The Next Generation of Cell-Based, Tissue Engineered Products for Wound Care.

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Stratatech's human skin substitute tissues

NIKS® cells

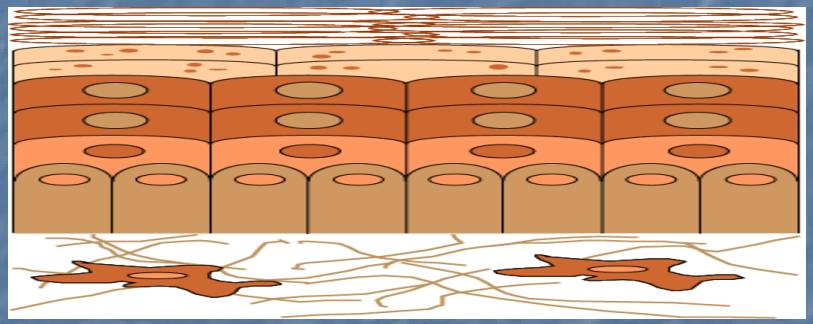
- Proprietary epidermal progenitors
- FDA CMC testing complete
- Master & working cell banks in place

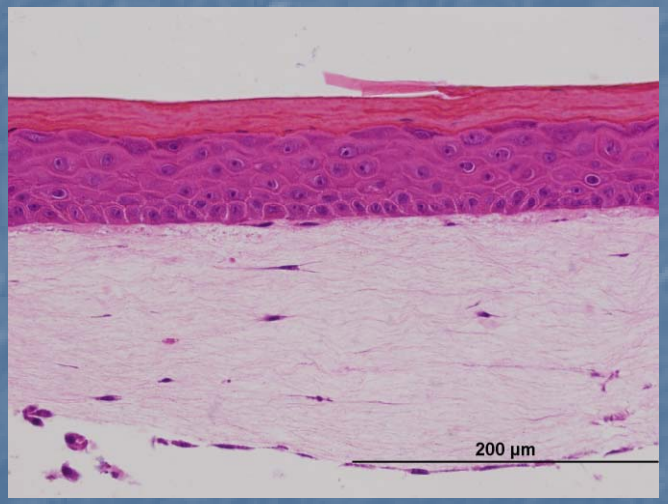
Epidermal layer:
NIKS® cells

+

Dermal layer:
human fibroblasts
and non-bovine collagen

Cultured under proprietary conditions





- Fully developed multi-layered human skin
- Physical barrier present
- Highly reproducible
- Biologically active
- Strong, durable, suturable
- Temporary tissue substitute

StrataGraft®
ExpressGraft®

Next Generation: Regenerative Medicine

Platform technology: Proprietary allogeneic NIKS® human keratinocyte progenitors

StrataGraft®

- Fast follower – regulatory path, customer base, education, reimbursement
- Better handling, storage, multiple formats
- Large characterized cGMP cell banks
- Scalable cGMP manufacturing process
- Positioned for lower COGS
- Completed Phase I/IIa clinical trial

ExpressGraft®

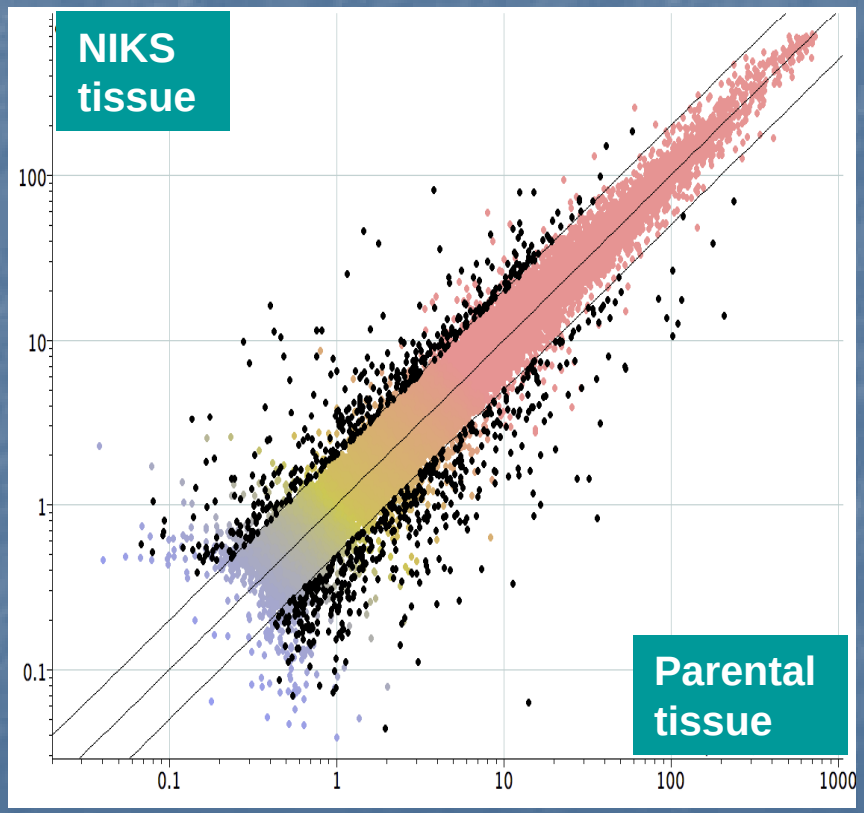
- First genetically enhanced tissue pipeline
- Engineered skin substitutes expressing therapeutic proteins to accelerate healing
  - Anti-infective
  - Pro-angiogenic
  - Anti-protease
- R&D through NIH STTR/SBIR, ATP, DOD
- MCB, WCB, EOP cell banks completed

Discovery of the NIKS® cell line

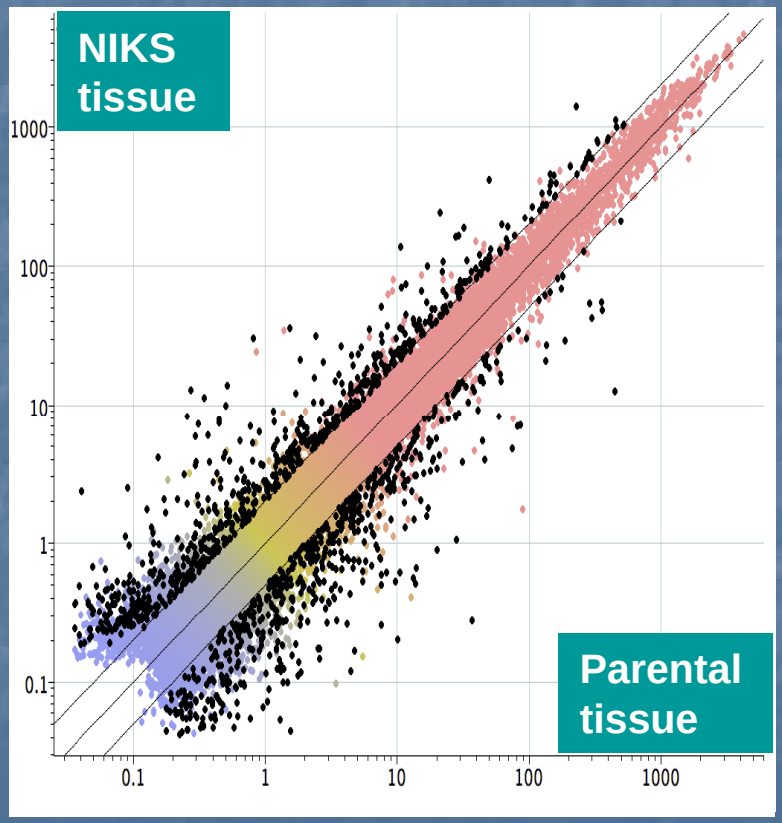
- Continuous source of long-lived human epidermal progenitors
- Adventitious agent-free (Regulated by CBER)
- Non-tumorigenic
- Low immunogenicity
- Can be stably genetically engineered with naked DNA
- Regenerative medicine applications
  - –Therapeutic skin substitutes
  - –Exploring NIKS-based induced pluripotent stem cells (iPS cells)
  - –Consumer products testing and discovery

Gene expression profile: NIKS® compared to parental keratinocytes

CodeLink Array



Agilent Array



NIKS®: consistent source of pathogen-free human epidermal progenitors

Test	Detection method	Result
Sterility	Broth culture	Negative
Mycoplasma	Broth culture/Hoechst stain	Negative
HIV-1	PCR	Not detected
HIV-2	PCR	Not detected
HTLV-1/HTLV-2	PCR	Not detected
Human Papilloma Virus	Hybrid capture detection	Not detected
Epstein-Barr Virus	PCR	Not detected
Cytomegalovirus	PCR	Not detected
Hepatitis B	PCR	Not detected
Hepatitis C	PCR	Not detected
Human B-19 Parvovirus	PCR	Not detected
Porcine Parvovirus	Indicator cell line + immunodetection	Not detected
Reverse transcriptase	PCR-enhanced Reverse transcription	Not detected
Adventitious agents-unidentified	Indicator cells + immunodetection	Not detected
Adventitious agents-unidentified	Inoculation of embryonated eggs and adult/suckling mice	Not detected

Waisman Clinical Biomanufacturing Facility

What are the problem wounds?

