imc FH KREMS UNIVERSITY OF APPLIED SCIENCES/AUSTRIA

Studying the international way

Characterization of anti-inflammatory substances from Cyanobacterias

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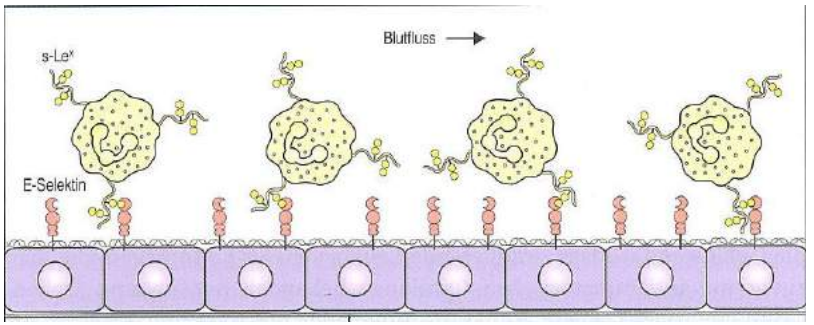
Cyanobacteria

- European Territorial Co-operation Czech Academy of Sciences and IMC Krems
- Rich in secondary metabolites showing biological activity
- Anti inflammatory activities
- Anti tumor activities

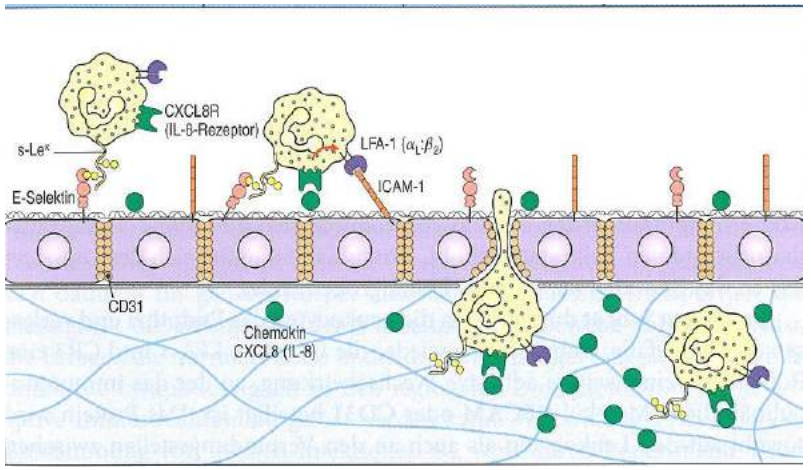
Roles of the vascular endothelium

- Transport of substances between blood and tissues
- Modulation of the vascular tone
- Control of blood coagulation
- Control of fibrinolysis
- Control of leukocyte **extravasation**

Non inflammatory conditions



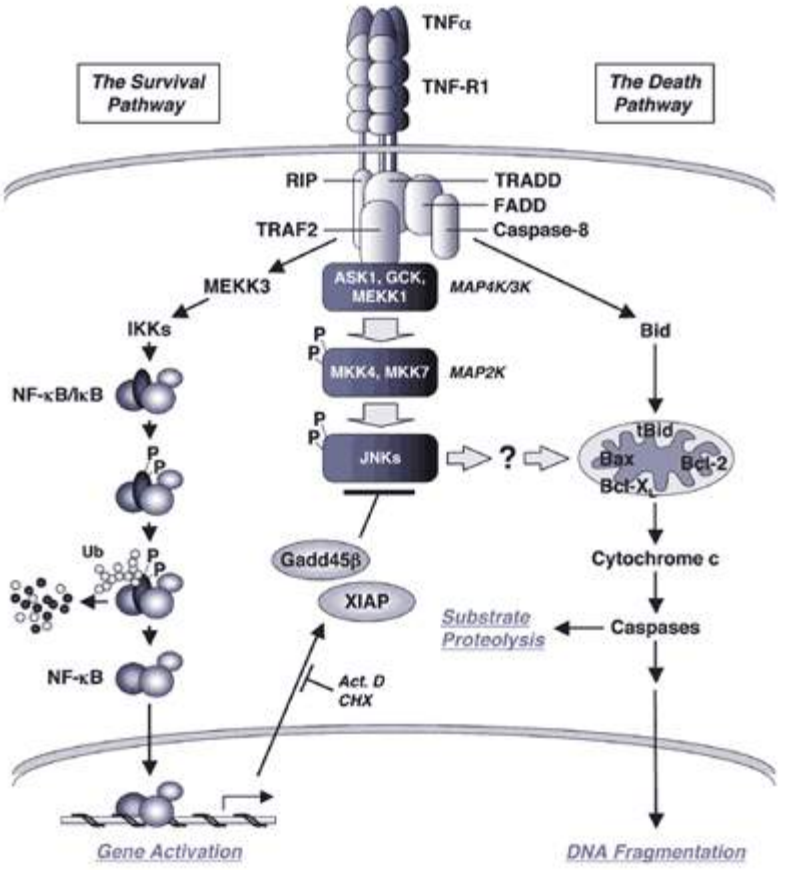
Pro-inflammatory conditions (TNF alpha)



