Drug Discovery Today • Volume 12, Numbers 9/10 • May 2007

Functional human tissue assays

Chris Hillier and David Bunton

Biopta Ltd, Weipers Centre, Garscube Estate, Bearsden Road, Glasgow G61 1QH, UK

Functional human tissue assays can be used to measure a vast range of physiological effects at the level of the organ, cell or even gene. In relation to drug discovery, such assays have been used in three main areas: discovery biology, *in vitro* efficacy pharmacology, and safety pharmacology. The most common area to which assays have been applied has been discovery biology, to investigate the mechanisms underlying a novel drug target or to validate that a target identified in a particular tissue is capable of eliciting a physiological response. Furthermore, as the available assays develop, they are considered an important adjunct to routine safety and efficacy pharmacology tests. Such approaches are often superior to extrapolation from animal data.

Introduction

Functional human tissue assays use fresh specimens of human tissue to measure the integrated physiological response following exposure to a drug. The intention is to prepare each tissue in such a way that the measurements made reflect one or more specific functions targeted by the test drug (Table 1). For example, vascular tissue can be used to screen an anti-hypertensive drug, or intestinal tissue can be used to screen drugs for inflammatory bowel disease. In a similar way, certain tissues are useful screening tools for the prediction of off-target effects, for example, cardiac cells can be used to screen for arrhythmic side effects caused by an anti-inflammatory drug.

There is a common perception in industry that using animal tissues provides a more convenient tool than using human tissues. The latter are considered difficult to obtain, of poor quality and difficult to standardize, and it is this perception that has hampered the universal adoption of human tissue assays by industry [1,2]. However, animal tissue is often proven to be a poor predictor of the human response to drugs [3]. In reality, the preclinical phase does not always accurately predict the outcome of clinical trials. Indeed, a series of late-stage failures, post-registration withdrawals, and declining discovery pipelines has highlighted the over-reliance on animal studies and the lack of quality human-derived data. Furthermore, there is growing evidence that human tissue testing can provide a reliable, sensitive, convenient and economic alternative.

Corresponding author: Bunton, D. (davidbunton@biopta.com)

Vascular studies

The most commonly studied human tissue is vascular tissue. Large blood vessels become available following major surgery, for example, limb amputations or organ removal; and veins are commonly available from removal for varicosities. Cardiac blood vessels are of major interest but their supply is dependent upon the transplant program; therefore, they tend to be difficult to source. Small cardiac vessels can be obtained from the atrial tip, removed to permit access to cannulas during cardiac surgery that requires bypass. The smallest capillary-sized blood vessels from neural tissue, which are extremely valuable for studies concerning the blood–brain barrier, are also difficult to source.

With respect to drug discovery, smaller vessels are of greatest interest. Tiny arteries, between 150 μm and 300 μm in diameter, are the primary regulator of blood flow to all organs [4]. These are ubiquitous and can be obtained in sufficient numbers from small biopsies of tissue from a range of organs, including the skin, kidneys, mucosa, heart and skeletal muscle [5–7].

Methods and apparatus for the study of isolated human blood vessels

Functional vascular assays usually require blood vessels to be assessed as ring preparations of a few millimetres, by means of either 'strain gauge' or 'pressure–flow' methodologies. The strain gauge system (Figure 1) uses two metal wires, placed through the lumen of the vascular ring, that are fixed to a micrometer and a

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2007.03.012

TABLE 1

Methods, tissue types and primary measured outputs from the most common functional human tissue assays

Tissue type	Preparation	Methods	Primary measurement
Blood vessels	Isolated arterial strips and ring segments	Organ bath and/or myography	Contractility
Cardiac	Purkinje fibres Atrial appendage	Electrophysiology Organ baths	Behaviour of ion channels Contractility
Respiratory	Lung slices Isolated airways	Perfusion system Organ baths	Release of local signalling factors Contractility
Neural	Brain slices	Electrophysiology	Behaviour of ion channels and transmitter-gated channels
Uterus	Isolated smooth muscle strips	Organ bath	Contractility
Gastrointestinal mucosa	Mucosal sheet	Ussing chamber Organoculture	Membrane transport Release of cytokines
Skin	Intact epidermis and dermis Culture of full-thickness skin	Franz diffusion cell Organoculture	Transport of substances through the skin Release of local factors, such as cytokines and growth factors
Adipose tissue	Intact adipose tissue	Organoculture	Release of adipokines and cytokines

transducer. The wires are able to transmit changes in force to a transducer when the walls of the vessel pull on them following drug application. The tissues, submerged in physiological solution, remain functional for up to two weeks (for some responses) if oxygenated at 37 °C; however, in most cases, experiments last no more than 8–12 h. The technique is robust and reliable, but important differences exist between this assay and the *in vivo* scenario. The isolation of the artery dissects the nerves; therefore, the central neural influence is abolished. A measure of sympathetic drive can, however, be assessed by using field-stimulation, where the blood vessel is placed between two platinum or silver plates, through which a small voltage is applied. The resulting electrical field stimulates the sympathetic nerves on the blood vessel to release their catecholamine stores, producing a measurable vasoconstriction response.

