Characterizing MDSC Migration After Functionalization with Targeted Microparticle Drug Delivery Systems

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[1]Massachussetts Institute of Technology, [2]Koch Institute for Integrative Cancer Research,
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ABSTRACT

Myeloid derived suppressor cells (MDSCs) are proliferate in the bone marrow of tumor bearing hosts and are currently thought to play a role in the suppression of the anti-tumor immune response (Srivasta et al. 2012) and facilitation of tumor metastasis (Srivasta et al. 2012). Tumors release chemokines that cause MDSC proliferation and chemotactic migration towards the site of the tumor (Sinha et al. 2010). This investigation looks into harnessing the tumor specific tropism of MDSCs in conjunction with layered polymer microparticle backpacks to act as a vehicle for targeted drug delivery. MDSCs are isolated from spleens of tumor-bearing mice and magnetically separated to isolate CD11b+/GR1+ MDSCs. Successful separation was evaluated through the use of flow cytometry. These MDSCs were then incubated with biotinylated backpacks to attach them via a streptavidin bridge and biotinylated anti-GR1 antibodies. The efficacy of backpack attachment was analyzed through confocal microscopy. Cellular migration assays were used to test the effects of backpack attachment on the motility of MDSCs. These measurements showed that the backpacks caused a 30% decrease in the migration of the MDSCs and future studies will be done to investigate strategies by which migration and cellular viability of MDSCs with attached backpacks can be improved both in-vitro and in-vivo.

INTRODUCTION

Cancers are responsible for nearly 7.6 million deaths per year and unfortunately most leading chemotherapeutic treatments are highly toxic to healthy cells. In an attempt to combat this issue, controlled drug delivery systems are used in which drugs are transported to the site of the tumor, thereby limiting toxicity and dosage concentration (Srivasta et al. 2012). We propose to use myeloid derived suppressor cells (MDSCs) attached to polymer backpacks as a drug delivery vehicle to achieve targeted drug delivery to the tumor site. .

MDSCs are found in bone marrow and are commonly known for suppression of the anti-tumor response and facilitation of tumor metastasis. Tumors overexpress colony stimulating factors such as GMCSF, MCSF, and IL-6 which in turn stimulate growth and development of MDSCs. In response to these chemokines, MDSCs migrate to the tumor site where they inhibit the anti-tumor immune response.

This tumor-specific tropism of MDSCs is harnessed in order to deliver drugs directly to the tumor site. Essentially, microparticles are attached as drug-carrying backpacks onto MDSCs. The specific particles chosen for use in this investigation were layered polymers with a cell-adhesive region, a payload region, and a release region for efficient cellular attachment and drug release (Swiston et al. 2010). The end goal is to use MDSCs to transport the drug loaded backpacks to the site of the tumor and directly unload the therapeutic agent.

METHODS AND MATERIALS

Myeloid Derived Suppressor cells (MDSCs) were isolated from spleens of tumor-bearing mice using magnetic-activated cell sorting (MACS). CD11b+/GR1+ MDSCs were used for this experiment. The isolated MDSCs were incubated with polymer backpacks coated with anti GR-1 antibodies for attachment to GR-1 on the MDSC surface (Figure 1A). To test the effect of backpack attachment on MDSC migration toward tumor cell conditioned media (TCCM), transwell migration assays were performed (Figure 1B).

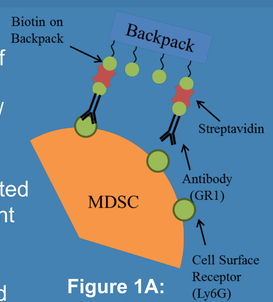

Figure 1A: Antibody Attachment: Biotinylated Backpacks Attach to Cells Using A Streptavidin Bridge and Biotinylated Antibody

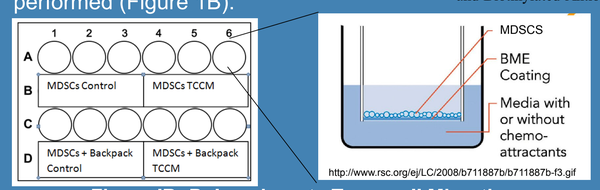

Figure IB: Polycarbonate Transwell Migration Assays

RESULTS

CD11b+/GR1+ MDSCs were isolated by MACS. The purity of the isolated MDSC population was evaluated using flow cytometry. MDSCs were stained with anti-CD11b FITC and anti-GR1 PE antibodies and analyzed. Flow cytometry results (Figure 2) showed that 99.4% of the MDSCs expressed both CD11b and GR-1. These MDSCs then were conjugated to rhodamine labeled polymer backpacks. This conjugation was observed through the use of fluorescnce microscopy (Figure 3A). MDSCs functionalized with backpacks were then run through a transwell polycarbonate membrane and the resultant migration was compared to control MDSCs. After the migration, the MDSCs that flowed through the transwell membrane were again observed under the fluorescent microscope. There was a fewer number of MDSCs with red fluorescence observed post-migration (Figure 3B). The migrated cells were then counted and these values were compared to the known amount of cells originally plated for the migration assay to calculate a percent migration. There was a significant difference between the percent migration of MDSCs with and without backpacks in tumor conditioned media and there was a large significant difference between the percent migration in control versus tumor conditioned media in both MDSCs only and MDSCs with backpacks (Figure 4).