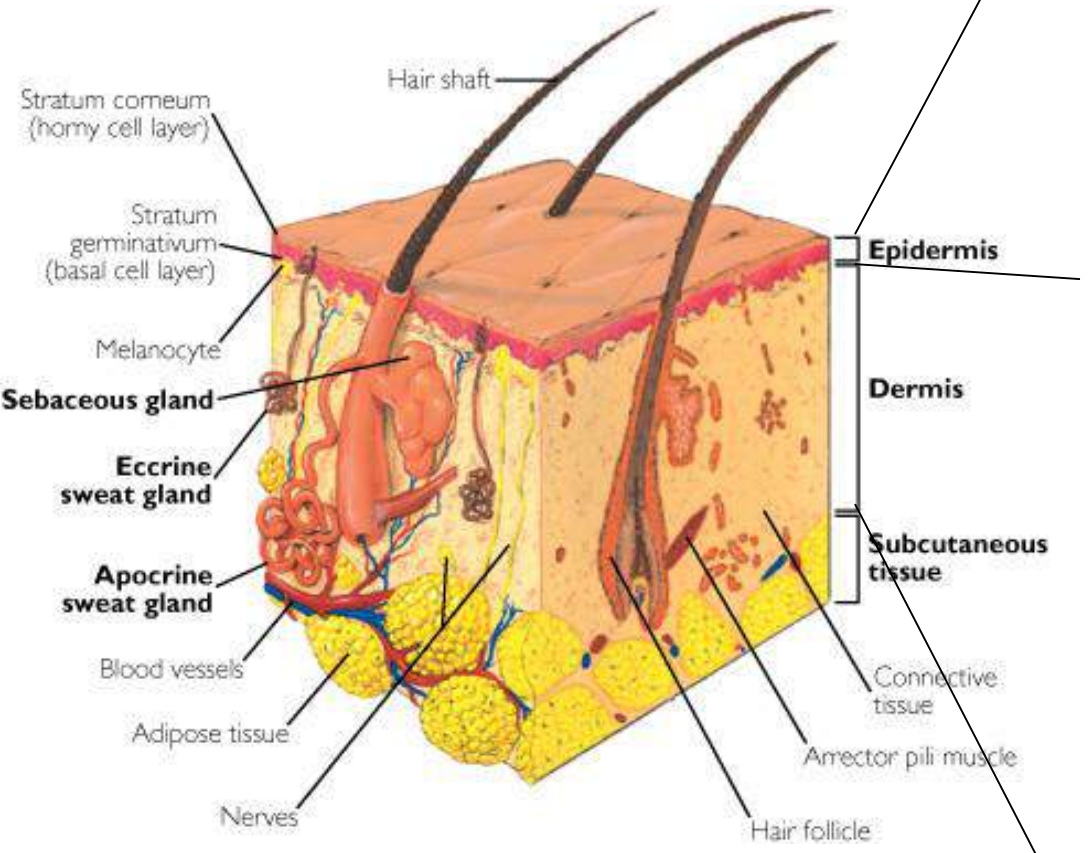
Cell-Signalling during inflammation

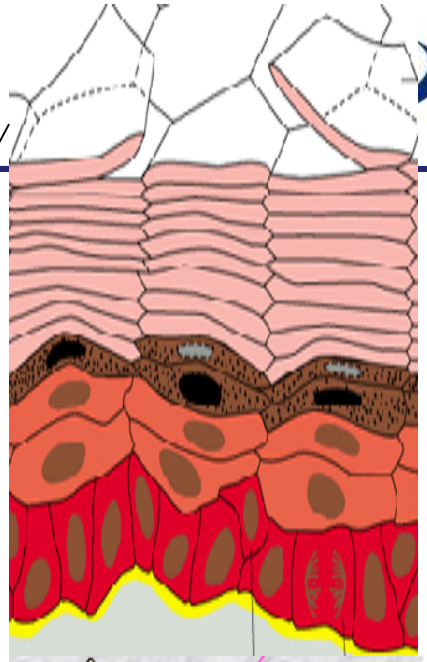
- Activation of Nuclear factor κB (NF-κB) leads to the transcription of pro-inflammatory genes (ICAM-1)

- LPS or TNFα are strong activators of this pathway



Skin





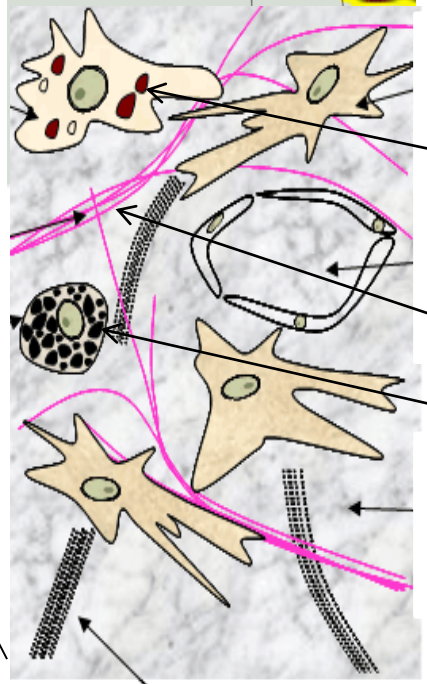
Stratum corneum

Stratum lucidum
Stratum granulosum

Stratum spinosum

Stratum basale

basal cell layer



Fibroblasts

Macrophage

Capillary
Elastic fibres

Mast cell

GAGs

Collagen fibres

Wound healing

- *In vivo* all wounds heal following a specific sequence of phases which may overlap.

- The process of wound healing depends on the type of tissue which has been damaged and the nature of tissue disruption.

