Supporting information

Lysozyme Activated Co-delivery of Latanoprost-Timolol from Mucoadhesive Chitosan Nanocomposite to Manage Glaucoma

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1. Preparation of simulated tear fluid (STF)

To maintain the physiological conditions for the experimental setup, we have prepared the simulated tear fluid (STF). The chemicals were used as received without further purification. In one litre beaker containing distilled water, 6.78 g/L of NaCl, 2.18 g/L of NaHCO$_3$, 1.38 g/L of KCl, and 0.084 g/L of CaCl$_2$.2H$_2$O were added and stirred for a few minutes followed by adjusting the pH to 7.4 by using 2 M HCl and NaOH (1).

2. HPLC analysis procedure

For the simultaneous quantification of the drugs, a high-performance liquid chromatography (HPLC) study has been performed as per previous reports (2,3). The chromatographic separation of simultaneous loading and release was analyzed by using Thermoscientific hypersil C18 (300 x 4.6 mm), 5μ reverse-phase analytical column. An injection volume of 20 μL with a 1 mL/min flow rate was maintained. The mobile phase consisted of Acetonitrile: Buffer (3.4 g potassium dihydrogen phosphate in 1000 mL WFI and pH adjusted to 2.8 with orthophosphoric acid) in the ratio of 50:50. The run time of the instrument was specified for 20 mins with the column over a temperature of 30 °C. Further, the detection of TM and LP was carried out at two different wavelengths such as 254 nm and 210 nm respectively.

3. Band-gap analysis

The emission spectral peak variations have been expressed in band-gap, which was calculated by using the plank's quantum energy equations (4).

$\Delta E = hc / \lambda_{cut\ of}$

Where 'h' denotes planks constant, 'c' represents the speed of light, $\lambda_{cut\ of}$ considered as the wavelength of emission of GQDs, wavelength as 377.2 nm, and 401.77 nm for GQDs and drug nanocomposite.

4. Application of *in vitro* drug release data on mathematical models

Zero order model: The release of drug can be expressed by the equation (5,6):

$$C_0 - C_t = K_0 t$$

$$C_t = C_0 + K_0 t$$

C_t refers to the amount of drug released at time t, C_0 is the initial concentration of drug at time t=0, K_0 is the zero-order rate constant. Here, the slope of the cumulative drug release vs. time plot gives the correlation coefficient (R^2) value.

First order model: The release of drug can be determined by the equation:

$$DC/dt = -K_1 C$$

K_1 refers to the first-order rate constant, expressed in $time^{-1}$ or per hour After rearranging and integrating the equation,

$$\text{Log } C = \log C_0 - K_1 t / 2.303$$

C_0 is the initial concentration of the drug, C is the percent of the drug remaining at time t. Here, the slope of the log % of drug remaining vs. time gives the R^2 value.

Korsmeyer-peppas model: Korsmeyer-peppas model is represented as:

$$M_t / M_\infty = K_{kp} t^n$$

$$\text{Log} \left(M_t/M_\infty\right) = \log K_{kp} + n \log t$$

M_t/M_∞ is a fraction of the drug released at time t, M_t is the amount of drug released in time t, M_∞ refers to the amount of drug released after time ∞, n is the diffusional exponent or drug release exponent, K_{kp} is the Korsmeyer release rate constant. Here, the graph is plotted between log cumulative % drug release vs. log time and the slope gives R^2 value.

Hixson-Crowell model: Hixson-Crowell equation can be expressed as,

$$W_0^{1/3} - W_t^{1/3} = \kappa \, t$$

Where, W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and K (kappa) is a constant incorporating the surface-volume relation.

Figure legends:

Fig.S1. Time-resolved intensity decay curves of GQD and drug nanocomposite performed at λ_{ex}=295 nm and λ_{em}=401 nm.

Fig.S2. FT-IR spectral data of prepared drug nanocomposite.

Fig.S3. FE-SEM image of the drug nanocomposite at A. 300 nm and B. 200 nm magnification.

Fig.S4. EDS image of A. GQD, B. Latanoprost, C. Timolol, and D. Drug nanocomposite.

Fig.S5. Elemental mapping (C, O, N, & S) of A. GQD, B. Latanoprost, C. Timolol, and D. Drug nanocomposite.

Scheme.S1. Diagrammatic representation of the experimental setup to evaluate the samples for ^{1}H-NMR studies.

