In Vitro Characterization of Skin Constructs with a Structural Role

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FDA/NIST - Sponsored Workshop on

“In Vitro Analyses of Cell/Scaffold Medical Products”

December 6, 2007, Washington, DC

Goals of *In Vitro* Analysis

- **Insight**
  - "Breakthrough" is defined as productive insight
- **Definition**
  - What the product should be
- **Mechanism**
  - The "how" and "why"
- **Safety**
- **Scientific Validation**
- **Reduction of Risk**

Structure/Function

- **Structure isn’t just man-made**
- **Biological processes modify and give rise to structure**
- **Structure**
  - **Imparts function**
  - **Influences other functions**

Cells and Function

- **Biological character**
  - **Cell type**
    - **Identification**
    - **Purity of cell type (FACS)**
  - **Character of the population**
    - **Growth-related parameters**
    - **Biosynthesis**
  - **Behavior in process**
    - **Cell response and interaction**
      - **Proliferation/Differentiation (range that defines component/product)**
      - **Character (apoptotic, proliferative, differentiating, biosynthetic)**

For details on basic testing, QA/QC aspects see: Wilkins and Parenteau (2001) "Bioengineered Skin: Manufacturing, Safety and Quality Control," in: Cutaneous Wound Healing, V. Falanga, ed. Informa Health Care.

Biochemistry and Function

- Biological character
- **Biochemical character**
  - **Composition**
  - **Biosynthesis**
    - **Changes to matrix (over time)**
    - **Production of factors**

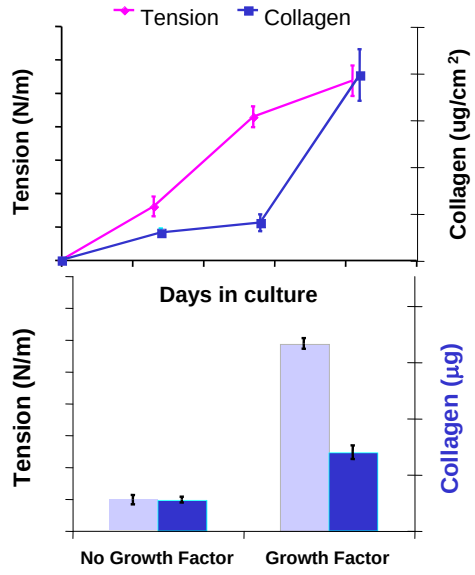
Mechanics and Function

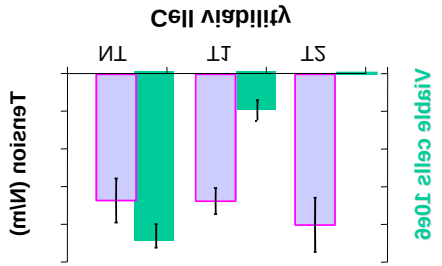
- Biological character
- Biochemical character
- **Physical character**
  - **Burst strength, Membrane Inflation**
  - **Degree of Contraction**

    (collagen gels)

Answers from mechanical testing

- **Change over time**
- **Configuration comparisons**
- **Growth factor influence**
- **Cellular contribution**





Integrated Function

- Biological character
- Biochemical character
- Physical character
- **How they come together**

Parenteau et al., Cytotechnology, 1992. 9:163.

Morphology

- **Morphology of the epidermis reveals**:
  - Character of the cell population
  - Indicator of normalcy
  - Verification of process
- **Stratification is assessed against time**

  For quality of the tissue development
    - Representation of differentiated strata
    - Presence of stratum corneum
- **Validated with immunohistochemistry and biochemistry**

Bilbo et al. J. Toxicol.–Cut.&Ocular Toxicol., 1993 12:183.

