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Human Pharmacological Services

Accelerating Development, Reducing Risk

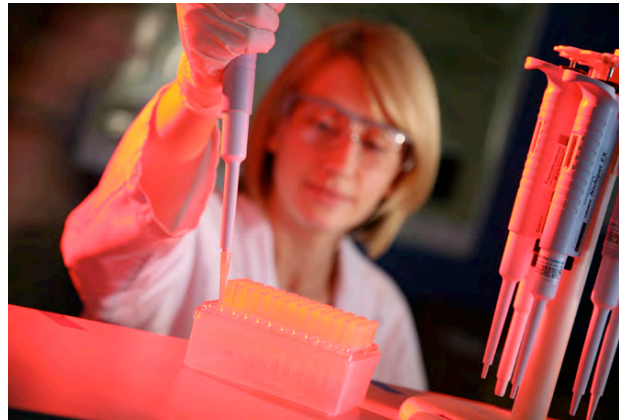
Efficacy, Potency and Sensitivity: The Use of Isolated Human Tissues

Prof Chris Hillier
School of Life Sciences

Early Drug Development

Animal tissues:

- used extensively in early development
- generally a poor predictor of the human response
- animal-derived preclinical data is a poor predictor of the outcome of clinical trials
- We might speculate on a correlation between an over-reliance on animal data and late stage failures, post-registration withdrawals and declining discovery pipelines



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Human Tissue Assays in Drug Development



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The Early Development Cycle



Isolated animal tissues

- fast & cost effective
- number of validated assays
- potency & efficacy

Isolated human tissues

- potency & efficacy
- duration of action

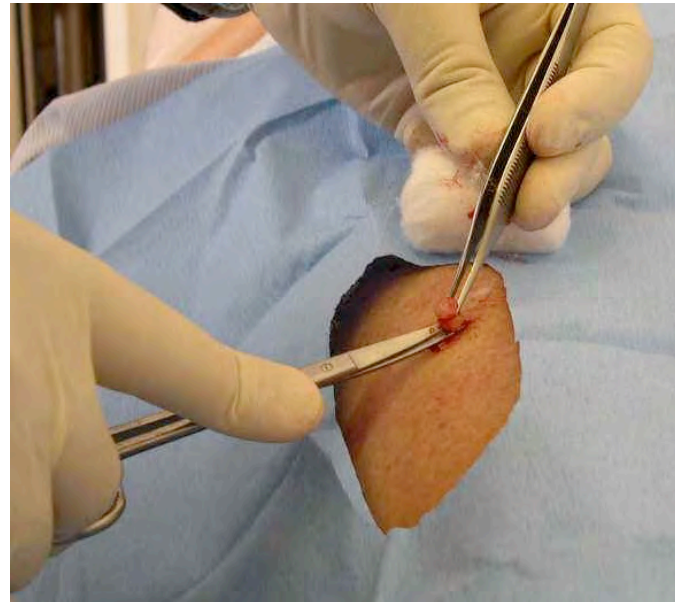
Isolated human tissues

- human safety, incl human cardiac tox



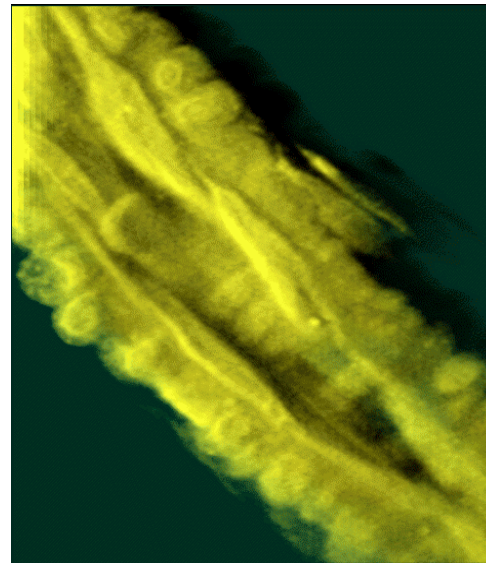
Functional Human Tissue Assays

- samples of living “isolated” human tissue
- different cell pops; phenotypes; genotypes
- expose to novel drug candidates
- integrated physiological response measured
- assay for efficacy, potency, sensitivity, off-target effects
- growing range of tissues and tests available