Fig.S6. Represents *in vitro* HCE cell counts of drug nanocomposite (DC) and Lyz-treated drug nanocomposite (L-DC) of the bright field imaging study with the concentrations such as 20, 60, and 100 µg/mL. (Statistical significance is plotted in comparison to control; p-values: ns-non significant, $**p<0.01$, $***p<0.001$, $****p<0.0001$).

Fig.S7. *In vitro* AO/EB staining images of Lyz-treated drug nanocomposite against HCE cell lines with concentrations of 20, 60, and 100 µg/mL. Images were captured after 24 h of drug treatment (RFI-Red field image, GFI-Green field image, Image magnification-100 µm).
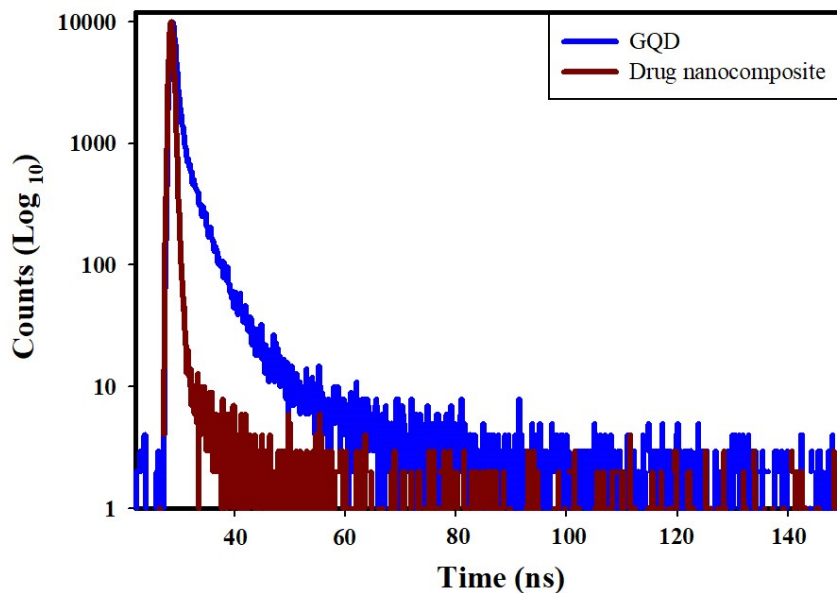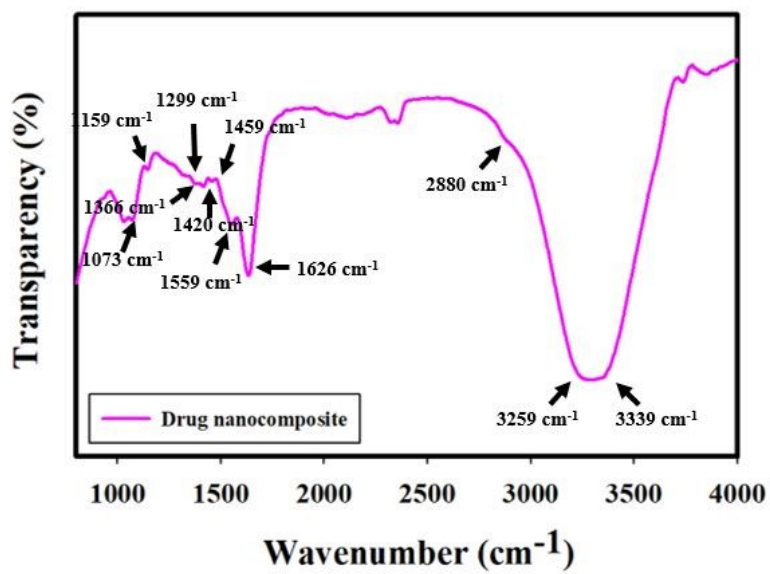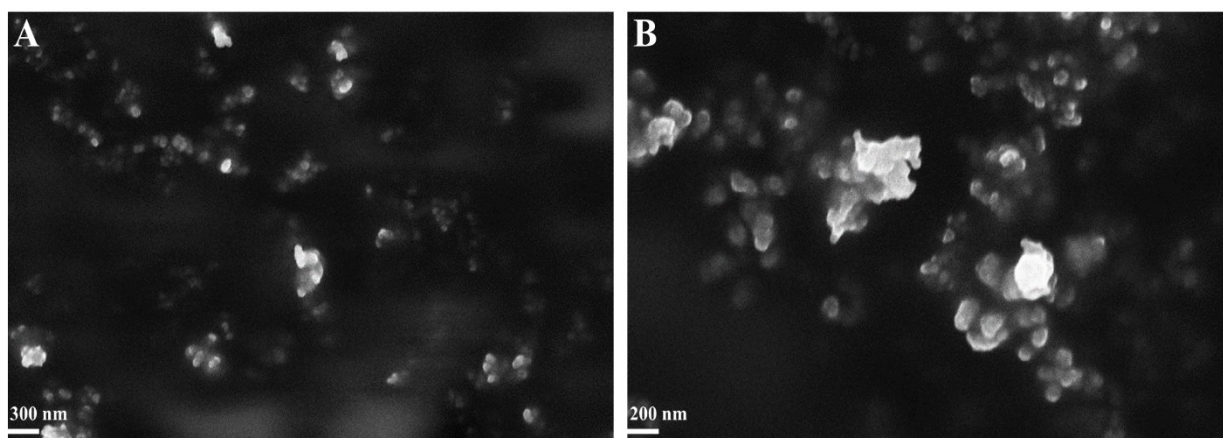
Figures:

