CURSO AVANÇADO EM ENGENHARIA DE TECIDOS E REOLOGIA

ADVANCED COURSE ON TISSUE ENGINEERING AND RHEOLOGY

Scientific Programme – 27 de Janeiro de 2006

PROGRAM

08:30-08:45 h	**Registration**
08:45-09:00 h	**Opening**
09:00-09:30 h	**Introduction to regenerative medicine and biotherapies** Prof. Jean-François Stoltz
09:30-10:00 h	**Ex-vivo expansion and differentiation of human hematopoietic stem cells supported by human stromal-based culture systems** Prof. C. Lobato Faria
10:00-10:30 h	**Discussion** Coffee Break
10:30-11:00 h	**Matrix metalloproteinases as modulators of tissue response to artificial matrices.** Dr. Henrique S. do Rosário
11:30-12:00 h	**Mechanobiology and cartilage tissue engineering** Prof. Sylvaine Muller
12:00-12:30 h	**Discussion**
12:30-14:00 h	Lunch Break
14:00-14:30 h	**Neuronal production *in vitro* from embryonic stem cells** Prof. Doutor Domingos Henrique (FML/IMM)
14:30-15:00 h	**Oxygen therapeutics: current issues and new challenges** Prof. Patrick Menu
15:00-15:30 h	**New fluorescence molecular tools of bio-imaging to monitor membrane potential in multiphoton mode** Prof. Dominique Dumas
15:30-16:00 h	**Discussion**
16:00 h	**Closing**

INTRODUCTION TO REGENERATIVE MEDICINE AND BIOTHERAPIES

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SUMMARY

Most human tissues do not regenerate spontaneously, this is why cell therapies and tissue engineering are promising alternatives. The principle is simple: cells are collected in a patient and introduced in the damaged tissue or in a tridimentional porous support and harvested in a bioreactor in which the physico-chemical and mechanical parameters are controlled. Once the tissues (or the cells) are mature they may be implanted.

In parallel, the development of biotherapies with stem cells is a field of intensive researches given the hopes for clinical applications that it brings up. Embryonic stem cells are potentially more interesting since they are totipotent, but they can only be obtained at the very early stages of the embryo. The potential of adult stem cells is limited but isolating them induces no ethical problem and it has been known for more than *40 years* that bone marrow does possess the regenerating functions of blood cells. The properties of blood cells from the umbilical cord are forerunners of the haematopoietic system but the ability of these cells to participate to the formation of other tissues is more problematic. Finally, gene therapy, has been nourishing high hopes but few clinical applications can be envisaged in the short term, although potential applications are multiple (haemophilia, myopathy…).

Examples of this new regenerative therapeutic medicine are developed in this work.

1. REGENERATIVE MEDICINE, CELL THERAPY AND ENGINEERING

For more than 10 years, both medical practice and health sciences have benefited from advances in the fields of fundamental biology, physics/chemistry, mechanics and their engineering applications.

Most human tissues do not regenerate spontaneously and healing is a defective option that may be associated with a contraction probably capable to thwart regeneration. This is why "preparation of biotissues" or development of biotherapies represent promising alternatives. Their principle is simple: cells collected from a patient or a donor are either introduced with or without modifying their properties (introduction of genes) into a support primarily made of three-dimensional porous polymers, or cultured inside a bioreactor in which physicochemical parameters and mechanical stress are controlled. Once the tissue is fully mature or the number of cells adequate, engraftment may be performed. *In vitro* preparation of tissues such as cartilage, bone, blood vessels, cardiac muscle, skin, ligaments, inspires great hopes for the next decades, with perspectives of new therapies to restore tissue functions.

A large number of potential methods with many variants exist for each tissue or type of therapy. For example, the amounts of tissue produced by *in vitro* cultures are generally higher when three-dimensional porous supports are used than with monolayer cultures. Moreover, as well as biochemical parameters, mechanical stress influences the differentiation of cells that are used. Such changes are now considered as critical not only for understanding pathophysiological mechanisms (osteoarthritis, inflammation, atherosclerosis…) but also for tissue reconstruction. For example, the quantities and quality of cartilage obtained depend on the intensity, magnitude, and frequency of mechanical stress. This fact seems natural since it is well known that prolonged immobilization, implying absence of mechanical stress, weakens cartilage, bone, and muscles and decreases their respective mass.

In other respect, biotherapies have also major applications in the field of cancer therapies (i.e. leukemia, melanoma, prostate cancer....) and degenerative diseases (myopathies, neurological diseases…).

