Chapter 1

Wound care

A. Wounds and wound healing

I. Wound healing and tissue transplantation

Adult wound healing

Wound repair proceeds through several stages that overlap:

- Inflammation (coagulation, inflammation).
- Proliferation (re-epithelialization, fibroplasia).
- Remodelling (maturation).

Inflammatory phase (days 0–6)

Coagulation/haemostasis – after wounding, bleeding occurs and haemostatic cascades are activated leading to the formation of a thrombin–platelet plug (clot), adherent to type II collagen exposed by endothelial disruption. This platelet clot is a source of:

- Growth factors – **platelet-derived growth factor** (PDGF), transforming growth factor alpha and beta (TGF-α and –β).
- Inflammatory vasoactive and chemotactic cytokines.
- Fibrinogen, fibronectin, thrombospondin and von Willebrand factor.

Subsequent formation of a **fibrin–thrombin mesh** traps more platelets to continue the cycle. After an initial period of vasoconstriction there is active vasodilatation due to the inflammatory mediators (histamine, kinins, complement).

Inflammation – activation of mast cells (release histamine) and influx of **neutrophils** (production of inflammatory mediators and cytokines), **macrophages** (remove debris, release TGF-β) and **T-lymphocytes** (recruit and activate fibroblasts), conversion of differentiated keratinocytes to immature cells that migrate over the wound surface. There is initial vasconstriction of injured vessels followed by vasodilatation (and increased permeability) due to histamine and other vasoactive substances.

- Within **12 hours** of wounding, cells appear in the wound – **neutrophils** and **monocytes** are attracted by chemotaxins including fibrin degradation products, complement proteins, leukotrienes, TGF-β and PDGF.
- Translocation of marginating neutrophils (24–48 hours) through capillary endothelium and basement membrane is facilitated by secretion of collagenase. Unless there is a continuing inflammatory stimulus, the neutrophil response and population declines after a few days, whereupon debris is removed by macrophages.
- **Macrophages** are attracted to the wound (48–96 hours) where they synthesize and release further **cytokines and growth factors**; they are **vital to wound healing**.
- Dermal **fibroblasts** migrate into the wound by forming cell–matrix contacts with matrix

proteins such as fibronectin, vitronectin and fibrinogen.
- **T-lymphocytes** migrate into wounds following the influx of macrophages (5–7 days) and persist for up to 1 week – a reduced response may lead to poorer wound healing. Their primary role seems to be to mediate in **fibroblast recruitment** and activation.

Proliferative phase (4 days to 3 weeks)

Re-epithelialization begins within hours of wounding with **migration** of marginal keratinocytes over the matrix but beneath the forming eschar. There is a phenotypic conversion of differentiated keratinocytes into non-polarized cells expressing basal cytokeratins similar to cultured cells; increased mobility comes from dissolution of anchoring junctions and reorganization of the cortical actin cytoskeleton to form lamellipodia. Cells stop migrating when they form a contiguous layer due to **contact inhibition**. Restitution of the basement membrane then induces the cells to adopt their previous morphology and form anchoring junctions with fibronectin.

- **Epidermal growth factor** (EGF) mRNA levels increase rapidly after wounding to promote re-epithelialization. Abnormalities of EGF expression are thought to impair wound healing whilst glucocorticoids suppress EGF expression in cutaneous wounds but have less effect on EGF receptor levels.
- **Melanocyte growth-stimulating activity**, or growth-related gene (MGSA/GRO), is normally expressed by suprabasal, differentiated keratinocytes and is up-regulated in regenerating human epithelium. It is a ligand for the type B IL-8 receptor which is also up-regulated in proliferating keratinocytes (as well as dermal fibroblasts, macrophages and smooth muscle), suggesting that this cytokine may act as an autocrine or paracrine factor-mediating cutaneous wound repair.
- **Insulin-like growth factor-1** (IGF) and IGF-binding protein-1 have been demonstrated to act synergistically to accelerate the healing of adult skin wounds.

Fibroplasia – there is an influx of fibroblasts over the fibronectin scaffold; they are activated by PDGF and TGF-β. These cells synthesize type III collagen, which with ongoing neovascularization forms granulation tissue. Wound **tensile strength** increases during this fibroblastic phase.

