Johns Hopkins Engineering

Immunoengineering

Immunoengineering—Allergy and Autoimmunity

Tolerance through scaffolds

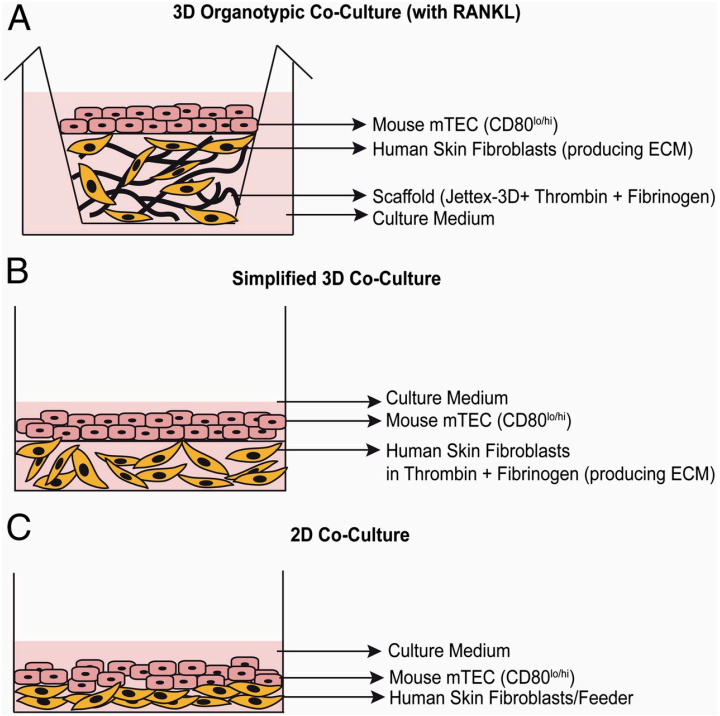
JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

Biomaterial Case Studies to Illustrate Design Principles

- Replicating tolerance at the cellular level - particles
- **Replicating tolerance at the organ level – scaffolds**
  - Recreating the thymus for antigen-specific modulation
  - Immunodeficiencies and Autoimmunity applications
    - Lose T cells in HIV, cancer, or bone marrow transplantation
    - Tissue transplantation
  - Biologic modeling
- Delivery of allergen immunotherapy

Importance of 3D environments

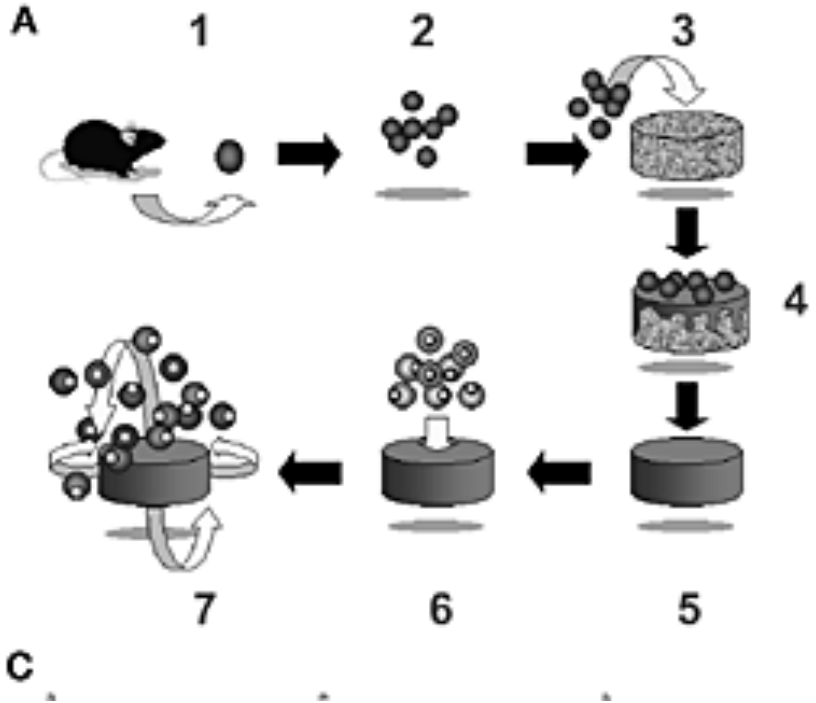
- mTEC (medullary thymic epithelial cells) regulate auto-antigen specific tolerance
- Lose gene expression in 2D
  - e.g. AIRE, FOXN1
- Produce matrix remodeling in 3D condition with co-culture



Pinto, Sheena, et al. "An organotypic coculture model supporting proliferation and differentiation of medullary thymic epithelial cells and promiscuous gene expression." *The Journal of Immunology* 190.3 (2013): 1085-1093.