Isolated Human Blood Vessels

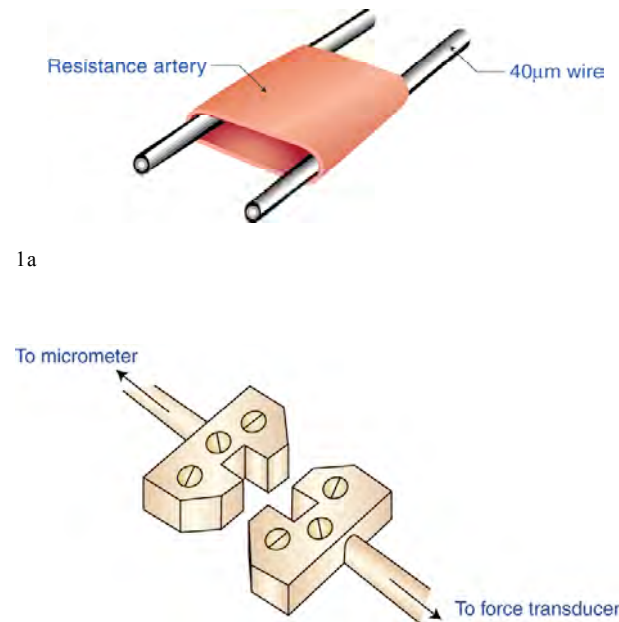
- Large blood vessels available following surgery
- Small vessels via surgery or biopsy programme
- Site-specific vessels (arteries/veins):
 - cardiac (heart failure)
 - gut mucosa (ulcerative colitis)
 - skin (blushing)
 - placental (pre-eclampsia)
 - uterine (post-partum haemorrhage)
 - cerebral (blood-brain barrier)



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Resistance Artery Wire Studies

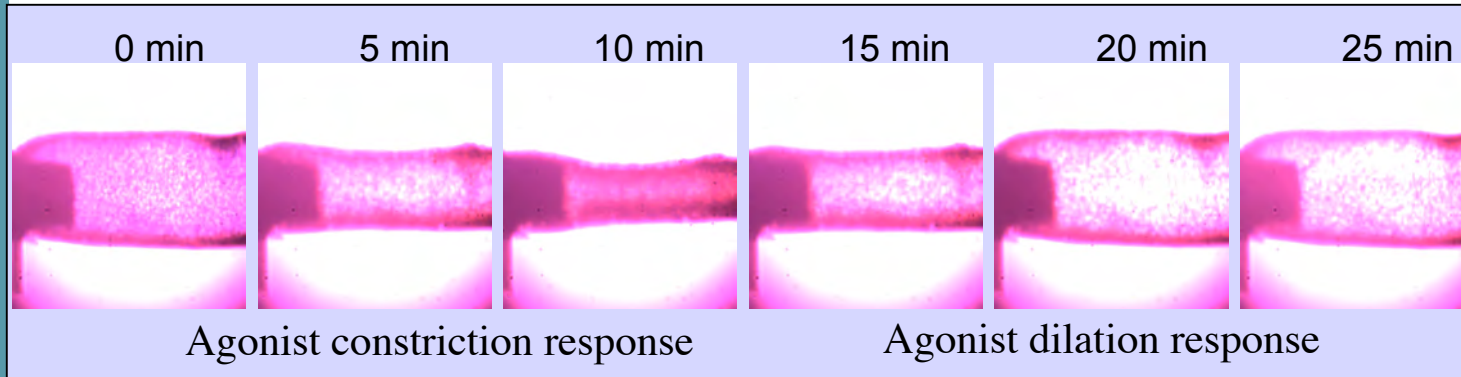
- small “resistance” arteries (150 μ m-300 μ m)
- primary regulator of blood flow to all organs
- responsible for peripheral vascular resistance
- ubiquitous
- obtained from very small biopsies
- ring preparations in ‘strain-gauge’ or ‘pressure/flow’ systems



Perfusion Studies



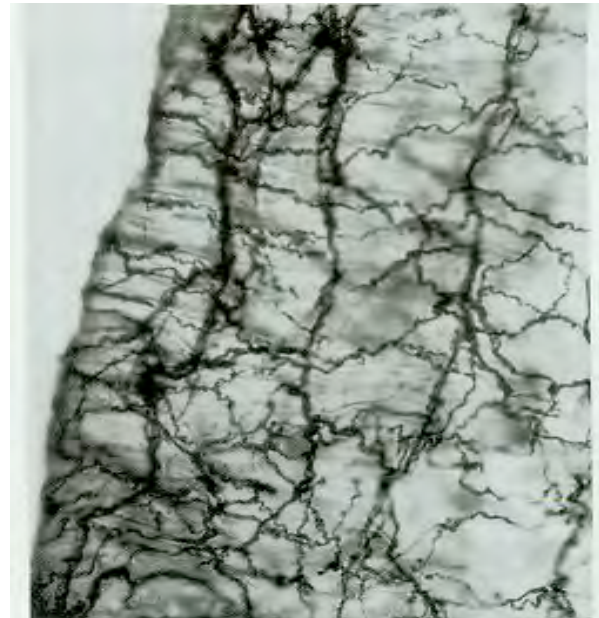
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- Allows intraluminal delivery of the drug
- 10x sensitivity & closely mimics in vivo situation
- Compare endothelial & smooth muscle effects
- Utilise automated, standardised technology
- Useful for testing of delivery reagents (e.g. RNAi)

