

The Use of Human Cells in Biomedical Research and Testing

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Summary — The ability to use human cells in biomedical research and testing has the obvious advantage over the use of laboratory animals that the need for species extrapolation is obviated, due to the presence of more-relevant morphological, physiological and biochemical properties, including receptors. Moreover, human cells exhibit the same advantages as animal cells in culture in that different cell types can be used, from different tissues, with a wide range of techniques, to investigate a wide variety of biological phenomena in tissue culture. Human cells can also be grown as organotypic cultures to facilitate the extrapolation from cells to whole organisms. Human cell lines have been available for many years on an *ad hoc* basis from individual researchers, and also from recognised sources, such as the European Collection of Animal Cell Cultures (ECACC) and, in the USA, the Human Cell Culture Centre (HCCC). Such cells have usually been derived from tumours and this has restricted the variety of types of cells available. This problem has been addressed by using primary human cells that can be obtained from a variety of sources, such as cadavers, diseased tissue, skin strips, peripheral blood, buccal cavity smears, hair follicles and surgical waste from biopsy material that is unsuitable for transplantation purposes. However, primary human cells need to be obtained, processed, distributed and handled in a safe and ethical manner. They also have to be made available at the correct time to researchers very shortly after they become available. It is only comparatively recently that the safe and controlled acquisition of surgical waste and non-transplantable human tissues has become feasible with the establishment of several human tissue banks. Recently, the formation of a UK and European centralised network for human tissue supply has been initiated. The problems of short longevity and loss of specialisation in culture are being approached by: a) cell immortalisation to generate a cell type possessing the properties of both primary cells and cell lines; b) the inhibition of intracellular activities resulting in oxidative stress; and c) the use of stem cells, both of embryonic and adult origin.

Key words: *biomedical research, cultured human cells, human cells, human research tissue banks.*

Non-animal Methods Used in Biomedical Research and Testing

There are several non-animal methods currently used in biomedical research and testing: a) prior information (databases); b) computer modelling (expert systems); c) biokinetic modelling (for example to predict systemic toxicity); d) the use of new technologies via the application of molecular biology (genomics, proteomics, metabonomics); e) *in vitro* models (cells, tissues, organs); and f) human studies (volunteers and patients in a prospective way, and by the analysis of exposed populations [epidemiology] in a retrospective manner; 1–5).

Of all the above approaches, cell cultures have perhaps been the most widely used, especially as they have many advantages: a) different cell types can be obtained from different tissues (for determining organ-specificity of effects, for example); b) a wide range of cell endpoints are available; c) numerous well-characterised methods and protocols exist (e.g. the use of cell-free systems, cell lines, primary cultures, subcellular fragments, tissue slices, and perfused cells and organs); d) it is possible to investigate cell proliferation, as well as sublethal and lethal

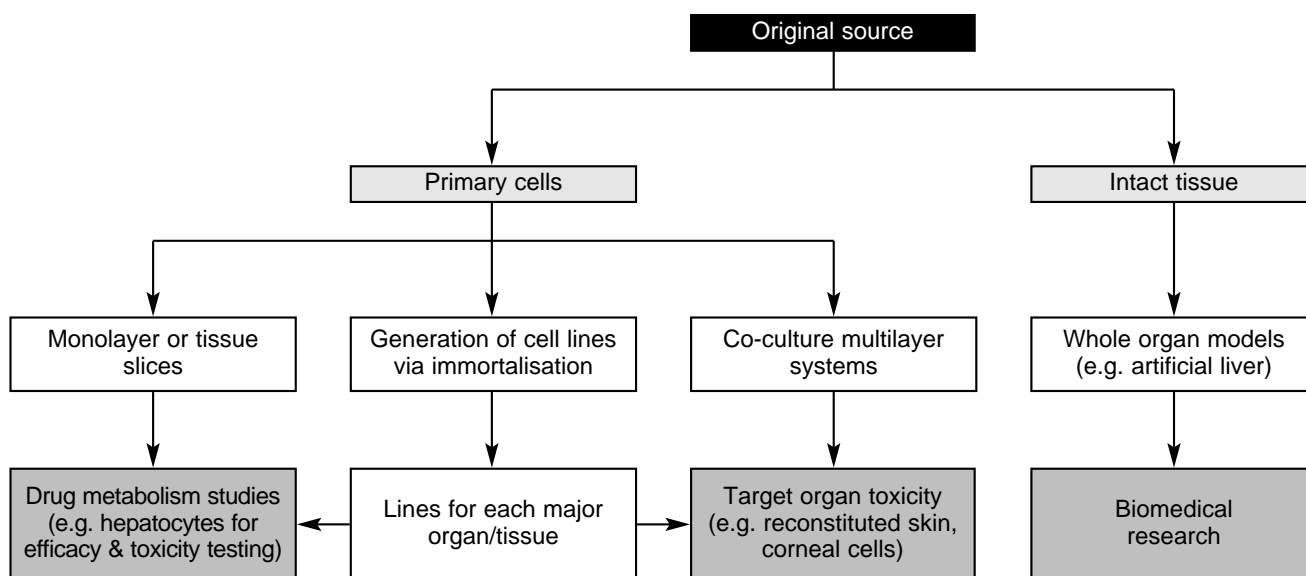
effects of various exposures, and the extent of cell recovery from such treatments; and e) although cells tend to senesce and de-differentiate in culture, there are ways of minimising these effects (6–12). Therefore, it is feasible to culture differentiating, as well as differentiated, cells (Table 1).

