Supporting Information

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Nanoparticle-Based Targeting and Detection of Microcavities

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Chemical Characterization:

Table S1. X-ray Photoelectron Spectroscopy results for unmodified and modified starch nanoparticles, highlighting the efficiency of the cationization reaction by analysis of Nitrogen content.

	StNP-1 (Unmodified)	StNP-5 (Anionic)	StNP-2 (Cationic)	StNP-3 (Zwitterionic)
O (532 eV)	36.4% (45.5%)	31.7% (50%)	31.4% (31.6%)	26.3% (38.7%)
C (285 eV)	63.3% (54.5%)	59.7% (50%)	65.7% (63.2%)	56.5% (58.1%)
N (401 eV)	0% (0%)	0% (0%)	1.5% (5.3%)	2.0% (3.2%)
Na (1071 eV)	0% (0%)	3.9% (0%)	0.4% (0%)	2.9% (0%)
Cl (198 eV)	0% (0%)	4.7% (0%)	0.3% (0%)	7.1% (0%)

X-Ray Photoelectron Spectroscopy (XPS) Results:

The unmodified (1), cationic (2), anionic (5), and zwitterionic (3) starch nanoparticles were analyzed by XPS to determine the chemical composition, in particular examining the presence of Nitrogen which is unique to the cationic functional group, as is common in the literature for analysis of starch cationization.[15, 20, 21] Results shown indicate the measured atomic percentage of Oxygen, Carbon, Nitrogen, the theoretical expected maximum (in brackets) and the measured most common impurities Sodium and Chlorine, resultant from the presence of these chemicals in both the oxidation and cationization reactions. The variations in the ratio of carbon and oxygen from the theoretical limit occur because of the assumption in the calculations that the starch is a linear polymer, though it is known to be highly branched and cross-linked. It was noted that Nitrogen was not detectable in the unmodified and anionic StNP samples, but was present in the cationic and zwitterionic StNP samples on the order of 1.5-2.0 atomic percent, which corresponds to a DS of approximately 0.28, consistent with similar studied reactions for highly cationic starch.[15,20] This could conceivably be stretched further using techniques in Pi-Xin et al.,[21] and future studies can examine how the modification of particle charge impacts the ability of the particle to adhere to carious lesions.

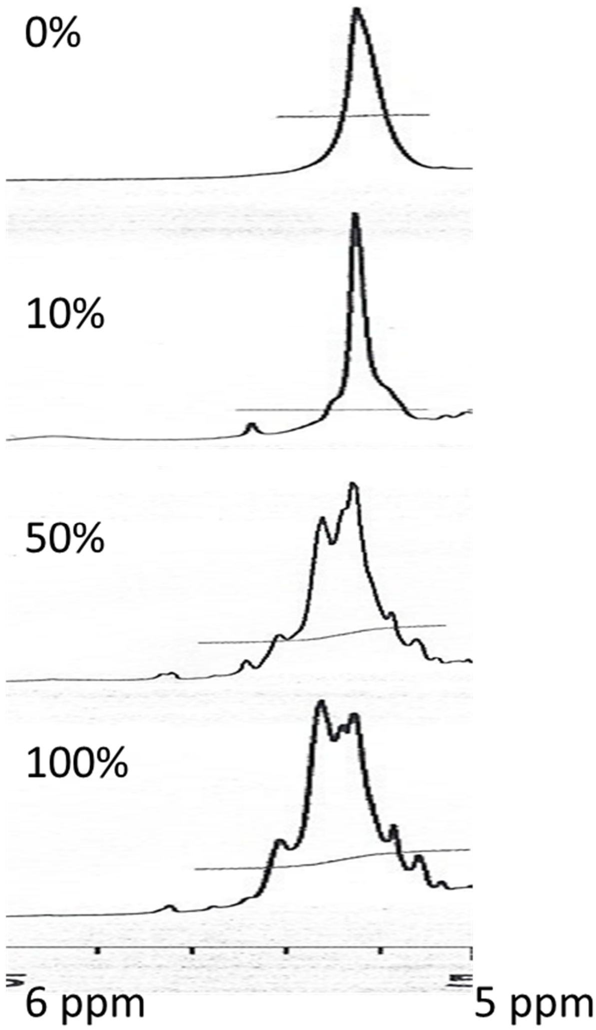

Figure S1. (H'-NMR results for TEMPO oxidation reaction, showing the peak shift corresponding to conversion of the C6' hydroxyl to a C6' carboxyl (5.2→5.4ppm) with increasing amount of added sodium hydroxide (percentage on left))

Starch nanoparticles were modified by TEMPO oxidation as per Kato et al.[16] Similar to this paper, H'-NMR results were measured for increasing amounts of added sodium hydroxide, shown as a percentage, with 100% corresponding to a 1:1 molar ratio of NaOH : Starch glucose units. This allowed for a determination of the DOS for the reaction by noting the peak shift of the C'6 hydroxyl to a C'6 carboxyl, from 5.2→5.4 ppm. Measuring the relative peak integrals determined that a maximum DOS of approximately 55% was obtained. This reaction is limited from reaching full conversion because of the cross-linking and branched nature of the starch, resulting in fewer chemically and sterically available C'6 hydroxyls. Furthermore, as discussed in Kato et al., small levels of C'6 aldehyde or hemiacetal intermediates can be formed, which explain the additional peaks visible in the H'-NMR spectra.

