Adult Wound Healing

Introduction

The ability to heal wounded tissue is an evolutionary survival mechanism. It allows wounded individuals to continue to contribute to the gene pool (chicks dig scars!). As you might expect from such a highly conserved process, there are multiple (and highly complex) interactions between various organ systems – all of which have not been fully elucidated as yet. Despite the complexity, only a brief understanding is required to be able to make a clinical difference to a wounded patient (which of course, is the point of this topic).

Topic Overview

Adult wound healing takes place in three broad, overlapping phases. The whole process is quite complicated, so refer back to this overview often.

- **1. Inflammatory Phase**
  - Haemostasis
    - Vasoconstriction
      - Neural
      - Humeral
    - Coagulation
      - Fibrinogenesis
      - Platelet aggregation and activation
  - Acute Inflammation
    - Vasodilatation and Increased permeability
    - Cellular Infiltration
      - PMN
      - Macrophage

- **2. Proliferative Phase**
  - Fibroplasia
    - Fibroblast proliferation
    - Deposition of ECM
      - Formation of the Provisional Matrix
      - Formation of the Collagenous Matrix
  - Angiogenesis
  - Epithelialization
    - Epidermal Cell Proliferation
    - Epidermal Reconstitution
  - Wound Contraction
- **3. Remodeling Phase**

Classification of Wound Healing Types
There are generally 4 types of wound healing which one may encounter:

1. Wound Healing by **1st Intention**. This occurs when the edges are brought into close approximation such as sutured surgical incision wounds. It requires very little extracellular matrix (ECM) formation and similarly limited epidermal regeneration.
2. Wound Healing by **2nd Intention**. This occurs when a gap exists between the wound edges, such as wounds left open to heal by contraction. This requires a large amount of ECM and significant epidermal regeneration – generally the presence of granulation defines this type of wound healing
3. Wound Healing by **3rd Intention**, also called **delayed primary closure**. This occurs when a contaminated wound is **intentionally left open** to allow drainage and improvement in tissue defenses before being closed primarily a few days later. The ultimate tensile strength of these wounds is identical to primary wound healing.
4. **Partial thickness** wound healing. This occurs by epidermal regeneration such as in partial thickness burns. It does not require the formation of any ECM.

