Electronic Supplementary Material (ESI) for Toxicology Research
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Supplementary Data

Cytotoxicity and potency of mesocellular foam-26 in comparison to layered clays used as haemostatic agents

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Supplementary Materials and Methods

LDH assay

Cells were seeded at a density of 50,000 cells/cm^2 in 96-well microplates and cultivated for 24h. Cells were then treated overnight with the test compound before LDH leakage was determined. The CytoTox-ONETM Homogeneous Membrane Integrity Assay kit was purchased from Promega (Madison, WI, USA) and the assay was performed according to the manufacturer's instructions. Fluorescence (560 nm excitation and 590 nm emission wavelengths) was measured using a microplate reader (Tecan Safire2TM, Männedorf, Switzerland).

MTS assay

The CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) reagent was purchased from Promega (Madison, WI, USA) and the assay was performed according to the manufacturer's instructions. Cells were seeded at different densities and were cultivated for 24 hours before the assays were performed in order to generate the data shown in figure S3. Otherwise, cells were seeded and treated similarly as for the NRU and LDH assays. Absorbance was measured at 490 nm using a microplate reader (Tecan Safire^{2TM}, Männedorf, Switzerland).

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Supplementary figures

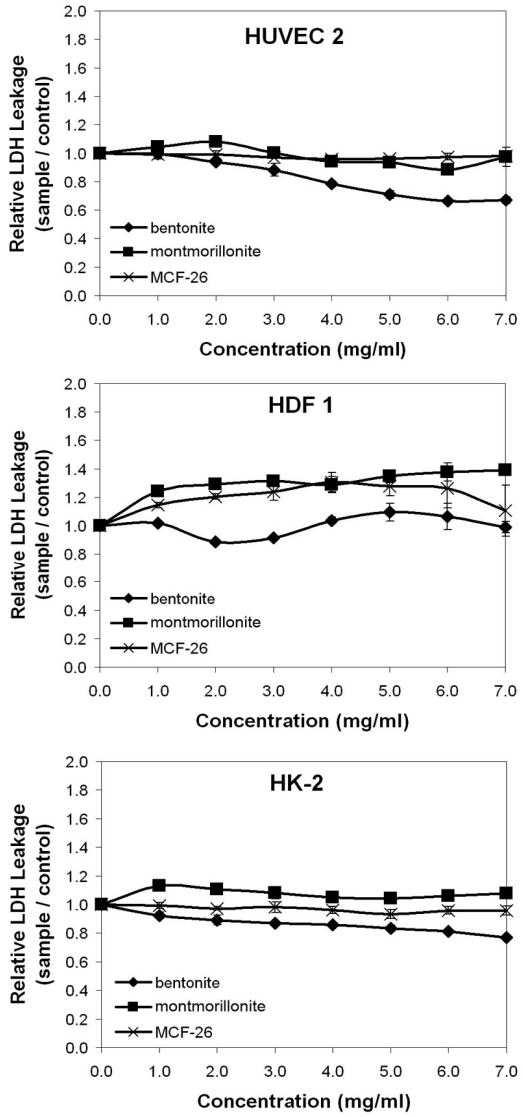

Figure S1: Dose-dependent effects of bentonite, montmorillonite and MCF-26 on LDH leakage. The compounds were applied at concentrations of up to 7 mg/ml to the cell types indicated. All values were normalized to the values obtained with untreated control cells. Error bars show the s.d. (n = 3).