- Large full thickness wounds
  - Lack of donor site availability
  - Temporary coverage with cadaver skin
- Serial donor site harvests
- Autologous cultured keratinocytes (Epicel)
  - 3 week delay to establish initial cultures
  - 4 cell-layer thick keratinocyte-only grafts have poor durability

What are the problem wounds?

- Deep second degree burns
  - Prolonged healing with hypertrophic scar formation
  - Rab et al, Burns 2005: Should dermal scald burns in children be covered with autologous skin grafts or with allogeneic cultivated keratinocytes?
    - Faster healing
    - Less pain
    - Better cosmesis
    - No donor site
  - No consistent source of allogeneic keratinocytes with full barrier function

The solution: Next generation regenerative medicine approaches

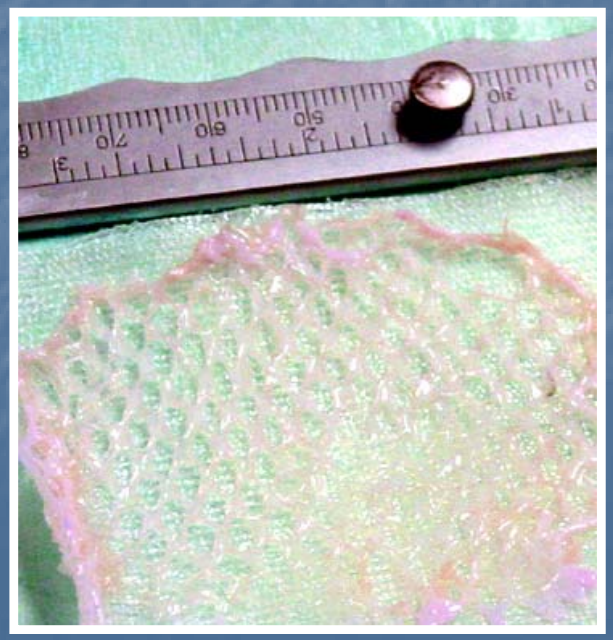
- Cultured skin substitutes
  - Living tissue interacts with the wound bed
  - Immediate wound coverage
  - Barrier function
  - Controls pain
  - Genetic engineering to address:
    - Bacterial bioburden
    - Poor vascular ingrowth
    - Abnormal proteases

Clinical application number 1

- Stratagraft can be used as a temporary wound cover in patients with large TBSA skin defects
  - Bench and animal work completed
  - IND with the FDA
  - Phase I/II clinical trial completed

Phase I/II Clinical Trial

- *NIH/NIAMS R44-AR47499 Translational Clinical Grant*
- Full-thickness wounds ≥5% TBSA requiring serial debridement and grafting
- Temporary coverage for 7 days with StrataGraft® and cadaver allograft
- Primary efficacy outcome was autograft take at 2 weeks



Patient demographics

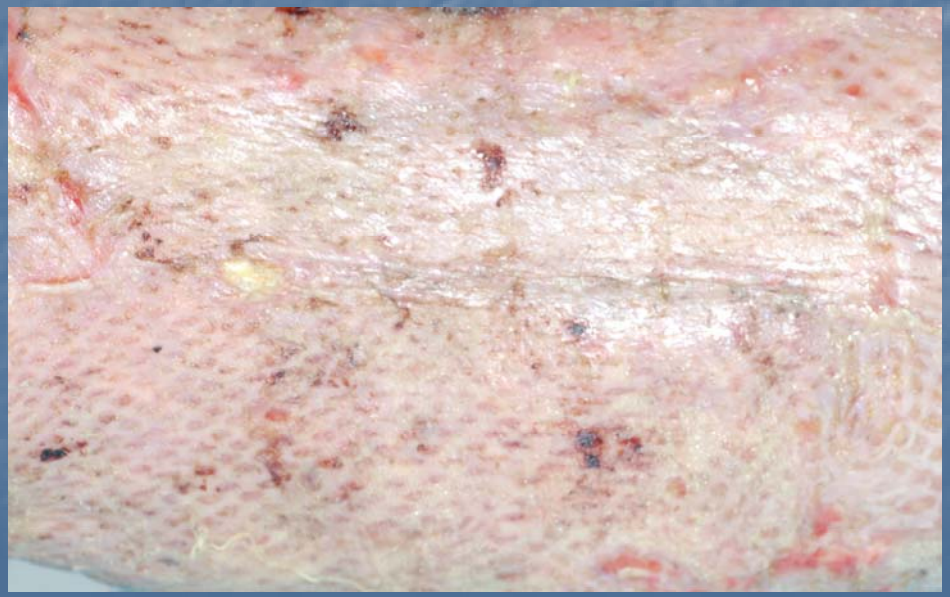
	Age (yr)	Gender	% TBSA	MOI	Treated TBSA
	64	M	17.5	MVC - burn	0.3
	65	F	6	Thermal burn	0.3
	52	M	16	Thermal burn	0.3
	21	M	13	Thermal burn	0.3
	40	M	5	MVC	0.6
	52	F	8	Necrotizing Fasciitis	0.6
	66	M	25	Explosion - burn	0.6
	46	F	15	Necrotizing Fasciitis	0.9
	29	M	73	Explosion - burn	0.9
	38	M	12	Explosion - burn	0.9
	48	F	13	Thermal burn	0.9
	20	M	17.5	Electrical burn	1.2
	34	M	42.5	Explosion - burn	1.5
	38	M	31	Thermal burn	1.5
	38	M	41.5	Thermal burn	1.5
Mean ± SD	**43 ± 15**	**73 % M 27 % F**	**22 ± 18**		**0.8 ± 0.5**

Complex wound management

Clinical trial results

Cadaver allograft, day 7

StrataGraft®, day 7



Autograft: 2 weeks post allograft

Autograft: 2 weeks post StrataGraft®

StrataGraft® clinical trial overview

Clinical Trial – Complex Skin Defects (>5% TBSA)

- Phase I/IIa (15 patients)
- Funded in partnership with NIH (NIAMS)
- Evaluate safety of StrataGraft® skin tissue
- Dose escalation
- Early efficacy

Primary Endpoints

- Autograft take @ 2 wks post placement
- Wound appearance, graft take

Results

- StrataGraft® was pink, adherent, remained intact
- Autograft take unaffected by StrataGraft®
- *In vitro* immunology assessments show low immunogenicity

Primary endpoints achieved

No adverse or serious adverse events

Journal of Trauma (2009) 66:866-874

Clinical application number 2

- Stratagraft can be used as a temporary wound cover in patients with deep second degree burns after operative debridement
  - Bench and animal work completed
  - Clinical trial design proposed

