# Supporting Information

**Tough Composite Hydrogels with High Loading and Local Release of Biological Drugs**

*Jianyu Li, Eckhard Weber, Sabine Guth-Gundel, Michael Schuleit, Andreas Kuttler, Christine Halleux, Nathalie Accart, Arno Doelemeyer, Anne Basler, Bruno Tigani, Kuno Wuersch, Mara Fornaro, Michaela Kneissel, Alexander Stafford, Benjamin R. Freedman, David J. Mooney**

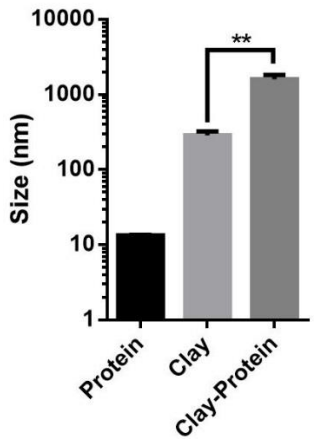


**Figure S1**. Sizes of the protein drug, initial clay and drug-laden clay particles measured with dynamic light scattering. Data represents the mean ±SD; N=3 per group. P values were determined by a student t test; **P≤0.01.

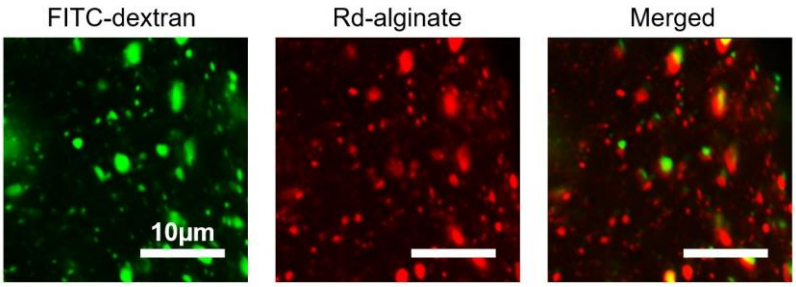


**Figure S2**. Confocal images of the composite hydrogels containing Rhodamine (Rd)-labeled alginate and Fluorescein isothiocyanate (FITC)-labeled dextran bound to the clay microparticles. The scale bar is 10 μm.

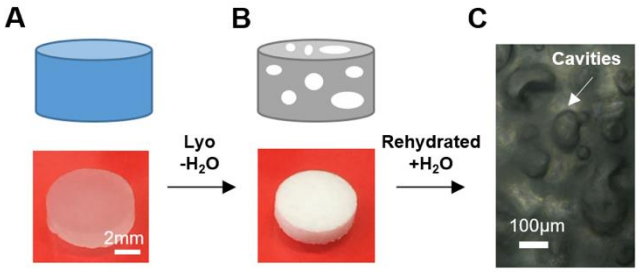


**Figure S3.** Lyophilization and rehydration of composite hydrogels. An alginate hydrogel loaded with clay nanoparticles and drugs (A) is frozen and lyophilized to remove the water for storage (B); the dry scaffold is rehydrated with water before implementation. C) Bright field image of the composite hydrogels after rehydration in which the micro-sized cavities are visible.

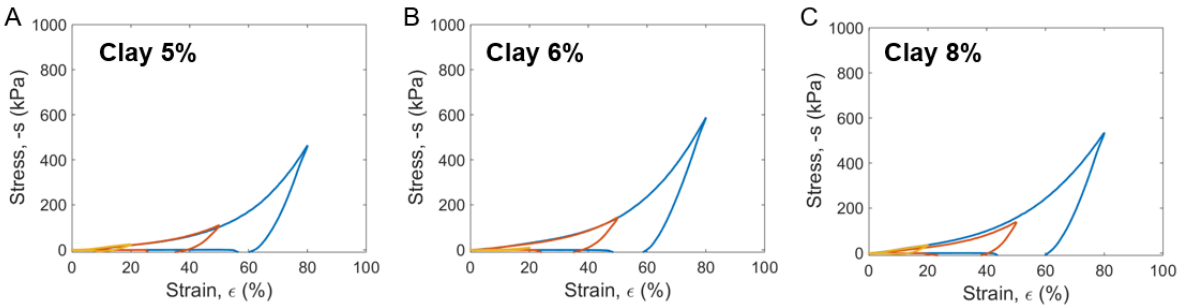


**Figure S4.** Loading-unloading tests of the composite hydrogels. The hydrogels of varying clay contents (A 5%; B 6%; C 8%) are subject to three levels of maximum compressive strains (10%, 50% and 80%).

