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Consistency and Safety of Cell Banks for Research and Clinical Use: Preliminary Analysis of Fetal Skin Banks

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Current restrictions for human cell-based therapies have been related to technological limitations with regards to cellular proliferation capacity, maintenance of differentiated phenotype for primary human cell culture, and transmission of communicable diseases. We have seen that cultured primary fetal cells from one organ donation could possibly meet the exigent and stringent technical aspects for development of therapeutic products. We could develop a master cell bank (MCB) of 50 homogenous ampoules of 4–5 million cells each from one fetal organ donation (skin) in short periods of time compared to other primary cell types. Safety tests were performed at all stages of the cell banking. MCB ampoules could create a working cell bank to be used for clinical or research use. Monolayer culture of fetal skin cells had a life span of 12–17 passages, and independent cultures obtained from the same organ donation were consistent for protein concentration (with 1.4-fold maximal difference between cultures) as well as gene expression of MMP-14, MMP-3, TIMP-3, and VEGF (1.4-, 1.9-, 2.1-, and 1.4-fold maximal difference between cultures, respectively). Cell cultures derived from four independent fetal skin donations were consistent for cell growth, protein concentration, and gene expression of MDK, PTN, TGF-β1, and OPG. As it is the intention that banked primary fetal cells can profit from the potential treatment of hundreds of thousands of patients with only one organ donation, it is imperative to show consistency, tracability, and safety of the process, including donor tissue selection, cell banking, cell testing, and growth of cells in upscaling for the preparation of cell transplantation.

Key words: Fetal cells; Fibroblasts; Cell banking; Safety; Tissue engineering

INTRODUCTION

Establishment of cell banks is a crucial step in the process of many vaccines, medicinal products, or tissue-engineering products (TEP). The need for TEP is largely increasing as the population ages. Regeneration capacity of mammalian tissue gradually disappears from the embryo to adult state, where healing response leads to the formation of fibrosed tissue and loss of organ function. One solution has been to replace deficient organs by grafts. Whereas transplantations of kidneys, bone marrow, liver, lungs, and heart are well-established procedures, the demand for organ transplantation cannot be fulfilled due to limited availability of graft and immunocompatibility problems. On the other hand, tissues with high mechanical functions have been replaced with prostheses, including artificial hips, knees, intervertebral discs, and teeth. However, long-term survival of implants is limited due to mechanical mismatch between the implant and tissue (16,19,24).

Within this context, TEP can provide biological substitutes to replace, restore, or repair damaged tissue in a wide range of areas (1,2,4,12,18,27,29). Skin, however, is the most active subject of research and clinical application (18). The design of TEP includes a biologically active substance and a delivery system. Scaffolds provide a three-dimensional support for cell attachment, proliferation, and differentiation as well as for new tissue formation. Combination of material, three-dimension architecture, and degradation rates allow the conception of various scaffolds customized to specific applications. Regarding cell-based TEP for skin, the

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choice of cell type is of primary importance. Cells used for TEP need to be readily expanded in vitro and to retain biological activity for restoring and maintaining organ functions. Autologous cells are usually preferred as they avoid rejection and treatment with immunosuppressive drugs, but these are limited to the patient and the limitations to this technique reside in the in vitro expansion of the cells (7).

Biopsy from severely burned adjacent skin can be of poor quality and/or cannot yield enough cells for in vitro expansion (13). Additionally, these biopsies on the patient create a second surgical wound, which is generally painful and slow healing. Allogenic cells (not from the same individual) have been investigated as an alternative source of cells coming from human or animal (xenogeneic) sources. Again, skin is one main area of allogenic TEP (9,32), but similar to autologous cell transplantations, in vitro expansion and maintenance of a differentiated state are major limits (18,34).

Stem cells have garnered the hopes of scientists because they have high self-renewal capacity and can generate multiple cell lineages (29). They can be isolated not only from bone marrow but also from many tissues, such as amniotic fluid, brain, skin, heart, kidneys, and liver. Although widely distributed, stem cells represent only a small fraction of a tissue cell population, which requires an extensive in vitro expansion step. Another source of stem cells is blastocytes. These embryonic stem cells (ESC) can be isolated from early-stage embryo and present the advantage of being pluripotent, unlike those from adult stem cells, which can differentiate into a restricted number of cell lineages.

Cultures of stem cells are technically very demanding. Maintenance and expansion of adult stem cells in an undifferentiated state require the addition of many specific growth factors (1), and so far culture of ESC has not been possible without feeder layers (which are usually formed by animal cells), which is in some part responsible for the inconsistent colony cell growth (20). The necessity to use exogenous growth factors as well as animal products is a limiting factor for the scale-up of stem cell cultures for clinical applications. Unlike stem cells, fetal cells are differentiated cells with high expansion, regeneration, and low immunogenicity properties (2,4). They can be isolated from fetal tissues, which follow embryonic stage at 9 weeks of development.

