Iron nanoparticle composite hydrogels for studying effects of iron ion release on red blood cell in vitro production

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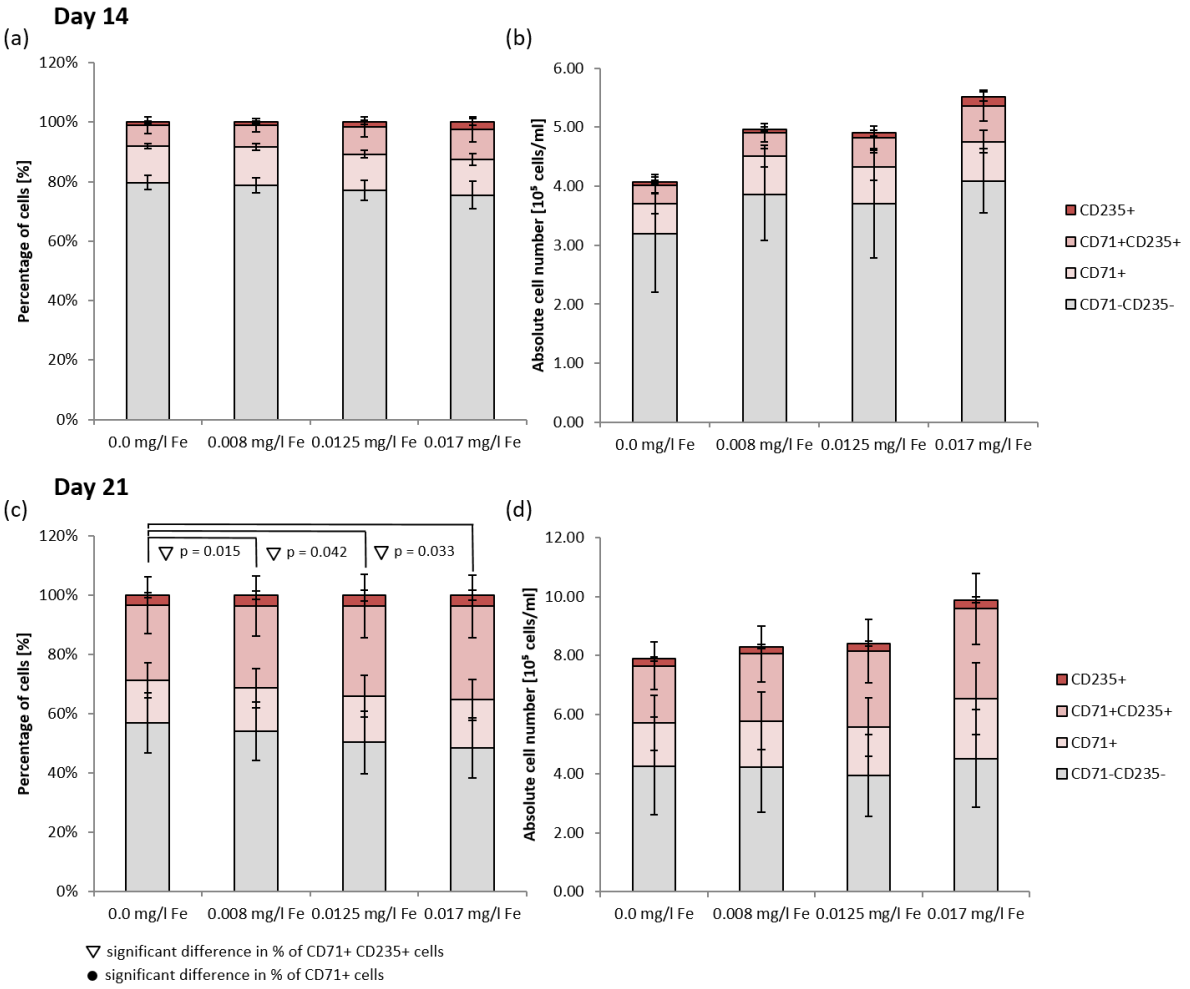

Figure S1. Percentages and absolute cell numbers of erythroid progenitor cells in 0.008 mg/L, 0.0125 mg/L and 0.017 mg/L iron spiked cell culture medium after 14 (a,b) and 21 (c,d) days of erythroid differentiation. While no significant difference in the abundance of cell populations was detectable after 14 days of culture, a significant ($p \leq 0.05$) increase of the $CD71^{+}/CD235a^{+}$ population in iron spiked media compared to the cell population in culture media without iron supplementation was observed after 21 days of cultivation.