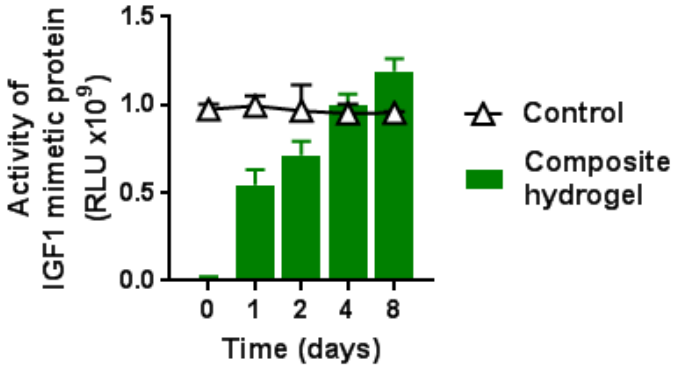


**Figure S5.** Bioactivity assessment of the IGF1 mimetic protein. Cumulative bioactivity of the IGF1 mimetic protein following continuous 8-day in vitro release from the composite hydrogels and bioactivity of buffer-reconstituted IGF1 mimetic protein during the same time course (Control). The bioactivity of the protein was assessed based on phosphorylation of the type I Insulin-like growth factor receptor (IGF1R) on NIH-3T3 fibroblast cells, detected by ELISA. Data represents the mean ±SD; N=2 per group.

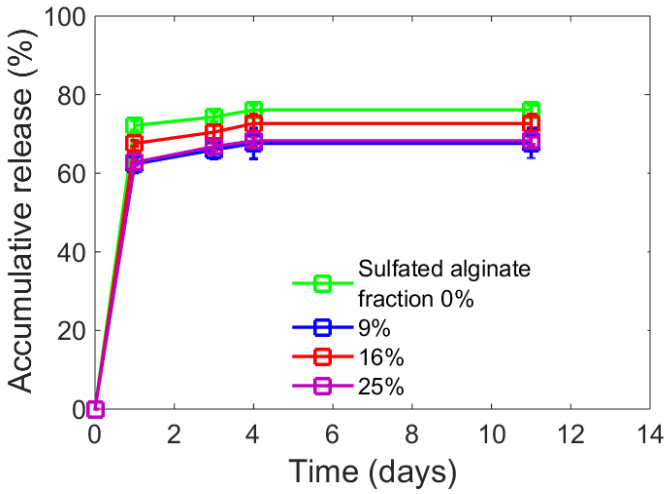


**Figure S6.** In vitro release profiles of the IGF1 mimetic protein from sulfated alginate hydrogels. The hydrogels were formed with a mixture of unmodified alginate and sulfated alginate as the content of the sulfated alginate varied from 0% to 25%. Data represents the mean ±SD; N=3 per group.

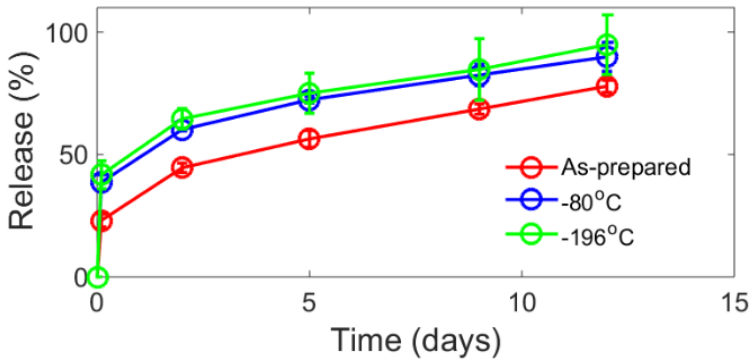


**Figure S7.** In vitro release profiles of the IGF1 mimetic protein. The composite hydrogels have been treated with and without lyophilization (As-prepared). -80°C and -196°C are the temperature at which the hydrogels were frozen before lyophilization. Data represents the mean ±SD; N=3 per group.

