# Could Fresh Human Tissues Play a Key Role in Drug Development?

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**Summary** — Biopta was founded in 2002, to provide human tissue-based drug development and testing services to the pharmaceutical industry. Although animal tissues are readily available and are relatively inexpensive, they frequently fail to faithfully predict the results seen in the clinic. Human tissues can provide integrated responses to test drugs in a manner more representative than individual cell types or cell lines alone, and more-directly relevant to the species of interest — *Homo sapiens*. In order to expand the use of human tissues, however, an improved infrastructure for the collection and distribution of fresh, functional tissues is highly desirable. Moreover, where there is the potential to obtain tissue from various locations, it becomes possible to test tissue that is specific to the site of drug activity. This is important, as differences may occur between the same tissue types in different locations in the body. The detection of adverse effects is greatly helped by knowledge of how existing drugs behave in the human body. These drugs can act as reference compounds, so that new compounds can then be compared, by using standard concentration–response type studies, in a huge variety of tissues, and their effects extrapolated from what is known of the reference compounds.

**Key words:** *angiogenesis, biopsy, cardiac, HRT, human tissue, neural, respiratory, vascular, Vioxx®.*

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## Introduction

Biopta was founded in 2002, following in the footsteps of Pharmagene, the first company in the world to really focus on human tissue for drug discovery and development. The premise underlying Biopta was to commercialise the wide range of human tissue assays that were being developed and performed by academic units at Glasgow Caledonian University.

Animal tissue is the method of choice for most academic and commercial users, because it is convenient, inexpensive and readily obtained. However, Biopta's experience, particularly within the commercial sphere, is that it is generally a poor predictor of the human response, in some cases bearing no resemblance whatsoever to human-derived data. It is therefore not surprising that there has been much speculation about whether or not there is a correlation between an over-reliance on animal data and the current problems that the pharmaceutical industry is suffering, including late-stage failures, post-marketing withdrawals, and a decline in drug discovery pipelines.

Novel technologies that focus on human tissue-derived data, such as those developed by Biopta and others, are able to significantly assist in the lead selection process. Human tissues are ideally suited for use at this stage, as the responses obtained can help researchers to decide which of a group of leads is most likely to be successful in the clinic.

It is important to understand what is meant by human tissue. In this context, human tissue does not refer to cell lines, individual cell types, primary or secondary cells, but groups of different cell types together as a working tissue, e.g. an isolated blood vessel or airway. The interaction between different cell populations is critically important to the overall behaviour of the tissue and its response to drugs under test; therefore, an intact section of human cardiac muscle, whilst not as valuable as a whole organ, is clearly a more relevant test system than an isolated cardiomyocyte. Although data derived from isolated tissues cannot reveal everything about the whole organism, they can be much more useful than data from separate cell lines. Thus, Biopta can expose a tissue to a candidate drug and then measure the integrated response of

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all the cells working together, in order to find out whether the drug behaves as intended in human tissue, which is the ultimate goal. Biopta can perform a range of assays to assess efficacy and potency, and there is a growing range of different tissue types available. However, perhaps the most rapidly growing application of human tissues is in the field of safety pharmacology.

In the early days, Biopta focused on vascular and respiratory assays, and these remain a particular strength. Isolated blood vessels can be obtained after many types of surgery, but with respect to drug development and discovery, the smallest of these are of the greatest interest. Tiny arteries, between 150μm and 300μm in diameter, are the primary regulator of blood flow to all organs (1). These are ubiquitous, and can be obtained in sufficient numbers from very small biopsies of tissue, from a range of organs including the skin, kidneys, mucosa, heart and skeletal muscle (2–4).

The ability to use residual surgical tissues from various locations means that test blood vessels can often be used that are specific to the site of drug action. For example, Biopta is developing assays on very small blood vessels from neural tissue, for use in studies concerning the blood–brain barrier.