- **Physiology of Wound healing**
  Haemostasis (Vasoconstriction, Platelet response, Biochem. response)

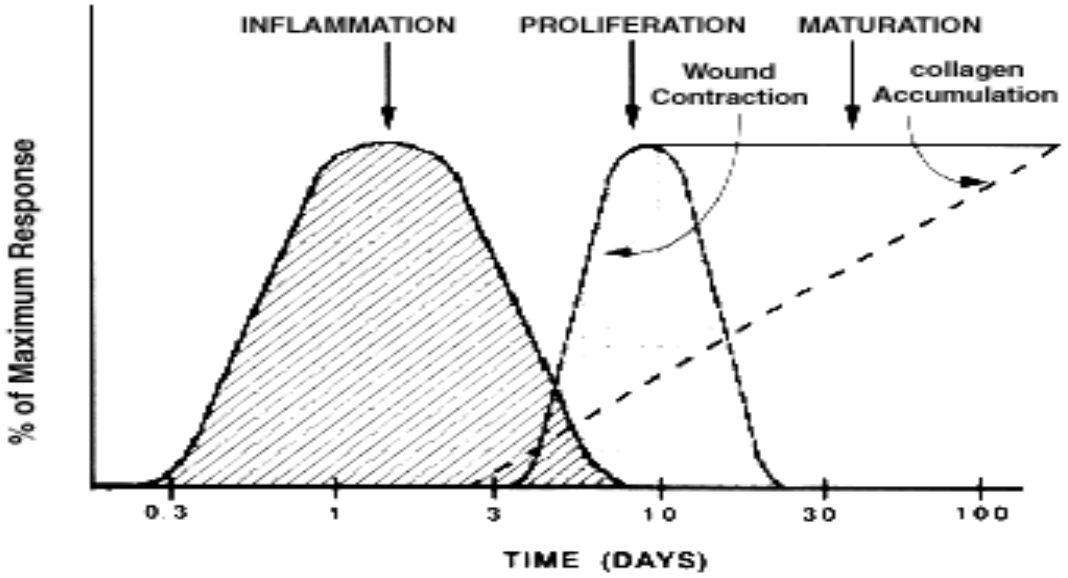
Tissue Repair

-Inflammation

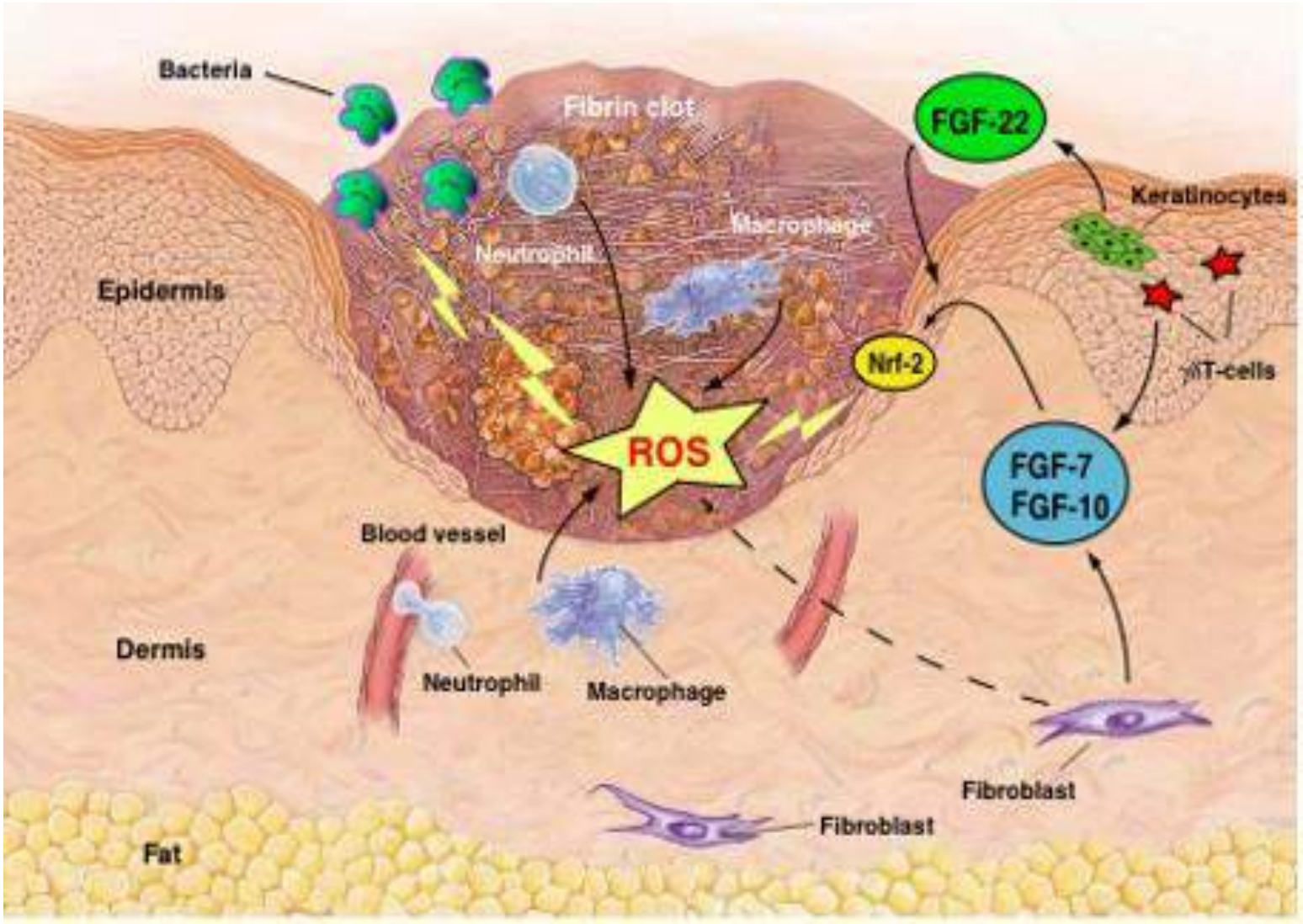
-Reconstruction

(Angiogenesis, Cell migration, Wound contraction…)