Clinical application number 3

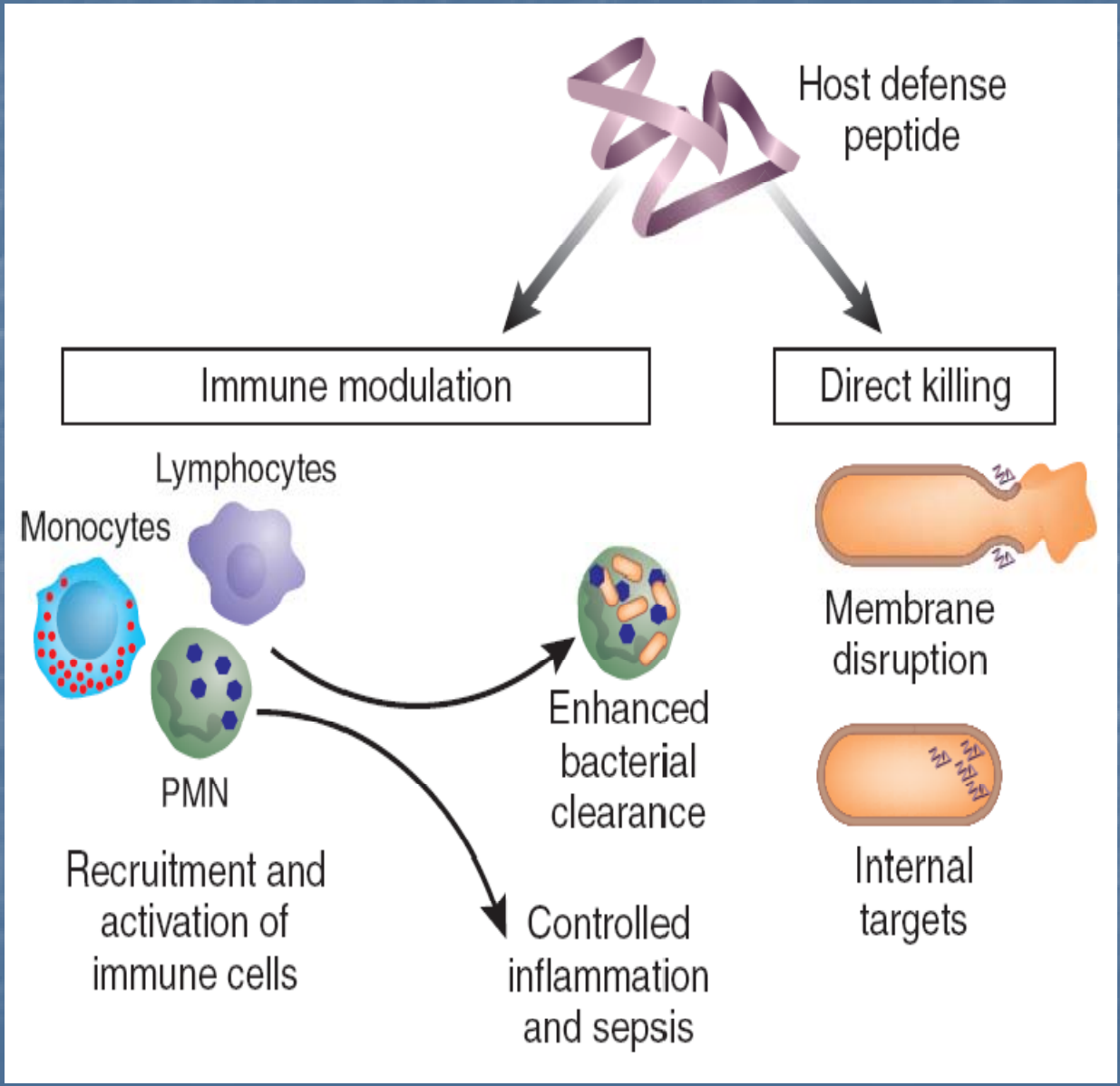
- ExpressGraft can be used as a delivery vehicle for therapeutic agents through genetic engineering to control bioburden
  - Bench work completed
  - Preclinical animal studies in progress
  - Master and working cGMP cell banks
  - Pre-IND meeting in 2009
  - NIH Recombinant DNA Advisory Committee unanimously approved in 2009

Key technology trends in wound care

- **Improve efficacy and reduce treatment costs**
  - Faster healing rates found for products with dermis and epidermis
  - Address pathophysiology of the wound
  - Health care reimbursement – “price per unit” of increasing importance
- **Biologically derived products to reduce rate of infection**
  - *Pseudomonas aeruginosa*
  - Methicillin-resistant *staphylococcus aureus*
  - Vancomycin-resistant *enterococcus*
  - Multidrug-resistant *Acinetobacter baumanni*
- **Cosmetic outcome of wound treatment**
  - Reduction or elimination of scar formation
- **Enhance shelf life**

Human Defense Peptides (HDPs)

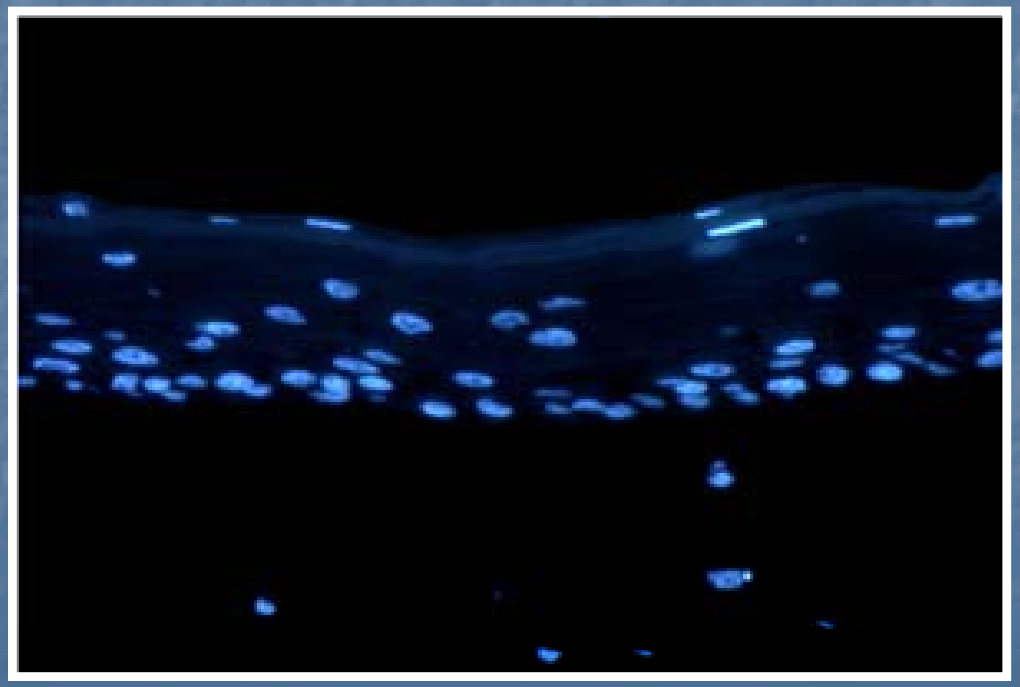
- Defensins
  - α, β
  - epithelial tissues
- Cathelicidin
  - Antimicrobial
  - Chemotactic
  - Angiogenic
  - Work in synergy with other HDP

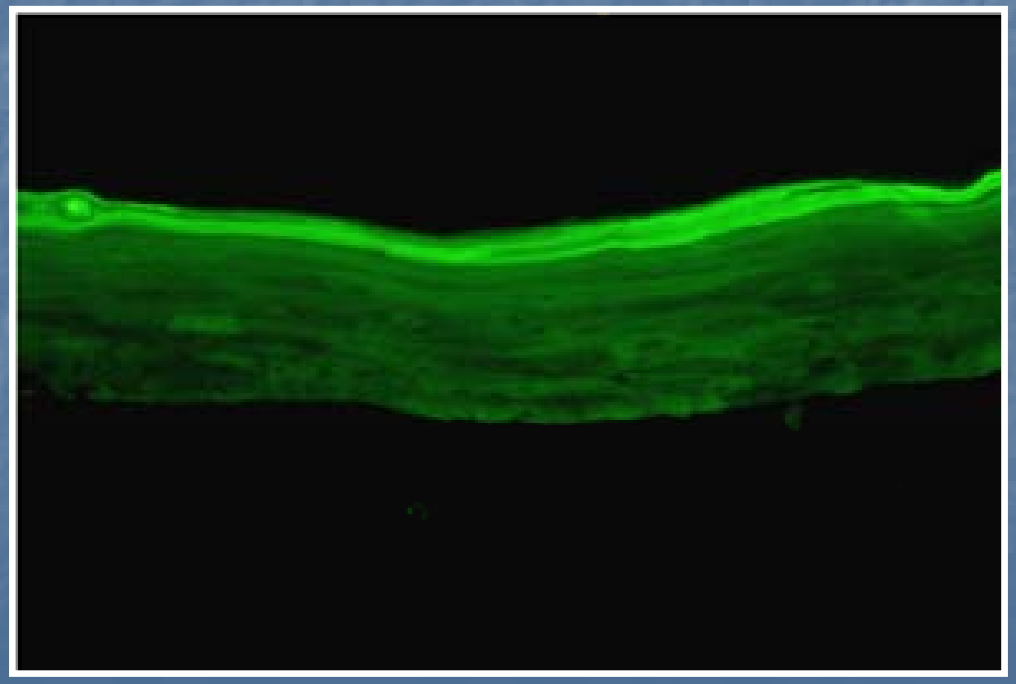


Hancock & Sahl Nature Biotech 2006

Genetically enhanced NIKS®

- **Genetically modified tissue therapeutics**
- **Non-viral vectors**
- **Green fluorescent protein-expressing NIKS®**
- **Normal tissue architecture generated**