A pressure-flow system is an alternative methodology, where the vascular tube is cannulated at each end, enabling fluid to be perfused at physiological levels. In this assay, the transducers measure pressure and flow rather than force. The technique also uses imaging microscopy to measure changes in the lumen diameter that correlate directly with vasoconstriction or relaxation (Figure 2).

The choice of whether to choose a strain gauge test or a pressure-flow system depends on the level of sensitivity required. The pressure-flow systems offer up to ten times more sensitivity than the strain gauge. For higher throughput, the strain gauge is preferred because cannulation of small vessels is technically difficult and therefore limits the number of arteries that can be studied simultaneously. Consequently, to acquire the most sensitive physiological data, one might need to sacrifice throughput by using the perfusion method, which more accurately mimics the *in vivo* environment. Strain gauges also alter the natural orientation and environment of the tested tissue, whereas pressure-flow systems are much less disruptive.

Isolated human blood vessels and drug discovery

The strength of using isolated human blood vessels in discovery biology is evidenced by achievements including the identification of the vascular endothelium as a major modulator of vascular function [8], and the discovery of the nitric oxide pathway [9], the endothelin pathway [10], vascular endothelial growth factor [11] and endothelium-derived hyperpolarizing factor [12].

In drug discovery, one of the major applications of these assays is the prediction of off-target effects that can lead to hypotension during clinical use. Blood vessels sourced from within the same tissue produce similar responses but there can be differences in receptor populations and functional pathways in blood vessels from different tissues. This means that several vascular beds should be screened to compile a full vascular screen for the purposes of predicting blood pressure changes. The skin, gut and skeletal muscle together represent over 90% of blood flow in the body; therefore, the screening of small arteries from these three tissues constitutes a comprehensive screen.

Up to twenty small arteries can be obtained from a single skin biopsy; therefore volunteer 'control' groups can be studied in addition to patients. This enables direct *in vitro* clinical trials-type comparisons during preclinical or clinical phases. These studies can reveal small changes that could not be observed otherwise, and would not be revealed by short-term observation during clinical trials. Small, measurable effects that are repeated and accumulated throughout the body in a chronic manner could feasibly produce the kind of serious adverse responses that can result in post-registration drug withdrawal.

Angiogenesis, the process of new blood vessel formation, is involved in several disease processes, including tumour growth, gastrointestinal disorders and macular degeneration [13,14]. In cancer, therapeutic strategies are being developed for anti-angiogenic purposes, to prevent blood flow to a growing tumour, and for pro-angiogenic purposes, to enhance the availability of cytotoxic drugs to tumour cells [15]. Tumour tissue is readily available from most surgical removal procedures, although this is highly sought-after; therefore, commercial and academic researchers must often compete for the available tissues.

A different assay can predict the responses of tissue to angiogenic drugs following the manipulation of growth-promoting factors, including VEGF, FGF-2, PDGF and TGF-β. These assays use 2 mm sections of explant blood vessel placed into a 96-well culture plate. Nutrients, endothelial growth media and antibiotics are applied before storage in a humidified incubator. Each segment is then tested with different concentrations of a test

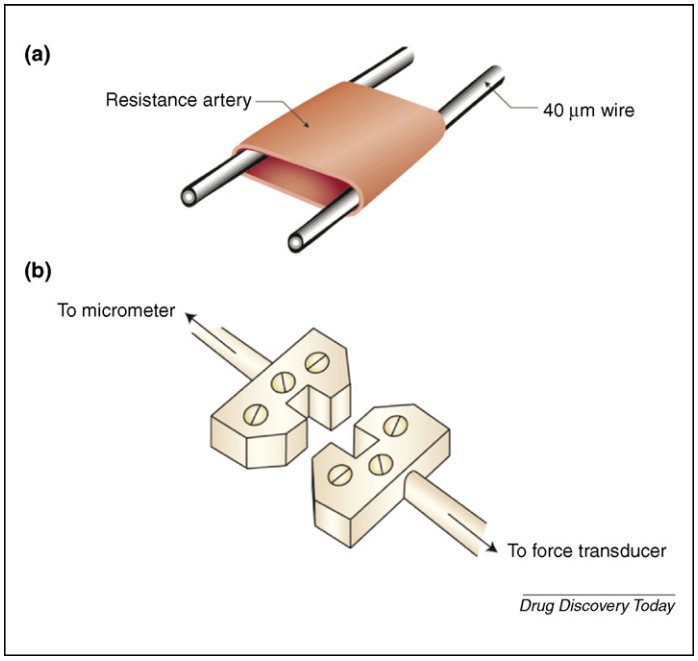

FIGURE 1

Strain gauge systems have been developed for the detection of minute (milli- and micro-Newton) forces generated by contractile tissues. **(a)** An isolated resistance artery with two 40 μm diameter wires placed through its lumen. The wires are then attached to the strain gauge by the device shown in **(b)**, where the wires are connected to two metal 'jaws'. One jaw produces deflections of the strain gauge, whereas the other is connected to a micrometer that enables the resting stretch on the tissue to be controlled.

anti-angiogenic compound as well as both negative and positive controls (e.g. heparin–steroid treatment). The vessel ring cultures are then examined at various time-points, up to two weeks, for endothelial 'sprouting', using light microscopy [16].