Biological bandages composed of fetal skin fibroblasts showed convincing results in regenerating skin in wounds and second and third degree burned children (8,17). Development of fetal cell treatments shows promising results in other areas, such as in orthopedics. In response to injury, bone yields a fibrosis tissue with no mechanical properties. Human fetal bone cells were shown to have high proliferation and differentiation ability and could be used as a source of cells for TEP for bone (23,26). Although application of fetal cells in TEP is broad, high levels of safety and consistency in cell banking are common features necessary for their development in clinics. Hence, in this article we present the criteria for the establishment of a fetal skin cell bank with emphasis on the consistency and safety aspects necessary for preclinical trials.

MATERIALS AND METHODS

Fetal Skin Tissue

For this study, we have acquired four organ donations of fetal skin with written consent from each mother donor as approved by the University Hospital Ethics Committee. Organ donations ranged from 13 to 17 weeks of gestation (FS1, 14 weeks female; FS2, 17 weeks female; FS3, 13 weeks male; FS4, 14 weeks male), and a 1–4-cm^2 biopsy from the abdominal region was excised. At this stage, observations concerning the tissue received were noted (color, size, consistence) and representative samples were taken and examined microscopically to confirm that the tissue was acceptable for subsequent expansion of cells. Tissue was also sent for pathological assessment.

For each organ donation, a specific record was prepared in relation to the donor and to the fetal tissue obtained. Relevant details relating to the health of the mother who donated the fetal tissue were specifically recorded as part of the record for the original tissue. The patient name was not recorded; the patient was given a reference number for traceability following Good Clinical Practice guidelines. The mother donors were tested for infectious diseases (status of the donor for HIV, HBV, and HCV) at the time of tissue donation and again 3 months after to ensure negative sero-conversion (Fig. 1, donor testing). Testing and antibodies used were as follows: HBsAg-Cobas Core HbsAgII EIA (Hoffmann-La Roche AG); anti-HIV-1/HIV-2 (Hoffmann-La Roche AG); Cobas Core anti-HIV-1/HIV-2 EIA DAGS II (Hoffmann-La Roche AG); anti HCV-Cobas Core anti-HCV EIA (Hoffmann-La Roche AG); AXSym anti-HCV EIA, version 3.0 (Abbott Diagnostika); PCR-HCV-Cobas Amplicor, version 2.0 (Hoffmann-La Roche AG); *Treponema pallidum*: Serodia-TP.PA (Fujirebio, Almedica AG); anti-CMV-Vidas CMV IgG (BioMérieux SA); ETI-CYTOK-M reverse (Sorin Diagnostics S.r.L); *Toxoplasma gondii*-Toxo-Screen DA (IgG) and Toxo-ISAGA (IgM) (BioMérieux SA).

For the tissue, information concerning the age of the fetus, date, time, and place of acquirement were recorded. Types and amounts of tissue received and into

how many containers were also recorded. The containers were then labeled with the identification code before transfer to the laboratory. Details of the medium into which the tissues were collected (DMEM, Gibco), together with supplier, batch number, and certificates were attached to the record. Sterility and microbiological testing of the media were performed and specific containers with medium were provided to the medical doctor responsible and kept in a designated refrigerator. Prior to transfer to the laboratory, the tissue was stored for less than half a day in the designated refrigerator, under lock and key, that was accessed only by the medical doctor.

Processing of the Fetal Skin Tissue

Specific cell culture from biopsy material has been previously described elsewhere (8,17) and is represented, in part, in Figure 1. Briefly, fetal skin biopsies were cleaned of adherent tissue, washed three times for 15 min each in phosphate-buffered saline (PBS: NaCl 6.80 g/L, Na_2HPO_4 1.48 g/L, KH_2PO_4 0.43 g/L), dissected, and put into culture in tissue culture grade plates (60 cm, Falcon). Once the adherent cells reached 80% confluence, they were trypsinized (EDTA and trypsin solution, Gibco), amplified, and ampoules of cells in 1 ml of freezing medium (50% DMEM, 40% FCS, 10% DMSO) were stored in liquid nitrogen. Cell banks were stored in the vapor phase of alarm-fitted (to assure sufficient liquid nitrogen) liquid nitrogen storage vessels (Carbagas) rather than in the liquid phase, and portions of each cell bank were split between different vessels in separate locations for security.

Although very extensive data tracking is essential for GMP records, for our laboratory cell banks we prepared a certificate of analysis for each cell bank, giving its designation, number of ampoules prepared, date, tests performed, specification, and results. Ampoules were labeled with the cell bank code and each ampoule individually numbered. Logbooks were used to record all ampoule movements including the initial deposit of the cell bank and also each time an ampoule was removed, whether for testing or for experimental purposes. These changes were signed and dated in the cell bank logbook.

Safety Testing

The master cell bank (MCB) of one cell line destined for clinical use was characterized and extensively tested in the GMP facilities of Bioreliance in Glascow, Scotland to demonstrate the absence of viruses (Fig. 1, MCB testing). Testing requirements were based on those required for human diploid cells used for vaccine production (33) and for cell substrates used for biotechnological products (6). All the tests performed are listed in Figure 1.