Platform technology: stable genetic modification of epidermal progenitors

Antimicrobial factors (1 product)

Angiogenesis factors (2 products)

"ExpressGraft" pipeline of products

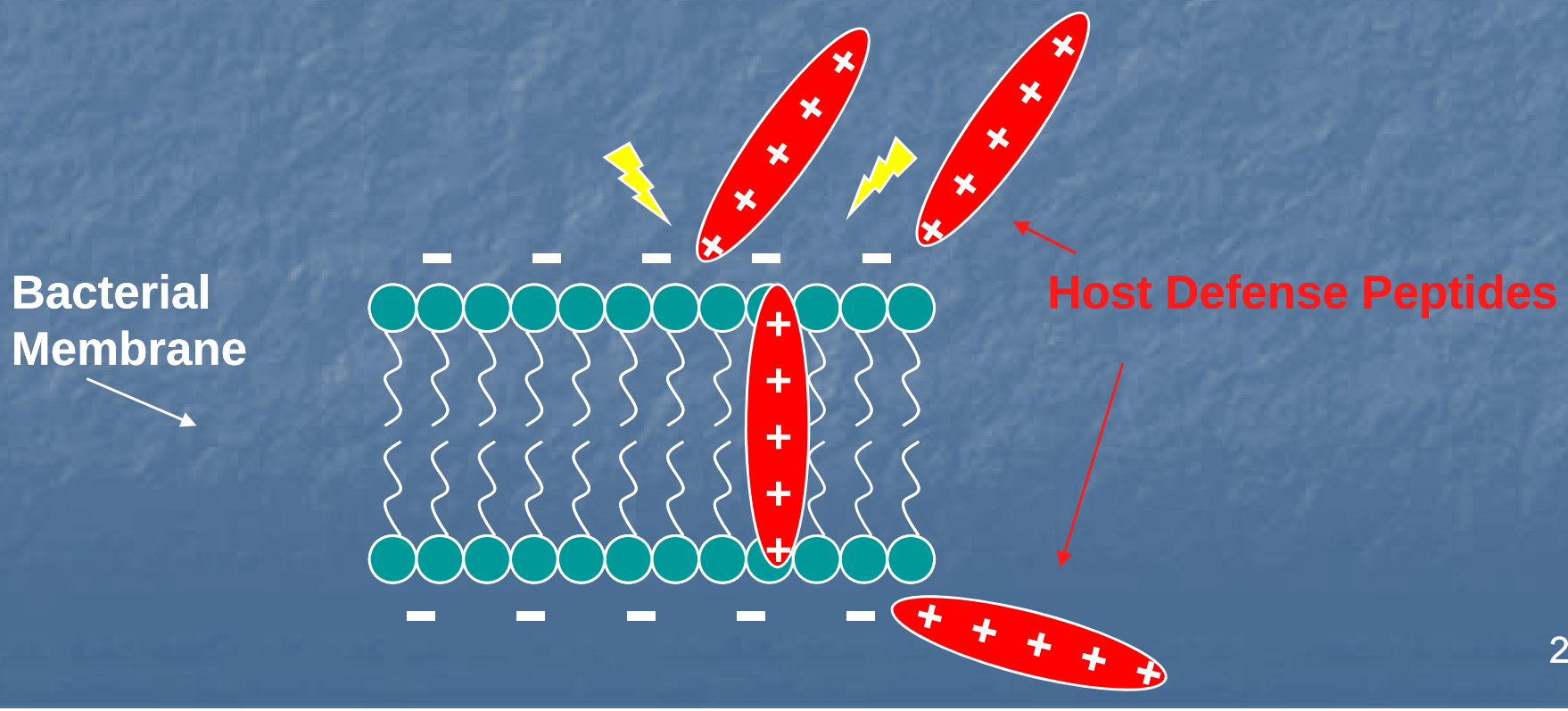
Protease inhibitors (1 product)

Pro-innervation factors (1 product)

Growth factors (1 product)

ExpressGraft®: Anti-infective human tissue

- Cathelicidin (hCAP18/LL-37) - broad spectrum and fast acting (Gram +/- bacteria, fungi, yeast, viruses, HIV)
- Suppressed levels in burn wound fluid and diabetic ulcers
- Local, sustained delivery of hCAP18/LL-37 while providing a biologically active temporary tissue replacement

