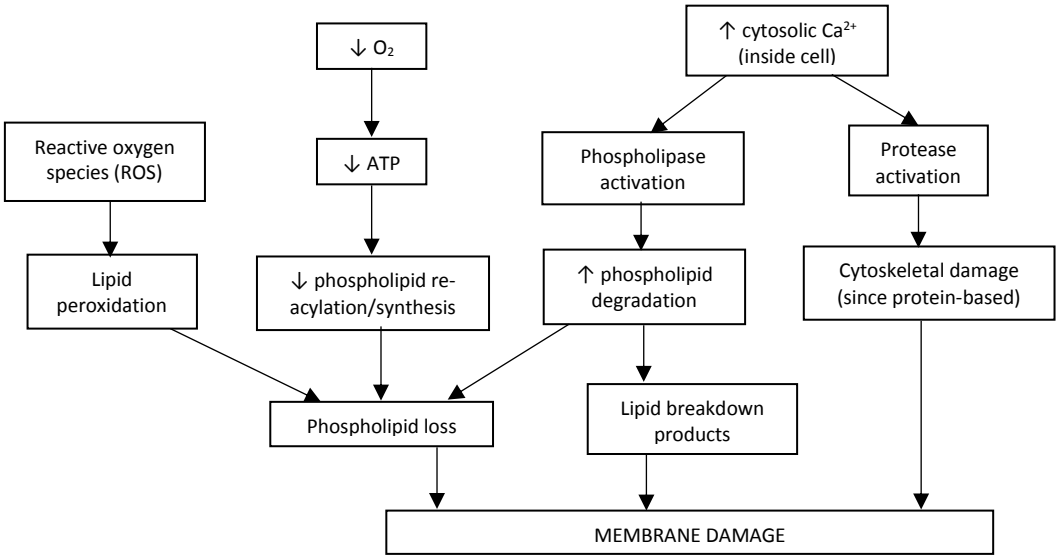
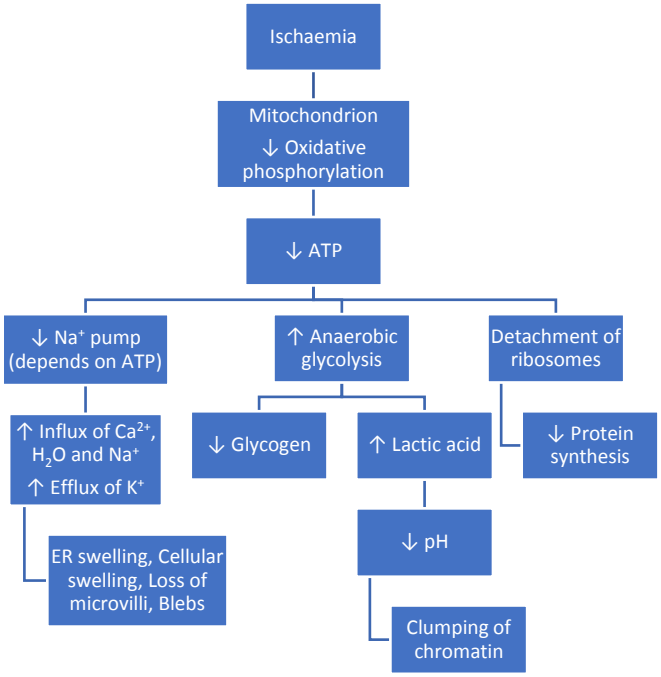
Cell Injury

- Reversible and irreversible injury depend on time and severity of injury
- Irreversible injury can lead to cell death (either necrosis or apoptosis)
- Some cells can adapt to injury
- Unregulated cell death (necrosis) is pro-inflammatory
- There are many regulated cell death mechanisms other than necrosis and apoptosis
- The earliest manifestation of almost all types of cell injury is cellular swelling
- ATP depletion and decreased ATP synthesis are frequently associated with hypoxia and chemical injury

Cell Injury

- Reduced ATP production due to mitochondrial damage
- Loss of calcium homeostasis due to cell membrane damage
- Disrupted membrane permeability of organelles and cell membranes
- Free radicals
- Protective mechanisms in response to cell injury
    - Heat shock response genes
        - Produce proteins which are upregulated in the face of cell stress
        - Protects proteins from stress-related damage
        - Clean up damaged proteins
    - Many tissues and organs can survive significant injury if they are 'pre-stressed' (adaptation)
- Morphological alterations
    - Decline in cell function
    - Biochemical alterations leading to cell death
    - Ultrastructural changes – cell swelling, fragmented nuclei, etc.
        - Pyknosis, karyorrhexis, karyolysis
    - Light microscopic changes – cytoplasmic changes, nuclear changes, fatty change
    - Gross morphologic changes
- Hypoxia – most common injury
    - Insufficiency of oxygen due to reduced blood supply
    - Triggers –
        - Ischaemia – blockage in blood supply (e.g. embolus, cardiac failure)
        - Hypoxaemia – reduced oxygen (e.g. high altitude, anaemia)
        - Oxidative phosphorylation inhibition (e.g. cyanide poisoning)
    - Effects
        - Decreased ATP – metabolite substrates limited, wastes accumulate
        - Free radicals are highly reactive and damage macromolecules
            - Sudden increase in oxygen can also increase free radicals (reperfusion injury)
            - Can cause lipid peroxidation → membrane damage
            - Can cause protein modifications → breakdown, misfolding
            - Can cause DNA damage → mutations
    - Outcome depends on balance between reversible and irreversible injury (time-dependent)
        - Tissue type also important
    - Reperfusion (restoration of blood flow) generally results in cell recovery if substrates are provided





