Aptamer-Conjugated Gold Nanoparticles Targeting Human Epidermal Growth Factor Receptor 2 (HER2) for Cancer Theranostic, *In Vitro* Assays.

Paola Y. Carrión García [1], Celia N. Sánchez Domínguez [1], *, Hugo L. Gallardo Blanco [2], Elsa N. Garza Treviño [1], Jesús R. Delgado Balderas [3], Jorge A. Roacho Pérez [1] and Margarita Sánchez Domínguez [4]

1 Universidad Autónoma de Nuevo León, Facultad de Medicina, Departamento de Bioquímica y Medicina Molecular. Av. Dr. José Eleuterio González 235, Mitras Centro, 64460 Monterrey, Nuevo León, México.
2 Universidad Autónoma de Nuevo León, Facultad de Medicina, Departamento de Genética. Av. Dr. José Eleuterio González 235, Mitras Centro, 64460 Monterrey, Nuevo León, México.
3 Delee Corp, California, United States; Universidad Autónoma de Nuevo León, Facultad de Ciencias Químicas. Avenida Universidad s/n, Cd. Universitaria, Ciudad Universitaria, 66455 San Nicolás de los Garza, Nuevo León.
4 Group of Colloidal and Interfacial Chemistry Applied to Nanomaterials and Nanoformulations, Centro de Investigación en Materiales Avanzados, S.C. (CIMAV), Unidad Monterrey, Alianza Norte 202, Parque de Investigación e Innovación Tecnológica, 66628, México.
* Correspondence: celia.sanchezdm@uanl.edu.mx

INTRODUCTION

The human epidermal growth factor receptor 2 (HER2) is a transmembrane glycoprotein overexpressed in solid tumors such as breast and prostate, associated with aggressive behavior and resistance [1].

The monoclonal antibody Trastuzumab used as standard targeted therapy has been associated with resistance, low response and cardiotoxicity [2].

Advances in nanotechnology have highlighted the potential of its application in biomedicine for diagnosis and treatment, materials called "theranostics" [3].

We investigated the potential of aptamer-functionalized gold nanoparticles (AuNPs) as a theranostic tool, comparing its effect with trastuzumab and the ability to detect HER2 overexpressing cells.

METHODS

- AuNPs-PEG 5 kDa were conjugated with AptHer2 aptamer through a maleimide-tiol link and characterized with UV-Vis, DLS, ζ Potential, and TEM.
- Celular viability was tested with the MTT assay on Vero CCL-81 cell line (non-cancerous), LNCaP (low HER2 expression), ZR-75-30 (HER2 overexpression) and HCC1954 (HER2 overexpression, resistance to trastuzumab).
- Hemolysis test was performed in treatment with nanosystem and trastuzumab.
- Binding capacity of nanosystem was tested on Vero CCL-81 and ZR-75-30 cell suspension with fluorescence detection at 664 nm.

RESULTS AND DISCUSSION

Characterization: AuNP-PEG-AptHer2 nanosystem absorption spectrum (Figure 1a) showed three main peaks corresponding to nucleic acid absorption, localized surface plasmon resonance (LSPR) of AuNPs, and fluorophore ATTO647N absorption. TEM micrography (Figure 1b) demonstrated spherical morphology with average core diameter of 19.21 nm. The nanosystem possessed a hydrodynamic diameter of 65.30 nm and a superficial negative charge of -17.4 mV (Table 1). This increment in the diameter of 20 nm core AuNPs could be due to the coating of PEG 5 kDa and conjugation with AptHer2 aptamer to the surface [4].

Table 1. Dynamic light scattering (DLS) and ζ Potential measurements of AuNP-PEG-AptHer2.

Sample	Hydrodynamic diameter (nm ± SD)	ζ Potential (mV ± SD)
AuNP-PEG-AptHer2	65.30 ± 1.12	-17.4 ± 0.68