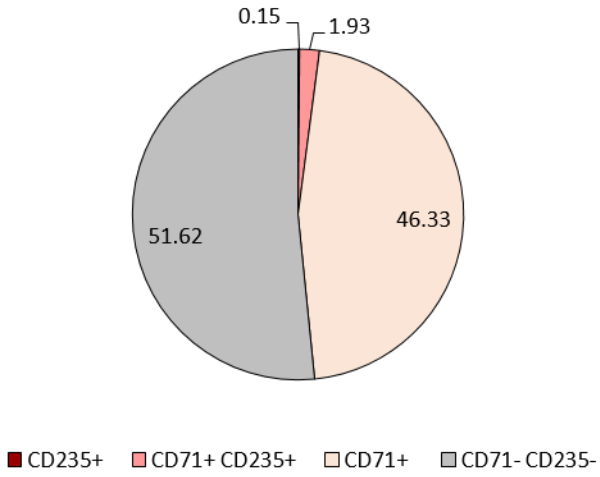

Figure S2. Analysis of CD71 and CD235 expression after 7 days of pre-expansion. The percentages of single and double positive as well as double negative cell populations are given as a pie chart. The colour code for the different populations is indicated at the bottom of the figure, percentages are indicated within or beside the respective pie chart sections.

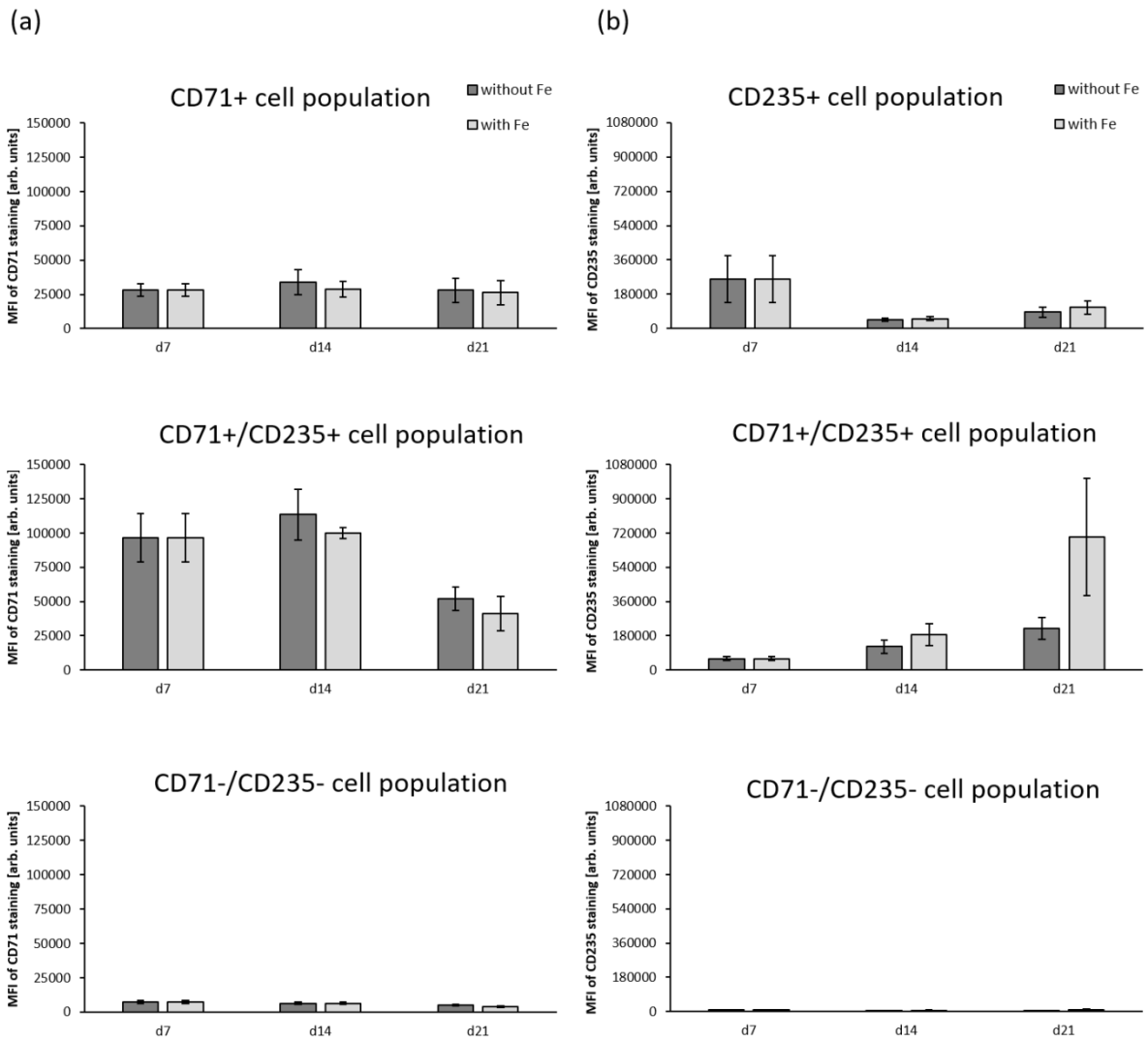

Figure S3. Median fluorescence intensity (MFI) of CD71/CD235 stained cell population over a time course of 21 days of erythroid differentiation. The median fluorescence intensity (MFI) of the CD71 staining (a) or the CD235 staining (b) are shown on the y-axis, the