half volume of PBS in the culture medium decreased cell proliferation to 78%, compared with full medium (nonsignificant), and that the presence of poly(ethylene glycol) (PEG) and SA in PBS provided similar proliferation results (80%). This shows that there was no inhibition of cell proliferation compared with PBS alone and thus PEG and SA could be considered noncytotoxic to C2C12 cells, as expected. Next, the effective concentration range of BMP-7 was determined in terms of cell proliferation and alkaline phosphatase (ALP) activity. Results are presented in Supplementary Figure S6 and show that proliferation of C2C12 was similar or superior to controls up to 1.4 μg/mL of BMP-7. ALP activity was significantly upregulated for 0.7 μg/mL and increased further for 1.4 μg/mL. An excessively



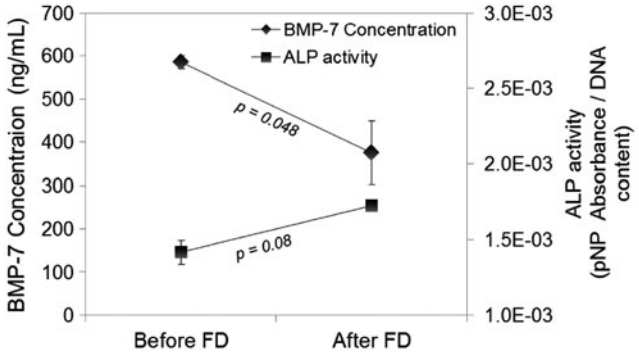
SUPPLEMENTARY FIG. S5. The effect of medium on C2C12 proliferation. **(A)** DNA concentration (ng/mL) and **(B)** cell proliferation% for different media. C2C12 cells were cultured for 5 days and treatments were subjected to cells twice on days 1 and 3. Mean ± SE, $n = 6$ ($p = 0.051$).

higher dose of BMP-7, 3.3 μg/mL, was shown to reduce both proliferation (40%) and ALP expression. This is due to the downregulation of cell receptors on the surface of cells with higher amounts of GFs in solution, which lead cells to senescence or death. In this study, the effective concentration range of BMP-7 to be used with C2C12 is thus found to be between 0.2 and 1.4 μg/mL.[31]



SUPPLEMENTARY FIG. S6. The effect of BMP-7 concentration on **(A)** proliferation and **(B)** ALP expression of C2C12 cells cultured for 5 days. Treatments were subjected to cells twice on days 1 and 3. Mean ± SE, $n = 6$.

The Effect of Freeze-Drying on BMP-7



SUPPLEMENTARY FIG. S7. The effect of BMP-7 freeze-drying on BMP-7 detection by ELISA ($n = 3$) and ALP expression of C2C12 cells ($n = 6$). Mean ± SE.

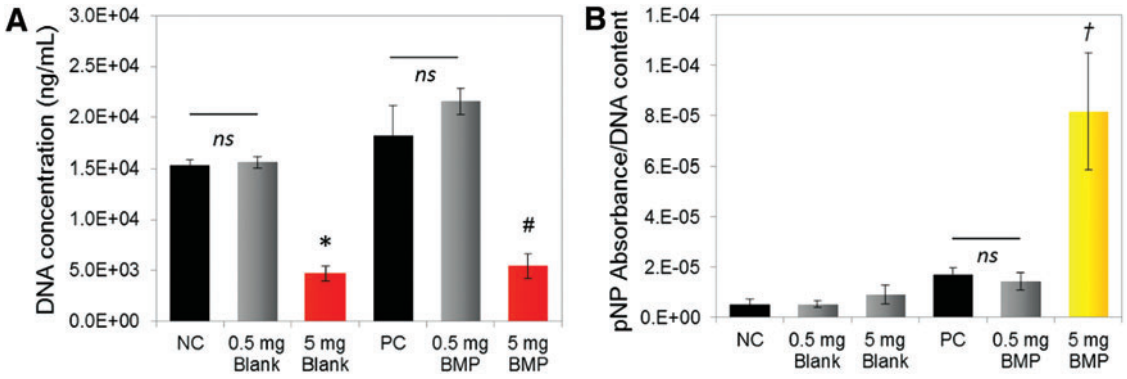
In Vitro Microparticle Direct Culture

Preliminary culture

The *in vitro* effect of microparticle amount on proliferation and differentiation of MC3T3-E1 cells was assessed for the BMP-7-loaded particles (BMP formulation) and unloaded particles (Blank formulation). Concentrations of 20,000 cells/well (500 μL/well) were seeded in 24-well plates and cells were let to adhere for 24 h before treatments, which are presented in Supplementary Table S1. Cells were grown for 7 days before analysis of DNA content and ALP expression. Half the volume of media was changed twice before analysis. Results are presented in Supplementary Figure S8.

