

SUPPLEMENTARY SECTION
to

**Exposure to silicone oil endotamponades induces VEGF antibody aggregation
and loss of functionality.**

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Materials

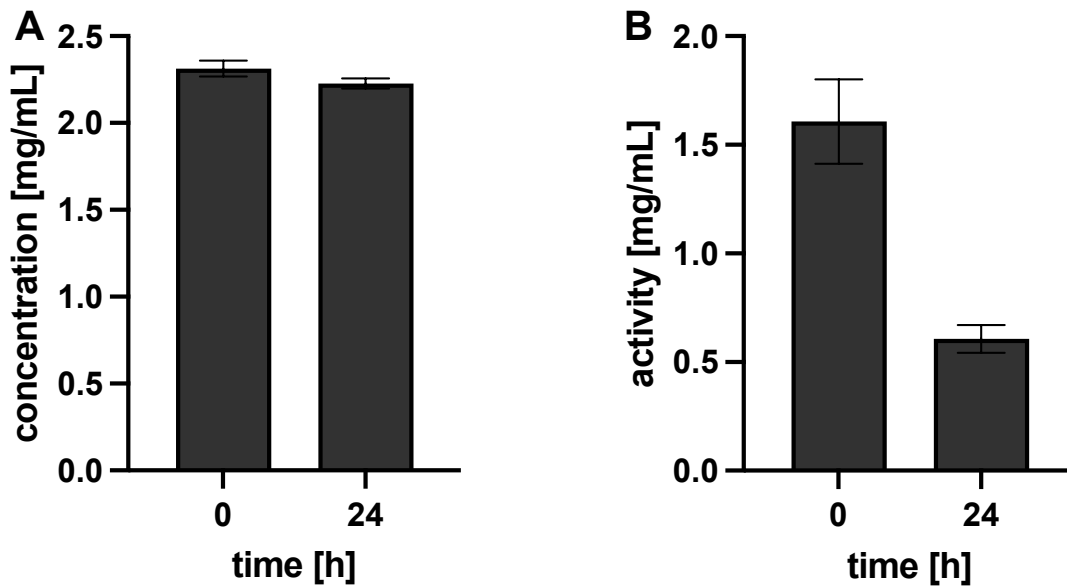
Bevacizumab (Avastin™ 25 mg/mL) and trastuzumab (Herceptin™ 21 mg/mL) were purchased from Roche Pharma AG (Grenzach-Wylen, Germany). Balanced salt solution (BSS) was purchased from Beaver-Visitic Internation Ltd. (Halifax, UK). Tween80, polylysine, sodium bicarbonate and phosphate-buffered saline (PBS) were purchased from Merck KGaA (Darmstadt, Germany). Siluron 2000 (S2000), Densiron 68 (D68), F4H5 and F6H8 were provided by Fluoron GmbH (Ulm, Germany). Atto488-NHS was purchased from Atto-Tec GmbH (Siegen, Germany). The raw materials for the synthesis of the third-generation hydrogel 4ARM-SH-10K (M = 10.000 g/mol) and 4ARM-MA-10K (M = 10.000 g/mol) were purchased from JenKem Technology (Tianjin, China). SK-Br-3 cells (ACC 736) were purchased from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany). Non-protein blocking buffer was obtained from Pierce Biotechnology (Waltham, Massachusetts, USA), NucRed Live 647 from Invitrogen (Carlsbad, US) and Mounting Medium from Carl Roth GmbH (Karlsruhe, Germany).

Cell culture and activity assay with trastuzumab

Coverslips were sterilized with 70% ethanol and placed into 12 well plates after drying. They were incubated with 0.1 mg/ml polylysine diluted in PBS. After 30 min the solution was aspirated, and the coverslips were dried under laminar flow. SK-Br-3 cells were seeded (500.000 cells/well) and grown until adherence (12 h). The medium was aspirated, the cells were washed with PBS-T and blocked for 2 h at RT with non-protein blocking buffer. Following a washing step with PBS-T, the atto-labelled trastuzumab with and without previous contact to silicone oil was added and incubated for 1 h. Afterwards the cells were washed two times for 10 min with PBS-T. Then, cells were fixed with 4% PFA for 20 min at RT followed by a PBS-T wash. NucRed Live 647 was added according to the manufacturer's protocol and followed by another wash with PBS-T and one wash with PBS. The coverslips were then mounted onto microscopic slides and sealed with nail polish. The cells were imaged with a Leica confocal microscope (Leica Camera AG, Wetzlar, Germany).

Results

Parts of the experiments were reconducted with a second batch of bevacizumab to assure consistency of the results. Supplementary material Figure 1 depicts the results of these analyses. In short, results of the first batch were comparable suggesting a good reproducibility. Contact with silicone oil led to a drastic decrease of bevacizumab activity measured using an ELISA while protein content did not decrease using Nanodrop measurements.



Online Figure 1: While protein concentration did not decrease in the hydrophilic phase containing bevacizumab after contact with silicone oil for 24 hours (A), the activity of bevacizumab drastically decreased quantified using ELISA. (B)