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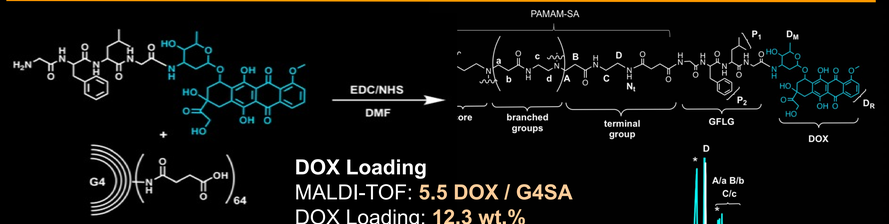
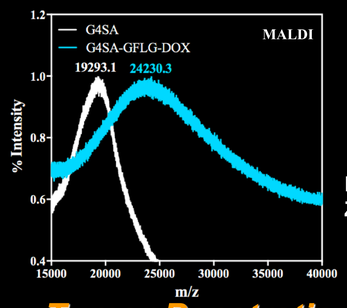
Abstract

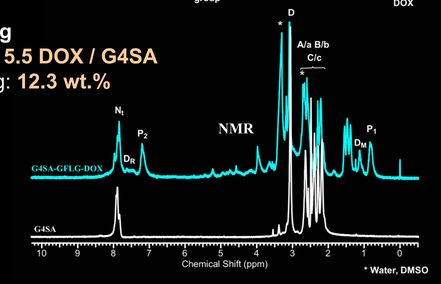
Lung cancer is the leading cause of cancer death in the United States[1]. Different from gains in survival for other types of cancers, chemotherapy has done little to improve outcomes for lung cancer patients in the past decades[2]. The inability of certain powerful chemotherapeutics such as doxorubicin (DOX) to penetrate deep into the complex tumor microenvironment (TME)[3] is an important issue to be addressed in the treatment of primary and secondary lung tumors. The purpose of this study was to design a nanocarrier capable of modulating the interaction of DOX with the extracellular matrix (ECM) present in the TME so as to promote the penetration of DOX and enhance its cytoreductive ability. An *in-vitro* 3D lung adenocarcinoma coculture model was established. The expression of collagen and fibronectin (ECM) was ascertained with immunofluorescent staining. DOX was conjugated to succinamic acid terminated, generation 4, poly (amido-amine) dendrimers (G4SA) through a degradable peptide bond (GFLG) resulting in a carrier with 5.5 DOX, 10nm hydrodynamic diameter and -17mV zeta potential. The penetration of conjugated DOX was investigated as a function of time by measuring its Mean Fluorescence Intensity using confocal microscopy, and cell kill using Caspase 3/7 activity. DOX in the form of G4SA-GFLG-DOX conjugates was seen to penetrate at a greater rate and extent into the co-culture spheroids as compared to free-DOX. Its ability to induce apoptosis and reduce the rate of growth of the co-culture spheroid also increased when in conjugate form.

Nanocarrier Design: G4SA-GFLG-DOX



DOX Loading
MALDI-TOF: **5.5 DOX / G4SA**
DOX Loading: **12.3 wt.%**

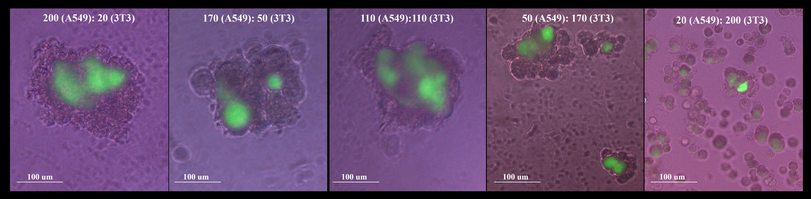




Hydrodynamic Diameter: 10 nm
Zeta Potential: -17 mV

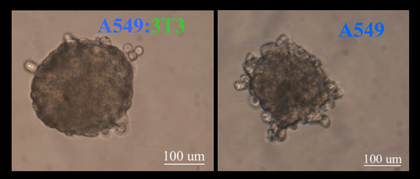
G4SA = SA(COOH)-modified PAMAM dendrimer
DOX = doxorubicin **GFLG** = degradable peptide

Tumor Model: 3D Co-culture Spheroids



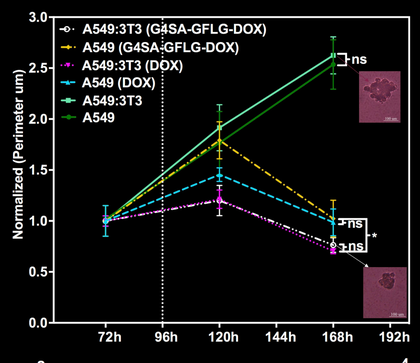
Impact of Seeding Ratio A549:3T3:
200:20 & 170:50 → single tight spheroids
≥ 110:100 → multiple loose spheroids

NIH-3T3 (3T3): mouse fibroblast
A549: human alveolar carcinoma

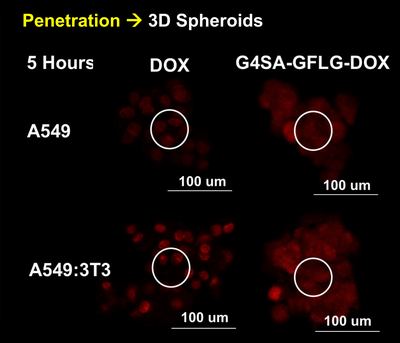


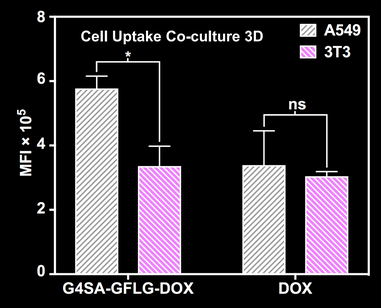
- **Single Culture Spheroids:** 220 A549 cells
- **Co-culture Spheroids:** 200:20 A549:3T3 cells

