FMRI RET 2016-Nanofibers Used as Tissue Scaffolds for Wound Healing

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Abstract

This research involves electrospinning polystyrene and cactus mucilage solutions in varying concentrations to create nanofiber membranes that will be used to test for cell proliferation. Electrospinning involves placing a polymer solution into a syringe to which an electric field is then applied, which overcomes the surface tension of the polymer solution. A polymer jet is then ejected from the syringe needle tip, undergoes plastic stretching, and is deposited onto the collector as extremely thin fibers that range in diameter from nanometers to a few microns [1]. Incorporating different substances into the polymer solution gives the resulting nanofibers different properties. Our research focuses on determining the effect of cactus mucilage gelling extract from the prickly pear plant, *Opuntia ficus-indica,* on cell proliferation in the nanofiber membrane for its use as a tissue scaffold in wound healing.

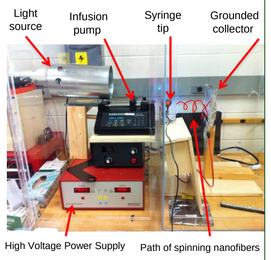

Figure 1. Electrospinning Equipment

Background

Polymeric scaffolds to promote wound healing have been used successfully in the last few years, replacing the standard tissue graft that causes the patient to have a second injury as tissue is removed from another part of the body. The patients own cells can be grown on the scaffold in vitro, thus preventing immune responses. By using biodegradable polymers, the scaffolds will degrade in time in the patient's body at the same rate that tissue regeneration occurs. The topography of the nanofiber membrane mimics the body's own extracellular matrix. This feature, in addition to the high surface area to volume ratio of the nanofibers and the porosity of the nanofiber membrane, allows for cell adhesion, migration and proliferation [3].

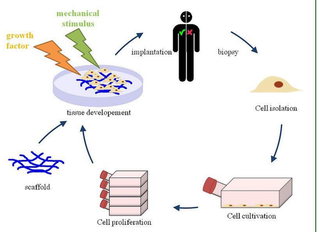

Figure 2. Tissue Engineering Process[2]

Objectives

To fabricate polymer and cactus mucilage electrospun nanofibers to be used for wound healing and tissue scaffolding applications.

Approach

Electrospinning is performed to create nanofiber meshes of 3 different polymer solutions: a) 70:30 of 20% polystyrene in d-Limonene, b) 0.5% mucilage in DI water at a 70:30 w/w ratio with 20% polystyrene in d-Limonene (Figure 3), and c) 1% mucilage in DI water. at a 70:30 w/w ratio with 20% polystyrene in d-Limonene (Figure 4).

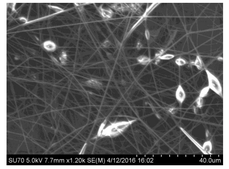

Figure 3. SEM image of 70:30 polystyrene and 0.5% GE mucilage nanofibers

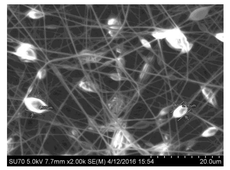

Figure 4. SEM image of 70:30 polystyrene and 1% GE mucilage nanofibers

ATCC L929 mouse fibroblast cells are cultured in T25 flasks, incubated at 37˚C and 5% CO_2 and passaged in 1:8 ratios once they are at or near 100% confluence. Cells are then counted using a hemacytometer. 5 x 10^3 cells are placed into 12 wells of a 24 well plate that contains each of the above types of nanofiber scaffolds created in triplicates. The last set of wells contain just cells in medium to be used as a control.

After four days of incubation, the nanofiber scaffolds are washed with DPBS to retain only adherent cells. These scaffolds are then placed into new wells with 0.5 mL medium and 50uL MTT. We performed this MTT assay because metabolically active cells reduce MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) forming an intracellular purple punctate precipitate named formazan (Figure 5), which can be quantified by a spectrophotometer. In figures 6-8 below, Column 1 contains 20% polystyrene in d-Limonene. Column 2 contains a 70:30 solution of 20% polystyrene in d-Limonene and 0.5% gelling cactus mucilage. Column 3 contains a 70:30 solution of 20% polystyrene in d-Limonene and 1% gelling cactus mucilage. Column 4 contains cells in medium. Column 5 contains just medium. After two hours of incubation, the medium was aspirated from each well and 0.25 mL of DMSO was added. A plate shaker was used to mix the solution in each well until a uniform color was achieved. Then 0.2 mL of solution was transferred from each well in the 24 well plate to a 96 well plate and put in a plate reader The higher the absorbance, the greater the cell proliferation on each scaffold. We read the absorbance at 570 nm and 660 nm as according to the ATCC protocol for performing an MTT assay.

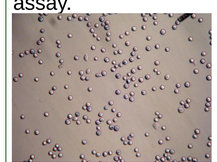

Figure 5. Purple punctate precipitate formed in metabolically active cells

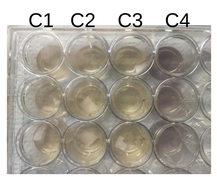

Figure 6. The 24 well plate after one hour of incubation with MTT

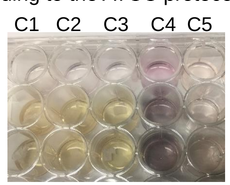

Figure 7. Results after two hours of incubation with DMSO added to the first row of wells

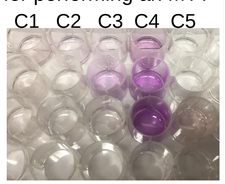

Figure 8. Results after DMSO was added to all of the wells.

Since the wells containing the nanofiber membranes did not have cells proliferate successfully, there was a concern about the toxicity of the fibers. Therefore, the fibers were detoxed by being soaked in DPBS for one hour and then in medium for 48 hours while incubating. The fibers were moved to a new well plate and reseeded with cells the following day with the previous procedures.

Conclusions

The data table below shows the absorbance information for the first trial of growing cells on the nanofiber scaffolds. When comparing the absorbance of the three well plates that contained the polystyrene (PS) fibers, there is evidence that mucilage does increase cell proliferation. However, these three well plates also show a decrease in absorbance when compared to the well plate that contained only medium, which warrants further investigation of the toxicity of the materials used to fabricate the nanofiber membrane and exploration of other bio polymers.

Table 1. Absorbance for PS/Mucilage Nanofiber Membranes

Trials	Blank	70:30 20% PS	70:30 20% PS and 0.5% mucilage	70:30 20% PS and 1% mucilage	Medium	
A	0.001	0.133	0.139	0.145	0.749	Delta
B	-0.001	0.069	0.058	0.708	1.399	Delta
C		0.065	0.071	0.078	1.576	Delta

We received similar data from a second investigation where the nanofibers where first soaked in DPBS for one hour and then medium overnight to allow the release of toxic substances before seeding cells on them. Cells were not able to grow in this medium after the fibers were removed. When moving the fibers to a new well plate, cells were not able to grow in these wells either. Overall, polystyrene does not allow for cell adhesion or viability.

Future work will involve using biodegradable, biocompatible polymers to continue cell culture testing on mucilage membranes.

References

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