The Use of Human Cells

The advantages of using human cells

Human cells, as well as animal cells, can be used in tissue culture, and this can provide both scientific and welfare benefits, compared with tests involving whole animal surrogate species. The former benefit relates to the fact that species extrapolation problems are obviated. This is because human cells are better models than animal cells for phenomena occurring in humans (since they possess human enzymes and targets such as receptors). The obvious welfare advantage is that there is no need to use donor animals. Human cells can be used in a variety of forms and ways (Figure 1).

Figure 1: Potential uses of human cells and tissues



Types of human cells used in tissue culture

The principal clinical sources of human cells and tissues for research are: a) patients undergoing routine surgical operations (living donors); b) patients who die in intensive care; c) patients who die in Accident and Emergency units (brain-stem dead, heart-beating donors); and d) post-mortem examinations. Cells are derived from tissue that is deemed unsuitable for transplantation (surgical waste). The tissues most frequently obtained for research in this way are liver, kidney, lungs, tracheobronchial tract, skin and heart (13; see also Table 2).

Issues concerned with acquiring and using human cells

In recent years, there has been interest in developing controlled ways for the procurement and subsequent distribution of human cells via the establishment of human research tissue banks (HRTBs).

In view of the importance and advantages of using human cells as potential alternatives to animal experiments, there have been several recent workshops on the subject. The first ECVAM workshop on human cells (14) made the following key recommendations: a) human tissue banks would be the best intermediaries between hospitals, industrial and academic organisations to supply human material for research safely, ethically and efficiently; b) staff at tissue banks should prepare educational material for the public and health professionals on the benefits of human material for research; c) human tissue is a precious resource that should be donated with genuine and informed consent; d) standardised consent

Table 1: Differentiating and differentiated mammalian cell cultures

Bone marrow stromal cells
Blood-brain co-culture
Brain re-aggregate culture
Cardiomyocytes (beating)
Chondrocytes
Embryonic micromass cultures (limb bud; mid-brain)
Embryonic stem cells
Isolated proximal tubules
Monolayer and multilayer cultures (e.g. human keratinocytes and corneal cells)
Rat hippocampal culture
Umbilical vein endothelial cells

Table 2: Human cell material used in biomedical research and testing

Non-transplantable, surgical waste
Cadaveric
Biopsy material (e.g. waste tumour tissue; diseased tissue)
Buccal cavity smears
Skin strips
Peripheral blood
Hair follicles
Buccal cavity cells

forms should be used for this purpose; and e) better methods for cryopreserving and culturing human cells are needed.

Legal issues

In some countries, there is an opt-in system for donation, which means that informed consent is required from donor (patient) or next-of-kin. However, other countries operate an opt-out system, in which consent is presumed unless otherwise expressly forbidden. In some countries, there are calls for individuals to carry donor cards, indicating their consent for tissues and cells to be taken for research purposes, in addition to medical purposes. The overriding principle of all of these systems is that any usage for medicine (transplantation) takes precedence over research usage. In the past, and in many countries at the present, human tissue has often been obtained by informal *ad hoc* arrangements made between researchers and surgeons. However, this has led to a number of problems due to lack of control, and it is gradually being realised that a formalised process of acquisition is better than such informal arrangements.