Although the donor was obviously a human source, isoenzyme testing to show Caucasian human origin was necessary for documentation. Other tests were sterility, mycoplasma, and retroviral reverse transcriptase activity (FPERT assay). Examinations for viruses, virus-like particles, mycoplasmas, fungi, yeasts, and bacteria were done with a minimum profile of 200 cells with quantitative transmission electron microscopy. In vitro testing of picornovirus, orthomyxovirus, pramyxovirus, herpesvirus, adenovirus, and reovirus was accomplished with several control cell lines. In vivo virus testing was completed using suckling mice, adult mice, guinea pigs, and embryonated eggs. Human virus detection was screened using Q-PCR for all of the following viruses: HepB, HepC, HIV-1, HIV-2, HTLV-1, HTLV-2, HHV-6, HHV-7, HHV-8, EBV, hCMV, SV40. B19 parovirus was also screened with Q-PCR (Fig. 1, MCB testing). All of the extensive tests were accomplished and validated in the GMP facilities of Bioreliance (Glasgow, Scotland). Safety testing of the working cell bank (WCB) for preclinical and research projects can then be somewhat limited compared to the MCB safety testing because each MCB ampoule results in a full WCB (Fig. 1, WCB testing). Safety testing for laboratory research-developed cell banks need to be continually controlled for mycoplasma and bacterial contamination.

Consistency of Processing of Tissue

We derived three MCB and WCB from one initial fetal skin tissue to look at variability of fresh tissue processing. The initial fetal skin tissue was divided into three equivalent sections. Each section was treated as an independent tissue according to the procedure described above. Protein concentration and mRNA level of MMP-14, MMP-3, TIMP-3, and VEGF were measured at passage 3 in triplicate in each of the three independent cultures designated as A, B, and C. Protein concentration was measured in fetal cell extract obtained by freeze–thaw cycles with Bradford Protein Assay (Biorad, CA, USA) using albumin from bovine serum fraction V for the standard curve. Total RNA was isolated from cultured cells using the NucleoSpin, RNA II kit (Marchery-Nagel, Düren, Germany) as described by the manufacturer. One microgram of total RNA was reverse transcribed using the 50 units of StratScript reverse transcriptase enzyme (Stratagene, San Diego, CA, USA) in a volume of 50 µl containing 1× first-strand buffer (Stratagene), 3 µl of random primers (100 ng/µl) (Promega, Madison, WI, USA), 40 units of RNasin (Promega), and 2 µl of dNTP mix 100 mM (Promega) as described by the manufacturer. mRNA expression of matrix metalloproteinase-3 (MMP-3), MMP-14, tissue inhibitor of metalloproteinase-3 (TIMP-3), vascular endothelial growth

factor, and β-actin were measured by real-time PCR using TaqMan® Gene Expression Assays (Applied Biosytems). Measurements were performed in triplicates. Relative gene expressions were analyzed with the $2^{-\Delta\Delta CT}$ method and normalized to β-actin gene.

Life Span of Cell Lines

The entire life span of each cell line was determined to define limitations of cell bank usage. Cellular life span was defined as the number of in vitro passages until the growth rate was reduced to 25% of the initial maximum growth rate. Three independent cell cultures (A, B, and C) were serially cultured in monolayer from passage 2 to 18. Each week using the previous passage, three new plates with 3,000 or 6,000 cells per cm^2 were prepared for a total of 18 weeks. Cell number was manually counted after 7 days of culture for each passage.

Once the cell life had been determined for each fetal skin fibroblast cell line, some cells were retained to provide cells for testing beyond the production limit (extended passage). These cells were stored as an "extended-life cell bank" and were tested to confirm that they still possess the same characteristics as those from the WCB with respect to karyology, morphology, identity, and biological activity.

Consistency Between Biopsies of Different Tissue Donations

We derived master and working cell banks from four fetal donors (FS1, 14 weeks; FS2, 17 weeks; FS3, 13 weeks; FS4, 14 weeks) to investigate interindividual variation possibilities at several gestational ages. Protein concentration was measured with the Bradford Assay (Biorad) in fetal cell extract obtained by freeze–thaw cycles. The concentration of basic fibroblast growth factor (bFGF) was measured in fetal cell extract with the human bFGF EIA Kit (Chemicon Internationale, CA, USA).

mRNA expression of Midkine (neurite growth promoting factor 2 NEGF2 or MDK), transforming growth factor-β1 (TGF-β1), osteoprotegerin (OPG), pleiotrophin (PTN), and β-actin were measured by real-time PCR as described above.

Biological Activity of Fetal Cell Extracts

As an additional consistency test, the biological activity of the cells was tested. Fetal cell extract obtained as described above was added to the culture medium of aged adult skin cells (NP/CHFR, male, 76 years old). The number of NP/CHFR skin cells was counted manually in triplicate using a hemocytometer and trypan blue exclusion for viability after 4 days of stimulation with fetal cell extracts (concentrations ranging from 0 to 4×10^5 cells/extract) to determine if fetal cell extracts at different concentrations could stimulate aged skin cell growth and with higher concentrations to look at potential toxicity ($1–2 \times 10^6$ cells/extract).