- **Activin** is strongly expressed in wound skin. Overexpression in transgenic mice improves wound healing and enhances scar formation; activin A has been implicated in stimulating formation of granulation tissue whilst activin B mRNA has been localized to hyperproliferative epithelium at the wound edge.
- Secretion of glycosaminoglycans (hyaluronic acid, chondroitin sulphate, dermatan sulphate), which become hydrated to form an amorphous **ground substance** within which fibrillar collagen is deposited.
- Zinc, vitamins A (retinoids) and C are also required for normal collagen synthesis.

Angiogenesis – induced by ***vascular endothelial growth factor*** (VEGF).

Remodelling phase (3 weeks to 18 months)

The extracellular matrix appears to modulate fibroblast activity through changes in composition during healing. When fibronectin initially predominates, fibroblasts actively synthesize hyaluronic acid and collagen, but in a maturing wound, when the amount of collagen reaches a certain, abundant level, fibroblast proliferation and collagen production ceases irrespective of any stimulation by TGF-β. At this point, usually ~10 days, the wound becomes a relatively acellular scar.

- Residual fibroblasts mature into **myofibroblasts** and form cell–matrix and cell–cell contacts that **contract** the wound (scar contracture). Type III collagen is gradually replaced by **type I collagen** by the activity of metallomatrix proteins released by macrophages, keratinocytes and fibroblasts, slowly returning to normal type I : III ratio of 3 : 1. Collagen is initially disorganized but then becomes **lamellar** (and aligned along lines of stress) by the activity of fibroblasts and collagenases with permanent cross-links.
- The abundant capillaries regress.
- Peak wound tensile strength is achieved at ~60 days and is a maximum of ~80% of unwounded skin strength.

Factors affecting wound healing

Discussions generally divide these into patient factors and wound factors. Most factors impairing wound healing may be attributed to a lowering of the oxygen concentration in the wound including radiotherapy

and diabetes. An oxygen tension of more than 40 mmHg augments **fibroblastic activity** and is required for the hydroxylation of proline and lysine residues to form **cross linkages** in the collagen α-chain. Oxygen also facilitates **cell-mediated killing** of pathogens in the wound.

Wound infection causes a prolonged inflammatory phase; reduced oxygen tension affects fibroplasia, causes collagen lysis and inefficient keratinocyte migration.

- Tissue expansion – increased rate and strength of healing.
- Low serum protein – prolonged inflammatory phase and impaired fibroplasia.
- Increased ambient temperature (30 °C) – accelerated wound healing.

Patient factors

- Age – there is a reduction in the cellular multiplication and production rate with age. The tensile strength and wound closure rates also decrease with age – the stages of wound healing are protracted.
- Nutrition – malnutrition is associated with impairment of fibroblast function and reduced wound tensile strength.
  - Vitamin C – essential for hydroxylation of collagen.
  - Vitamin E – antioxidant actions that neutralize lipid peroxidation (and thus cell damage) caused by ionizing radiation, for example.
  - Minerals – many are cofactors in collagen production, e.g. zinc influences re-epithelialization and collagen deposition.
- Systemic illness – many impair oxygen delivery and collagen synthesis (fibroblasts are oxygen-sensitive and collagen production is reduced if PaO_2 is below 40 mmHg). Examples include anaemia and pulmonary disease.
  - Smoking (multifactorial – the nicotine in one single cigarette causes vasoconstriction that lasts 90 minutes, cyanide impairs oxidative enzymes whilst carbon monoxide impairs the oxygen-carrying capacity of haemoglobin). Stopping will improve:
    - Carbon monoxide (12 hours).
    - Free radicals (1 week).
    - Nicotine effects (10 days).
  - Diabetes – multiple factors. These patients are prone to infection, whilst vascular disease reduces oxygenation and neuropathy increases vulnerability to ischaemia.
- Drugs.
  - Steroids – anti-inflammatory actions affect wound healing in many ways including macrophage and fibroblast function, reduced angiogenesis and contracture. Vitamin A is usually said to reverse steroid effects and increases collagen synthesis.
  - Non-steroidal anti-inflammatory drugs (NSAIDs) – almost halve collagen synthesis in some studies, which is related to the reduction in prostaglandins.
  - Chemotherapy – e.g. cyclophosphamide is anti-inflammatory and methotrexate potentiates infections.
- Genetic conditions e.g. Ehlers–Danlos/cutis hyperelastica, progeria etc.