Ethical issues

Legal and ethical issues are closely related, and it is crucial that sufficient and comprehensible information is provided on how and why the cells are going to be used when informed consent is being sought. An example of the kind of forms used for this process is provided in Boxes 1–3. It is also very important that care is taken to ensure that cells are distributed only to researchers who have local ethical approval for their work. There is also a need for all those involved in acquiring and using human cell material to be accountable by using fully transparent and documented processes.

Safety issues

These refer mainly to the safety of researchers and anyone handling cells, for example, during their transport and processing. The main potential threat to health is the possibility of contamination of tissues with disease-causing viruses, such as hepatitis and HIV. However, implementing safety measures can be exacerbated in countries where consent is required to screen potential donors for such viruses.

Logistical and cultural issues

One of the most important of these relates to efficiency, as tissues can become available at any time.

This means that decisions can have to be made rapidly as cells quickly die and/or become unsuitable. It is, therefore, important to use an efficient, streamlined system, which also takes account of transportation needs. Tissue has to be preserved carefully, transported safely, checked, and distributed, often over large distances. A further need is for a tracking system to be used, involving the recording of the details of tissue (e.g. time of collection, sex, disease-status and age of donor). Lastly, there is an important need for the production and distribution of educational material to explain the above issues and how they can be overcome in a responsible and effective manner. This is because personal attitudes to donation vary widely, and the public needs to be informed of the benefits and controls involved in using human cells. This need is especially acute during the present high level of distrust of individuals about the use of clinical material.

Sources of mammalian and human cells and tissues

Mammalian and human cells and tissues are available from a wide range of sources, many of which operate on a commercial basis (Table 3). In recent years, however, there has been interest in developing controlled ways for the procurement and subsequent distribution of human cells. The favoured means for this is via the establishment of HRTBs, as recommended in the ECVAM workshops on the use of human cells (14, 15, and see also 16).

The HRTBs provide a formalised way of ensuring the efficient and safe acquisition, transport, storage, tracking, checking, processing and distribution of human cells to bona fide researchers. At least one HRTB has a call centre operating round the clock that works in conjunction with transplant coordinators and tissue bank staff. The latter include scien-

Table 3: Some sources of animal and human cells

European Collection of Animal Cell Cultures (ECACC, UK)
Human Cell Culture Centre (HCCC, USA)
International Institute for the Advancement of Medicine (IIAM, USA)
UK Human Tissue Bank (de Montford University, Leicester, UK)
Peterborough Human Tissue Bank (Peterborough General Hospital, UK)
Roehampton Hospital Skin Bank (London, UK)
LICR/UCL Breast Cancer Laboratory, University College (London, UK)
Netherlands Brain Bank (Amsterdam, The Netherlands)
Central Tissue Bank (Warsaw Medical School, Warsaw, Poland)

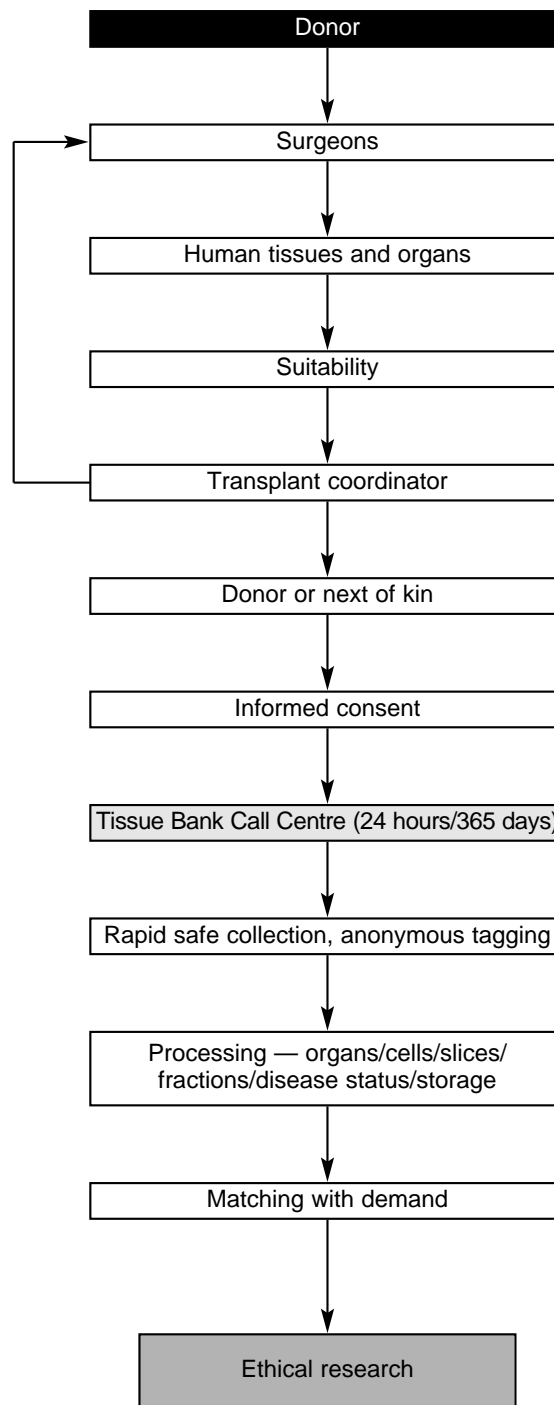
Box 1: Example of a patient donor information sheet