SUPPLEMENTARY TABLE S1. EXPERIMENTAL CONDITIONS

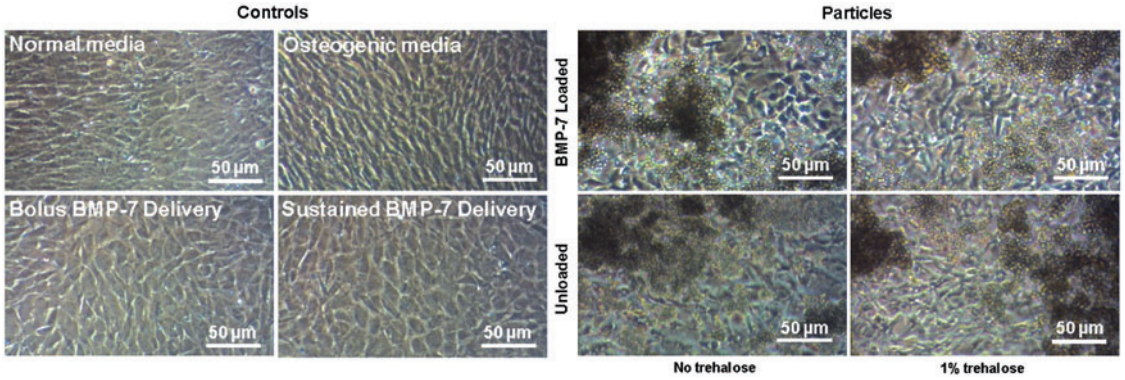
Negative control	NC	Normal media
Positive control	PC	Osteogenic media
Condition 1	0.5 mg (Blank)	0.5 mg/well unloaded particles
Condition 2	5 mg (Blank)	5 mg/well unloaded particles
Condition 3	0.5 mg (BMP)	0.5 mg/well BMP-7-loaded particles
Condition 4	5 mg (BMP)	5 mg/well BMP-7-loaded particles



SUPPLEMENTARY FIG. S8. **(A)** Proliferation and **(B)** differentiation results of MC3T3-E1 cells after direct contact with microparticles. Proliferation was assessed by PicoGreen® ($n=4$) and differentiation was assessed by ALP expression normalized to DNA content ($n=4$). (* and # indicate $p<0.05$ compared with NC and PC, respectively, and † indicates $p<0.05$ compared with PC). ns, non-significant (i.e., $p>0.05$).

Proliferation was enhanced when the osteogenic media were used ($p=0.004$). There were no statistical differences on proliferation between the loaded and unloaded particle groups ($p=0.142$). The 0.5 mg/well groups performed equally than their respective controls ($p>0.7$), but 5 mg/well significantly lowered proliferation ($p<0.001$). ALP expression, indicative of the effectiveness of BMP-7 released from microparticles, was significantly higher for the 5 mg/well group, but no differences were observed for 0.5 mg/well. In conclusion, the use of 5 mg/well was sufficient to trigger significant ALP expression from MC3T3-E1; however, cell proliferation was significantly impaired. In addition, with such a high amount of particles in wells, it was impossible to observe cell monolayers with optical microscopy due to heavy particle coverage. Because 0.5 mg/well was not sufficient to induce significant ALP expression, an intermediate value of 2.5 mg/well was recommended to ensure significant ALP expression, while minimizing proliferation damage and allowing cell imaging.

Final culture



SUPPLEMENTARY FIG. S9. Optical microscopy images of tissue culture wells after 14 days of MC3T3-E1 culture for controls (*left*) and treatments (*right*) with 2.5 mg particles per well.