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Supplementary materials for

Anthracene dimer crosslinked, washing and sterilization-free hydrogel films

for multicellular spheroid generation

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Fig. S1. Photographs of an un-crosslinked PHEMA film (A) and a crosslinked PHEMA film (B) soaked in methanol. Both films were prepared by solution casting of PHEMA-AN-7.79. To crosslink the film in B, it was irradiated with 365 nm UV light for 15 min. The films were stained with methylene blue for clarity.



Fig. S2. Measurement of water contact angle of the crosslinked PHEMA film. The water contact angle of the as-prepared film was measured to be $39.8\pm2.9^{\circ}$. After swelling in water, the water contact angle of the patterned film was measured to be $105.4\pm4.4^{\circ}$.



Fig. S3. Determination of bacteria growth by Alamar blue assay. PHEMA films were first fabricated in the wells of 12 well plates via photo-dimerization or one-step UV curing. Without washing and sterilization of the films, cell culture media were added into the wells. The plates were incubated at 37 $^{\circ}$ C and 5 $^{\circ}$ CO₂ for 168 h. At predetermined intervals, the culture media were sampled and mixed with alamar blue reagent. The

fluorescence intensity (excitation wavelength: 540nm, emission wavelength: 590nm) was determined after 24h incubation at 37 °C.



Fig. S4. Relative viability of Hela cells in 2D monolayers (A) or 3D multicellular spheroids (B) after 24 h culture in the presence of various concentrations of 5-FU, DOX, or OXP.



Fig. S5. Relative viability of A549 cells in 2D monolayers (A) or 3D multicellular spheroids (B) after 24 h

culture in the presence of various concentrations of 5-FU, DOX, or OXP.