Thus, the issue of *in vitro* tissue or cell culture is multifaceted as it involves genetics for the choice of initial cells (progenitor cells, differentiated cells or genetically modified cells), biochemistry for the choice of the polymeric scaffold and of the culture medium and mechanics. To the previous matters must be added practical considerations such as optimization of the size and shape of materials produced that must allow the physiological medium to circulate everywhere during the growth period. Finally, once good quality tissue or cells have been obtained, one must be able to use them to replace the damaged tissue while ensuring adequate compatibility.

2. PROGENITOR CELLS AND BIOTHERAPY

Progenitor cells currently represent an expanding area of research, stimulated by their potential clinical applications in the fields of cell therapy and tissue repair.

Embryonic progenitor cells are potentially the most interesting as, by contrast with those found in adult human tissues, they are the only ones to be "totipotent", i.e. able to differentiate into all varieties of adult cell types. One can easily envision the ethical issues generated by the collection and utilization of such cells. For instance, it must be emphasized that using human embryonic progenitor cells is banned in many countries.

Although adult progenitor cells have a more limited potential (they are multipotent) but their collection poses no ethical issues. Since the 1960s, it is also known that bone marrow has a function of blood cell regeneration which involves its hematopoietic progenitor cells. Progenitor cells present in the bone marrow include hematopoietic progenitors and other cell populations such as endothelial and mesenchymal stem cells. The latter play a role of growth support for hematopoietic progenitors through cytokine secretion and cellular contacts they create either directly (production of adhesion molecules) or indirectly (production of extracellular matrix components). Mesenchymal stem cells may differentiate into a host of tissue cells, including osteoblasts, chondroblasts, myoblasts…

Finally, cells of the umbilical cord blood are fetal progenitors. Despite their properties of precursors of the hematopoietic system, their capacity to participate in the formation of other tissues is not well documented today.

Two main options are currently available for repairing a damaged organ or tissue:

– graft replacement: despite continuous technical refinements offering an increasingly improved quality of life to the patient, there is a cruel lack of material available for transplantation.
– injection of cells or preparation of biotissue to replace destroyed cells represent the so-called cell or tissue therapies. Although very appealing, this approach has its drawbacks that must be overcome. Collected cells must be available in adequate amounts or grown in culture. Then, they must be transferred into the tissue to be repaired where they must multiplicate and appropriately exert their function.

Cell plasticity is also important, although its mechanisms are not perfectly known. Indeed, it has been shown that bone marrow cells collected from a patient and injected into another tissue may participate in tissue regeneration. Thus, when stem cells are injected into a myocardial infarct lesion, it seems reasonable to hope that this area will be "repaired" and will in part recover its contractile properties, although no one knows precisely which cell types are regenerated and how the cardiac function is restored. Is "plasticity" revealed in this way or does this correspond to injection of an adult pluripotent stem cell population? Further research will be needed to elucidate those mechanisms.

3. WHICH TISSUES OR ORGANS CAN BE REGENERATED, OR WHICH DISORDERS CAN BE TREATED?

Many therapeutic domains are candidate in the mean term, including treatment of hematological, oncological, cardiac, vascular, osteoarticular, derma-

tological, ophthalmological, degenerative neurological disorders…. However, exploitation of most adult stem cells because they are unipotent, i.e. able to produce only one type of cells. Only bone marrow, brain, and probably adipose tissue appear to be able to provide multipotent cells. They are scarce and difficult to access, we still do not know how to localize them *in vivo* and they cannot be directly utilized. Furthermore, To date, only those hematopoietic and mesenchymal stem cells that can be collected from the bone marrow have been used for therapeutic applications.

3.1. Blood – Hematopoiesis

The bone marrow was the first organ in which progenitor cells were identified. Such cells were used in the treatment of hematological disorders for more than half a century. Not only are hematopoietic progenitor cells widely used nowadays in the treatment of malignant hematological disorders and immune deficiencies; their indications also encompass the treatment of autoimmune disorders and some solid tumors. Hematopoietic progenitor cells are used in both the autologous and allogeneic settings. In the autologous setting, infusion of hematopoietic progenitor cells allows the use of myeloablative intensification chemotherapy protocols, before autologous transplantation enables hematopoietic recovery. Although autologous transplantation of hematopoietic progenitors does not exert in its own right a therapeutic effect on the disease, it corrects a major side effect of antitumoral chemotherapy. Available data from both animal studies and the first clinical studies conducted in humans show that concomitant treatment with mesenchymal stem cells accelerates the post-transplantation hematopoietic recovery.