Barrier Function

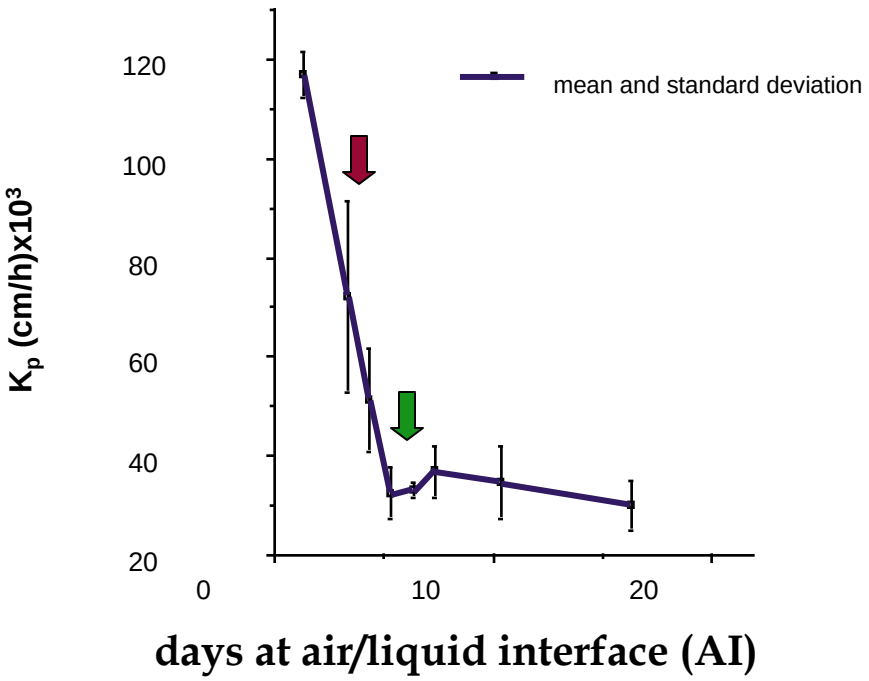
- **Measured by**
  - **Percutaneous absorption (Kp (cm/h)x10^3)**
  - **Trans-epidermal water loss (TEWL)**
    - ***In vitro***
    - ***In vivo***
- **Functional measure of differentiation with time**
- **Supports morphological findings**

Cornification adds a structural/functional component

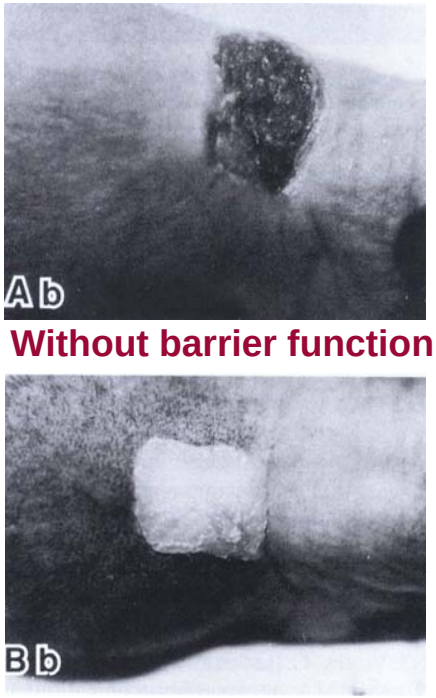
- **Protects underlying living layers**
- **Allows clinical meshing**
- **Provides physiological feedback**
- **Impacts ability to survive on animals**

Structure/Function

Percutaneous Absorption



30 days post graft



Nolte et al., Arch Dermatol Res, 1993. 285: 466;
Parenteau et al., 1996 Biotech. Bioeng. 52:3.

Bioequivalence

The concept in practical terms

- Consistent biological elements
- Consistent behavior in process
  - proliferation
  - differentiation
- Consistent biological character

Bioequivalence of the Skin Construct

- **Keratinocyte and fibroblast strains are tested for performance in skin equivalents**
- **Skin equivalents made from test strains are tested for:**
  - Ability to meet morphological criteria
  - Time to maturation
  - Barrier function
  - Basic cytokine profile
  - *In vivo* performance in athymic mice