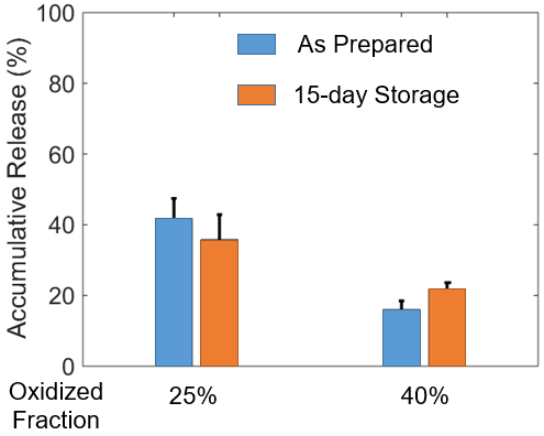


**Figure S8.** Burst drug release as a function of the storage and the fraction of oxidized alginate. The dry scaffolds after lyophilization were either rehydrated immediately (as prepared) or stored at +4$^{o}$C for 15 days before the in vitro release studies. The initial burst release within 24 hours was recorded. Data represents the mean ±SD; N=3 per group.

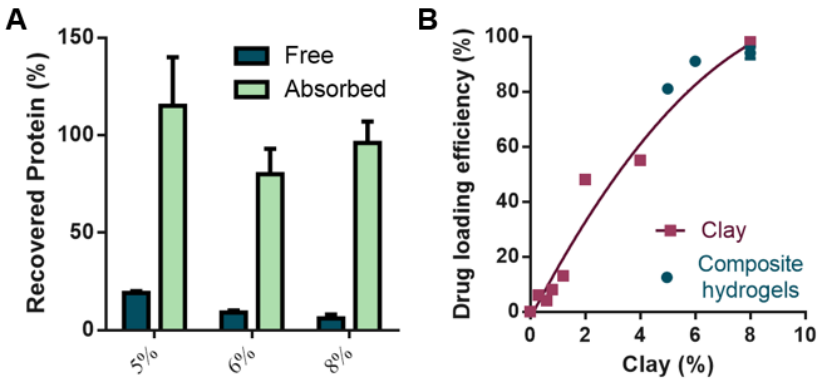


**Figure S9.** Forced release studies of the model drug as a function of the clay content in the composite hydrogels. After synthesis, the composite hydrogels were digested right away with a combination of EDTA and alginate lyase, and then treated with polyallylamine (PAA) to force the protein to dissociate from the clay nanoparticles. A) The percentages of the protein drug detected after digestion (denoted as Free, $P_F$) and PAA treatment (denoted as Absorbed) were plotted as a function of the clay content. Data represents the mean ±SD; N=3 per group. The recovered protein drug is represented as the % of protein initially encapsulated in the hydrogels. B) The drug loading efficiency of the composited hydrogels, consisting of clay nanoparticles and alginate hydrogels, was compared with that of the clay nanoparticles. The drug loading efficiency is calculated by 100%-$P_F$.

