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Perspectivas e desafios regulatórios no uso de células-tronco em métodos alternativos ao uso de animais

Perspectives and regulatory challenges for the application of stem cells in alternative methods to animal testing

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ABSTRACT

Introduction: The use of stem cells for toxicological evaluations seems to be a promising strategy since it allows a greater prediction of human effects. However, in Brazil, there is no specific legislation regulating the use of stem cells for nontherapeutic purposes, technological development, diagnosis or as an alternative method to animal testing. **Objective:** To review the literature and to provide an overview of the current situation of the nontherapeutic use of stem cells in Brazil and in the world. **Method:** A non-systematic bibliographic survey was carried out bringing together scientific articles and legislation. **Results:** This review brings the current approach of Brazilian legislation regarding the nontherapeutic use of human material and briefly discusses international regulatory approaches that allow the nontherapeutic use of stem cells. On the other hand, the Brazilian legislation for use of blood and blood products is quite broad and mature and could serve as a model for the nontherapeutic use of stem cells or other materials of human origin. **Conclusions:** The encouragement of the debate by the interested bodies and entities is the first step to initiate the development of specific legislation that could allow the scientific and technological development of Brazil in order to follow the world's biotechnological advances.

KEYWORDS: Stem Cells; Biotechnology; Health Legislation; Regulation; Alternative Methods

RESUMO

Introdução: Utilizar células-tronco para avaliações toxicológicas parece ser uma estratégia promissora para permitir uma maior predição de efeitos em humanos. Entretanto, no Brasil, não existe legislação específica que regulamente o uso de células-tronco para fins não terapêuticos de desenvolvimento tecnológico, diagnóstico ou como método alternativo ao uso de animais. **Objetivo:** Revisar a literatura e fundamentar um panorama da situação atual do uso não terapêutico de células-tronco no Brasil e no mundo. **Método:** Realizado levantamento bibliográfico não sistemático reunindo artigos científicos e legislação. **Resultados:** Essa revisão traz a abordagem atual da literatura científica e da legislação brasileira e discorre brevemente sobre abordagens regulatórias internacionais no que concerne ao uso não terapêutico de células-tronco. Em contrapartida, a legislação brasileira é bastante abrangente e madura na regulamentação de sangue e hemoderivados e pode servir de modelo para o uso não terapêutico de células-tronco ou outros materiais de origem humana. **Conclusões:** O incentivo do debate pelos órgãos e entidades interessadas é o primeiro passo para iniciar o desenvolvimento de uma legislação específica que permita o desenvolvimento científico-tecnológico do Brasil de maneira a acompanhar os avanços biotecnológicos mundiais.

PALAVRAS-CHAVE: Células-tronco; Biotecnologia; Legislação Sanitária; Regulamentação; Métodos Alternativos

INTRODUCTION

Stem cells are distinguished from other cell types because of two main characteristics: their self-renewal potential and the undifferentiated condition that enables their maturation into several cell types (Figure 1). The ability of stem cells to replicate leads these non-specialized cells to be able to renew themselves through cell division even after long periods of inactivity[1]. Furthermore, under certain physiological or experimental conditions, these cells may be induced to differentiate into specific cells of distinct tissues or organs[2]. In some organs like intestines, skin and bone marrow, among others, resident and tissue-specific stem cells divide regularly to keep the organ or tissue under physiological conditions. However, in more complex organs like the pancreas and the heart, this potential of cellular self-replication and differentiation is reduced and occurs only under special conditions[3].

Stem cells play a key role in the formation of organs and tissues during the development and throughout human growth, dividing and differentiating whenever necessary to replace dead cells and contributing to tissue homeostasis[4].

