

MINIREVIEW

Regulatory and microbiological safety issues surrounding cell and tissue-engineering products

Daniel N. Galbraith¹

BioReliance Corporation, Todd Campus, West of Scotland Science Park, Glasgow G20 0XA, Scotland, U.K.

Cell therapies and tissue-engineered products that contain living cells are potentially some of the most exciting of the novel therapeutic products currently under development. These products, however, present a number of important safety issues, particularly with respect to the transmission of human viruses. In addition, the short shelf life of these products precludes the normally extensive characterization performed on other biotherapeutic products. Careful examination of the risks and extensive testing of the raw materials have been used in place of product testing to ensure safety.

Introduction

With the advent of human-somatic-cell therapies and tissue-engineered products, many new opportunities are presented to clinicians to help patients with conditions that were thought to be difficult or impossible to treat in the past [1]. Many such products are currently being marketed that contain living human cells, having already passed clinical trials and having received regulatory approval (Table 1). These products are designed mainly for the treatment of serious skin lesions that are not responsive to other forms of treatment.

The success with these relatively simple forms of tissue is encouraging, and has raised the potential for the procedure to be repeated with more complex tissues such as kidney or liver. The importance of these products to many millions of potential patients cannot be underestimated. Currently the only means of treatment for many conditions is by a transplant of tissues or organs. The ever-increasing number of patients requiring these treatments, and the fact that the donated organs come from an ever-dwindling supply, means that many individuals will never be treated. Cell therapies in some cases can offer appropriate treatment for these conditions from materials produced *in vitro*, thereby reducing or eliminating the need for donated organs. Although the potential for these products appears great, they come with sometimes difficult manufacturing challenges

and regulatory problems that need to be overcome to deliver safe and effective products. All of these products require, as part of their make up, living human cells that have been cultured and expanded *in vitro* for a period of time. Cell populations from harvested tissues have an inherent variability between donors, and therefore the manufacturing challenge of assuring consistency and potency of product batches can prove difficult. However, perhaps the main regulatory concern for these products to date has hinged on their microbiological safety. The products, being composed of living cells, cannot be 'terminally sterilized' in the way that other therapeutic products are. Therefore any microbe contaminating the donated material or being introduced as an adventitious agent during the manufacturing process has the potential to multiply during the processing and could compromise the product, leading to serious consequences for the treated patients. The source of the cells used in these products is frequently allogenic; however, in some instances autologous cells are used. It could be considered that autologous-cell therapies may be low or zero risk with respect to contaminating microbes; however, there is the potential to increase the concentration of a microbial contaminant during the culture of the cells. This will almost certainly put patients at risk and should not be disregarded.

Microbiological risk assessment of cell-therapy products

With respect to the donor of the tissue, a number of screening methods may be applied that will reduce or eliminate the risk of contamination with some agents. In the past, donors have been screened for human immunodeficiency viruses (HIV) and human T-cell lymphotropic virus (HTLV) and for hepatitis viruses, mainly B and C (HBV and HCV) [2].

Key words: contamination of tissue-engineered products, human cell therapy, viral risk.

Abbreviations used: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HBV, hepatitis virus B; HCV, hepatitis virus C; HSV, herpes simplex virus; HTLV, human T-cell lymphotropic virus; WNV, West Nile virus.

¹ email dgalbraith@bioreliance.com

Table 1 Tissue-engineered products currently marketed

Product name	Manufacturer and address	Cell type	Clinical use
Epicell®	Genzyme (Cambridge, MA, U.S.A.)	Keratinocytes	Burns
Apligraf®	Organogenesis (Canton, MA, U.S.A.)	Fibroblasts/keratinocytes	Ulceration of skin
Epidex®	IsoTis (Lausanne, Switzerland)	Keratinocytes	Ulceration of skin
Transcyte®	Smith & Nephew (Memphis, TN, U.S.A.)	Fibroblasts	Burns

These tests have been carried out using the currently available protocols that are based on the detection of antibodies reactive against synthetic viral proteins. Research has shown that the detection of antibodies in screened individuals runs the risk of an antibody-negative window period (the absence of reactive antibodies) where an individual has been exposed to viral infection and indeed can be viraemic [3]. HCV is potentially the most concerning of agents in this category, where individuals have been shown to be negative for the presence of reactive antibody, but are viraemic for a number of months before seroconversion [4]. This finding has led to the introduction of molecular amplification techniques, such as PCR, which will assay for the presence of virus by means of targeting the viral genome [5]. In this way, infection can be detected at a much earlier stage than would have been possible with the antibody-based techniques. The molecular or antibody-based techniques also suffer from a common disadvantage in that the specificity of the tests does not allow for a wide number of microbes to be detected.