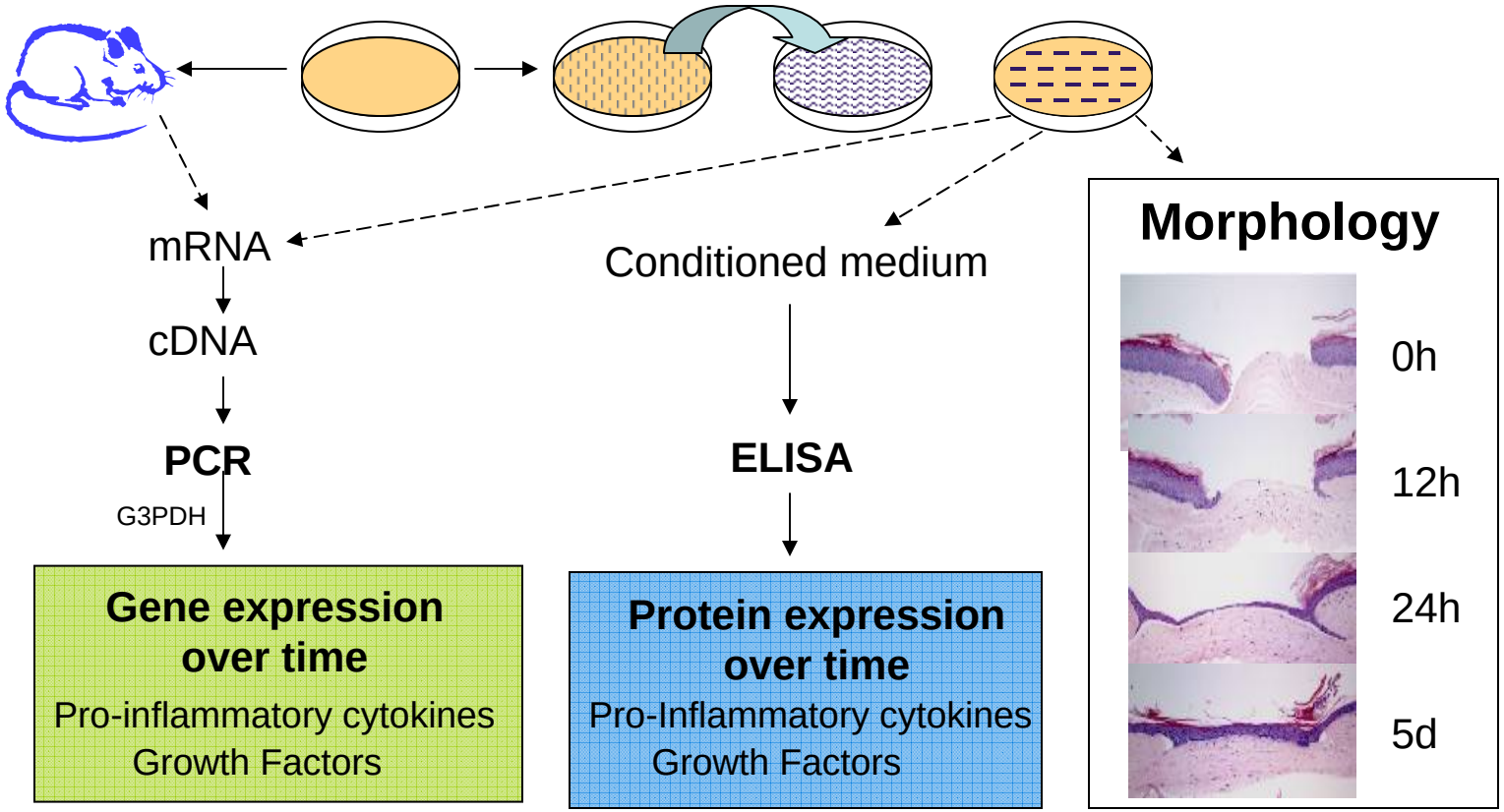
Practical Benefits

- **Sets product parameters**
- **Helps achieve reproducibility**
- **Targets mechanism of action**
  - Further functional assessment
  - For safety assessment

