

Supplementary data

An electric field responsive drug delivery system based on chitosan-gold nanocomposite for site specific and controlled delivery of 5-fluorouracil

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Schematic representation of the preparation of modified electrode is shown in figure S1. Modified and control ITO plates were placed in a 60 mm sterile petri dish and a poly dimethyl sulfoxide (PDMS) mould was positioned over the plate to make uniform distribution of cells. The mould was prepared with 2 cm diameter and 0.5 cm height. SiHa cells were seeded into the PDMS well on ITO plate.

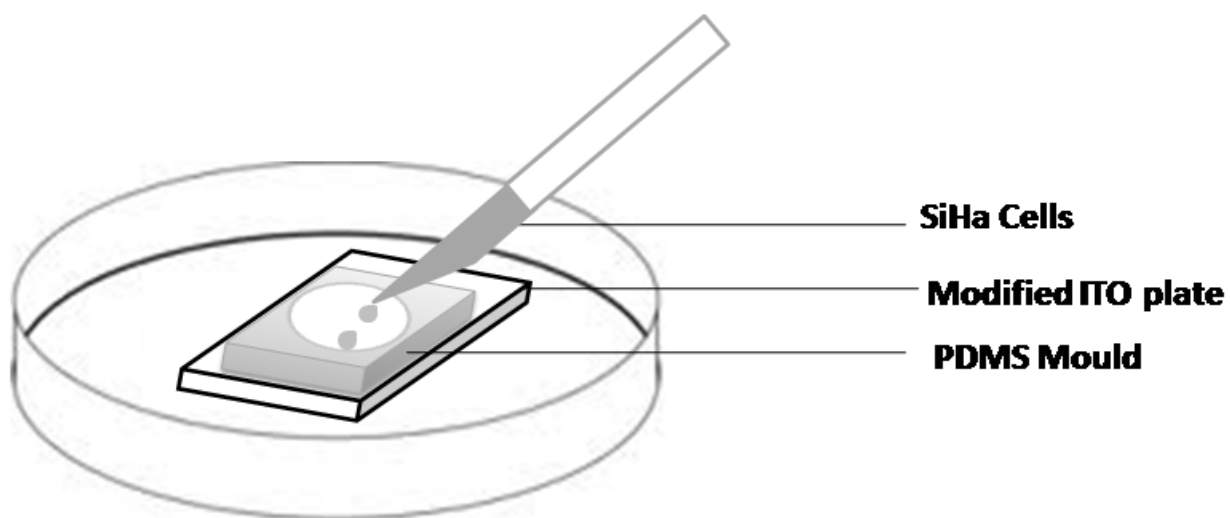


Figure S1. Schematic representation of the preparation of modified electrode

Figure S2 shows bright field optical microscope image that confirms the reversible sol-gel transition of CGNC with pH variation. The images were taken by dropcasting CGNC-FU onto ITO plate. Figure S2 (A) corresponds to CGNC-FU dropcasted after gelation at pH 6. To this electrode a potential of 1.5 V was applied for 15 minutes using a DC power supply. The modified ITO plate shown in figure S2 (A) was kept as anode and a bare ITO plate was kept as cathode. In response to the electric field, pH around anode decreases and CGNC loses its gel-like structure as evident in figure S2 (B). Thus at a higher pH of about 6, CGNC forms a porous gel like structure into which the drug molecules get encapsulated. The drug can be released by lowering the pH and thereby facilitating the gel to sol transition.

