From Bench to Bedside

Lee Ann Applegate at the University Hospital of Lausanne (CHUV/UNIL) and Dominique P Pioletti at the Swiss Federal Institute of Technology Lausanne (EPFL) investigate how translational medicine can bring safe cell therapies to the patient

Although we need to continue our efforts in basic sciences and research for the discovery of fundamental knowledge, we also need to focus on how this knowledge can improve human health. Often, it is not a single view or approach that will lead to discoveries, but rather the interface of disciplines that combine multiple fields of knowledge such as engineering, biology and medicine. An interdisciplinary approach, allowing for a new way of thinking, can benefit the future of human health and enable new therapies to reach the clinic more rapidly.

This approach can be used for cellular therapies combined with tissue engineering to provide delivery systems for efficient use in the clinic. However, it is first necessary to have an understanding of the scope of new FDA and EU regulations for cellular therapies, adequate fundamental knowledge in order to make the correct cell choice, and close interdisciplinary collaboration to assure the best developed delivery system and the manner in which this will be applied to the patient. Translational medicine is the process where the interdisciplinary biomedical community focuses on moving research discoveries from the laboratory into routine clinical practice in order to diagnose and treat patients (see Figure 1).

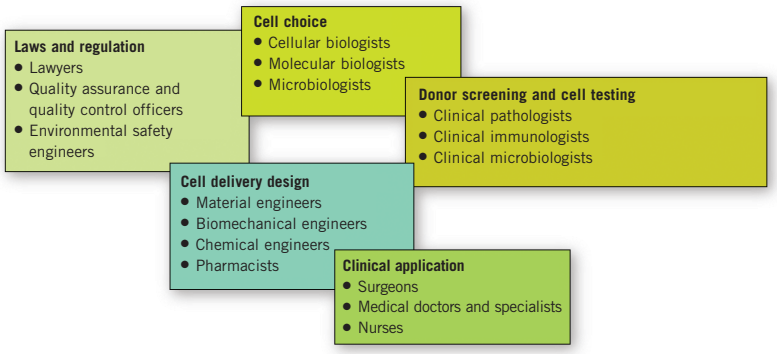
CELL CHOICES, DELIVERY AND REGULATION OF CELLULAR THERAPIES

Cellular therapy is becoming an interesting addition to medical therapies for repairing, restoring or ameliorating the function of tissues. Some cell choices are more adaptable to cellular therapy in patients. Tissue choices from animals and humans at all stages of development can be evaluated with the advantages and disadvantages for each final cell type (see Figure 2, page 32). There is some confusion between the terminology and potential of embryonic, foetal and adult stem cells. This is understandable since the terms are used for different contexts. In legal aspects, the term 'embryo' denotes the earliest stages following fertilisation of an ovum by a sperm. A zygote would include early stage cleavage embryos produced by cell division up to 50- to 60-cell stage (each cell which is a blastomere) and the blastocyte for the 60-cell stage to the point of implantation at about two weeks post-fertilisation. Pathology would classify the embryonic stage as up to nine weeks after gestation, and thereafter until birth would be classified as the foetal stage.

From each of these tissues, different cell lines can be established, but with a varying

Figure 1: Translational medicine: interdisciplinary approach for cellular therapies

Multiple fields of expertise are necessary to develop a successful platform for translational medicine of cellular therapies: lawyers for interpreting regulatory issues; biologists for the most appropriate cell choice; engineers for delivery and tissue engineering design; and medical doctors for donor screening and clinical application of final defined therapeutic cellular products.



range of complications in tissue culture techniques. Embryonic stem cells are developed from pre-implantation embryos from the inner-cell mass before the first two weeks of development. These cells are frequently obtained from extra embryos developed by *in vitro* fertilisation techniques to aid couples for fertility purposes. There has been a moratorium on the use of embryonic stem cells since 1975, with new laws in 1993 permitting their use under certain circumstances. These particular cells form the centre of an ethical debate, and other researchers have begun using foetal, embryonic cells derived from voluntary interruption of pregnancy between five and eight weeks. Cell lines are normally developed from the genital ridge of the foetus. As this tissue is considered an organ donation in most countries, it bypasses the major problems that have been raised with regard to embryonic stem cells. These cells are indeed covered by federal funding which was re-instated rather quietly by the Bush administration in 2002.

Most foetal cell research is developed from specific tissues obtained at the latter end of the first trimester (11 to 14 weeks) following voluntary interruption of pregnancy, which is considered legal in most countries. Cell lines at this stage are tissue-specific and cells are therefore differentiated with specific functions. Federal funding for these types of cells was also affected by the moratorium but re-instated in 2002, as the cells are derived from a legal organ donation, as long as the mother donor gives informed consent, the tissue is a donation and not paid for, and that there is no change in timing or method of pregnancy interruption for the sake of research.

