Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Bacteria-triggered degradation of nanofilm shells for release of antimicrobial agents

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Information
Supporting Information presents light and fluorescence micrographs of model drug loaded microcapsules with varying ionic strength and probes with varying charge.

A few of the dark spots on the fluorescence microscope pictures are dirt on the lens; however, the probe -filled microcapsules are seen as either lighter spots than the background ($I_{core} > I_{solution}$) or as spots with the same color as the background ($I_{core} = I_{solution}$).

Acronyms
VB = Vancomycin BODIPY
EDC = N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
Sulfo-NHS = N-hydroxysulfosuccinimide sodium salt
PAH = polyallylamine hydrochloride
PLGA = poly-L-glutamic acid
HA = hyaluronic acid
PLL = poly-L-lysine

Light microscopy of $(PAH/PLL)_3$ and $(HA/PLL)_3$

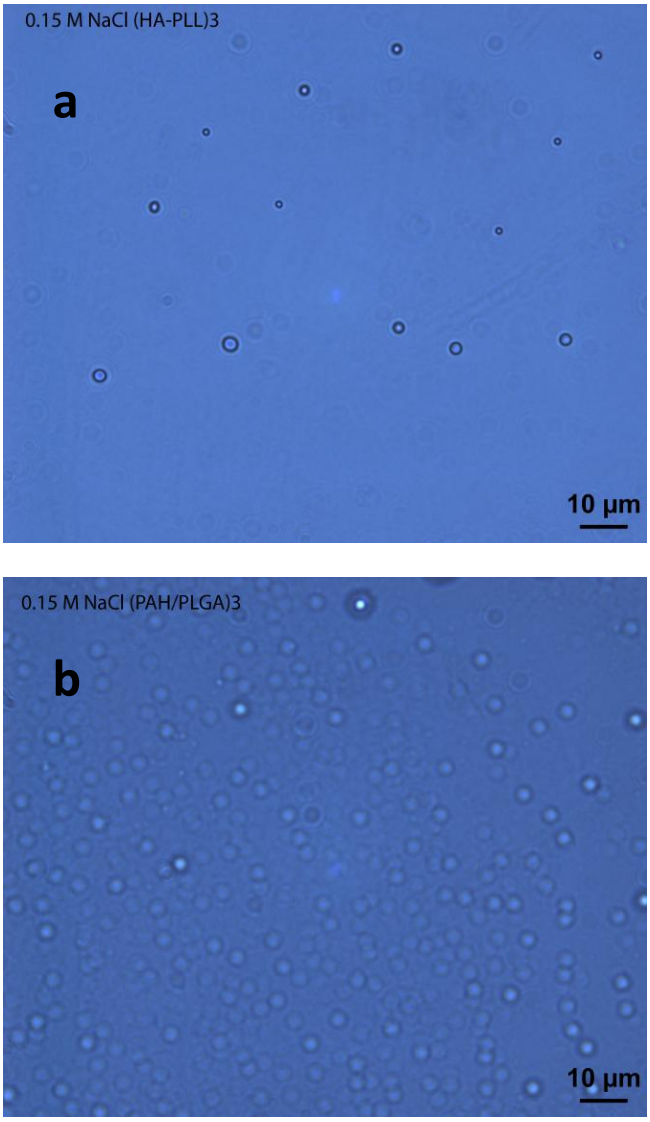

Figure S1. Optical microscopy images of microcapsules. (a) $(HA/PLL)_3$ microcapsules in solution. (b) $(PAH/PLGA)_3$ microcapsules in solution. The approximate size of the capsules is 3µm.