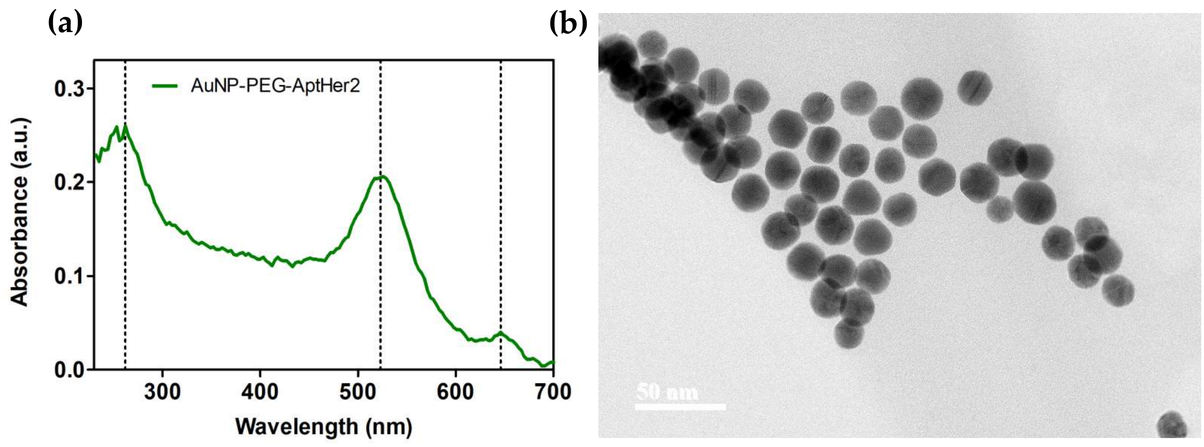

Figure 1. Characterization of AuNP-PEG-AptHer2. a): UV-Vis absorption spectrum. b): TEM micrography.

Cell viability: Trastuzumab treatment demonstrated non-specific cytotoxic effect on Vero CCL-81 cell line (Figure 2a) and LNCaP cell line (Figure 2b). ZR-75-30 cell line (Figure 2c) demonstrated a reduction in viability when treated with nanosystem and trastuzumab. Treatment of HCC1954 resistant cell line with nanosystem (Figure 2d) showed significant cell viability reduction at all evaluated concentrations.