Biological Response and Interaction

- **Response to wounding**
- **Immunology**

Response to Injury *In Vitro*



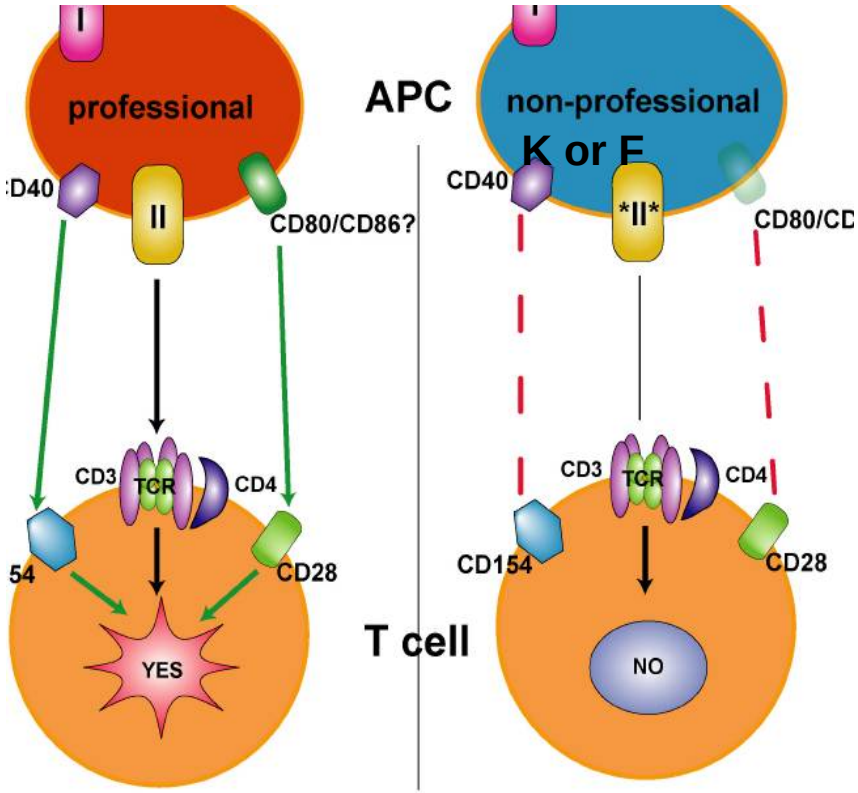
Falanga et al. J Invest Dermatol, 2002. 119: 653.

Biological Response and Interaction

- **Response to wounding**
- **Immunology**

Immunology *In Vitro*

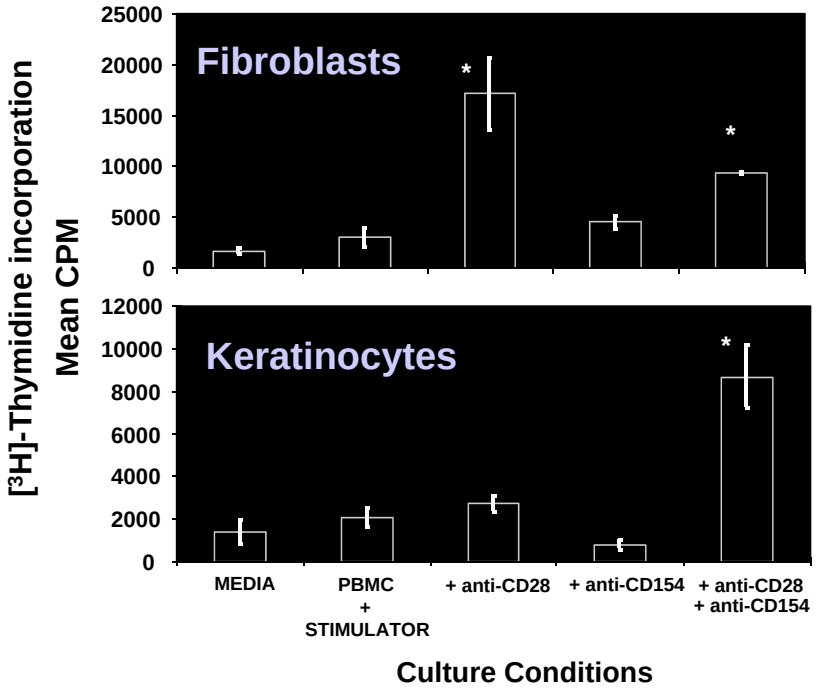
- **T cell proliferation assay**
- Used to:
  - **Determine T cell reactivity to alloantigen on target cells (Keratinocytes and Fibroblasts)**
  - **Monitor patient response**
  - Illuminate mechanism of poor co-stimulation
  - Determine likelihood and impact of sensitization to alloantigens
  - Determine impact of cytokines on immune response



Theobald et al. 1993 Transplantation 55:128;
Nickoloff et al. 1993 Am. J. Pathol. 142:1029.