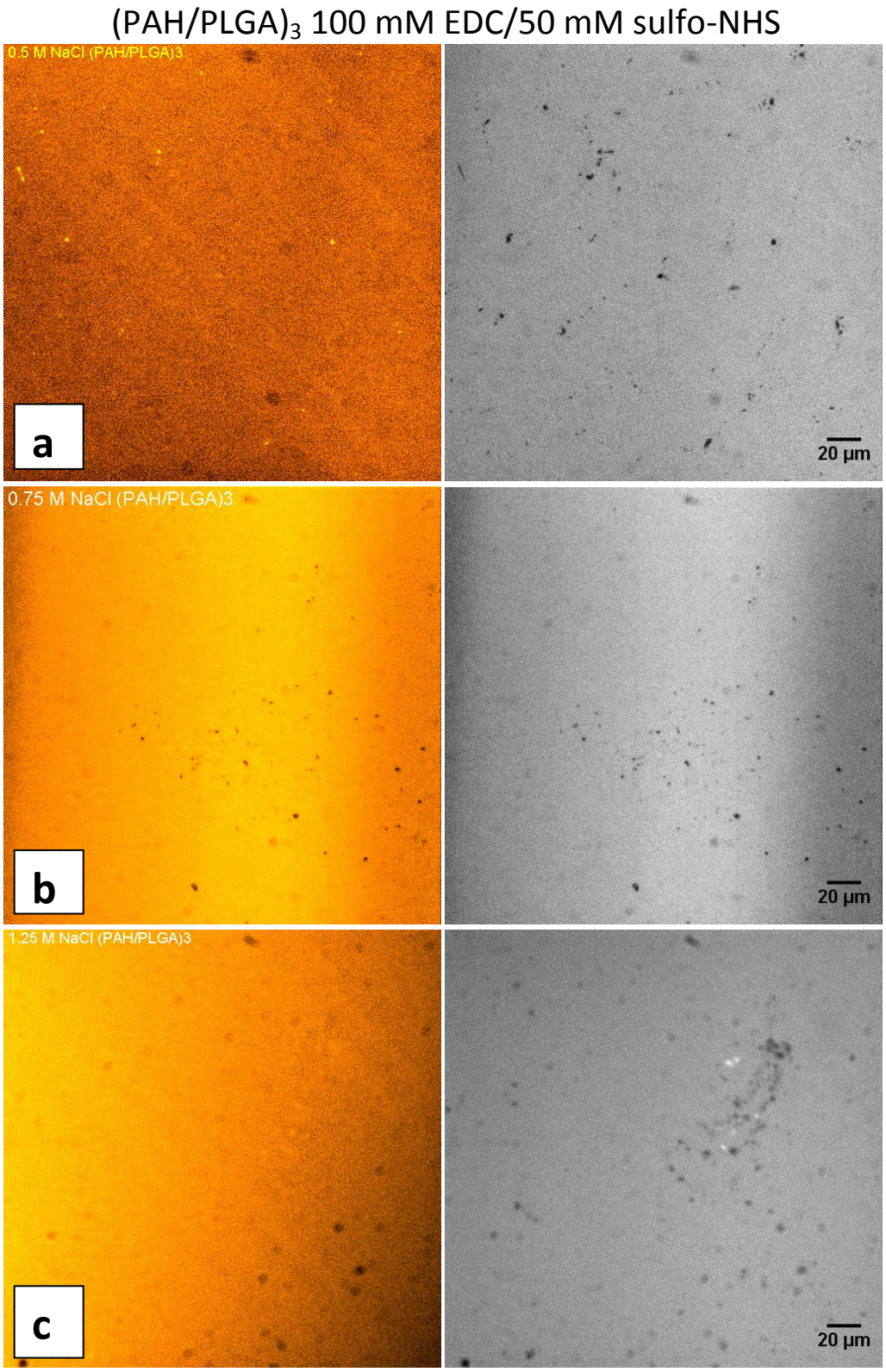

Figure S2. Fluorescence micrographs taken 30 minutes after loading 100 mM EDC and 50 mM sulfo-NHS crosslinked $(PAH/PLGA)_3$ microcapsules with 4 kDa FITC-dextran at (a) 0.5 M NaCl, (b) 0.75 M NaCl and (c) 1.25 M NaCl. The corresponding optical microscopy images taken in the same frame as the optical microscopy images are seen to the right. The loading was successful at 0.5 M NaCl and the aggregation was found to increase with increasing ionic strength.