Any individual may well harbour an extensive number of microbes and viruses at one particular time. Viruses such as herpesviruses [e.g. herpes simplex virus (HSV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV)] and polyomaviruses (JC and BK virus) are known to remain latent and detectable in humans from early childhood and are potential contaminants of cells from normal healthy individuals [6]. Most of these viruses can be easily screened for by simple PCR tests on the product. As these viruses are so ubiquitous, there may be no significance in these agents being present in the product for the majority of patients, and perhaps we should disregard these viruses even when present. However, in some instances these agents can prove to be of concern. In the case of human heart-transplant patients the transfer of an organ from a CMV-positive individual to a recipient who has never been exposed to the virus can prove to be fatal, owing to the high degree of immunosuppression required for these patients to avoid rejection. The CMV produces a viraemia and frequently results in pneumonitis, which, even when treated with antiviral agents, may prove fatal [7]. Therefore every agent should be considered as a potential pathogen, since every patient poses different risks.

There are some viruses where there is more concern with the risk of contaminating a product. Recently, in the United States, there has been a dramatic increase in the

incidence of West Nile virus (WNV) [data for 2002: 4156 cases reported with 284 fatalities (Centre for Disease Control, Atlanta, GA, U.S.A.; <http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount02.htm>)]. This is considerable, as the likely reported cases will only represent < 1% of infections [8]. The remaining cases are asymptomatic or have only mild clinical signs; therefore many individuals will not know they are infected when donating the tissue. The potential exists, therefore, for harvested tissues to be infected with this type of agent. WNV can replicate in most human cells, and therefore the virus would extensively compromise the product [8]. It is noteworthy that WNV is not the only arthropod-borne virus that can infect humans, and caution should be used in the isolation of suitable donor material [8]. Viral or other microbial infection may be present at an insignificant titre in the original donated material and thus too low to initiate an infection if directly transferred to the recipient. However, during the *in vitro* culture period, where the cells are expanded, any contaminating microbe has the opportunity to achieve much higher titres than would be possible *in vivo*. The reason for this is the lack of any effective immune system in the *in vitro* culture where viruses can rapidly increase in titre under these conditions. The consequence of this increase in quantity may be to achieve a titre of virus that is at a level damaging to the product and therefore the patient. Irrespective of the quantity of contaminating microbes, however, placement of product in immunologically privileged sites may severely affect the patient. The products that are currently marketed for the treatment of leg lesions caused by diabetes mellitus and vascular disorders typically treat areas of skin that are poorly supplied by the circulatory system; therefore the immune system would have little if any effect on a graft placed in this area. With cell-therapy products, the only opportunity of detecting the presence of contaminating viruses is by observation of the health of the cells during processing or during the screening of the original donated material, therefore the most sensitive and comprehensive tests should be applied at this stage.

As well as the potential for infection from known viruses, the 'emerging' viral risks have to be accounted for. The very recent outbreak of severe-acute-respiratory-syndrome ('SARS') virus in humans shows we are continually being challenged with new pathogens for which we have no means of detection [9]. Clearly any new entity that arises

should be considered in the risk factors for contamination, and specific tests should be developed rapidly to ensure the product is not compromised.

A subset of the risks from the donor are the risks from different cell types. With each cell type there are a number of safety issues that should be considered. The tissue type is clearly of significance with relation to the potential microbes that could contaminate the harvest. Samples of tissue from sites such as the skin or bone present a multitude of microbes, both bacterial and viral, that are significantly different from those in tissues harvested from internal organs such as the colon or kidney [10]. Harvested tissue from the lung, for example, should be considered to be screened from respiratory pathogens such as rhinoviruses, parainfluenza viruses, coronaviruses, influenza viruses, adenoviruses and respiratory syncytial virus, to name a few.