RESULTS

Cell Banking

With our fetal tissue we deviate from a conventional process of cell banking, which is usually started with an ampoule of frozen cells. Our MCB was not from frozen cells, but derived from the fresh fetal tissue itself. A MCB of 50 ampoules of 5 million cells at passage 1 could be established from each fetal skin biopsy. Each ampoule of cells in the MCB was mixed to assure consistency and that each was identical. Ampoules of cells from the MCB were then expanded by serial subculture up to passage 2 and pooled for distribution into ampoules to form the WCB, for which vials were then used for specific research projects or preclinical trials. The WCB generated consisted of 50 ampoules each with 5 million cells in each one for each fetal tissue at passage 2 and 4, respectively (Fig. 1).

Life Span of Fetal Cell Lines

Actual cell counts were conducted each week with two media changes on triplicate plates of cells after 7 days of cell growth for two different initial concentrations of cells: 3,000 and 6,000 cells/cm^2. Each week using the previous passage, three new plates with these concentrations were prepared for a total of 18 weeks. For the concentration of 3,000 cells/cm^2, stable populations were seen up to passage 12 where cell number decreased to 25% of initial cell number. When cells were cultured at 6,000 cells/cm^2, it was possible to continue several additional passages to 17 where the growth was then reduced to 25% of the growth seen in early passages (Fig. 2). It is necessary to establish the most efficient cell life by using the minimum number of initially seeded cells, which will permit the maximum production of a finished product at the passages permitted (less than 2/3 the life of the cell line under specific conditions).

Testing for Clinical and Research Purposes

All of the tests listed in Figure 1 for MCB testing will need to have a negative result to be cleared for further use. There has to be absolute surety for the absence of contaminants, whether microbiological or viral, for a product that is destined for clinical use. All passages used for direct application will need to be batch tested with specific criteria fulfilling a batch release record. These cell lines will also need to be checked routinely for contamination just before application to the patient. Regarding research use, testing fetal cells that have been banked in a WCB should have routine testing of myco-

