TECHSCALE

Biological Evaluation of CD44-Targeted Graphene Oxide Nanocarriers for Selective Doxorubicin Delivery

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INTRODUCTION & AIM

Targeted drug delivery can improve the selectivity of anticancer therapy by exploiting specific interactions between nanocarriers and tumor-associated receptors. Hyaluronic acid (HA) is a natural ligand of CD44, a cell-surface receptor highly expressed in various cancer cells.

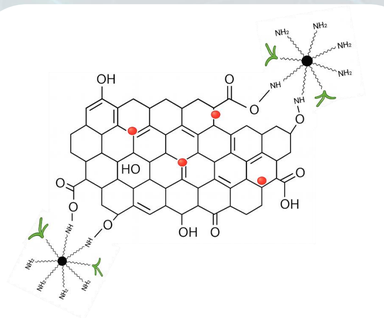
Here, we developed a PEGylated graphene oxide (GO) nanoplatform functionalized with HA and loaded with doxorubicin (DOX). We investigated whether HA surface density and cellular CD44 expression influence nanocarrier uptake, intracellular localization, and anticancer activity.

NANOPLATFORM DESIGN

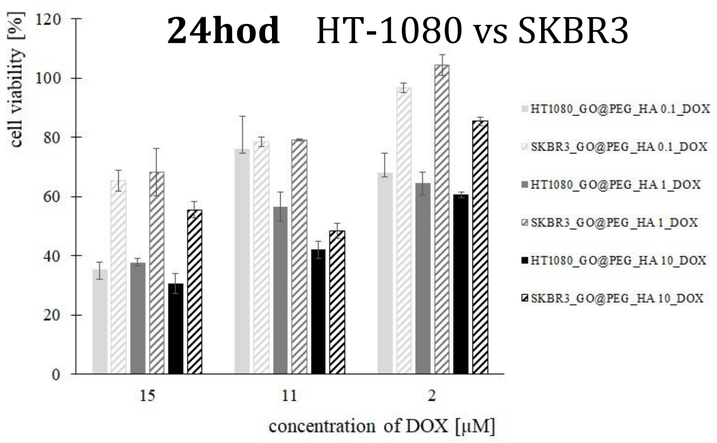
Nanosized GO was sequentially functionalized with 8-arm PEG-NH_2 and HA (0.1, 1, or 10 mg/mL), followed by non-covalent loading of DOX.

Final GO@PEG–HA–DOX nanoplatform

- 88% of analyzed flakes <400 nm
- HA-mediated CD44 recognition
- DOX loading efficiency: 11%
- DOX loading through non-covalent interactions
- unloaded GO@PEG showed minimal cytotoxicity



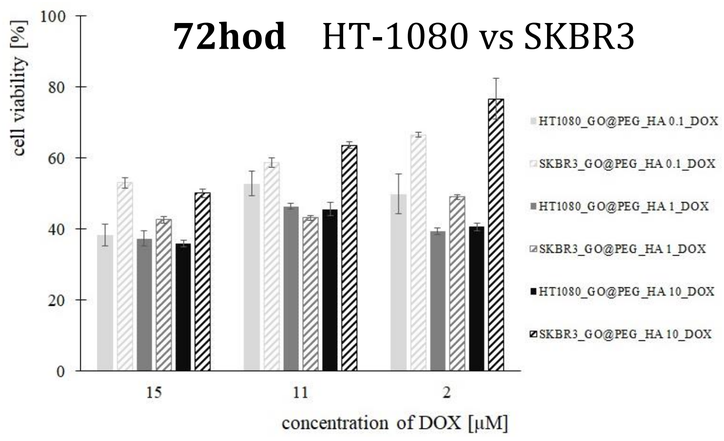
BIOLOGICAL RESPONSE: CD44-DEPENDENT SELECTIVITY



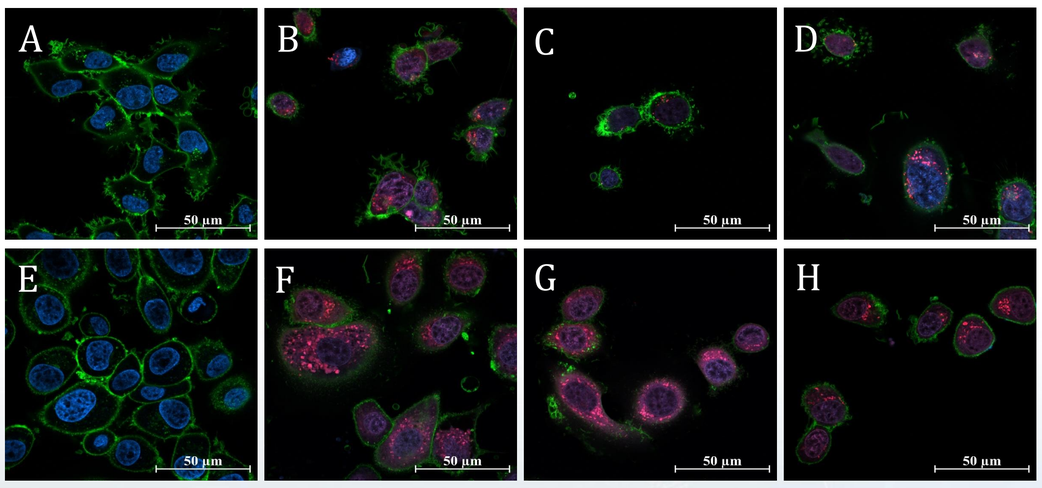
After 24 h, HA-functionalized GO@PEG–HA–DOX nanocarriers induced a stronger cytotoxic response in $CD44^+$ HT-1080 cells than in $CD44^-$ SKBR3 cells.

