Interactive Tissue Culture Systems: Innovative Tools for Toxicity Testing

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Interactive tissue culture systems were designed as a novel approach to utilize the organotypic differentiation state and the specific composition of tissues for drug research in vitro. In the intact organism, tissue is characterised by specific interactions of the involved cell types and by tissue-specific extracellular matrices. Both features are major prerequisites for proper tissue differentiation, physiology and function. Interactive tissue culture systems offer the unique opportunity to employ these tissue properties ex vivo to monitor the effects of test substances with unprecedented accuracy. Substances that cause no toxic damage in conventional cell culture because side effects are the result of cellular interactions within a tissue, can be quickly excluded from more expensive tests, thereby reducing the total number of tests and hence, costs. In addition, interactive tissue culture systems allow for the use of human tissues in vitro, thereby effectively supplementing the existing cell culture-based drug screening procedures. Interactive tissue culture systems thus offer an intriguing perspective to provide data relevant to humans. This technology has applications in pharmacotoxicity and biocompartibility tests as well as in molecular targeting studies.

KEYWORDS

biotolerability, vascular system, development, kidney, pharmacological test, tissue culture

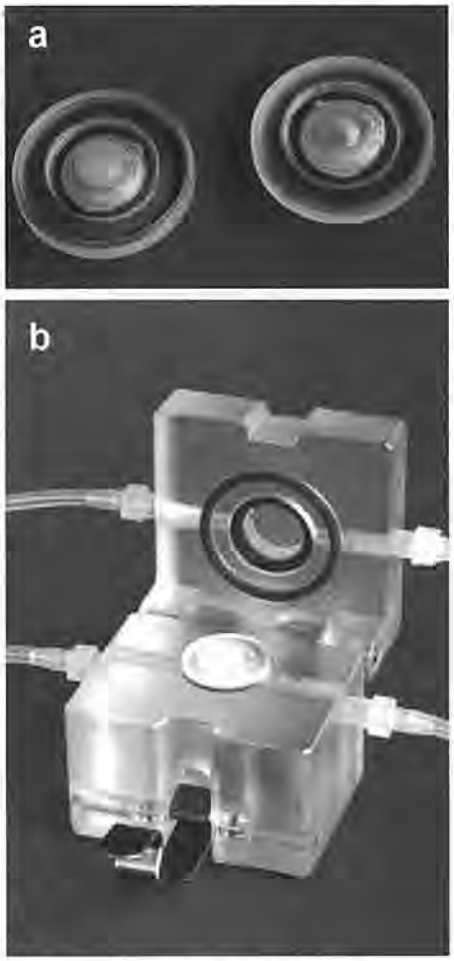

Fig. 1: Tissue preparation and culture
Fig. 1a: The picture shows tissue carries with mounted tissue samples. The tissue carrier has an outer support and inner O-ring. The mounted tissue sample can be simply transferred from a culture container to an analysis unit. In addition, the system ensures an optimal exchange of nutrients and metabolites.
Fig. 1b: A gradient perfusion chamber was used for the toxicity tests. This perfusion chamber consists of two compartments that can be perfused independently. The tissue sample separates the upper from the lower chamber compartment. This construction allows the unilateral application of the test substance, as encountered when the test substance is applied on the body surface or epithelial tissue.

The development of new drugs requires extensive tests in order to verify that these substances are harmless for the user. Initially, these substances are tested in tissue culture systems [1, 10] and microorganisms [9], before their tolerance is tested in higher animals, and finally in humans in clinical studies. This costly stepwise procedure is supposed to guarantee that the newly developed substance is well tolerated by the consumer and free of undesired side effects. For ethical and economic reasons, it is therefore necessary to develop test procedures that are safe and easy to evaluate.

In the past, some active substances without toxic effects in tissue culture systems turned out to have unexpected side effects in animals. There are various possible explanations for these seemingly paradox findings. An organism consists of a multitude of different cells linked by complicated physiological processes. For example, one could imagine that the test substance itself does not have< a toxic effect, but rather the products of biotransformation are toxic. With these considerations in mind we have developed a novel test system in which complex tissues can be confronted with the test substance. Since this test system is based on the interaction of the different cell types of the tissue, we call it an "interactive tissue culture system". The cells are embedded in a tissue specific matrix and constitute a structured architecture thereby retaining tissue specific functions, allowing the effects of the test substance on physiological intercellular interactions to be studied.

