General Introduction

During severe burns, cells are damaged (release ITM into area and blood), stem cell (e.g. hair follicle) areas can be entirely destroyed (bad for regrowth). So there is increased inflammation, bacteria,

It is likely we will need to create a model that is spatio-temporal, as the position and distribution of the damage in a wound dramatically effects the dynamics of the inflammatory response. Non-healing, or chronic, wounds result from one or more phases of the natural, or acute, wound healing process. These overlapping phases involve an inflammatory response and associated cellular migration, proliferation, matrix deposition, and tissue remodelling.

Alkaline Phospatase and it's role in the Immune System (from previous IAS paper)

Alkaline Phospatase (AP) can detoxify bacterial lipopolysaccharides (endotoxins) by removing the two phosphate groups found on LPS carbohydrates (Bates et al., 2007). It may function as an adaptive mechanism to help the host manage the potential toxic effects of gram-negative bacteria. It also dephosphylates extracellular nucleotides, such as ATP. These nucleotides have a different effect/role once they are outside of the cell, e.g. signaling molecules, modulation of vascular tone, electrolyte transport, mast cell degranulation, cell death, and synaptic transmission (Brake and Julius, 1996). Clearly effects such as cell death are part of the ischemic insult that results from severe burns, contributing to the damage of blood vessel tissues, reducing blood supply to the area. But, we should pay attention to whether some roles (signaling) of the ECN should not be eradicated.

AP's other key role is the maintenance and restoration of physiological barriers. During an ischemic shock (and inflammation triggering insult) this barriers tend to become hyper-permeable and/or dysfunctional. Humans have four distinct AP isozymes: tissue-nonspecific
AP (liver/bone/kidney type AP)

This paper models systemic insult (massive amount of ITMs) from multiple sources and therefore the "tissue" represents the entire body. It is compartmentalised into: liver, blood stream, and tissue. For severe burns, and the minimisation of scarring, we most likely need to introduce a cell agent based model that is coupled to the HIIS model. There are several interlinked phases of burn wound healing, each phase of the process is dependent on the preceding spatio-temporal action of the IS. E.g. the ECM cannot be remodelled until there has been sufficient angiogenesis.

Possible: suggests ATP and H202 may establish a pro-inflammatory permissive environment to potentiate the recruitment of immune cells into the inflamed tissue. Some evidence that atp enhances response to attractant by generating a signal amplification loop (Weavers et al, 2016).

Deep (third degree) **burns** are so serious largely because they destroy the many hair follicles and sweat glands that invaginate deep into the dermis and serve as efficient sources of epithelial regrowth after more superficial injury.

Wound Repair and Regeneration

Eming, Sabine A et al. "Wound repair and regeneration: mechanisms, signaling, and translation." ***Science translational medicine*** **vol. 6,265 (2014): 265sr6. doi:10.1126/scitranslmed.3009337**

Hypertrophic Scar	Keloid Scar
Rapid Growth	Constant Growth
Generally regress <6 months	No spontaneous regression
A SMA myofibroblasts	Extend beyond margins of tissue damage
Collagen fibers parallel to skin surface	Genetic predisposition (younger & darker skin)
Vertically orientated blood vessels	Thick, haphazardly orientated collagen bundles

Wound healing after skin injury involves extensive communication between the different cellular constituents of the diverse compartments of the skin and its extracellular matrix (ECM). (Emig et al, 2014).

Chronic Wounds (*Barrier defects that have not proceded through orderly and timely reparation to regain structural and functional integrity)*

- Show hyperproliferative and nonmigratory epidermis
- Unresolved inflammation
- Presence of infection
- Biofilm formation (*Perpetuates the inflammatory phase of wound healing)*
- Increase in inflammatory cells (*neutrophilis and macrophages)* but not all are properly funcitioning
- Uncontrolled proteases (*e.g. Amylase. Enzymes that break down proteins and peptides)* that interfere with essential repair mechanisms
- Some fibroblasts becomes senescent (*biologically ageing)*
- Reduction of angiogenesis (*formation of new blood vessels from pre-existing ones)*
- Reduction in stem cell recruitment and activation
- Reduction in ECM remodeling

Inflammatory Response to Wounds (*Natural acute wound healing procedes through several largely overlapping phases that involve:*

- Inflammatory response
- Cellular Migration
- Cellular Proliferation
- Matrix deposition
- Tissue remodeling

Interruption or deregulation of one or more phases may lead to non-healing/chronic wounds.

Inflammation helps to fight infection, clear debris and induce the proliferation phase (fibroblasts entering, then angiogenesis), but may lead to tissue damage if it lasts too long. This is often the case when: unable to clear debris, excessive detritus, devitalised tissue, or microbial biofilm is present.