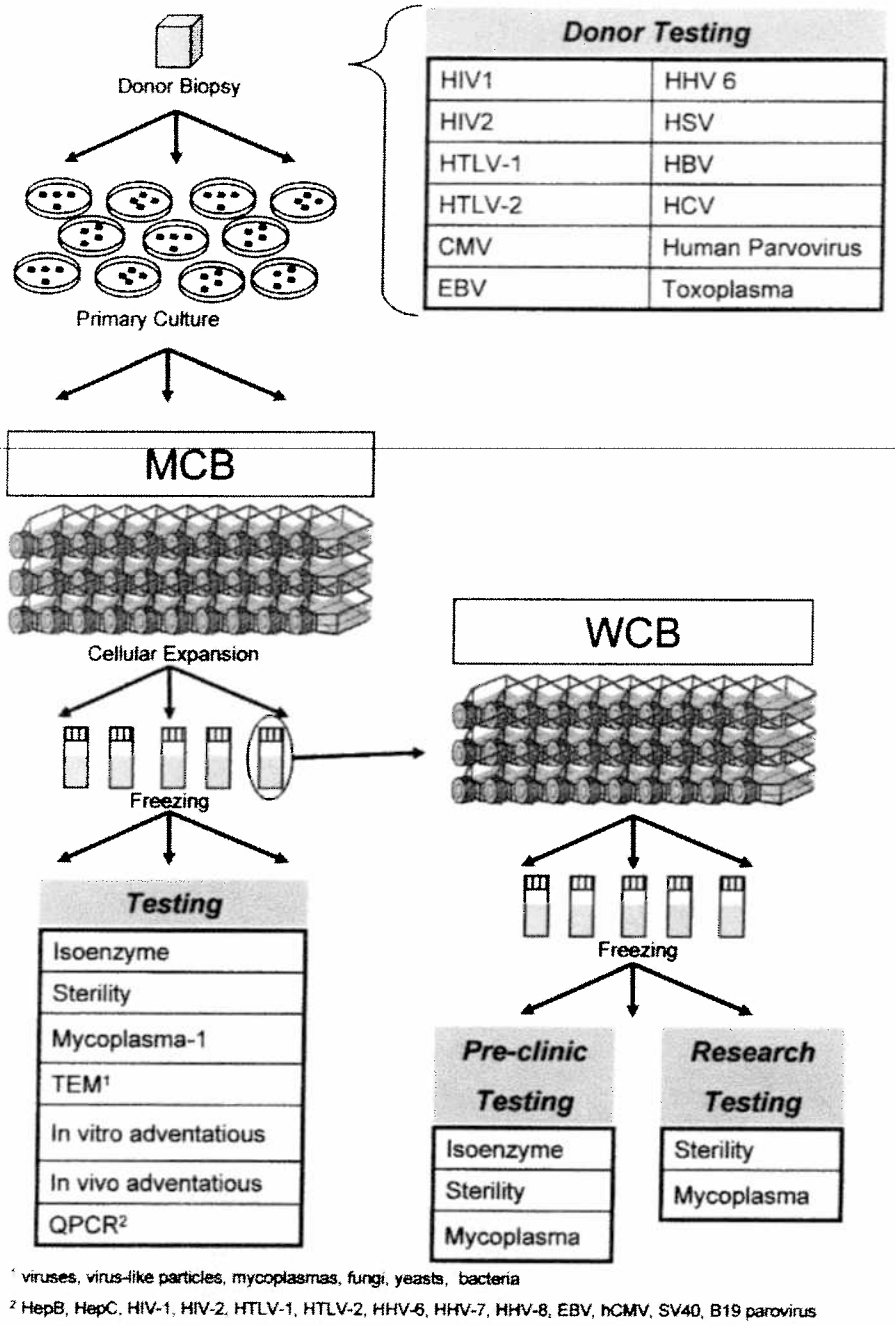

Figure 1. MCB and WCB processing with associated safety testing.

plasma and microbial contamination conducted every month on the cell lines in use (Fig. 1, WCB testing).

Consistency of Tissue Processing

From the original fetal tissue, three different cultures were developed and each of these cultures was tested in triplicate for protein concentration and gene expression (Fig. 3). Protein concentration was measured in the freeze–thaw extract of the three independent cell cultures (A, B, C) at 3,000 cells/cm^2 from passage 1 to 11. The range of mean protein concentration was 1.7 ± 0.2 µg/µl at passage 0 and 3.9 ± 0.5 µg/µl at passage 5. When looking at the variability between the three independent cultures (A, B, and C), fold difference in protein concentration between A, B, and C was the lowest at passage 0 (1.1-fold difference) and the highest at pas-

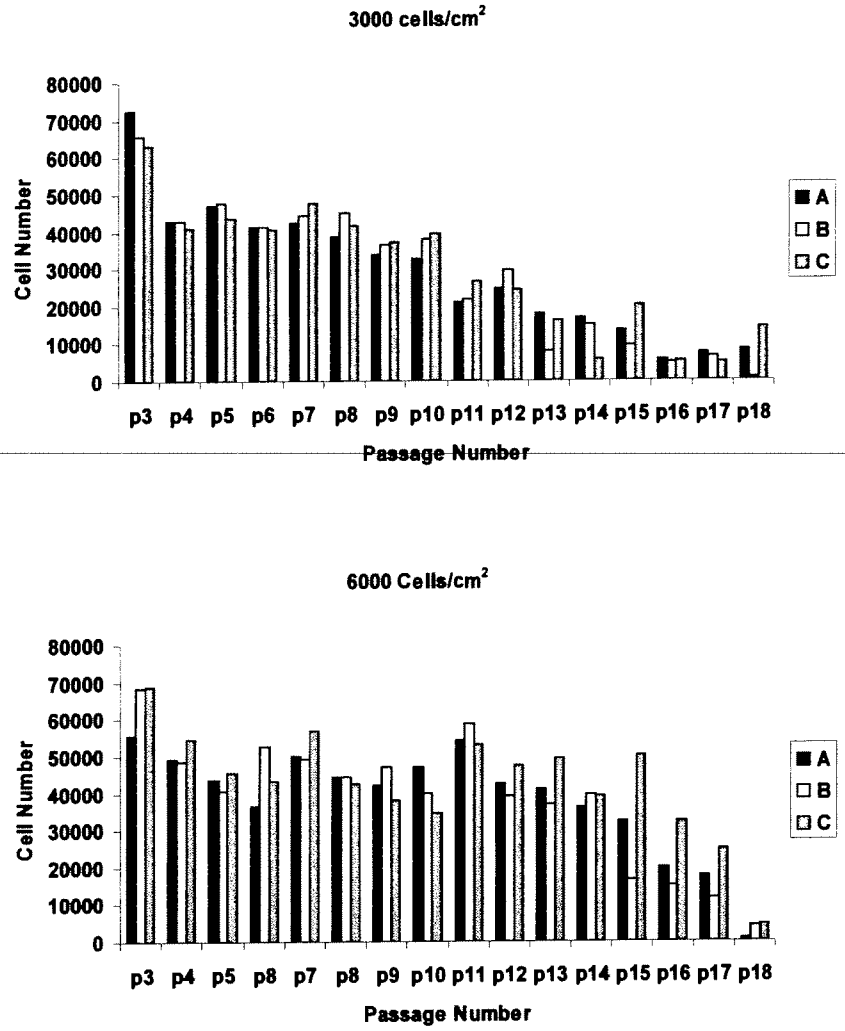

Figure 2. Cellular life span of fetal cells in culture. Number of fetal skin cells counted at each passage up to 18 passages for each of three independent cultures from the same tissue at 3,000 or 6,000 cells/cm^2 initially seeded.

sage 10 (1.4-fold difference). No significant differences were noted throughout the cell handling for the same number of cells per extraction. In addition, the three independent cultures showed relatively consistent mRNA level of genes involved in skin regeneration, MMP-14, MMP-3, TIMP-3, and VEGF, with maximal fold difference between the three independent cultures of 1.4, 1.9, 2.1 and 1.4, respectively.

