Supplementary information

Experimental section

Platelet-rich plasma (PRP) preparation: The platelet concentrates were provided by the blood bank of the Kantonsspital Graubünden in Chur in accordance with the current ethical laws of Switzerland. PRP was prepared by the classic centrifugation procedure as soon as the plasma was received. Briefly, the plasma was subjected to two 10 min centrifugation steps, each at 2000 g. After centrifugation the platelets were accumulated at the bottom with the platelet-poor plasma (PPP) on top. The platelets were collected and re-dispersed in a PPP volume 20 times lower than the initial plasma volume. All procedures were performed under sterile conditions. The platelets were activated by freeze-thawing and aliquots were stored at -20ºC.

Preparation of PRP/alginate and PRP/alginate/silica hydrogel macro-beads: Sodium alginate, calcium chloride ($CaCl_2$), sodium metasilicate nonahydrate (Na_2SiO_3, $9H_2O$) and pentaethylenehexamine (PEHA) were purchased from Aldrich. 50 UI working bovine thrombin in 900 mM NaCl was obtained by 1:10 dilution of 500 UI bovine thrombin in 70 mM NaCl (kind gift from Baxter, Austria) with further addition of NaCl. Sodium Alginate 0.5% (w/v) in Iscoves Modified Dulbecco's medium (IMDM) containing or not Na_2SiO_3, $9H_2O$ and a PRP/Thrombin solution were mixed in equal volumes. The mixture was added dropwise with an auto-injector (Harvard Apparatus) injection rate 27.5 ml/min, 5 ml syringe and a 18 gauge needle) into a 102 mM $CaCl_2$ solution (pH 7) containing 50 mM PEHA. Gels were formed immediately and left maturating for 10 min in the $CaCl_2$ solution at room temperature.

The microstructure of the beads and the presence of silica were characterized by scanning electron microscopy and infrared spectroscopy. Lyophilized and calcined (700ºC) samples were fractured and coated with a 10 nm thick layer of gold-platinum (80:20) (Baltec MED 020, Balzers, Liechtenstein). Imaging was performed using a Hitachi (Tokyo, Japan) S-4100 field emission scanning electron microscope operated at 5.0 keV. Potassium bromide discs containing the sample were prepared by grinding 5 mg of lyophilised sample with 100 mg of potassium bromide and compacted in a press at 8 tons of pressure. Mid-IR spectra were recorded on a Perkin-Elmer 2000 FTIR instrument (Beaconsfield, Buckinghamshire, England) with a minimum of 12 scans.

Measurement of silica concentration in the macro-beads: A molybdic acid solution (20 g/l ammonium molybdate tetrahydrate and 60 ml/l concentrated hydrochloric acid in deionized

water) and a reducing solution (20 g/l oxalic acid, 6.67 g/l 4-methylaminophenol sulphate, 4 g/l anhydrous sodium sulphite and 100 ml of concentrated sulphuric acid completed with deionized water, all products from Aldrich) were prepared. In a typical experiment, 20 mg samples were digested in 2 ml of boiling 2 M sodium hydroxide for 1 h. The digested sample (200 µl) was reacted with 2 ml of molybdic acid solution for 15 min followed by the addition of reducing solution (8 ml). The blue colour was left to develop for 2 h at room temperature before measuring the optical density at a wavelength of 810 nm. Calibration was performed using a prepared 100 ppm SiO_2 standard solution. The silica release kinetics was performed in triplicate. Average values and standard deviations are reported.

Measurement of the total released proteins: 4 beads of the materials (PRP/Alginate and PRP/Alginate/silica 100 mM) were added to 3 ml of 0.1 M HEPES *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid] solution. Aliquots of solution (100 µl) were withdrawn every 2 h during the first 12 h and every 24 h for the next 4 days. A fixed volume (5 ml) of Coomassie reagent solution (Quick Start Bradford Protein Assay Kit from Bio-Rad) was added to the sample and absorbance at a wavelength of 595 nm was measured at room temperature after an incubation time of 15 min. Blanks consisted of 100 µl of 0.1 M HEPES solution and 5 ml of Coomassie reagent solution. A bovine serum albumin (BSA) solution (2 mg/ml) was used for the calibration. The protein release kinetics was performed in triplicate. Average values and standard deviations are reported.