In the allogenic setting, hematopoietic progenitors have a therapeutic impact on the disease via their GvL (Graft versus Leukemia) or GvT (Graft versus Tumor) immunological effect. This effect particularly marked in leukemias of myeloid origin is now strongly enhanced by non-myeloablative pregraft conditioning. Many studies are being conducted to try to dissociate the GvL effect from the GvHD (Graft versus Host disease) effect which is a detrimental sequel of hematopoietic stem cell allograft. The first trial of mesenchymal stem cells injected in a hematopoietic stem cell allograft context has shown that such cells improve graft tolerance (with less rejection **and** GvH reactions). In addition, they differentiate inside the body into fat, bone, and cartilage and hence potentially have the capacity to repair bone and cartilage lesions. That is why a first clinical trial was conducted in children suffering from osteogenesis imperfecta, a disease that produces a brittle skeleton. Following total bone marrow allograft, mesenchymal stem cells cultured from cells provided by the same donor as for the hematopoietic stem cell allograft were injected in 2 steps and were able to differentiate into osteoblasts, thus contributing to spectacular improvement in the growth of treated patients.

Finally, it should be noted that a French team was recently able to produce functional human red blood cells *in vitro* from CD34+progenitor cells, which makes it reasonable to hope a

future alternative to transfusion with *in vitro* cell preparations of red blood cells for patients with rare erythrocyte phenotypes.

3.2. Heart failure

Heart failure is still a major public health problem. Limitations of both drug therapy and heart transplantation are now well known and new approaches are being contemplated. Cell therapy could be an alternative to conventional therapies. The principle consists in injection of cells into the destroyed area in order to restore some function. Experimental research has shown that transplantation of myoblasts collected from skeletal muscle improves left ventricular function in post-infarct patients. However, myotubes differentiated in this manner retain a phenotype of skeletal muscle and do not seem physically connected to the host cardiac myocytes. These characteristics might reduce the functional efficacy of this type of graft or even facilitate the development of ventricular arrhythmias. In parallel, other studies suggest that because of their plasticity, some bone marrow cells can acquire the characteristics of the tissue into which they are implanted. Several studies have confirmed the value of such bone marrow progenitor cells in the treatment of ischemic heart disease.

Current investigations are conducted to identify those bone marrow cells which are best suited for cell transplantation and cardiomyogenic differentiation. Results obtained in animals have demonstrated the capacity of bone marrow cells to differentiate without purification nor pre-treatment into cardiac cells, albeit with a low rate of differentiated cells. In parallel, a significant improvement in cardiac function after transplantation of hematopoietic progenitor cells like CD133+ into an infarcted myocardium has been reported. Current research has 3 main objectives : (1) to characterize the cardiomyogenic potential and/or endothelial differentiation of stromal cells; (2) to determine the functional effects of transplantation of these cells, as compared with skeletal muscle myoblasts, in animal models; and (3) to investigate the mechanisms responsible for an improvement in cardiac function following stem cell transplantation. These preclinical studies, while determining to what extent recovery of the contractile function can be attributed to angiogenesis or paracrine processes, will characterize the differentiation potential of bone marrow cells into the myocardium. In a recent work, we have assessed by using a Pinhole Gated single photon emission tomography (Pinhole Gated SPECT) the presence, extent and localizations of 3-months old myocardial infarction in rats, the in vivo localization of intramyocardial implanted Bone Marrow mesenchymal cells and the impact of this cell therapy on myocardial perfusion defect.

3.3. Cartilage

Development of a biocartilage by engineering is an expanding domain which needs not only characterization of cells required for the neosynthesis of a replacement matrix (chondrocytes or stem cells), but also the choice of a scaffold.

In animals, it has been shown that cartilage lesions can be improved by implantation of biomaterials that contain chondrocytes. For that reason, the use of adult chondrocytes harvested from cartilage tissues is currently being assessed in humans. In addition to the fact that a piece of healthy cartilage must be collected by biopsy, other drawbacks of this method are the use of mature cells with limited proliferation potential and of monolayer cultures which might induce a dedifferentiation of chondrocytes into fibroblasts. Moreover, it appears that fibrocartilage predominates. Several works have recently shown that mesenchymal stem cells collected from bone marrow can differentiate *in vitro* into chondroblasts thanks to the addition of TGFβ, IGF, BMP2 or FGF, thus enabling the synthesis of type II collagen. Thus, because of their proliferation and differentiation capacities, mesenchymal stem cells seem to represent an interesting pathway to obtain cartilage cells.

