Cell Culture of Human Dermal Fibroblasts and Efficacy of Lipid Nanoparticles Used in COVID-19 Vaccines

Introduction:

For the VKURA internship, I worked in Willerth Labs run by Dr. Stephanie Willerth over the summer. Their specialty is tissue engineering and therapeutics [1]. During my internship, I helped in culturing human dermal fibroblasts (HDFs) for scientific research and wrote sections of a review paper on nanotechnology used in COVID-19 vaccines and therapeutics. One of the sections I worked on was about the efficacy of lipid nanoparticles used in mRNA vaccines and what current research may entail for the future of nanotechnology in medicine.

Culturing Human Dermal Fibroblasts

What are They?

Dermal fibroblasts are the most common cell type found in the dermis layer of the skin [2]. They aid in wound healing [3] and produce proteins to strengthen the dermal layer and interact with epidermal cells [2]. In stressful conditions where wound-healing is involved, they also aid in regulating inflammation and cellular growth [4]. Under a microscope, they have the appearance of flat cells with elongated threads that connect to other cells.

Method:

-For culturing HDFs, I referenced the provided HDF protocol by Cell Applications, INC [5].

-Steps described below are for educational purposes only and should not be used for instruction. The method below only briefly summarizes the process, and I recommend reading the HDF protocol [5] for a thorough explanation of the process. Always talk to your supervisor should any questions arise and follow proper safety protocol.

Step 1 (Seeding):

To start the culturing process, we thawed a fresh batch of HDFs from the liquid nitrogen tank and prepared flasks for the cells to grow. Once appropriate flasks were sterilized and filled with 15 ml of Fibroblast Growth Medium (FGM), the cells were be seeded into the flasks [5]. The cells then needed to be stored in the incubator for 24 hours before the first media change meant for removing traces of DMSO [5]. Since I was still training, I shadowed my colleague as she seeded the first batch of HDFs. Because newly grown cells can be extracted and seeded for more batches of HDFs, I seeded later batches using cells extracted from the passaging process instead of frozen cells.

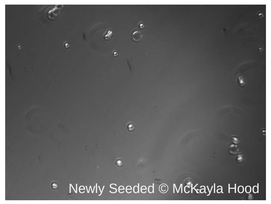
Step 2 (Maintenance):

To keep the cells healthy, I changed the media every two days [5] and took pictures under the microscope to ensure the cells were growing well and that they were not contaminated.

Cell Growth

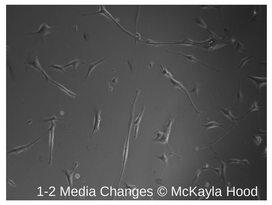
-The images below depict human dermal fibroblasts (HDFs) at different stages of growth during the culturing process.

Newly Seeded



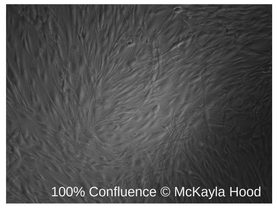
-When HDFs are newly seeded, they have a spherical shape without threads. There is also lots of room in the flask for new cells to grow.

Around 1-2 Media Changes



-Overtime, the cells produce threads that reach out to other cells, and new cells start to fill up the flask. The growth rate of cells is highly variable depending on the batch of cells seeded and the seeding density.

Ready For Passaging



-Once cells have grown to around 80-100% confluence, they are ready to be passaged and stored in liquid nitrogen [5].

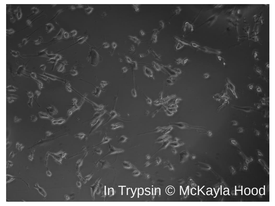
Step 3 (Passaging):

Passaging is the process where cells grown in the flasks are extracted and stored in the liquid nitrogen tank. For the HDFs, we extracted the cells using Trypsin to detach the cells from the bottom of the flasks [5]. After the cells were extracted, we counted the cells we had using trypan blue and a cell counter. Once we had a good estimate of how many cells we had, we seeded a portion of the new cells into new flasks and stored the remaining cells in a cryovial with freezing media.

Before Trypsin

-Cells are at around 80% confluence, so they were ready for passaging.

Cells in Trypsin



-During the passaging process, cells are removed from the flask by soaking them in Trypsin. The Trypsin detaches them from the flask and induces them to form clumps. Once the cells form tight clumps, they can be tapped and removed from the flask [5].

Efficacy of Lipid Nanoparticles in COVID-19 mRNA Vaccines

To stop the spread of COVID-19, multiple vaccines have been put in development and observed for their efficacy against the virus. With these developments have come the introduction of new mRNA vaccines [6]. They function by using host cell mechanisms to produce viral proteins [6]. Perks of these formulations are that they are non-infectious and can be rabidly produced [7] which is highly beneficial during the time of a pandemic where a deadly virus is widely spread. While these benefits show a lot of promise, there are challenges that come with mRNA formulations. These include present enzymes that degrade the mRNA molecules and the negatively charge of mRNA that makes it difficult to penetrate negatively charged cell membranes [7]. This is where lipid nanoparticles (LNPs) are beneficial as they aid in preserving mRNA molecules and the penetration of cells [7]. However, when implementing LNPs into mRNA vaccines for medical practice, it is important to observe the longevity and toxicological aspects of these vaccines.

- Below is a quote from a section I wrote for the COVID-19 nanotech review. During the making of this poster, the review was still in progress and this section has already been altered and incorporated with another student's work. To avoid using parts of that student's section, I decided to share an older version of the section.

"Two vaccines currently in use are the Moderna and Pfizer/BioNTech vaccines [6]. Both vaccines showed efficacies of around 95% [8]. This indicates that similar formulations for future vaccines may be great advancements for preventative medicine. However, there are issues with these formulations that must be considered. For example, due to the unstable nature of these vaccines, they must be stored at extremely low temperatures [8]. Another aspect to consider is the longevity of the vaccines. According to a study where the antibody and T-cell responses of 122 individuals were analyzed for six months after two doses of the Pfizer vaccine, after six months post second dose, the median levels of S-RBG IgG dropped to 7% of their peak potencies [9]. This may be an indicator that overtime the immunity brought by these vaccines may have short lifespans and require constant boosters. Evidence shows that a gradual decrease in efficacy of the vaccines may be due to the lipid nanoparticles used. A study examining interactions between lipids and mRNA found mRNA impurities caused by ionizable lipids which disrupt mRNA translation and can negatively impact the efficacy of mRNA-lipid based products [10]. There are also possible health effects of lipid nanoparticles that need to be considered. A study where empty lipid nanoparticles formulated with phosphate buffered saline was injected in mice found intense inflammation caused by the lipids which could possibly lead to side-effects in mRNA vaccines [11]. This may be an indicator that for future uses of lipid nanoparticles in vaccines, more studies need to be made to find ways to combat the negative effects of using nanoparticles while preserving its effects. This may be in forms of improving upon current formulations using lipid nanoparticles or creating secondary drugs and devices for lessening the negative effects." [12]

Acknowledgements

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Sources

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