Multi-Walled Carbon Nanotube Filter for Activating Effector T-cells

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Abstract: This project explores the interaction between T-cell suspensions and multi-walled carbon nanotube (MWCNT) and amine-functionalized (NH_2)-MWCNT filters. This filter aims to induce T-cell activation by mechanical forces. Cell viability and T-cell activation experiments were performed on the filtered T-cell suspensions using fluorescence microscopy and enzyme-linked immunosorbent assays, respectively.

1. Introduction

Effectively treating cancer remains a challenge given its ability to easily evade and suppress immune responses. Recently, adoptive T-cell transfer (ACT) has gained popularity as a promising cancer immunotherapy that enhances the effectiveness of white blood cells [1]. T-cells are a type of white blood cell that proliferate and differentiate into effector T (T_{eff}) cells which play a critical role in killing cancer cells. The goal of this project is to enhance the efficacy of ACT technology by developing a functional filter that aims to induce T_{eff} cell activation by mechanical forces. Multi-walled carbon nanotubes (MWCNTs) are used as the filter material given their attractive material properties such as high structural integrity, surface area and mechanical performances [2]. To improve the biocompatibility of the MWCNT filters, ammonia plasma functionalization was performed to graft amine functional groups.

2. Methods

2.1. MWCNT synthesis: Thermal chemical vapor deposition was used to synthesize a homogenous coating of MWCNTs on 316L stainless steel mesh. Acetylene was used as gaseous hydrocarbon precursor and the MWCNT growth on the mesh was achieved at 700 ˚C.

2.2. Plasma functionalization: Plasma-enhanced chemical vapor deposition (PECVD) was used to graft amine functional (R-NH_2) groups on the MWCNT filters. The system consisted of a continuous wave capacitively-coupled radiofrequency (13.56 MHz) reactor operating at ~1 Torr and 5 W in Ar/NH_3 mixtures.

2.3. Spleen suspension: A suspension of spleen cells was created to have access to the T-cells. The spleen cell suspension was dispensed normally using a syringe through the MWCNT and NH_2-MWCNT filters. The filtered suspensions were further analyzed by fluorescence microscopy and enzyme-linked immunosorbent assays (ELISA).

3. Results and Discussions

3.1. Cell viability: A fluorescent indicator, calcein-acetoxymethyl, was added to the filtered suspension after being dispensed through the MWCNT and NH_2-MWCNT filters. Fluorescence microscopy was used to capture the images. The green fluorescence indicates 46% and 36% cell viability for the MWCNT and NH_2-MWCNT filters, respectively.

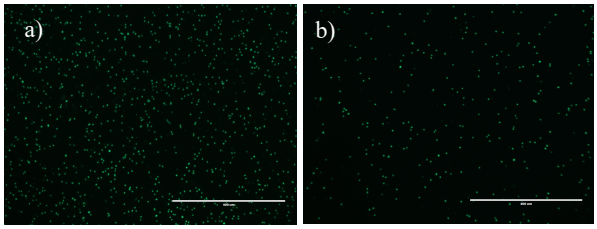

Figure 1: a) Fluorescence microscopy images of filtered suspension through filters (a) MWCNT (b) NH_2-MWCNT.

3.2. ELISA: A mouse interferon ELISA kit was used to measure the amount of IFN-γ secreted by the spleen cells. IFN-γ is a cytokine released when T-cell activation occurs. Phytohemagglutinin (PHA)-L was used as the positive control to stimulate the secretion of IFN-γ. Low quantities of IFN- γ were detected after being filtered through the MWCNT and NH_2-MWCNT filters.

Table 1: Concentrations of IFN-γ in spleen cell suspensions

Spleen suspensions	Concentrations of IFN- γ (pg/mL)
PHA-L	443.35
MWCNT	2.58
NH_2-MWCNT	1.39

4. Conclusion

Mechanical forces alone did not induce the activation of T-cells. The NH_2-MWCNT filters will act as the foundational material for future studies including the immobilization of non-physiological agonists. Target antibodies can potentially be immobilized on the amine functionalized MWCNT surfaces.

References

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