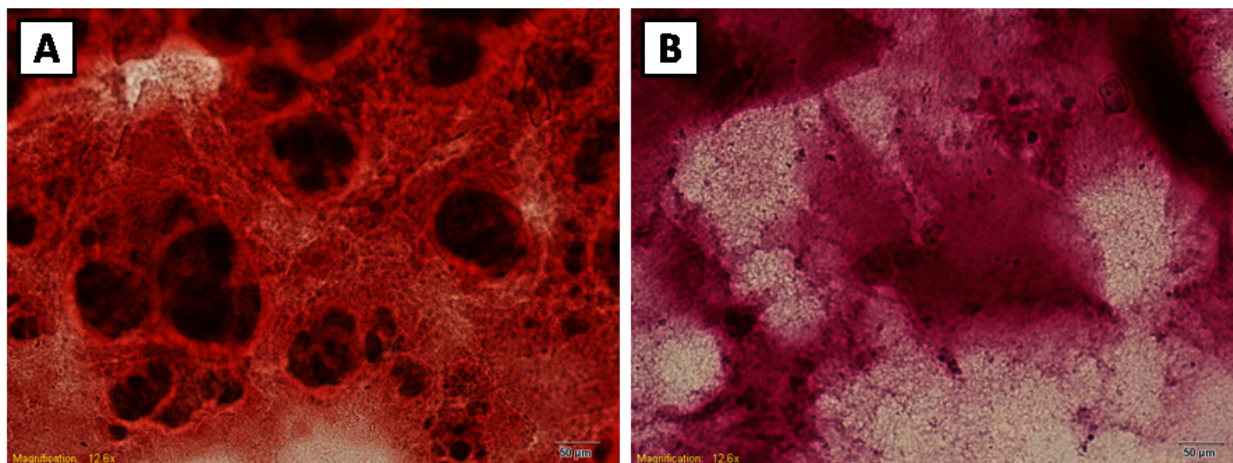


Figure S2. Bright field optical microscope image of CGNC dropcasted onto ITO plate. (A) CGNC-FU at high pH (B) CGNC-FU after applying potential (Scale bar-50 µm).

Size distribution of CGNC (figure S6) and CGNC-FU at pH 6 (figure S7) by DLS is shown below.