Artificial Thymus to Develop CD4+ and CD8+ T cells

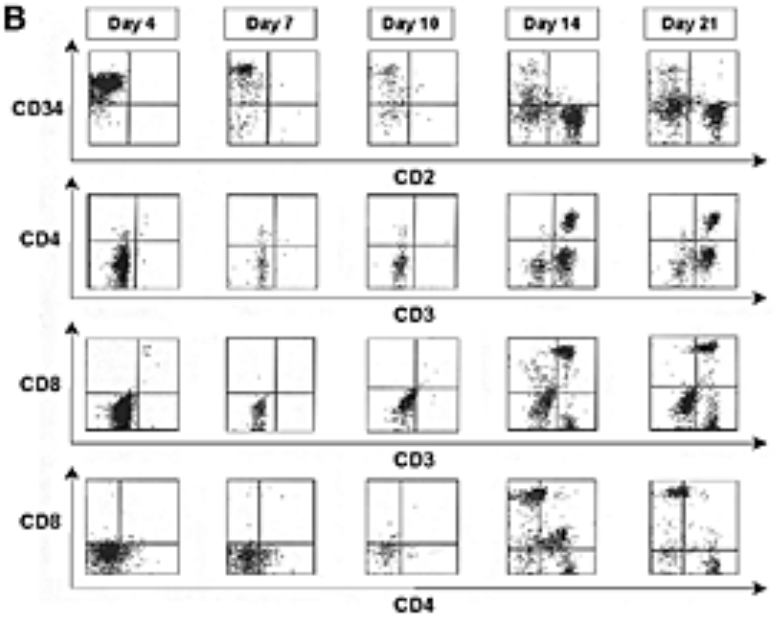
- Deriving T cells from CD34+ stem cells requires cytokines and chemokines
- CellFoam – tantalum coated matrix
  - Pore density and size
  - Thymic stroma
  - T cell density



Poznansky, Mark C., et al. "Efficient generation of human T cells from a tissue-engineered thymic organoid." *Nature biotechnology* 18.7 (2000): 729.

