Streamlined ex-vivo engineering of human T cells: a single-step approach to activation and lentiviral transduction

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Abstract

Efficient T-cell engineering is crucial for the success of CAR T-cell therapy but requires multiple labor-intensive steps, including T-cell isolation, activation, and transduction. Traditional methods are often complex and time-intensive, requiring materials from various suppliers and involving distinct activation steps with antibody-coated beads or soluble antibody complexes, followed by transduction processes that commonly rely on spinoculation with enhancers like polybrene or LentiBOOST. These approaches often suffer from variability and inefficiencies that can impact cell yield and therapeutic efficacy. We present an innovative approach to enhance the ex vivo engineering of human T cells by integrating cell activation and lentiviral transduction into a single, efficient step, reducing time and resource requirements while maintaining high performance. Our approach leverages a lyophilized alginate-based sponge that is embedded with activation reagents, including anti-CD3 and anti-CD28 antibodies, as well as interleukin-2. This sponge provides a convenient platform where users can directly apply a mixture of virus and unstimulated T cells or PBMCs for transduction. The hygroscopic nature and 20–300 µm pore size of the sponge help to spatially constrain cells and virus to a small area, facilitating efficient transduction, as well as ensuring uniform exposure to activation signals. The release of activated and transduced cells is facilitated by a chelating release buffer that depolymerizes the sponge, ensuring the rapid recovery of high-quality transduced cells with minimal handling. This design simplifies the workflow compared to spinoculation with polybrene, reducing the process by nine steps and saving 60 minutes, while enhancing transduction efficiency through centrifugation-free and enhancer-free microfluidics. The reduced handling steps also minimize contamination risks and variability between batches. Our data across multiple donors and operators consistently indicate strong activation and transduction efficiencies (%CV <15%; n = 54), with no adverse effects on growth rates, cell phenotypes, or exhaustion marker expression compared to standard protocols. This reproducibility underscores the robustness of our approach. Furthermore, our ex vivo lentiviral T-cell transduction sponge is user-friendly and scalable, accommodating cell numbers ranging from 2 x 10^6 to 1 x 10^7 per sponge, making it suitable for both research and preclinical applications. By providing a streamlined and user-friendly system for T-cell engineering, we strive to drive progress in T-cell therapies and expand their availability to a wider range of patients.

1 Lenti-X™ T-Cell Transduction Sponge workflow

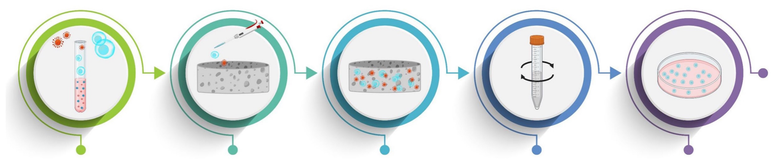

Figure 1. Simple transduction workflow using the Lenti-X T-Cell Transduction Sponge. Unstimulated T cells or PBMCs and virus are mixed prior to application to the sponge. The transduction mixture is added to the sponge and incubated for 1 hr, followed by the addition of media and incubation for 48 hr. During this time, T-cell activation and transduction occur. No spinoculation is required. Healthy, transduced T cells are released using an optimized Release Buffer that depolymerizes the alginate matrix. T cells are then ready for subsequent analysis or continued culture.

