Supplementary Materials

Toxicity and efficacy of green tea catechin derivative-based micellar nanocomplexes for anticancer protein delivery

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Supplementary Figure Legends

Figure S1. Schematic drawing of the microfluidic set-up. The reservoir (1) contained 20 ml of cell culture medium (complete endothelial cell medium was used for HUVEC/BT-474 co-cultures and HUVEC monocultures), which was pumped at a rate of 4 ml/minute into gas-permeable silicone tubings (2) using a peristaltic pump (3). The flow direction is indicated by arrows. The tubings were connected to a µ-slide by polypropylene luer connectors (4) inserted at the inlets and outlets. A closed circuit was used and a sterile filter (5) was inserted into the reservoir bottle for gas exchange. HPTCs were seeded in an in-house-developed microfluidic chip and were perfused with renal cell medium.

Figure S2. Schematic drawings of (a) the structure and synthesis of OEGCG and PEG-EGCG, and (b) the structure and two-step assembly of the Herceptin-MNC. Herceptin was complexed with OEGCG to produce the MNC core. Complexing with the PEG-EGCG shell gave rise to the MNC. Details of the synthesis of the different compounds and of MNC formation and characterization are described previously.[1] Herceptin was replaced by BSA in the generation of BSA-MNCs.

Figure S3. Size distribution of (a) Herceptin-MNC and (b) BSA-MNC. (c) Integrity of the MNC in dilution. The results are reported as mean values and the bars represent s.d. (n = 3).

Figure S4. Images of HUVECs taken after treatment with Herceptin-MNCs or their individual compounds. Static cultures of HUVECs were treated with Herceptin-MNCs or individual compounds as indicated. Free or MNC-complexed Herceptin was used at 1000 µg/ml, and the other compounds were used at equivalent concentrations. Untreated cells and the vehicle (0.5% DMSO) and positive (100 µg/ml puromycin) controls are shown. HUVECs

were stained with DAPI (blue) and WCS stain (green). Some of the cytoplasmic vacuoles observed after treatment with OEGCG (100 μM / 48 μg/ml) are indicated by arrows. Scale bar: 50 μm.

Figure S5. γH2AX detection by immunostaining. Static cultures of the indicated cell types were treated with 1000 μg/ml gold chloride (positive control) or Herceptin-MNCs (1000 μg/ml Herceptin, other compounds at equivalent concentrations). γH2AX was detected by immunostaining (red), and cell nuclei were counterstained with DAPI (blue). γH2AX-specific fluorescence was only detectable in the positive control. Scale bar: 50 μm.

Figure S6. Nuclear γH2AX-specific fluorescence determined by HCS. HPTC, BT-474 and MCF7 cells were treated with Herceptin-MNC or BSA-MNC or their individual compounds, as indicated at the top of each diagram. The same compound concentrations as shown in Fig. 1 were used (except OEGCG, which was used here only at MNC-equivalent concentrations). γH2AX was detected by immunostaining, and cell nuclei were counterstained with DAPI before samples were imaged by HCS. Image analysis was performed with the MetaXpress® image analysis software for determination of nuclear intensities of γH2AX-specific fluorescence. All data were normalized to vehicle controls (n = 4, mean ± s.d.).

Figure S7. Untreated BT-474/HUVEC microfluidic co-cultures. Untreated co-cultures (no compound, no vehicle) were kept under microfluidic conditions for 7 days. Arrowheads indicate some of the regions where the HUVEC endothelium was disrupted. Platelet endothelial cell adhesion molecule (CD31; green) and human epidermal growth factor receptor 2 (HER2/neu; red) were detected by immunostaining. Cell nuclei were stained with DAPI (blue). Scale bar: 100 μm

References

1. J. E. Chung, S. Tan, S. J. Gao, N. Yongvongsoontorn, S. H. Kim, J. H. Lee, H. S. Choi, H. Yano, L. Zhuo, M. Kurisawa and J. Y. Ying, *Nat. Nanotechnol.*, 2014, **9**, 907-912.