Major blood vessels are often the focus of investigative studies. However, the blood vessels in which Biopta is primarily interested are only about the diameter of a human hair. They are the major determinants of peripheral vascular resistance, and hence have a critical role in regulating blood pressure. In addition, specialised techniques are required to study such small blood vessels, and in order to achieve a sufficiently high-throughput to make human tissue tests a routine part of drug development, Biopta has developed expertise in miniaturised pharmacological techniques. These assays usually require blood vessels to be assessed as ring preparations of a few millimetres in length, by means of either 'strain-gauge' or 'pressure/flow' methodology. The strain-gauge system involves two metal wires, placed through the lumen of the vascular section, that are fixed to a micrometer and a transducer, which is able to measure changes in force as the walls of the vessel pull on the force transducer following drug application. The tissues are submerged in physiological solution, and can remain functional for up to 24 hours (for some responses), if kept at 37°C and oxygenated. The technique is robust and reliable; however, important differences exist between this assay and the *in vivo* scenario. The isolation of the artery involves dissection of the nerves, so central neural influence is abolished. A measure of sympathetic drive can be assessed, however, by using electrical 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, thus producing a measurable vasoconstriction response.

An example of the value of such a vascular assay comes from tests of hormone replacement therapy (HRT), which has an effect on the cardiovascular health of post-menopausal diabetic women. Both women with diabetes and post-menopausal women have independently increased cardiovascular risks. These factors act synergistically to create a greater risk in post-menopausal women with diabetes. Small biopsies of skin or residual skin and subcutaneous tissue from surgery are donated by patients; in this study biopsies were taken before and after the patients were given HRT (5). Tissue can also be obtained from age-matched subjects as controls. Our data showed that, in controls, a normal response is measured to the agonist acetylcholine, which essentially is a marker of vascular health. Prior to HRT, post-menopausal women with diabetes showed an impaired response to acetylcholine, as expected from information in the literature. However, results from the second post-HRT period, in the same patients, showed that it had markedly improved.

This example demonstrates the power of this technology, i.e. multiple biopsy data can be derived from the same patients or residual surgical tissues from groups of patients known to be taking the drug of interest. This type of study can even be extended to perform further longitudinal studies over, for example, a year or more. This may help to identify small chronic changes that are invisible in short-term clinical trials, but are revealed only after a drug has been marketed, as was recently seen with Vioxx® and other drugs. However, this type of study is time-consuming, and simpler, more-rapid tests using tissue residual to surgery are more commonplace, where no invasive biopsies are required, yet the current medication and medical status of the patient is known and patients can still be "recruited" to the project by the use of defined exclusion/inclusion criteria.

An alternative technique to the wire system is the perfusion/pressure system, in which the vascular tube is cannulated at each end, which allows fluid to be perfused through the vessel. In this case, the transducers do not measure force, but pressure, flow and changes in diameter or vascular permeability.

The choice of whether to choose a strain gauge test or a pressure/perfusion system depends on the level of sensitivity required. The pressure/perfusion systems offer up to ten times more sensitivity than the strain gauge, but for higher-throughput, the strain gauge test is preferable.

The strength of this approach in discovery biology is evidenced by many achievements, including: the identification of the vascular endothelium as a

major modulator of vascular function (6), and the discovery of the nitric oxide pathway (7), the endothelin pathway (8), vascular endothelial growth factor (9), and endothelium-derived hyperpolarising factor (10).

One of the major applications of these assays is the prediction of off-target effects that may lead to hypertension or 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 a number of vascular beds should ideally be screened to predict blood pressure changes. The skin, gut and skeletal muscle represent over 90% of the body's circulatory system, so the screening of small arteries from these areas constitutes a fairly comprehensive screen. Skin is the most easily accessible, and is also easy to use. Up to twenty small arteries can be obtained from a single skin sample obtained from cosmetic procedures, or in some instances, from biopsies, and both 'control' (healthy) and patient groups can be studied. These often allow direct *in vitro* 'clinical' trial-type comparisons to be made during the preclinical or clinical phases.

Vascular permeability is also of interest. *In vivo* measures of permeability currently involve injecting animals with a dye, then killing them and looking at the tissues to see how the dye has escaped from the vascular compartment. However, this can be done by using isolated blood vessels *in vitro*: the dye is injected through the lumen, then the leakage of the dye out of the cannulated vessel because of changes in endothelial tight junctions, can be imaged. This can be analysed and quantified both before and after the application of a drug, so a measure of how a compound alters vascular permeability can be obtained.

Angiogenesis, the process of new blood vessel formation, is involved in a number of disease processes, including tumour growth, gastrointestinal disorders, and macular degeneration (11, 12). 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 (13). Tumour tissue is readily available from surgery, although it is highly sought after by researchers.