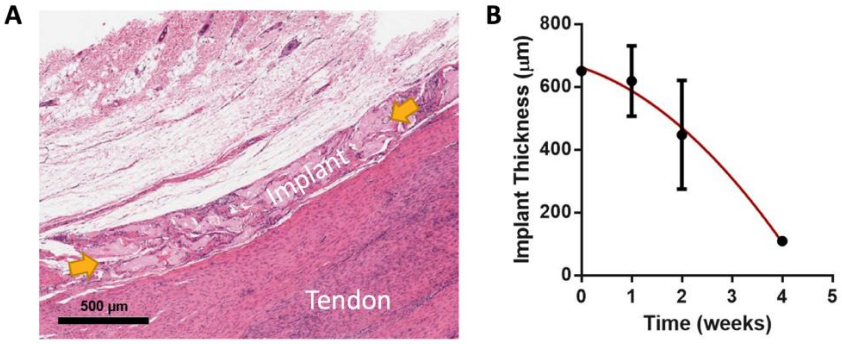


**Figure S10**. A) Histological section of the composite hydrogel and surrounding tissues after 4-week implantation. B) Measured thickness of the implant decreases with time. The data was interpolated with a curve fit to a second-order polynomial regression (red line). Data represents the mean ±SD; N=4 per time point.

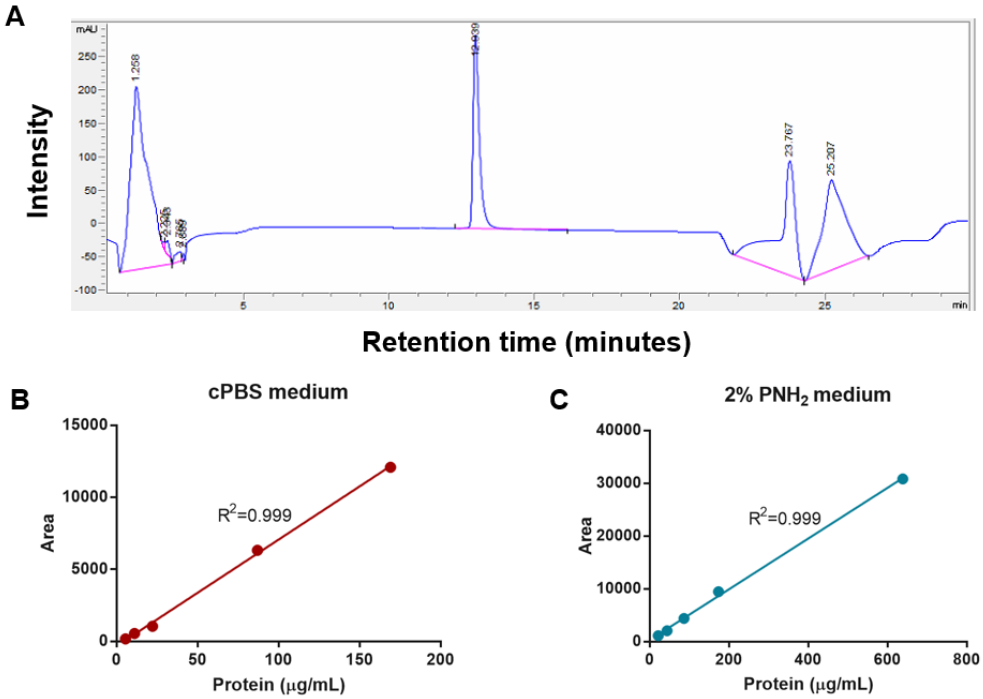


**Figure S11.** HPLC analysis of the model drug. A) A representative intensity-retention curve where the peak at 12 minutes refers to the model protein. The standard curves of the protein dissolved in (B) cPBS and (C) 2% polyallylamine solution.

**Table S1.** Gradients of the mobile phases in the HPLC analysis.

| Time [min] | MPA [a] [%] | MPB [b] [%] | Flow rate [mL/min] |
|---|---|---|---|
| 2 | 85 | 15 | 1 |
| 10 | 40 | 60 | 1 |
| 19 | 20 | 80 | 1 |
| 19.1 | 0 | 100 | 1 |
| 21 | 0 | 100 | 1 |
| 21.1 | 95 | 5 | 1 |
| 22 | 95 | 5 | 1 |
| 24 | 0 | 100 | 1 |
| 26 | 0 | 100 | 1 |

[a] MPA refers to 90% water, 10% acetonitrile (ACN) plus 0.1% (v/v) trifluoroacetic acid (TFA); [b] MPB refers to 20% water, 80% ACN plus 0.1% TFA.