Measurement of growth factor-β1 (TGF-β1) release: 20 beads of the materials containing 0.450 ml of PRP were placed into 10 ml of PBS solution at 37ºC. Aliquots of solution (100 µl) were withdrawn at 0.5, 8, 24 and 48 h. Aliquots of the solution containing alginate beads were withdrawn at the same time points and used as controls to ensure that no interference occurred with the biochemical assay. The TGF-β1 concentration in PRP was also determined. TGF-β1 released in solution (or present in PRP) was quantified using a DuoSet ELISA development kit (#DY240, R&D Systems, Minneapolis, MN). The assay was performed following the manufacturer protocol. A dilution series of human TGF-β1 standards (#840118, R&D Systems, Minneapolis, MN) was prepared. Sample activation was performed by mixing 100 µl solution (or PRP) with 100 µl 2.5 N acetic acid/10 M urea, followed by a 10 min incubation at room temperature and neutralization by addition of 100 µl of 2.7 N NaOH/1 M HEPES. Samples were diluted with reagent diluent (1.4% delipidized bovine serum albumin, 0.05% Tween 20 in Phosphate Buffered Saline (PBS) pH 7.4) prior to measure (1:40 for PRP

and 1:4 for the samples). Absorbance was recorded at 450 nm and corrected for optical imperfections by subtractions of the absorbance at 560 nm. The quantity of TGF-β1 in the soaking solution was calculated from the amount of TGF-β1 measured by the ELISA assay in 100 µl sample volume, corrected by the appropriate dilution factor and the exact volume of PBS soaking solution. The average TGF-β1 concentration measured in the PRP used for this study was 220 ± 57 ng/ml; therefore the TGF-β1 concentration in the experimental volume was 99 ± 25 ng. The difference in the TGF-β1 released from the silica containing beads and PRP/alginate beads at the various time points was calculated and plotted as a function of time (Figure 2B). Average TGF-β1 and standard deviation values reported correspond to a minimum of 3 measurements.

Encapsulation of human mesenchymal stromal cells (hMSCs): Bone marrow aspirates were obtained from two patients undergoing routine orthopaedic surgery, after informed consent in accordance with the Swiss national guidelines. hMSCs were isolated according to the procedure reported by Meury *et al*. hMSCs were suspended in alginate, alginate/silica, PRP/alginate with thrombin, PRP/alginate/silica with thrombin, at a density of $3x10^6$ cells/ml. Three different concentrations of sodium metasilicate in beads were tested: 5, 25 and 50 mM and the PRP/alginate volume ratio was set as 0.9. The pH and the ionic strength of the solutions were adjusted respectively to 7.4 and 330 mOsm or as close as possible in the case of the 50 mM silicate concentration. A 100 mM silicate solution was not used for the cell study since the high osmolarity of this solution would negatively affect the cell survival. Beads were formed by dropping cell suspensions with a 18G needle into a 102 mM $CaCl_2$ solution containing 50 mM PEHA. After 10 min, the beads were washed in 0.9 M NaCl first and then in complete culture medium (IMDM supplemented with 10% FCS-1% NEAA, all products from Gibco). The beads were transferred to a 48 well-plate (2 beads and 800 µl of media per well) and cultured for 3 days in an incubator set at 5% CO_2, 90% humidity and 37ºC. Amount of live hMSCs in beads was assessed by the trypan blue exclusion assay at 1 and 3 days of culture after bead dissolution according to the procedure reported by Perka *et al.* The amount of viable cells was normalized to the amount of seeded cells. Data were tested for normality using the Kolmogorov-Smirnov test. The non-parametric Mann-Whitney U test or a one-way ANOVA and a post hoc Bonferroni test were performed to determine differences between days of culture and silicate concentrations using SPSS 18 software (SPSS Inc., Chicago, Illinois, USA).