However, other factors appear to play a role in the differentiation of those cells into chondrocytes. Indeed, cartilage is an avascular connective tissue able to meet the biomechanical requirements of the locomotor apparatus but its regeneration capacities, if any, are limited. Furthermore, it is submitted to pressure forces that are a key regulator of its functional capacity. Chondrocytes synthesize an extracellular matrix that contains several types of collagen (in particular type II at initial stages and type X at later stages), proteoglycan, more specifically aggrecan, and hyaluronane (HA). Quantitative and qualitative consistency of those components is required for maintenance of the cartilage structural and functional integrity. It also noteworthy that the extracellular matrix is in contact with the synovial fluid, whose redox status and contents in both ionic charges and protein can influence chondrogenesis. Thus, arthritis and any inflammatory state of the synovial fluid may elicit secretion of pro-inflammatory cytokines such as IL-1, which exert a detrimental action in terms of cartilage synthesis. By contrast, hypoxia seems beneficial.

The mechanical forces and strain to which joints in motion are submitted *in vivo* (0-20 MPa at the level of the hip) results from a complex combination of tension forces, shear stress, and compression, the latter being most important for the cartilage. Chondrocytes are particularly responsive to these forces which in extreme cases may disturb their metabolism, thus adversely changing the mechanical properties of the extracellular matrix and the interactions between cells and the matrix (focal adhesion phenomena). Thus, when immobilized, cartilage looses its mechanical resistance properties. In addition, excessive work of cartilage, without any rest phase, accelerates its degeneration. It would therefore appear that a compromise between compression intensity and time on load has to be found when developing a repair tissue. It is now acknowledged that mesenchymal stem cells of articular joints use mechanical signals to regulate their metabolic activity. Application of a mechanical stress such as pressure interferes with cell physiology as it deforms chondrocytes and triggers cellular and subcellular mecanotransduction events. The impact of characteristics of the mechanical

stress, including its frequency, length, and magnitude, on essential properties of the chondrocyte such as its differentiation potential and ability to synthesize the extracellular matrix is now well documented.

Quite interestingly, some authors have reported that differentiation of mesenchymal stem cells into chondroblasts was more efficient when mechanical stress was imposed to cells. To this aim, harvested mesenchymal stem cells were placed in presence of TGFβ on three-dimensional scaffolds made of hyaluronane gel. Cells were then submitted or not submitted to daily mechanical pressure for several weeks. After a 3-week culture, it was shown that the mechanical stress accelerated differentiation of mesenchymal stem cells into chondroblasts, which was reflected by enhanced synthesis of aggrecan and type II collagen.

Different synthetic, organic, and hybrid biomaterials have been proposed for cartilage engineering. Examples of synthetic or biological compounds include polymers of polylactic and polyglycolic acids, collagen-based and fibrin-based supports and polysaccharidic polymers such as hydrogels. Hydrogels are made of reticulated polymers with a specific capacity to absorb large quantities of water. From a mechanical point of view, hydrogels have the advantage of utilizing water in the same manner as cartilage. Secondary to compression, water is expelled from hydrogels, which enables them to "adsorb" the shock. As soon as the pressure stops, water returns into the material which recovers its initial volume. From a biological point of view, hydrogels represent a three-dimensional environment that is porous enough to permit cell proliferation and nutriment transport. Sodium alginate hydrogels are a reference model for studies on cell morphology, proteoglycan and collagen synthesis or biomechanics of articular chondrocytes. Sodium alginate is not a natural component of the extracellular matrix but its structure is similar to that of cartilage glycosaminoglycan and it has been proposed as a valuable material to maintain the chondrocyte phenotype. An MIT team in BOSTON has recently developed an injectable polysaccharide-based gel intended for treatment of cartilage lesions. This gel contains a photosensitive molecule that is photopolymerized when exposed to UV light.

The search for a biomaterial-made support with optimal biomechanical characteristics is one of the major current issues of cartilage engineering. Several lines of research are currently followed:

- cell selection (autologous chondrocytes or progenitor cells)
- cell biomechanics, in particular investigation of the influence of mechanical stimulation to articular joints and characterization of inflammation processes in presence of biomaterials
- development of porous materials with properties interacting with cells.