Field-Stimulation Assays

- highly innervated
- provide measure of vascular sympathetic tone
- generate electrical field using platinum or silver electrodes
- stimulates release of catecholamines
- neurotoxicity
- neuropathy studies



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Vascular “Profiles”



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- prediction of off- target effects
- vascular “profile”
 - skin, muscle, gut
(~90% of blood volume)
- up to 20 small arteries from single biopsy
- both ‘control’ and patient groups can be studied



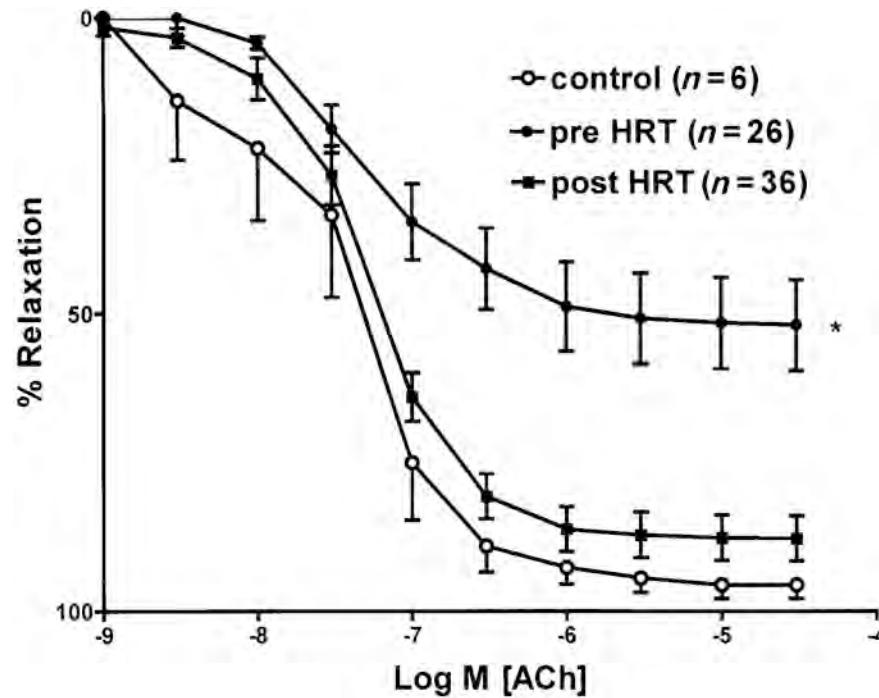
“Phase II” - in vitro

Post-menopausal women with diabetes:

- 1st biopsy pre-HRT
- 2nd biopsy post-HRT
- direct comparison

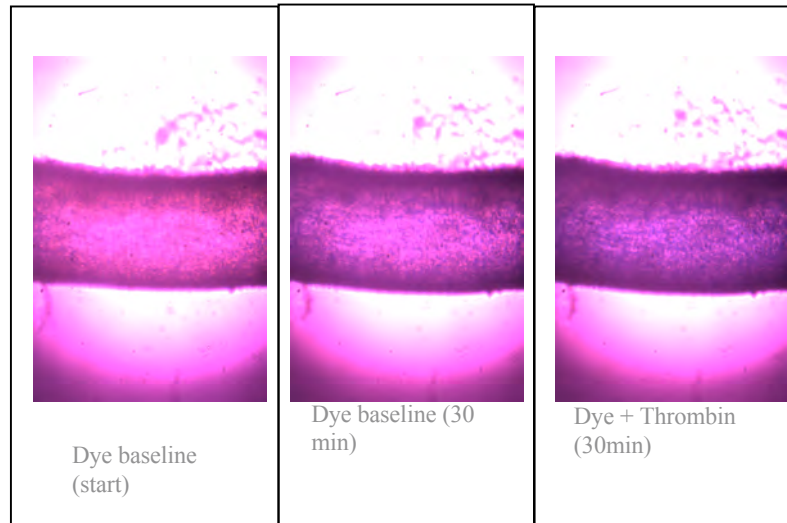
Longitudinal studies:

- reveal chronic changes not observable during clinical trials

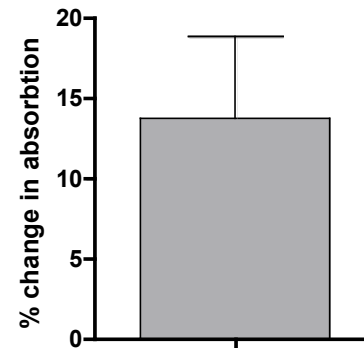


Perera, M. et al. Hum. Reprod. 2002 17:497-502;

Vascular Permeability



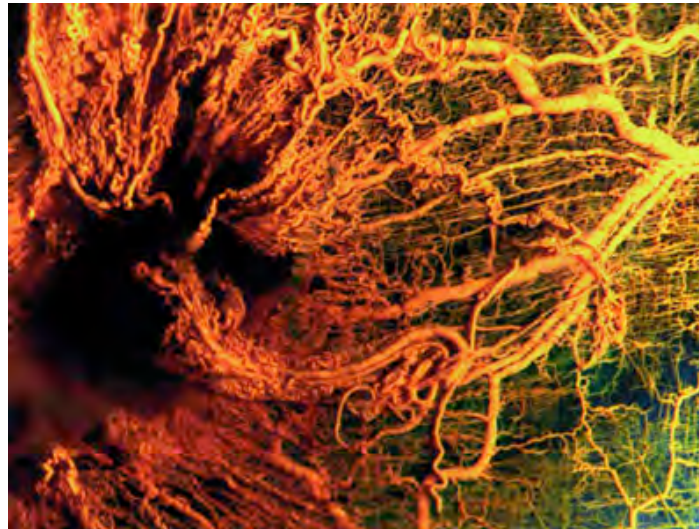
Thrombin-induced change in Vascular Permeability



- In vivo: injection of dye - conjugates to plasma proteins
- Dye measured through wall after sacrifice of animal
- In vitro: real-time imaging and analysis of permeability
- Allows multiple test conditions and time point analyses

Angiogenesis

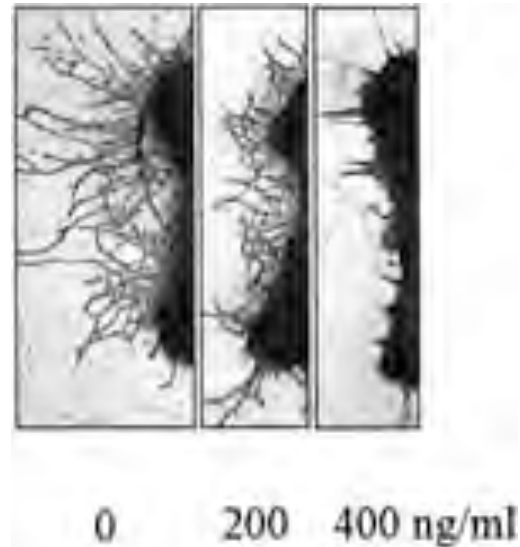
- process of new blood vessel formation
- involved in many diseases:
 - tumour growth
 - gastrointestinal disorders
 - macular degeneration
 - retinopathy
- therapeutic strategies for both anti- and pro-angiogenic purposes



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Organoculture

- Tissue response to angiogenic drugs:
- 2mm tissue sections
- 96-well culture plate
- time-points up to 2 wks
- endothelial 'sprouting' using light microscopy
- applied to target selection and lead optimization studies



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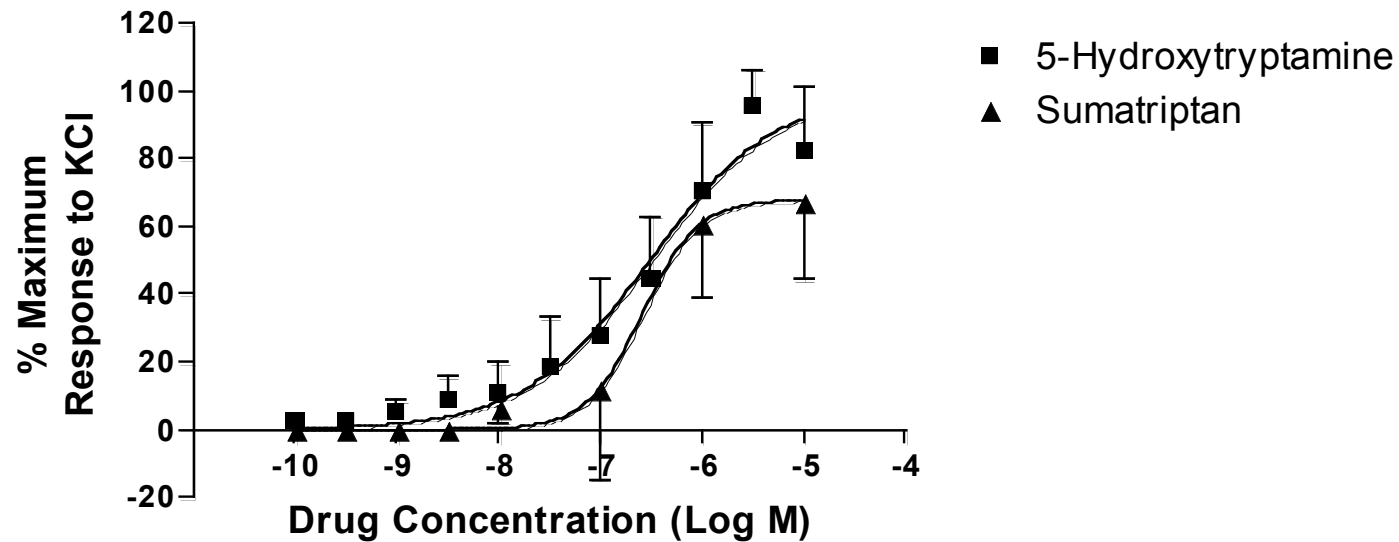
Adverse Effects – validation against reference compounds