Injury:

- Leads to immediate activation of the clotting cascade which:
- through the assembly of a fibrin clot
- Assures hemostasis

- Provides the matrix architecture to initiate the invasion and recruitment of inflammatory and other cells
- Platelets trapped in the clot also release growth factors and chemokines into the local wound environment *(Clinical use of platelet-rich plasma to promote healing)*
- early inflammatory response mobilises local and systemic defense responses to the site

Recent investigation of chronic wound tissue and fluid indicate a continual competition between inflam and anti-inflam signals leading to a misbalanced environment for proper wound healing to occur. (Eming et al, 2010; Beidler et al, 2009)

- Increased proinflammatory cellular infiltragtes composed largely of neutrophils and macrophages contribute to delayed healing
- Deregulation of key proinflam cytokines (*IL-1β TNFα)* prolong the inflam phase
- These cytokines increase in chronic wounds, due to them causing elevated metalloproteinases that degrade the local ECM and thus impair cell migration (*Tarnuzzer and Schultz, 1996)*
- Continued presence of a high bacterial load in wounds result in a sustained influx of pro-inflammatory cells and increased imflammation
- ***The epidermis up-regulating and secreting antimicrobia peoptides early in response to barrier damage and exposure to microbes***
- Recruitment of bone marrow and endothelial progenitors to the site of injury coordinated by specific chemokines *(Stem cell modulation is being explored for potential therapy but in burns the hair follicle, sebaceous gland and basal layer of the epidermis may be damaged / destroyed)*
- **Angiogenesis** ***(****sprouting of capillaries from existing blood vessels)* **and vasculogenesis** *(mobilization of bone marrow–derived endothelial progenitors)* **contribute to new blood vessel formation during tissue repair**
- Inadequate local angiogenesis would lead to increased ischemic insult?
- Reduced angiogenesis leads to elevated cell death
- Macrophages are stimulated by low oxygen content to induce angiogenesis
- endothelial cell damage may lead to slower capillary growth
- members of the vascular endothelial growth factor (VEGF) family are proangiogenic factors
- **Modulating the balance between pro and antiangiogenic molecules?**
- Cellular senescence: oxidative stress may drive uncontrolled fibroblast proliferation and keloid formation. Senescent fibroblasts and keratinocytes secrete MMPs 2,3,9, maspin (antifibrotics)
- If epithelisation is slow, scarring is likely.

Inhibition of Alkaline Phosphatase by L-Phenylalanine (Fernley and Walker, 1970)

Computational Systems Mechanobiology of Wound Healing
Tepole 2016

- Wound Healing process relies on co-ordinated action of cells and spatiotemporal profile of cytokines
- Production and diffusion of chemical signals (PDGF and TBF-β) attract macrophages and fibroblasts
- **Macrophages maintain production of TBF- β and release other factors:**
  - **TGF-α** is a chemoattractant for **keratinocytes** (**part of the reepithelisation process; epidermal keratinocytes CAN CONTRIBUTE TO DE NOVO HAIR FOLLICLE FORMATION during large wound healing)**

- **Fibroblast growth factors** (1,2,4 regulates endothelial cells during angiogenesis)
  - **Fibroblasts** (deposit and remodel the new ECM; also release FGF; assemble ECM by acute tissue contraction and fiber realignment)
- **VEGF** (regulates endothelial cells during angiogenesis)

(http://dx.doi.org/10.1016/j.cub.2016.06.012) Systems Analysis of the Dynamic InflammatoryResponse to Tissue Damage Reveals SpatiotemporalProperties of the Wound Attractant Gradient

-Wound signal is released from the wound edge for 30 minutes and diffuses at 200 μm^2/min
-Failure in normal wound repair can be used as an early prognostic marker of a non-healing wound, perhaps after fluorescent activated cell (FACS) sorting of immune cells
-?extensive injury to skin also promotes the early trafficking of a unique subclass of leukocytes (circulating fibrocytes) to the injured region?

We can use the innate model as a system effect depending on blood flow, so that processes such as angiogenesis allow for further anti inflammatory action

Burn Surgery

Scar Formation

Cells like macrophages release soluble factors that stimulate fibroblasts to lay down connective tissue, including collagen and glycosaminoglycans. Fibrosis is the formation of excess fibrous tissue in a tissue during reparation ("normal" healing is replacement of same kind of cell). It's function is to hold the tissue together, it has four stages: Angiogenesis, migration and proliferation of fibroblasts, deposition of ECM, remodeling of ECM. Before the process begins EPC (*endothelial precursor cells,* found in bone marrow and vessels) must be present,
Body forms collagen fibers (parallel instead of "basket-weave" as per) during wound repair.
Collagen helps to

Social Impact on Inflammatory Markers

Castagné, Raphaële et al. "A life course approach to explore the biological embedding of socioeconomic position and social mobility through circulating inflammatory markers." Scientific reports vol. 6 25170. 27 Apr. 2016, doi:10.1038/srep25170

- Stress hormones alter biological mechanisms including inflammatory and immune responses.
- Lower education andor income is associated with a greater burden of inflammation.
- Many studies use C-Reactive protein (CRP) as a general proxy to characterise inflammatory status (others used interleukin 6 IL6, or fibrinogen, or tumour necrosis factor α (TNF-α)). **However, this is limited.**
- **This paper instead used, a "*wide range of cytokines, chemokines and proliferation factors.***
- *Groups found with high inflammation: manual work father, upward social mobility (compared to remained socially advantaged)*

Questions

1. "Additional Phase III clinical trials are currently on the way to confirm the beneficial effects of AP previously reported in CABG and valve surgery." - What was the result of the 3rd phase clinical trials ?

References