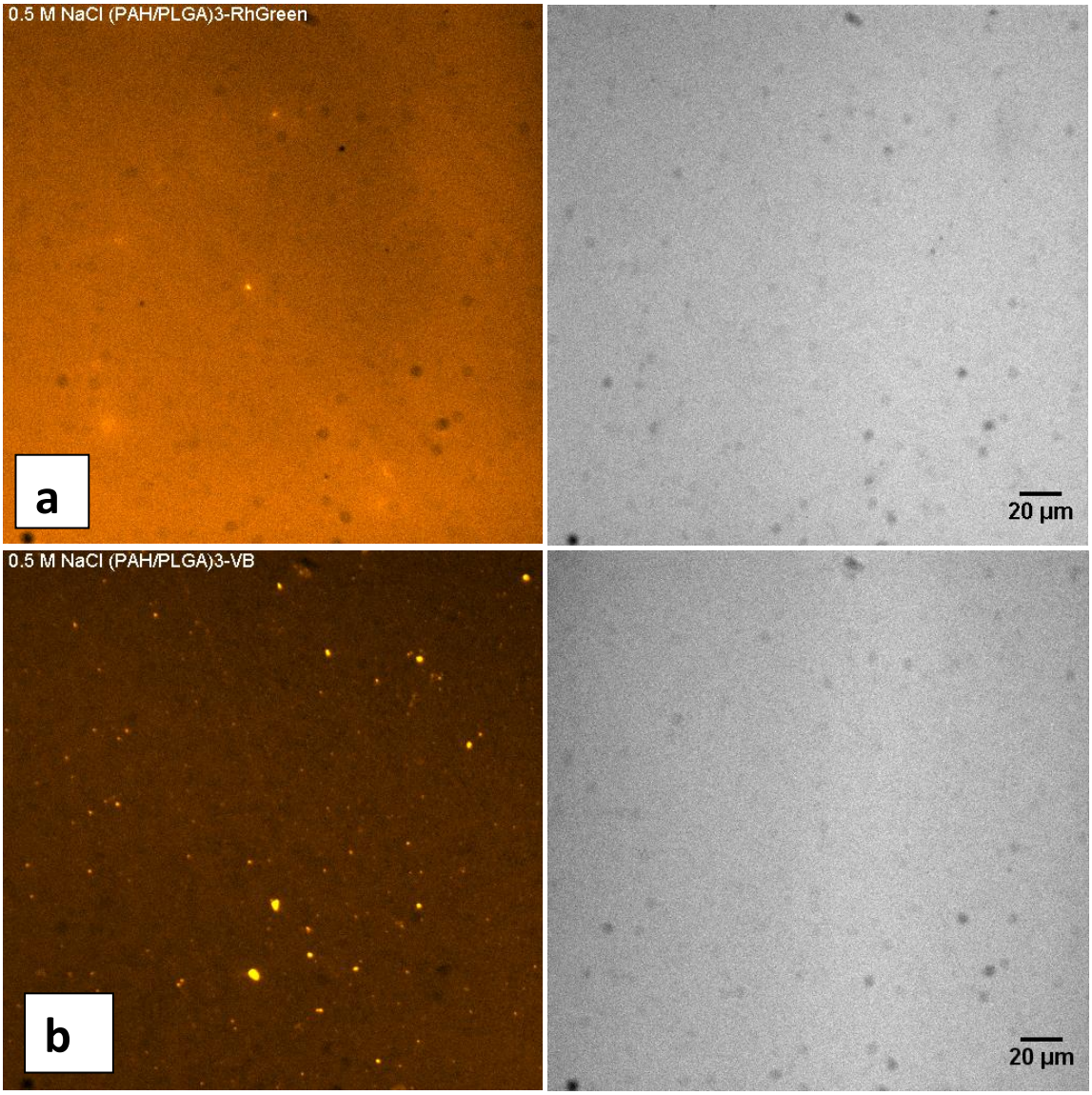

Figure S3. Fluorescence micrographs taken 60 minutes after loading 100 mM EDC and 50 mM sulfo-NHS crosslinked $(PAH/PLGA)_3$ microcapsules with (a) cationic Rhodamine green (3 kDa) and (b) neutral Vancomycin-BODIPY (VB, 1.7 kDa) at 0.5 M NaCl. The corresponding optical microscopy images taken in the same frame as the optical microscopy images are seen to the right. Both VB and Rhodamine Green were successfully loaded into the microcapsules at 0.5 M NaCl.