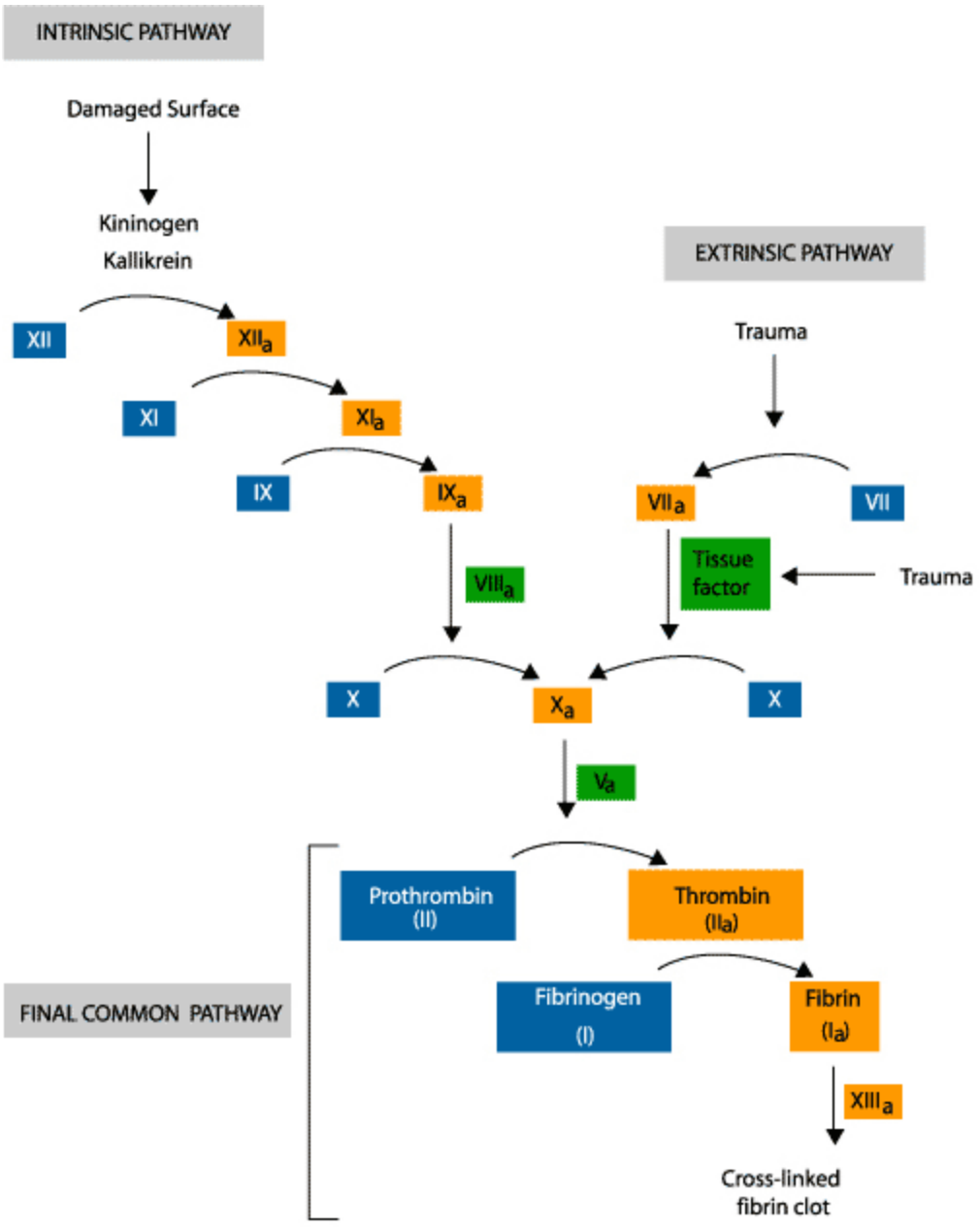
Adult Wound Healing

1. The Inflammatory Phase
There are 2 (a good number to remember…as you will see) components to this phase: haemostasis and acute inflammation.

1.1 Haemostasis
Any injury beyond the epidermis will result in disruption of blood vessels and thus bleeding. Clearly, healing cannot proceed until bleeding has stopped. This occurs via 2 complementary pathways.

Vasoconstriction (0 – 15 minutes)
Arteries and arterioles undergo vasospasm under the influences of both humeral and neural factors. **Neural regulation** is via **sympathetic stimulation** and its control over local noradrenalin secretion. **Humeral factors** include circulating adrenaline and vasocontrictive prostaglandins such as thromboxane A_2.

Coagulation (0 – 6 hours)
Again, 2 complementary pathways are activated: Fibrinogenesis and platelet aggregation.
1. Fibrinogenesis
Trauma to blood vessels exposes the sub-intimal tissues to the circulation. Both intrinsic and extrinsic pathways are stimulated, but the **extrinsic pathway** is essential, resulting in generation of **fibrin** from fibrinogen.



2. Platelet aggregation

The damaged vessel walls cause **platelets to adhere** to the wall and to each other. The platelets are also **stimulated to degranulate**, releasing the contents of both α and dense granules:

α granules contain essential clotting and wound healing substances (**the raw material**).