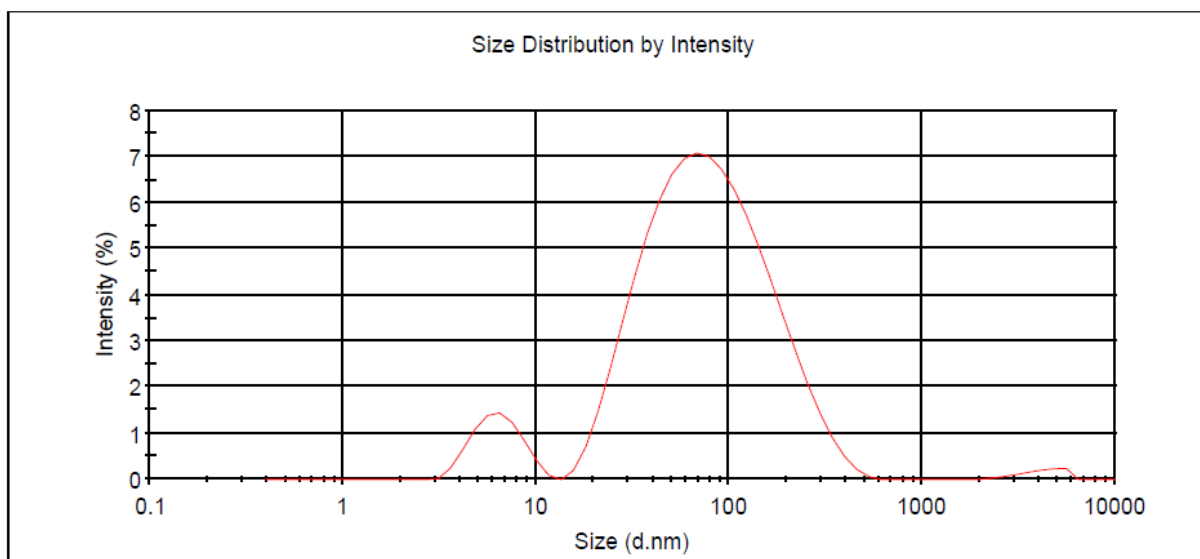


Figure S3. Size distribution report and curve obtained for CGNC by DLS

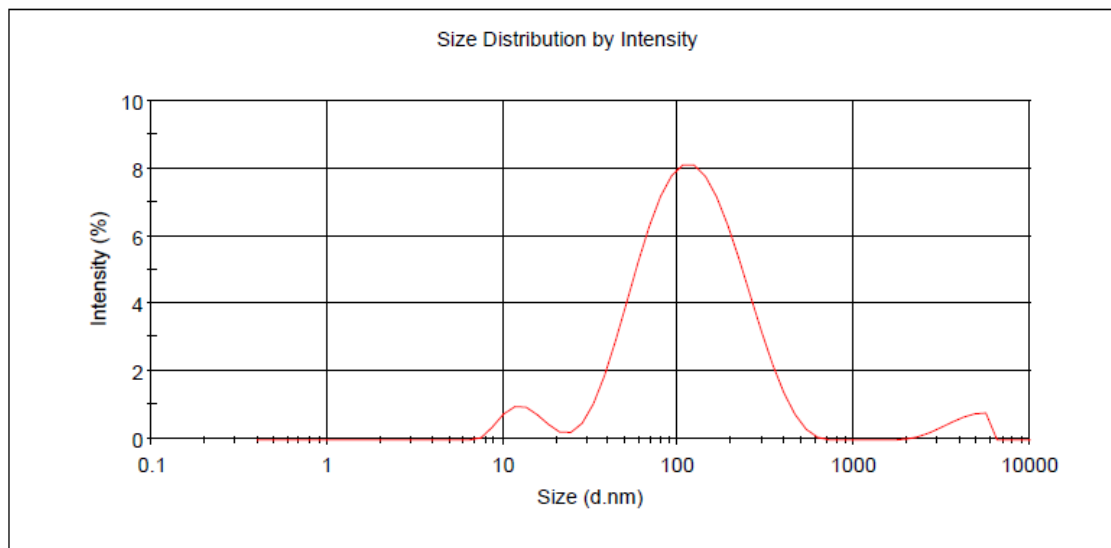


Figure S4. Size distribution report and curve obtained for CGNC-FU at pH 6 by DLS

Figure S5 shows the cell viability percentage with respect to concentration evaluated by SRB assay, where X-axis represents the concentration of CGNC and 5-FU and Y-axis represents the cell viability percentage. Comparing with cells treated with CGNC and 5-FU alone, CGNC-FU has shown a prominent cytotoxic effect at same concentrations due to enhanced internalization of chitosan and cytotoxic effect of 5-FU. The cytotoxicity was not studied in normal cells, since the system is meant for a site specific drug delivery. In the cytotoxicity analysis it was observed that the CGNC alone also causes toxicity to the SiHa cells at higher concentration which may be due to the pH sensitivity of the nanocomposite. We observed that the pH of the cell culture medium (MEM) lowered after treating with CGNC and CGNC-FU conjugate. The lower pH of the medium may lead to the less viability of SiHa cells at higher concentration of CGNC. However, we observed that SiHa cells could grow on a CGNC deposited film (see figure S6). Thus it is believed that the cytotoxicity observed with CGNC alone in the SRB assay is purely due to the change in pH of the medium. Moreover, the system is suggested for the use in the site specific drug delivery and hence the observed cytotoxicity of CGNC does not limit the application of the present system.