-Maturation



Wound healing



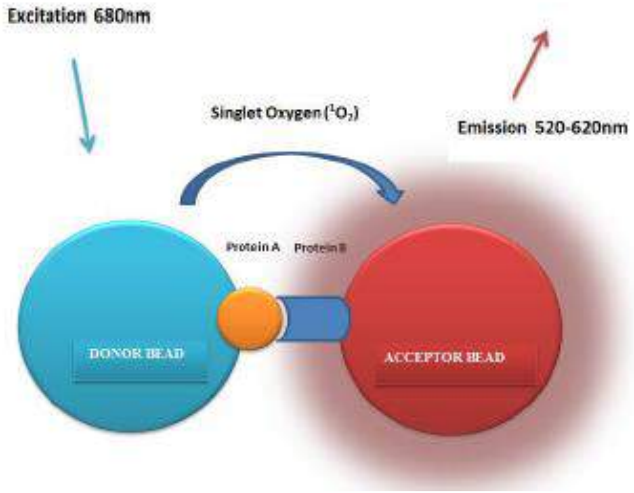
Cytokines

- TGF-βs
- PDGF
- EGF
- TGF-α
- VEGF
- IGF-I
- FGFs
- Angiopoietin
- FGF-7/KGF
- Endothelin
- TNF-α
- IL-1β
- IL-6
- IL-4
- IL-8
- IL-10
- SLPI
- MCP-1
- MIP-1α
- MIP-2
- IL-18
- IFN-α/β

AlphaLisa technology for detection of molecules

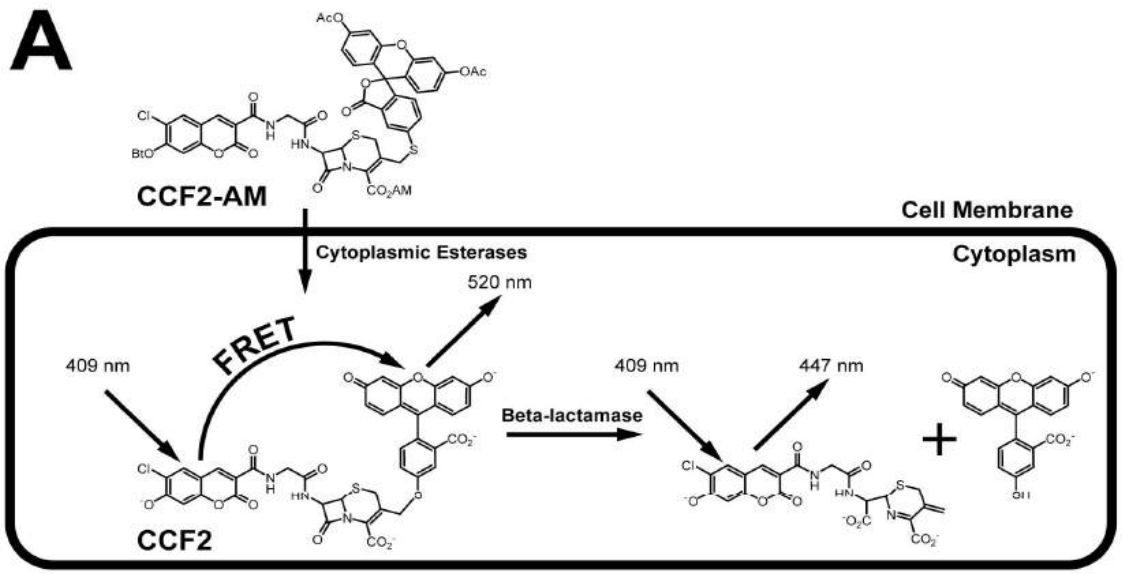
- **Features of the Alpha technology**

- **High sensitivity**: signal is a **cascade reaction** and is triggered through a **high concentration of photosensitize**r in the Donor Bead
- Each donor Bead can **releases up to 60000 singlet oxygen** molecules
- Acceptor beads contain a **high concentrations of thioxene derivative** which are stimulated to produce light upon activation through singlet oxygen.
- The amplification cascade may **detect molecular interactions** in the **femtomolar range** of individual binding partners.
- **Low background** and high **signal- to background ratio**
  - Due to a time resolved reading mode