Requirements of an interactive tissue culture system

Tissue culture systems designed for pharmacological and toxicological testing need to:

- provide reproducible culture- and test results
- allow easy handling
- allow the unilateral application of test substance
- allow the option of automated test evaluation

Reproducibility

There are two main preconditions for the reproducibility of culture- and test results: First of all, the organotypic state of cell differentiation in the cultivated tissue has to be maintained, and secondly, the organotypical tissue composition has to be preserved. In addition, it is desir-

able for the system to be independent of undefined medium additives such as serum or cellular extracts. Another important prerequisite for the reproducibility of culture results is the absolute necessity to avoid necrosis during tissue culture.

In our culture system, organs or parts of organs are prepared without proteolytic disintegration of the tissue, thereby maintaining the organotypic composition of tissue. Tissue can be prepared using the following techniques: Vibratome sectioning, stripping off distinct tissue parts, or using simply sufficiently thin tissues such as retina, cornea, and skin.

After preparation, the tissue samples are inserted in a tissue carrier in order to avoid curling and to facilitate an optimal nutrient supply (Fig. 1a). Once set up, the tissue samples are cultivated in a perfusion chamber (Fig. 1b, [7]). The chamber is continuously perfused with fresh culture medium. Permanent perfusion of the tissue culture with medium ensures the rapid removal of metabolic products, guarantees an optimal oxygen supply for the tissue, and helps to counteract the danger of necrosis. Using this culture technique, there ist no need for the addition of fetal calf serum or tissue extracts to the culture medium. These culture conditions allow the maintenance of complex organotypical tissue structures, such as tissue explantates from kidney [3] or stomach mucosa [5].

Handling

The handling of this system is simple. Straightforward sectioning or dissecting techniques are applied for tissue preparation. The tissue samples are mounted on a tissue carrier (Fig. 1a) and can thus be easily inserted in or removed from the culture container. The culture container is locked by snap locks (Fig. 1b).

Application of test substance

The organism can take up liquid or gaseous substances via skin contact, inhalation, swallowing or injection. In each case, there is only a unilateral contact of the test substance with various tissues of the body. Accordingly a gradient culture chamber is used which allows for unilateral substance exposure under culture conditions (Fig. 1b). The gradient chamber consists of an upper and a lower compartment. The compartments are separated from each other by the tissue sample. The test substance can thus be applied to one side of the tissue,

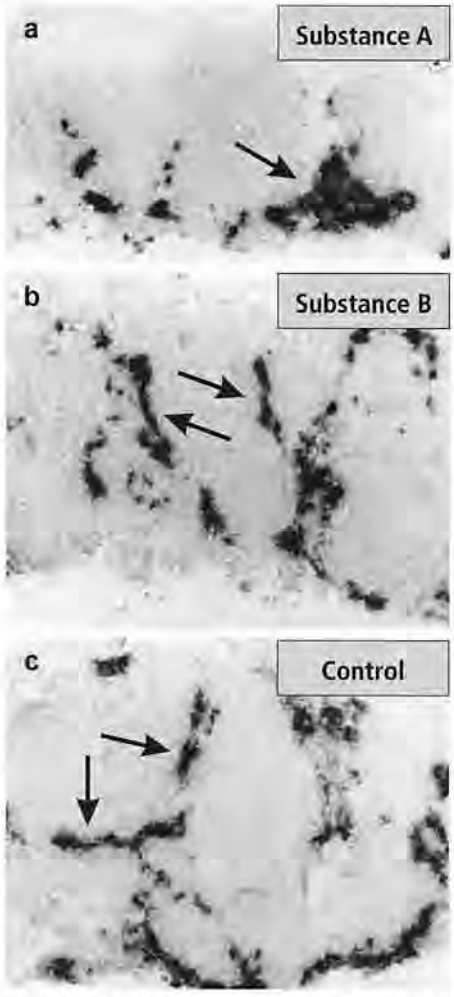

Fig. 2: Test of vitreous body substitute on renal explants; renal tissue explants were perfused continuously with serum free medium. Vessels and endothelial cells were detected immunochemically with the antibody EC1 [3].
Fig. 2a: Substance A showed a dramatic toxic effect upon the vascular network of the renal explant already after 24 h of cultivation. Only aggregates formed by the endothelial cells (arrow) were detectable. The architecture of the network was destroyed. By contrast, Substance B showed no toxic effect within 24 h of culture (Fig. 2b, arrows). In the control experiment, the upper compartment of the chamber was filled with culture medium. Likewise the architecture of the renal vascular network was preserved (arrows). Magnification x 350.

while the other chamber compartment is perfused with culture medium.