day of analysis on the x-axis. Results obtained from staining of cells differentiated on iron-free hydrogels are shown in dark grey, results from cells cultured on iron-releasing hydrogels in light grey. The population, for which the MFI is shown, is indicated on the top of each graph.

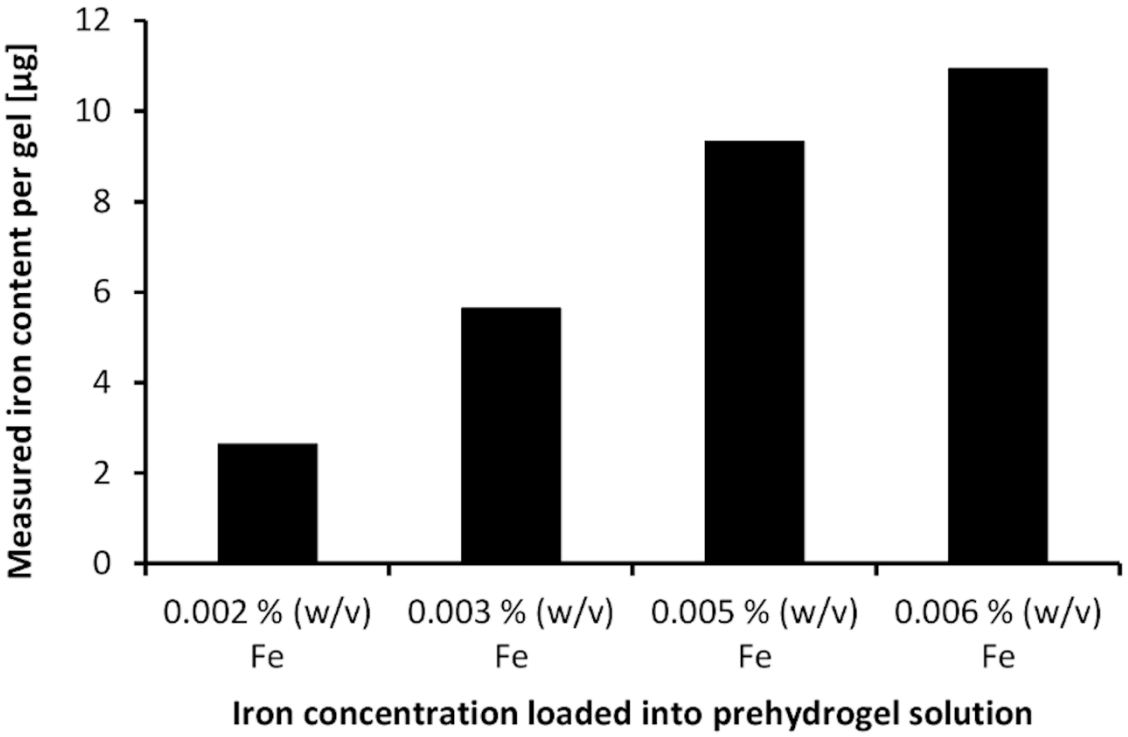

Figure S4. The iron content (mass of iron per gel) of 0.002% (w/v), 0.003% (w/v), 0.005% (w/v) and 0.006% (w/v) 3D hydrogels. With increasing concentrations of iron nanoparticles included into the composite 3D scaffolds during production, the mass of iron measured by ICP-OES increased accordingly from 2.628 µg/gel, 5.637 µg/gel and 9.3245 µg/gel to a maximum of 10.93 µg per gel.