Wound factors

- Infection – this prolongs the inflammatory phase. Endotoxins reduce tissue oxygenation, stimulate phagocytosis and release of collagenases and radicals that may damage normal tissue.
- Oedema – reduces tissue perfusion.
- Denervation – prone to ulceration (also increased collagenases).
- Radiation – endarteritis obliterans.

Collagen

Collagen forms about one-third of the total protein in the human body. The aminoacids hydroxyproline and hydroxylysine are important components of (pro)collagen; deficiencies of vitamin C and iron inhibit their hydroxylation.

- Procollagen – three polypeptide chains as triple helix form tropocollagen.
- Tropocollagen units form collagen filaments.
- Filaments form fibrils, which form fibres.

There are at least 16 different types of collagen.

- Type I – most common and predominates in bone, tendon and skin.
- Type II – hyaline cartilage, cornea.
- Type III – immature scar, blood vessels, bowel, uterus.
- Type IV – basement membrane.
- Type V – fetal and placental tissue.

Dystrophic epidermolysis bullosa is associated with mutations of collagen VII, which form anchoring fibril-specific proteins.

Contraction versus contracture

Contraction is a physiological process which is part of wound repair, whilst contracture is a pathological process which may be related to undesirable healing, fibrosis and tissue damage, which causes shortening, distortion, deformity and limitation of movement.

Myofibroblasts are the source of contraction in wounds; they are found dispersed throughout granulating wounds and appear on the third day, peaking on the tenth day. They are specialized fibroblasts with contractile myofilaments and cellular adhesion structures and their action leads to contraction of the entire wound bed.

Adjuncts to healing

- **Negative wound pressure** – the exact mechanism of action is unclear but negative pressure may help by reducing oedema and interstitial pressure, improving tissue oxygenation and removing inflammatory exudate/mediators and bacteria. See also VAC®.
- **Hyperbaric oxygen** – increases oxygen delivery to wounds. It may be useful in selected wounds e.g. ischaemic (acute arterial insufficiency, crush injuries), radionecrosis, gas gangrene and diabetic ulcers (Medicare covers its use if there are 'no measurable signs of healing for at least 30 days of standard wound therapy').
- **Growth factors** – use is mostly experimental. Recombinant PDGF B-chain (becaplermin) marketed as Regranex, the only agent shown to be efficacious in double-blind studies. It has FDA approval, however, there is a warning that there is an increased cancer mortality in patients who used three or more tubes.
- **Laser biostimulation**.
- **Ultrasound** – results equivocal.

Debridement

Debridement of necrotic, non-viable tissue is an important part of wound management.

- Non-selective – mechanical e.g.
  - Scrubbing.
  - Wet-to-dry dressings.
  - **Hydrogen peroxide** (usually 3%) – this is highly reactive and a source of reactive oxygen species. When applied to tissues it bubbles due to the reaction with tissue catalase releasing water and oxygen; Staphylococci tend to be catalase positive whilst Streptococci do not have catalase and are thus said to be more susceptible to peroxide. It is commonly used as a wound antiseptic and whilst in vitro it shows broad activity, the few studies on its clinical efficacy generally show that it is relatively ineffective in reducing bacterial count but does appear not to delay wound healing. The AMA (Roderheaver GT. In Krasner D, Kane D (eds). *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*, 2nd Edn. 1997;97–108) suggested that the effervescence may have some mechanical benefit in loosening debris and necrotic tissue in a wound.
  - Pulsed lavage systems, Versajet®.
- Selective debridement.
  - Sharp/surgical.
  - **Enzymatic** – selectively digest dead tissue/slough e.g. Iruxol Mono® which is a collagenase, clostridiopeptidase A, but takes several days. Others include Debridase (bromelain, derived from pineapple stems) which seems to work quicker (Rosenberg L. *Burns* 2004;**30**:843–850).
    - Autolytic – the combination of moist dressings e.g. hydrocolloids and endogenous enzymes can lead to the breakdown of necrotic tissue.
  - Biological – maggots of certain species, e.g. *Lucilia sericata*, can cause benign myiasis – i.e. the larvae only eat dead tissue. They also have antimicrobial action and promote healing to a certain extent.