Test evaluation

Test evaluation can be performed using imaging procedures, and biochemical and molecular biological techniques. The evaluation is considerably simplified by measuring suitable parameters in the culture supernatant. Used culture medium is permanently drained from the culture chamber. After applying the test substance, the concentration change of a typical secretional or metabolic product can be recorded as a parameter to assess tissue damage. Online recording of these parameters can be achieved by inserting a probe in the culture medium stream.

An example application: Toxicity testing of vitreous body substitutes

Background: Vitreous body substitutes are applied in ophthalmology, when the vitreous body is injured or damaged [2]. In the following example, two such substitutes were investigated. The toxicity of both substitutes had previously been tested in cell culture systems [8], but no toxic effects were detected. When tested on rabbits' eyes, however, Substance A resulted in extensive pathological changes of the blood vessel system of the retina, while test Substance B showed a considerably better tolerance in animal experiments.

Selection of test tissue

In our experiments, we used kidney tissue. This organ is not fully developed at birth. For tissue function, however, a well developed vascular system is essential. Pathological changes of the vascular system caused by toxicity, also evoke considerable damage to organ function. Hence, the kidney's developing vascular system was used as a model system for in vitro toxicity tests. Tissue explantates from neonatal rabbit kidney were obtained by stripping off the thin organ capsule [6]. This tissue area is characterized by a typical architecture of the blood vessel system [4]. The maintenance of this typical structure was used as an analysis parameter when assessing the toxicity of test substances. When toxic substances are absent, the vascular system can be maintained for as long as 13 days in perfusion culture [3].

A gradient perfusion chamber was used for vitreous body substitute toxicity tests. This perfusion chamber consists of two compartments that can be perfused independently. The tissue preparation separates the upper from the lower chamber compartment. The upper compartment was filled with the test substance and was not perfused. The lower compartment was continuously perfused with culture medium. Thus, one side of the piece of tissue had contact to the test substance, while the other tissue side was provided with nutrient solution. In the control experiment, the upper chamber compartment was filled with medium and was not perfused. The nutrient solution remained stationary in the upper chamber, while the lower compartment was perfused with medium. Using the perfusion culture technique, there was no need for additional fetal calf serum in

the culture medium (Iscove's Modified Dulbecco's Medium, IMDM, Gibco-BRL Life Technologies, Eggenstein, supplemented with $1x10^{-7}$ M Aldosterone and $1x10^{-9}$ M 1.25 Dihydroxyvitamine D3). In the presence of these hormones, blood vessels in the renal tissue remain completely intact over the two week culture period [3]. Vessels and endothelial cells were identified immunohistologically, using the antibody EC1 [3].

Results: The experiments were supposed to assess whether the effects of test Substance A on the vascular system could already be detected in vitro.

As a control, tissue was exposed to a non-toxic test substance (Substance B) or to the pure culture medium. Renal explants exposed to test Substance B for 24 hours retained an optimal vascular network, as did the control explantate (Figs. 2 b,c). In contrast, the vascular network was destroyed when the tissue was exposed to test Substance A for 24 hours (Fig. 2a). Only small groups of endothelial cells, remained detectable.

In contrast to the tissue culture system, the application of test Substance A in the cell culture system had shown no toxic effect [8]. This discrepancy between the two systems emphasizes the importance of the physiological interactions between cell populations in the tissue when performing toxicity testing. Cell culture systems are excellent tools for the detection of acute toxic effects. However, other test systems are needed when the toxic effect is dependent on intercellular interactions.

The culture conditions described in this example can be widely varied to meet the specific requirements. Variations of the perfusion rate, different cultivation protocols, as well as different culture media, as required by different tissues, are possible and easily implemented. This newly developed model system is suitable for use in a broad spectrum of differing applications. It is suitable for pharmacological tests, for the analysis of drug targeting, for the introduction of molecular probes, and for biocompatibility tests. One special advantage of this method lies in the possibility to use human tissue in tests.

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