Since the 1930s, medical doctors and scientists have used tissue from voluntary pregnancy interruptions not only for understanding cell biology but also as an important entity in the development of vaccines by using defined tissue-derived cell lines. The Nobel Prize for Medicine in 1954 was awarded to American immunologists who developed the polio vaccine based on cultures of human foetal cells. Since this time, many other necessary vaccines (rubella, chicken pox, hepatitis A and so on) have been developed with the use of foetal cell lines including two primary human diploid cell lines which were originally prepared in the 1960s. The first cell line, WI-38 (Wistar Institute 38) was developed by Leonard Hayfleck in 1964 from foetal tissue from a voluntary pregnancy interruption and later given the ATCC (American Type Culture Collection) number of CCL-75. This cell line was used for the historical production of vaccine RA 27/3 against rubella (1,2).

Adult stem cells, frequently referred to as mesenchymal stem cells, have raised the hopes of scientists for new treatments because they have high self-renewal capacity and can generate multiple cell lineages. They can be isolated not only from bone marrow but also from many other tissues such as amniotic fluid, adipose tissue, brain, skin, heart, kidneys and liver. Although widely distributed, adult stem cells represent only a small fraction of a tissue cell population (often only one for every 10^4 to 10^5 cells), which requires an extensive *in vitro* expansion step.

However, stem cell cultures are technically very demanding. Maintenance and expansion of stem cells in an undifferentiated state require the addition of many specific growth factors. Culture of these cells without feeder layers (which are usually formed by animal cells) is difficult and feeder layers are responsible for some aspects of inconsistent colony cell growth. The need to use exogenous growth factors as well as animal products is a limiting factor for the scaling out of stem cell cultures for clinical applications (3,4).

Unlike stem cells, foetal cells are differentiated cells with high expansion, regeneration and low immunogenic properties. They can be isolated from foetal tissues, which follow the embryonic stage after nine weeks of development. Foetal cells have extensive expansion possibilities and cell culture requirements are minimal compared to stem or mesenchymal cell types. As the foetal cells are already differentiated and do not need to be directed or altered, the vast number of additional growth factors normally necessary are not needed for cell culture and expansion (5-12).

Cell choice is of utmost importance and each element of processing needs special attention to produce a successful cell therapy and to verify safety and consistency before entering clinical trials.

Whole cell bioprocessing and adaptable procedures to Good Manufacturing Processes (GMP), which make it possible to develop extensive master cell banks

Figure 2: Cellular source and development stage

Cellular source can come from human and animals at different stages of development including embryonic, embryonic-foetal, foetal and adult involving different beginning tissue sources ranging from zygotes to specific tissues (bone marrow, adipose, amniotic fluid, skin, liver, bone, cartilage and so on)

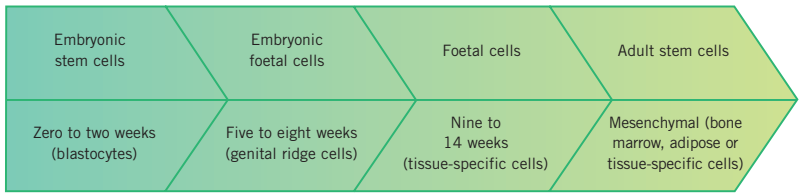
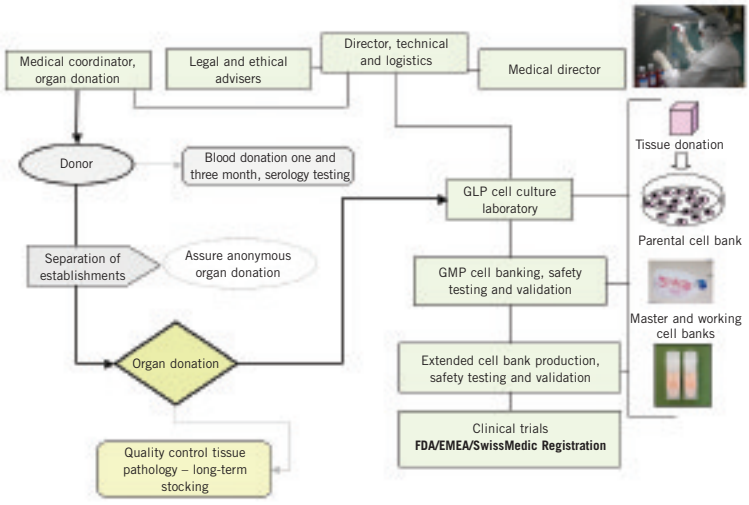


Figure 3: Organisation of a cell therapy platform

Director of technical and logistics coordinates the programme with essential legal and ethical advisors and a medical director for interpretation of medical quality assurance (serology and pathology reports). The separation of hospitals for the organ donation and all other aspects of the platform including serology, pathology and the GLP cell culture laboratory can assure complete anonymous and coded organ donations. Importantly, the Director of Technical and Logistics is not involved in any manner in the organ donation process as required by law. Final approval for use of validated cell banks for human therapy is coordinated and approved with both Hospital Ethics Committees and national regulatory agencies (such as EMEA, FDA and SwissMedic).