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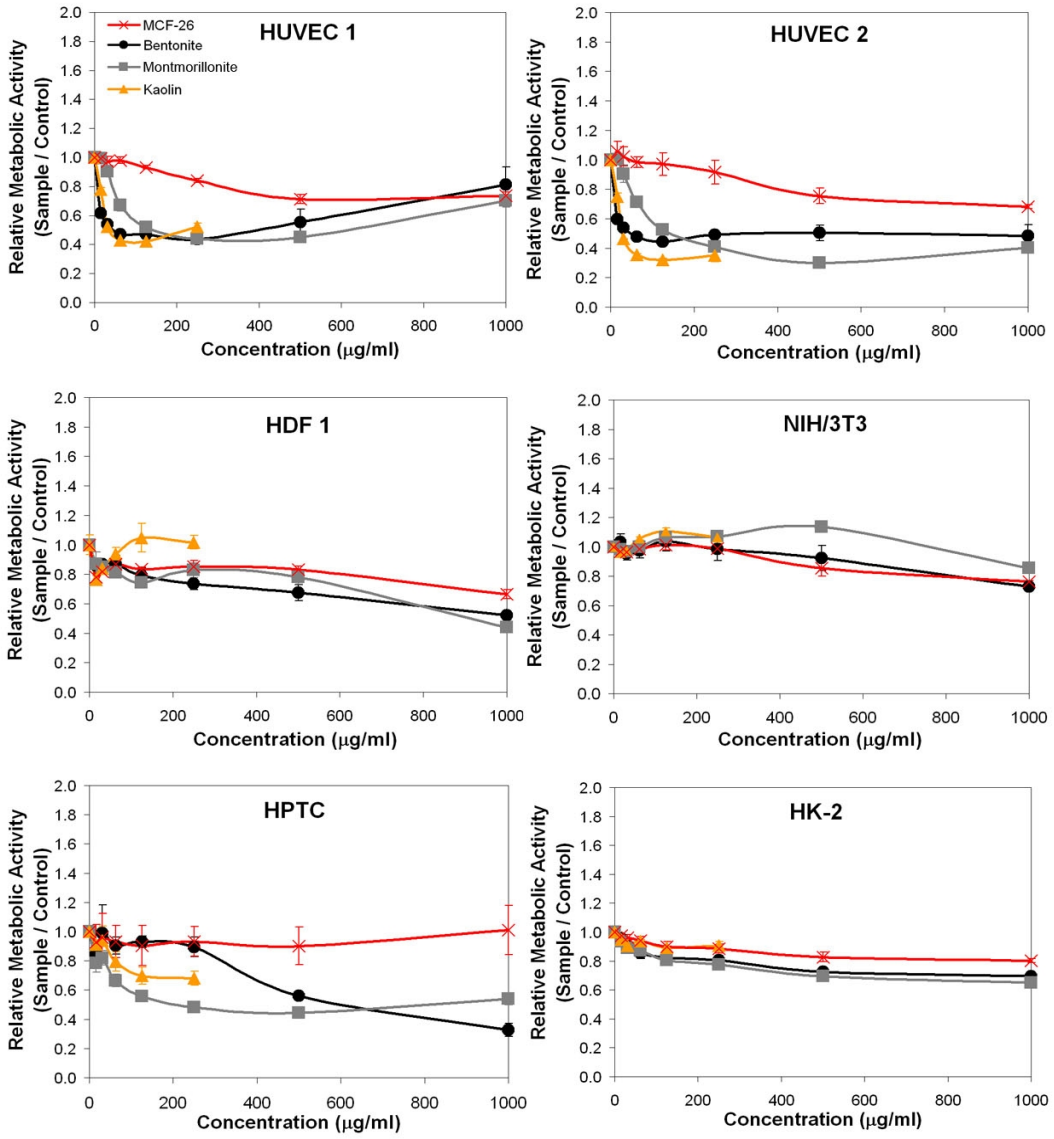

Figure S2: Dose-response curves determined with the MTS assay. The cell types indicated were treated with up to 250 µg/ml of kaolin (orange graphs) or with up to 1000 µg/ml of MCF-26 (red graphs), bentonite (black graphs) or montmorillonite (grey graphs). Relative metabolic activity was determined by using the MTS assay and all values were normalized to the values obtained with untreated control cells. Error bars show the s.d. (n = 3).

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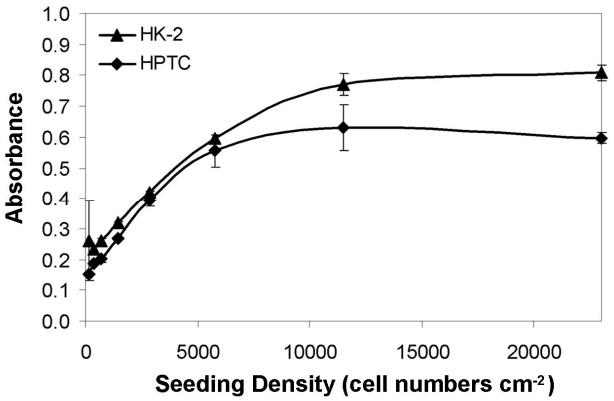

Figure S3: Relationship between absorbance measured with the MTS assay and cell numbers. HPTC and HK-2 cells were seeded at different densities and the MTS assay was performed after 24 hours. The absorbance readings obtained with the MTS assay (mean +/- s.d., n = 3) were plotted against the seeding densities.