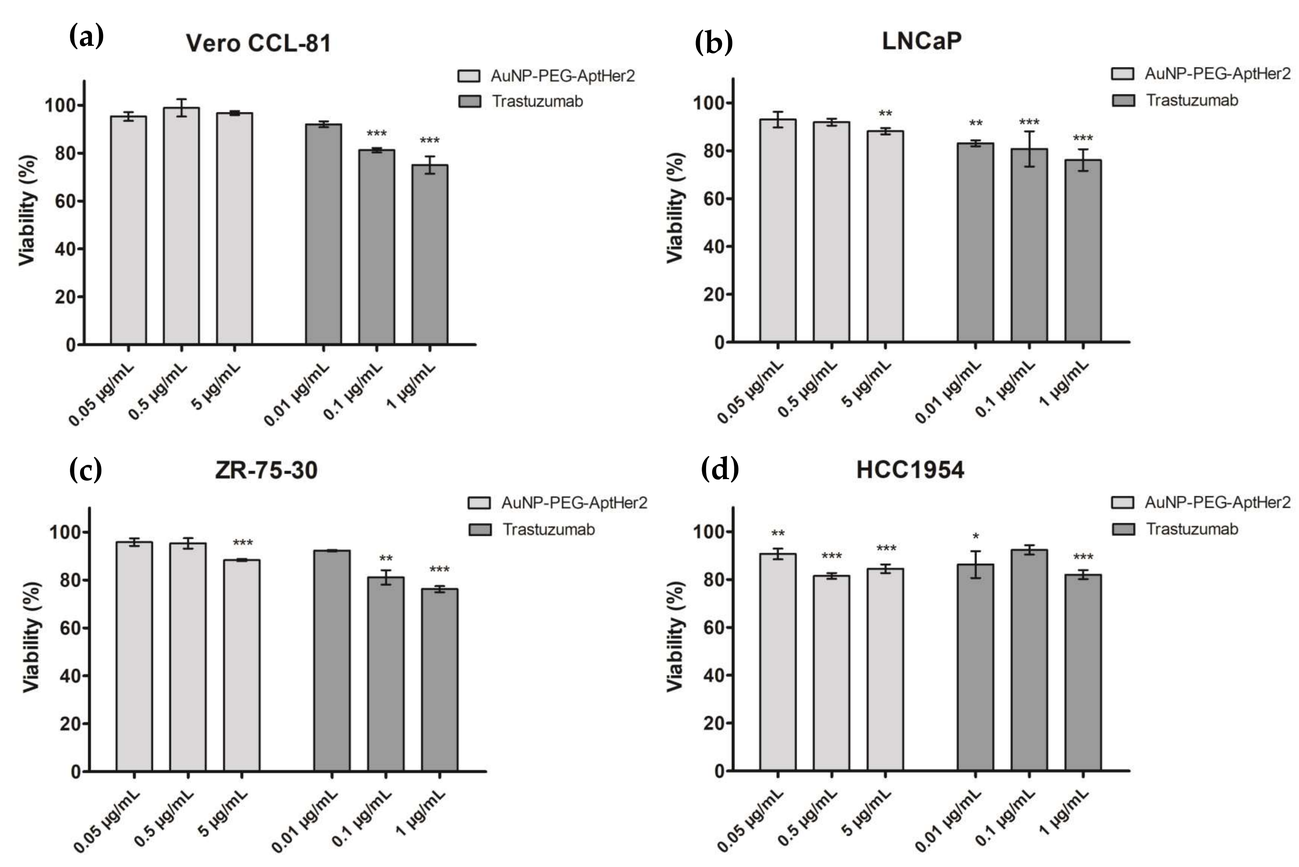

Figure 2. MTT viability assays in: (a) Vero CCL-81 cell line (b) LNCaP cell line (c) ZR-75-30 cell line (d) HCC1954 cell line. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significant vs. non-treated control.

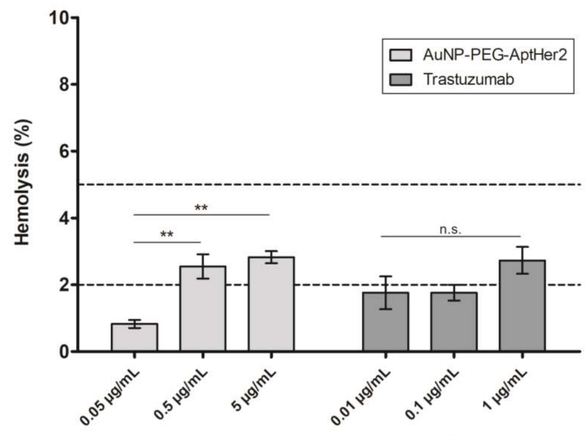

Figure 3. Hemolytic activity of AuNP-PEG-AptHer2 and trastuzumab. ** $p < 0.001$ significant vs. non- treated control, n.s.: no significance.

Hemolysis assay: Based on the ASTM-F756-17 standard practice for the evaluation of the hemolytic properties of materials, treatment with AuNP-PEG-AptHer2 nanosystem and trastuzumab (Figure 4) demonstrated slight to non-hemolytic activity of < 3% hemolysis.

Fluorescence detection: Incubation of Vero CCL-81 cells with nanosystem (Figure 4) demonstrated a low fluorescence intensity of 8%, while incubation of ZR-75-30 cells with nanosystem demonstrated high fluorescence intensity of 90%, suggesting target specific binding [5].

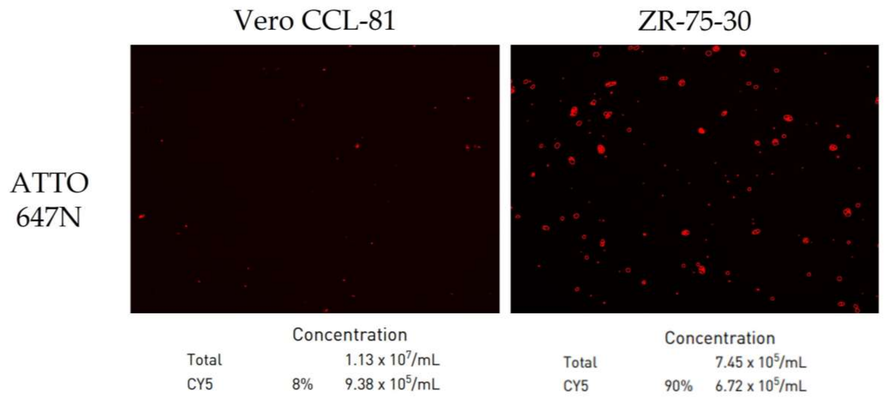

Figure 4. AuNP-PEG-AptHer2 fluorescence detection of Vero CCL-81 and ZR7530 cell lines.

CONCLUSION

AuNP-PEG-AptHer2 nanosystem selectively reduced cell viability in HER2 overexpressing cell lines and showed slight to no hemolytic activity at the evaluated concentration. The fluorescence detection suggests that nanosystems can specifically bind to HER2 targeted cells. These results provide insight into the potential of targeted gold nanoparticles in cancer theranostics.

ACKNOWLEDGEMENTS

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