2 Easy-to-handle, convenient format

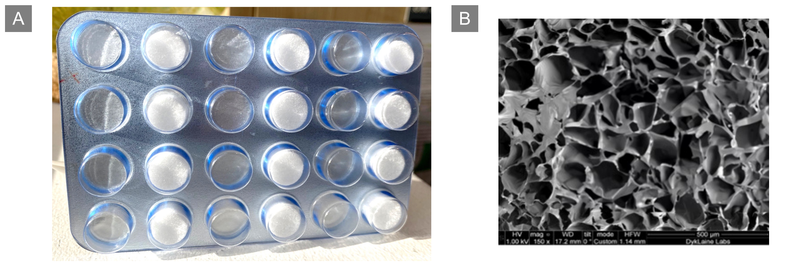

Figure 2. Convenient format for use with a 24-well tissue culture plate. Panel A. Each kit comprises 12 individual sponges, each capable of facilitating transduction of up to 1 x 10^7 T cells. **Panel B.** Every sponge features a complex microfluidic pore structure with pore sizes ranging from 20–300 μm. The SEM image shown is at 150X magnification. For T-cell activation, the pores contain anti-CD3 and anti-CD28 antibodies along with rhIL-2. **Panel C.** Simple placement of the transduction sponge into a well before the application of the sample. **Panel D.** The transduction mix, consisting of 2 x 10^6–1 x 10^7 T cells and virus (max volume of 150 µl) is added to the sponge and incubated for one hour before the addition of media and incubation for 48 hr. **Panel E**. T-cell release is facilitated by transferring the sponge to a 15 ml conical tube and adding Release Buffer, followed by a short wash step.

3 The T-cell sponge generates potent activation signals

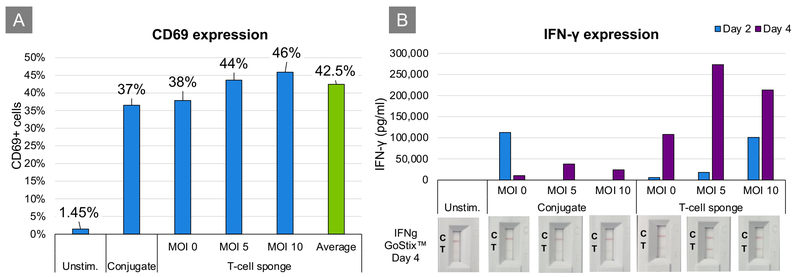

Figure 3. Efficient T-cell activation with the Lenti-X T-Cell Transduction Sponge. Panel A. 4 x 10^6 human primary T cells were mixed with a lentivirus expressing ZsGreen1 at the indicated multiplicities of infection (MOIs) and added to the Lenti-X T-Cell Transduction Sponge or treated with CD3/CD28 Immunocult reagent ("Conjugate"). After 48 hr, treated cells were assayed for CD69 expression by FACS. **Panel B**. Activation was further assessed by IFN-γ production at the times indicated using IFN-gamma GoStix™ Plus. The IFN-gamma GoStix Plus cassettes for each condition from Day 4 are shown. C denotes "control" and T denotes "test".

4 T-cell transduction efficiency and viability

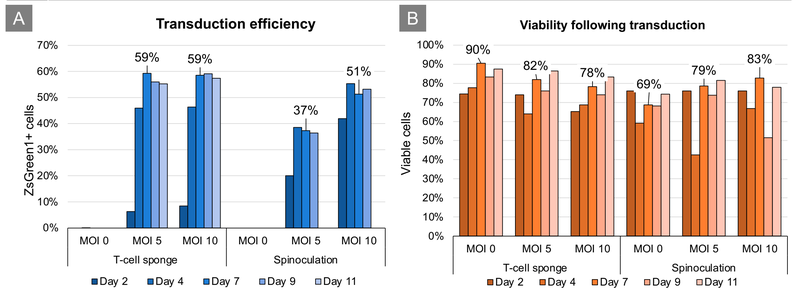

Figure 4. The Lenti-X T-Cell Transduction Sponge enhances primary T-cell transduction efficiency while preserving viability. 4 x 10^6 human primary T cells were mixed with lentivirus expressing ZsGreen1 at the indicated MOIs and added to the Lenti-X T-Cell Transduction Sponge. Spinoculated samples were activated with CD3/CD28 ImmunoCult reagent for two days, then exposed to the lentivirus with polybrene (8 µg/ml) and centrifuged at 1,500*g* for 90 min. **Panel A.** Transduction efficiency was assessed by FACS analysis for ZsGreen1 expression at the indicated time points. **Panel B.** Viability was assessed at the indicated post-transduction timepoints by 7-AAD staining and FACS analysis.