Artificial Thymus to Develop CD4+ and CD8+ T cells

- Require 2-3 weeks to differentiate and acquire CD4+ and CD8+ markers



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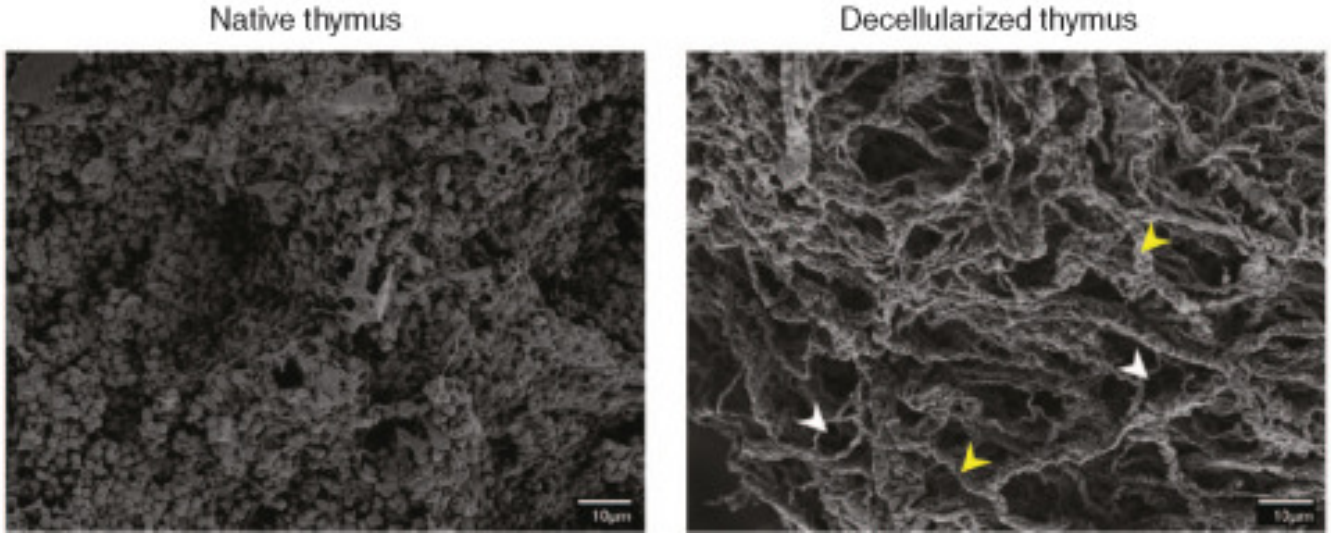
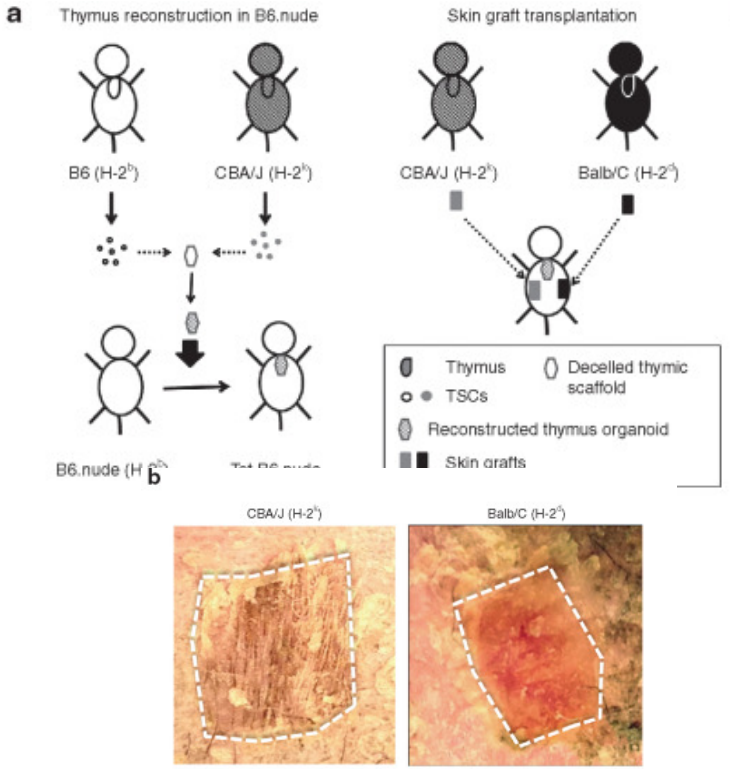
Scaffold Source for Developing the Artificial Thymus



Fan, Yong, et al. "Bioengineering thymus organoids to restore thymic function and induce donor-specific immune tolerance to allografts." *Molecular Therapy* 23.7 (2015): 1262-1277.

Scaffold Source for Developing the Artificial Thymus

- Decellularized matrices enable effective maintenance of TECs and development of T cells
- In vivo thymic organoids recruit immune cells which can initiate antigen-specific immune response
- Further mixing TECs from donor and recipient allow acceptance of skin graft