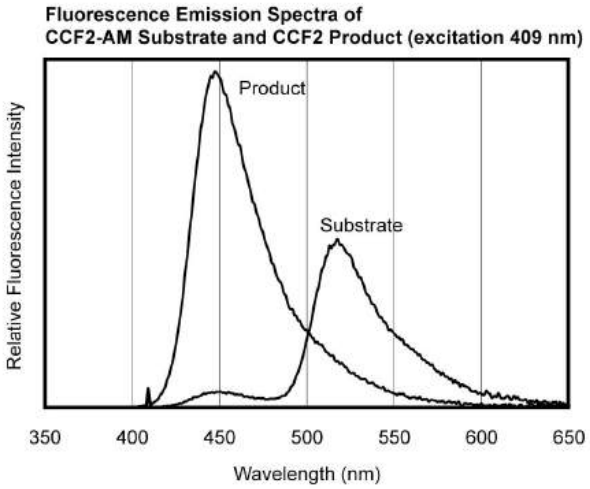


***A**mplified **L**uminescence **P**roximity **H**omogenous **A**ssay* (Alpha Screen™)

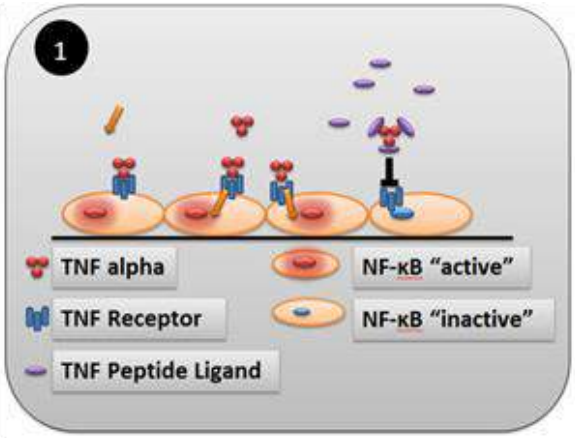
GeneBLAzer® technology

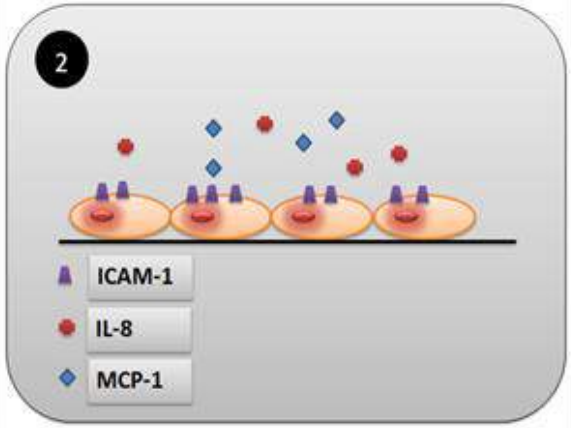


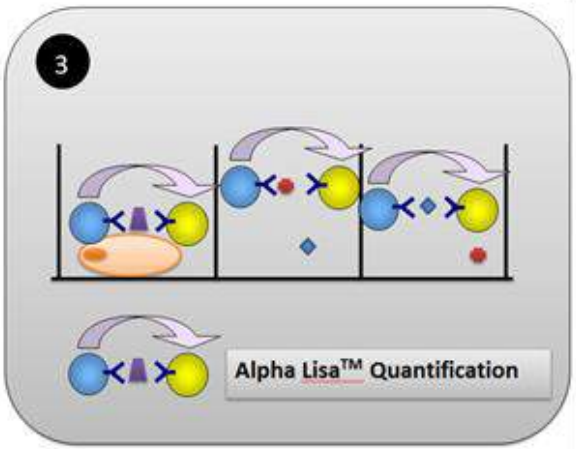
- beta-lactamase reporter gene under control of the gene response element stable integrated
- CCF2-AM substrate: a cephalosporin core linking a 7-hydroxycoumarin to a fluorescein