(MCB) and working cell banks (WCB), will facilitate thorough testing (see Figure 3). Once MCBs are produced, WCBs can be created to establish individual batches of treatments for high numbers of patients. Further, these cell banks can be thoroughly tested for safety with regard to sterility, pathogens and adventitious agents and tumorigenicity. Once safety is assured, efficient cell presentation with biocompatible delivery systems can be assessed for specific tissues. For delivery systems, biocompatible biomaterials need to be available in order to provide an extracellular matrix environment for cell differentiation, delivery and release. The cell and materials need to be tested together, not only to assure biocompatibility, but also their interactions, cellular stability and possible degraded by-products of combination and degradation or absorption. Ease of applicability of the final product will be of importance for clinical use.

All cellular products must be in compliance with GMP guidelines with respect to medicinal products and investigational medicinal products for human use. The EU regulation on advanced therapy medicinal products (ATMP) was adopted in all European Member States on 30th December 2008, and the FDA recently also proposed regulations on human cells, tissues and cellular and tissue-based products. The main scope of the regulations is to establish clear classification criteria for many new cell-based medicinal products. For the EU, it makes reference to the 2004/23/EC directive on donation, procurement and testing of human cells and tissues, as well as the directive 2002/98/EC on human blood and blood components. Together, these directives dictate that human cells have to be in compliance with the quality requirements therein described, and that all ATMP have to be prepared under GMP conditions. Key elements, including identity, purity, sterility, stability, safety and efficacy, are recommended for cellular-based products. These new regulations impose a strict criteria for the production and environment used for the production of cell-based products for clinical trials and treatments (13-15).

CLINICAL APPLICATION

Risk assessment of final cellular products is of utmost concern. Self-renewal of undifferentiated cells presents a potential for tumour formation. Certain techniques, such as cellular cloning or encapsulation of cellular products, are alternatives that can assure safety. Many cellular-based therapies will not be consistent regarding the total cell population. Associated accessory cells raise additional questions for potential risk, as well as their physiological role after administration. Cellular death of transplanted cell populations, ectopic tissue formation or migration from the site of administration could be problematic when cellular therapies are within anatomically sensitive areas, such as for the central nervous system, joint regions or myocardium. Preclinical animal models for specific pathologies or tissue repair are an important step, and special care in the interpretation of pertinent safety and biological activity needs consideration. Appreciation of relevant advantages and limitations needs to be well assessed for animal model choice.

CONCLUSION

Cell-based therapies are being developed and introduced for all types of tissue repair including skin, bone, cartilage, muscle and spine, among others (5-12, 16). They offer promise for repairing and replacing damaged tissue and restoring lost functionality. One of the major challenges for assuring that more patients will benefit from cell-based therapies in the future will be the optimisation of the choice of cell type, as well as their isolation and proliferation. Equally important will be the delivery system of the cell choice and their interaction with these scaffolds to assure biocompatibility. The development of MCB from the cell choice provides a major advantage for the creation of a therapeutic biological agent. Careful selection of donors and extensive screening of both the donor and cultured cells avoids transmissible viral, fungal or bacterial disease and therefore can provide a safe and secure use of cells for therapeutic purposes. Clear regulatory affairs of cellular use, particularly for organ donation of embryonic and foetal cells, will be necessary to help researchers and clinicians in future therapies (17-19). Overall, cooperative interdisciplinary efforts and cooperation to form successful translational medicine platforms in universities and hospitals will help to ensure further patient safety.

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About the authors

Lee Ann Applegate acquired her BS at South Dakota State University, and PhD at the University of New Mexico with post-doctoral appointments at Lovelace Medical Center, MD Anderson Cancer Center and Baylor College of Medicine. She moved to Switzerland for an International Fellowship for Cancer Research Award (IARC) in 1989 and is the Director of Cellular Therapies in the Department of Musculoskeletal Medicine at the University Hospital (CHUV) in Lausanne, Swizerland. Professor Applegate has developed cellular therapies for different clinical applications using progenitor cells from musculoskeletal tissues, particularly for the treatment of burn patients. Email: lee.laurent-applegate@chuv.ch

Dominique Pioletti received his MSc in Physics from the Swiss Federal Institute of Technology Lausanne (known as EPFL) in 1992. He pursued his education in the same institution and obtained his PhD in Biomechanics in 1997. He developed original constitutive laws, taking into account viscoelasticity in large deformations. He then spent two years at the University of California, San Diego as a post-doctoral fellow acquiring a greater understanding of cell and molecular biology, with particular interest in gene expression of bone cells in contact with orthopedic implants. Since April 2006, Dominique has been appointed Assistant Professor tenure-track at the EPFL and is Director of the Laboratory of Biomechanical Orthopedics. Email: dominique.pioletti@epfl.ch