These assays were applied for target selection and lead-optimization studies, and have proved to be a valuable adjunct to the primary discovery pathway.

Respiratory studies

Respiratory tissue assays are technically extremely difficult to set up *in vitro* and, therefore, can only be studied by trained technicians. Respiratory tissue is easily damaged, and handling must be precise and gentle. Moreover, despite the large number of surgical resections that take place, there is still a shortage of lung tissue available. Lung tissue is most often removed for the treatment of lung cancer; and there is a responsibility on the pathologist to confirm the identity of the specific tumour type present. Tumour-type identification usually involves inflating the lung or lobe with formalin, to fix the tissue in preparation for histological sectioning, which renders the tissue useless for organ bath studies.

Functional assays using lung tissue involve dissecting bronchial rings or thin strips of parenchymal tissue. To counter the delicate nature of these tissues, the techniques used for blood vessels have been adapted to enable sensitive studies in small smooth muscle airways [17,18].

Thin strips of parenchyma, which appear to be free of blood vessels or bronchi upon gross examination, can be dissected free from macroscopically normal fresh human lung tissue and used as an isolated tissue preparation. Lung parenchymal tissue has contractile properties, and strips of it can be mounted in an organ bath for the measurement of contraction through force generation, using a modified strain gauge technique. These assays provide insights into bronchoconstriction or dilation effects [19] in much the same way that the observation of changes in arterial tone elucidates probable blood pressure changes. Isolated bronchial rings dissected within 24 h of surgery retain the ability to respond to nerve stimulation [20] and can generate an IgE-mediated sensitization of the responses to bronchoconstrictors (Figure 3) [21]. This makes them a useful model for atopic asthma.

Lungs can also be filled with agarose to allow slices to be prepared. Slices that contain airways in profile, but which do not contain agarose and contain beating cilia are chosen in a pre-screening stage. The slices containing these functional sections of airway are then cultured under a video microscope, and changes in airway diameter are assessed using video microscopy. It is a distinct advantage that this methodology retains the normal anatomical relationships and structures [22,23].

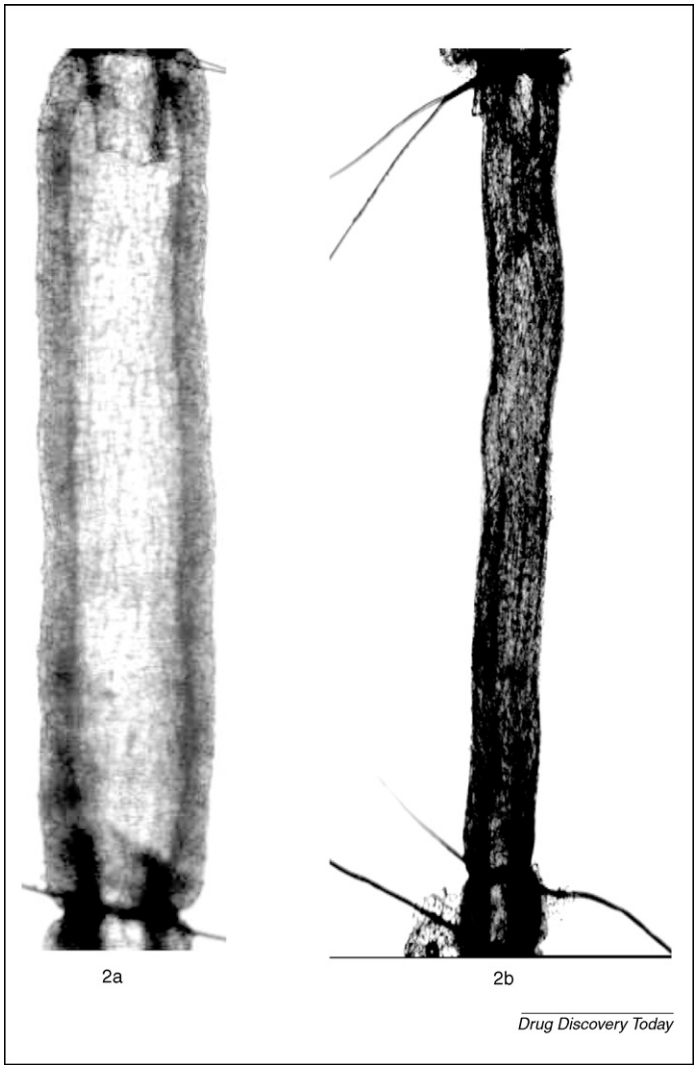

FIGURE 2

An isolated resistance artery in its passive state **(a)**, with approximately 175 μm diameter, and following agonist exposure in its fully activated state **(b)**. Reproduced with permission from [48].