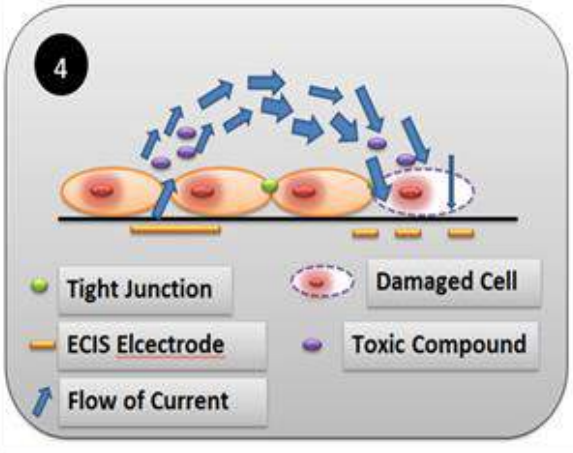


Anti Inflammatory Screening Assays





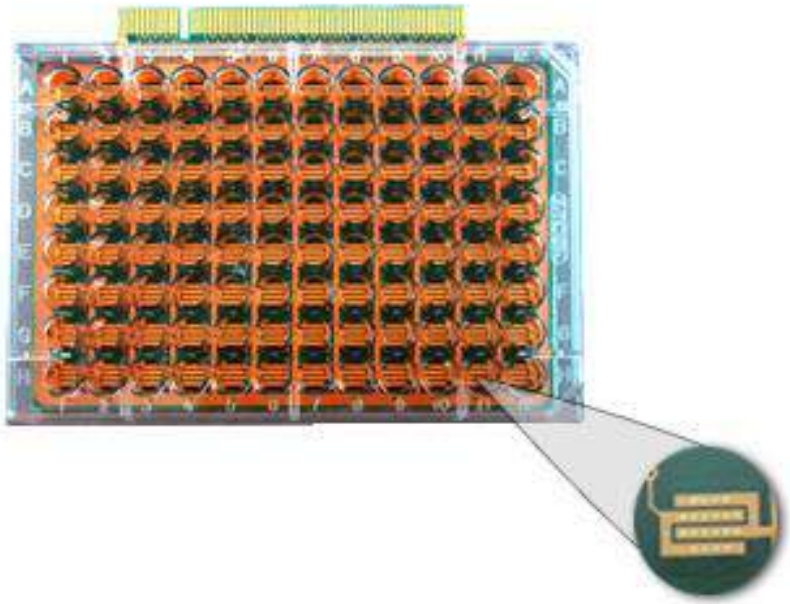


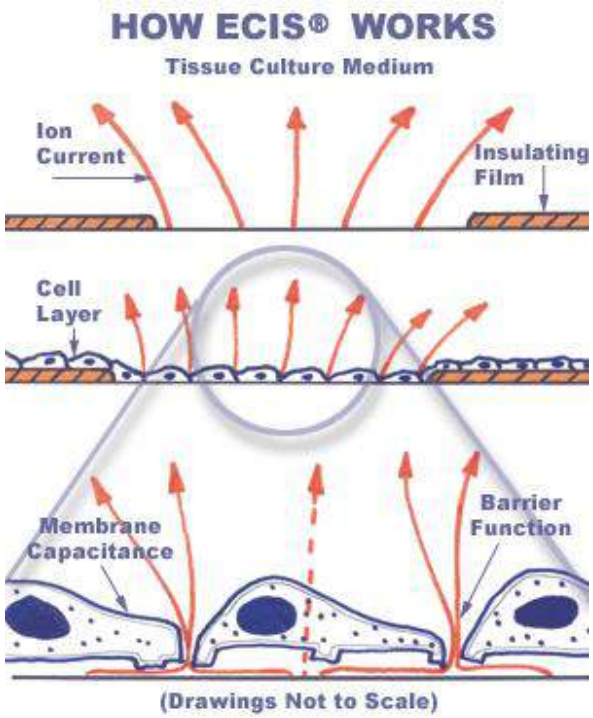


1. Activation of the NFkB pathway with human Tumor necrosis factor alpha
2. Production of cytokines (e.g. ICAM-1, IL-8)
3. AlphaLisa technique
4. Detection of toxic substances with impedance measurement

ECIS technology

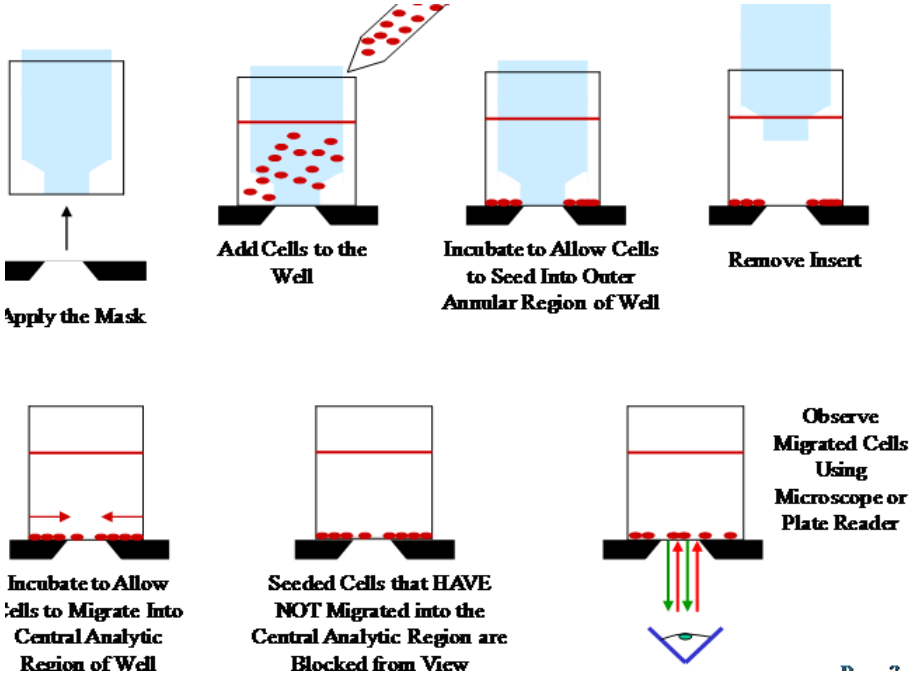
- **E**lectrical **C**ell Substrat **I**mpedance **S**ensing Method