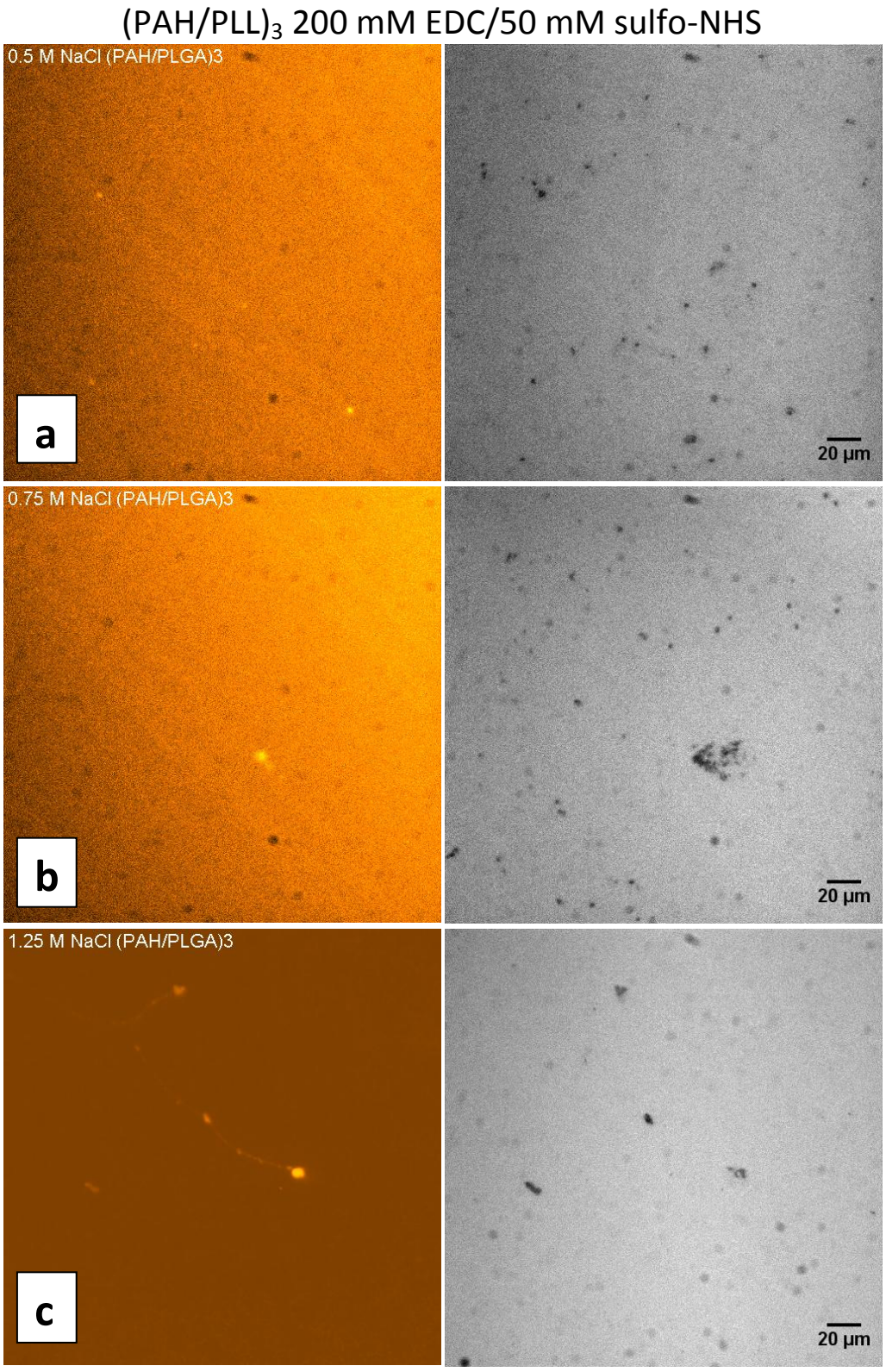

Figure S4. Fluorescence micrographs taken 30 minutes after loading 200 mM EDC and 50 mM sulfo-NHS crosslinked $(PAH/PLGA)_3$ microcapsules with 4 kDa FITC-dextran at (a) 0.5 M NaCl, (b) 0.75 M NaCl and (c) 1.25 M NaCl. The corresponding optical microscopy images taken in the same frame as the optical microscopy images are seen to the right. The higher crosslinking degree resulted in less aggregation with increasing ionic strength. The loading was successful despite the changes in the surrounding solution; however, fewer probe-loaded microcapsules were found than for microcapsules crosslinked with 100 mM EDC (Figure S1a). The microcapsules crosslinked with 200 mM EDC were not loaded with VB or Rhodamine Green.