Fig.S1.

Fig.S2.

Fig.S3.

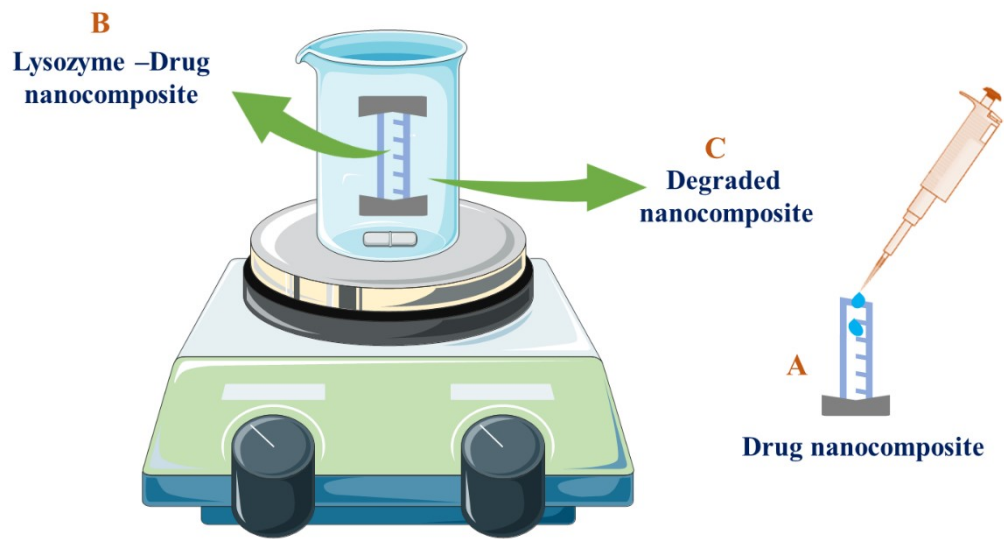**Fig.S4.**
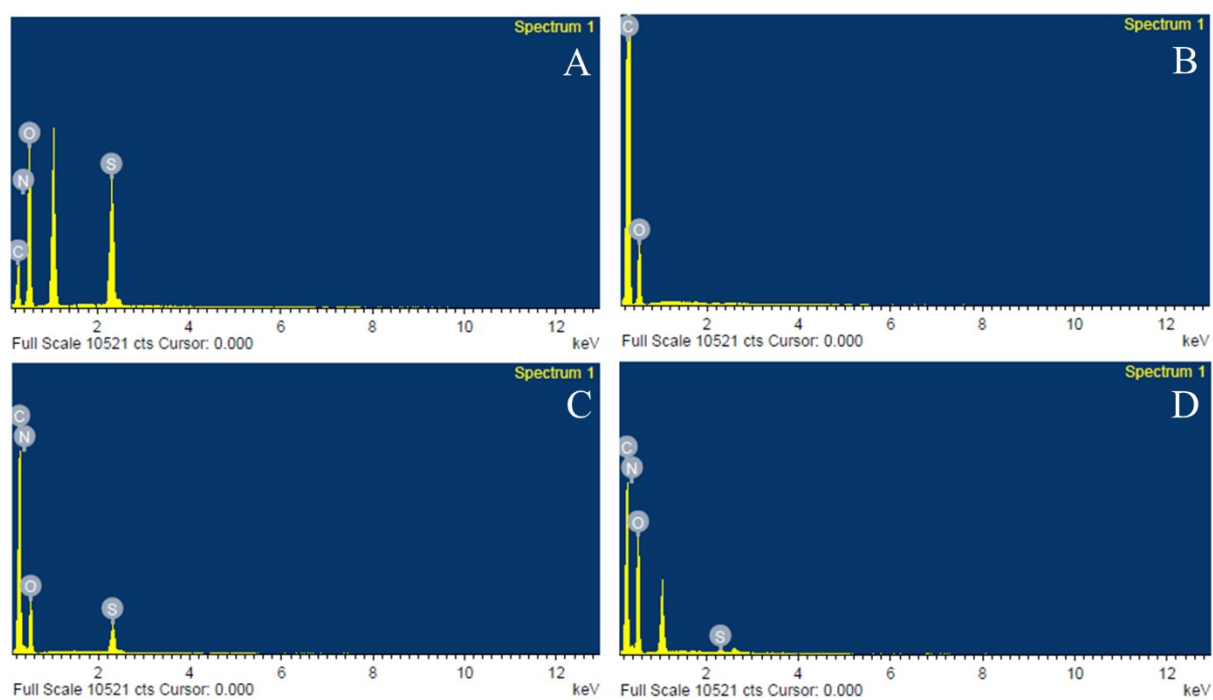