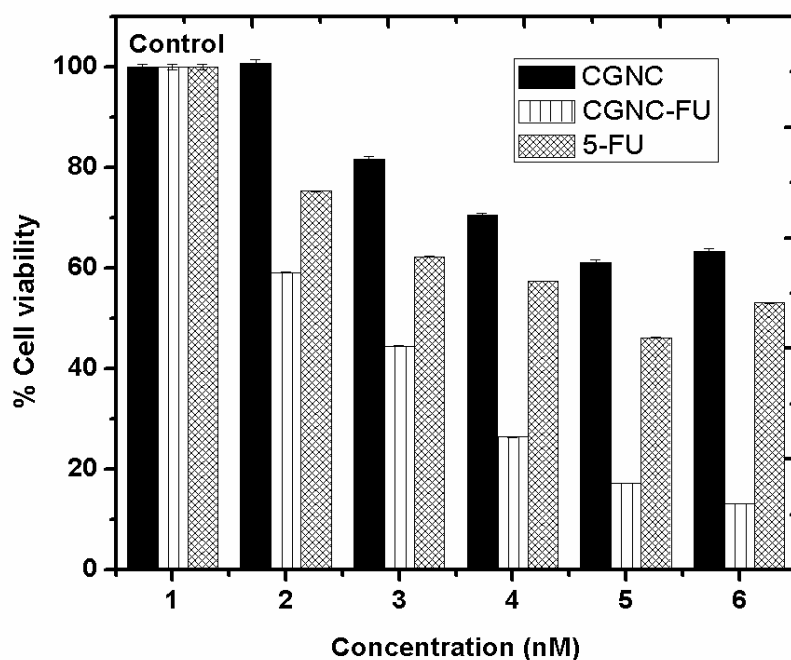


Figure S5. SRB assay of CGNC, CGNC-FU and 5-FU in SiHa cells after 48 h incubation. Concentrations of CGNC in CGNC-FU is same as that in CGNC and the concentrations of 5-FU in CGNC-FU is equal to the concentration of 5-FU taken (In X-axis '1' corresponds to the control cells without samples, '2' corresponds to the concentration of CGNC as 0.03 nM and that of 5-FU as 125 nM, '3' corresponds to concentration of CGNC as 0.06 nM and that of 5-FU as 250 nM, '4' corresponds to concentration of CGNC as 0.12 nM and that of 5-FU as 500 nM, '5' corresponds to concentration of CGNC as 0.25 nM and that of 5-FU as 1000 nM and '6' corresponds to concentration of CGNC as 0.5 nM and that of 5-FU as 2000 nM).

Figure S6 corresponds to the bright field optical images under lower magnification of SiHa cells grown on ITO plate, ITO plates modified with CGNC and CGNC-FU before and after applying potential (1.5 V). The images show that the cells grow well on ITO plate and ITO plate modified with CGNC. But the growth of SiHa cells is inhibited while growing on ITO plate modified with CGNC-FU conjugate even before applying potential. After applying potential most of the cells have been shown to lose their morphology. Thus the proposed drug delivery system possesses cytotoxic effect on SiHa cells and the effect can be improved by triggering drug release by applying an electric field.