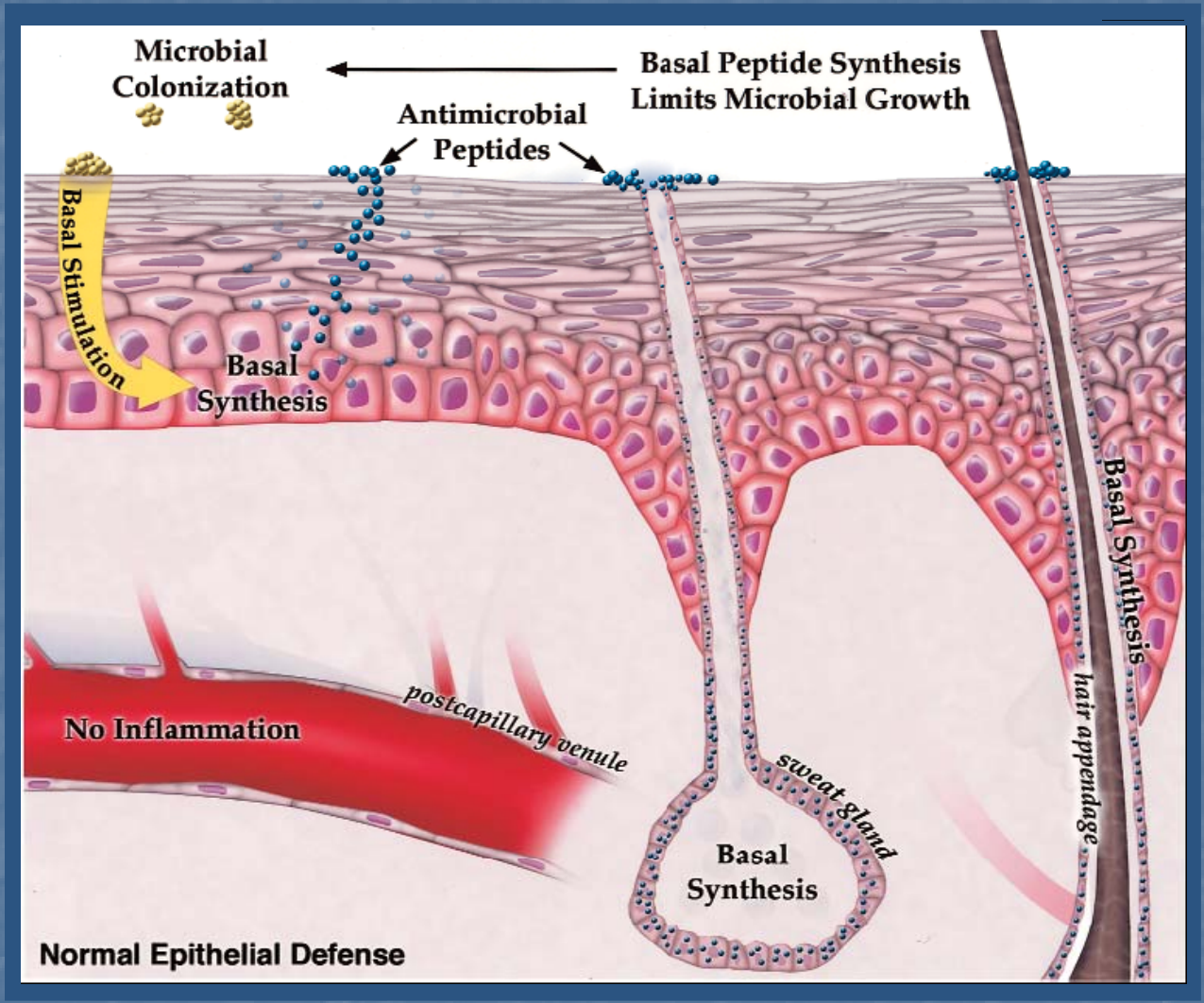


Biological activities of LL-37

(*Adapted from Nijnik and Hancock, Current Opinions in Hematology 16:41, 2009)*

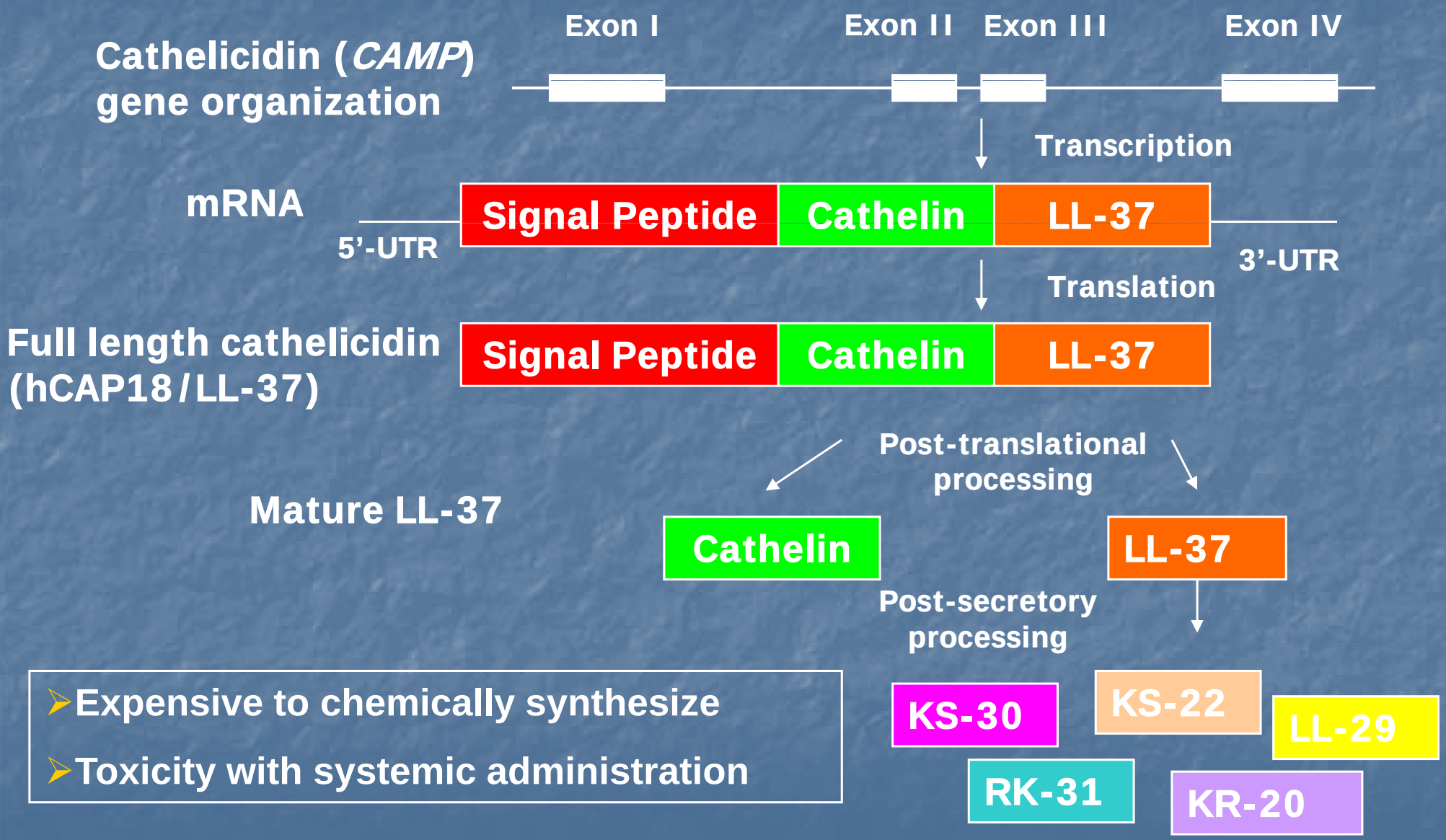
Activity	Description	Citation
Microbicidal activity	Acts on broad spectrum pathogens	Bowdish et al, J Leukoc Biol, 77:451, 2005; Durr, et al., BBA 1758:1408, 2006
Inhibits biofilm formation	Inhibits *P. aeruginosa* biofilms	Overhage et al., Infect Immun 76:4176, 2008
Induces virulence genes	Induces group A *Streptococcus* resistance to opsonophagocytic killing by leukocytes	Gryllos et al., PNAS 105:16755, 2008
Chemotaxis	Acts as chemokine for neutrophils, monocytes, mast cells, T cells	Soehnlein et al., Blood 112:1461, 2008
Mast cell degranulation	Release histamines, prostaglandins from mast cells	Niyonsaba et al., Eur. J. Immunol 31:1066, 2001
Induction of immune mediators	Induces chemokines in monocytes, IL-8 in keratinocytes	Braff et al., J. Immunol. 174:4271, 2005
Regulation of inflammation	Suppresses LPS-induced inflammatory cytokines	Lande et al., Nature 449:564, 2007
Apoptosis	Induces neutrophil secondary necrosis	Zhang et al., J. Leuko. Biol. 84:780, 2008
Wound healing	Promotes keratinocyte migration and wound healing	Carretero et al., J. Invest. Dermatol. 128:223, 2008
Angiogenesis	Promotes vascularization by effects on vascular endothelium	Kuczulla et al., J. Clin. Invest.111:1665, 2003