5 The T-cell sponge supports cell growth and expansion

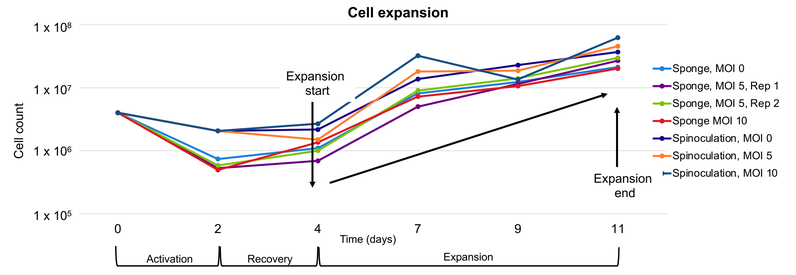

Figure 5. T cells transduced with Lenti-X T-Cell Transduction Sponge or using a spinoculation protocol expand and grow similarly. 4 x 10^6 human primary T cells were mixed with a lentivirus expressing ZsGreen1 at the indicated MOIs and added to the Lenti-X T-Cell Transduction Sponge. Spinoculated samples were activated with CD3/CD28 ImmunoCult reagent for two days, then exposed to the lentivirus with polybrene (8 µg/ml), centrifuged at 1,500*g* for 90 min, and incubated overnight. All T cells were transferred to a G-Rex 24-well plate ("Expansion start") and cultured for the indicated duration. T cell counts were assessed at the specified time points.

6 Sponge-transduced T cells exhibit a normal phenotype

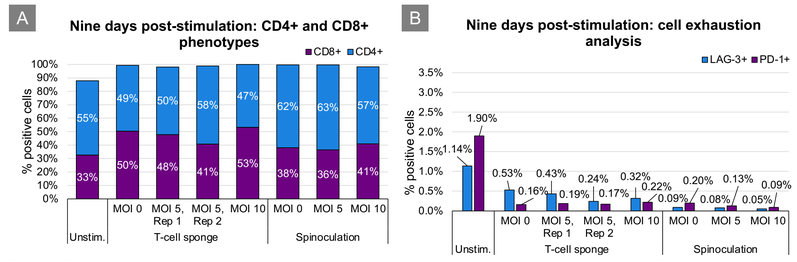

Figure 6. T cells transduced with the transduction sponge are phenotypically the same as spinoculated cells. 4 x 10^6 human primary T cells were mixed with a lentivirus expressing ZsGreen1 at the indicated MOIs and added to the Lenti-X T-Cell Transduction Sponge. Spinoculated samples were activated with CD3/CD28 ImmunoCult reagent for two days, then exposed to the lentivirus with polybrene (8 µg/ml), centrifuged at 1,500*g* for 90 min, and incubated overnight. **Panel A.** Activated and transduced cells were expanded out for nine days using a G-Rex 24 Well Plate and then analyzed for CD4 and CD8 expression using FACS analysis. **Panel B.** Activated, transduced, and expanded cells were also analyzed for the presence of exhaustion markers PD-1 and LAG-3 using FACS analysis.