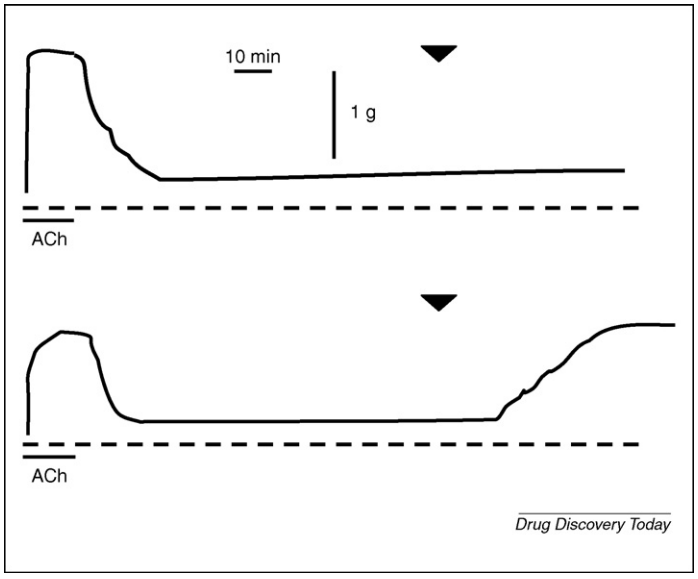

FIGURE 3

Examples of contractile responses to immunological stimuli in human isolated bronchi following a maximal contraction to acetylcholine (1 mM). Response of a pair of airways (from the same patient) to *Dermatophagoides pteronyssinus* (house-dust mite). The airways are incubated in normal serum (upper trace) or atopic serum (lower trace). Exposure of the isolated airway to atopic serum causes the tissue to constrict in response to *D. pteronyssinus exposure*. Reproduced from [21] with permission of the European Respriratory Society.

Lung assays provide useful screens for compounds that are designed to produce changes in airway tone; they can also act as a screen to rule out potential lung off-target effects for therapeutics targeting other tissues that share similar pathways. They are also useful for inflammation studies, where the mode of delivery is likely to be inhalation.

Studies on cardiac tissue

Cardiac tissue is one of the most difficult human tissues to source. Human cardiac ventricular muscle is donated following cardiac resection, transplant or post-mortem, whereas atrial tissue is more frequently available from atrial appendage removal during cardiac catheterization [24]. Cardiac tissue requires specialized dissection and handling techniques to maintain its unique electrical properties and needs to be used rapidly following removal from the patient, ideally within minutes, or be placed in an 'arresting' solution to reduce metabolic activity.

For assaying, cardiac tissue is cut into strips that are placed in an organ bath and stimulated using platinum or silver electrodes. Test drugs are added, and the resulting changes in cardiac muscle contractility recorded. Electrical activity recording is a read-out of the effect of drugs on particular areas of isolated cardiac segments. Despite being developed primarily for diagnostic purposes, advanced imaging techniques, including NMR [25], radiolabeled ligands [26], magnetic resonance imaging (MRI) [27], positron-emission topography (PET) and microPET [28], are proving useful for *in vitro* tissue assessment because they are able to identify and image ligands, receptors and their interactions precisely, thereby providing multiple read-outs from single, isolated tissues. In addition, these techniques are able to provide information on discrete metabolic changes that are impossible to identify by other methodologies. For example, in isolated atrial appendages, NMR was used to measure intracellular ATP and pH, while force recordings were simultaneously captured using a strain gauge [25]. However, such methods require significant capital investment and, therefore, are still not commonly available.

These functional assays of cardiac contractility can offer an insight into potential cardiac changes caused by a test therapeutic in much the same way that HERG (human *ether a-go-go*-related gene) assessment provides a screen that reveals potential cardiac arrhythmias.

Membrane transport

Membrane transport assays are carried out on skin, respiratory and gastrointestinal epithelium and glandular tissues. The availability of skin and lung tissue has been discussed above. Tissue from the colon and rectum are readily available following surgical resection for cancer and chronic inflammatory conditions; however, upper-tract tissues, such as duodenum and stomach, are more difficult to source.

Tissue sheets are analysed using specialized tissue culture techniques, whereby a test compound is added to the apical surface, and its effects (e.g. absorption or secretion) are measured at the basolateral surface.

Human skin, the most abundant residual fresh tissue available from surgery, is used in several assays. In tests using full-thickness skin, a diffusion cell (most commonly a Franz cell) is used to create a sealed system where drugs can be applied to a suspended portion of tissue, with the active compound passing through the dermis. This technique is limited, however, to the use of, essentially, dead skin; this has led to modified versions that use fresh functional full-thickness skin in a modified organ culture system, which enables the simultaneous measurement of functional changes such as cytokine and matrix metalloprotein (MMP) production [29].

In addition to fresh human skin, engineered 'humanized' skin, where primary human fibroblasts and keratinocytes are grown together to produce an organotypic sheet of human skin, is now commercially available for the testing of permeability, using the Franz cell method [30]. Whether the engineered skin possesses all of the phenotypic characteristics required is not yet clear; however, the morphology and phenotype seem to be similar to actual human skin.

The Ussing chamber, a 40-year-old methodology, is currently proving extremely useful in human tissue *in vitro* drug testing using membranes [31]. Skin or gastrointestinal mucosa, obtained from resection owing to gastrointestinal tumours or inflammatory bowel disease, is most frequently studied using this assay. The Ussing method consists of two chambers, which lie on either side of each other and are separated by a small insert, in which the test tissue is placed. Electrodes for voltage and current record the potential gradient between the two chambers, which provides important information on potential side effects from the transepithelial transport of drugs.