Fetal wound healing

Rowlatt U. *Virchows Archiv* 1979;**381**:353–361. This was the first report of scar-free healing in humans.

The term fetal wound healing is used to describe the regenerative process that occurs with minimal or no scar formation; it only occurs in the skin and bone of the fetus, but not nerve or muscle i.e. it is organ specific. The exact reasons for this are unclear though

some have postulated on the significance of various findings including:

- Environment: fetal wounds are rich in hyaluronic acid and fibronectin.
- Wounds are conspicuous by an absence of inflammation and angiogenesis; healing is largely controlled by fibroblasts rather than macrophages.
- Reduced levels of TGF-β, PDGF, bFGF.
- Type III collagen is deposited in a more organized manner close to normal structure.
- Tenascin is a modulator of cell growth and migration in fetal wounds.

Bone

Bone is composed of 25% organic material (mostly type I collagen), 60% mineral (mainly hydroxyapatite) and 5% trace elements.

- Endochondral bone – laid down as cartilage first, usually at an epiphysis, followed by ossification. This occurs in long bones.
- Membranous bone – osteoid is laid down directly by osteoblasts without a cartilaginous stage – this occurs in facial bones.
- Cortical – concentric lamellae around a Haversian canal.
- Cancellous – made up of lamellar bone but in loosely woven spicules/trabeculae. It is not the same as immature/woven bone.

Fracture healing

There are four phases similar to wound healing described above:

- **Haemorrhage, inflammation and proliferation** (1–7 days) – activation of clotting cascade to form a fibrin coagulum between the bone ends which is invaded by neutrophils, then by macrophages and fibroblasts to form granulation tissue.
- **Soft callus stage** (3–4 days) – capillaries from the periosteum invade the fibrin clot. Undifferentiated periosteal mesenchymal cells differentiate to become chondrocytes that form a cartilaginous external or **bridging callus** (its extent increases when there is movement of the bone ends) with further differentiation of chondrocytes into osteoblasts with **endochondral ossification** of callus to form woven bone (hard callus stage about 3 weeks after injury).
- **Remodelling** (years) of woven bone to mature lamellar bone, orientated along lines of stress.

Primary cortical union/bone healing occurs when bone ends are anatomically reduced and there is rigid fixation (also for membranous bone or for vascularized bone flaps). Bone regeneration occurs from within the opposed Haversian canals with little or no external callus reaction; it takes about 6 weeks i.e. typically longer than secondary bone healing described above (for fractures that are not rigidly fixed or have a small gap).

Bone reconstruction

A good blood supply and stability of the bony ends is essential for healing. Blood supply:

- Nutrient artery which enters medulla and usually supplies the inner two-thirds of the cortex.
- Periosteal artery which supplies the outer third of the cortex.
- Metaphyseal, apophyseal (at tendon/ligament attachments) and epiphyseal.

Healing of bone grafts

- **Osteoinduction** – pluripotential cells in the recipient site (pericytes) are 'induced' to become bone cells; this is controlled by bone morphogenic proteins.
- **Osteoconduction** – bone graft acts as scaffold for the ingrowth of cells and capillaries. Old bone is reabsorbed and new bone deposited i.e. 'creeping substitution'.
- **Osteointegration** – new bone formation by surviving cells within vascularized bone graft.

Vascularized bone grafts i.e. bone flaps are recommended for defects larger than 5–6 cm. It can take over a year for stable union. Distraction osteogenesis can be used for defects > 10 cm.

Factors affecting healing of bone grafts

- Patient factors e.g. age, nutrition, immunosuppression, diabetes, obesity, drugs.
- Bone graft factors.
  - Intrinsic properties – there is usually less resorption if the periosteum is intact and in membranous bone. Preparation, i.e. fresh or treated, will make a difference.
  - Placement – orthotopic (graft placed in position normally occupied by bone) grafts are less prone to resorption whilst heterotopic

Table 1.1. The characteristics of types of bone grafts related to their healing and strength over time.