Host defense peptides (HDP) in human skin



J Allergy Clin Immunol (2002) 110(6):823-31

Post-translation modifications activate cathelicidin



- Expensive to chemically synthesize
- Toxicity with systemic administration

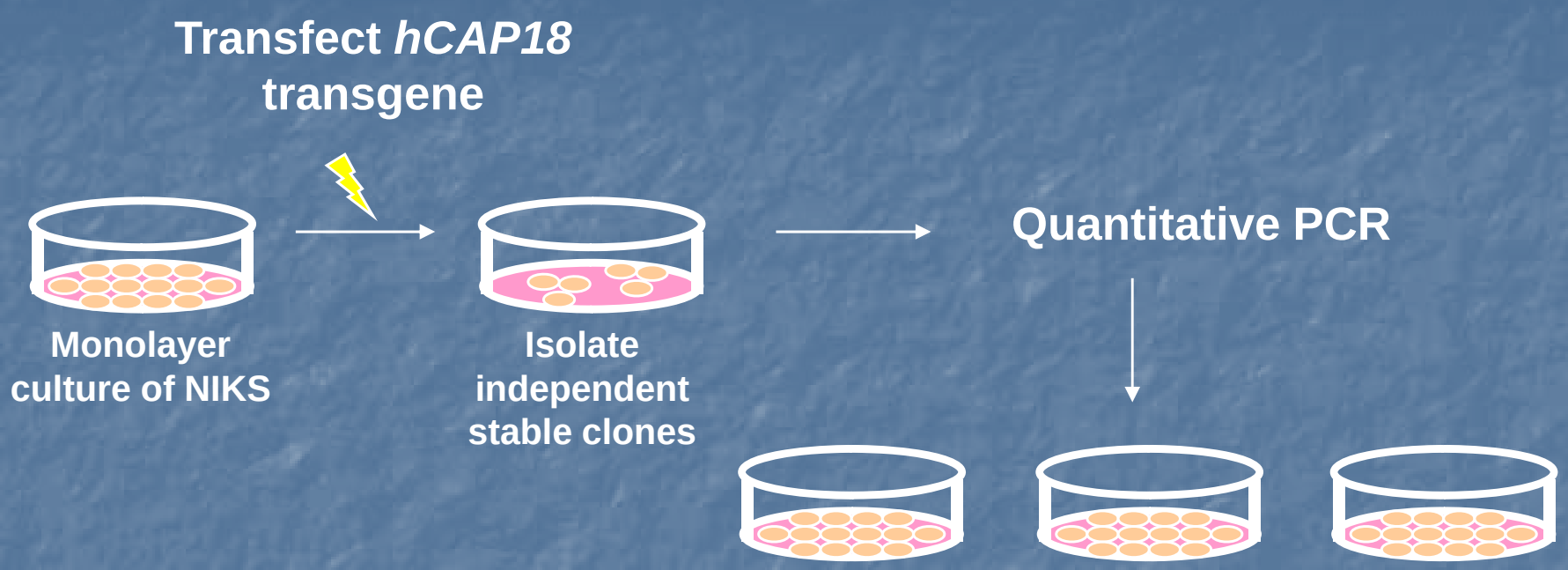
ExpressGraft$_{Enhance}$ tissue

UbC Pro	Bsd	Inv Pro	hCAP-18	globin

Mammalian hCAP-18/LL-37 expression fragment

- Hypothesis-based research funded by NIH
- Stably transfected, clonal cell line
- ~20 copies stably integrated on chr 19q
- Non-tumorigenic, karyotypically stable
- Master and working cGMP cell banks
- hCAP-18/LL-37 concentration in tissue
  - By volume: 1.8 μM (9 μg/ml)
  - By surface area: 227 ng/cm^2

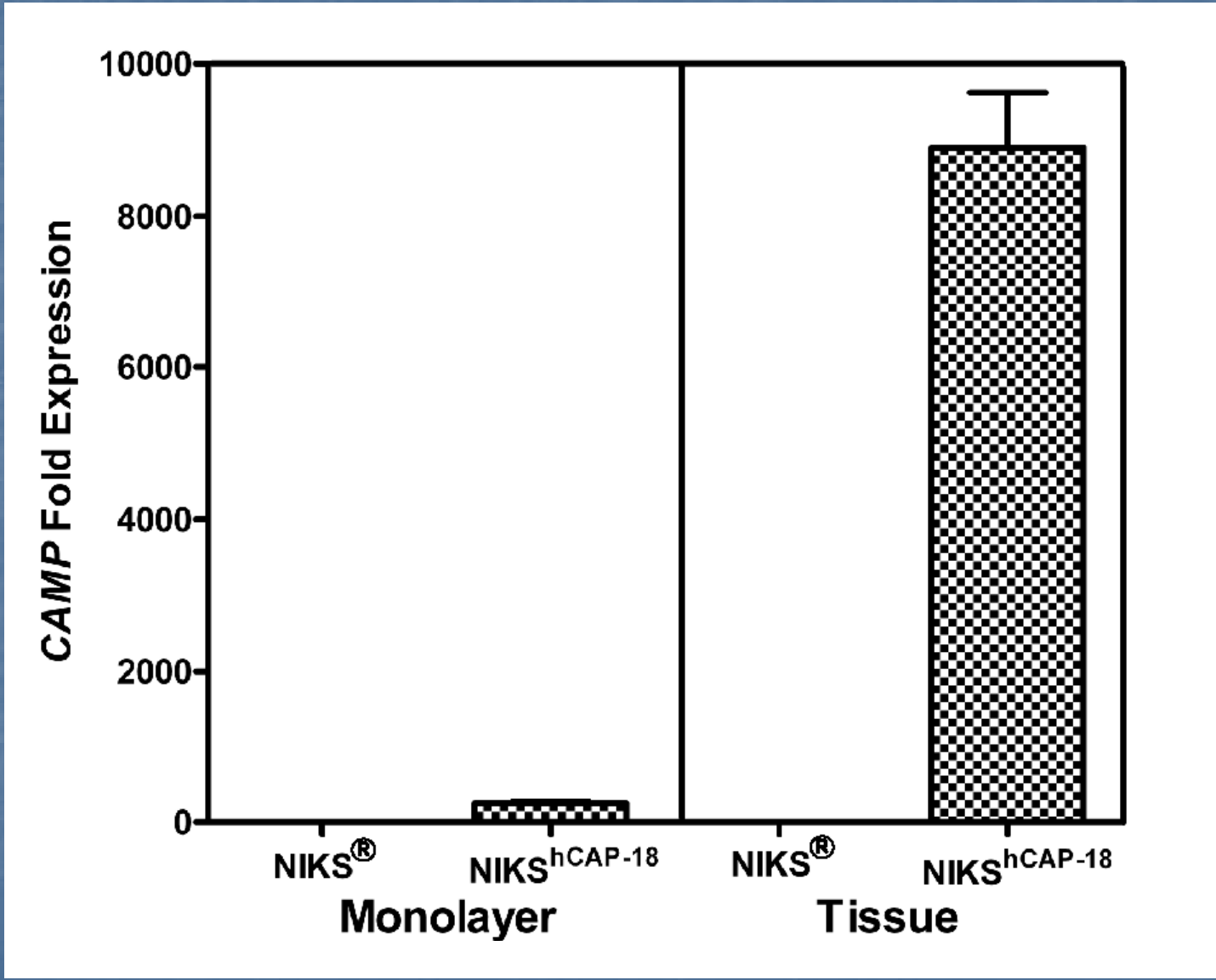
Independent clonal isolates expressing *hCAP18*



Expand clones and screen

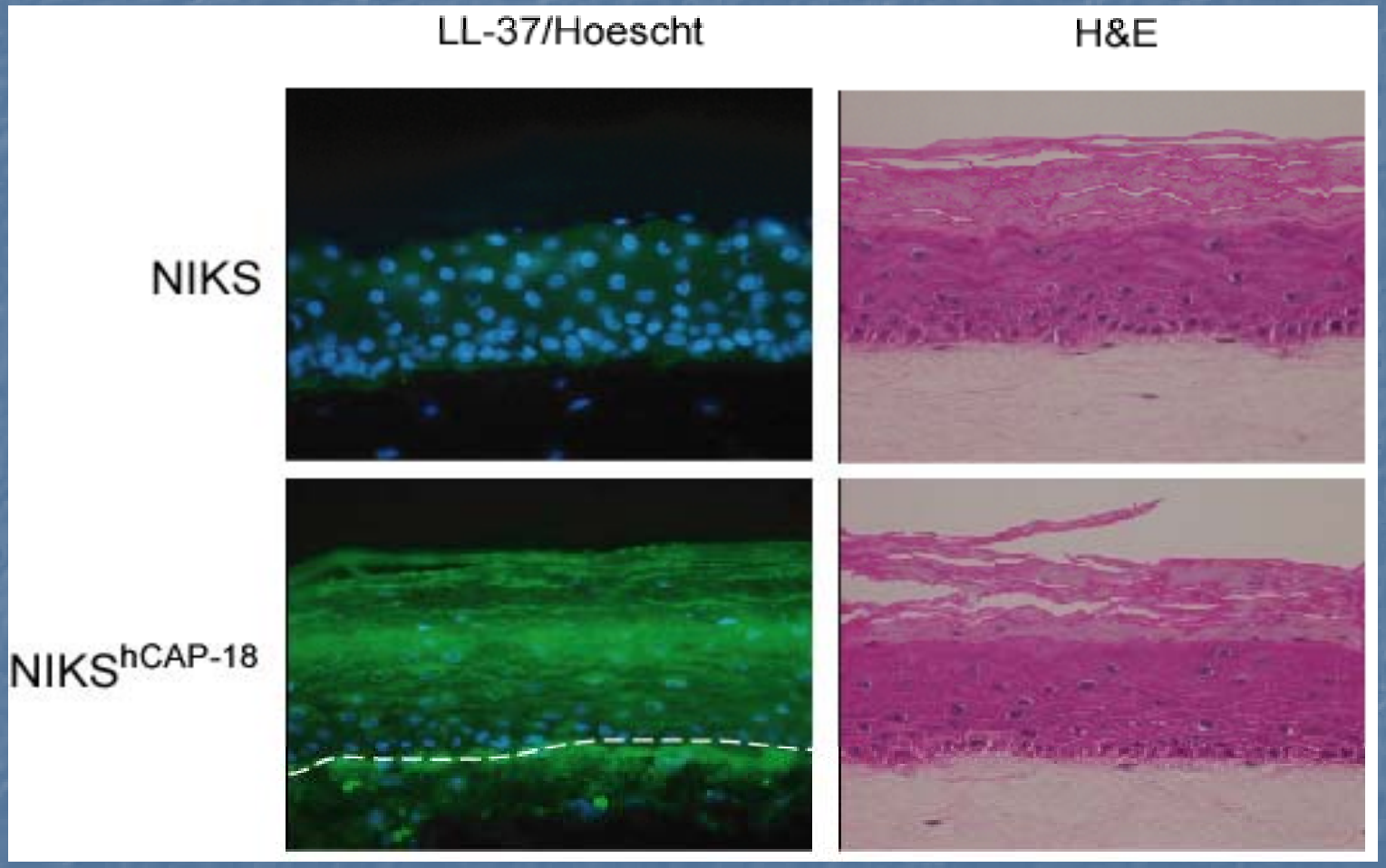
- Biological activity
- Cell growth
- Karyotype
- Tissue histology
- Transgene copy and insertion site
- Tumorigenicity
- Potency