The differentiation potential of stem cells varies according to their stage of development and is directly related to their ontogeny. The totipotent stem cells appear in the early stages of embryonic development (in the morula phase) and are able to differentiate and form the entire organism, including extra-embryonic annexes, such as the placenta. Embryonic stem cells, however, are derived from the internal mass of the blastocyst and, because they are pluripotent, they have the ability to become cell types of the three embryonic leaflets, except extra-embryonic annexes[5,6]. Somatic stem cells derived from adult tissues are called multipotent, oligopotent or unipotent and have a more restricted differentiation capacity, usually into the cell types of their organ of origin[7].

The pluripotency of embryonic stem cells is a very interesting characteristic because of its potential of application in the search for treatment for several diseases. Moreover, these cells are an excellent model of study in the investigation of new drugs[5], toxicological studies or alternative methods to the use of animals[8,9]. However, studies have shown that the use of embryonic stem cells may be related to tissue rejection and the risk of tumor formation due to their high differentiation and proliferation capacity[10]. In addition, ethical issues associated with how these cells are obtained (involving the use and destruction of human embryos) are relevant. There is no global consensus and legislation varies in each country, as reviewed[11]. In Brazil, they must be used pursuant to Law n. 11.105, of March 25, 2005, known as the Biosafety Law[12].

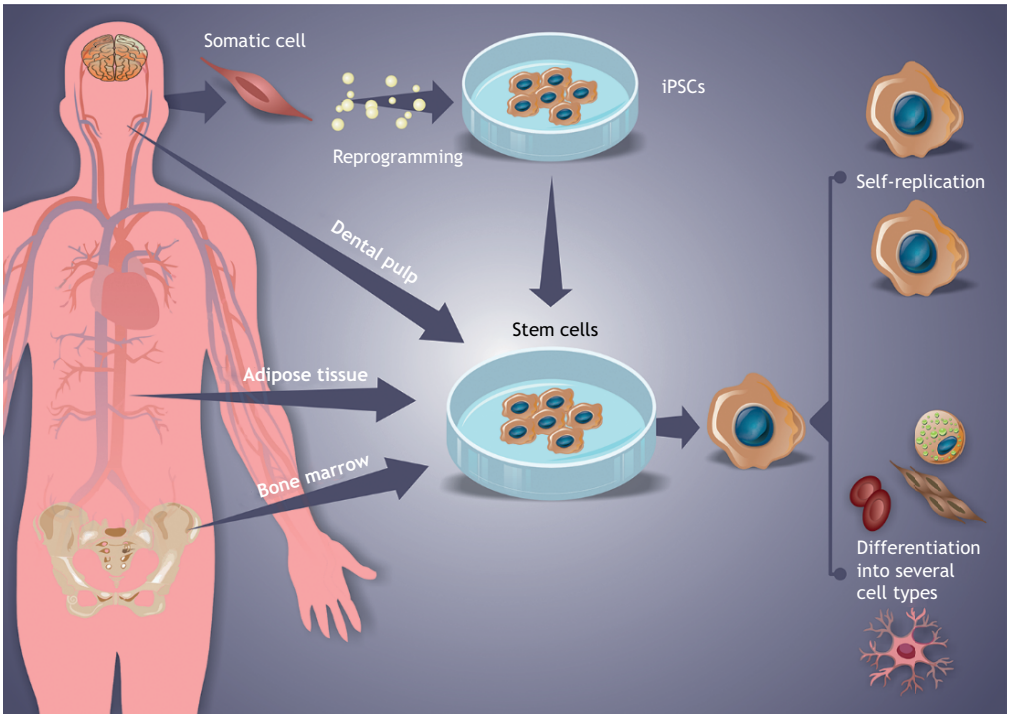
In 2006, searching for alternatives to the use of embryonic stem cells, researchers Kazutoshi Takahashi and Shinya Yamanaka presented a new source of human pluripotent stem cells with the development of induced pluripotent stem cells (iPSC) from differentiated adult cells. By changing the expression levels of four transcription factors (Oct3/4, Sox2, c-Myc and Klf-4), they were able to induce the reprogramming of an adult somatic cell so that it returned to its undifferentiated state[10]. This breakthrough enables the generation of pluripotent stem cells from adult cells of the patient himself/herself. This has a great potential for application in cellular therapy and regenerative medicine, with significant advantages, like: immunological tolerance, since these cells can be derived from cells of the patient himself/herself, as well as the absence of ethical questions associated with the use of embryos[13,14]. Furthermore, iPSCs derived from patients with diseases that are not yet fully elucidated can be an effective model in the research of the pathophysiology of these diseases and as a biotechnological tool in drug screening[15]. The discovery and the possibility of reprogramming generated such an impact that in 2012 researchers Shinya Yamanaka and John B. Gurdon shared the Nobel Prize in Medicine[16].

