Electrospun Poly(dimethyl siloxane)-based Meshes as a Platform for T Cell Expansion

Alex Dang, Sarah De Leo, Danielle Bogdanowicz, Helen H. Lu, and Lance C. Kam
Columbia University, Department of Biomedical Engineering, New York, NY 10027, USA

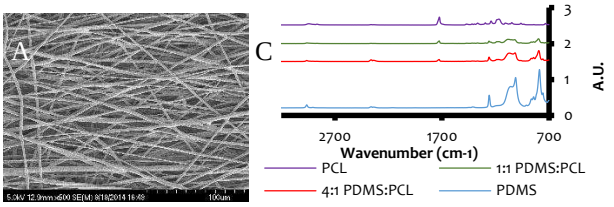
Introduction: Recent advances in the genetic manipulation of T cells have led to striking successes in immunotherapy of cancer. However, advances in cell production and manufacturing are needed to fully realize the power of this approach. The current gold standard for clinical, ex vivo expansion of T cell populations consists of rigid (GPa) polystyrene beads which present antibodies to CD3 and CD28, thus activating T cells upon binding. Recent work by our lab demonstrated improved human T cell stimulation can be achieved using on much softer poly(dimethyl siloxane) (PDMS) substrates (~15 kPa – 2 MPa) [1],[2].

In this report, we leverage this mechanosensing property of T cells via a fiber-based mesh system to improve T cell expansion. This format preserves the high surface area provided by beads while greatly reducing the risks associated with incomplete removal of these structures from the final cell product. We herein examine the effect of composition, rigidity, fiber densities, and pore sizes of electrospun polymer fibers on long-term (17 – 19 days) T cell expansion.

Materials and Methods: Fibrous meshes were synthesized via co-electrospinning of either Sylgard 184 or NuSil MED-4086 PDMS mixed with poly(ε-caprolactone) (PCL) in a 3:1 v/v dichloromethane (DCM) / N,N-dimethylformamide (DMF) solution at 8 – 10 kV onto a grounded collection with a working distance between 8 – 12 cm. Total polymer concentration was varied from 19 to 50% wt/v. Coating of meshes with activating antibodies was performed with goat-anti-mouse IgG linkers followed by a 1:4 ratio of mouse IgG-anti-human CD3 (OKT3) to mouse IgG-anti-human CD28 (CD28.6) (2 h, 23 °C). Cells were treated with carboxy-fluorescein succinimidyl ester (CFSC) in order to track cell division and proliferation. Cell proliferation studies were performed with human $CD4^+$ / $CD8^+$ T cells isolated from whole blood using the Rosette Sep Human T Cell Enrichment Cocktail (StemCell Technologies) system, and monitored for at least 17 days. Flow cytometry (BD FACS-Canto II) was performed on days 3 and 5 for all meshes.

Results and Discussion: FTIR revealed incorporation of PDMS and PCL components. Materials properties were assessed through tensile testing (Instron) scanning electron microscopy (SEM) (see fig. 1). Long-term culture for 17-19 days coupled with at least 8 maximum doublings for Sylgard PDMS/PCL meshes indicated a robust system for expansion of T cells (see fig. 2). Expansions on NuSil PDMS/PCL meshes yielded up to 1.5x doublings compared to Sylgard meshes. Flow cytometry revealed proliferation indices comparable to or significantly higher than that of the current gold standard and pure PCL controls for experimental meshes (see fig. 3).

Conclusion: We have shown that electrospinning PDMS with PCL produces micro- and nano-scale fibers with properties desirable for T cell expansion and substantially higher population doublings than the current gold standard, particularly in NuSil PDMS/PCL meshes. Additionally, we have demonstrated the feasibility of long-term culture of T cells in our system.



B

PDMS:PCL	Fiber Diam. (µm)	Pore Radius (µm)
1:1	2.28 ± 0.39	11.71 ± 2.12
3:1	0.74 ± 0.27	3.07 ± 0.73

Figure 1: (A) NuSil PDMS/PCL meshes. SEM images were used for (B) fiber diameter and pore size analyses. (C) FTIR analysis for Sylgard PDMS/PCL meshes.

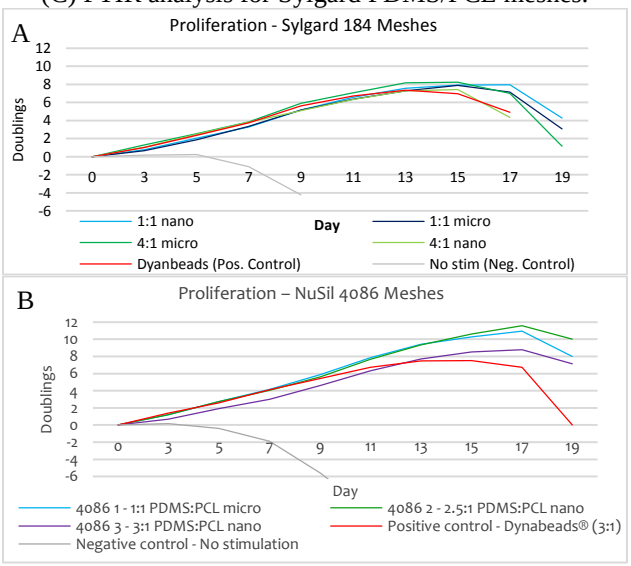


Figure 2: Expansion profiles for $CD4^+$/$CD8^+$ T cells stimulated via electrospun Sylgard (A) and NuSil 4086 (B) meshes over 19 days.

Sample (Sylgard) PDMS:PCL	Prolif. Index	Sample (NuSil) PDMS:PCL	Prolif. Index
1:1 micro	1.55	1:1 micro	1.80
1:1 nano	1.49	2.5:1 nano	1.66
4:1 micro	1.75	3:1 nano	1.78
4:1 nano	1.72		
Dynabeads®	1.53	Dynabeads®	1.57
No stimulation	1.0	No stimulation	1.0

Figure 3: Three-day proliferation indices for meshes

Acknowledgements: This work is supported by the NIH (PN2EY016586, R01AI088377) and the Wallace H. Coulter Foundation. De Leo is supported by the NSF GRFP.

References:

[1] O'Connor et al, J. Immunology, 2012, 189: 1330-1339.
[2] Judokusumo et al, Biophysical Journal, 2012, L05-L07.