Membrane-type assays are useful measures of biochemical changes, particularly those involving transport and diffusion, and are thus useful for testing drugs that require application on the skin or absorbance through a mucous membrane. The ability to make detailed assessment of Na^+/K^+ balance is vitally important for testing antiperspirant medication, and the assessment of skin

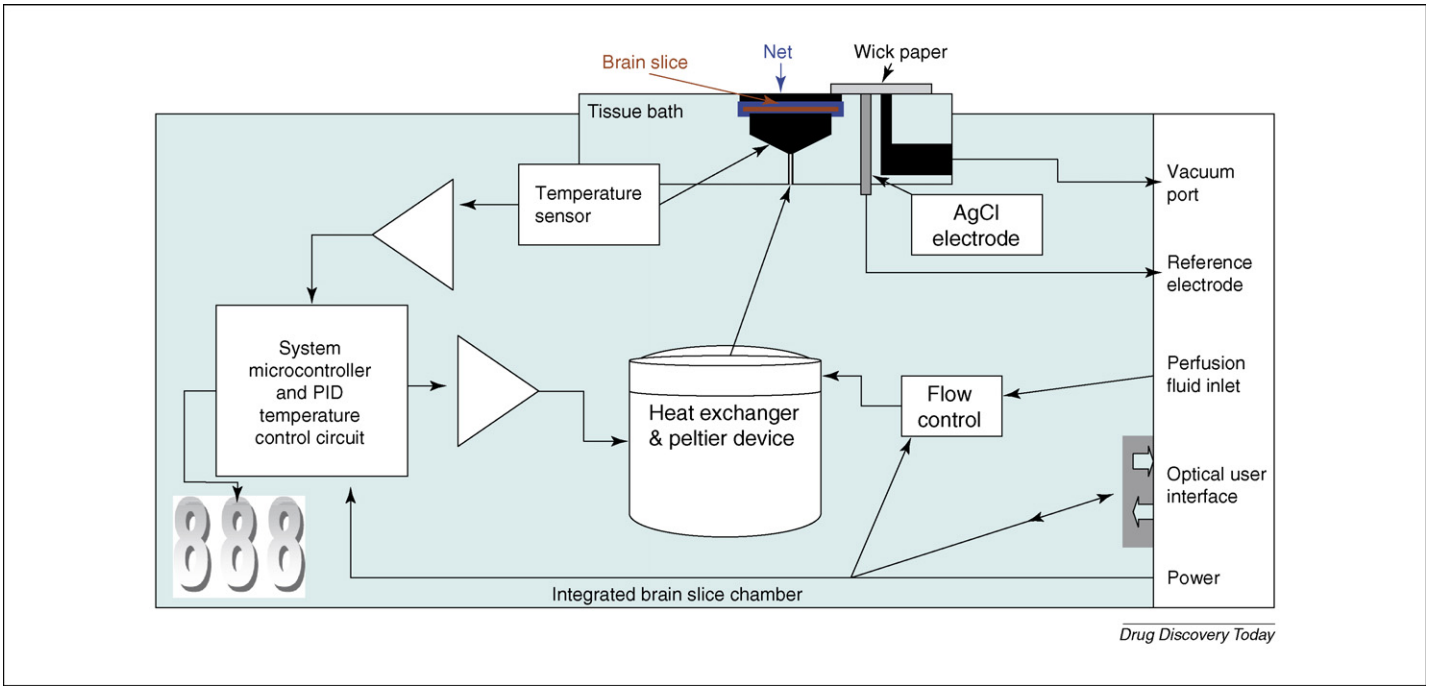

FIGURE 4

Schematic diagram of the integrated brain slice chamber. The integrated brain slice chamber (IBSC) integrates the slice chamber, control electronics, display, flow control and user interface within one unit. The tissue bath incorporates a vacuum suction port, integrated reference electrode, isolated temperature sensor and quick connect–disconnect contacts. The design ensures system variables are minimized, providing an identical and independent environment for each brain slice. Reproduced with permission from [34].

application is used as much by companies developing cosmetics and toiletries as by those developing pharmaceuticals [32].

Neural tissue

Probably the most difficult human tissue to obtain functional data from *in vitro* is neural tissue. This is from the perspective of supply (residual brain tissue is available from surgery for drug-resistant epilepsy or tumour resections) as well as from the perspective of technical difficulty in preparation; however, some *in vitro* functional bioassays are available. In these assays, brain slices of ~300–500 μm, which are constantly perfused with specialized media within incubation chambers (Figure 4), are used to take electrophysiological measurements of synaptic activity following pharmacological or physical stimuli [33,34]. With the help of an imaging device, a carbon-fibre, glass or metal electrode is carefully positioned within the slice for direct recording of neuronal activity. Stimulation electrodes can also be used to activate afferent or efferent pathways, and measurement of the subsequent neurotransmitter release can be made by pre-incubation of the tissue slice with a radiolabelled neurotransmitter [33]. Despite the vulnerability of human neural tissue to time-dependent degeneration, due to its high metabolic activity, studies on neuronal behaviour in the hippocampus, hypothalamus and cortex have successfully been conducted. Although there are several bespoke brain slice assays in use in academic institutions, these are generally not commercially useful; however, Stopps *et al.* [34] have recently made advances in this area by developing a system that enables electrophysiological recordings from multiple brain slices (Figure 4). Careful preparation and perfusion of the slices means that they can remain viable for long enough (>3 h) to investigate synaptic plasticity, although this has, to date, been conducted in brain tissue from mice [34]. Finding functional human tissue assays that correlate to diseases such as Alzheimer's, Parkinson's, psychosis or depression has become a major target and, as such, brain slice experiments are a valuable tool for discovery biology in the central nervous system.