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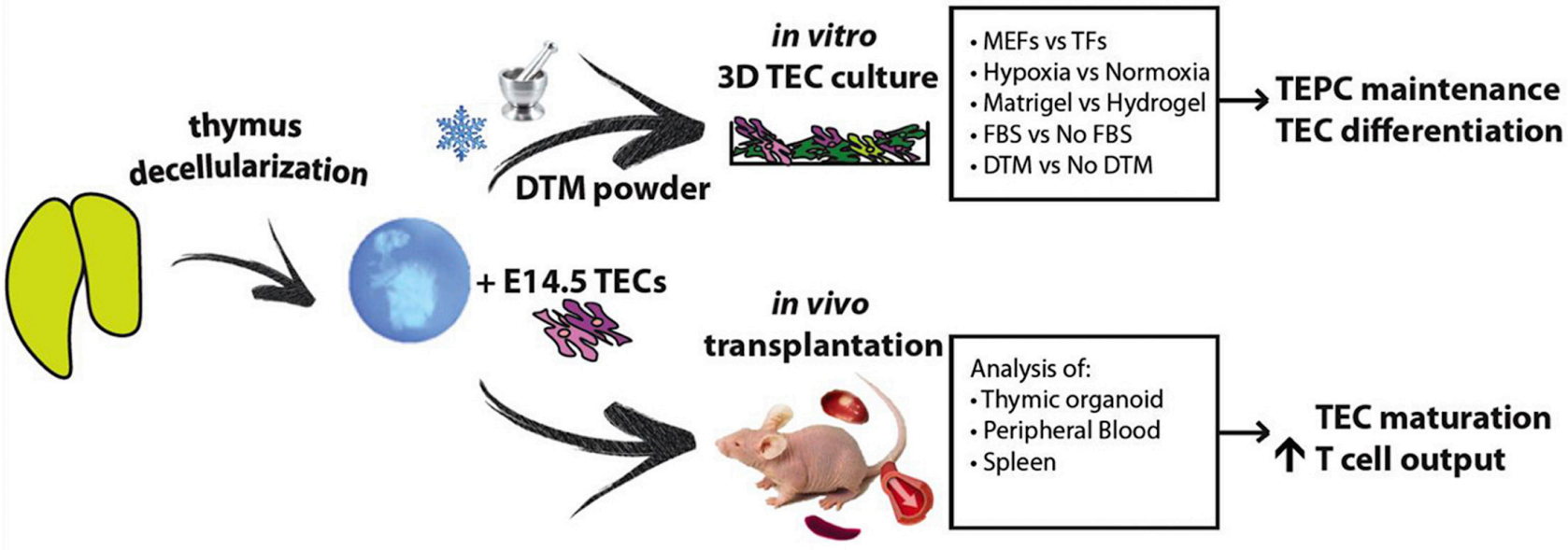
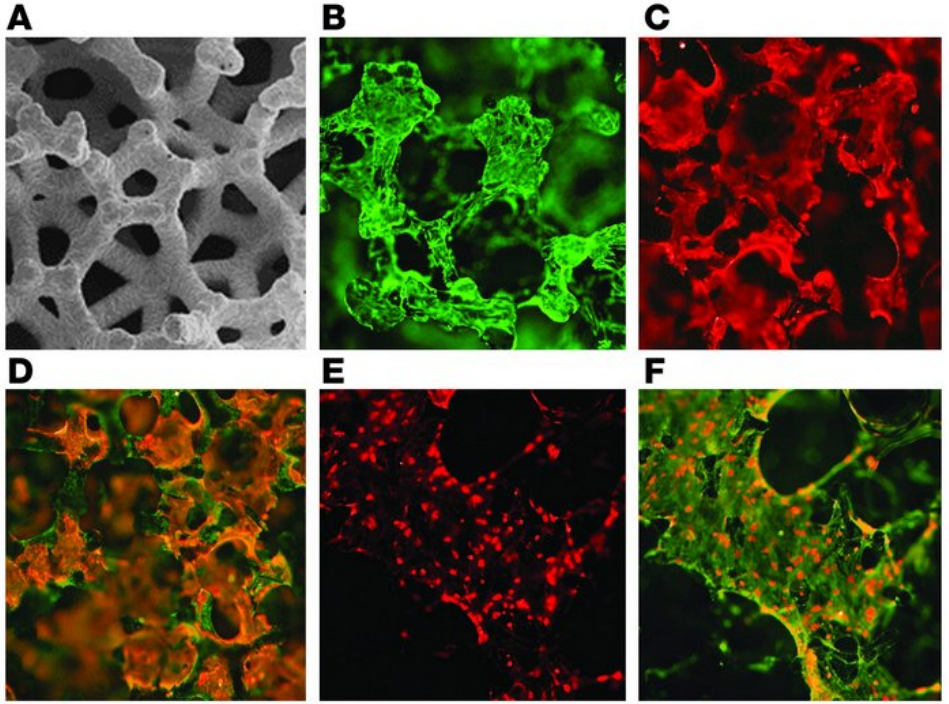
Immune Organs



Hun, Michael, et al. "Native thymic extracellular matrix improves in vivo thymic organoid T cell output, and drives in vitro thymic epithelial cell differentiation." *Biomaterials* 118 (2017): 1-15.