However, the iPSC generation process involves technologies that, to date, do not seem to be ideal for therapeutic application. Initially, retroviruses and/or lentiviruses were used to transduce the genes of interest. However, this approach allows for the occurrence of potentially malignant insertion mutations and transformations on the host chromosome or the induction of undesired immune responses[17,18]. New methodologies involving plasmids[19], synthesized ribonucleic acids (RNA)[20] and proteins[21] have been developed and tested, showing promising results. However, the risks of genetic damage and uncontrolled cell growth cannot yet be completely ruled out, nor can the fate of these cells be effectively predicted when applied *in vivo*[22].

Adult stem cells, although not pluripotent, but multipotent, oligopotent or unipotent, are capable of originating a significant number of specific cell types, which makes them attractive as a therapeutic and/or technological tool[23]. Among adult stem cells, we can highlight mesenchymal stem cells (MSCs), which form a heterogeneous population of stromal cells. The main advantage of these cells is that they can be isolated from several adult tissues[24]. These cells are progenitors, self-renewing and have differentiation potential into cells of mesodermal origin like adipocytes, osteocytes and chondrocytes[6]. In most tissues, the self-renewing capacity of MSCs is directly related to tissue regeneration capacity[1]. It is believed that, in addition to the ability to differentiate, these cells act in the modulation of tissue repair responses in a paracrine manner by releasing growth factors and other mediators interacting with the tissue niche[24,25,26].

MSCs can be isolated from virtually any tissue (Figure 1) and, although they are more commonly isolated from bone marrow[27], studies demonstrate the effective isolation of MSCs from other sources, like adipose tissue[28], placenta[29], dental pulp[30] and umbilical cord[31,32]. These sources have the advantage of forming easily obtainable material, which is often discarded and does not usually require additional invasive procedures to be obtained.

Bone marrow-derived MSCs are the most widely studied and currently used type; however, these cells are usually collected from the iliac crest of voluntary donors and the harvest process is considered invasive and of poor yield[33]. The dental pulp is considered a promising source of MSCs for orthopedic and maxillofacial reconstructions,



Source: Elaborated by the authors, based on literature review.

Figure 1. What are stem cells? Schematic representation of the main tissues from which mesenchymal stem cells (MSCs) can be extracted, like bone marrow, adipose tissue and dental pulp, not neglecting other sources like umbilical cord and menstrual blood. Furthermore, somatic cells can be reprogrammed to obtain induced pluripotent stem cells (iPSC). Once obtained, stem cells from both sources can be maintained in culture through their self-replicating characteristic or induced to differentiate into several cell types, according to the potentialities of each type of source cell and the inducers used.

since it can generate mineralized tissue, extracellular matrix and other connective tissues[34]. Cells derived from the placenta and umbilical cord have the advantage of greater self-renewal ability when compared to bone marrow MSCs[35]. Adipose tissue, on the other hand, has been considered one of the most abundant and easily accessible sources of MSCs. These MSCs have the same multipotent characteristics of the MSCs isolated from other sources[36,37].

Considering the use of human stem cells for nontherapeutic purposes, in the process of selecting the type of stem cell to be used, characteristics such as cell differentiation potential (multipotent or pluripotent) should be evaluated, as well as the convenience of obtaining the source tissue of these cells. For example, disposable tissues or from non-invasive procedures tend to be more interesting.