Necrosis

- Death of groups of contiguous (localised) cells in tissue or organ
- Causes inflammation due to spilling whereas apoptosis does not
    - First cells to come in are neutrophils
- Coagulative (ischaemic necrosis)
    - Cells died but basic shape and architecture of tissue remains
    - Maintains solid consistency
    - Necrotic cells ultimately removed by inflammatory cells
    - Regenerated or replaced by scar (fibrosis)
- Gangrene (coagulative necrosis of the lower limbs)
- Liquefactive (e.g. brain ischaemic necrosis)
    - Complete dissolution of necrotic tissue
    - Massive infiltration by neutrophils (abscess formation) – release ROS and proteases
- Caseous (e.g. TB and fungal infections)
    - Accumulation of amorphous (no structure) debris within an area of necrosis
    - Tissue architecture abolished
- Infarct (red or white)
    - Area of ischaemic necrosis
    - White – arterial occlusion in solid tissues
    - Red/haemorrhagic – venous occlusion in loose tissues

Apoptosis

- Programmed cell-autonomous suicide
- Requires energy expenditure
- Triggers
    - Withdrawal of growth stimuli such as growth factors
    - Death signals (e.g. by Fas and TNF)
    - DNA damage (e.g. p53)
    - Unfolded protein response (causing ER stress)
- Normally occurs during development (limbs) and for removal of certain cells (self, infected)
- Gross morphological changes
    - Cytoplasm shrinks, blebbing of plasma and nuclear membranes, membrane-bound bodies (no inflammation) are phagocytosed
- Intrinsic (mitochondrial) pathway – internal cell injury results in apoptosis of the cell
    - Activation of pro-apoptotic factors (BH3-only proteins – Bim) → cytochrome c leakage → caspases
    - BCL2, an anti-apoptotic protein antagonises pro-apoptotic factors to oppose apoptosis

- Extrinsic (death receptor) pathway – receptor-ligand interactions induce cell death
    - E.g. FasL on cytotoxic T cell binds to Fas on target cell, breakdown occurs via caspases
    - E.g. TNF binds to target cell and induces apoptosis

Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome-size fragments
Plasma membrane	Disrupted	Intact; altered structure, especially orientation of lipids
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiologic or pathologic role	Invariably pathologic (culmination of irreversible cell injury)	Often physiologic, means of eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA damage

Autophagy

- Regulated death mechanism
- Stress or starvation can result in autophagic vacuolisation within the cell
- Outcomes – autophagic survival or autophagic cell death (depends on Beclin-1 levels)

Mitochondria

- Necrosis: low O2 → mitochondrial damage → low ATP, high ROS → Necrosis
- Apoptosis: more pro-apoptotic proteins (BH3-only), less anti-apoptotic proteins (BCL2) → leakage of mitochondrial proteins (cytochrome c) → Apoptosis