Another risk presented by cell or tissue products is the harvesting procedures and the culture conditions under which the cells are grown. During the harvesting of cells there are normally some procedures included for the selection of the cell type required for the patient's treatment. One method of cell selection is the use of antibodies reactive against specific cell-surface markers; the cells are then sorted by a second procedure such as FACS [11]. Antibodies can be obtained from one of two sources: cultured antibody-producing cells (predominately murine) or animals previously immunized with specific antigens. Both cases clearly present a risk of contamination of microbes from the antibody reagent to the cells during the cell-sorting procedure [12]. The risks vary depending on the source of the antibody and also with the procedures used in the preparation of the reagent (i.e., if there are any virus-inactivation steps used in the production of the reagent). After harvest and selection, cells are normally grown in a defined medium that is enhanced with growth supplements. A very common supplement for use in recently isolated cells is bovine serum. New guidelines describing the screening of bovine serum prior to its use in the manufacture of a human biological product have recently been introduced [13]. These guidelines are designed to control the quality and safety of bovine serum used during the manufacture of human biological medicinal products. The European regulatory agencies have identified the need for a risk assessment for transmissible spongiform encephalopathies ('TSEs') in all products derived from ruminants, and this requirement is included here. In addition, serum should not contain any detectable bacteria, mycoplasmas or fungi. The virus-testing list is not exhaustive, but includes known bovine pathogens, including bovine viral diarrhoea virus, bovine polyomavirus, bovine parvovirus, bovine adenovirus, blue tongue virus, rabies virus, bovine respiratory syncytial virus, reovirus and rabies virus. Manufacturers

should be aware of the current guidelines regarding bovine safety testing and the emerging zoonotic agents that may be transmitted by bovine serum. In an enlightened step the authors of the guideline also makes clear that manufacturers using bovine serum should be aware of emerging bovine viruses and are encouraged to investigate the presence of these agents in serum. This testing can be completed by the serum supplier, manufacturer or a contract testing organization; however, the responsibility for compliance lies with the manufacturer of the medicinal product.

The remaining component aspect of the cell-therapy or tissue-engineered products are the scaffolding materials used as matrices to support the growth of the living cells. Products already mentioned use collagen, sourced usually from humans or animals to provide this material. Collagen is useful in that it is not immunostimulatory and is slowly biodegradable and therefore meets a number of requirements for the products. The biological source of this material is clearly of concern, and steps are required to be in place to ensure that any microbial contamination is reduced or eliminated from the product.

Testing strategies for cell-therapy products

As has been mentioned, for many of the microbes already recognized there are tests available that are already described in the European or United States Pharmacopoeias [14–16]. Tests such as those for sterility or mycoplasma are very simple to carry out and have been shown to be successful at eliminating contaminated products, preventing patients being put at risk. However, the disadvantage of these tests is that they take longer than one month to carry out. For many of the cell or tissue products where living cells are the critical component, this would clearly make these non-viable as products if we were to apply the strict criteria. Therefore other alternative methods require to be applied to facilitate the expedient use of these products. Already there is acceptance of the use of the PCR techniques to detect mycoplasma [17]. This technique uses a number of different primer sets that will identify the vast majority of common mycoplasma contaminants in a matter of hours as compared with the culture techniques, which require weeks of culture [18,19]. The PCR tests can be run alongside the traditional techniques, but the product would be released after the rapid screen. The sterility test presents a much more difficult challenge, as this test is capable of detecting a large number of bacterial and fungal species. There are a small number of reports of automated methodologies to identify the bioburden (the number of contaminating organisms in a certain amount of product); however, these still remain to be fully expanded and used

in a routine environment for cell therapies. PCR technology has been used to identify specific viruses, and, owing to the increased sensitivity achieved by this methodology, this will help eliminate contaminated materials before they reach the patient. There are two tests that are included in the screening of other biological products that are used to screen for a wide range of viruses: the *in vitro* cell culture assay and the study in animals [20]. These are long-standing techniques that rely on the ability of many viruses to cause cytopathic changes in cell culture or clinical changes in animals. These tests can take a number of weeks, and therefore can prove difficult to implement in the scheduling for release testing of time-critical products. The only useful alternative solution today is to expand the number of PCR tests to include all viruses that we know pose a risk to the product. In the future, technologies such as the use of chip hybridization [21] may provide a useful and rapid means of identifying contaminants.