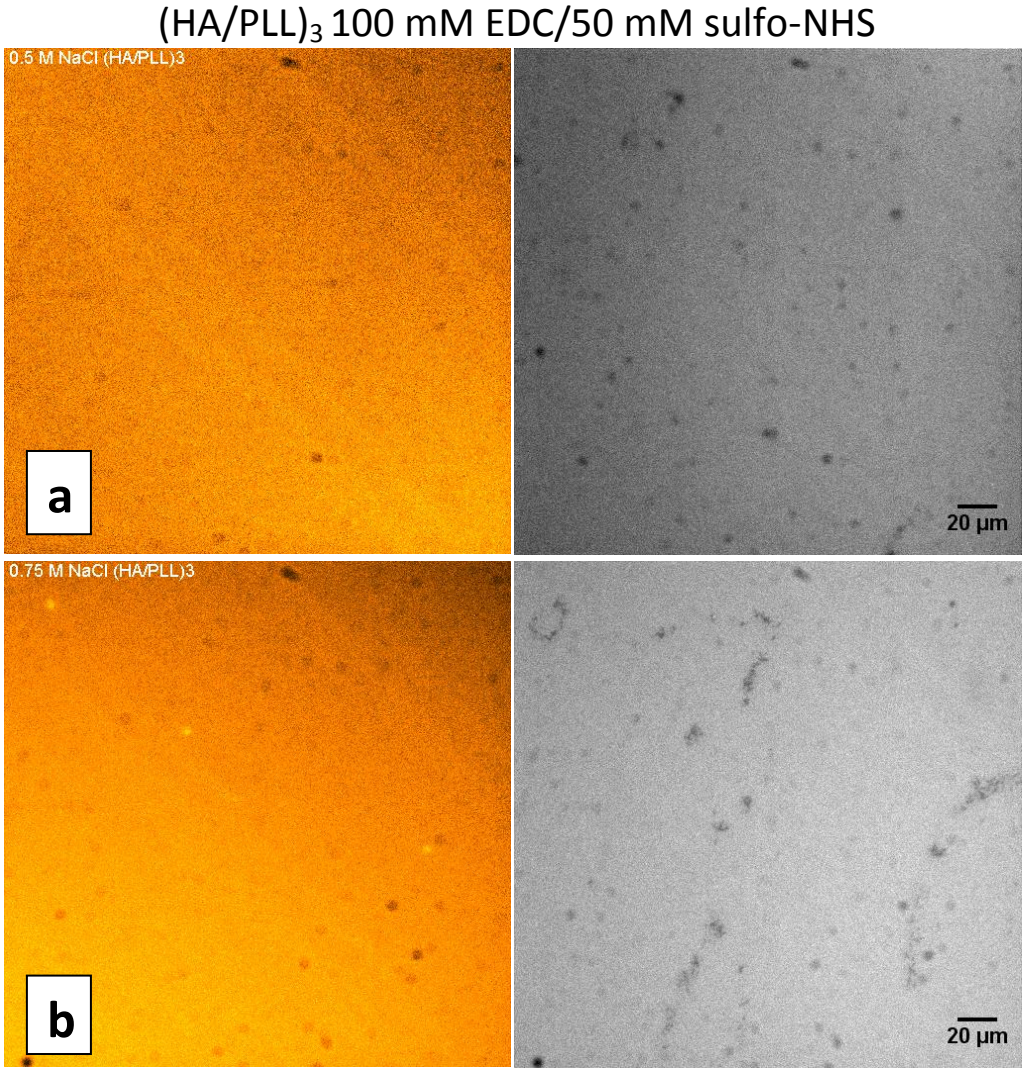

Figure S5. Fluorescence micrographs taken 30 minutes after loading 100 mM EDC and 50 mM sulfo-NHS crosslinked $(HA/PLL)_3$ microcapsules with 4 kDa FITC-dextran at (a) 0.5 M NaCl and (b) 0.75 M NaCl. Anionic FITC-dextran could not easily penetrate the nanofilm. Thus, 1.25 M NaCl in ionic strength was not tested. However, as the ionic strength was increased in (b), a few loaded capsules were discovered. The corresponding optical microscopy images taken in the same frame as the optical microscopy images are seen to the right. Also, since HA gels in water, the HA/PLL microcapsules were difficult to discover in the optical microscopy due to their transparency.

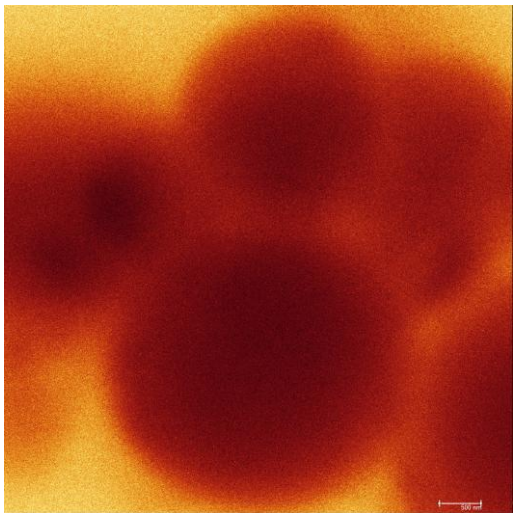

Figure S6. CLSM image of $(HA/PLL)_3$ with FITC-dextran (4 kDa) and 0.5 M NaCl taken 30 minutes after loading. As described in Fig. 4, the probe had difficulty penetrating the nanofilm; however, with CLSM as technique, the intensity of the probe could be measured in the microcapsules. Thus, despite HA electrostatically repelling the probe, the capsule could penetrate the nanofilm with time due to the cationic component, PLL. The microcapsules in Figure 4c were allowed 16 hours for loading, which gave successfully loaded capsules crosslinked with 100 mM EDC and 50 mM sulfo-NHS. Hence, it was concluded that the loading was time dependent.