There are two types of assay for angiogenesis. One involves the rapid and automated immunohistochemical identification of vascular endothelial cells in tissue slices, by using antibodies against von Willebrand factor, a specific marker of vascular endothelial cells. This methodology involves classical fixation, labelling, and imaging, but can now be carried out in a plate-based format that significantly increases throughput (14).

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-beta. These assays involve the use of thin, 2mm sections of explant tissue, which are placed into a 96-well culture plate. Each segment is then tested with different concentrations of an anti-angiogenic test compound, with both negative and positive controls. The organotypic cultures are then examined for endothelial 'sprouting' at various time-points, for up to two weeks, by using light microscopy (15). This type of study is well-suited to target selection and optimisation studies.

## Respiratory Tissue

Lung tissue is regularly removed in surgery, but much of it is required by pathologists for diagnosis. At this stage, it frequently undergoes processes which render it useless for live tissue studies, which limits its availability for that purpose. Working with the respiratory system is also technically difficult. It is a low pressure system with very fragile tissue, so special skills are required. The techniques used in blood vessel analysis are therefore altered and specifically designed for use with lung tissue.

Lung bronchial tissue has contractile properties, and can be tested as prepared strips or rings for measurement of force generation, by using a modified strain-gauge technique. The assays used provide insight into bronchoconstriction or dilation effects (16), in much the same way that the observation of changes in arterial tone elucidates likely blood pressure changes. Isolated bronchial rings, dissected within 24 hours of surgery, retain the ability to respond to nerve stimulation (17). They can generate an IgE-mediated sensitisation of the responses to bronchoconstrictors (18), which makes them a useful *in vitro* model for the study of allergies in patients with powerful allergic responses compared to control subjects. Initially, acetylcholine is applied, in order to measure the basic contraction response. Subsequently, one preparation is incubated in blood serum from a non-allergic individual, whilst another is incubated in serum from a patient with a hyper-immune response, before challenge with, say, house dust mites, resulting in constriction of the tissue incubated with serum from the asthma sufferer. Drugs can then be tested in an attempt to modulate this response.

Although lung assays provide useful screens for compounds designed to produce changes in airway tone, Biopta is often asked to do this kind of study for companies with no particular interest in respiratory physiology, but who need to know that there are no respiratory issues associated with their drug delivery methodology.

## Cardiac Tissue

The most readily-available source of cardiac tissue is the atrial appendage, which is often removed when a patient is connected to a perfusion machine during bypass surgery (19); however, ventricular muscle, which is of greater interest for safety pharmacology studies is also available through heart tissues not suitable for transplant. Cardiac tissue requires specialised dissection and handling techniques for the maintenance of its unique electrical properties, and needs to be used either extremely rapidly following removal from the patient, or to be placed in an 'arresting' solution to reduce metabolic activity. The trabeculae (round muscular columns that exist throughout the cardiac tissue), papillary muscles or strips from within the ventricles can be studied for their contractile and electrophysiological properties. They have similar inotropic (force of contraction), lusitropic (rate of relaxation) and chronotropic (rhythm) responses to drugs as the whole heart, except with the obvious loss of central nervous system control. An example data trace from a trabeculae study might include contraction/relaxation over time or the response to parasympathetic neurotransmitters or catecholamines. Currently, only hERG testing is regulated and required by the FDA for cardiac drug safety relating to prolongation of QT intervals; however, it could be argued that these other data, especially from ventricular muscle, also provide valuable information.

Human tissue assays are not restricted to contractile tissues, or tissue that can generate a force. Valuable information can also be obtained from tissue sheets from skin, or respiratory, gastrointestinal, and some glandular tissues. These types of assays may involve the addition of a compound to one surface of the tissue sheet, and the measurement of a response at the other surface, such as the production of cytokines or other local hormones. These measurements may also include measures of absorption, secretion, or electrochemical changes.

## Transmembrane Studies

An abundance of skin tissue is now available through cosmetic surgery, as well as reconstituted skin preparations made by companies such as MatTek and L'Oréal. In tests on full-thickness skin, a diffusion cell (most commonly, a Franz cell) is used to create a sealed system, whereby drugs can be applied to a suspended portion of tissue, with the active compound passing through the epidermis and dermis. However, this technique is limited by the use of dead skin, which has led to modified versions that use functional full-thickness skin in an organ culture system. These modified techniques permit the simultaneous measurement of functional changes such as cytokine and MMP (matrix metalloproteinase) production (20). Biopta uses this technique to model the inflammatory changes found in atopic dermatitis and psoriasis and by achieving a throughput of 20–30 "biopsies" per skin sample, means that, for the first time, human tissue can be viewed as a secondary screening tool.