Measure test compounds against known reference compounds



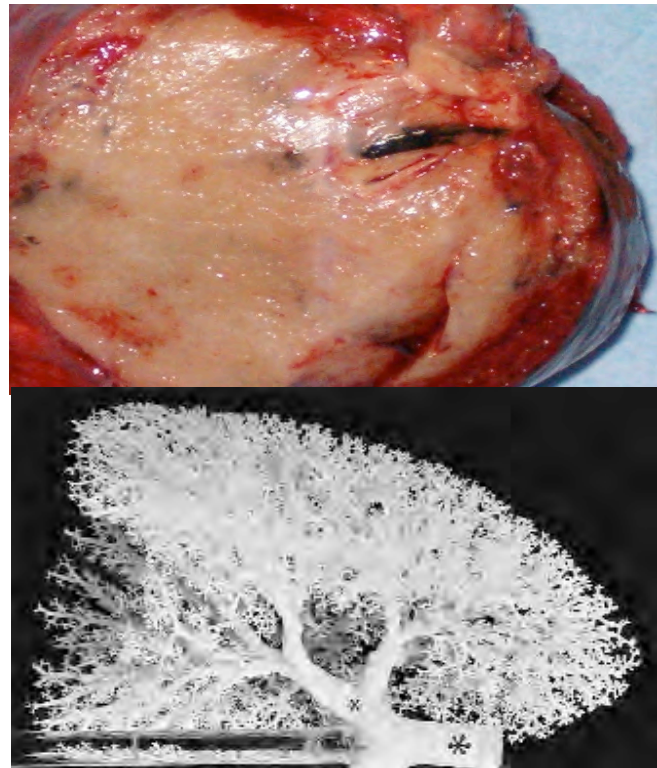
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Contractile Responses of Isolated Human Coronary Artery



Isolated human lung tissue

- Technically extremely difficult
- very easily damaged
- bronchial rings
 - changes in airway tone
- parenchymal strips
 - strain gauge
- nerve stimulation
- screen lung off-target effects for therapeutics targeting other tissues but which share similar pathways



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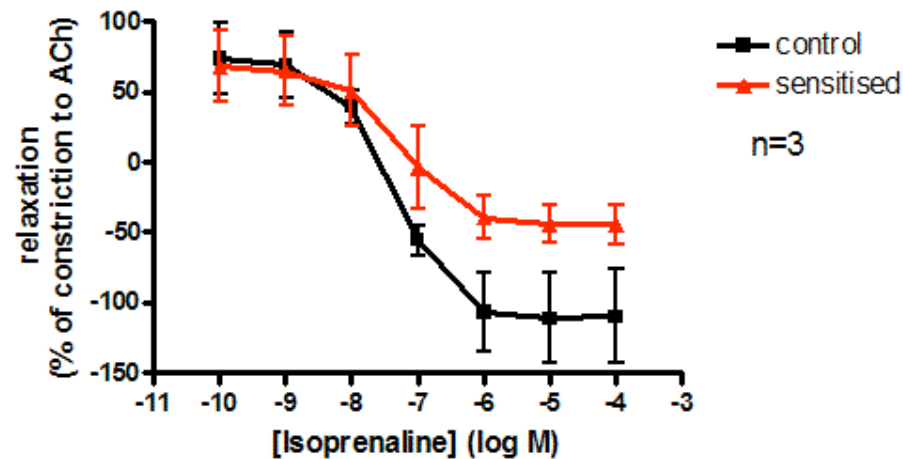
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In vitro allergy models