4.4. Progenitor cells and ophthalmology

The type of epithelial cell therapy represented by autografts has already reached the stage of clinical evaluation. Limbic epithelium is collected from the

healthy eye, cultured on an amniotic membrane, and grafted after expansion onto the diseased eye. In terms of endothelial cell grafts, modest developments have been achieved at the stages of *in vitro* and animal experiments, whereas cultures of human corneal endothelial cells have been performed for about 10 years. Preparation of artificial corneas from human corneal cells has been considered.

One out of 500 retinal cells might be able to differentiate into all cellular types of this tissue. These so-called progenitor cells are present from birth up to the age of 70 and have raised great expectations. They have been shown to survive when injected into eyes of mice or day-old chicks. Furthermore, they migrate, integrate, and finally differentiate, in particular into photoreceptors. In the future, their collection and culture should be possible and their use in the treatment of degenerative diseases of the retina could be considered. Recently, it has also been reported that total recovery of vision was obtained in a woman with retinitis pigmentosa who received a graft of progenitor cells. In that case, cells were collected from an aborted human fetus. However, current investigations seem to focus on the utilization of cells from adult individuals.

3.5. Cell therapy and neurodegenerative diseases

The paradigm that newborns hold their definitive stock of neurons is currently challenged. Initially identified in rats, neuronal progenitor cells have been found in the olfactive bulb of hippocampus of adult human brains. This discovery opens potential therapeutic possibilities for disorders such as Parkinson's disease, Alzheimer's disease and Huntington's chorea. For about 15 years, efforts have been made to develop new therapies against degenerative neurological disorders. Two therapeutic approaches can be considered. Attempts can be made to substitute the missing neurons with homologous neurons able to replace their function. This is a technique of substitutive therapy via transplantation of nerve cells of fetal origin. Treatments that reinforce natural defenses of neurons can also be considered. The first trials of substitutive therapy in patients with Parkinson's or Huntington's disease have been encouraging. Human neurogenesis is complex. Our olfactive bulb hosts progenitor cells able to divide and produce neurospheres (nerve cell precursors) but their functions remain elusive. Experimental grafts into several areas of mice brains have apparently failed to induce the creation of neurons. Outside of their native milieu, they tend to produce another type of cells, namely glial cells. To remediate to this situation, several options have been considered, such as injecting substances able to stimulate progenitor cells into the damaged brain areas, or harvesting progenitor cells, allowing them to multiplicate in culture, and differentiating them into neurons before grafting.

3.6. Other applications

a) Diabetes and Langherans, islets: transplantation of insulin-producing b cells is the only means to obtain glycemic control in insulin-dependent

diabetic patients. While giving satisfactory results, transplantation of the whole organ cannot be generalized because of the severity of the intervention. That is why transplantation of islets of Langerhans, an authentic cell therapy of diabetes, is promising despite the scarcity of available results. Other approaches can be considered, such as using progenitor cells of the pancreas excretory system to obtain islets, or obtaining insulin secretion by non-pancreatic tissues via gene transfer.

b) Skin substitutes

Since the 1970s, different approaches to skin reconstruction have been developed but little is known about their actual efficacy. Three main methods are currently proposed: culture of keratinocytes on a fibroblast layer; culture of fibroblasts in a collagen mesh, leading to formation of a dermis equivalent that can be coated with a culture of keratinocytes; and culture of fibroblasts on nylon.

With regard to graft, techniques have been designed to obtain either an epidermis or a dermis. They should be distinguished since both tissues have their specific functions. For epidermis, a technique of keratinocyte culture on fibrin support has been developed that preserves the clonogenic potential of keratinocyte stem cells. Although the epidermis has a barrier function (keratinocytes), grafting a dermis is also required. A distinction should be made between the immediate benefit (contribution to epidermis engraftment), for which the extracellular matrix plays the main role, and the long-term benefit, for which fibroblasts are more important (participation in the synthesis and remodeling of the extracellular matrix and in the growth and differentiation of epidermis). No approach is currently totally satisfying.

4. GENE THERAPY AND THERAPEUTIC CLONING: REALITY OR DREAM ?

Therapeutic cloning is another approach. As early as the end of the 19th century, WEISMANN affirmed that inherited characters were transported by specific "biophores": myocytes had specific myocyte biophores, red blood cells had specific RBC biophores, etc. Owing to the scheme imagined by WEISMANN, each cell contained biophores corresponding to all tissues but only some of them could function in a given type of cells. If the term "biophore" is replaced by "gene", this reasoning is relatively close to modern concepts. But the question is, what mechanisms select active and inactive genes in a cell ?