Consistency Between Tissues of Different Donors

Cell cultures derived from four biopsies from different tissue donations yielded 4.1 (FS1), 4.4 (FS2), 2.9 (FS3), and 3.6 (FS4) million cells at passage 4. In addition, protein concentration measured in extracts processed by freeze–thawing three times was not different among the four cell cultures (FS1: 4.2 ± 0.7; FS2: 4.6 ± 0.4; FS3: 4.5 ± 0.2 ; FS4: 4.0 ± 0.2 μg/μl) as well as β-FGF concentration, normalized to total protein concentration. mRNA expression of MDK, PTN, TGF-β1, and OPG had a similar level among the four different cell cultures. The maximal fold differences in gene expression between the four cell cultures were 2.2, 2.2, 1.5 and 3.1, respectively, for MDK, PTN, TGF-β1 and OPG (Fig. 4).

Biological Activity

When added to aged fibroblasts, NP/CHFR, in cell culture, fetal cell extracts had a stimulatory effect on the cell growth of NP/CHFR cells (Fig. 5). The effect was dose dependent, with a 2.6 increase in growth stimulation of NP/CHFR cells seen with fetal extracts compared to controls where only buffer was added. At higher

doses, the growth stimulation remained at the maximum height seen. Much higher doses of fetal cell extract—five times what was used in experimentation for growth stimulation (2×10^6 cells/extract)—were necessary for some toxicity (20–30%) of target cells and this may have also been due, in part, to the increased volume necessary to deliver this dilution of the extract.

DISCUSSION

Fetal skin cells show qualities required for the establishment of a cell bank to be used for TEP and medicinal products. The life span and the proliferation rate of the cells allowed to build a MCB of 50 ampoules containing 4–5 million cells each from only one organ donation. Then each ampoule from the MCB can be derived into a WCB to be used for clinical or research purposes. MCB and WCB have been prepared from fetal skin tissue in short periods of time compared to other primary cells, which saves not only time but is interesting financially because GMP sterile facilities are usually rented by the day. Cell banking offers advantages for safe cell therapy application for skin TEP. Clinical uses have extended significantly for a wide variety of indications including burns, acute and chronic wounds, skin loss, surgical wounds, and bullous diseases (5,11,12,14,25,28). This means that stringent controls and testing are necessary to ensure proper safety (6).

For maximum security, it would be best to have extensive testing on cell lines used in preclinical studies. Thus, fetal cells have a major advantage because extensive material for therapeutic usage can be developed from only one organ donation. However, due to the impeding costs, cell banks destined only for research purposes are difficult to test thoroughly.

Until now, there have been no reported biopharmaceuticals derived from continuous cell cultures that have been implicated in the transmission of infectious agents to humans. Most sources of contamination are adventitious, which means that the contamination is introduced from an external source such as the medium, trypsin, or serum used in the culture process. Several points of interest in cell culture that are to be used in preclinical