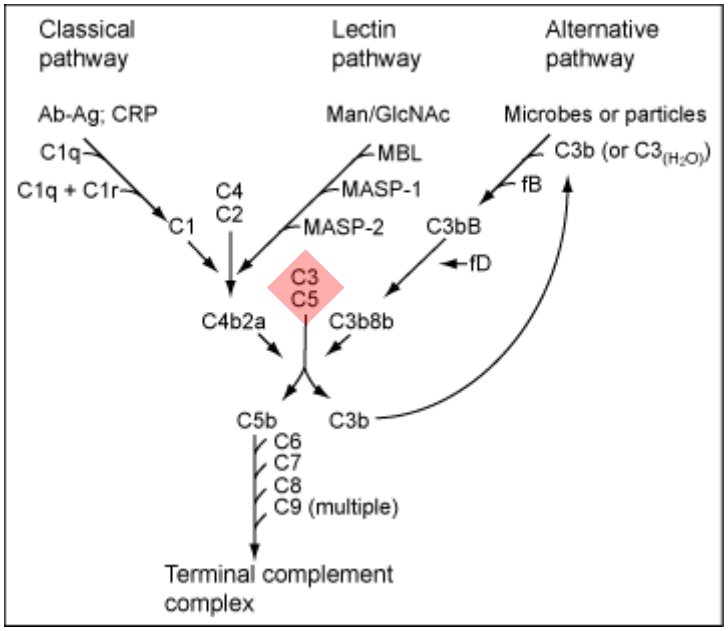
Dense granules contain the **"fuel"** to continue the process: ATP, calcium and serotonin.

The combination of the above processes results in the formation of a **stable clot**, which seals the vessel ends and also provides what is known as **"initial matrix",** on which the **"provisional matrix"** will be built. Very importantly, the formation of the clot also

initiates the next component phase, due to **activation of complement** and **chemotaxisis of macrophages** due to the by-products of clot formation (So…the clot thickens ☺).

1.2 Acute Inflammation
This sub-phase has 2 components (spot the trend?).

1. Vasodilatation and Increased Permeability (15 min – 6 hours)
This phase is initiated by **platelet degranulation products** and **complement** (C3a and C5a). The C3a/C5a complex is responsible for 2 further actions: **opsonization** of bacteria and stimulation of **tissue mast cells.** All of the above result in disruption of tight endothelial cell junctions (making the vessel walls **"leaky"**).



2. Cellular Infiltration (6 hours – 3 days)
Circulating **PMNs** are attracted to the area and adhere to the injured endothelium, a process called **margination.** This occurs by way of **cell wall components** called **selectins** (much like intercellular Velcro!). The PMNs migrate through the "leaky" endothelium by **diapedesis** and are then **activated** by **CD4+ lymphocytes**. The PMNs **scavenge** tissue debris, foreign bodies and opsonized bacteria. This "cleans" and prepares the wound for healing, but is not an essential element (which is why chemotherapy and AIDS patients can still heal, albeit somewhat compromised). Once in the ECM, some neutrophils are bound to the matrix by **integrins**, others travel freely, elaborating proteases to form O_2 free radicals to kill bacteria and clear the ECM.

Between 48 and 72 hours the inflammatory **cell population changes**. Circulating **monocytes** undergo a similar migration into the tissues where they are now called **macrophages**. Although **phagocytosis** is also a macrophage function, there are 2 (the trend continues…) other essential functions:

1) **Degradation of the "initial matrix"**
2) More importantly, the **secretion of cytokines and growth factors**.

Growth Factors are **large protein** mediators that act on **non-haemopoetic cells** to modulate wound healing by stimulating protein production, ECM synthesis and cellular death. They are secreted by both fibroblasts and macrophages. The most important growth factors are Fibroblast Growth Factors (**FGF**), Platelet Derived Growth Factor (**PDGF**), Transforming Growth Factors (**TGF**), Vascular Endothelial Growth Factor (**VEGF**) and many, many, many others !

Cytokines are extremely potent **small regulatory proteins** released by nucleated cells. They have **haemopoeitic cells for targets** – they can be subcategorized as chemokines, lymphokines, monokines, interleukins, interferons, colony stimulating factors (depending on their specific target). They have complex and finely **tunable autocrine and paracrine** regulatory functions (appropriately termed **"cytokine networks"**).
This function makes the macrophage an **essential cell** which orchestrates the wound healing process (The "first key").

2. The Proliferative Phase

This phase overlaps the previous phase and consists of 4 complementary parts.