7 Transcriptomic analysis of transduced cells

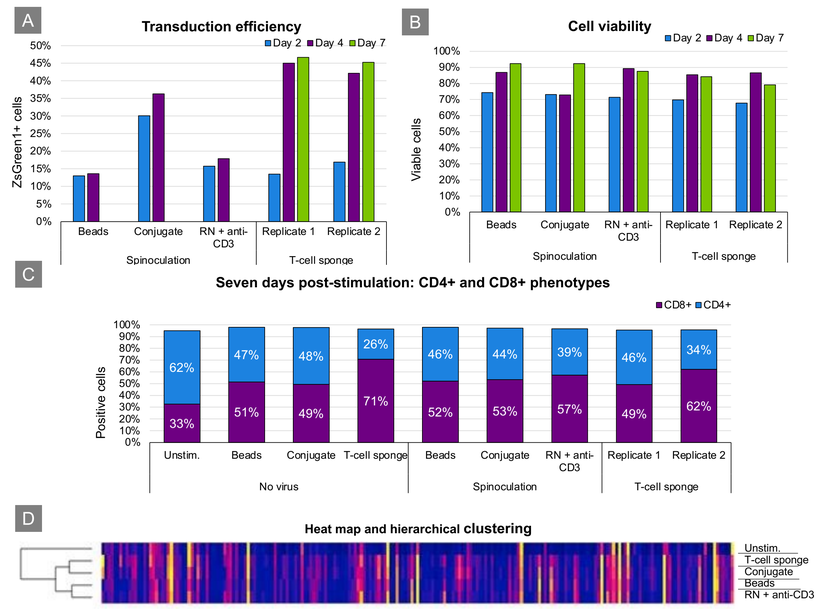

Figure 7. Cells transduced with the Lenti-X T-Cell Transduction Sponge are phenotypically similar to those transduced by standard methods. 4 x 10^6 human primary T cells were activated using one of the following methods for two days: anti-CD3/CD28-coated beads ("Beads"), CD3/CD28 ImmunoCult reagent ("Conjugate"), or RetroNectin® reagent + anti-CD3 ("RN + anti-CD3"). Cells were then transduced with a lentivirus expressing ZsGreen1 at an MOI of 5 in the presence of polybrene (PB, 8 µg/ml), followed by centrifugation at 1,500*g* for 90 min. For the T-cell sponge transduction, 4 × 10^6 T cells were mixed with the same lentivirus (MOI = 5), incubated within the sponge for two days, and then released. **Panel A.** Flow cytometry analysis of ZsGreen1 expression at the indicated timepoints post-transduction. **Panel B.** Cell viability at 48 hr post-transduction assessed by 7-AAD staining and flow cytometry. **Panel C.** Activated and transduced cells were expanded out for seven days using a G-Rex 24 Well Plate and then analyzed for CD4 and CD8 expression using FACS analysis. **Panel D.** Heat map and hierarchical clustering of differentially expressed genes in activated and transduced cells at 48 hr post-transduction. RNA was isolated using the SMART-Seq® mRNA LP kit and sequenced on an Illumina® NextSeq® instrument. Heat map and clustering are based on the top 200 most-differentially expressed genes for each activation/transduction condition.

8 Efficient T-cell transduction within the PBMC population

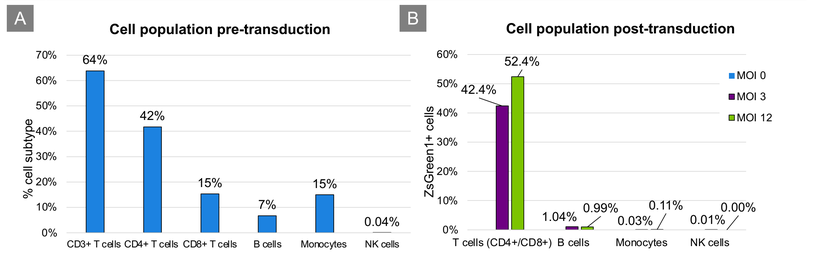

Figure 8. T cells can be specifically activated and transduced when starting with PBMCs with the Lenti-X T-Cell Transduction Sponge. 1 x 10^7 unstimulated PBMCs were combined with lentivirus encoding ZsGreen1 at an MOI of 3 and 12, and then directly applied to a transduction sponge containing anti-CD3 antibodies, anti-CD28 antibodies, and IL-2 (100 IU/ml) for T-cell activation. After 48 hr, the cells were released from the sponge for further culture and analysis. **Panel A.** The composition of the PBMC population was assessed by FACS prior to lentiviral transduction with the sponge. **Panel B.** Transduction efficiency and cell phenotypes were analyzed at 48 hr post-transduction by FACS for ZsGreen1 expression and cell phenotypes.

Conclusions

- We present a streamlined workflow that combines activation and transduction in a single step, minimizing T-cell handling and hands-on time
- The T-cell sponge enables the transduction of T cells from either pre-isolated T cells or PBMCs
- The T-cell sponge delivers potent activation signals, achieving transduction levels comparable to or better than standard methods like spinoculation
- Transduced T cells maintain a phenotype and expansion profile similar to standard methods
- The T-cell sponge supports activation and transduction of 2 x 10^6–1 x 10^7 T cells per sponge, facilitating applications that require large numbers of transduced cells

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