In vitro cell imaging, biochemical responses and inflammation in tissue engineering

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Overview

• Tissue engineering

• Why anti-inflammatory approaches?

• What is so important about inflammation?
  1 Skin
  2 Nerve
  3 Heart

• Anti-inflammatories
  • α–MSH
  • Asprin
  • BMS

• Surface modification of biomaterials & medical devices
Tissue Engineering

The development and manipulation of laboratory grown:

- molecules
- cells
- tissues
- organs

...to replace or support the function of defective or injured body parts
Tissue Engineering

Implantation of autologous or allogeneic cell-containing devices that induce the regeneration of functional human tissues

- novel scaffolds created in 3-D in which cells grow and reconstitute tissue
- purification & large scale production of signal molecules (growth factors)
- bioreactors
- e.g. bone regeneration
- e.g. skin regeneration

Autologous – from self
Allogeneic – from donor
Doctors re-grow severed thumb

• The first step involves growing cells from the patient's own bone tissue.
• A coral matrix is shaped to look like the old bone.

• After 6 weeks in laboratory culture, the patient's own cells are injected into the coral scaffold.
• The 'engineered' thumb is sewn back on to the patient's hand.
Inflammation

Arises in response to injury or infection of body tissue

4 classical signs

• **Redness** - increased blood flow
• **Heat** - increased blood flow
• **Pain** - sensory nerve activation
• **Swelling** - oedema / leukocyte accumulation
What is inflammation for?

- Inflammation is required for a normal wound healing response (+ kills bugs)
- Stimulates cellular proliferation and matrix production
- Excessive inflammation can compromise cell survival:
  - Inflammatory rejection
  - Hyperproliferation
  - Scar tissue formation
  - Cancer
Inflammation

The inflammatory response is triggered by small soluble molecules called proinflammatory cytokines.

These are released by immune cells and damaged cells:
- Interleukins - IL-1β, IL-4, IL-6, IL-8, IL-12, IL-18
- Tumour necrosis factor - α
- γ-Interferon
- Lipopolysaccharide
Inflammation

1. Tethering
2. Rolling
3. Adhesion
4. Migration
Why anti-inflammatory strategies?

- To improve the success of existing approaches / strategies to tissue engineering where inflammation is a problem
  - E.g. Chronic wound healing - Skin tissue engineering

- As a novel approach to delivering a therapeutic agent together with a scaffold or cells where inflammation is a problem
  - E.g. Nerve injury repair

- As an approach to improve ‘standard’ forms of local drug delivery
  - E.g. Coronary restenosis
Skin tissue engineering
Commercially available tissue engineered products

• Skin is one of the few tissue engineered products already in the market place
• Over 1 million burn injuries per year (USA)
• 45,000 hospital admissions per year, 50% to specialised burn treatment centres
• Products available to treat superficial and deep burns

Epidermal
Epitel

Dermal
Alloderm
Integra
TransCyte

Composite
Apligraf

Image of TransCyte (tm) courtesy of Advanced Tissue Sciences, Inc.
Skin tissue engineering
Research in novel scaffolds and bioreactors

[Diagram showing a flowchart and images of tissue engineering processes]
1. Lack of vascular supply
   – Oxygen & nutrient deprivation

2. Viability of cells in the donor graft
   – Donor source, age of cells, proliferative potential

3. Infection
   – Bacterial / viral

4. Inflammation
   – Grafted donor cells have to survive an acute inflammatory environment in the wound bed

Epidermal
Epithelial autograft procedures are reported to have approximately a 50% acceptance rate of donor graft material

Dermal
Matrix graft procedures are reported to have approximately an 80% acceptance of donor graft material

Composite
Epidermal / dermal graft procedures are reported to have approximately 60-70% acceptance of donor graft material
Skin tissue engineering

A basis for improvement using anti-inflammatories?
Nerve injury

- 245,000 people with SCI in the USA
- Annual SCI incidence is 11,000
Nerve injury

- Immediate delivery of methylprednisolone is needed following SCI
- High levels of inflammatory cytokines detected locally after SCI
- Intense inflammatory reaction thought to be contributory to scar tissue formation
- Local delivery of anti-inflammatory agents reported to improve repair and functional recovery
- Use of anti-inflammatory drugs in vivo shown to be proregenerative
Nerve injury

A basis for improvement using anti-inflammatories?
Coronary Heart Disease

- PTCA
  - 2 million performed worldwide
  - Increase of 8% annually (estimated)
- Stents
- Restenosis
  - Immune system response
- Drug eluting stents
Coronary Heart Disease

A basis for improvement using anti-inflammatory?
Anti-inflammatory compounds

Steroids
NSAIDS
Peptides

Systemic delivery
Oral delivery
Local delivery

Melanocortins
\( \alpha \)-MSH

**Proopiomelanocortin**

\( \gamma \)-MSH
\( \alpha \)-MSH
CLIP
\( \gamma \)-LPH
\( \beta \)-EP

**\( \alpha \)-Melanocyte Stimulating Hormone (\( \alpha \)-MSH)**
- 13 amino acid peptide
- potent anti-inflammatory activity
- produced in pituitary, gut and skin
- Activity arises by binding to the melanocortin-1 receptor (MC1R)

