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Synthesis, purification and characterization of aptamer peptides to synthesize anticancer bioconjugated

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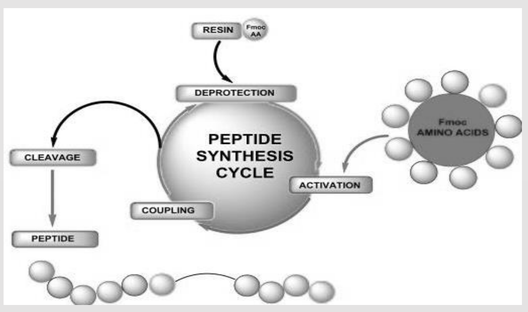
INTRODUCTION

Conventional cancer treatments have side effects, decreased quality of life of patients and may contribute to multidrug resistance.[1,2,3] An alternative to the current anticancer treatments is the use of antitumor peptides, such as Melittin (sequence GIGAVLKVLTTGLPALISWIKRKRQQ), because they are molecules that inhibit the proliferation of tumor cells and are selective to tumor targets. [4,5,6,7] However, they have low specificity and high toxicity relative to normal cells, leading to the need to work with bioconjugated antitumor aptamers peptides, which are more selective and lead to greater efficacy in killing tumor cells. [8] Thus, the goal of the research is the synthesis of bioconjugates that have specific molecular targets, as well as the evaluation of the antitumor potential of the bioconjugates and the toxic potentials in normal cells.

MATERIALS AND METHODS

In this work, three peptides, LO1904 (sequence FNLPLPSRPLLR), LO1908 and LO1908oxi (sequence NPGTCKDKWIECLLNG) were synthesized by Solid Phase Peptide Synthesis [9] and purified by High Performance Liquid Chromatography. The strains worked on were MCF-7, MRC-5 and A549. Membrane labeling of the aptamers peptides was performed by In Cell Analyzer and flow cytometry. Cell viability was performed by MTT assays.

Solid Phase Peptide Synthesis



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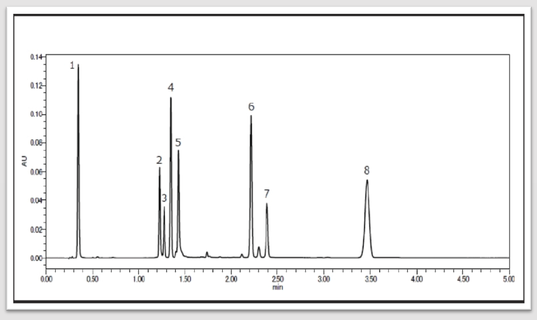
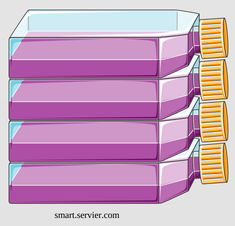
High Performance Liquid Chromatography



www.chromatographyonline.com

Cell culture



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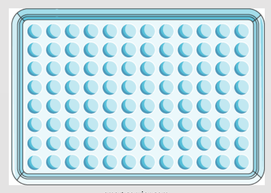
In Cell Analyzer



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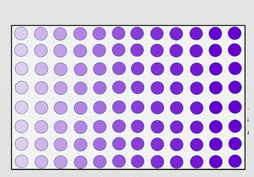
Cell viability assay



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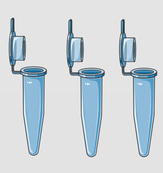
Flow cytometry