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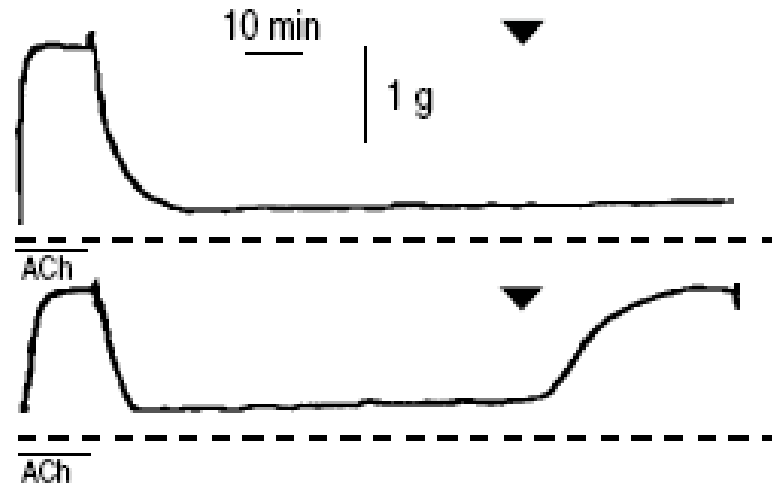
Effect of IgE sensitisation on relaxations responses of isolated airways



Isolated human airways respond to immunologic challenges in a similar way to the in vivo response

- Incubate with IgE, (associated with allergy)
- Reduces dilation to b-adrenergic agonist, isoprenaline

In vitro allergy models



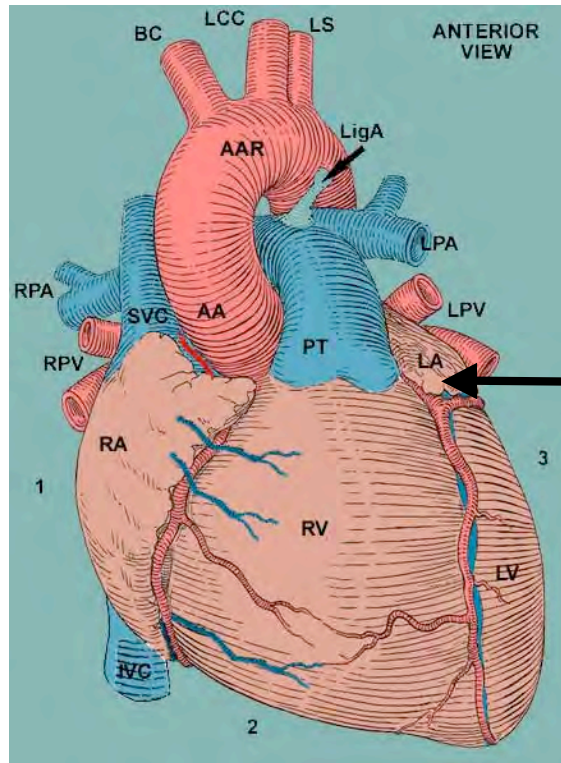
Control response followed by response to house dust mite

Airways from same patient incubated in normal (upper) or atopic (develops immediate allergic reaction because of presence of antibody) serum (lower)

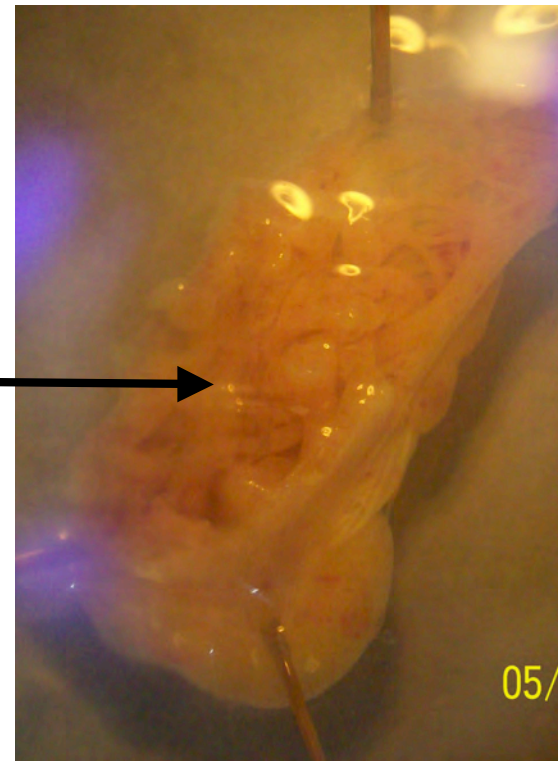
Exposure to atopic serum causes airway constriction