The Importance of Cell Source

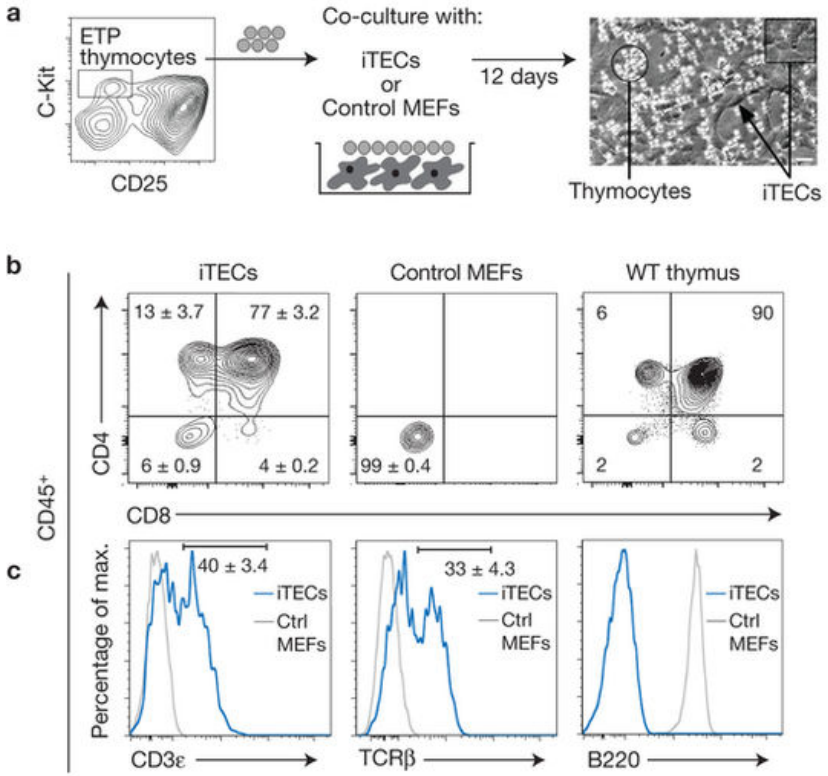
- Usually systems used xenogenic tissues or human fetal thymus
  - No MHC or not MHC matched to patients
  - Limits understanding of specific disease or generating therapeutic T cells
- Keratinocytes and fibroblasts were able to replace thymic epithelial cells
  - Skin is accessible tissue



Clark, Rachael A., et al. "Human skin cells support thymus-independent T cell development." *The Journal of clinical investigation* 115.11 (2005): 3239-3249.

The Importance of Cell Source

- iTECs are fibroblasts transduced to express FOXN1
- Just aggregating iTECs and implanting into adulty mice form functional thymus
  - Develop T cells in vivo
  - But require fetal thymic mesenchyme



Bredenkamp, Nicholas, et al. "An organized and functional thymus generated from FOXN1-reprogrammed fibroblasts." *Nature cell biology* 16.9 (2014): 902.

Immune organs

Table 1 Characteristic features, applications, advantages, and limitations of engineering approaches for T lymphopo

Engineering approaches	Applications	Advantages (+) and limitations (–)
1) Reconstruction of TSC's 3-D network		
• Fetal thymic organ culture (FTOC) • Reaggregate thymus organ culture (RTOC) • Artificial scaffolds • Decellularized thymic scaffolds	• To study T cell tolerance and MHC restriction in vitro • To study thymopoiesis in vivo upon grafting into an ectopic locations of athymic mice	• Simple and straightforward design (+) • Absolute dependency on biopsy and isolation of thymus or thymic cells (–) • Limited culture sizes of 3-D platforms (–) • Limited number of T cells that can be generated in vitro (–)
2) Cellular Engineering		
• Differentiation of stem cells into TSCs • Genetic introduction of effector molecules that define TSC functions to cell lines or somatic cells • Cell reprogramming	• To use human pluripotent stem cells for regeneration of thymus or induction of immune tolerance • To generate T cell precursors and functional T cells using robust 2-D culture platforms in vitro	• Use of clinically relevant, endogenous stem cell sources (+) • Use of readily available 2-D culture platforms for recapitulation of T lymphopoesis in vitro (+) • Potential xenogenic cross-contamination (OP9-DL1) (–) • Ineffective positive selection of CD4 + T cells (OP9-DL1) (–) • Need for complex genetic modifications and related risk of viral contamination (–)
3) Biomaterials-driven artificial presentation of developmental signaling molecules		
• Plate- or bead-bound Notch ligands for differentiation of T precursors from various stem cells • Use of pMHC tetramer to induce antigen specificity on developing T cells	• To generate T precursors from various stem cells in vitro, which later can be employed in adoptive cell therapies. • To induce or selectively expand antigen-specific T cells	• Potential realization of purely biomaterial-based T lymphopoesis ex vivo (+) • Requirement for expensive recombinant proteins (–) • Generation of potentially self-reactive T cells due to lack of negative selection (–) • Limited T cell expansion (–)

Roh, Kyung-Ho, and Krishnendu Roy. "Engineering approaches for regeneration of T lymphopoiesis." *Biomaterials research* 20.1 (2016): 20.

Summary

- Scaffolds enable the three dimensional architecture found within the thymus
  - This allows growth of important regulatory epithelial cells to developing T cells
  - This enables migration and communication from T cells to epithelial cells
- Applications in immunosuppression and autoimmunity to generate functional T cells
- Important considerations for scaffold design of an artificial thymus
  - Pore size
  - Material and source
  - Strength
  - Cell concentration
  - Cell choice and source
  - Bioreactor
  - Cell maintenance – cytokines, chemokines, growth factors, etc.