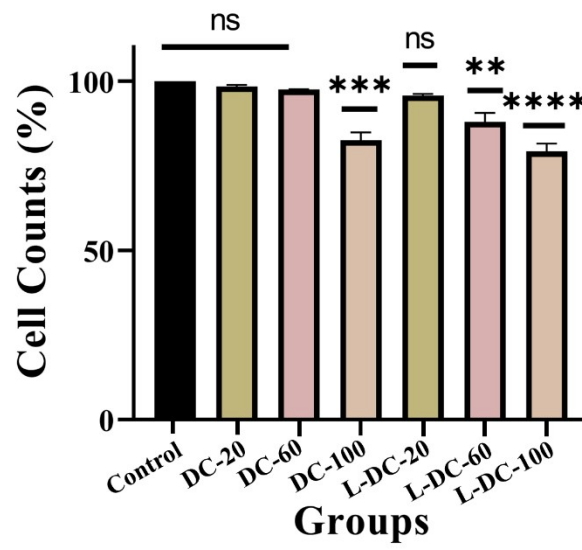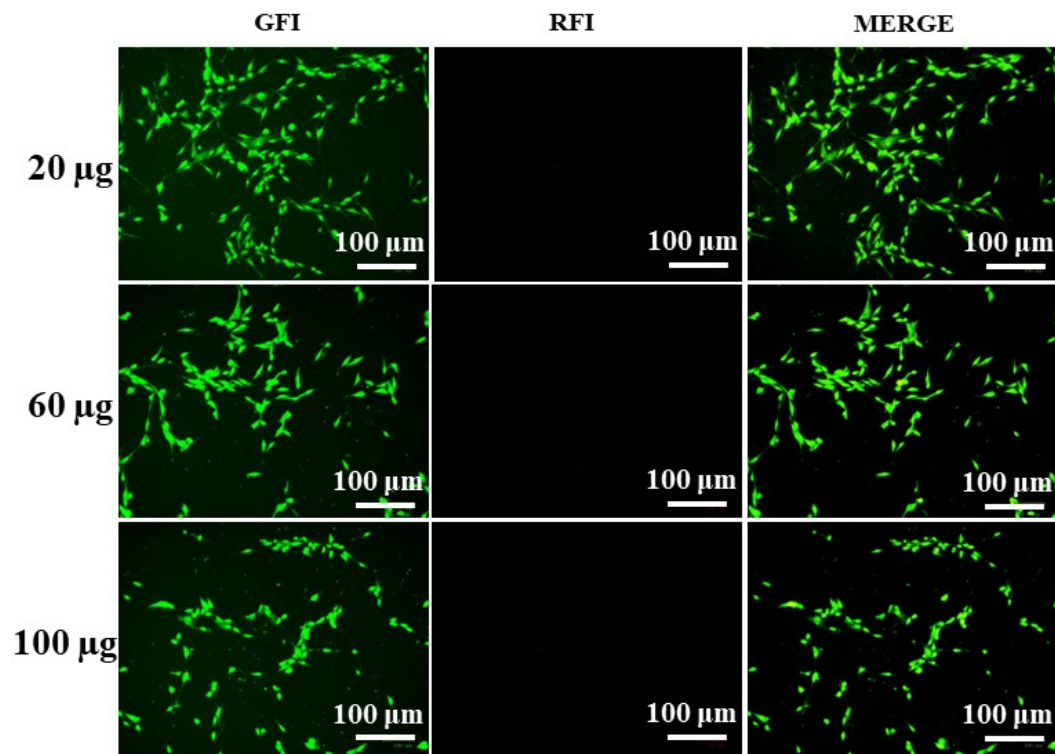
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