Although stem cells are a very promising biotechnological tool, this application is not yet a concrete reality in Brazil. In this review, we give a brief description of the nontherapeutic potentialities of stem cells in the development of methodologies for evaluating toxicity and alternative methods to animal testing and describe the current regulatory situation of this type of application in Brazil. Additionally, we cite international regulatory models for the use of human material and national legislation governing the use of blood and blood products as examples that could support the discussion and development of specific regulations to support the nontherapeutic use of stem cells in Brazil.

METHOD

The present study was done in a narrative literature review format. To do so, the bibliographic survey was carried out in a non-systematic way, bringing together scientific articles and legislation documents that could provide an overview of the current situation of the nontherapeutic use of stem cells in Brazil and worldwide.

The search for bibliographic references was done on PubMed and Google Scholar databases, with keywords like: stem cells (*células-tronco*), regulation (*regulamentação*), alternative methods (*métodos alternativos*). Brazilian legislation was consulted through publications of the Official Gazette and the portals of the National Sanitary Surveillance Agency (Anvisa). Furthermore, whenever necessary, some references (publications in the media, papers and scientific reviews) were directly searched to complement the discussion of the topics we addressed.

RESULTS AND DISCUSSION

Stem cells in alternative methods to animal testing

The use of stem cells in cell therapies has been considered a major milestone in the advancement and development of human medicine and the regulation of its use in Brazil has recently been revised[38]. Another great possibility of application, not yet

explored but very promising, is the use of stem cells in the development of alternative methods to the use of animals, with focus on the application of the 3Rs principle: reduction, refinement and replacement[39]; and also in the development of more relevant human toxicity assessment and prediction assays[8].

There is an estimate that only in Europe more than 12 million animals were used in animal experiments in 2005[40]. It is estimated that between 50 and 100 million animals are used in animal experiments worldwide every year[41]. There is a pressing global demand for reduction and refinement of the use of animals in research and development. In Brazil, this reality became more evident after the creation of the National Network of Alternative Methods (RENAMA) in 2012 and the Brazilian Center for Validation of Alternative Methods (BraCVAM) in 2013. Furthermore, with the publication of Normative Resolution (RN) n. 17, of July 3, 2014, by the National Council for the Control of Animal Experimentation (Concea), which provided for the recognition of alternative methods to the use of animals in research in Brazil[42], and in the same year, with the publication of RN n. 18, of September 24, 2014, 17 alternative methods to the use of animals in research have been recognized and must be applied to replace the original methods within 5 years[43]. In 2016, a new resolution, RN n. 31, of August 18, 2016, added seven other recognized alternative methods to the original list[44].

One of the areas of greatest demand for the introduction of alternative methods to animal testing is toxicology, since analyses of the toxicological potential are crucial both in the development phase and during the regulatory and validation processes of new products. In addition to the ethical issues surrounding the use of experimental animals, there is a great economic appeal for the development of new tests that are more predictable, since studies demonstrate that apparently non-toxic substances in non-human models may present high toxicity when applied in humans during clinical trials[45]. A classic example is the case of thalidomide, which for years of research did not show any reproductive toxicity in mice, but when administered in humans, it caused devastating teratogenic effects in the 1950s[46,47]. This disparity in the effects of a substance between humans and animals occurs due to the great genetic, metabolic and physiological diversity between species, even if they have evolutionary proximity[48].

Therefore, studies involving human cells and tissues are fundamental in the attempt to meet this demand. Within this context, in 2012, Meganathan et al. conducted a study evaluating the effect of thalidomide on human embryonic stem cells (hESC). Thalidomide showed toxic effects on these cells during induction of differentiation. Thus, the authors proposed this model as an alternative for the detection of teratogenic effects of drugs[49]. The same approach was proposed by other authors through the evaluation of the teratogenicity of thalidomide and of substances like ethanol, caffeine, retinoic acid and lithium[50].