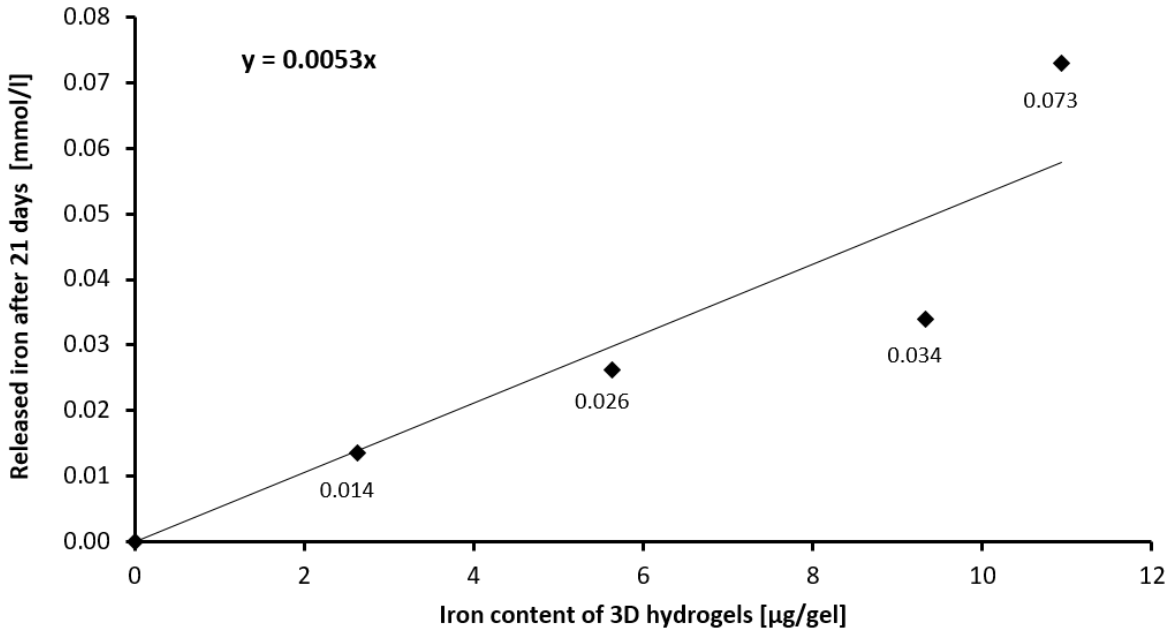

Figure S5. Linear correlation of measured iron content in the 3D composite scaffolds and the total amount of iron released into water after 21 days. With increasing iron concentrations in the 3D hydrogels the amount of released iron into the supernatant is elevated. The insertion of the measured iron content of a flat hydrogel pads (4.17 µg/gel) into the linear equation of the plotted graph reveals an iron release of 0.022 mmol/l after 21 days for a 3D hydrogel with the same iron content.

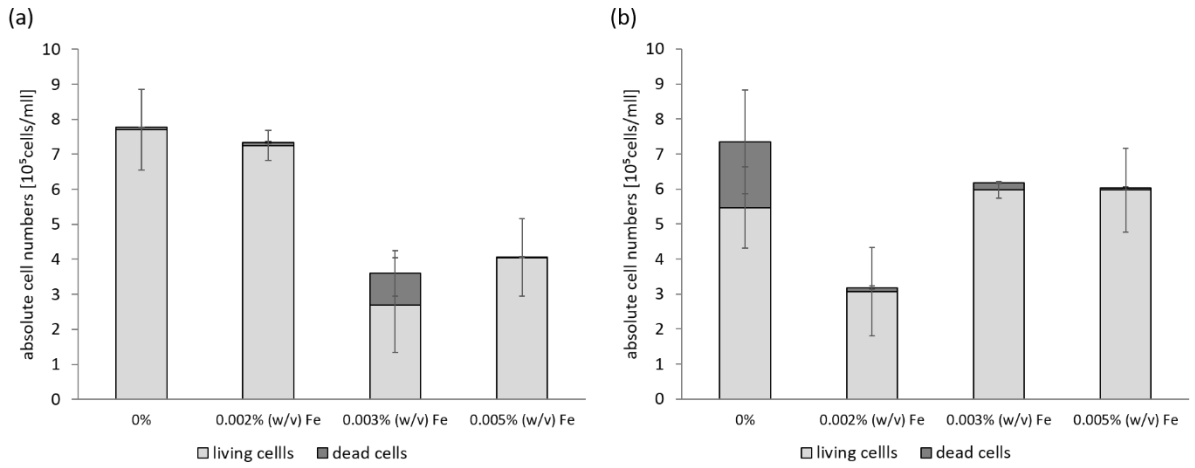

Figure S6. Cell viability of erythroid progenitors after 14 days (a) and 21 days (b) of culture including differentiation in 0.002 % (w/v), 0.003% (w/v) and 0.005% (w/v) iron containing 3D composite scaffolds. The error bars represent the SE. N=3 independent experiments for all iron nanoparticles scaffolds.