Stable tissue-specific expression of hCAP-18 mRNA



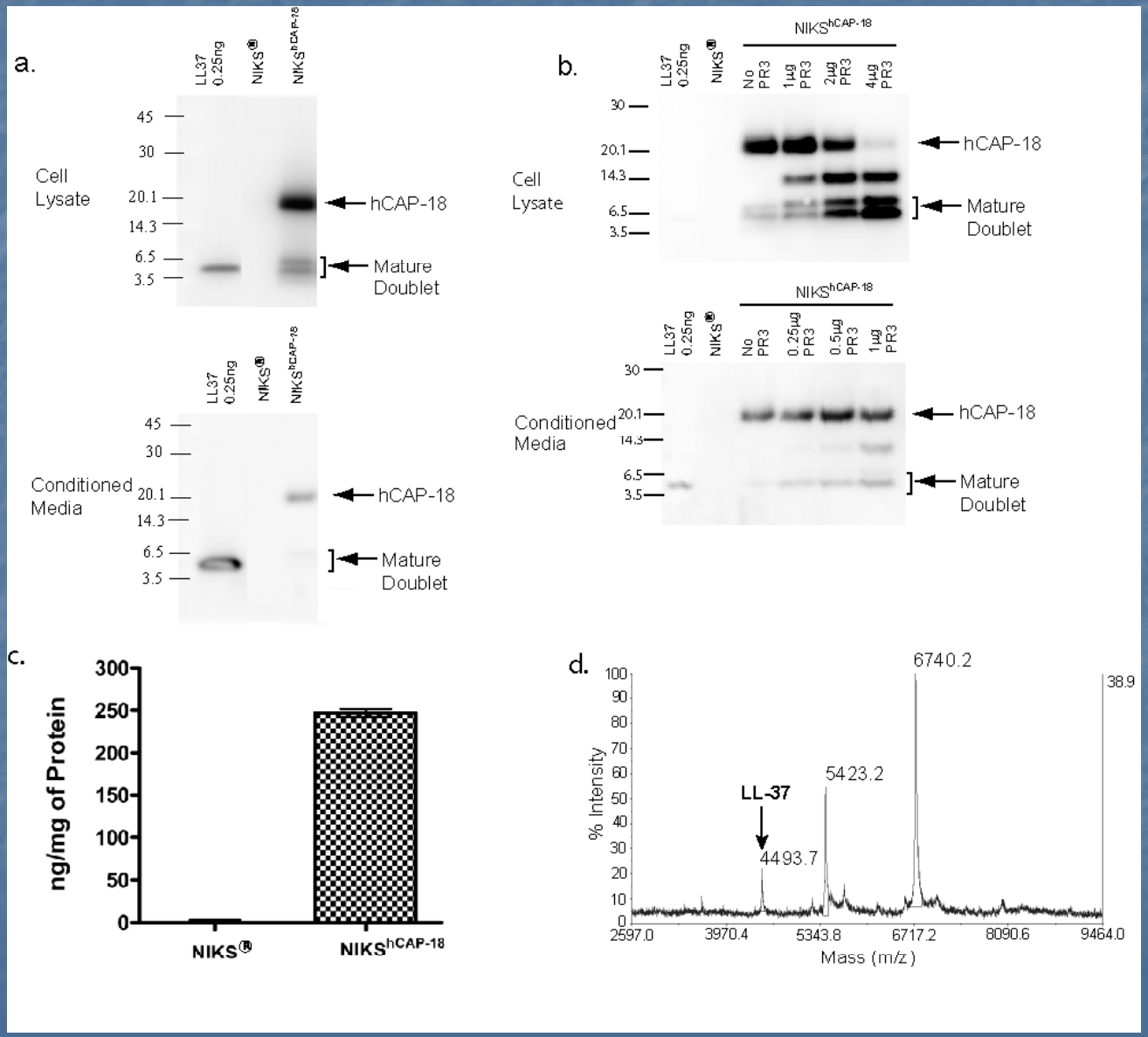
Molecular Therapy (2009) 17(3):562-569

Epidermal hCAP18/LL-37 production



Molecular Therapy (2009) 17(3):562-569

hCAP18 is processed to active forms (LL37)

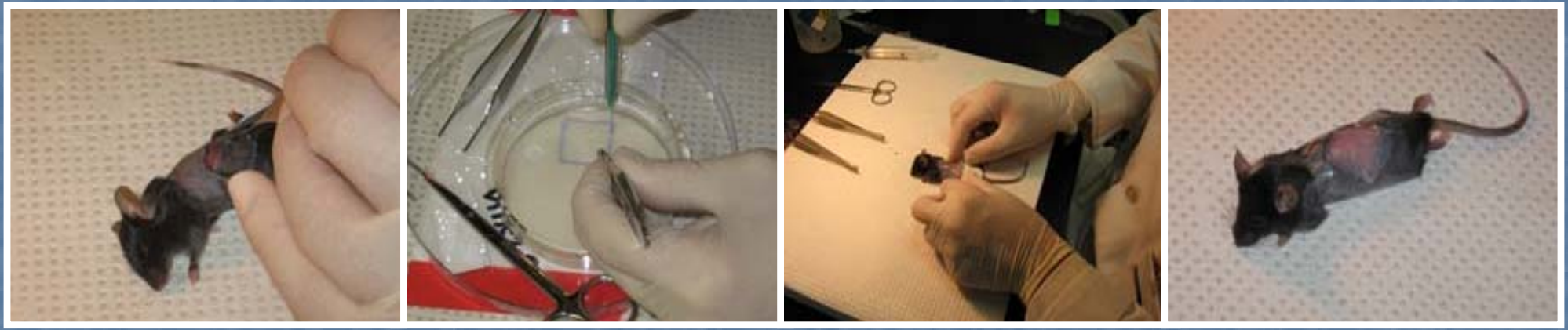


Molecular Therapy (2009) 17(3):562-569

In vivo activity of ExpressGraft$_{Enhance}$ tissue

Day 1 – Innoculate 3rd degree burn with 10^4 multidrug-resistant *Acinetobacter baumannii*