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RESULTS

High Performance Liquid Chromatography

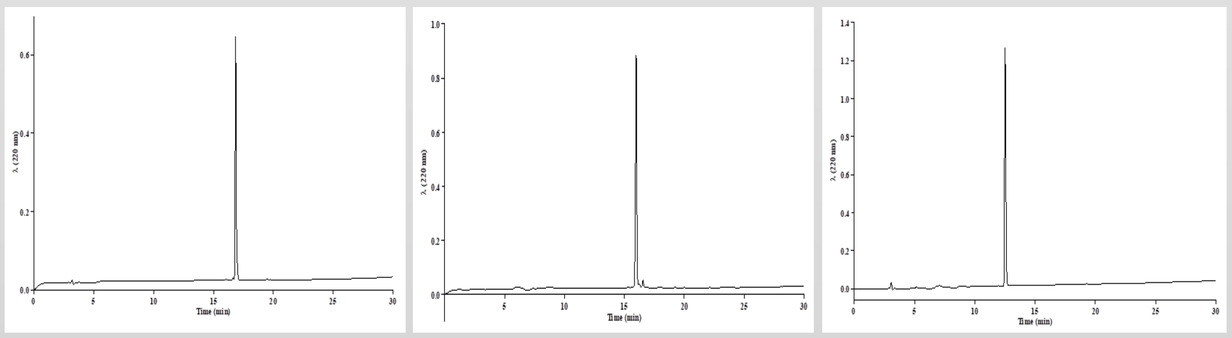

Figure 1: Chromatographic profile of pure LO1904, LO1908 and LO1908oxi peptide

In Cell Analyzer

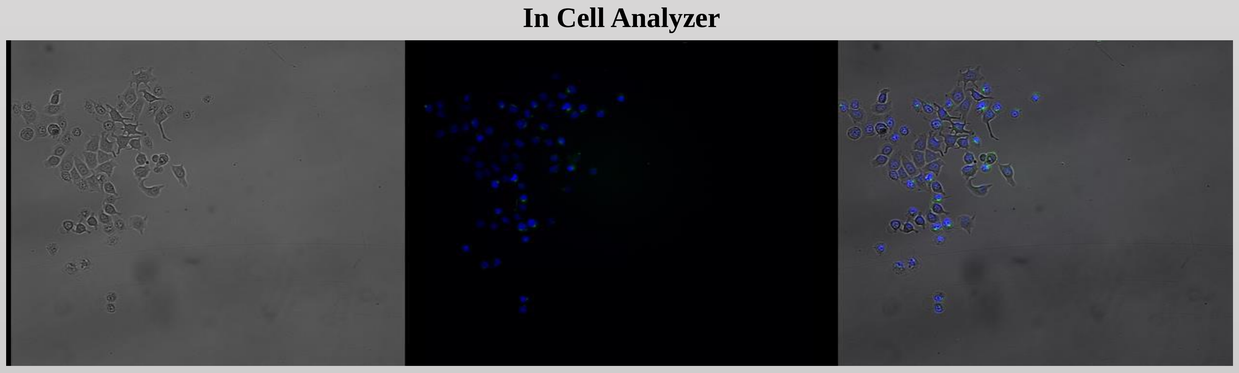

Figure 2: Automated microscopy of cell line culture plate analysis for peptide LO1904 at concentration 32µg/mL. Light field of MCF-7, dark field peptide labeling, light field of MCF-7 with peptide labeling

Cell viability assay

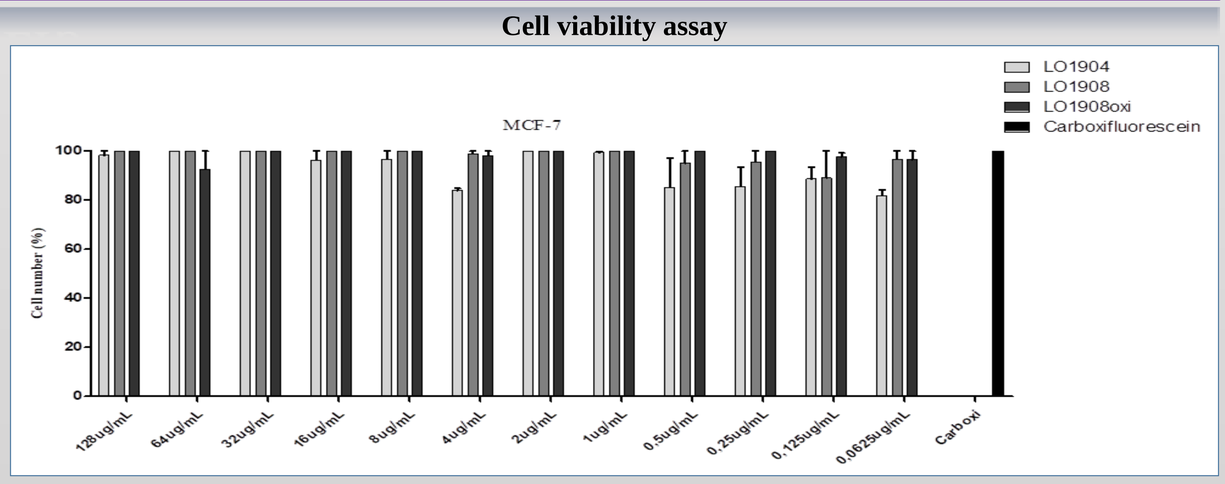

Figure 3: Cell viability assay in MCF-7 strain for peptides LO1904, LO1908, LO1908oxi and carboxyfluorescein