2.1 Fibroplasia (3 days – 3 weeks)
This is the development of extracellular matrix (ECM) and the formation of collagen by fibroblasts. 2 Processes (again…!) are at work: initially an increase in the number of fibroblasts occurs **(Fibroblast Proliferation)**, followed by the **deposition of ECM** by these fibroblasts.

2.1.1 Fibroblast Proliferation (3 – 5 days)

To increase the number of fibroblasts in the area (increase the "workforce"), they are generated from 2 sources:

1. **Mitosis** of existing fibroblasts
2. **Differentiation** of tissue mesenchymal cells

No protein synthesis occurs during this phase in adults. This means that there is NO increase in wound strength – the wound is totally reliant on sutures.

2.1.2. Deposition of ECM

This is the formation of the matrix *(resist inserting movie quote here)* and consists of 2 (surprise!) processes.

2.1.2a Formation of the Provisional Matrix

The "**initial matrix**" (see earlier) formed in the first phase is degraded and is replaced by a new **"provisional matrix"**, which consists of:

1. **Glycosaminoglycans** (GAGs), such as **hyaluronic acid (HA)**
2. **Glycoproteins** (GPs), such as **fibronectin**.

2.1.2b Formation of the Collagenous Matrix (or "Final Matrix")

Some of the HA in the provisional matrix is degraded to make way for the deposition of collagen. **Collagen** is secreted by fibroblasts onto the scaffold of the

"provisional matrix" (like concrete being laid onto steel mesh during bridge construction). Initially this is mainly **type III collagen**.

2.2 Angiogenesis (Day 2 onwards)

All these cellular processes require **oxygen and nutrients**. These can only be delivered by a capillary network and thus angiogenesis is essential. Under the influence of the **cytokine factors**, capillaries at the periphery of the wound undergo **sprouting** to form **endothelial tubes** which penetrate the "provisional matrix". The endothelial tubes later become canalized to become new blood vessels. These new blood vessels within the matrix of fibronectin, HA and collagen as well as macrophages and fibroblasts constitute what is known as **"granulation tissue"**.

2.3. Epithelialization (hours onwards)

The reformation of the epithelium occurs in concert with and on top of the previously described processes for ECM development. It also consists of 2 (ha!) processes:

2.3.1. Epidermal Proliferation

This process occurs within the first few hours post wounding. The **basal cell layer** at the periphery of the wound **flattens** and undergoes **increased mitosis** to provide a population of cells for migration. Once migration of a cell begins, it does not undergo further mitosis. Migration begins with the **disappearance of desmosomes** (freeing the cells from the basal lamina and each other), followed by **sheet migration** of a monolayer of cells. Migration does not occur randomly (like leaves on a pond) – the cells are directed by the underlying **fibronectin and tenascin** of the matrix, which act as "railroad tracks".

2.3.2. Epidermal Reconstitution

Once the various cell sheets meet, further migration is stopped by a process of **contact inhibition**. The cells become **columnar** and begin dividing again, to reconstitute the multilayer epidermis. The basement membrane is reconstituted by the laying down of very specific PGs such as **laminin, tenascin and vitronectin**, as well as **collagens type IV and VII**. The neo-epidermis is thinner, flatter and without rete pegs, thus full-thickness scar epidermis can never replicate normal epidermis.

2.4. Wound Contraction (5 days until remodeling phase)

Wound contraction is a **cell mediated** process and can occur without a collagen lattice. The **myofibroblast** (a fibroblast-like cell with α-actin microfilaments in its cytoskeleton) is thought to be the responsible cell. It is responsible for **centripetal wound contraction** at a **rate of 0.6 mm** per day. The rates of fibroplasia and angiogenesis and most especially epithelialization determine the degree of wound contraction. This means that early flap cover or skin grafting results in much less contraction – important for areas such as eyelids or flexor surfaces.

3. The Remodeling Phase

The ECM is **dynamic** and is constantly being remodeled (like the parliament!). The remodeling phase is characterized by a rate of synthesis equal to the rate of degradation.

