Designing Stiffness-Tunable Polyacrylamide Hydrogels to Enhance CAR-Macrophage Manufacturing and Performance

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Introduction

Cancer remains the second leading cause of death in the United States,[1] with current treatments limited by drug resistance and relapse. Immunotherapy offers a promising alternative but has been effective primarily for blood cancers. Chimeric antigen receptor macrophage (CAR-M) therapy, engineered via gene delivery, shows potential for treating solid tumors by enhancing macrophage infiltration and cancer cell engulfment.[2,3] However, challenges in manufacturing and efficacy hinder its clinical application. Hydrogels provide a tunable platform that can improve gene delivery through optimized mechanical and biochemical cues.[4-6] Despite existing studies on macrophage behavior, the impact of hydrogel stiffness on macrophage gene delivery efficiency and phagocytosis ability is not well understood. Addressing these gaps is critical for advancing macrophage-based immunotherapies.

Objectives

To design a process that will improve CAR-M manufacturing and performance by using stiffness-tunable polyacrylamide (PAM) hydrogels as culture substrates to provide desired biophysical cues to the macrophages, via the completion of two specific aims:

Aim 1: Investigate the effect of substrate stiffness on **macrophage transfection efficiency/gene delivery** to generate CAR-Macrophages that target a specific cancer antigen.

Aim 2: Demonstrate that CAR-Macrophages generated via the hydrogels with optimal stiffness have enhanced **phagocytic capacity** of tumor cells compared to those generated on conventional plastic culture substrate.

Materials and Methods

The experimental workflow involved a 7-day process, shown in **Fig. 1.** Polyacrylamide (PAM) hydrogels at 2, 20, and 100 kPa were formulated and coated with 0.05 mg/ml Collagen I using UV crosslinking. RAW 264.7 mouse macrophage cells were seeded at a density of 200k/cm², and Lipofectamine 3000 was used to deliver CAR-antiCD19-GFP (green fluorescent protein) plasmids. After 48 hrs, flow cytometry quantified % transfection, while cells were reseeded to run a phagocytosis assay with Nalm6 (CD19+ B cell leukemia) co-culture. Phagocytosis was quantified using flow cytometry.

Methods and Results

EXPERIMENTAL WORKFLOW

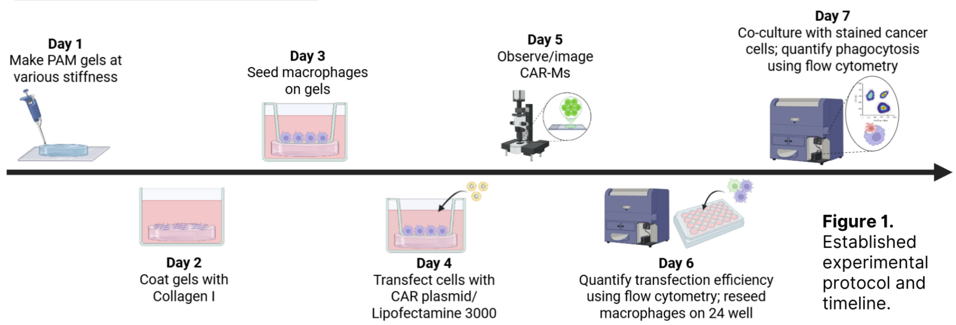


Figure 1. Established experimental protocol and timeline.

TRANSFECTION EFFICIENCY

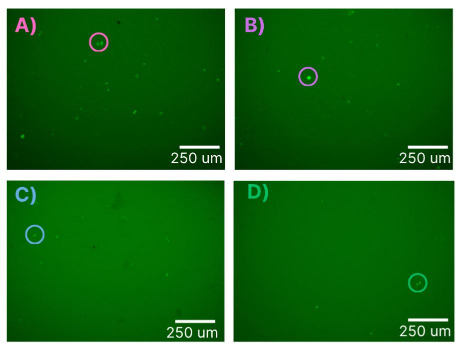
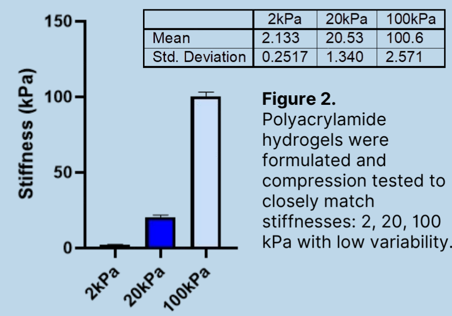


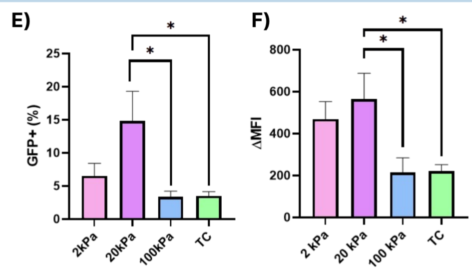
Figure 3. Transfection efficiency varies with substrate stiffness. Representative GFP images 24 hrs post-transfection for macrophages cultured on **A)** 2 kPa, **B)** 20 kPa, **C)** 100 kPa hydrogels, and **D)** tissue culture plastic (TC, ~1GPa). Cells on 2–20 kPa substrates showed higher GFP+ fractions than on stiffer conditions. Flow cytometry 48 hrs after transfection expressed thorugh **E)** GFP+ percentages and **F)** normalized mean GFP fluorescence intensity (MFI) across five independent experiments.

HYDROGEL VALIDATION

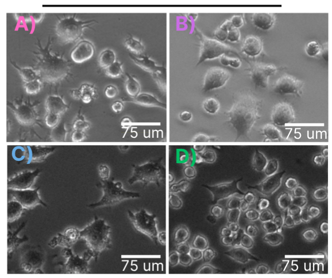


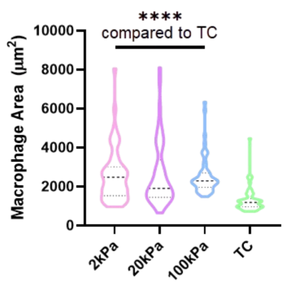
	2kPa	20kPa	100kPa
Mean	2.133	20.53	100.6
Std. Deviation	0.2517	1.340	2.571

Figure 2. Polyacrylamide hydrogels were formulated and compression tested to closely match stiffnesses: 2, 20, 100 kPa with low variability.



PHAGOCYTOSIS ASSAY





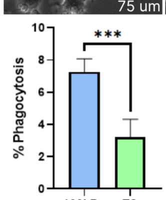


Figure 4. Macrophage phagocytosis of Nalm6 cells after 72 hours of culture on **A)** 2 kPa, **B)** 20 kPa, **C)** 100 kPa hydrogels, and **D)** tissue culture plastic (TC). **E)** Macrophage area was significantly greater for cells cultured on hydrogels compared to TC. **F)** Although data are incomplete due to experimental issues, flow cytometry analysis indicates that phagocytosis rates were higher on 100 kPa substrates compared to TC.

Future Directions

- Understand the effects of substrate stiffness on CAR-M phagocytosis and tumor killing
- Understand the effects of substrate stiffness on macrophage polarization/phenotype to decode mechanisms behind stiffness-dependent behavior changes

QR code for references and further resources on CAR-M therapy.