Inflammation

The study of inflammatory processes can identify and elucidate targets involved in diseases such as rheumatoid arthritis, Crohn's disease and autoimmune diseases. It can also help to understand the inflammatory responses associated with delivery and formulation problems.

The inflammatory process begins with the release of mediators such as histamine, prostaglandins and cytokines from the affected tissues; therefore, selective inhibition of pro-inflammatory cytokine expression is a major strategy in anti-inflammatory drug development.

Many of the tissues we have already discussed are used for inflammation studies. The tissues used most are lung, skin, gut mucosa, synovium, cartilage and adipose tissues. Skin and adipose tissue are removed regularly for cosmetic surgeries, and joint replacements provide synovium and cartilage.

Any of these tissues can be studied using organ culture systems, designed to measure changes in the production and release of the inflammatory mediators following incubation with a test substance. Because injured tissue itself is a producer of many of these inflammatory mediators, careful handling and preparation of tissue is important, and validated controls are vital for the correct interpretation of results. In brief, the tissue segments are cultured in tissue-specific media containing antibiotics, and the media is assayed for the molecule of interest. Once validated, this system

enables test compounds to be applied, and cytokines, adipokines, [35], intracellular signaling [36], growth [37] and cell migration [38] can all be measured. The results are usually expressed according to the wet weight of the biopsy specimen (pg/mg tissue/ml culture medium), which correlates well with protein content of tissue homogenates. In addition to force generation, drug candidates have been assessed in lung tissue *in vitro* by measuring the release of cytokines, inflammatory mediators or metabolites, by radioimmunoassay from parenchymal strips incubated with a test substance [39]. These are compared with the responses in other important cellular components of respiratory tissues, such as mast cells or pulmonary epithelial tissue [40]. Because these methods preserve the normal behaviour and growth of primary human tissue, they provide a more relevant screening system than available artificial tissue models [41].

Tissue acquisition

Access to fresh human tissue can be difficult, and the costs, time and logistical problems that exist when using fresh tissue have not yet been overcome. The provision of surplus tissue for drug discovery is, correctly, of secondary importance to the surgeon or pathologist; therefore, there might be a conflict of interest between the pathologist and the researcher that ultimately limits the availability of fresh tissue for researchers and tissue suppliers.

On a positive note, the recent increase in the number of tissue banks, although primarily focused on creating banks of frozen or fixed tissues, might, in the near future, provide a more structured system for the supply of fresh tissues. Several commercial companies offer to supply processed, fixed or frozen human tissue; however, those seeking fresh human tissue for their research need to either create a bespoke tissue supply network or outsource the experimental work to a contract research organization (CRO) with a developed tissue network.

Consideration must also be given to the quality of tissue obtained. The available experimental time for individual tissues varies from organ to organ, and, in general, reflects the level of metabolic activity *in vivo*, that is, cardiac and brain tissues have the shortest 'experimental window' and therefore present the greatest difficulties when carrying out functional assays. In animals, cardiac tissue can be used within seconds or minutes of collection, whereas human cardiac tissue must be transported in an arrested state, to reduce metabolic activity and avoid degradation. The inevitable consequence of this is that the attrition rate of such experiments is higher in human than in animal tissues. Collection and transport procedures must therefore be optimized, and the research team must be flexible, to use tissue as and when it becomes available.

Several studies have examined the use of various transplant solutions for the preservation of tissue function; however, little improvement has been made on standard physiological solutions. Tissue $>2\ mm^3$ exceeds the limits of diffusion and, therefore, supplementary methods, such as continuous gassing, perfusion or cooling at $\sim 4\ ^{\circ}C$, are often necessary. Recent innovations, such as artificial haemoglobin and haemoglobin cross-linked to superoxide dismutase might help to prolong tissue function but this has not yet been demonstrated. Optimization of cryopreservation procedures for fresh tissues offers one further potential route for increasing the experimental window. Lung and vascular tissues retain many of their functional responses when thawed [42].