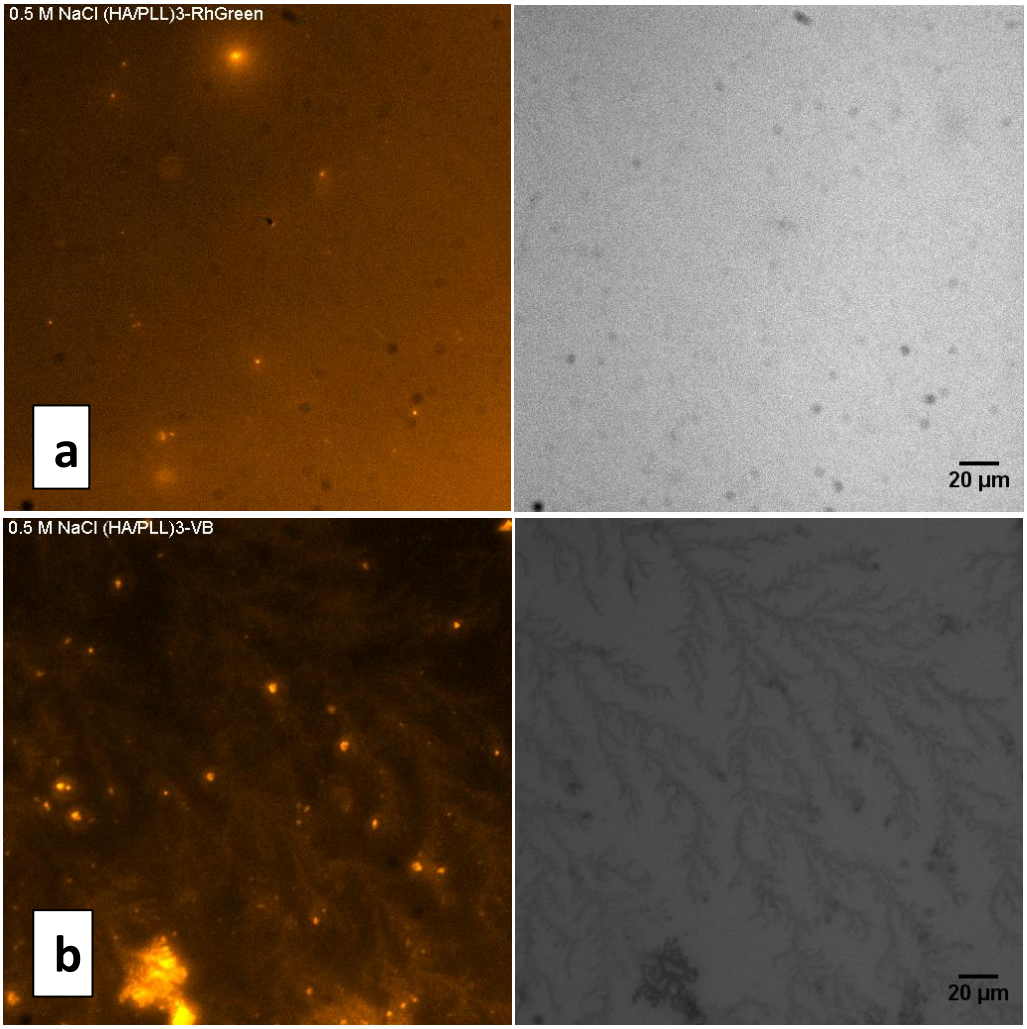

Figure S7. Fluorescence micrographs taken 90 minutes after loading 100 mM EDC and 50 mM sulfo-NHS crosslinked $(HA/PLL)_3$ microcapsules with (a) cationic Rhodamine green (3 kDa) and (b) neutral Vancomycin-BODIPY (1.7 kDa) at 0.5 M NaCl. The sample in (b) had dried and attached to large salt crystals, which also contributed to the aggregation. The corresponding optical microscopy images taken in the same frame as the optical microscopy images are seen to the right. As seen in the micrographs above, by changing the charge of the probe, the microcapsules with the lower crosslinking degree were successfully loaded.