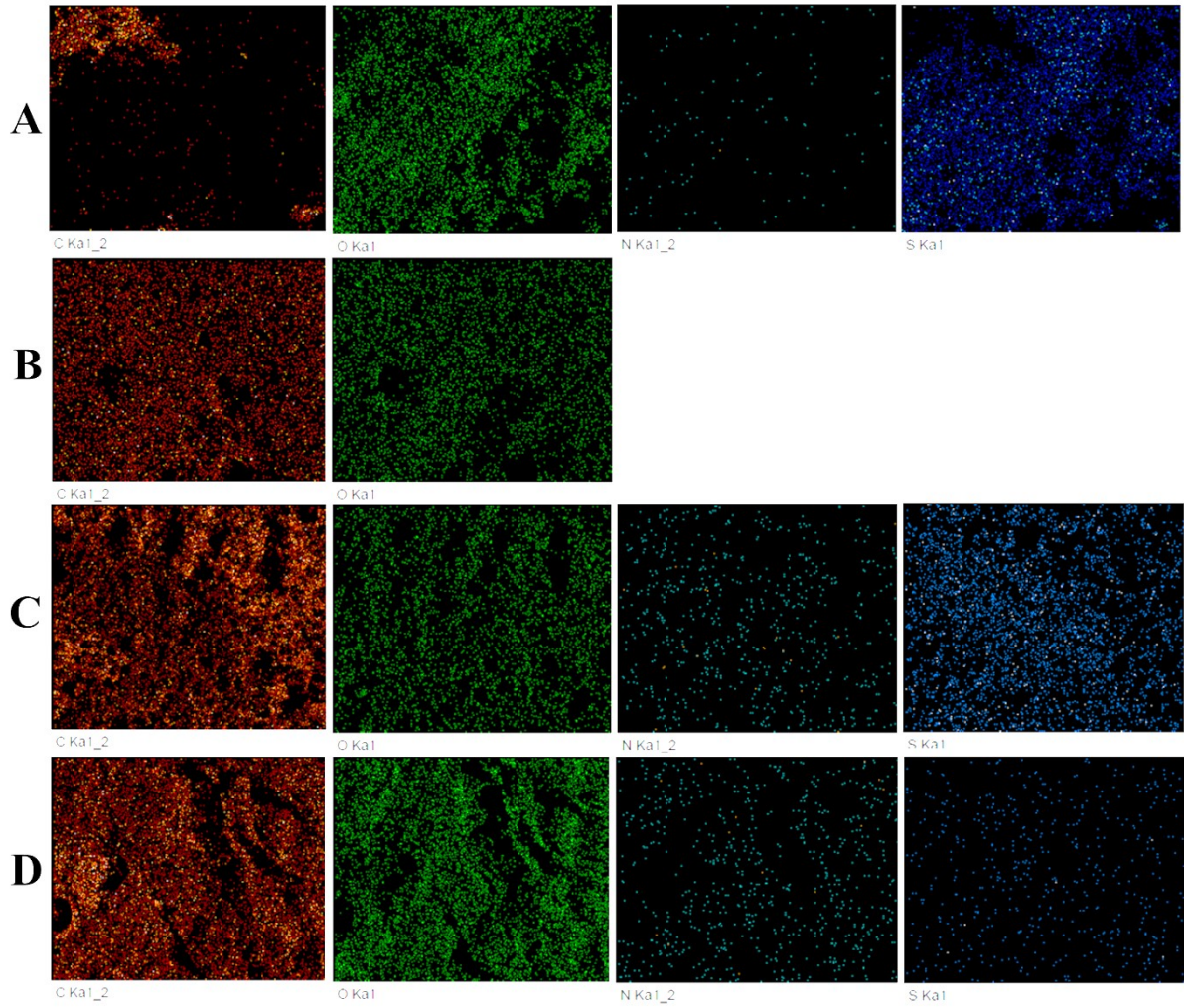
Scheme.S1.

Fig.S6.

Fig.S7.

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