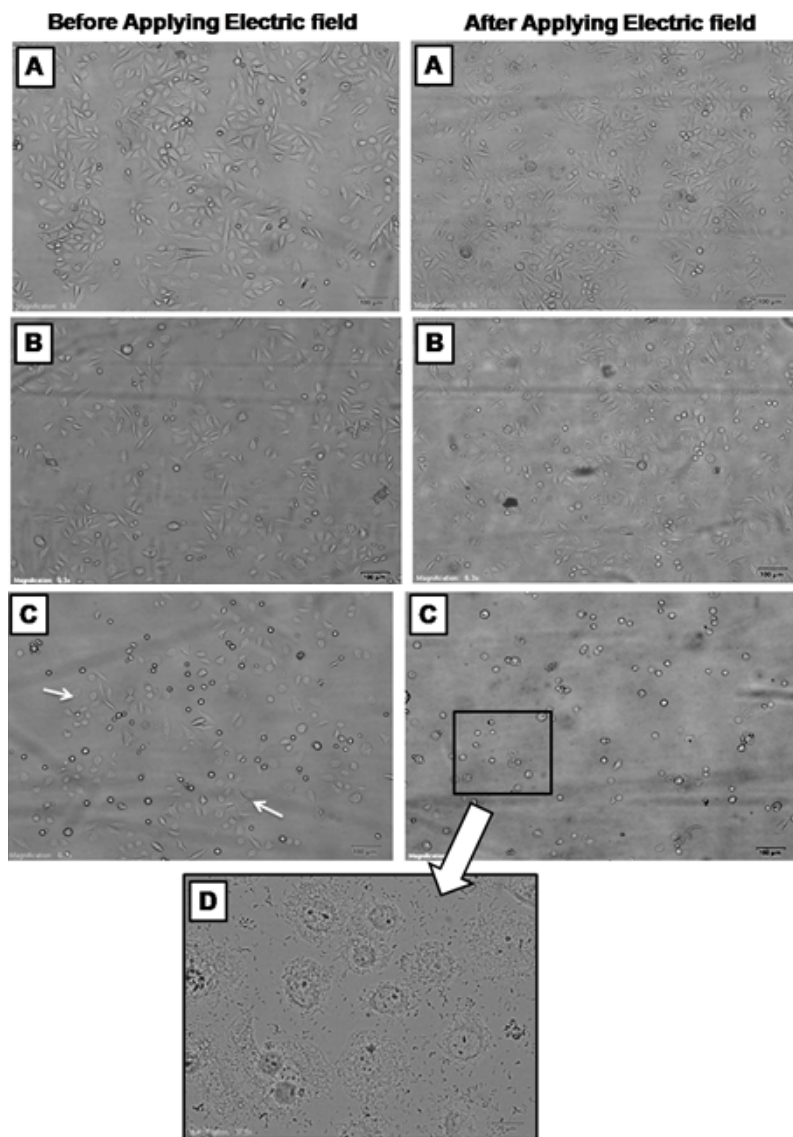


Figure S6. Bright field optical microscope images of SiHa cells grown on modified and control ITO plates before and after applying potential. 'A' shows SiHa cells on ITO plate, 'B' shows SiHa cells on ITO modified with CGNC and 'C' shows SiHa cells on ITO modified with CGNC-FU conjugate (Scale bar-100 μm). The arrow marks shown in 'C' before applying potential point out the viable cells which are absent after applying potential. 'D' shows the magnified image of 'C' after applying potential.

Figure S7 represents the drug release study on gold electrode at pH 7.4 and 5.3 in the presence and absence of external stimulus. Here we have modified the gold electrode using CGNC-FU conjugate in order to provide a biocompatible surface for drug deposition. As in the case of modified ITO plate, gold electrode also gives a high drug release percentage at pH 5.3 upon triggering by electric field.

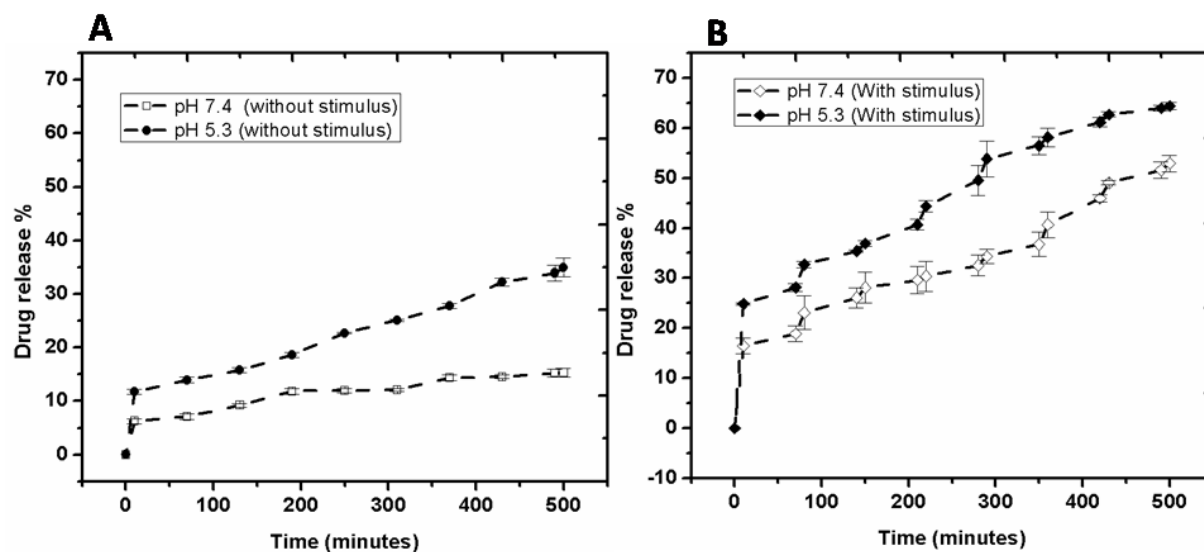


Figure S7. Plot showing drug release on gold electrode at pH 7.4 and 5.3 (A) without external stimulus and (B) with external stimulus