Cytokine Supplementation for T Lymphocyte Expansion Using Magnetic Mineral-Coated Microparticles

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Statement of Purpose: Adoptive cell therapy (ACT) is rapidly evolving and improving to become a key weapon in the immunotherapy arsenal. Through expansion +/- engineering of a patient's immune cells, ACT seeks to manufacture cells that are capable of specifically targeting and destroying tumors. *Ex vivo* manufacturing of these cells requires frequent and costly supplementation with immunostimulatory growth factors called cytokines (Dudley, M, et al. *Nature Reviews Cancer*. 2003;(3)**:**666–675). Interleukin-15 (IL-15), a T lymphocyte and natural killer cell activator, has been shown to improve bioactivity of chimeric antigen receptor (CAR)-T cells by maintaining a less differentiated phenotype and reducing cellular exhaustion (Alizadeh, D, et al. *Cancer Immunol Res.* 2019 May;7(5):759–772.). There is a need for improved cytokine delivery that will minimize cost and labor associated with IL-15 media supplementation. Here we evaluate magnetic mineral-coated microparticles (MCMs) as a delivery system that can slowly and locally release bioactive IL-15 into cell culture, and then be removed with an external magnet prior to cell infusion into patients. Our lab has developed a protocol for creating nanostructured calcium phosphate coatings on various materials that can trap biologics within their porous structure and release cargo slowly as the coating degrades (Yu X, et al. *Adv Mater*. 2017;29(33):1-18). Magnetite (iron(II,III) oxide or Fe_3O_4) is a highly magnetic mineral that, when coated with this calcium phosphate matrix, demonstrates the dual properties of electrostatic protein binding and magnetism. These qualities make it an ideal candidate for IL-15 delivery during *ex vivo* immune cell expansion. We showed that magnetite powder can be functionalized with a calcium phosphate coating, the resulting magnetic MCMs can be loaded with IL-15 to promote T cell proliferation, and a neodymium magnet can pull the particles out of suspension. This work aims to make ACT a more affordable and accessible option for cancer patients.

Materials and Methods: Magnetic MCMs were prepared according to our lab's protocol by suspending magnetite powder in modified simulated body fluid (mSBF) under rotation at 37°C for 7 days (ref). MCMs were collected and resuspended in fresh mSBF each day, then washed in deionized water and freeze dried. Proper mineral coating formation was assessed using scanning electron microscopy. To bind proteins, MCMs were rotated in solutions of IL-15 (NCI Preclinical Repository) or other reporter proteins at varying concentrations for one hour. Loaded magnetic MCMs were incubated in elution buffer (400 mM ammonium phosphate dibasic/200 mM ammonium bicarbonate) to quickly elute proteins for analysis within 30 minutes, or in regular SBF to mimic longer term *in vivo* release over the course of days. Supernatants were collected to quantify released proteins at different time points using techniques such as luminescence readings and enzyme-linked immunosorbent assays (ELISA, R&D Systems). CTLL-2 mouse T lymphocytes (ATCC) served as a cell model for proliferation experiments. Proliferation and viability were assessed using Cy-QUANT (Thermo Fisher) and CellTiter-Blue assays (Promega), respectively.

Results: A plate-like calcium phosphate coating was deposited on the surface of the magnetite particles, as shown on scanning electron microscopy (Figure 1A). For protein binding experiments, magnetic MCMs performed at least as well as regular MCMs that use beta tricalcium phosphate powder as a core material instead of magnetite. Magnetic MCMs successfully bound and released both luciferase and IL-15 in a sustained manner *in vitro*. When applied to T cell culture, MCMs promoted proliferation using a low dose (<1 ng/mL) of IL-15. A neodymium magnet was able to pull the magnetic MCMs out of solution for collection (Figure 1B).

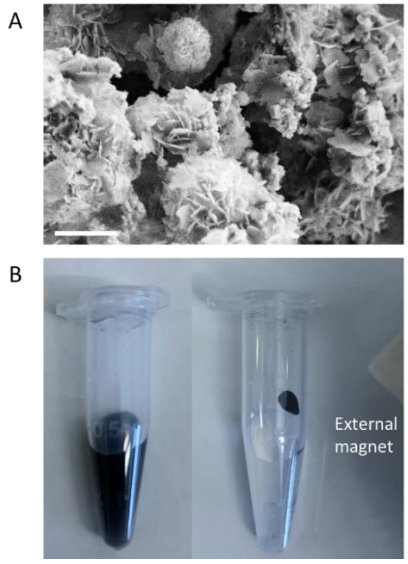

Figure 1. Magnetic mineral-coated microparticles (MCMs) as a removable cytokine delivery vehicle. **(A)** Scanning electron microscopy image of the surfaces of magnetic MCMs (scale bar 2 um). **(B)** Use of an external magnet to pull magnetic MCMs out of solution.

Conclusions: Magnetite particles coated with a calcium phosphate matrix were successfully loaded with IL-15 for supplementation of T cell culture. Our results suggest that this delivery approach could be applied to ACT expansion to reduce costs and time spent on media changes. This could make ACT a more viable treatment strategy for cancer patients.