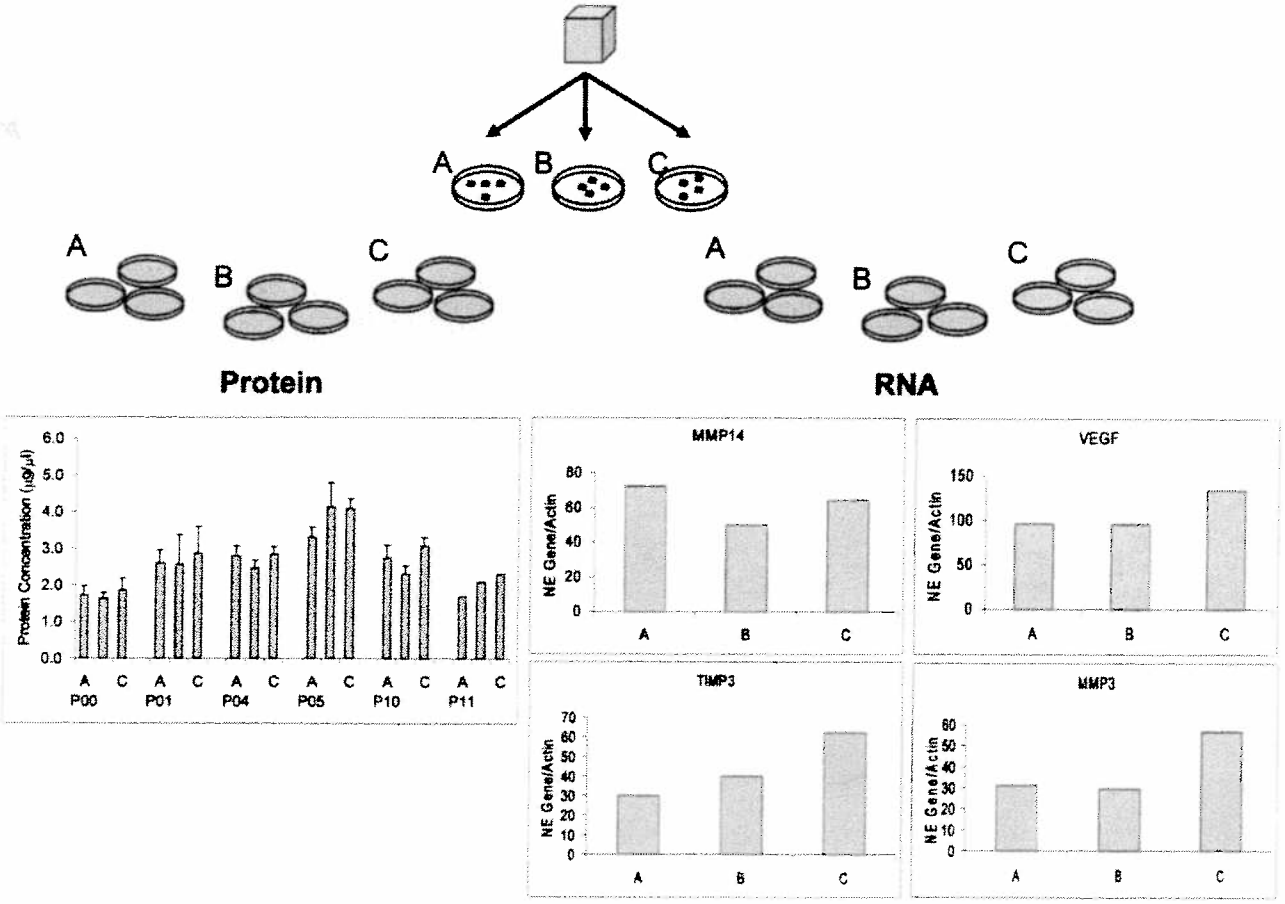

Figure 3. Consistency of the processing of tissue. Protein concentration and RT PCR of specific genes in the three independent cultures A, B, and C derived from one biopsy.

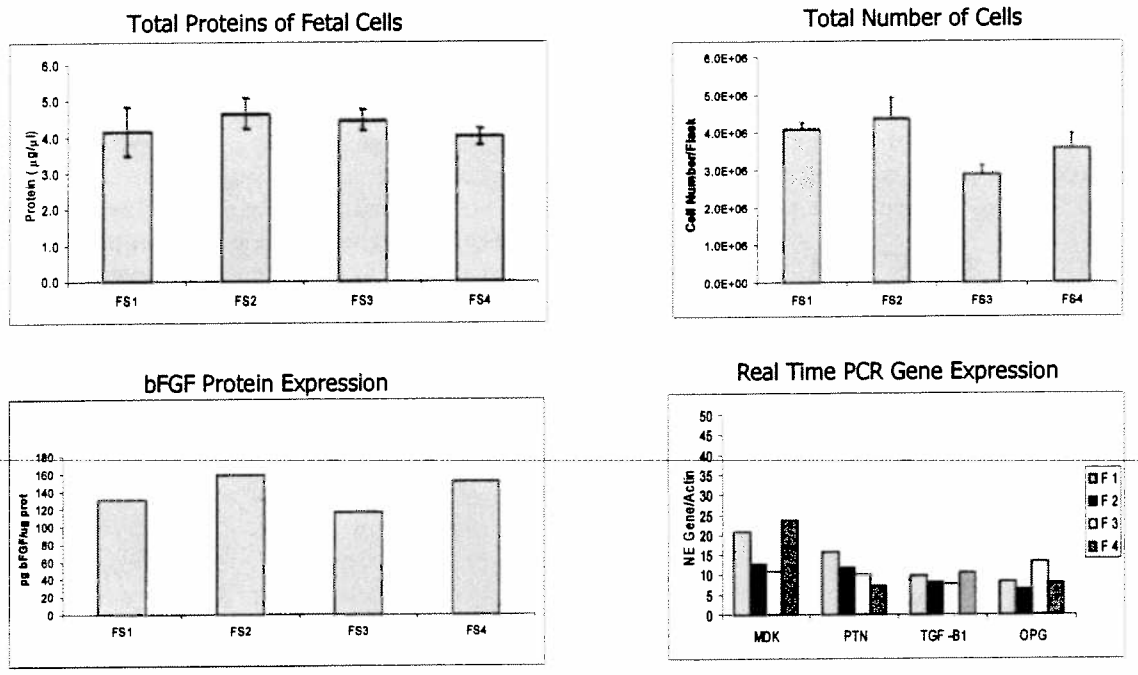

Figure 4. Cell growth, protein concentrations, and fibroblast growth factor and RT-PCR of specific genes in different donors of fetal cells.