As early as 1959, Russell and Burch indicated cellular models for assessing toxicity as an alternative to animal testing. Since then, many *in vitro* studies have been established and tested, and there are currently several cellular models used in toxicology. These models use both primary and transformed cells, both of which have some type of limitation, like low proliferative rate and accumulation of gene mutations, respectively[48].

Stem cells are considered targets for the development of models with a high potential for application in toxicological tests due to their self-renewal and differentiation characteristics[51]. Due to their ability to differentiate, they have the potential to originate a wide variety of tissues that could replace or complement animal models while avoiding interspecies differences. Thus, these cells have been extensively evaluated for their applicability in the creation of toxicity models. Stem cells can be used both in their undifferentiated and differentiated state and, therefore, have a greater range of application, which imparts several advantages when compared to other cell types[52].

Stem cell-based toxicology assays are classified into three categories: acute toxicity assessment through cell viability/survival (cytotoxicity) determination; evaluation of the inhibitory effect on cell differentiation (developmental toxicological tests); and evaluation of inhibitory/stimulant effects on specific cellular functions (functional assays). In this way, many models for toxicological evaluation can be created through the application of stem cells in culture[53].

Induced pluripotent stem cells and *in vitro* toxicity prediction assays

There is currently a single validated toxicity test with stem cells, the Embryonic Stem Cell Test (EST). This assay uses murine embryonic stem cells as the substrate and evaluates both cell viability compared to BALB/c 3T3 cells and the differentiation process to cardiomyocytes after exposure to test substances (ECVAM n. 113)[54]. However, the cells used come from mice and this method only contemplates the process of cardiomiogenic differentiation. In this way, the development and validation of new methods using stem cells as substrate is essential[54]. After the publication of the EST, some groups worked on the improvement of this protocol, aiming at greater accuracy in the toxicological evaluation. These improvements are quite promising for a better predictive evaluation of toxicity, since they enable the analysis of the expression of molecules involved in the process of cell differentiation[54,55,56].

The potential of differentiation of pluripotent stem cells enables the toxicological evaluation in several cell lineages and the evaluation of specific cellular functions like albumin secretion and glycogen storage, as well as the ability to metabolize substances during differentiation into hepatocytes, molecular causes of arrhythmias during cardiac differentiation and dysfunctions during neurogenic differentiation[53,57]. The pluripotency is a great advantage, but we should bear in mind that the standardization of differentiation processes in different cell lineages is still the object of study in many groups. Lineages of pluripotent cells may have a greater or lesser degree of ease of differentiation. The iPSCs typically maintain epigenetic marks from the cell of origin, which

may facilitate or hinder the differentiation process in a given lineage[58,59]. Because of these challenges, many Brazilian and foreign researchers are focusing their efforts in unraveling and understanding the mechanisms that lead to the process of cellular differentiation[60,61,62,63,64]. Pluripotent cells can be maintained in culture for long periods, which enables their use in *in vitro* repeated dose toxicological studies. This makes them more similar to *in vivo* models and is a major breakthrough in non-animal tests[65]. The drawback of this cell is the value invested in cell culture. The prices of the media used for the maintenance of these cells are still high, which results in rather expensive culturing when compared to that of adult stem cells. For this reason, some companies have already created more affordable culture media that are as efficient as the traditional media. In spite of some drawbacks, the use of pluripotent stem cells, in particular iPSC[10,66], represents a major advance for the development of toxicological assays. These assays can work as models for the evaluation of genetic diseases without the ethical issues associated with the use of hESC and provide the possibility of personalized toxicology and pharmacology[47,57].

In 2014, Pistollato et al. carried out a comparative study using embryonic stem cells and iPSC for the evaluation of neurotoxicity. The study demonstrated similar results between the two cell types, proving that reprogrammed cells also represent a promising model in the toxicity evaluation[67].