Regulatory issues

Human tissue provision and handling are subject to legislative control designed to protect the right of the donor to decide whether their own tissue is destroyed, stored or used for medical research. In Europe, the current prohibition of animal testing for cosmetics, toiletries and household products [43], and the prohibition of *in vivo* genotoxicity tests for cosmetics ingredients due in 2009, have been powerful drivers in the uptake of alternative methodologies using human tissue. EU legislation provides guidance on the standards for quality and safety surrounding all aspects of human tissue use, including donation, procurement, testing, processing, preservation, storage and distribution of all human tissues and cells, excluding blood (which is currently covered by other legislation). In the UK, the Human Tissue Authority (HTA) and Human Fertilisation and Embryology Authority (HFEA) act as the national authorities responsible for implementing the EU Directive. The use of post-mortem tissue is the focus of the Human Tissue Act [44], which although still broadly covering surgical samples from living donors, delegates the responsibility for surgical tissue control to the NHS and individual biobanks. In the US, both the NIH and the FDA provide guidelines for researchers wishing to use human tissue; however, these are, again, broadly defined and in the case of the NIH, relate only to NIH-funded researchers [45–47]. Therefore, both the EU and the US are working towards developing a monitored and highly structured framework for all participants working with human tissue for whatever purpose; however, increased supply networks and greater commercialization of the methods developed within academia is required before functional human tissue assays will become a routine part of drug discovery.

Summary

Functional *in vitro* assays can define efficacy and safety at defined targets in both healthy and diseased human tissues. The use of fresh tissues by the pharmaceutical and biotech industries has been sporadic and has taken place on a project-specific basis, usually during the discovery stage rather than during safety pharmacology studies, for example, through the use of isolated coronary arteries or cardiac muscle. However, the recent increase in demand has driven the development of better instruments and more suitable approaches, and improved organ culture methods for isolated tissues or organs are making these methodologies more attractive to drug developers.

References

1 Soldin, S.J. (1992) Drug receptor assays: *quo vadis*? *Ann. Clin. Biochem.* 29, 132–136

2 Garratini, S. (1985) Toxic effects of chemicals: difficulties in extrapolating data from animals to man. *Annu Rev Toxicol Pharmacol* 16, 1–29

3 Olson, H. *et al.* (1998) The predictivity of the toxicity of pharmaceuticals in humans from animal data – an interim assessment. *Toxicol. Lett.* 102–103, 535–538

4 Halpern, W. *et al.* (1978) Mechanical properties of smooth muscle cells in the walls of arterial resistance vessels. *J. Physiol.* 275, 85–101

5 Buus, N.H. *et al.* (2000) Nitric oxide, prostanoid and non-NO, non-prostanoid involvement in acetylcholine relaxation of isolated human small arteries. *Br. J. Pharmacol.* 129, 184–192

6 Johannssen, V. *et al.* (1997) Alpha 1-receptors at pre-capillary resistance vessels of the human nasal mucosa. *Rhinology* 35, 161–165

7 Mulvany, M.J. *et al.* (1990) Structure and function of small arteries. *Physiol. Rev.* 70, 921–961

8 Furchgott, R.F. *et al.* (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288, 373–376

9 Palmer, R.M. *et al.* (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327, 524–526

10 Yanagisawa, M. *et al.* (1988) A novel peptide vasoconstrictor, endothelin, is produced by vascular endothelium and modulates smooth muscle Ca^{2+} channels. *J. Hypertens.* 6, S188–S191

11 Sagar, I.K. *et al.* (1981) The role of trace elements and phosphates in the synthesis of vascular-permeability factor by *Vibrio cholerae*. *J. Med. Microbiol.* 14, 243–250

12 Taylor, S.G. *et al.* (1988) Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol. Sci.* 9, 272–274

13 Danese, S. (2006) Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. *Gastroenterology* 130, 2060–2073

14 Orosz, K.E. *et al.* (2004) Delivery of antiangiogenic and antioxidant drugs of ophthalmic interest through a nanoporous inorganic filter. *Mol. Vis.* 18, 555–565

15 Phung, M.W. *et al.* (2006) *In vitro* and *in vivo* assays for angiogenesis – modulating drug discovery and development. *J. Pharm. Pharmacol.* 58, 153–160

16 Jung, S.P. *et al.* (2003) Effect of human angiostatin protein on human angiogenesis *in vitro*. *Angiogenesis* 6, 233–240

17 de Jongste, J.C. *et al.* (1987) Comparison of human bronchiolar smooth muscle responsiveness *in vitro* with histological signs of inflammation. *Thorax* 42, 870–876

18 Hulsmann, A.R. *et al.* (1993) Studies of human airways *in vitro*: a review of the methodology. *J. Pharmacol. Toxicol. Methods* 30, 117–132

19 Armour, C.L. *et al.* (1985) The lung parenchymal strip as a model of peripheral airway responsiveness. *Bull. Eur. Physiopathol. Respir.* 21, 545–549

20 Back, M. *et al.* (2006) Effect of cold storage on cholinergic responses induced by electrical field stimulation in human bronchi. *Pulm. Pharmacol. Ther.* 19, 297–302

21 Tunon de Lara, J.M. *et al.* (1995) IgE-induced passive sensitization of human isolated bronchi and lung mast cells. *Eur. Respir. J.* 8, 1861–1865

22 Opazo Saez, A.M. *et al.* (2000) Peripheral airway smooth muscle mechanics in obstructive airways disease. *Am. J. Respir. Crit. Care Med.* 161, 910–917

23 Finney, M.J. *et al.* (1985) Effects of bronchoconstrictors and bronchodilators on a novel human small airway preparation. *Br. J. Pharmacol.* 85, 29–36