Research title	Reconstruction of human skin for toxicological research.
Site	Title of organisation or laboratory undertaking the work.
Investigators	Individual names listed.
Preamble	<p>You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information and discuss it with friends, relatives and your medical doctor, if you wish.</p> <p>During the operation you are undergoing, a small amount of skin will be removed. Normally this waste skin is disposed of by incineration. However, we would like to ask you to allow us to use that piece of skin. The waste skin would be taken to the laboratory and treated to release individual skin cells (which are the building blocks of skin).</p> <p>The skin cells would be used to reconstruct the human skin <i>in vitro</i> (outside the body). To be able to grow skin cells in the laboratory, they must be undamaged and alive, so fresh skin samples are needed.</p> <p>If you decide to take part [in the study], you are still free to withdraw at any time and without giving a reason. We have to assure you that you are under no obligation to participate in this study. If you choose not to participate, it will not affect the standard of medical care you receive.</p> <p>All information that is collected about you will be strictly confidential.</p>

tists who work in tissue culture laboratories in which tissue and cells are processed and maintained in a frozen state. HRTBs have now been established in several countries, especially in the USA and Europe. Thus, HRTBs play a central and crucial role in allowing the use of human cells and tissues for research purposes (Figure 2).

The latest workshop on human tissue banking (17) defined an HRTB as follows: an intermediary to legally, ethically and safely acquire, store and distribute human tissue (cell material containing DNA) and body fluids, using a cost recovery/not for profit mechanism, for bona fide research purposes.

Figure 2: The central role of research human tissue banks



In Europe, a European Network of Research Tissue Banks (ENRTB) is being established, as a result of recommendations made at two workshops (15, 17). The ENRTB is designed to promote the use of human cells and tissues in research, and to provide a forum for issues and problems to be discussed and shared among those involved in acquiring and using human cells. The ENRTB has the mission to estab-

Box 2: A second example of a patient donor information sheet

Description of the project

The aim of the project is to use the reconstructed human skin to test the effects of skin creams, formulations, sunscreens and other chemicals that may come into contact with skin. The results of the research can produce a routine test for safety of a product.

This is ideal as such compounds are tested on human tissue and this can also act as a relevant alternative to animal testing.

lish a sustainable network for sharing information to guide in the establishment and running of human tissue banks with the ultimate goal of sharing human tissues/information derived from use of these donations across this network under harmonised guidelines and agreed best practice to promote the use of human tissue. The group will also seek funding, and organise the production of educational material and further meetings to formally establish the network. The next meeting of the ENRTB is scheduled to be held in The Netherlands during 2003.

The use of human cells and tissues in toxicology

Toxicologists have used human tissues whenever they can, but their ability to do this has been severely limited by the poor availability of cells from internal organs. For example, human cells have been used in organotypic, multi-layered cell culture models. These models are useful because they can have more than one cell type present, with

Box 3: An example of a volunteer questionnaire

Preamble	To aid us in the analysis of the experimental data obtained using the skin sample, please complete this short form:
General	Age, sex, ethnic group, hair colour.
State of health	Any regular medication, any family incidence of skin cancer, any personal skin allergies to specific products (e.g. to cosmetics or sun-tan lotion).
Skin type	On hot summer days in Britain, do you: (a) always burn, never tan; (b) burn easily, tan slowly; (c) burn occasionally, tan easily; (d) never burn, always tan?

spatial differentiation, and communication between cells in three dimensions. Also, they can either be cultured submerged in liquid media or raised to the air-liquid interface growing on inert membranes in inserts (18–21).