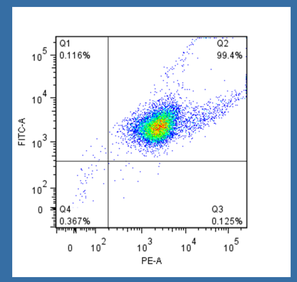

Figure 2: Flow Cytometry of MDSCs (CD11b+/GR1+)

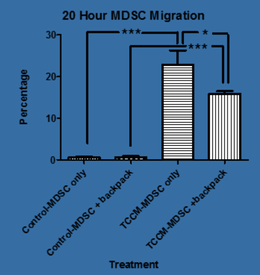

Figure 4: Percent MDSC Migration after 20 Hours

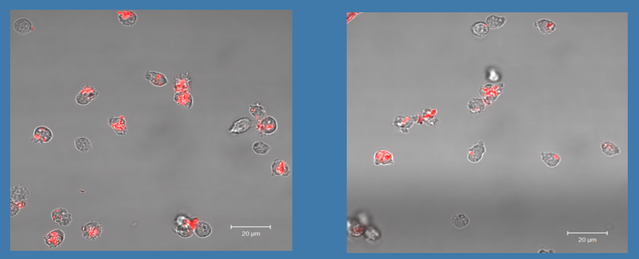

Figure 3A: MDSCs with Backpacks Prior to Migration Assay.

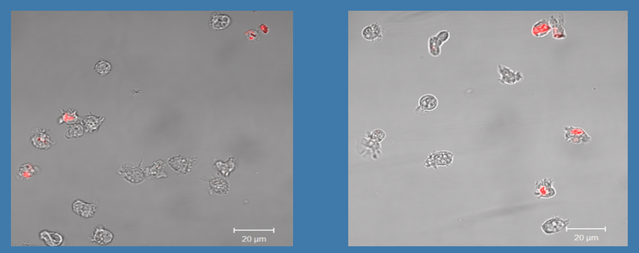

Figure 3B: MDSCs with Backpacks After Migration Assay

DISCUSSION

Flow cytometry analysis confirmed the isolation of CD11b+/GR1+ MDSCs and fluorescence microscopy confirmed the attachment of backpacks to these MDSCs. The results from the migration assay demonstrate that the tumor conditioned media performed far better than the control media (IMDM) in terms of encouraging migration. However, there was also a significant decrease of ~30% migration when backpacks were added to MDSCs in TCCM. This indicates that the backpacks are in fact impeding the motility of the MDSCs and affect tropism towards growth factors released by tumor cells. This could be caused by the backpacks either blocking chemical receptors on the MDSCs or by the increased size of the MDSC-backpack complex.

CONCLUSIONS

These experiments have shown that although the MDSCs successfully attach to the backpacks and are able to migrate towards tumor conditioned media alone, when combined with the microparticle backpacks, their motility is decreased by 30%. Future studies will investigate mechanisms through which migration and cellular viability can be improved both in-vitro and in-vivo.

REFERENCES

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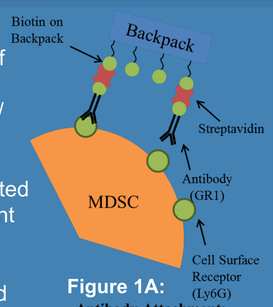

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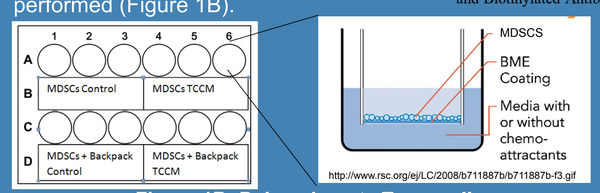

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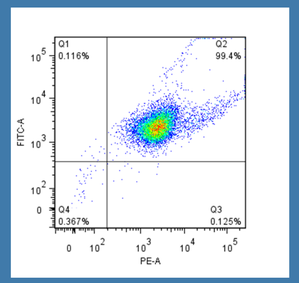

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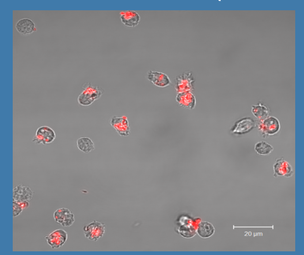

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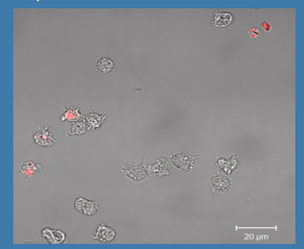

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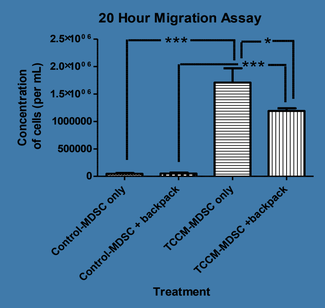
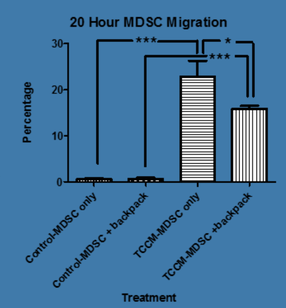


Figure 5A: Cell Count of MDSC Migration after 20 Hours



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