As collagen is cross-linked, the wound undergoes a progressive increase in strength from **3%** of original at **1 week** (the weakest time) to **80% at 3 weeks**. Remodeling of the collagen bundles adds only a small percentage to the total strength (which continues to improve for 1 year) but never attains full strength. This is due to the smaller amount of HA and the lack of reticular collagen pattern which makes the scar more brittle than unwounded skin. The features of scar are related to its healing pattern which are :

- Lack of dermal appendages
- Parallel thick collagen bundles instead of fine reticular bundles
- Decreased elasticity due to collagen pattern and less HA
- Contour deformity

The degradation of ECM is an essential feature of the remodeling phase. The matrix is degraded by **Matrix Metalloproteinase (MMPs)** which are enzymes secreted by fibroblasts. There are about 25 MMPs. The rate of degradation is controlled by **Tissue Inhibitors of Metalloproteinase (TIMPs)**. It is the ratio of MMPs to TIMPs which controls the degradation rate. **Chronic wounds** have many time definitions, but the **common feature** is the increased degradation of ECM by **excessive MMPs**.

Initially the collagen laid down is all type III but later much more **type I collagen** is secreted. Initially haphazard, the collagen becomes re-organized into thick parallel bundles during the remodeling phase. Wound healing is complete (and the wound considered mature) when the **ratio of Type I collagen to type III reaches 4:1** – this may take up to **18 months**. In general, it is best to leave a wound to progress to maturity before considering a scar revision. This will often result in a better final cosmetic outcome than operating too soon. Assuming that the process is unfolding normally and no vital structures are being compromised the earliest time before another surgical intervention can be considered is 3 months, although waiting 1 year is the usual recommendation.

Factors Affecting Wound Healing

These can be divided into local and systemic factors:

A. Local Factors	B. Systemic Factors
1. Arterial Insufficiency **2. Venous Insufficiency** **3. Oedema** **4. Infection** **5. Pressure** **6. Radiation** **7. Foreign Material** **8. Poor Wound Care**	**1. Diabetes Mellitus** **2. Malnutrition** **3. Vitamin deficiency** **4. Chemotherapy** **5. Smoking** **6. Ageing** **7. Steroids** **8. Inherited Collagen Disorders**

Local Factors

1. Arterial Insufficiency
Local ischaemia inhibits collagen production as oxygen is a vital co-factor in the hydroxylation step. ABPI and arteriography may be required before bypass surgery.

2. Venous Insufficiency
Increased venous pressure leads to protein extravasation (dermatoliposclerosis) as well as oedema both of which increase the diffusion distance.

3. Oedema
This causes ischaemia by 2 mechanisms:

- Increased extracellular volume and thus increased diffusional distance
- Decreased oxygen concentration in the ECF.

4. Infection
Quantitative counts of 10^5 organisms/g are associated with infection which is a clinical diagnosis. Counts of 10^3/g may be required in the presence of foreign material. β-haemolytic streptococci in any concentration may be deleterious. Bacteria inhibit wound healing by many mechanisms, including increased collagen breakdown, decreased epithelialization and angiogenesis and metabolic competition with lowered tissue oxygen concentrations. Additionally skin grafts and flaps are more likely to fail. A more recent evolution in the understanding of wound healing and bacterial exits around the concept of 'biofilm' – see later.

5. Pressure
Prolonged and unrelieved pressure above 32mmHg occludes the microcirculation and leads to local ischaemia. In paraplegic patients, denervation *per se* does not have an adverse effect on epithelialization, but tissue perfusion due to altered auto-regulation is affected and tissues are more susceptible to local temperature changes.

6. Radiation
This impairs wound healing by two mechanisms:
Microcirculatory ischaemia as a result of fibrotic end-arteritis obliterans.
Fibroblastic proliferation (and thus collagen production) are inhibited due to DNA damage in the fibroblasts resulting in delayed or absent mitosis.

7. Foreign Material
This decreases the bacterial counts required to produce clinical infection and also prolongs the inflammatory phase as PMNs and Macrophages phagocytose this material.