Atrial Appendages

- conical muscular pouch connected to atria of heart
- access trabeculae (round muscular columns)
- throughout whole inner surface of heart chambers
- similar inotropic and chronotropic characteristics



Left atrial appendage



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Cardiac Trabeculae

Isolated trabeculae
beat spontaneously
in physiological
solutions

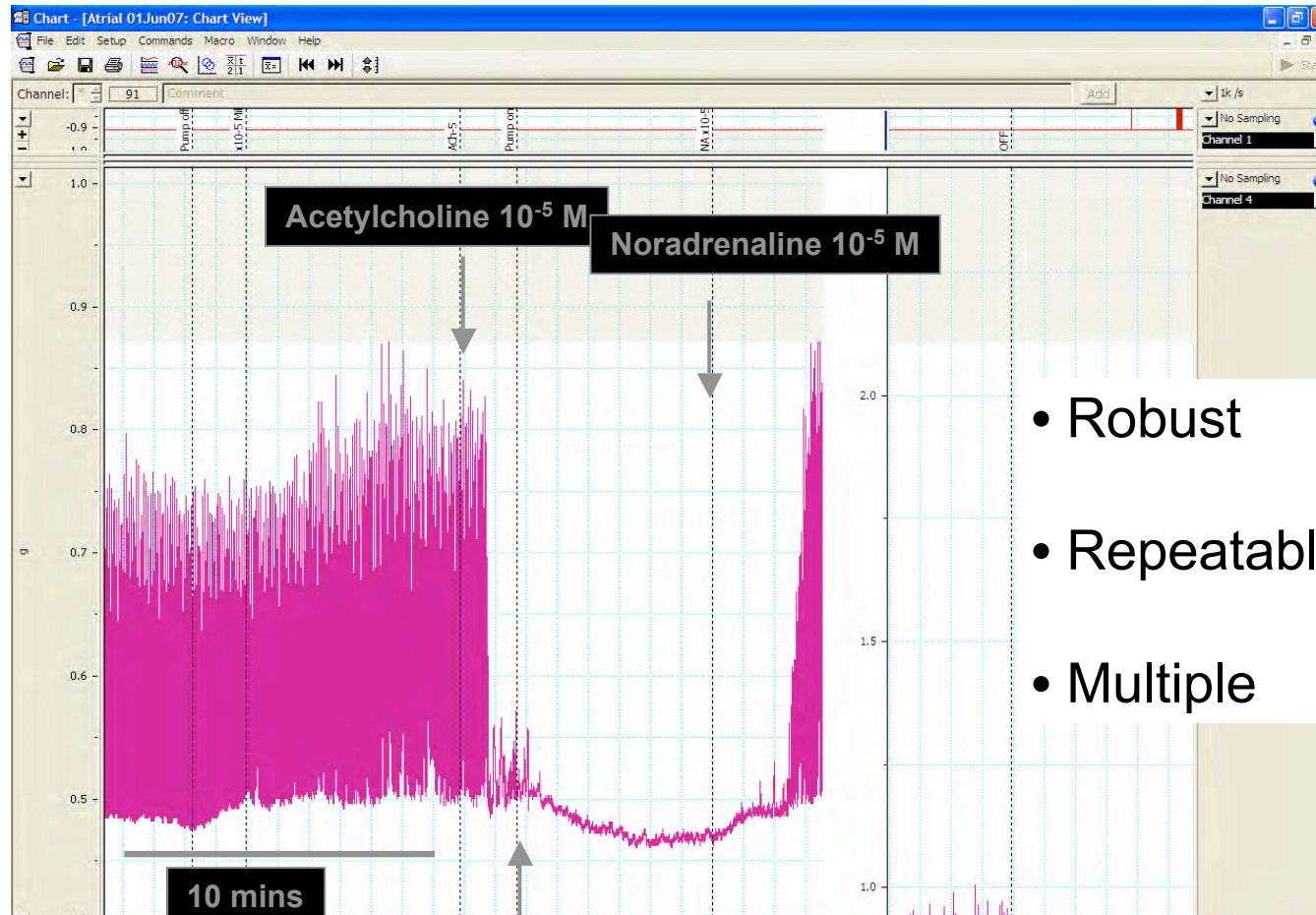
Measurements of
cardiac contractility
can be made

The tissues are also
abundant in coronary
micro-arteries



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Functional Assays of Cardiac Contractility



- Robust
- Repeatable
- Multiple

Identifies cardiac changes by test therapeutic similar to HERG test revealing cardiac arrhythmicity.