Human skin products

Human skin products are currently indicated for use in wound healing [22]. The majority of products are manufactured *in vitro* from neonatal human foreskin cells. The cells are harvested from the donor material and expanded in culture to a sufficient level to achieve a cell bank of the cell types required. When a product is being manufactured, the cells are recovered from frozen from the cell bank and are allowed to engraft on a biological matrix, forming layers of cells with a profile similar to that of normal skin. The cultured skin forms a dermal layer consisting of human fibroblasts and an upper epidermal layer that becomes organized, as in normal epidermis, to produce a superficial cornified stratum. The nature of the final product offers major therapeutic advantages, but, as has been mentioned above, raises important issues in safety evaluation because living human cells and material of bovine origin are placed in apposition to the recipients' tissues. The safety of these products depends on several independent processes:

1. Selection and screening of the donated material
2. Establishment and microbiological testing of master cell banks ('MCB') and working cell banks ('WCB') prepared from the donated material
3. Safety screening of the human and animal components used in the preparation or manufacture of the product
4. The short shelf life of the final product precludes conventional final product testing

In the case of the human skin products, as the sample is taken from a neonate, the primary focus of donor selection is on the health status of the mother. The reason for this is that microbes, and particularly viruses, of concern are either transmitted across the placenta or perinatally. Such viruses include the retroviruses HIV 1 and 2, and HTLV 1 and 2, the hepatitis viruses, particularly HBV and HCV, CMV and EBV [23–25]. The donor-selection procedure aims to specifically exclude certain viruses through serological screening of the donor's mother. Other agents can be eliminated through the use of a health and lifestyle questionnaire.

Cell-bank tests form the second major arm of the safety evaluation of human skin products. These tests act as a further assurance that human viruses are not present in the cells derived from the donor material. In addition, viruses introduced into cell-culture media and supplements, or introduced during the handling of the cells during the production of the cell bank, may be detected at this stage. The tests employed on the cell banks should be conducted in accordance with the principles laid down in the International Conference on Harmonisation Consensus Guideline [20]. This guideline covers the testing of cell banks and human and animal components. The testing involves the screening for adventitious agents, as well as recognized human pathogens such as HIV, the hepatitis viruses HBV and HCV, and human herpesviruses.

The final assurance of product safety, final product testing, is not performed on human skin products, as the shelf life is typically less than 28 days for these products and sometimes less than 14 days. Other than PCR tests for specific viruses, there are currently no traditional virology or sterility tests which can be usefully applied to yield results before the products are used in patients. However, new technologies are progressing in this area of rapid analysis [21]. Therefore the emphasis for product safety is placed on the prior screening of materials and validation of the process to ensure a safe product is manufactured. To date there has been no serious incidence of infections resulting from these products currently used. Therefore it would appear that the safety assessments already in place are sufficient to prevent serious contamination. However, it should be noted that relatively small numbers of patients are sampled in these clinical trials or in already-marketed products, and should the use of these materials become more widespread, the potential risks should be regularly reviewed and testing should be amended as necessary.

Conclusion

Tissue-engineered products such as replacement skin therapies have already been established as an effective and

viable treatment for indications that are unresponsive to other therapies. The regulatory hurdles have, with some difficulty, been surmounted, and safe products are now licenced and are routinely in use. Although skin therapies are perhaps one of the simplest of the potential tissue-engineered products to be produced, the testing matrices required to produce a safe product are directly applicable to other more complex tissues. In the future, owing to the advances in rapid and sensitive testing methodologies many of the current critical safety issues will become less onerous. However, it should be noted that, as with the discovery of prion disease, there is always the potential for new agents to cause safety concerns in biotechnology products. The artificial-tissue products are particularly sensitive to new infectious agents, because of their incorporation of living cells. The continual vigilance of manufacturers being aware of any new risk will be essential for a safe product and continuing public confidence.

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