**Supplementary material**

**Biocompatibility and characterization of polyglycerol-based thermoresponsive nanogels designed as novel drug delivery systems and their intracellular localization in keratinocytes**

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**Methods**

**Supplement 1. Cultivation of HaCaT cells**

The cell culture work was carried out as described in previous publications (Ahlberg *et al.*, 2014, Lohan *et al.*, 2015). In general, HaCaT cells were cultivated in RPMI 1640 medium (Gibco, Invitrogen, Carlsbad, CA, USA) with supplements (1% penicillin/ streptomycin (Biochrom, Berlin, Germany), 2% glutamine (Biochrom) and 10% FCS (PAA Laboratories, Vienna, Austria)). The cells were cultivated in 75cm2 flasks at 37°C, 5% CO2 and 100% humidity. Until a confluence of about 80% was reached, the cells were harvested by trypsination (0.5% trypsin and 0.2% EDTA, Gibco, Invitrogen, Carlsbad, CA, USA), counted and seeded in new 75cm2 flasks, and/ or were used for further investigations.

**Supplement 2. Cellular uptake of tNGs by HaCaT cells**

HaCaT cells were seeded on an iBidi® µ Slide 8 Well chamber slides (ibidi, Planegg-Martinsried, Germany) at 1.5 × 104 cells per well and cultured for 24 h prior to the experiment. The medium was exchanged with medium containing a final concentration of 200 µg/ml of tNG\_dPG\_tPG-IDCC or tNG\_dPG\_pNIPAM-IDCC for another 4 h (data not shown) and 24 h. 1 h prior to the measurement, the medium was replaced by fresh medium containing a final concentration of 100 nM LysoTracker® Green DND-26 (Thermo Fisher Scientific, Darmstadt, Germany). The internalization was observed by cLSM (Leica SP8, Wetzlar, Germany) and analyzed by Leica Application SuiteX software.

**Supplementary Figures**

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**Figure S1**

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**Figure S2**

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**Figure S3**

**Supplementary Figure captions**

**Figure S1: Cellular uptake of tNGs by HaCaT cells.** Cells were exposed to 200 µg/ml of each tNG for 24 h. Then, colocalization study of (A) tNG-dPG-pNIPAM-IDCC (left panel) and (B) tNG-dPG-tPG\_IDCC (right panel) and lysosomes was performed. tNGs have been labeled with IDCC (red pseudo-color), lysosomes have been stained with Lysotracker® Green (green pseudo-color). Scale bars represent 25 µm.

**Figure S2: Cellular uptake of tNGs by NHK.** Cells were exposed to 200 µg/ml of each tNG for 3 h. Colocalization studies between tNG-dPG-pNIPAM\_IDCC (A,B) or tNG-dPG-tPG\_IDCC (C,D) and lysosomes was performed. The tNGs were labeled with IDCC (red pseudo-color), while the lysosomal compartments were stained with Lysotracker® Red (green pseudo-color). Cell nuclei were stained with DAPI (blue pseudo-color). Scale bars represent 25µm.

**Figure S3: Cellular uptake of tNGs by NHK.** Cells were exposed to 200 µg/ml of each tNG for 48 h. Then, colocalization study of tNG-dPG-pNIPAM\_IDCC (A,B) and tNG-dPG-tPG\_IDCC (C,D) and lysosomes was performed. tNGs have been labeled with IDCC (red pseudo-color), lysosomes have been stained with Lysotracker® Red (green pseudo-color) and cell nuclei have been stained with DAPI (blue pseudo-color). Scale bars represent 25µm.