Characterization by Electrical Impedance of an In Vitro Model Based on Tumor Cell Spheroids

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Abstract

The main objective of this work is to assess the loosening effect of blebbistatin, a specific inhibitor of myosin II activity, on glioblastoma spheroids. Electric impedance will be used to quantify the effect of this drug, with therapeutic potential for facilitating the access of other treatments into the tumor.

Introduction

Glioblastoma

Glioblastoma (GBM) is the most common type of brain cancer, and one of the cancers with a highest mortality rate, largely attributed to treatment resistance and propensity for recurrence post-surgery [1]. Furthermore, GBM tumors generate a hostile microenvironment for immune cells, decreasing their efficacy, and thereby hindering immunotherapy treatments [2].
The main objective of this work is to better comprehend the obstacles impeding immune cell action against GBM, with the ultimate goal of mitigating these barriers and improving the patient's innate defenses against the tumor.

Three-dimensional models

Thanks to the use of three-dimensional cell cultures (i.e. spheroids), the conditions of these tumors and their microenvironment can be reproduced in the laboratory. [3]. The aim is to better understand the mechanisms of immune system inhibition and to seek new strategies that enhance the effect of the cells that defend our body.

Electrical impedance

Tumor cells forming three-dimensional structures exert forces that allow the whole structure to stand together, making intercellular gaps thinner, and the spheroids more compact and denser [4]. To quantify the magnitude of these tensions, electrical impedance can be used, since current will be conducted differently according to the degree of closeness the cells present.
This differential opposition to the current depends on the composition of the cells and matrix [5], and the space between adjacent cells [6], which is the parameter that will be modified by the addition of the drug.

Materials & Methods

Spheroids

To recreate GBM microtumors, cells from the commercial U-251 MG cell line were used. The spheroid generation protocol was based on the "non-adherent surface" (NAS) method. This involves seeding an initial amount of 30,000 cells in round-bottom wells pretreated with an anti-adherent solution and accumulating all these cells at the bottom of the well through centrifugation.

Blebbistatin

To reduce the compaction that appears in these spheroids, blebbistatin, an inhibitos of myosin II activity that reduces cellular contractility, was used [7]. This compound was added to the pre-formed spheroids (two days after the spheroids sedding), at a final concentration of 10 µg/mL: Spheroids were maintained for five days and medium containing the drug was refreshed after three days.

Electrical impedance

To characterize the impedance, an indirect measurement is carried out by immersing the spheroid in a previously characterized medium. A

small cuvette was modified from a commercial electroporation cuvette (Bio-Rad) and connected to an impedance LCR analyzer by a manufactured holder.
This new cuvette has a 1 µL hole, which makes it optimal to host the spheroid inside with the minimum amount of medium surrounding it.

Results

Spheroids treatment

The NAS method resulted satisfactory, producing structures of a constant size and roundness. It was confirmed, as observed in previous experiments in our lab [8], that spheroids from U-251 MG cells reduce their volume, due to powerful intercellular forces that cause spheroid compaction (Fig. 1).

Electrical impedance

A 3D finite element (FEA) model has been developed and different culture media have been characterized, to estimate the electrical conductivity of the spheroids as a function of their size, independently of their position in the cuvette (Fig. 2).

Conclusions and future steps

Blebbistatin has proven to be a molecule capable of relaxing the intercellular forces present in GBM spheroids, thereby reducing the degree of compaction of these spheroids.
Electrical impedance tests may be appropriate for estimating the magnitude of physical changes in tissue structure as they may modify its electrical parameters.
The next steps following these experiments will involve the addition of immune cells, antibodies, and traceable small molecules, such as fluorescent dextran, to compare their penetration into the spheroid with and without the effect of blebbistatin.
In addition, new measures will be carried out with our impedance characterization system, including spheroids of different cell lines. This will allow further study of *in vitro* tumor models and their use for the evaluation of therapies such as electroporation [9].

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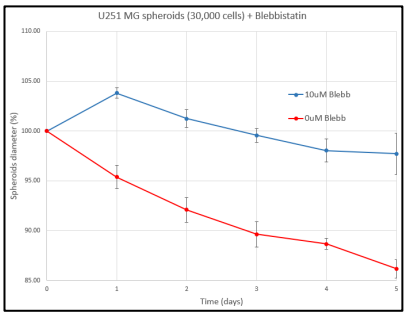

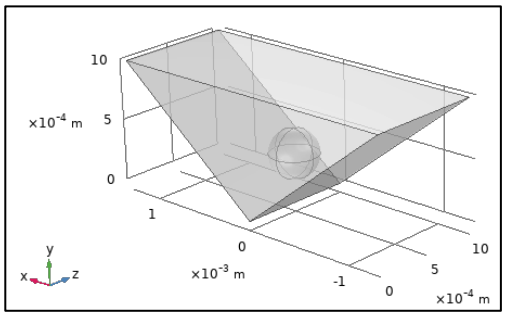

Figure 1: Evolution of spheroids generated from 30,000 U-251 cells
Figure 2: FEA model of the proposed impedance characterization system