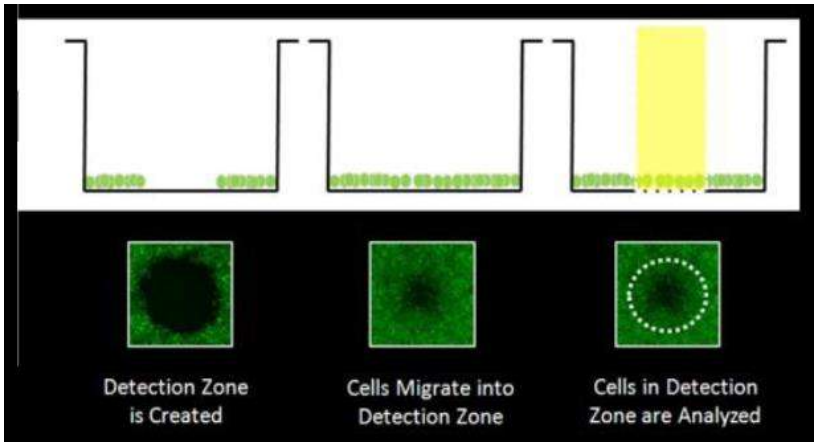




Applied Biophysics Inc., 185 Jordan Rd., Troy, NY 12180

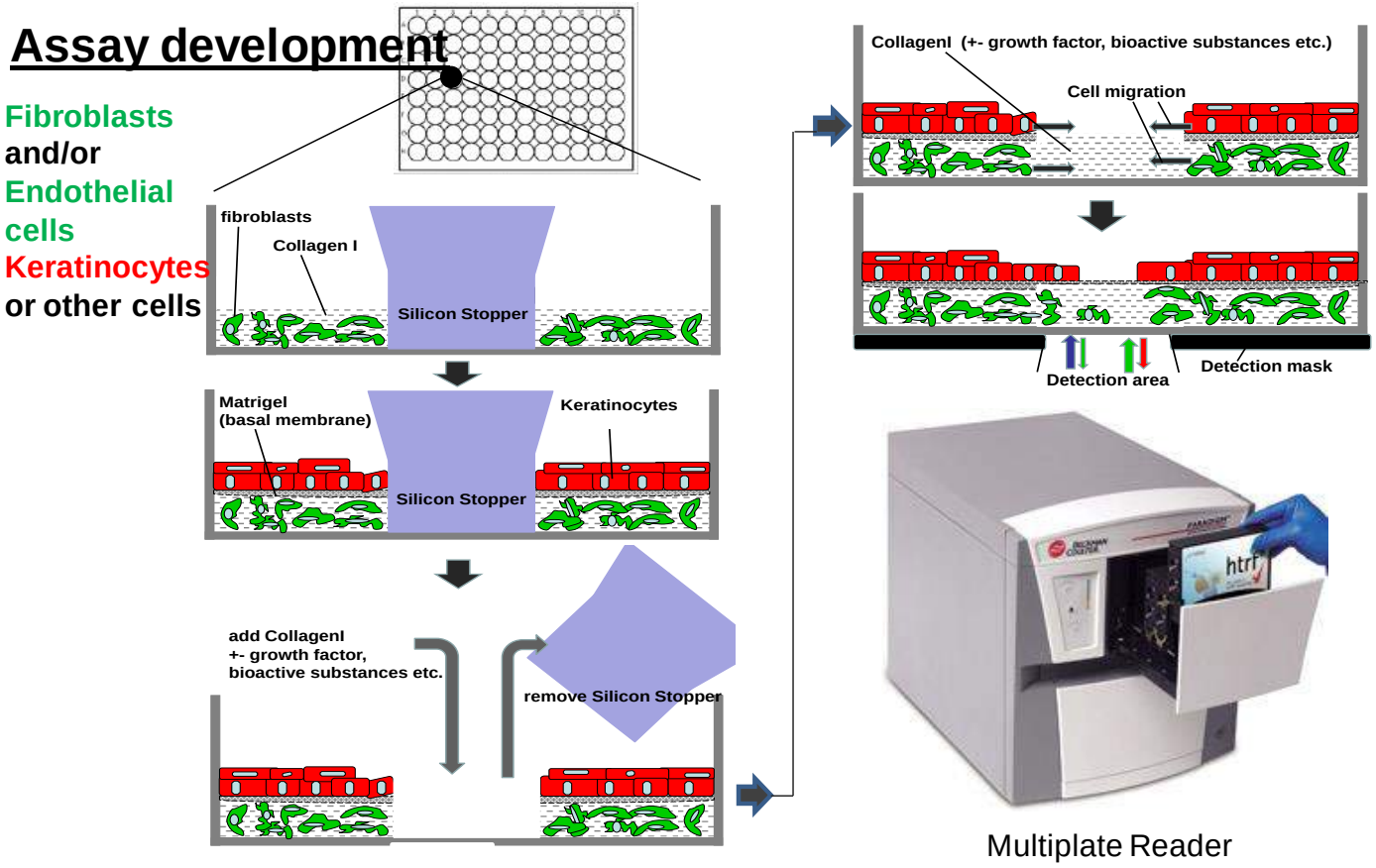
Oris™ Cell Migration Assay





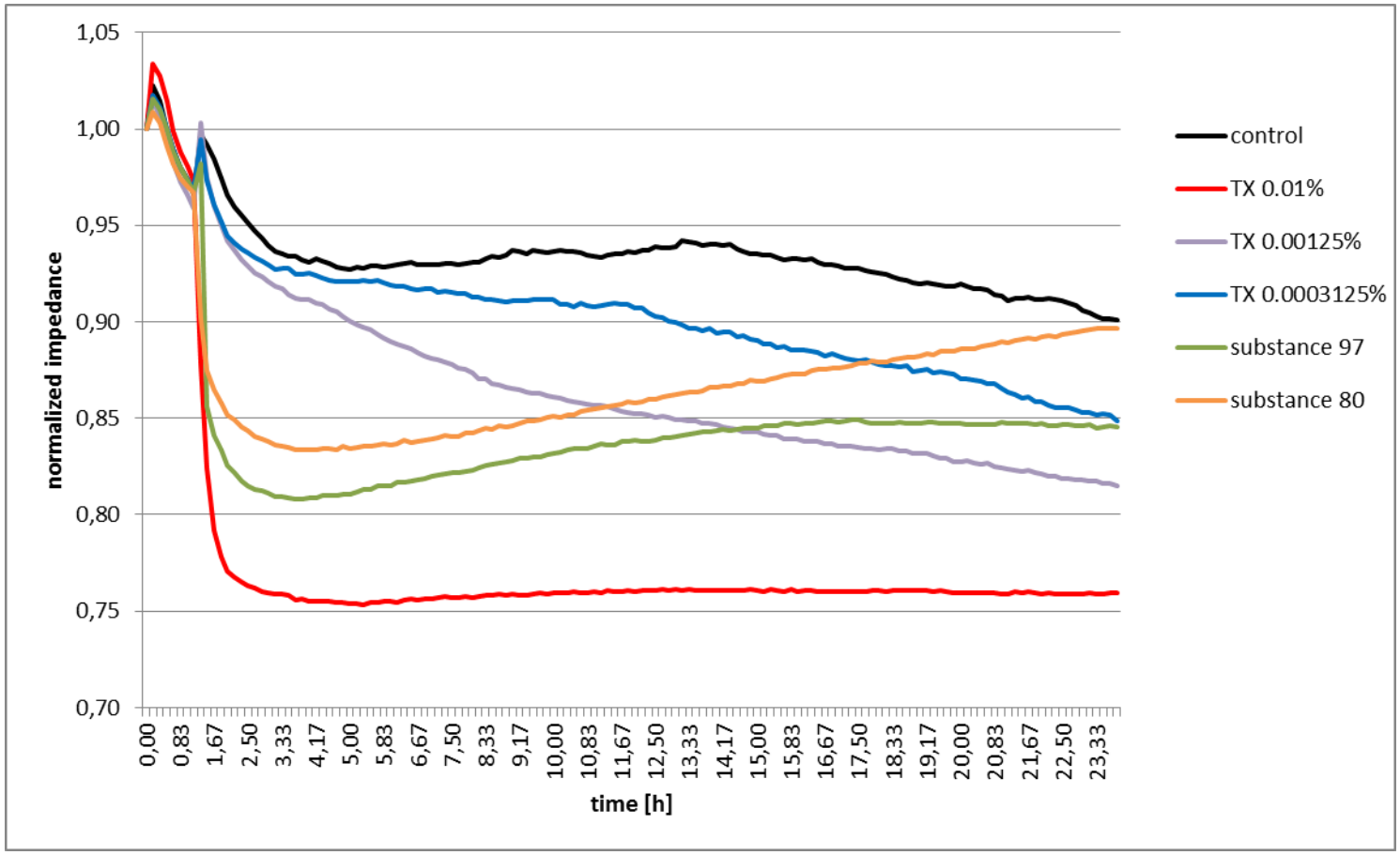
Oris™ Pro Cell Migration and Invasion Assays run on IN Cell Analyzer 2000; HaiGuang Zhang[1], Keren I. Hulkower[2] and Robert Graves[1], [1]GE Healthcare, [2]Platypus Technologies,