In comparison with conventional single cell, monolayer cultures, organotypic systems usually retain more of the *in vivo* characteristics of cells and tissues than are retained in normal culture systems. Also, organotypic systems can comprise cells that are targets for toxic chemicals. Examples include keratinocytes and corneal cells in models of the skin and eye, respectively, which can comprise both epithelial and endothelial cells in co-culture. A certain degree of barrier function, responsiveness to irritants (in the form of cytokine release) and xenobiotic metabolism occurs in these human cell models. These models can be composed of primary cells (more likely in the case of skin) or immortalised cells (usually necessary for the eye). When models are developed from primary cells, these can either be derived from individual donors, for example to investigate inter-individual variation in response, or they can comprise cells pooled from a series of donors.

FRAME has developed a human cell epithelial skin equivalent organotypic model (22). In this model, primary keratinocytes obtained with fully informed consent from cosmetic surgery patients are cultured under conditions that promote the differentiation of the keratinocytes into a stratified structure comprised of three main layers (cuboidal basal cells, spinous cells and heavily stained, granular flattened cells, the latter being equivalent to the stratum corneum). These are grown on collagen-coated insert membranes without dermal cellular components at the air-liquid interface to facilitate the testing of insoluble substances, such as creams and emulsions. It is also possible to grow such epithelial cultures on de-epidermalised dermis to produce reconstructed human skin, which retains even more *in vivo*-like characteristics than the epidermal equivalent (23).

Immortalisation of human cells, by introducing oncogenes into them, is a means whereby their differentiated status and longevity can be prolonged. In this way, cell lines can be obtained from primary human cell material. There are several immortalised cell lines that have been generated from a variety of cell types, and some of these have been used to develop organotypic models, comprising keratinocytes and corneal cells (21, 24, 25).

Disadvantages of using cultured human cells

Despite the advantages of using human cells in the various ways discussed above, there are several disadvantages that are inherent in using cell cultures,

particularly for toxicity testing, that exacerbate *in vitro* to *in vivo* extrapolation. These include a lack of: a) normal absorption, distribution, metabolism and excretion (ADME) processes and pathways occurring in the body, which are responsible for determining target organ levels of active chemical species and their removal from sites of action (leading to a need for exogenous metabolism); b) intact immune, transport, endocrine and nervous systems; c) normal biological barriers (e.g. blood–brain, blood–testis and skin barriers); d) cells from a complete range of tissues and organs; e) stability of cells in culture (due to a tendency for them to senesce and de-differentiate; and f) ability to accurately predict the occurrence of complex phenomena (e.g. behavioural effects). Due to some of the reasons above (e.g. no removal of toxic chemicals and an emphasis on metabolic activation rather than detoxification), it is common for *in vitro* toxicity assays to be oversensitive, prompting their use as screens, rather than as definitive tests.

There are, however, various ways of improving the relevance of cell cultures, including those comprised of human cells, for predicting the responses of whole animals. These improvements include the use of various methodologies for maintaining primary cells for extended periods in their differentiated state. Such techniques include the use of special growth factors; medium with different Ca²⁺ levels, the use of collagen gel sandwiches and co-culture systems, especially of hepatocytes (8, 12, 26). The latter help to maintain *in vivo* cell shape, attachments and enzyme activities. A further, increasingly-used technique involves developing genetically engineered cell lines (9). In this way, cell lines can be developed containing molecular targets (receptors) for toxicity from different species, including humans, as well as oncogenes that immortalise them in attempts to maintain primary cell characteristics in culture. Genes coding for mammalian metabolising potential (e.g. CYP isozymes and Phase II conjugating enzymes) can also be introduced into cultured mammalian cells. Lastly, biokinetic modelling is being used increasingly to facilitate *in vitro* to *in vivo* extrapolation (27).

Conclusions

There are major scientific and animal welfare advantages of using cultured human cells. The most important of these is that species extrapolation is obviated by using human cells in culture. In principle, therefore, if the problems of extrapolating from cell cultures to individual human beings can be minimised by using organotypic systems, comprising cells with extended viability and differentiated status, together with biokinetic modelling approaches, it should be feasible to use *in vitro* data for risk assessment purposes. This type of extrapo-

lation may well be no more uncertain than extrapolating data from systems involving surrogate whole animals, particularly if safety factors can be applied (as is done with *in vivo* data).

For the above reasons, it is crucial that efforts are continued to supply human cells and tissues to bona fide researchers in efficient, safe and ethical ways, via human tissue banks.

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