24 Dow, J.W. *et al.* (1981) Isolated cardiac myocytes. I. Preparation of adult myocytes and their homology with the intact tissue. *Cardiovasc. Res.* 15, 483–514

25 Lareau, S. *et al.* (1991) An NMR probe to study function and metabolism simultaneously in isolated human cardiac tissue. *Magn. Reson. Med.* 20, 312–318

26 Knapp, F.F. *et al.* (1997) Radiolabeled ligands for imaging the muscarinic–cholinergic receptors of the heart and brain. *Anticancer Res.* 17, 1559–1572

27 Koretsky, A.P. (2002) Functional assessment of tissues with magnetic resonance imaging. *(Repar Med). Ann. N. Y. Acad. Sci.* 961, 203–205

28 Johnstrom, P. *et al.* (2006) Imaging endothelin ET(B) receptors using [^{18}F]-BQ3020: *in vitro* characterization and positron emission tomography (microPET). *Exp. Biol. Med.* 231, 736–740

29 Companjen, A.R. *et al.* (2001) A modified *ex vivo* skin organ culture system for functional studies. *Arch. Dermatol. Res.* 293, 184–190

30 Ottaviani, G. *et al.* (2006) Parallel artificial membrane permeability assay: a new membrane for the fast prediction of passive human skin permeability. *J. Med. Chem.* 49, 3948–3954

31 Misfeldt, D.S. *et al.* (1976) Transepithelial transport in cell culture. *Proc. Natl. Acad. Sci. U. S. A.* 73, 1212–1216

32 Clunes, M.T. *et al.* (2004) Localisation of the vacuolar proton pump (V-H^+-ATPase) and carbonic anhydrase II in the human eccrine sweat gland. *J. Mol. Histol.* 35, 339–345

33 Freiman, T.M. *et al.* (2006) K(+)-evoked [(3)H]-norepinephrine release in human brain slices from epileptic and non-epileptic patients is differentially modulated by gabapentin and pinacidil. *Neurosci. Res.* 55, 204–210

34 Stopps, M. *et al.* (2004) Design and application of a novel brain slice system that permits independent electrophysiological recordings from multiple slices. *J. Neurosci. Methods* 132, 137–148

35 Lappas, M. *et al.* (2005) Leptin and adiponectin stimulate the release of proinflammatory cytokines and prostaglandins from human placenta and maternal adipose tissue via nuclear factor-kappaB, peroxisomal proliferator-activated receptor-gamma and extracellularly regulated kinase 1/2. *Endocrinology* 146, 3334–3342

36 Trujillo, M.E. *et al.* (2006) Tumour necrosis factor alpha and glucocorticoid synergistically increase leptin production in human adipose tissue: role for p38 mitogen-activated protein kinase. *J. Clin. Endocrinol. Metab.* 91, 1484–1490

37 Lateef, H. *et al.* (2004) All-*trans*-retinoic acid suppresses matrix metalloproteinase activity and increases collagen synthesis in diabetic human skin in organ culture. *Am. J. Pathol.* 165, 167–174

38 de Gruijl, T.D. *et al.* (2006) A postmigrational switch among skin-derived dendritic cells to a macrophage-like phenotype is predetermined by the intracutaneous cytokine balance. *J. Immunol.* 176, 7232–7242

39 Ghelani, A.M. *et al.* (1980) Response of human isolated bronchial and lung parenchymal strips to SRS-A and other mediators of asthmatic bronchospasm. *Br. J. Pharmacol.* 71, 107–112

40 Peters, S.P. *et al.* (1982) Dispersed human lung mast cells. Pharmacologic aspects and comparison with human lung tissue fragments. *Am. Rev. Respir. Dis.* 126, 1034–1039

41 Allen, D.D. *et al.* (2005) Cell lines as *in vitro* models for drug screening and toxicity studies. *Drug Dev. Ind. Pharm.* 31, 757–768

42 Muller-Schweinitzer, E. and Fozard, J.R. (1997) SCA 40: studies of the relaxant effects on cryopreserved airway and vascular smooth muscle. *Br. J. Pharmacol.* 120, 1241–1248

43 Pauwels, M. (2004) Safety evaluation of cosmetics in the EU. Reality and challenges for the toxicologist. *Toxicol. Lett.* 151, 7–17

44 Office of Public Sector Information. *Human Tissue Act 2004*, HMSO (http://www.opsi.gov.uk/acts/acts2004/20040030.htm)

45 Food and Drug Administration, Department of Health and Human Services (1991) Code of Federal Regulations, Title 45 *Public Welfare,* Part 46 US Food and Drug Administration (FDA) *Protection of Human Subjects* (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=50)

46 Office for Human Research Protections (2004). *Human Subject Regulations Decision Charts* (http://www.hhs.gov/ohrp/humansubjects/guidance/decisioncharts.htm)

47 National Bioethics Advisory Commission, (2001) *Ethical and Policy Issues in Research Involving Human Subjects*. NBAC In: http://www.bioethics.gov

48 Coats, P. and Hillier, C. (2000) Differential responses in human subcutaneous and skeletal muscle vascular beds to critical limb ischaemia. *Eur. J. Vasc. Endovasc. Surg.* 19, 387–395