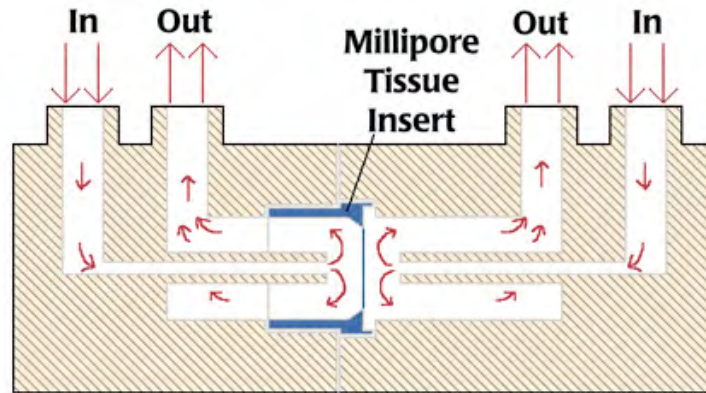
Tissue Sheets

- Skin
- Respiratory
- Gastrointestinal
- Glands
- Test compound added to apical surface
- Effects/absorption/secretion, measured at basolateral surface



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Skin



- Sealed system
- Active compound passed through epidermis & dermis
- Tissue engineering - 'Humanised' skin
- Fibroblasts & keratinocytes grown to produce skin sheet
- phenotype/morphology similar to human skin

Cosmetics

- Side effects from trans-epithelial drug transport
- Measures transport & diffusion
- Absorbance through mucous membrane
- Useful for skin application
- Cosmetics & toiletries as well as pharmaceuticals
- e.g. detailed assessment of Na^+/K^+ transport vital for antiperspirant medication



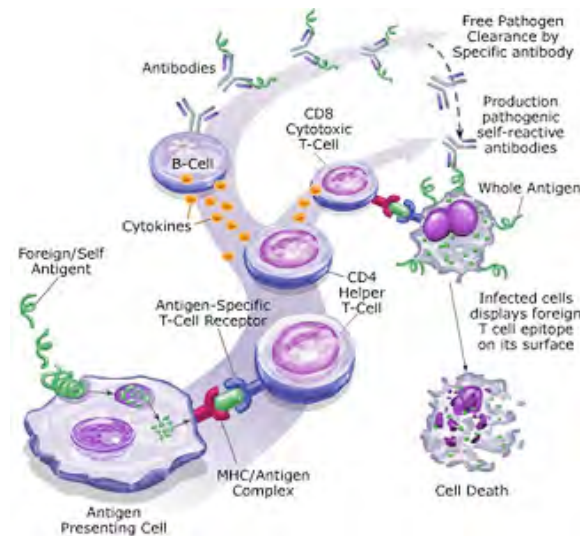
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Inflammation

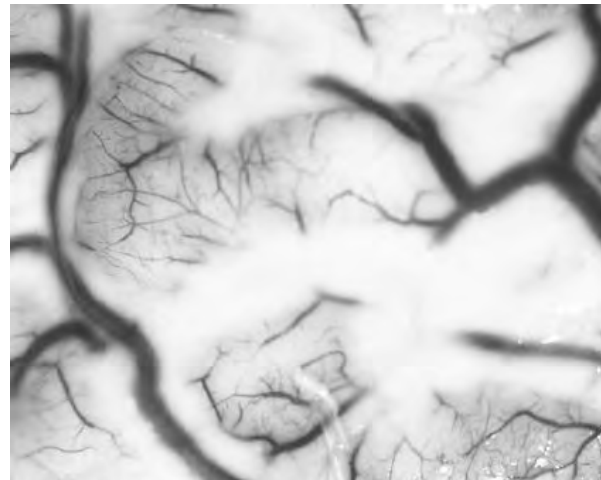
- Associated with delivery/formulation
- Rheumatoid arthritis, Crohn's, autoimmune diseases
- lung; skin; gut mucosa; synovium; cartilage; adipose tissues
- tonic changes (strain gauge)
- release of mediators (culture)
 - histamine, PGEs, cytokines (radioimmunoassay)

Selective inhibition of pro-inflammatory cytokines major strategy in anti-inflammatory drug development



Neural Tissue

- Functional human neural assays that correlate to Alzheimer's, Parkinson's, psychosis, depression etc.
- Brain slices: ~300-500 μ m
- Electrophysiology of neurons
- Stimulation electrodes
- Measure radiolabelled neurotransmitters
- Studies in hippocampus, hypothalamus & cortex but not commercially available
- Blood-Brain-Barrier



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- duration of action

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The Biopta Approach