Results

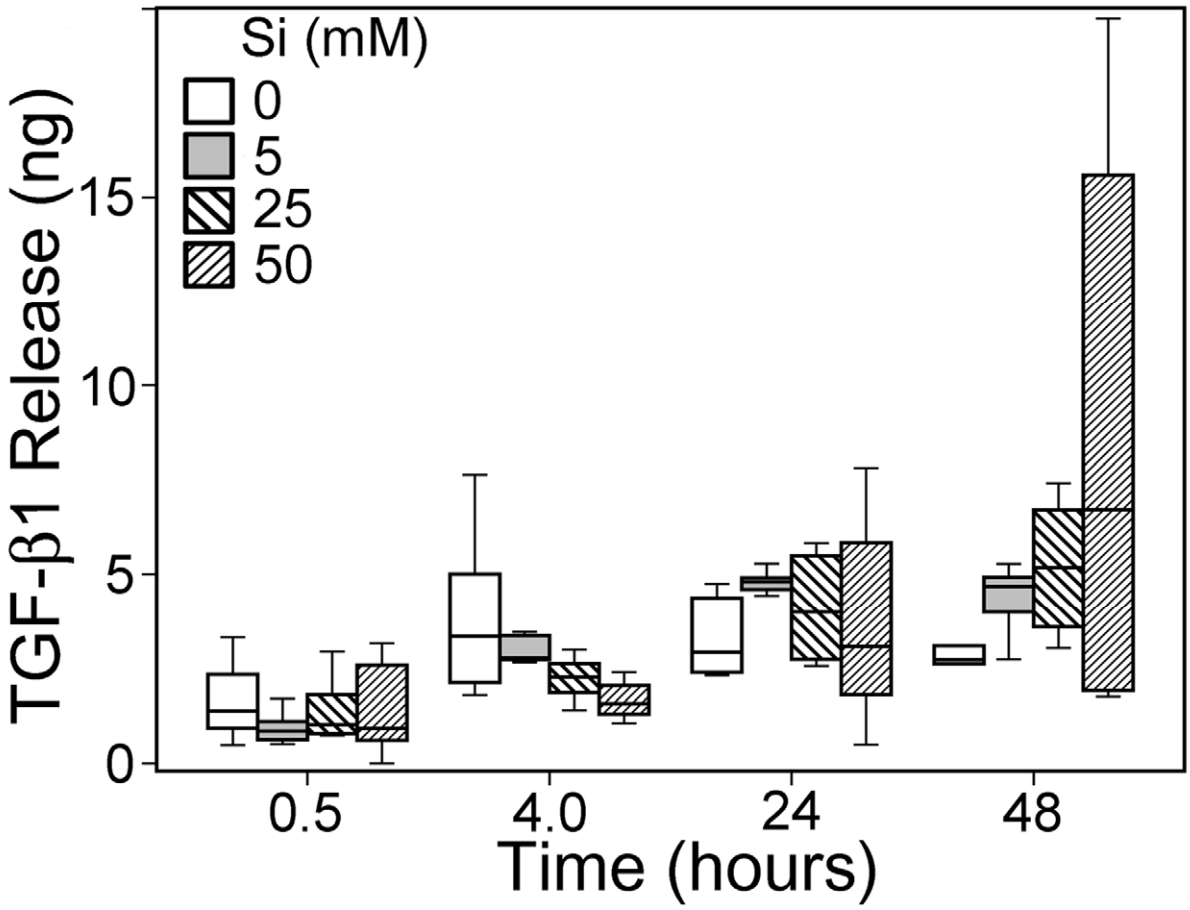

Fig. A Boxplot of the TGF-β1 release from PRP-alginate-silica beads 5, 25 and 50 mM silicate concentrations relative to the TGF-β1 release from PRP-alginate beads as a function of time in PBS solution at 37 ºC.