	Vascularized bone	Cortical graft	Cancellous bone
Osteoinduction	+/–	+/–	++
Osteoconduction	+	+	++++
Osteoprogenitor cells	++	–	+++
Immediate strength	+++	+++	–
6-month strength	+++	+++	++
12-month strength	+++	+++	+++

(in position not normally occupied by bone) are more prone to resorption.
- Recipient site – irradiation, infected or scarred.
- Fixation.
- Mechanical stress – physiological loading speeds up union and creeping substitution.

Tendon healing e.g. in the hand

Tendons consist of a dense network of spiralling collagen fibres that are predominantly type I; there is some type III collagen and a small amount of elastin. They are relatively acellular but do contain tenocytes, fibroblasts and synoviocytes.

- The endotenon holds tendon fascicles together and supports the sparse vessels and nerves; it is continuous with perimysium and periosteum.
- The epitenon is a vascular, cellular outer layer of a tendon which runs through a synovial sheath (zones 1 and 2) whilst in zones 3 and beyond, where there is no tendon sheath, the outer vascular layer is called 'paratenon'.

The blood supply in zones 1 and 2 comes via mesenteries ('mesotenons') called the vinculae – long and short, that enter the dorsal surface of the tendons from the transverse digital arteries at the level of the cruciate pulleys.

Synovial fluid also contributes to nutrition via imbibition and is the more important source in the hand; in the forearm, tendon nutrition is derived from vessels in the paratenon.

Tendon healing occurs by analogous processes of inflammation, fibroplasia and remodelling:

- Intrinsic healing by cells within the tendon itself.
- Extrinsic healing by cells recruited from synovial sheaths and surrounding tissues forming adhesions.

Studies have demonstrated that the strength and rate of healing are maximal in a tendon that is moving and stressed. A repaired tendon is weakest during period of collagen lysis at about day 14.

Cytokines, growth factors and plastic surgery
Rumalla VK. *Plast Reconstr Surg* 2001; **108**: 719–733.

Cytokines

Cytokines are proteins required for cell defence that are secreted predominantly by immune cells. They mediate in protective and reparative processes and also regulate cell growth and maturation.

Tumour necrosis factor-α

Tumour necrosis factor-α (TNFα) is released by macrophages/monocytes when stimulated by interaction with pathogens, tumour cells and toxins. It appears at wound sites after 12 hours of wounding and peaks at 72 hours.

- Mediates in chemotaxis of inflammatory cells.
- Up-regulation of cellular adhesion molecules at neutrophil target cell sites e.g. vascular endothelium (margination).
- Other effects include haemostasis, increased vascular permeability and collagen synthesis (although may impair wound healing if persists at high levels beyond natural peak and excess TNFα is associated with multi-system organ failure).

Interleukin-1

Interleukin-1 (IL-1) is produced by macrophages/monocytes and keratinocytes at wound sites and is detectable at wound sites after 24 hours, peaking around day 2 then rapidly declining.

- Neutrophil activation and chemotaxis.
- Increased collagen synthesis and keratinocyte maturation.
- High levels at chronic non-healing wound sites.

Interleukin-2

Interleukin-2 (IL-2) is produced by T lymphocytes.

- Sustains the post-injury inflammatory response via T-cell activation.
- Promotes fibroblast infiltration at wound sites.

Interleukin-6

Interleukin-6 (IL-6) is released by macrophages/monocytes, polymorphs and fibroblasts and is detectable at wound sites within 12 hours (as polymorphs arrive) and persists for up to a week.

- Promotes stem cell growth, B- and T-cell activation and mediates in hepatic acute phase protein synthesis (involved in the immune response).
- Stimulates fibroblast proliferation.
- High IL-6 increases scarring and high systemic IL-6 levels have been described as a marker of wound extent/severity (e.g. major burns) and a poor prognostic indicator.
- Low IL-6 in elderly patients with impaired wound healing and at scar-less fetal wound sites.

Interleukin-8

Interleukin-8 (IL-8) is released by macrophages and fibroblasts at wound sites.

- Neutrophil chemotaxis, adhesion (via up-regulated expression of endothelial cell adhesion molecules) and activation.
- Promotes keratinocyte maturation and migration.
- High levels in patients with psoriasis and low levels at fetal wound sites.