At 15 µM DOX, HT-1080 viability decreased below 40%, whereas SKBR3 viability remained above 60%.

The differences between the two cell models support preferential HA–CD44-mediated interaction and uptake of the nanocarrier in CD44-expressing cells.

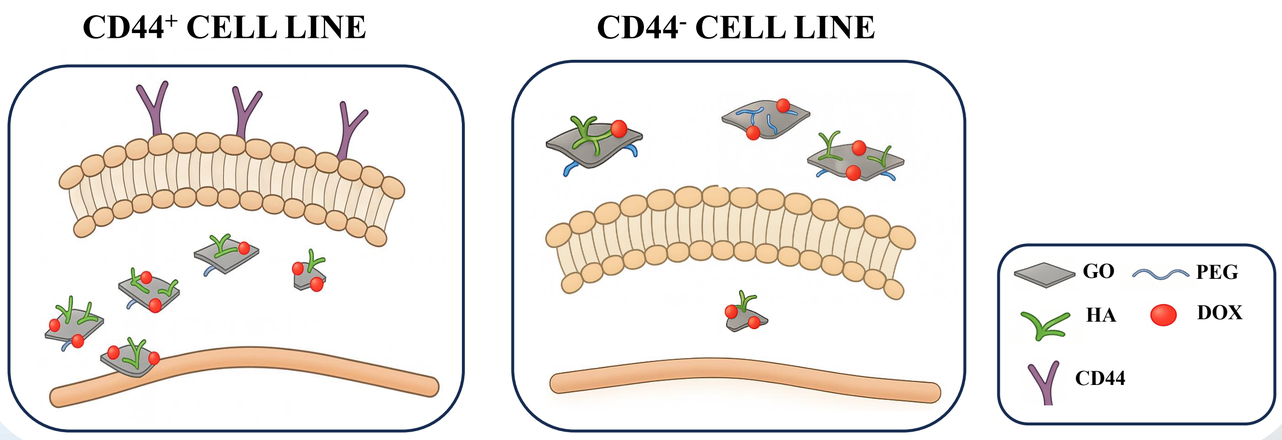


CONFOCAL MICROSCOPY



Higher intracellular DOX-associated fluorescence was observed in $CD44^+$ HT-1080 cells, with pronounced perinuclear accumulation. HT-1080 24h: A- control, B – GO@PEG_HA**0.1**_DOX c_{DOX}=2µM, C - GO@PEG_HA**1**_DOX c_{DOX}=2µM, D - GO@PEG_HA**10**_DOX c_{DOX}=2µM. SKBR3 24h: A- control, B – GO@PEG_HA**0.1**_DOX c_{DOX}=2µM, C - GO@PEG_HA**1**_DOX c_{DOX}=2µM, D - GO@PEG_HA**10**_DOX c_{DOX}=2µM.

MECHANISM OF ACTION



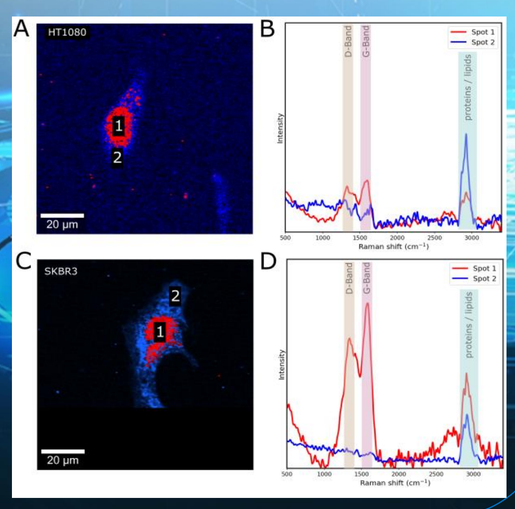
$CD44^+$ HT-1080 → enhanced HA-mediated interaction and uptake
$CD44^-$ SKBR3 → limited receptor-mediated interaction

CONFOCAL & RAMAN CONFIRM NANOCARRIER INTERNALIZATION

((A, B) HT-1080 ($CD44^+$): (A) confocal image; (B) corresponding Raman spectra from a DOX-positive intracellular region (Spot 1) and adjacent cytoplasm (Spot 2). **(C, D) SKBR3 ($CD44^-$):** (C) confocal image; (D) corresponding Raman spectra acquired under the same conditions.

DOX fluorescence → intracellular drug localization
GO D/G bands → direct detection of the nanocarrier

Co-localization of DOX fluorescence with characteristic GO D (~1350 cm^{-1}) and G (~1590 cm^{-1}) bands supports intracellular localization of the GO@PEG–HA–DOX nanoplatform.



CONCLUSION

- **HA-functionalized GO@PEG–HA–DOX showed preferential biological activity in $CD44^+$ HT-1080 cells, supporting CD44-mediated targeting.**
- **HA surface density critically influenced cellular response, highlighting the importance of ligand-density optimization.**
- **Confocal and Raman analyses supported intracellular localization of the GO-based nanocarrier and its potential for receptor-mediated DOX delivery.**

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MINISTRY OF EDUCATION, YOUTH AND SPORTS

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