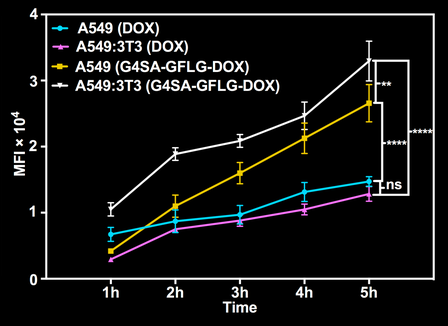
Tumor Penetration: G4SA-GFLG-DOX vs. DOX



Penetration → 3D Spheroids

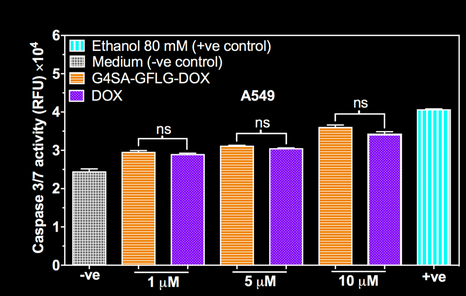


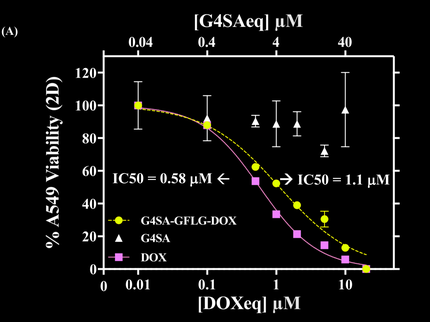


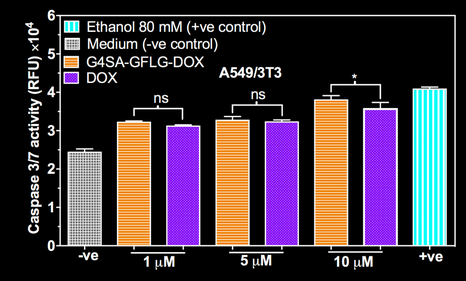


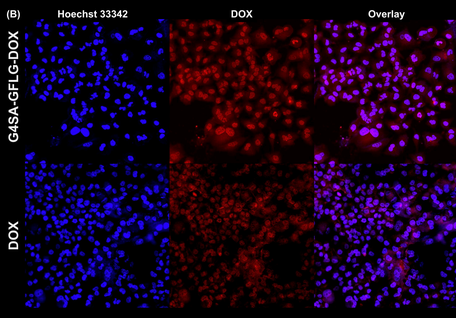
- **G4SA-GFLG-DOX**
  → Inhibits tumor growth as free DOX → effective conjugation strategy
  → Higher rate and extent of penetration in A549:3T3 → negative charge
  → Higher uptake in A549 than 3T3 in A549:3T3 → also affects penetration

Cell Kill: G4SA-GFLG-DOX vs. DOX



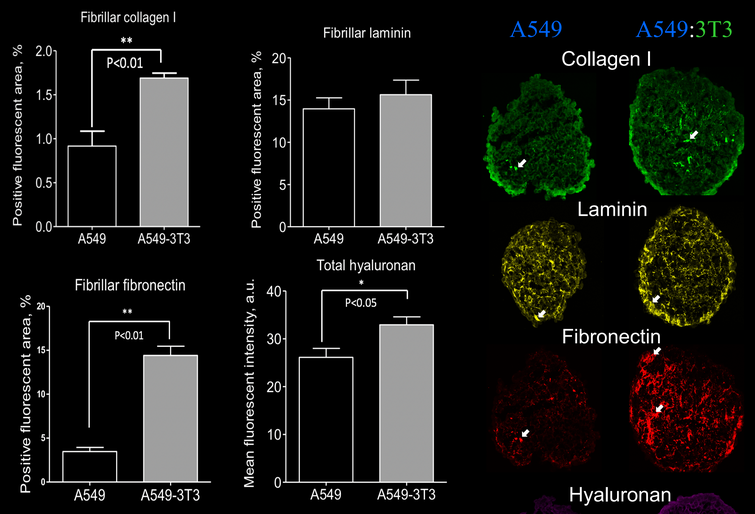






- **General trend → free and conjugated DOX → ↑ [DOXeq] → ↑ apoptosis / cell kill**
- **DOX in Conjugate → cell kill in 3D and 2D & accumulation in nucleus → good conjugation strategy**
- **G4SA-GFLG-DOX → apoptosis in A549:3T3 spheroid > free DOX (at 10μm)**

Tumor Model: ECM Expression



- A549:3T3 higher fibrillar ECM than A549
- Fibrillar ECM → barrier for transport of positive charge species

Conclusions

Nanoconjugate design → G4SA-GFLG-DOX with 5.5 DOX, 10nm and negative charge
3D Tumor model → single tight spheroids at 200 A549 and 200:20 A549:3T3
ECM expression → A549:3T3 > A549 → barrier for penetration of positive charge species
G4SA-GFLG-DOX → greater penetration in A549:3T3 than free DOX
Cellular uptake affects penetration → A549 greater uptake of G4SA-GFLG-DOX than 3T3
Conjugation strategy works → DOX released → nuclear colocalization & growth inhibition
Apoptosis correlates with penetration → ↑ penetration → ↑ apoptosis in A549:3T3 10μM

References

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3. Kohno et al. *J Cancer Res Clin Oncol* 1994, 120, (5), 293-7.

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The da Rocha Group