

## Supporting Information

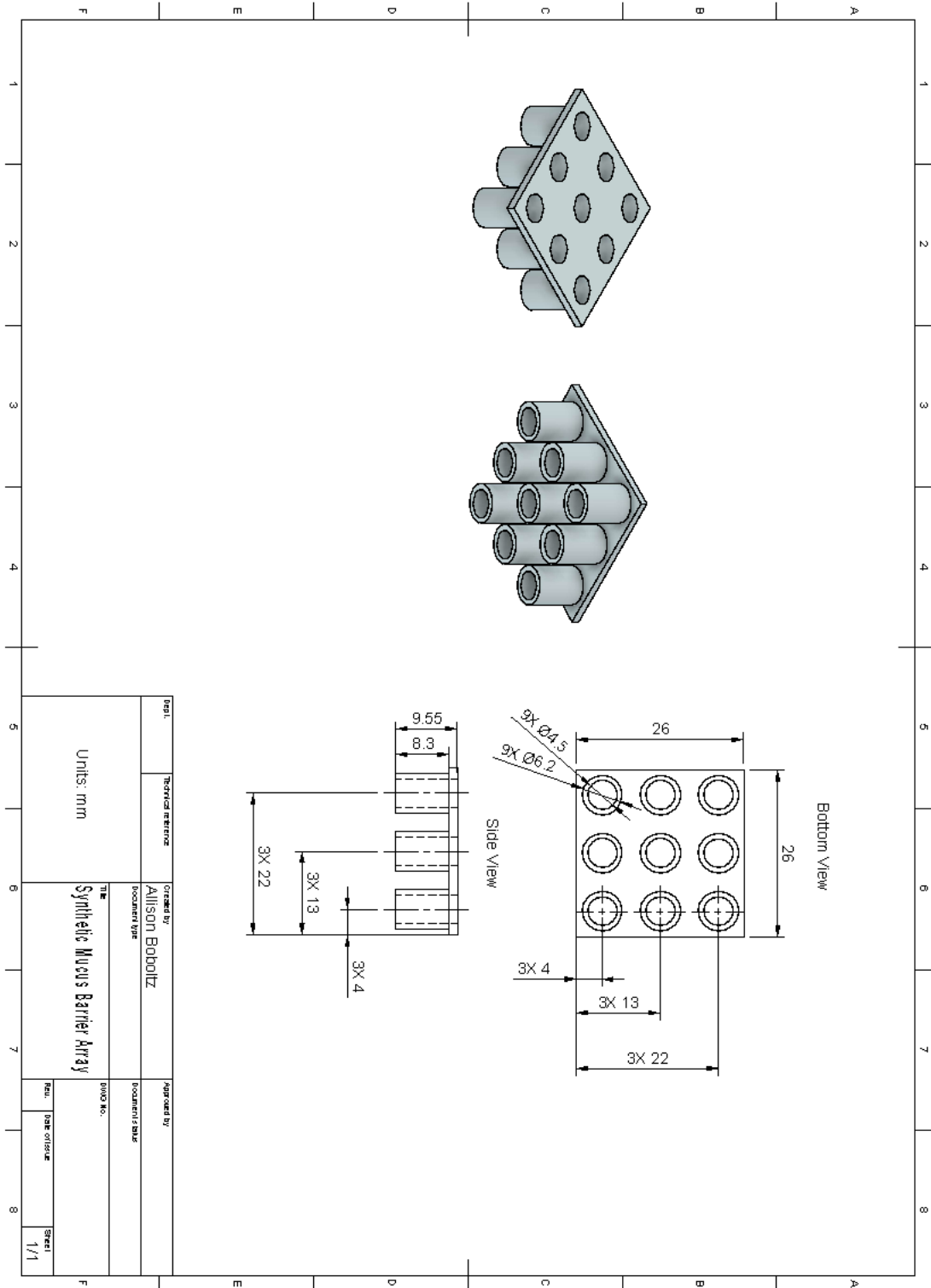
### **Synthetic mucus barrier arrays as a nanoparticle formulation screening platform**

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**Figure S1.** Engineering drawing with measurements of the synthetic mucus barrier array part that was designed using Autodesk CAD software. The array device is designed to fit snugly into the wells of a 96 well plate (Costar brand). All units are in millimeters. The STL file used to print this device is included as a supplemental file.

**Table S1. PEG density and conformation on the surface of 100 nm PS NPs.** The density of PEG groups attached to the NPs (% PEGylation) was found using a 1-pyrenyldiazomethane (PDAM) fluorometric assay, as previously described [see Reference 33]. The conformation of the PEG for each batch was determined using the ratio of the Flory radius ( $R_F$ ) to the distance between PEG chains ( $D$ ). The Flory radius ( $R_F$ ) of the linear 5 kDa PEG was calculated using the equation  $R_F = \alpha N^{3/5}$ , where  $\alpha$  is the size of the monomer (PEG is 0.35 nm) and  $N$  is the number of monomers within the polymer chain (113). The distance between PEG chains ( $D$ ) was found using the equation  $D = 2\sqrt{A/\pi}$ , where  $A$  is the area covered by a PEG chain, calculated using the inverse of the PEG density (% PEGylation). The PEG conformation was found to be a dense brush regime for each batch ( $R_F/D > 2.8$ ). The data shown represent the mean  $\pm$  standard deviation.

| <b>Batch Number</b>   | <b>% PEGylation</b> | <b>Conformation (<math>R_F/D</math>)</b> |
|-----------------------|---------------------|--|
| 100 nm PS-PEG Batch 1 | 69.26 $\pm$ 10.99   | 8.27 $\pm$ 0.66                          |
| 100 nm PS-PEG Batch 2 | 56.01 $\pm$ 7.10    | 7.44 $\pm$ 0.48                          |
| 100 nm PS-PEG Batch 3 | 71.96 $\pm$ 6.23    | 8.44 $\pm$ 0.37                          |
| 100 nm PS-PEG Batch 4 | 64.84 $\pm$ 0.23    | 8.01 $\pm$ 0.014                         |