Flow cytometry

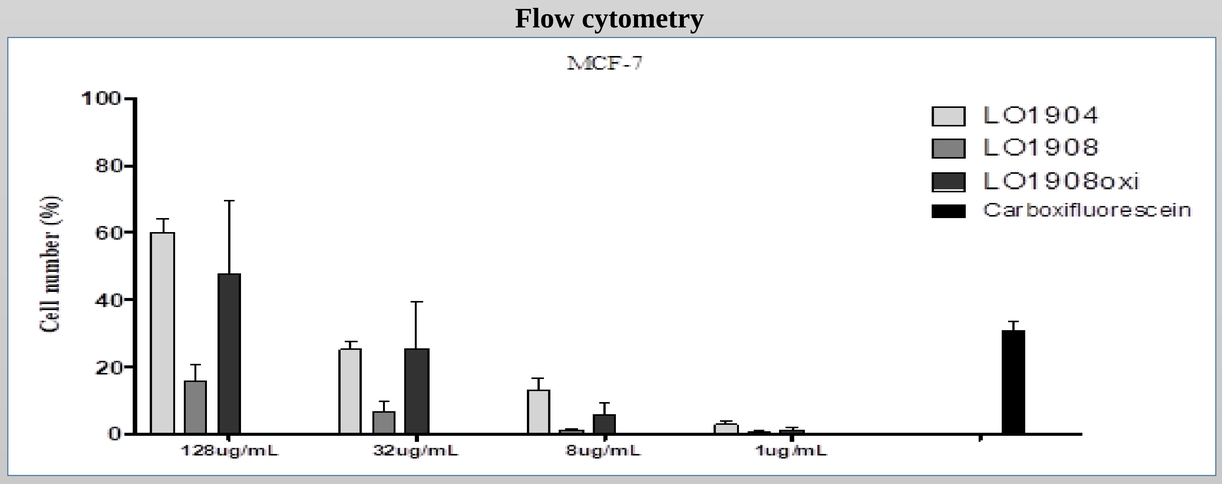

Figure 4: Labeling of the peptides LO1904, LO1908, LO1908oxi and carboxyfluorescein in the MCF-7 strain by flow cytometry

CONCLUSION

The results obtained demonstrate that the aptamer peptides did not cause the death of cell lines and that they present more evident markings on membrane proteins of the MCF-7 lineage. Melittin showed cytotoxic action in all cell lines. The results obtained demonstrate that there is a low selectivity of Melittin regarding tumor and non-tumor cells and that the aptamer peptides can bring a higher specificity and selectivity for anticancer molecules considering their binding to target proteins in tumor cells.

REFERENCES

1. BRAY, F. *et al*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. **CA: a cancer journal for clinicians**, Hoboken, v. 68, n. 6, p. 394-424, Nov. 2018.
2. OPAS. Organização Pan-Americana da Saúde. Folha informativa – **Câncer.** Organização Mundial da Saúde. 2018.
3. ACS. **American Cancer Society.** Treatment types. 2020.
4. ARMBRECHT, L. *et al*. Characterisation of anticancer peptides at the single-cell level. **Lab Chip**, v. 17, n. 17, p. 2933- 2940, 2017.
5. RADY, I. *et al*. Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy. **Cancer Lett**, v. 402, p. 16-31, 2017.
6. KLOCEK, G.; SEELING, J. Melittin interaction with sulfated cell surface sugars. **Biochemistry**, v. 47, n. 9, p. 2841-2849, 2008.
7. HEINEN, T. E.; VEIGA, A. B. G. Arthropod venoms and cancer. **Toxicon.**, v. 57, p. 497-511, 2011.
8. HOSKIN, D. W.; RAMAMOORTHY, A. Studies on anticancer activities of antimicrobial peptides. **Biochim. Biophys. Acta**, v. 1778, n. 2, p. 357-375, 2008.
9. MERRIFIELD, R.B. Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. **J. Am. Chem. Soc**., 85, 2149–2154, 1963.

ACKNOWLEDGMENTS

CAPES

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