8. Local Wound Care
Iatrogenic factors play an important role in the healing of a wound and appropriately tensioned sutures, accurate apposition, adequate debridement and haemostasis and moist dressings assist closure. Mechanical stress (excessive tension) has been shown to have

and adverse effect on fibroblast activation and collagen production. Repeated traumatic dressing changes are likewise implicated in prolonging the proliferative phase.

Systemic Factors

1. Diabetes Mellitus

Various components to the detrimental effect of this metabolic disease process on wound healing have been identified:

- Larger vessels rather than small vessels (as previously thought) are responsible for decreased peripheral perfusion (Macrovascular Disease). Histological evidence of thickened basement membrane and atherosclerosis of these larger vessels bear this out (Vasculopathy).
- Peripheral neuropathies either resulting from epineural oedema, intraneural toxicity of sorbitol and increased tendency to nerve entrapment syndromes renders these patients neurologically impaired. All divisions of the nervous system are affected :
    - Motor neuropathy : This affects principally the intrinsic muscles of the foot, leading to arch deformities, clawing and thus metatarsal head prominence.
    - Sensory neuropathy : This posterior tibial nerve and thus the sensory supply to the sole is responsible for both pressure ulceration and inadvertent injury
    - Autonomic neuropathy : This leads to decreased sweating and thus dry and cracked soles which provide portals for bacterial invasion
- Stiffened red cells (non-deformable) and increased blood viscosity also influence the microcirculation (Rheopathy).
- Lazy leukocytes and impaired phagocytosis allow detrimental bacterial proliferation (Immunopathy).
- High venous back pressure in the lower extremities increases transudation fluid and oedema.

2. Malnutrition

Human tissue requires both adequate protein and sufficiency energy to carry out the metabolic processes that healing entails. Protein of 0.8 to 2.0 g/kg/d and energy of 35 kcal/kg/d are required for the maintenance of normal tissue, injured tissue requires more. Malnutrition in animal studies have shown prolongation of the inflammatory phase and a decrease in fibroplasia and collagen production.

3. Vitamins and Minerals

Copper, Iron, Zinc, Vit C and Thiamine are essential to wound healing as they are co-factors in collagen production, but supra-physiological doses have not shown any increased benefit except where a deficiency exists.

Vitamin A supplementation may be beneficial even where deficiency does not exist as it supports and restores macrophage functioning. This effect may reverse the effects of glucocorticoid steroids.

Vitamin E is an antioxidant and cell membrane stabilizer and thus has adverse effects on the inflammatory phase of wound healing. Several clinical studies confirm the negative effect that topical Vit E has on scarring. Topical Vit E is thus NOT recommended.

4. Chemotherapy
This causes bone marrow suppression with a decrease in circulating leukocytes. This impairs the inflammatory phase of wound healing. Additionally neutropenia is associated with increased infection. Penacillamine prevents the formation of the aldehyde cross links which improve the strength of the collagen molecules. NSAIDS decrease collagen production even at therapeutic concentrations by impairing prostoglandin mediated inflammation – selective COX-2 agents may be better in this regard.

5. Smoking
Two effects are attributed to smoking :
Increased carboxy-haemaglobin which decreases oxygen off-loading at the tissues
Nicotine (also in non-smoking forms such as gum and snuff) is a powerful peripheral vasoconstrictor. Each nicotine "dose" causes profound peripheral vasoconstriction for 30 minutes.

6. Ageing
A decrease in the inflammatory phase is seen with ageing, and thus slowed wound healing (but hypertrophic scarring is unusual). Collagen production and cellular proliferation are also slowed, resulting in both normal skin and scar tissue being weaker. The upshot of this is that excellent scars usually result due to the low tension and decreased inflammation (which is good for facelifts).

7. Steroids
These inhibit both the inflammatory and proliferative phases of wound healing. Vitamin A may reverse these effects.