In the 1930s, SPEMANN was the first to propose the concept of cloning by nucleus transfer, which resulted later in the birth of the ewe Dolly. Dolly modified the fundamental concepts. Indeed, she originated from a non-fecundated ovule in which the gene-containing nucleus was substituted with the nucleus of a differentiated cell collected from the mammary gland of an adult animal. Transferred into the cytoplasm, this nucleus recovered the totipotency of an early embryo's cells. Since then, several groups have reported having cultured human embryonic cells *in vitro* and that immortal cell lines, a key element for future regenerative medicine, could thus

be obtained. In the current scientific and ethical context, clinical applications remain a remote prospect. The most common approach consists in providing "cure genes" to the cell to restore its missing function. Recent identification of a large part of the human genome represents a giant leap for potential strategies of gene therapy. In many cases, identification of genes provides information about the mechanisms of disorders due to either a single mutation (monogenic diseases) or multiple genetic alterations (multigenic diseases).

Advances in genetic knowledge also open the route for potential therapeutic possibilities, including the development of new treatments, production of proteins of interest by genetic engineering (antihemophilic factors VIII, IX; growth hormone) or the development of gene therapy when the gene sequence is known. Treatment in itself can be administered *in vivo* by injection of a vector carrying the therapeutic gene or by treating target cells *ex vivo* before reinjecting them to the patient (e.g., bone marrow). Gene therapy could be beneficial for many patients with various diseases, including monogenic inherited disorders, cancer, serious infectious diseases (HIV), degenerative disorders (atherosclerosis), and degenerative neurological diseases. This lists only some of the potential applications.

4.1. Myopathies

How could myopathies be treated gene therapies insistently popularized by the media? The principle consists in using either progenitor cells from a donor in whom the normal gene is expressed (which, however, will induce a rejection reaction if the recipient is not on immunosuppressive therapy) or cells of the patient that are re-injected after being genetically corrected *in vitro*. Those progenitors referred to as "satellites" are stored in the periphery of myofibers. However, both validation of the "progenitor" label and efficacy assessments are still needed. Current investigations, still in the domain of basic science, attempt to genetically manipulate mice to attach a fluorescent marker to their satellite cells. After extraction, those cells must be sorted and amplified before being reinjected. However, *in vitro* amplification can alter their capacity to replicate and even their properties. It is therefore necessary to develop culture conditions that will preserve their "progenitor characteristic" while increasing in the same time their survival rate and their capacity to target muscle tissues.

In a recent study, a French group successfully used the so-called "exon leap" technique in a mouse model of Duchenne's myopathy, the most frequent of chromosome X alterations among neuromuscular disorders. A clinical trial might be organized in the next 2-3 years.

It should be also noted that neuroprotective gene therapy has entered its initial step of evaluation in the treatment of Huntigton's disease. Finally, new gene therapies using hematopoietic or muscle progenitor cells are currently being developed (e.g. for Wiskott Aldrich's syndrome).

4.2. Atherosclerosis

Currently, the two main therapeutic options available for patients with atherosclerosis are angioplasty, with the asso-

ciated risk of restenosis, and bypass interventions. ISNER et al recently proposed a new approach consisting in a transfer of cells promoting VEGF production into the arterial wall. Prevention of intimal hyperplasia with gene therapy is currently intensively investigated. This approach relies either on a gene with toxic effects on proliferating cells (e.g., the gene coding for thymidine kinase) or on transfer of a gene of interest (e.g., coding for the NO synthase of endothelial cells). Atherosclerosis is an important therapeutic target that may also be reached via a lipid approach (hepatic transfer of the gene coding for LDL receptors) or by reducing intimal hyperplasia or thrombosis via the transfer of antithrombotic genes to vascular endothelial cells (tPA). Gene therapy of atherosclerosis might also aim at preventing plaque rupture, which is responsible for acute ischemic syndromes.

CONCLUSIONS

"This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning".

Sir Winston Churchill (1942)

The 21th century begins with perspectives of cell and tissue therapies and regenerative medicine. The potential indications of progenitor cells and therapeutic cloning open vast domains of research that have provoked an intrusion of ethics and religion into the scientific field. In France, therapeutic cloning is forbidden by law. In the US, the latest presidential campaign has shown the influence of religion on the choice of candidates, while in the same period the California State approved a proposal allocate grants over 10 years for that research. In the UK, a NEWCASTLE team was allowed in 2004 to start an experiment of therapeutic cloning. The scientific as well as ethical and religious debates have probably only started but the new regenerative medicine undoubtedly opens therapeutic horizons that can be hardly imagined today.

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