Transplantable Matrix Permits T Cell and Dendritic-Fusion Cell Interaction

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Statement of Purpose: The purpose of this project is to characterize a suitable matrix for allowing interactions between native T cells and a Dendritic-fusion cell line intended for use as a tumor cell therapy. A dendritic-fusion (DC/F) cell line has been created for the purpose of presenting tumor antigens and stimulating a native T-cell response. Due to drawbacks in either systemic of subcutaneous delivery, this project focuses on a suitable matrix that would allow interactions with native T cells, and be biocompatible and degradable. Alginate was evaluated for its ability to culture the DC/F cell line and allow for interactions with T cells in the surrounding media, as a basis for future work *in vivo*.

Methods: Sodium alginate (>65% guluronic acid monomer content; VLVG of M.W. w 30 kDa and LVG of w100 kDa NovaMatrix, FMC BioPolymer Drammen, Norway) was used to fabricate porous scaffolds with final concentration 1% alginate and 0.22% D-gluconic acid/hemicalcium salt as previously described.[1] Matrices were hydrated with a total volume of 40 μl containing $2.5x10^5$ DC/F cells, with $2.5x10^5$ T cells in the media. Cells were imaged every 15 minutes for 12 hours via cell trackers (in Green CMFDA and Red CMPTX Invitrogen Carlsbad, CA) or at 24 hours via dehydration and scanning electron microscopy (SEM) imaging to examine cell interaction. After six days within the matrix a live dead assay (Invitrogen. Carlsbad, CA) was performed to confirm viability of the matrix system. The ability of the scaffold to promote interactions leading to antigen specific immunity when loaded with cytokines IL-15 and IL-17 was evaluated by IFNγ production.[2,3] After 5 days of co-culture IFNγ production was tested in CD4 and CD8 cytotoxic T effector cells via intracellular FACs.

Results: Time lapse confocal imaging of the alginate scaffold demonstrated the presence of the DC/Fs in the scaffold. During the period viewed, cells appear to be capable of movement within the scaffold and adopt an extended conformation. Infiltration of T cells into the scaffold is observed in under one hour. After 2.5 hours multiple apparent interactions were readily visible between the T-cells and DC/F, with the number of interactions visible increasing during the entire 12 hours imaged. SEM images taken at 24 hours demonstrate the macroporous nature of the scaffold. Furthermore cell pairs were visible within niches of the alginate, with sizes ratios consistent with Dendritic and T cells.

Cell viability assays demonstrated that after 6 days, the cells were alive in the scaffold without significant differences between cells in the media surround the scaffold versus the scaffold itself.

FACs staining demonstrated that scaffold culture compared to simple co-culture was consistent with higher IFNγ production. Furthermore loading the scaffold with IL-15 and IL-17 further increased production when compared to an unloaded scaffold or co-culture with IL-15 and IL-17 in the media.

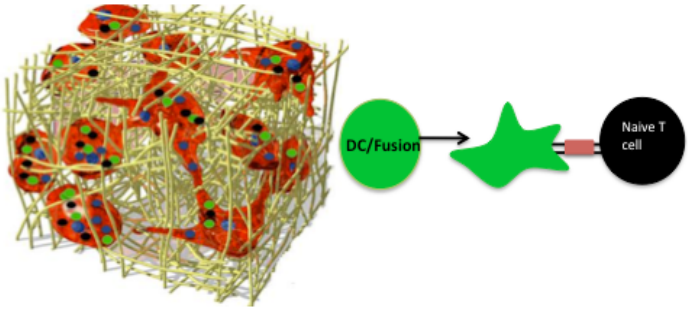

Figure 1. An alginate scaffold will be loaded with DC/F cells, in order to interact with and then activate naïve native T cells.

Conclusions: Results presented here indicate that alginate is a viable scaffold for the seeding of DC/F cells. It provides an environment in which D-F cells have the ability to migrate and stay viable over 6 days.

The scaffold also allows for T cell infiltration and interaction. The interactions required for T cell activation was confirmed by confocal (from 2.5-12 hours) and SEM after 24 hours in the scaffold.

The ability of the DC/F cells to stimulate the T cells was evaluated by IFNγ production. The greater production in the scaffold versus simple co-culture suggests that the 3D porous nature of the alginate provides for more efficient activation and interaction. Furthermore the loading of IL-15 and IL-17 enhanced the activation by DC/F, introducing another facet of T cell activation to explore. Future research will explore ways to modulate IL loading and characterize release, and then *in vivo* studies to evaluate efficacy in an animal model.

References:

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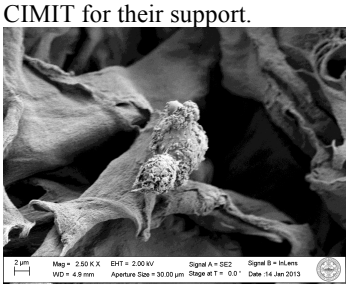

Figure 2. Imaging after 24 hours demonstrates interactions between cells in an alginate scaffold.