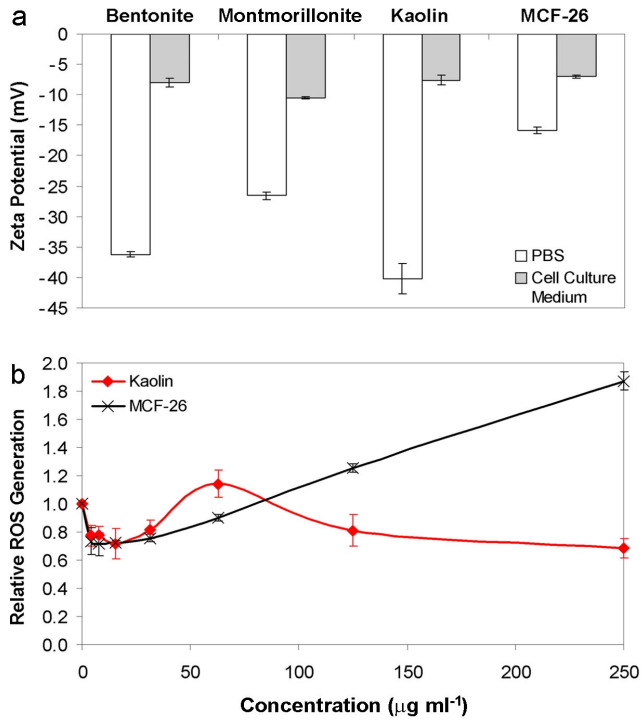

Figure S4: Zeta potential measurements and ROS generation. a) The zeta potentials of bentonite, montmorillonite, kaolin and MCF-26 were measured after suspension in PBS

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(white bars) or cell culture medium (grey bars). The bars show the mean +/- s.d. (n = 3). In cell culture medium the zeta potential of montmorillonite was significantly more negative than the zeta potentials of the other materials ($P < 0.05$). The zeta potentials of bentonite, kaolin and MCF-26 were not significantly different. After suspension in PBS the zeta potentials of bentonite, montmorillonite and kaolin were significantly more negative than the zeta potential of MCF-26 ($P < 0.05$). The zeta potentials of bentonite and kaolin were significantly more negative than the zeta potential of montmorillonite ($P < 0.05$). b) Kaolin (red graph) or MCF-26 (black graph) were applied at concentrations of up to 250 µg/ml to HUVEC (batch 2) and ROS generation was determined. All values were normalized to the data obtained with untreated controls and the error bars indicate the s.d. (n = 3).

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Table S1

IC50 values and cell viability (%) at the maximal concentrations of Ag NP and DMSO. The maximal concentration of Ag NP was 417 µg/ml and the maximal concentration of DMSO was 100 mg/ml. The table provides the mean values ± s.d. (n = 3).

Cell Type	Ag NP (10 nm)		DMSO	
	IC50 (µg/ml)	% at 417 µg/ml	IC50 (mg/ml)	% at 100 mg/ml
HUVEC 1	140 ± 49	32 ± 3	85 ± 3	41 ± 2
HUVEC 2	66 ± 19	29 ± 3	39 ± 4	32 ± 6
HUVEC 3	77 ± 5	38 ± 6	46 ± 2	31 ± 1
HDF 1	> 417	81 ± 8	> 100	63 ± 4
HDF 2	137 ± 12	22 ± 2	> 100	79 ± 6
HDF 3	347 ± 6	22 ± 3	> 100	88 ± 4
HEK 1	> 417	72 ± 13	> 100	108 ± 3
HEK 2	> 417	79 ± 19	> 100	103 ± 1
HEK 3	> 417	128 ± 14	> 100	117 ± 7
NIH/3T3	> 417	62 ± 10	> 100	52 ± 7
HK-2	84 ± 3	29 ± 6	25 ± 4	27 ± 4
HPTC	343 ± 19	39 ± 1	> 100	74 ± 5