8. Inherited Collagen Disorders
These disorders relate to collagen metabolic eg Pseudoxanthoma elasticum, characterized by increased collagen degradation and deposition of calcium and fat on the elastic fibres.

Biofilm and Wound Healing – A Bold New Frontier

Bacteria interact and often rely on each for mutual benefit. A number of bacteria may inhabit the wound simultaneously, but where each single bacterium is living independently, they are known as free-living **"planktonic"** bacteria. Where a number of bacteria in the same physical space begin interacting together for mutual benefit they are then known as a **"community"**. Bacteria in a community are morphologically and physiologically different from their planktonic counterparts.

Where a community of bacteria attach to a surface and secrete an **exopolymeric material** which encases the entire community, this is known as a **"biofilm"**. Biofilm provides the

bacterial population increased metabolic efficiency, enhanced substrate accessibility, enhanced resistance to environmental stress and inhibitors (such as antibiotics and leukocytes) and an increased ability to cause infection and disease. Dermal wounds often provide an ideal environment for bacteria to exist as a community, which may have a significant effect on wound healing.

Bacteria competent to form biofilm include (but certainly not limited to): *Prevotella melaninogenicus, Peptostreptococcus micros, Klebsiella pneumonia, Escherichia coli, Bacteriodes fragilis, Staphylococcus aureus* and *Pseudomonas*.

How does this biofilm form? Chronic wounds are invariably polymicrobial, with different micro-organisms from both endogenous and exogenous sources contaminating the wound surface. The properties of the wound surface and the host will predetermine which micro-organisms will attach, grow and remain components of a biofilm.

One species (the **"pioneering species"**) converts from free-living **"planktonic"** individual to an adherent group. These two groups are affectionately known as **"swimmers" and "stickers".** The metabolism of the adherent groups then encourages the growth and development of subsequent species groups. In this way **species complexity increases** in an unchallenged biofilm. This process continues until the **"climax community"** of stable residents emerges.

While **stable biofilm** may seem rather innocuous, it is in fact, a frighteningly **potent pathogenic entity**. The following properties of biofilm have been implicated in disease:

1. The stable biofilm is able to overcome insults and constraints (substrates, pH, oxygenation, antibiotics) that would be imminently fatal to planktonic forms of each resident bacteria. This protection is known as the '**indirect pathogenicity**'. This goes beyond a simple cloaking (bioslime) mechanism. In certain situations some pathogens may be found to be antibiotic sensitive on culture but are rendered 'resistant' by other members of the mixed infection.
2. An even more important role for biofilm is in the generation of symptomatic disease, as follows: A basic tenant of clinical microbiology is known as **Koch's postulates** and it states "a micro-organism must be present in every case of the disease, isolated and grown in pure culture, and then shown to cause the same specific disease when inoculated into a healthy host with the same micro-organism isolated again from the diseased host". The concept of biofilm in disease and impaired wound healing is problematic in the face of Koch's postulates, because a community of organisms are **collectively associated with an infection**. This means that the individual organisms may be unable to satisfy the requirements to cause disease but the combined effects of the biofilm may be able to do this.
3. In addition to above synergy, an even more advanced form of co-operation may evolve. The mechanism of **"quorum sensing"** involves communication strategies within a mixed community of organisms that ultimately enable them to **coordinate their activities** and enhance their pathogenicity and ability to cause

disease. This blurs the boundary between single cell organisms and multi-cellular "organism" as some residents take on highly specialized functions and are then dependant on other organisms for their basic survival needs.

4. Finally, as well as the production of **destructive enzymes and toxins** by the members of the biofilm, communities may also affect healing by promoting a chronic inflammatory state, resulting in the release of free radicals and numerous lytic enzymes. In addition, bacterial loading may cause increased local matrix **metalloproteinases (MMPs)** secretion by the wound fibroblasts.

Biofilm communities and their associated bacterial interactions are only just being understood and it is likely that their effect on infection and wound is significant. It represents a new frontier in the fight against disease and chronic wounds and is probably going to be an exciting new target for novel therapeutic agents.