Interferon γ

Interferon γ is produced by T-cells and macrophages.

- Macrophage and polymorph activation.
- Mediates in wound remodelling; reduces wound contraction.
- Possible role for decreasing scar hypertrophy but may decrease wound strength.
- Anti-inflammatory cytokine.

Interleukin-4

Interleukin-4 (IL-4) is produced by T-cells, mast cells and B-lymphocytes.

- Promotes B-cell proliferation and IgE mediated immunity and inhibits the release of pro-inflammatory cytokines by macrophages.
- Promotes fibroblast proliferation and collagen synthesis at wound sites.
- High levels are found in patients with scleroderma.

Interleukin-10

Interleukin-10 (IL-10) is produced by macrophages and T-cells.

- Inhibits production of pro-inflammatory cytokines at acute wound sites.
- If persistent at high levels at chronic wound sites, e.g. venous ulcers, it contributes to impairment of the wound healing response.

Growth factors

Growth factors are polypeptides whose primary role is in regulation of cell growth and maturation.

Platelet-derived growth factor

Platelet-derived growth factor (PDGF) is released from α granules within platelets and by macrophages.

- Recruitment and activation of immune cells and fibroblasts in the early post-injury phase.
- Later stimulates the production of collagen and glycosaminoglycans; reduced levels are found in non-healing wounds.
- Three isomers of PDGF (2 polypeptide chains 'A' and 'B'):
  - AA: elevated at acute wound sites.
  - BB: most useful clinically, used for chronic and diabetic ulcers (Regranex®).
  - AB.

Transforming growth factor β

Transforming growth factor β (TGF-β) is released by macrophages, platelets and fibroblasts.

- Fibroblast maturation, collagen and proteoglycan synthesis.
- Inhibition of proteases.
- There are three isomers: TGF-β1, TGF-β2 and TGF-β3.
  - TGF-β1 and TGF-β2 associated with hypertrophic and keloid scarring, and neutralizing antibodies decrease scarring at rat wound sites (Shah M. *J Cell Sci* 1994;**107**:1137–1157).
  - Low TGF-β at fetal wound sites.
  - TGF-β3 shown to decrease scarring.
  - Ratio of TGF-β1 and TGF-β2: TGF-β3 determines nature of scar.

Fibroblast growth factor

Fibroblast growth factor (FGF) is released from fibroblasts and endothelial cells.

- Regulates angiogenesis and keratinocyte migration at wound sites.
- Two main forms: acidic FGF (aFGF or FGF-1) and basic FGF (bFGF or FGF-2) that binds to the same receptors as aFGF but is ten times more potent.
  - Eight other isoforms complete the family: FGF-7 and FGF-10 known as keratinocyte growth factor (KGF) 1 and 2. KGF-1 is low in diabetics and steroid immunosuppression; recombinant KGF shown to improve re-epithelialization at wound sites.
- Application of exogenous bFGF to wound sites accelerates re-epithelialization.

Epidermal growth factor

Epidermal growth factor (EGF) is released from keratinocytes.

- EGF promotes epithelialization.
- Promotes collagenase release from fibroblasts (remodelling).
- Inhibits wound contraction at fetal wound sites.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is released mainly from keratinocytes, also macrophages and fibroblasts.

- Promotes angiogenesis at wound sites.
- Mediates in the formation of granulation tissue.

Insulin-like growth factor

At wound sites, insulin-like growth factor (IGF) is released by macrophages, neutrophils and fibroblasts; levels rise to a peak within 24 hours of wounding and persist for several weeks.

- Promotes fibroblast and keratinocyte proliferation, with possible role in angiogenesis.
- Two isoforms: IGF-1 and IGF-2.
- Low IGF levels are observed in diabetic and steroid-suppressed wounds.

Immunology

Major histocompatability antigens (called human leucocyte antigens – HLA, in humans).

- Type 1 – all nucleated cells and platelets.
- Type 2 – antigen-presenting cells: Langerhans cells, macrophages and lymphocytes.