The iPSCs can originate from several individuals, all of whom have different genetic backgrounds because of their origins and lifestyles. In this way, they open the door to the visualization of a more comprehensive toxicological action, since a cell derived from a single individual will not be able to capture this great genetic and epigenetic diversity that occurs in the dynamics of the populations. These cells may also enable personalized toxicological analysis, as they may originate from both healthy and ill people. In 2013, Liang et al. evaluated the cardiotoxicity in reprogrammed cells of patients with hereditary cardiac disorders, comparing them with healthy individuals. The study enabled them to observe that cells derived from the two groups of individuals had different susceptibilities to cardiotoxic drugs, indicating the possibility of personalized medicine and toxicology[68]. There are currently iPSC banks that can be used to verify the genetic profile of populations and also for toxicological tests, indicating how the substance would act in the population as a whole[69,70]. Therefore, iPSCs are a very promising model for toxicology.

Mesenchymal stem cells and alternative methods to animal testing

In addition to pluripotent stem cells, adult stem cells also represent an important source of cells to be applied in toxicology. Scanu et al. evaluated bone marrow-derived stem cells as a substrate for the Organisation for Economic Co-operation and Development (OECD) Method TG 129, an assay that evaluates cell viability through the capture of neutral red dye by viable cells and is recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[71,72]. The same group investigated the cytotoxic action of copper nanoparticles on this cell type[73]. Our group has recently demonstrated that adult stem cells derived from adipose tissue and submitted to the cytotoxicity assay advocated by OECD TG 129 are a suitable cell type for the evaluation of the cytotoxic potential of the panel of substances referenced by the ICCVAM[71]. In this work, adult stem cells demonstrated a similar response pattern as that presented by the reference cell line BALB/c 3T3[74], but with the advantage of being of human origin, which increases the predictability of the toxicity of these substances in humans. In another study of our group that corroborates the above mentioned data, we did the comparative evaluation of adult stem cells derived from different sources as substrate for toxicological evaluation. The results showed that the cells behave in a similar manner[75]. In addition, another advantage pointed out is that the tissues used as sources of these cells are usually disposal materials that could be used in toxicological tests. However, one of the major challenges for the regulatory application of adult stem cells is the lack of a legal framework that addresses the nontherapeutic use of stem cells in Brazil.

Recently, Xu et al. carried out a study evaluating the cytotoxicity of titanium dioxide nanoparticles using adult stem cells derived from adipose tissue and inducing them to adipogenic differentiation. The results demonstrated that low concentrations of these nanoparticles were able to promote changes in cell differentiation. They concluded that cytotoxicity assays based on specialized cellular functions, such as cell differentiation, provide greater sensitivity and reveal undetectable effects by traditional evaluation techniques such as quantification of reactive oxygen species and cell proliferation[76].

Stem cells are also an excellent tool to identify substances that cause epigenetic changes like changes in DNA methylation levels or histone acetylation, variants during mRNA processing, or unwanted post-translational modifications that could transform cells, causing them to lose control of fundamental processes like proliferation, differentiation, apoptosis and senescence[68,77]. This type of test, which evaluates functional alterations or changes in gene expression, would have the power to indicate even more harmful effects than those observed in differentiated cells. This approach highlights the importance of the model as a more sensitive tool in toxicological evaluations.

Development of alternative methods of greater physiological relevance

Despite the advantages we have presented, models that use traditional two-dimensional cell culture are limited and often criticized, since they do not have the ability to mimic the real conditions of a living organism. Although it is possible to observe several cellular processes that occur in human tissues, the interactions between cells, matrix, tissues and organs are not considered in the two-dimensional systems. In order to fill this gap, in the recent years, systems have been developed to integrate various cell types *in vitro*, using perfusion techniques through