and clinical trials are that any animal products used in the cell culture process, such as bovine serum and trypsin, should be virus tested and transmissible spongiform encephalopaties (TSE) certified (7). Reported cases of bovine spongiform encephalopathy (BSE) have made it necessary for stringent testing of serum. As only New Zealand or Australia have no reported BSE, these countries have interesting serum for research and clinical purposes (21). Other companies (i.e., Hyclone, Gibco) assure the contained and well-maintained herds for serum production and a guaranteed traceability for each bottle that is destined for cell cultures for preclinical and clinical usage. In addition, because of concerns about the transmission of bovine viruses it is recommended that the serum be inactivated (e.g., by gamma irradiation) prior to use (7). Stringent control of potential viral contamination is particularly important with this product as it is not subject to any purification steps and it is not certain what, if any, viral inactivation steps may be applied (7). If EDTA alone can be used for cell detach-

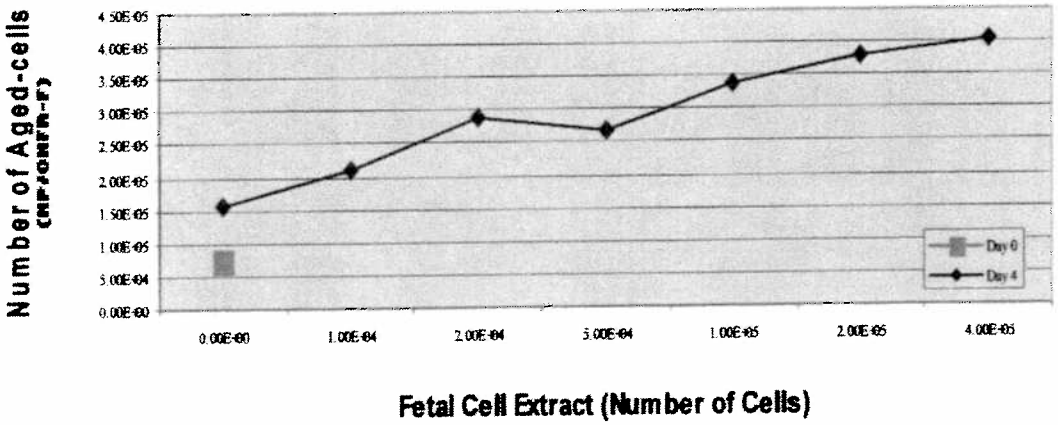

Figure 5. Biological activity of fetal skin extracts. Number of aged skin cells (NP/CHFR) activated as a function of various concentrations of fetal skin cell extract after 4 days of culture.

ment, it would be beneficial to avoid the use of trypsin because of viral safety concerns or the trypsin will need separate testing. In addition, the use of antibiotics in the medium is not an acceptable practice and should also be avoided. Thus, it is necessary to test both of the cell banks that have been produced but an increased testing should be emphasized on the MCB.

Once the MCB has been thoroughly screened and shown negative for all of the necessary testing, a less stringent testing platform should be established for the WCB that includes the isoenzyme assay assuring the identity, sterility, and mycoplasma. As extensive viral safety testing was performed on the mother donor when tissue was donated and at a 3-month interval to assure there was not sero-conversion; this provides the first security for laboratory employees. In vitro testing should be concluded to ensure no adventitious agents were introduced during the preparation of the bank either from the medium and its associated constituents used in the laboratory or via handling. These tests should be part of the routine quality assessment of cell cultures each month.

Regarding the use of the cells for only preclinical trials, it will be particularly important to ensure consistency of growth of the cells and consistency of the harvest obtained. High consistency in fetal cell banking can be achieved due to the minimum nutrient requirements of fetal cell cultures. In contrast with mesenchymal stem cells, fetal cells do not require feeder layers for growth nor growth factors for differentiation (10,29,30). For skin TEP, many products to date use a pool of multiple donors for a batch of cells. The differences between donors would not be as important because many donors could be used. We have seen herein low heterogeneity between four different fetal donors for skin cell banks established in the same manner (large-scale expansion). Cell counts, protein determination, and gene expression analysis are useful tools to assess the consistency of the cell banks when there is only one unique donor for skin TEP. Limits of variation for these assays specific for each cell type will need to be established internally to define the consistency of the process. These criteria would be sufficient for limited preclinical trials in a university situation. However, if the goal is to develop a "product" used on a large-scale or a multicenter basis, assessment of the cell-derived product should not be restricted to description parameters such as protein concentration but should include a functional assay (22). Furthermore, activity of the final product is likely to be due to synergistic effects of multiple proteins, which cannot be assessed by individual protein concentration or activity.

As can be seen, each element necessary to produce a successful TEP needs to verify safety and consistency in the development. Cell choice for TEP is of utmost importance, and progress to assure the consistency will be necessary before allowing preclinical trials. Fetal cell research was seriously impeded in the 1980s. Because academic programs could not easily continue fundamental research on fetal cells, the means to establish better primary cell cultures and study consistent processing lost at least a decade of advancement. It was research on stem cells that brought cell choice back into the center of study for TEP (3,10,15,27,30,31). Using only one fetal organ donation, it is possible to develop extensive MCB and WCB. Fetal cells, as already differentiated lineage, need low nutritional requirements (i.e., no feeder layers or external growth factors) to establish a fully defined consistent cell bank and can easily be assessed for safety, assuring an interesting cell choice for TEP.

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