Sequence

- Antigen-presenting cells present alloantigen to T-cells and express IL-1.
- IL-1 causes T-helper (CD4+) to produce IL-2.
- IL-2 causes clonal expansion of T-helper cells and B-lymphocytes.
- IL-2 also activates Tc-cells and NK cells (cellular immunity).
- B lymphocytes mediate antibody-mediated cell lysis (humoral immunity).

Allograft rejection

- Acute rejection occurs after 7–10 days, due to T-cell infiltrate (cellular immunity). It may be delayed in immunocompromised patients until the immunodeficient state has passed, e.g. recovery from a burn or stopping immunosuppressant drugs.
- Late rejection is due to antibody-mediated cell lysis (humoral immunity).
- Hyperacute rejection is due to preformed antibodies and the rejection response begins immediately.

Graft versus host reaction occurs when allograft containing lymphoid tissue reacts against an immunocompromised host.

Immunosuppressant drugs

- Cyclosporin blocks IL-2 which blocks clonal expansion of Tc-cells.
- Azathioprine inhibits T-cell-mediated rejection by preventing cell division.
- Prednisolone blocks the generation and release of T-cells.

Biomaterials

These are materials used to replace or augment tissues in the human body and can be classified as:

- Autograft – living tissue from host.
- Isograft – from a genetically identical twin.
- Allograft – tissue from same species.
- Xenograft – tissue from different species.
- Alloplast – derived from synthetic material.

The biological reactions to a foreign body include:

- Immediate inflammation with early rejection.
- Delayed rejection.

- Fibrous encapsulation.
- Incomplete encapsulation with continuing cellular reaction.
- Slow resorption.
- Incorporation.

Cultured epithelial autograft

A cultured epithelial autograft (CEA) begins with a full-thickness skin biopsy of several square centimetres taken from the patient for culturing. After 3 weeks' preparation time, there are enough cells to cover a 1.8 m^2 sheet five cells thick. The overall take is 80% under favourable conditions, though late loss can occur. The new skin separates easily from underlying dermis – these bullae contain high levels of thromboxane A2 (TXA2) and prostaglandin E2 (PGE2) suggesting on going inflammation.

- Cultured cells can be delivered as a sheet or as a suspension, which takes less time (cells are less mature) and less ligand-specific integrins/adhesion molecules are expressed facilitating graft take. CEA cell suspension may be used in combination with widely meshed split skin graft (SSG; e.g. 'sandwich' graft). It may also be used to speed up donor site re-epithelialization.
- A variant (mixed cell culture – ReCell®) is said to reduce pigmentary problems.

Bone grafts and cartilage grafts

Bone autografts

- Can be incorporated without host reaction, and is relatively resistant to infection.
- Donor site morbidity is a problem, as is the variable resorption rate in the graft – cortical grafts maintain their volume better than cancellous.

Cartilage autografts

- Relatively easy harvest and less donor site morbidity; infection and resorption is rare but may calcify.
- Has a tendency to warp and quantities are quite limited (septum, rib, conchal).

Allografts

These generally do not contain living cells due to processing to reduce antigenicity, though bone allograft may have osteoconductive and osteoinductive properties. They are **incorporated** into host tissues providing a structural framework for ingrowth of host tissues.

- Their advantages include a plentiful supply, a donor site is not required and operation time is usually reduced.
- Disadvantages include a potential for infection/disease transmission, and variable amount of resorption.

Examples include:

- Lyophilized fascia (dura mater, fascia lata) – risk of Creutzfeldt–Jakob disease (CJD) transmission in the former. Typically there is a 10% resorption rate.
- Homologous cartilage – greater tendency for resorption, replacement with fibrous tissue, ossification and more infection compared with autologous tissue.
- Homologous bone – acts generally as scaffold for formation of new bone; slower to become incorporated and revascularized.
- **AlloDerm** (Lifecell Corp.) – a homologous/cadaveric dermis that has been processed to remove cellular elements, it becomes incorporated into the host.

Cadaveric skin allograft

Allograft can be used by itself as a dressing (see above) or laid over 3 : 1 (or wider) meshed autograft (Alexander technique). Burns patients are immunosuppressed so rejection of allograft is usually delayed, in some cases up to 85% viability at 1 year.