Cellular Senescence and Ageing

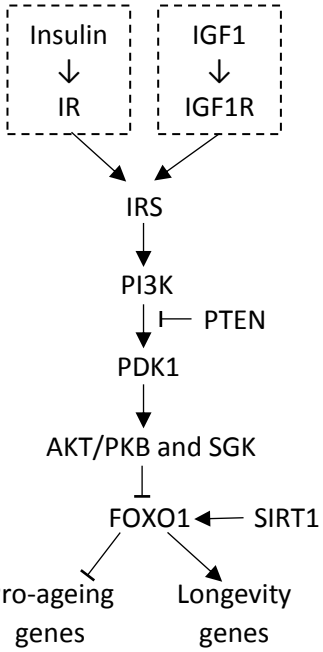
Ageing

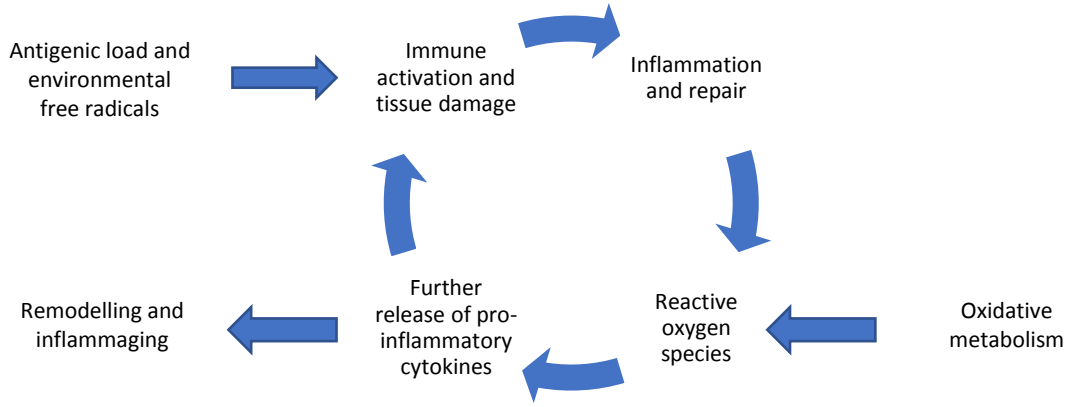
- Persistent and gradual decline in the age-specific fitness components of an organism due to internal physiological degeneration
- Universal risk factor for disease development
- Factors
    - Genetics – accumulation of mutations
    - Cell biology
    - Biochemistry – metabolism
    - Endocrinology
    - Regenerative medicine – way to replace ageing cells?
    - Demography and environment
- Cells decline in efficiency of cellular functions with advancing age
    - Mitochondria less efficient in performing oxidative phosphorylation
    - Synthesis of structural, enzyme and receptor proteins
    - Take up to nutrients and chromosomal damage repair impaired
    - Abnormally shaped organelles accumulate waste products
- Muscles atrophy due to loss of muscle fibres
- Organs decrease in size and activity
- Causes of ageing
    - Wear and tear theories – living and being exposed to the environment
    - Free radicals
    - Programmed ageing
    - Accumulation of gene mutations – predicts that genetic diseases should increase in frequency with age
- Telomerase – lengthens telomeres
    - Telomeres shorten with each cell division
    - The length of telomeres slows down the ageing process by allowing cells to divide
    - Cancer cells employ mechanisms that prevent telomere length shortening

Senescence

- Causes a loss of tissue repair capacity because of cell cycle arrest in progenitor cells
- Senescent cells produce proinflammatory and matric degrading proteins – Senescence Associated Secretory Phenotype (SASP)
    - Triggers senescence in surrounding cells
- Genetic factors and environmental insults combine to produce the cellular abnormalities that are characteristic of ageing
- Werner's syndrome – premature senescence
    - Inherited disease that causes premature ageing
    - Patients more susceptible to cancer, osteoporosis, diabetes and cataracts
    - Usually die in their 40s
    - Mutated WRN gene – encodes a helicase (unwinds DNA for replication, repair and transcription)
    - Improper DNA repair and rapid accumulation of mutations and improper transcription of genes needed to maintain vigour are possible causes
- Progeria
    - Children age rapidly and die as young as 12
    - Caused by dominant mutation in lamin A gene, which has a role in nuclear integrity (structure)
    - Patients have shorter telomeres than normal children
- Role of cytokines in replicative senescence
    - IL-6 and IL-8 contribute to maintain the senescent phenotype (SASP) via bystander senescence
    - These cytokines also inhibit cellular proliferation and promote senescence
    - Proof of bystander senescence – elimination of senescent cells (expressing p16 biomarker) delayed tumorigenesis and reduced age-related deterioration of several organs, including kidney and heart
- NFκB – central transcriptional regulator of stress/inflammatory response
    - NFκB contains 2 components – RelA and p50
        - P50 is an anti-transcription factor that stops proinflammatory protein expression

        - RelA is a pro-transcription factor that promotes expression of inflammatory proteins
    - Pathway can both promote and repress inflammation depending on the balance of RelA vs p50
- Chronic inflammation induces telomere dysfunction and accelerates ageing in mice
- Caloric restriction postpones senescence
    - Induces levels of some antioxidant enzymes which counteract ROS
    - Metabolic pathways interact with the insulin and IGF-1 (pro-ageing) pathways
        - Caloric restriction increases SIRT1
        - SIRT1 upregulates FOXO1 which upregulates longevity genes and inhibits pro-ageing genes
        - Insulin and IGF-1 pathways inhibit FOXO1
        - High PTEN levels make cells senesce more slowly
    - Reduction in IGF-1 receptor extends lifespan of mice
    - Mice lacking the insulin receptor in adipose tissue extends longevity
- Autophagy can be a survival mechanism in times of nutrient deprivation
    - Cell cannibalises itself and recycles the digested contents
    - Important in maintaining cell health as it clears cellular waste
    - When autophagy is inefficient, cellular waste accumulates and cells senesce faster
- Macrophages
    - More men were found to live past 100 on Sardinia than anywhere else in the world
    - Macrophages in these men appeared 'younger' and more active
- Ageing is a polypathology – multiple chronic diseases
- 'Inflammageing' – inflammation is the core of senescence





- Senotherapies prevent disease and extend healthy lifespan
    - Anti-ageing drugs = senolytics
    - Prevent senescence triggers
    - SASP inhibition
    - Senescent cell killing

Stem Cells

- Reservoir for replacing cells in nearly all tissues
- Progenitor cells – stem cell-like cells that can replace damaged tissue