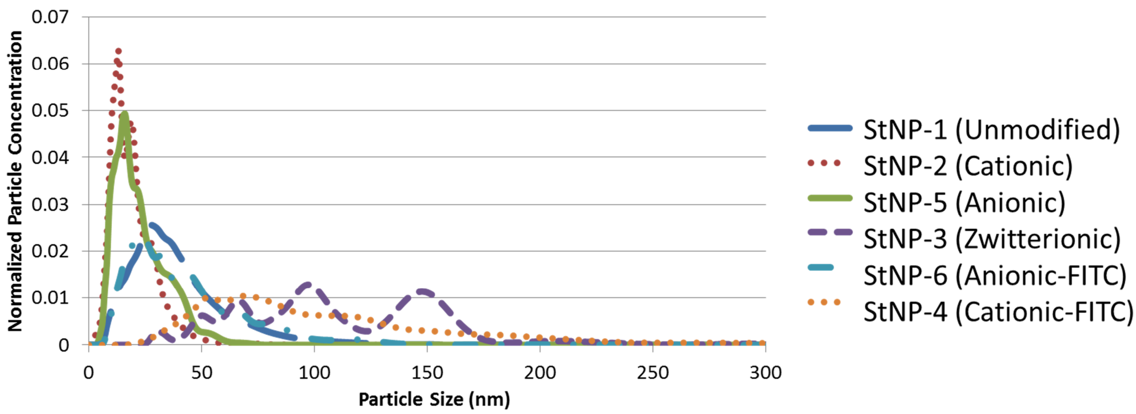

Figure S2 – Particle size distribution of modified StNPs by Nanoparticle Tracking Analysis (NTA). The particles show a median particle size less than 50 nm when unmodified, which is reduced to about 25nm with the addition of anionic or cationic charged functionalities (StNP-2 and StNP-5). The anionic fluorescent StNPs are similarly small, approximately 35 nm, while the lower overall charged zwitterionic

(StNP-3) and cationic fluorescent (StNP-4) StNPs result in slight particle aggregation, with average particle sizes of approximately 100 nm.

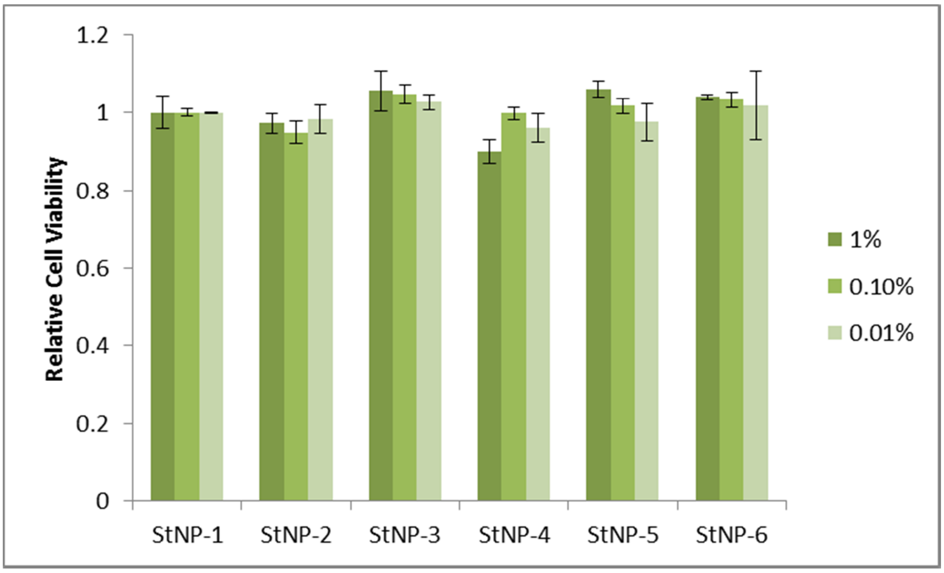

Figure S3. Tox8 cellular toxicity assay of modified StNPs after 2 hour exposure on HeLa cells. Even at high concentrations (1% by mass), all particles were non-toxic.

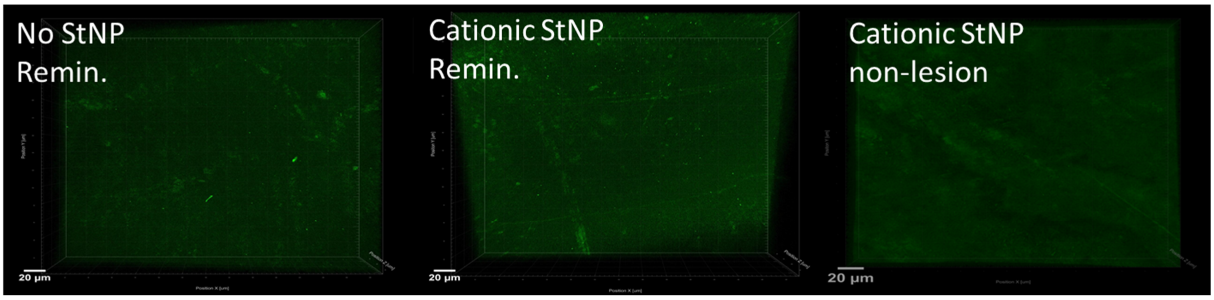

Figure S4. Two-photon micrographs of remineralized carious lesions with and without exposure to cationic fluorescent StNPs. Images most closely resemble the two-photon micrograph of a non-lesion surface, highlighting that remineralized lesions are “inactive” and from a surface perspective, healed.