- The skin must be retrieved within 24 hours of death from a refrigerated cadaver under aseptic conditions (screening serology for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and skin samples for culture of bacteria, yeast and fungi). It is stored in nutrient media at 4 °C for up to 1 week or cryopreserved (controlled freezing at 0.5–5 °C/min to –196 °C with liquid nitrogen and a cryoprotectant solution). When needed, it is rapidly rewarmed to 10–37 °C (~3–4 min).
- Donor exclusion criteria: high-risk categories for HIV i.e. homosexuals, drug abusers, those with tattoos, prostitutes and haemophiliacs, those with infection/sepsis, neoplasia and autoimmune disease. Only two cases of viral transmission in

3 million tissue transplants (including skin) have been described.

Skin banking in the UK: the need for proper organization
Freedlander E. *Burns* 1998;**24**:19–24.

The Sheffield skin bank was set up in 1991. They use similar inclusion/exclusion criteria as above (based on the guidelines of the American Association of Tissue Banks) using a one-page questionnaire requesting information on past medical and social history.

- Preservation media of 15% glycerol in phosphate buffer saline (PBS) with penicillin, streptomycin and amphotericin B added. Intracellular viruses are destroyed. Skin is banked in 15% glycerol at –80 °C (viable) or in 98% glycerol at room temperature.

Prepared in this way, the cadaveric skin has a 2-year shelf-life but is non-viable, effectively acting as a biological dressing. The paper goes on to describe the logistical difficulties in the running of the skin bank, and increasing demand coming from outside of the original catchment area, leading to the transfer of the service to the National Blood Service in 1996.

Xenografts

- Surgisis® – derived from pig small intestine mucosa. It is often used for fascia replacement; the acelullar matrix allows tissue ingrowth. There are no good clinical data for its effectiveness.
- Integra® – outer silicone layer covers bovine collagen cross-linked with shark chondroitin-6-sulphate that is revascularized and acts as a template for dermal regeneration (vide supra).

Alloplasts

Alloplasts have wide availability/supply without donor site morbidity, but tend to be expensive and elicit a host reaction of some sort as they are foreign materials.

- Metals e.g. stainless steel, vitallium alloy, titanium alloys (10 times stronger than bone and well tolerated but has low fatigue tolerance).
- Medpor (high density porous polyethylene) – allows vascular ingrowth and reduced tissue reaction, but it is expensive and can be difficult to remove.
- Hydroxyapatite – a calcium phosphate salt available in dense (high pressure compaction) or porous hydroxyapatite. A natural source of hydroxyapatite comes from coral. It allows a degree of vascular ingrowth but is brittle and can be difficult to shape. Also available for use as a tissue filler, Radiesse®.
- Silicone is a silicon polymer and its physical state depends on the amount of cross-linking. It is generally inert which means it tends to be encapsulated rather than incorporated.
- Expanded polytetrafluorethylene (ePTFE), Gore-Tex is available in many different forms. It shows reduced tissue ingrowth.

The following are resorbable (unlike the previous examples):

- Polylactide compounds e.g. Lactosorb plating system used in craniofacial surgery, poly-L-lactic acid (PLLA) tissue fillers (Sculptra®). These are completely resorbed and thus have fewer long-term risks.
- Polyglactin – is available as a suture (Vicryl), film or mesh.

II. Necrotizing fasciitis

Necrotizing fasciitis was first described by Wilson in 1952. It is a life-threatening infection (mortality rate up to 53%) that progresses along fascia and subcutaneous tissues. Some describe type I (polymicrobial) and type II (monomicrobial). In most, there is bacterial synergism as lytic toxins increase the spread of anaerobic organisms.

- **Mixed** anaerobe (*Escherichia coli*, *Bacteroides*) and aerobes (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis*). In most cases, *Streptococcus* and/or *Staphylococcus* are the initiating agent.
  - *Vibrio vulnificus* is often seen in those with chronic liver disease and may follow raw seafood ingestion as well as injuries in fishmarkets. This type of disease classically has subcutaneous bleeding.
- Group A beta haemolytic streptococcal (e.g. *Streptococcus pyogenes*) infection – group A *Streptococcus* is carried in the nose/throat of 15% of the population. It can contribute to type I infections or be responsible for type II (most cases are streptococcal but more recently methicillin-resistant *Staphylococcus aureus* (MRSA) has been