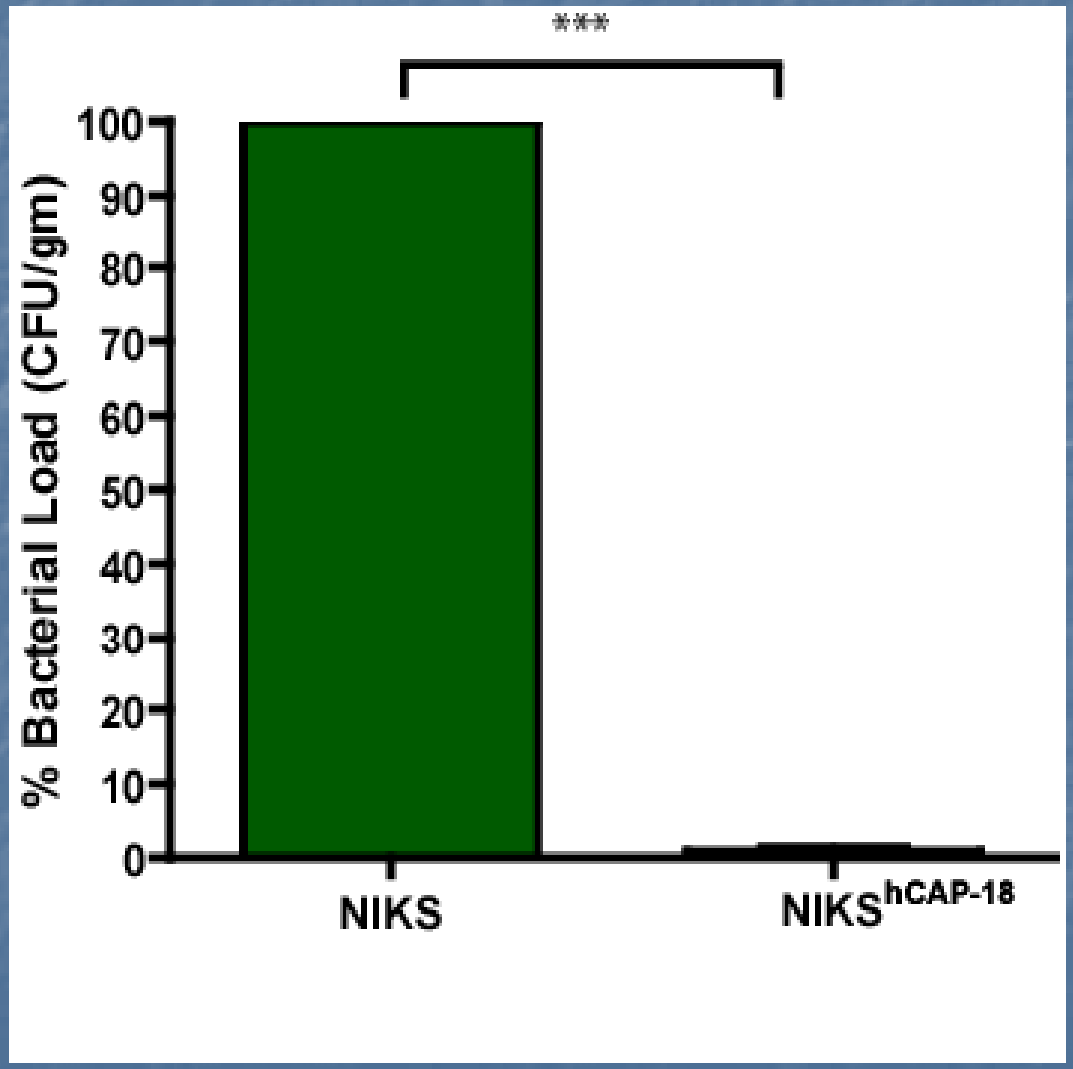
Day 2 – Place control and ExpressGraft skin grafts



Day 5 – Quantitative cultures of grafts/wound bed

Day 6 – Enumerate CFU/g tissue

Reduces bacterial load of a clinical isolate of multi-drug resistant *A. baumannii*



Molecular Therapy (2009) 17(3):562-569

“A paradigm shift in wound healing……”

See page 562

Bioengineered Human Skin: Working the Bugs Out

L Steinstraesser[1], S Al-Benna[1], M Kesting[2] and F Jacobsen[1]

doi:10.1038/mt.2009.14

Commentary

L. Steinstraesser, S. Al-Benna, M. Kesting, and F. Jacobsen. (2009) Molecular Therapy 17:405-408.

NIH Recombinant DNA Advisory Committee

- Non-viral vector with tissue-specific promoter
- Stable transfection, clonal selection
- Cell-based tissue therapy
- Sustained yet temporary local delivery
- First therapeutic use of cathelicidin
- Unanimous vote from NIH RAC to move into clinic

Clinical Trial Design

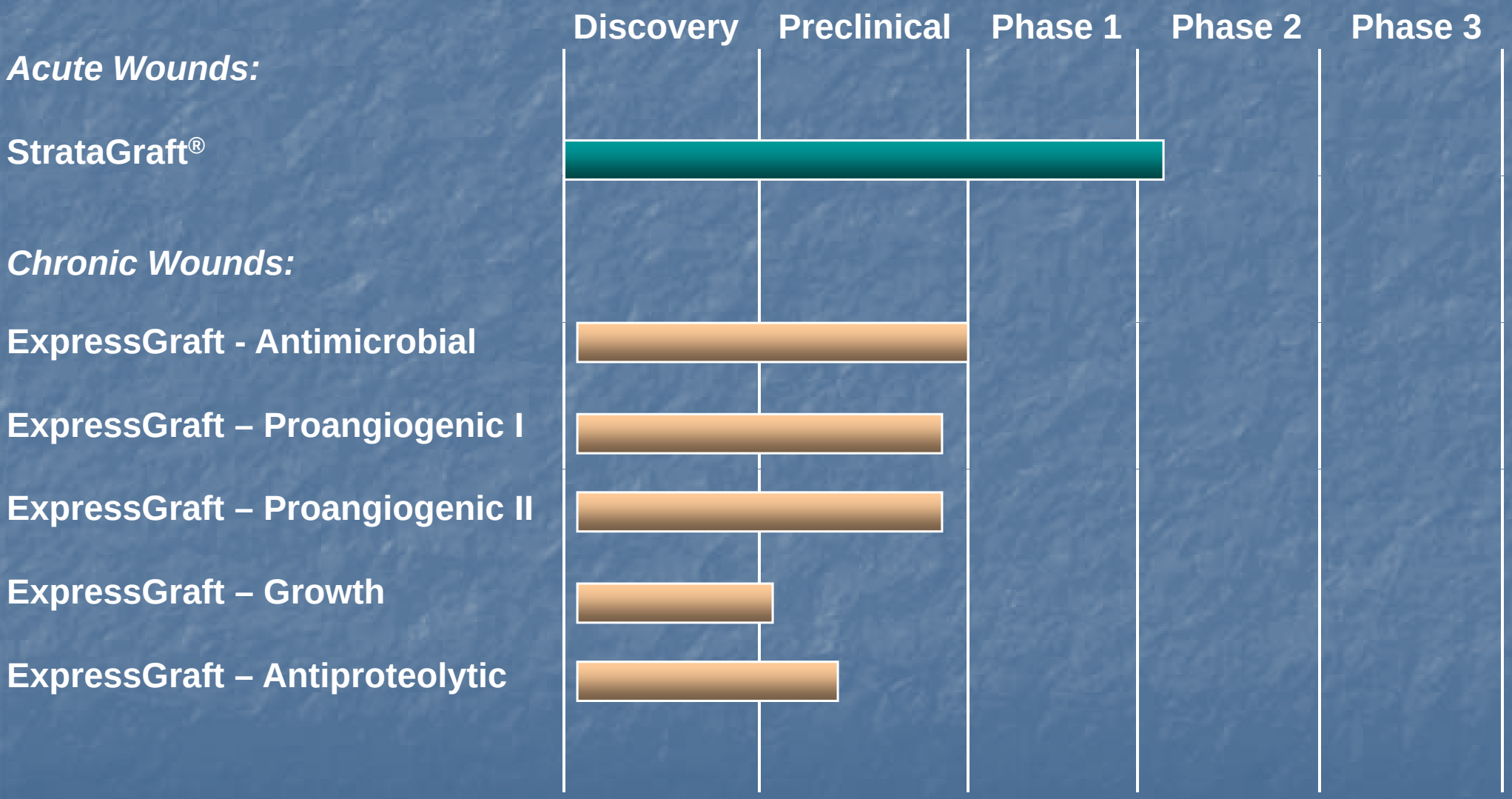
Rationale

- Risk vs. benefit
- Justified by particularly strong preclinical POC studies

Case-by-case consideration of proposed human trials

- Adequate product characterization, preclinical safety and proof-of-concept testing
- Appropriate trial design
  - Staged approach to Phase I studies
  - Dose escalation
  - Patient monitoring

Product Pipeline and Strategy



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Thank you!

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