3D Artificial skin model – wound healing

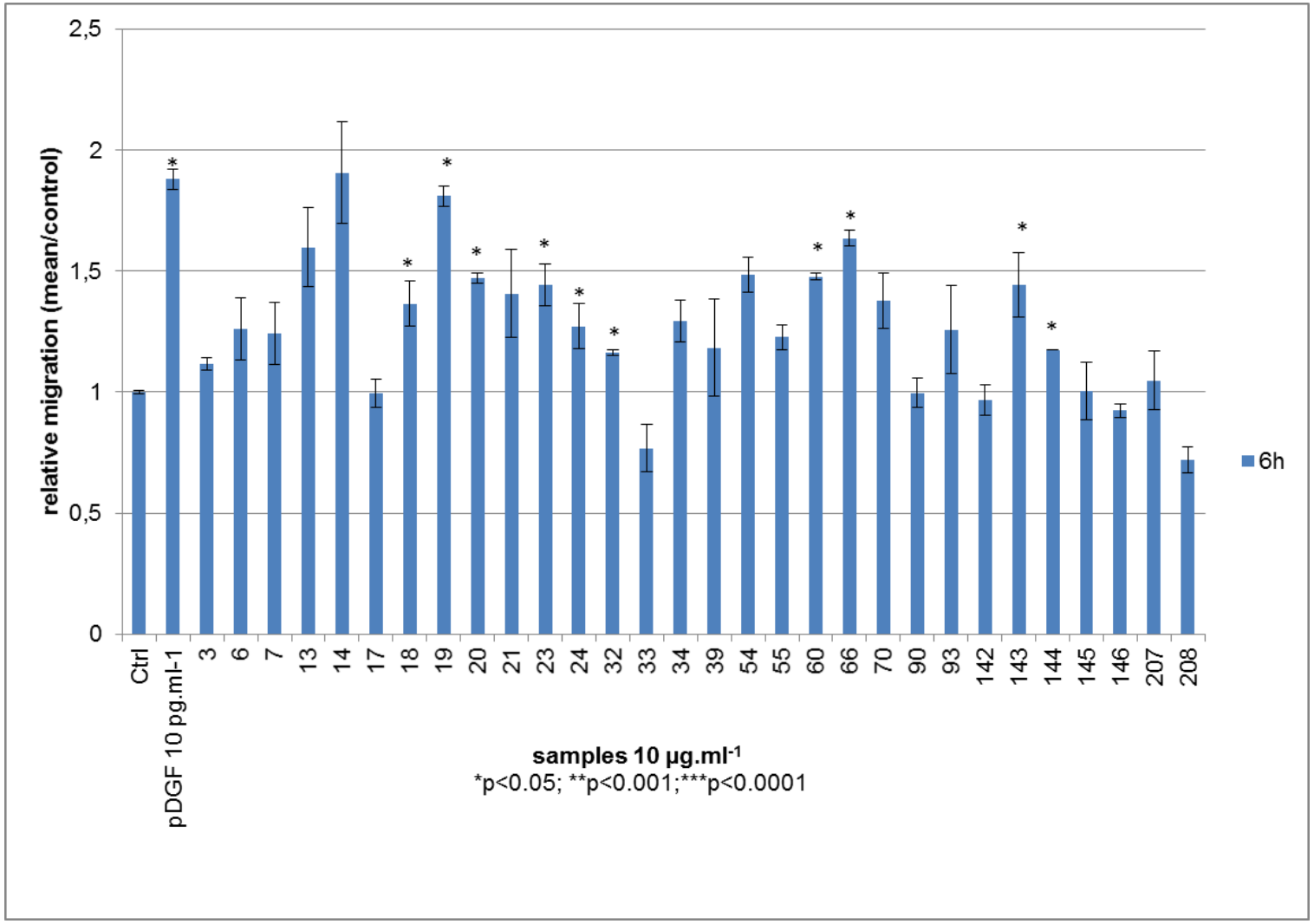


- Fluorescence, fast dual excitation/emission (simultaneous)
- 96, 384, 1536 well plates

ECIS technology



3D Artificial skin model – wound healing



Summary

- Cyanobacteria are a promising source of active metabolites
- AlphaLisa technique is a robust method for cytokine detection
- High reproducibility, because there are no washing steps necessary compared to „classical ELISAs“
- Advantage of the ECIS technology to detect cytotoxicity compared to other methods is the real-time observation of the substances

Acknowledgement

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