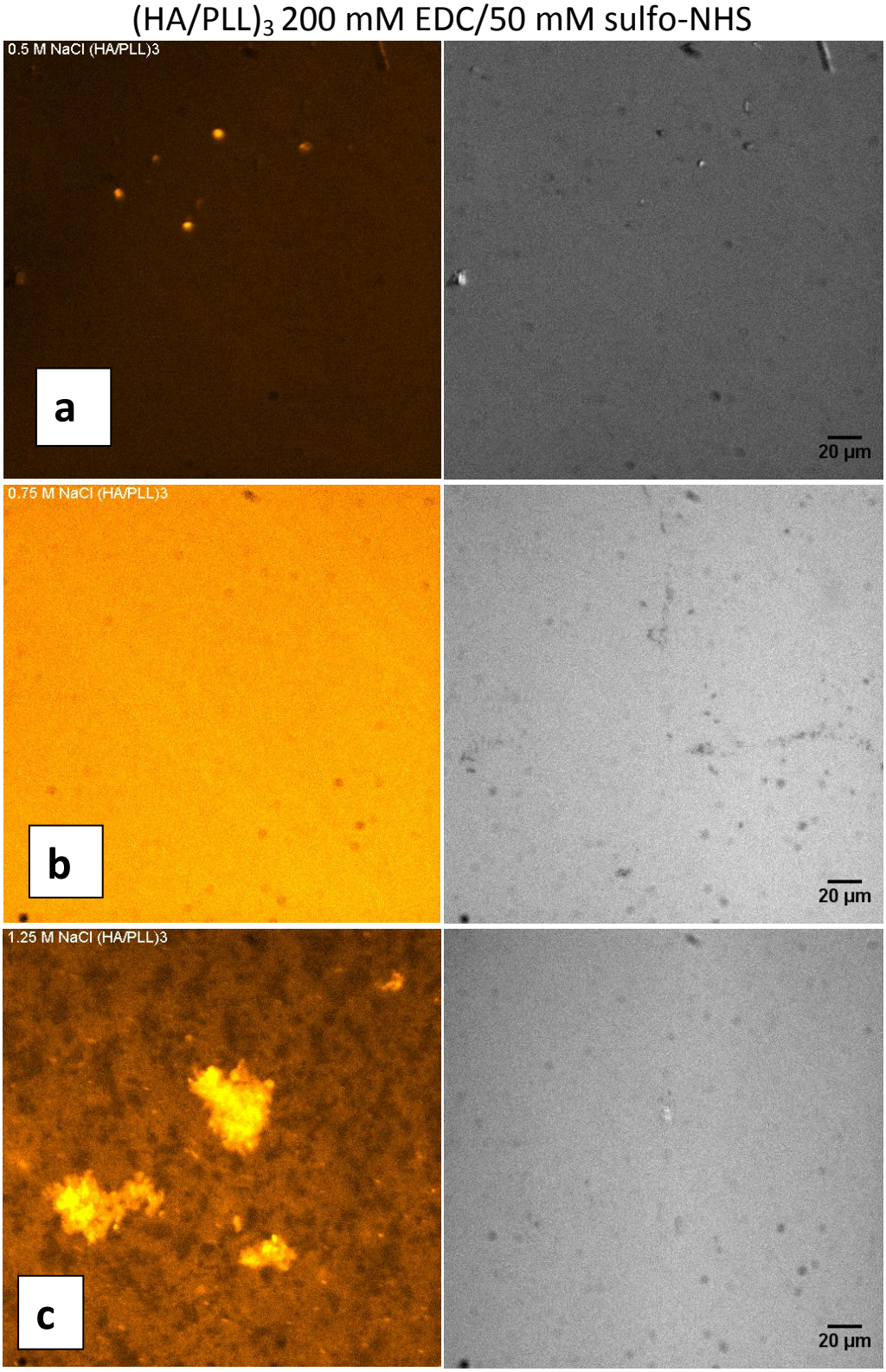

Figure S8. Fluorescence micrographs taken 30 minutes after loading 200 mM EDC and 50 mM sulfo-NHS crosslinked $(HA/PLL)_3$ microcapsules with 4 kDa FITC-dextran at (a) 0.5 M NaCl, (b) 0.75 M NaCl and (c) 1.25 M NaCl. The increased crosslinking degree enabled penetration of anionic FITC-dextran, most likely due to fewer negatively charged HA sites. Again, with increasing ionic strength, the aggregation increased. In (b) the microcapsules were difficult to discover, probably as a consequence of the probe concentration in the capsules being equivalent to the probe concentration in the solution. The corresponding optical microscopy images taken in the same frame as the optical microscopy images are seen to the right. Also, since HA gels in water,

the HA/PLL microcapsules were sometimes difficult to discover in the optical microscopy due to the transparency of the film.