The Ussing chamber, a 40-year-old methodology, is currently proving an extremely useful basis for *in vitro* drug testing with human tissue (21). Skin or gastrointestinal mucosa obtained from resection, because of GI tumours or inflammatory bowel disease, are most frequently studied. Two chambers are used together, separated by a small insert in which the test tissue is placed. Electrodes for voltage and current are used to monitor the potential gradient between the two chambers, which provides important information on possible side-effects produced by the transepithelial transport of drugs.

## Neural Tissue

The development of new assays concerning neural tissues is one of the focuses of research and development for drug testing companies, although there is still a long way to go. It is difficult (but not impossible) to access human neural tissue which is removed at surgery, for example, in the treatment of epilepsy or cancer. However, there are currently only a few good validated functional tissue assays that are relevant to the main challenges, such as Alzheimer's Disease, Parkinson's Disease, psychosis and depression. Assays are available for the study of brain slices (22, 23), and electrophysiology can be performed on neurons (22), by using stimulation electrodes similar to those employed in the field stimulation approach, but this time involving point-to-point stimulation and measurement of radiolabelled neurotransmitters.

Despite the vulnerability of human neural tissue to time-dependent failure, studies on neuronal behaviour in the hippocampus, hypothalamus and cortex have been successful. Whereas a number of assays are in use in academic institutions, they are generally not commercially useful. However, Stopps *et al.* have recently made advances in this area, by developing a system that allows electrophysiological recordings from multiple brain slices (23). The slices can remain viable long enough (over three hours) for investigations on synaptic plasticity, although, up to now, this work has been conducted on brain tissue from mice (23).

## Meeting Future Needs

Access to fresh human tissue can be difficult, and the cost, 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, and there may be a conflict between a pathologist's responsibilities and a researcher's need to place tissue in a physiological storage solution, thereby limiting the availability of fresh tissue for researchers and tissue suppliers. However, the major limitations on tissue availability are logistical, rather than reflecting a lack of sufficient tissue for the pathologist or a reluctance on the part of would-be donors; in fact, we estimate that less than 2% of surgical residual tissues that are suitable for research are made available to researchers despite over 95% of patients being willing to donate their residual surgical tissue to research (24).

Human tissue tests do present unique challenges. For many tissues, particularly those with the highest metabolic activity rates, such as the brain and heart, there is only a short experimental "window" during which they can be used. Improved transport and storage networks are therefore required, to ensure a ready supply of fresh tissue, in a state in which it can be exploited for functional assays. Studies to prolong the "shelf-life" of organs and tissues, by using various transplant solutions, artificial blood, etc. are under way, but little progress has been made so far.

The recent increase in numbers of tissue banks, although primarily focused on creating banks of frozen or fixed tissues, may soon provide a more-structured system for the supply of fresh tissues. A number of commercial companies offer to supply processed fixed or frozen human tissue. Lung and vascular tissues have been frozen, and upon thawing have retained many of their functional responses (25). However, those seeking fresh human tissue for their research need to either create a bespoke tissue supply network or to outsource the experimental work to a contract research organisation with a developed tissue network.

## The Role of Regulations

EU legislation provides guidance on the standards for quality and safety surrounding all aspects of human tissue procurement and use, except for blood (which is currently covered by other legislation). In the UK, the Human Tissue Authority (HTA) and the Human Fertilisation and Embryology Authority (HFEA) are responsible for implementing the EU Directive. The use of post-mortem tissue is the focus of the UK's *Human Tissue Act 2004*, which, although still broadly covering surgical samples from living donors, delegates the responsibility for surgical tissue control to the National Health Service and individual biobanks. In the UK, patients are asked to consent to the use of their tissues for specific research purposes, as outlined in the document accompanying the consent form. In Scotland, where separate regulations operate, the broader term "authorisation" is used, which implies that the donor (or their relatives) authorises the researchers to use their tissue for research as they see fit, potentially allowing for greater flexibility. We hope that the nation-wide regulation of human tissue donation will eventually lead to greater public trust in the process of tissue collection and distribution for research, and will ultimately make it easier for patients to donate tissues for research purposes.

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