Understanding Poor Immuno-reactivity

- **T cell proliferation assay**
- Used to:
  - Determine T cell reactivity to alloantigen on target cells
  - Monitor patient response
  - **Illuminate mechanism of poor immuno-reactivity**
    - **Cytokines**
    - **Co-stimulation**
  - Determine likelihood and impact of sensitization to alloantigens
  - Determine impact of cytokines on immune response

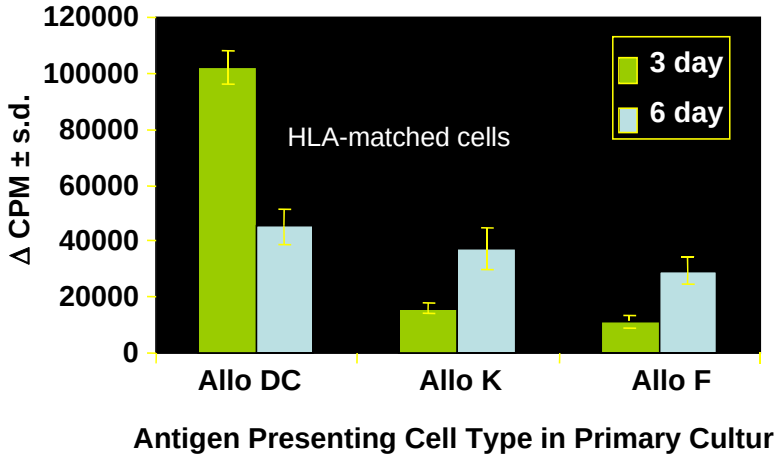


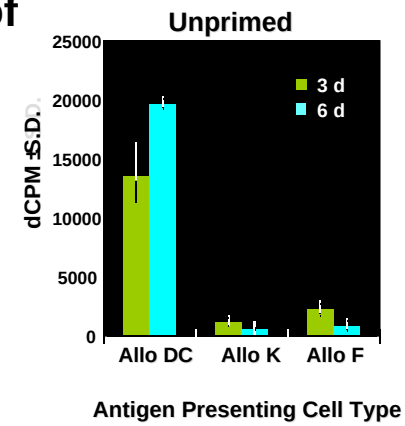
Laning et al. 2001 Transplantation. 71:1467;

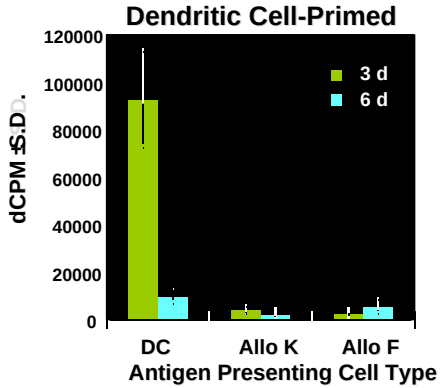
Laning et al. (1997) Cell. Immunol. 175:16-24.

Possibility of Sensitization

- **Reaction of primed and unprimed T cells**
- Used to:
  - Determine T cell reactivity to alloantigen on Keratinocytes and Fibroblasts
  - Monitor patient response
  - Illuminate mechanism of poor immuno-reactivity
  - **Determine likelihood and impact of sensitization to alloantigens**
  - Determine impact of cytokines on immune response







FDA *Summary of Safety and Effectiveness* for Apligraf® (Graftskin)

Take Home Message…

- **Acquire enabling knowledge, skills, and expertise early**
- **Develop a logical plan based on fundamental questions**
- **Use *in vitro* data to**
  - **gain insight for informed decisions**
  - **build a firm scientific rationale**
- **It doesn’t have to be fancy to be informative – quality trumps quantity**

The Ideal Preclinical Plan

- **Generates insight every step of the way**
- **Provides the information that:**
  - Determines Components
  - Defines the Product
  - Sets Process Parameters
- **Determines the “how” and “why”**
- **Creates the foundation for safety**
- **Lends support for probable efficacy**
- **Reduces strategic risk**
- **Continues to contribute**

***In vitro* and *in vivo* analyses work best in partnership.**

Each increases the value of the other.

The Path to Success Begins Day 1

- **Plan for success**
- **Prioritize efforts without ignoring the issues**
- **Develop a sense of what you need as early as possible**
  - Earlier is less costly than later
  - Earlier is less risky than later
  - Weaknesses in strategy can escalate
- **Second chances are rare**

Want to Learn More?

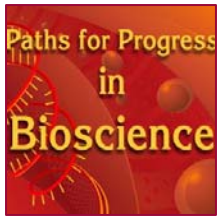
Special White Papers:

- ***Positioned for Success: Building a Biological Product Using a High-Value Preclinical Plan***
- ***Reducing Risk in Bioscience Development: Closing the Information to Knowledge Gap***

Additional White Papers online…

Educational resources:

- The Best of Bioscience Letter (*open access*)
  www.bestofbioscience.com
- Podcasts in Applied Bioscience:



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