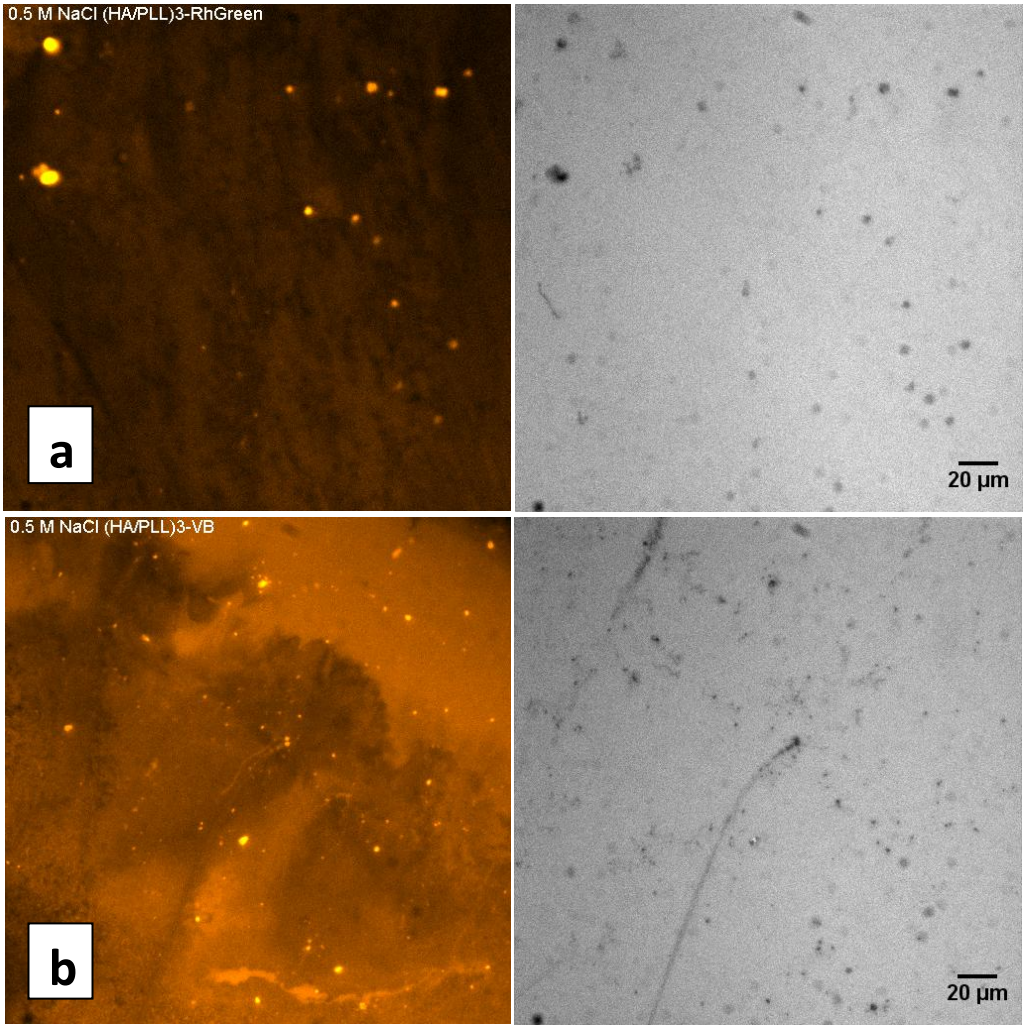

Figure S9. Fluorescence micrographs taken 60 minutes after loading 200 mM EDC and 50 mM sulfo-NHS crosslinked $(HA/PLL)_3$ microcapsules with (a) cationic Rhodamine green (3 kDa) and (b) neutral Vancomycin-BODIPY (1.7 kDa) at 0.5 M NaCl. The corresponding optical microscopy images taken in the same frame as the optical microscopy images are seen to the right. Again, both the VB and Rhodamine Green were successfully loaded into the microcapsules.