\( \alpha \)-MSH
Inhibition of pro-inflammatory pathway

IL-1, IL-6
TNF-\( \alpha \)
CAMP
Ca\(^{2+}\)
NF-\( \kappa \)B
ICAM-1

24 hours
1-2 hours

Upregulation of pro-inflammatory genes

Inhibited in regulation of T-cell binding
Melanocortin-1 Receptor

• G-protein linked to activate adenylate cyclase / cyclic AMP

• Melanocytes, melanoma cells, macrophages, monocytes, neutrophils, endothelial cells, fibroblasts, keratinocytes, Schwann cells, olfactory ensheathing cells

• Anti-inflammatory and down-regulation of immune responses

• Reduced production & action of proinflammatory cytokines by α-MSH

• Increased production of IL-10

• Inhibits activation of NF-κB
Human melanocortin-1 receptor

Ligand: α-MSH

Extracellular

Transmembrane

Intracellular

G-Protein

ATP

Adenyl cyclase

Cyclic AMP

Calcium
How does α-MSH act as an anti-inflammatory?

Proinflammatory cytokines

TNF-α

Adenylate cyclase

Cyclic AMP
If blocked, an increase in calcium signalling arises

α-MSH

MSH receptor

G

Phospholipase C

IP3

DAG

Endoplasmic reticulum

Calcium + calmodulin

Protein kinase

cAMP dependant

Inflamatory proteins
Cytokines / receptors

Adhesion molecules

Inflamatory mRNAs

ICAM-1

NF-κB

H2O2 / peroxide

GPx

TNF Receptor

G

Adhesio molecules

The University of Sheffield Department of
ENGINEERING MATERIALS
Anti-inflammatory signalling

Intracellular calcium release: Keratinocytes

Proving useful for biologically evaluating novel peptide ligands
Conduct your own α-MSH calcium experiment online…

http://www.sheffield.ac.uk/tissue-engineering
In vitro study of inflammatory vs. anti-inflammatory signals

- Skin cell
- Nerve cell
- Endothelial cell
Anti-inflammatory signalling

Inhibition of TNF-α stimulated NF-kB/p65 activation by α-MSH
HaCaT keratinocyte cell line

1 - Control
2 - α-MSH (10⁻⁹M)
3 - TNF-α + 100M cold oligo
4 - TNF-α + 100M mutant oligo
5 - TNF-α (2 ng/ml, 30 minutes)
6 - TNF-α (2 ng/ml, 60 minutes)
7 - α-MSH + TNF-α (30 minutes)
8 - α-MSH + TNF-α (60 minutes)
Anti-inflammatory approaches for nervous system injury

Acute inflammation arising after CNS injury causes further damage to spinal cord and inhibits repair. Similar inflammatory mechanism PN injury

MSH peptides already shown to improve CNS (& PNS) repair in vivo

Schwann & olfactory ensheathing cells used for therapeutic delivery

Combine delivery of cells, scaffold and drug

MC₁ expression identified on Schwann and OECs
α-MSH peptide found to inhibit the activation of NF-κB in Schwann and OECs
α-MSH inhibits TNF-α stimulated NF-κB activity in vascular cells

- **Control**
- **200 U/ml TNF-α**
- **200 U/ml TNF-α + 10-9 α-MSH**

[Images of endothelial and VSM cells]

**Graph:**
- Relative NF-κB/p65 activity (%)
  - VSM Cells
  - Endothelial Cells
  - O, T, T+M, T+F, M, F

- 
  - * for significance
  - ** for very significance
α-MSH inhibits TNF-α stimulated E-Selectin expression in vascular endothelial cells
Anti-migration properties

Inhibition of TNF-α stimulated migration by α-MSH in human melanoma cell lines: HBL and C8161

A HBL
B 0 hours

D C8161
E 4 hours

C 24 hours

HBL C8161

Control

TNF

TNF + MSH
Anti-invasive/adhesive properties

Inhibition of TNF-α stimulated invasion and adhesion by α-MSH in human melanoma cells: HBL

α3 integrin
α4 integrin
β1 integrin

Adhesion

Invasion

α3 integrin
α4 integrin
β1 integrin
Summary

- Potential anti-inflammatory applications in
  - Skin engineering
  - Nerve engineering
  - Heart engineering
  - Cancer research
- To improve existing strategies
- MSH peptides are anti-inflammatory and cytoprotective
- MSH compounds designed for ‘coating’ biomaterial surface
Can MSH peptides be attached to biomaterial surfaces?

Synthesis of MSH – calixarene conjugates

Hydrophilic rim
Rigid bowl
Legs

Rims bind to hydrophilic surface; bowls self assemble to leave a new surface made up of Y groups
Cells on Patterns

Royal Institution Christmas Lectures 2002: ‘Smart Stuff’
Prof. A. J. Ryan, Dept. Chemistry, Sheffield University
Keratinocytes on acrylic acid / octadiene pattern
Acknowledgements

- Richard Elliott
- Rebecca Hill
- Stephen Roberts
- Sarah Grubb
- Dr. Kathryn Smith
- Dr. Nick Williams
- Dr. Jon Kelly
- Dr. Kenneth Carlson
- Prof Kevin Shakesheff
- Prof. Sheila MacNeil