Proceedings of the 10th World Congress on Recent Advances in Nanotechnology (RAN 2025)
Barcelona, Spain - April, 2025
Paper No. NDDTE 116
DOI: 10.11159/nddte25.116

Gene-Activated Hydrogels for the Local Production of Immunostimulatory Proteins

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Extended Abstract

Glioblastoma multiforme is an aggressive brain tumor whose treatment is based on surgical resection followed by radiotherapy and chemotherapy. Despite this, the prognosis of these patients is poor, since only 5% of them survive 5 years after the diagnosis.

Current strategies focus on applying immunotherapy to combat the strong immunosuppression in the tumor microenvironment. This technique could synergize with the present therapies and boost their efficacy. However, the protective effect of the blood-brain barrier hinders the delivery of immunostimulatory proteins into the brain.

Gene-activated matrices are versatile platforms that enable the controlled and localized production of target proteins. Fibrin hydrogels, made from fibrinogen and thrombin, have been widely studied in regeneration medicine for their superior biocompatibility and biodegradability [1]. These hydrogels can polymerize at 37ºC, making them suitable for implantation in the brain cavity after tumor resection.

Here, we propose the use of gene-activated fibrin hydrogels for the in-situ delivery of immunostimulatory proteins for the treatment of glioblastoma multiforme. Our target protein is a viral protein called VP40. This protein participates in the assembling and budding of the Ebola virus and it can form virus-like particles or VLPs in the absence of other viral proteins. These VLPs mimic the native structure of the virus and possess immunogenic properties but lack replication capacity, providing a safer profile than the use of the virus. Hence, we hypothesize that the controlled administration of these VLPs could activate the innate immune response in these patients locally and, thus, help to revert the immunosuppression in the tumor microenvironment.

Consequently, we incorporated plasmid DNA codifying for the VP40 protein into fibrin hydrogels and studied the release of VLPs over time. To optimize the production of the VLPs, we incorporated different nanoparticles (two lipid-based nanoparticles and one polymer-based) for the delivery of the genetic material. We tested the cytotoxicity and transfection efficiency in two cell lines with a model plasmid. Then, we analyzed the supernatants of the hydrogels bearing the VP40 plasmid to assess the expression of the VLPs.

None of the prototypes showed cytotoxicity *in vitro* but different efficacy between the cell lines studied was observed. Besides, we detected VLPs similar in form to those from the Ebola virus on the cell supernatants, and the expression of VP40 in these supernatants lasted at least one week. Although there were no significant differences in the amount of VP40 released between the prototypes, it seems that the polymeric nanoparticles could be more retained in the matrix and the peak of the expression could be delayed.

Finally, we developed a platform that enables the production of immunostimulatory viral proteins during a week and whose release profile may be controlled with further modifications. This platform would be of interest as a coadjuvant treatment for glioblastoma patients. Future experiments will focus on evaluating their potential in glioblastoma models.

References

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