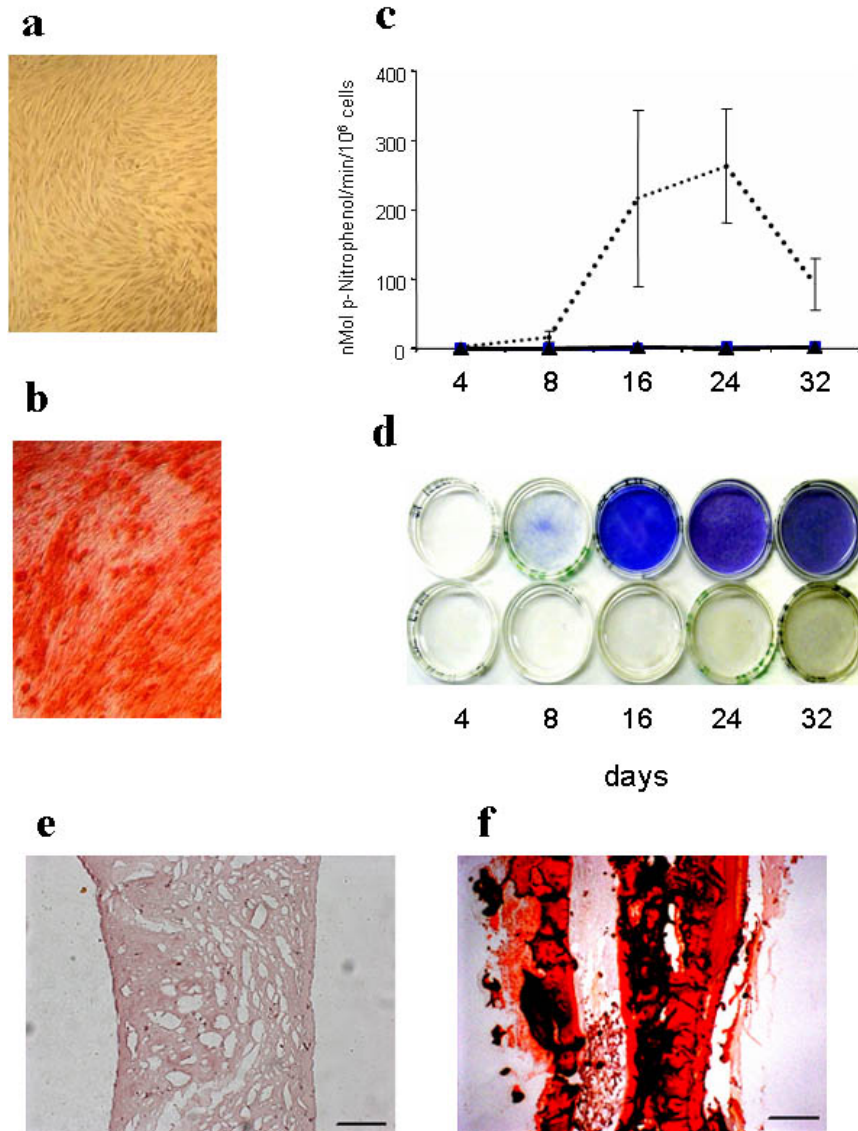




Supplementary Fig. S3. Osteogenic differentiation of human AFS cells

Staining for mineralized calcium with Alizarin Red S of AFS cells cultured on type I collagen for 25 days (a) in normal growth medium and (b) in osteogenic medium. (c) Secreted alkaline phosphatase activity of cells during osteogenic induction [dotted line] and in normal growth medium [solid line, triangles] was assessed in cell-conditioned medium by spectrophotometric measurement of release of p-nitrophenol after incubation with 2-amino-2-methyl-1-propanol and p-nitrophenyl phosphate. (d) Histochemical staining for alkaline phosphatase in differentiating [upper] and uninduced control [lower] cells. (e) Printed alginate/collagen scaffold containing AFS cells and cultured for 45 days in normal growth medium, stained with Alizarin Red S. (f) Equivalent printed construct cultured for 45 days in osteogenic medium, stained with Alizarin Red S.