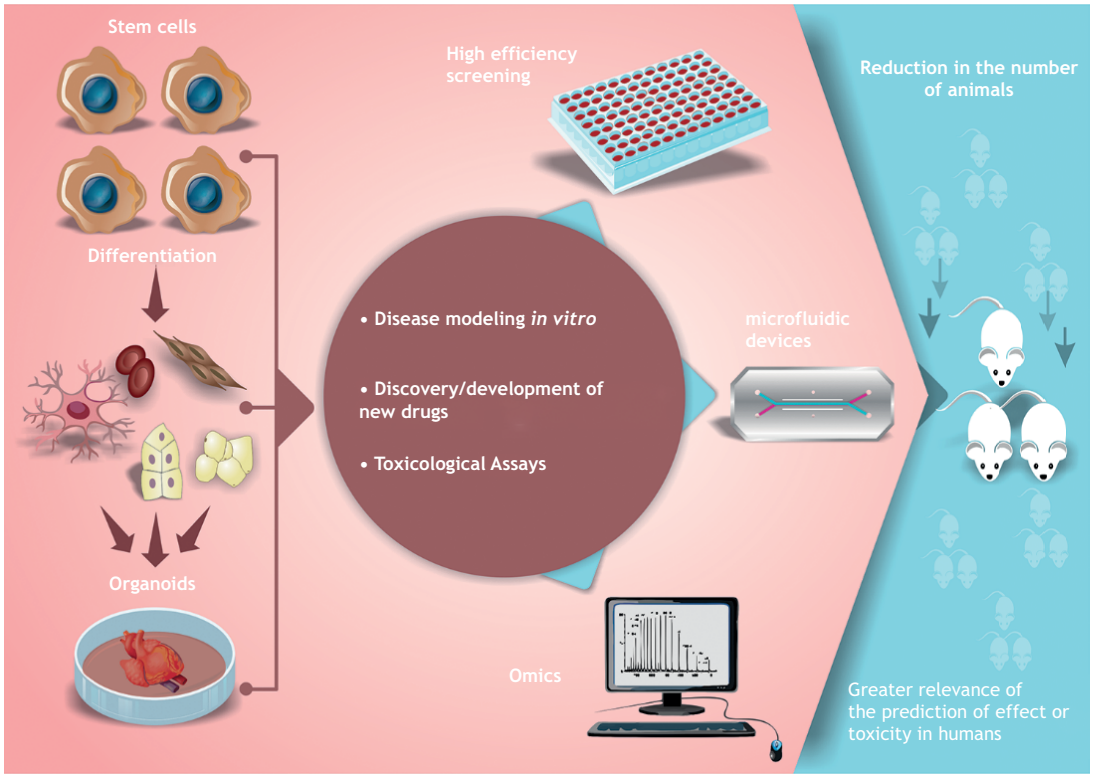
microfluidics. These systems allow the three-dimensional culturing of mini-organs, in addition to the coculture of different cells and structures, providing the best simulation of the physiological conditions in comparison with the traditional model[78]. Culturing in a microfluidic system in organ-on-a-chip platforms, where several physiological systems are interconnected, enables us to verify the action of a substance in different organs simultaneously[79]. By enabling the use of human cells, these systems should better predict the results that would occur in clinical trials.

The iPSCs are advantageous cellular systems to be applied in the microfluidic platforms, since they allow the development of patient-specific organs and systems and enable a more personalized toxicological evaluation[80]. Moreover, they allow the toxicological analysis of rare diseases for which there are no adequate study models, enabling screening and testing of substances in models of diseases that are difficult to treat and the selection of specific effective substances and the determination of non-toxic doses in a customized fashion[80,81].

Considering the advances observed in the use of stem cells as substrates in toxicological evaluation tests (Figure 2), there are countless advantages in using these cells. Toxicology that applies stem cells as substrate allows the determination of different forms of toxicity, like acute, embryonic, developmental, reproductive and functional. The tests performed with stem cells can therefore be quite comprehensive and answer not only one but several questions during the process of developing or regulating a new substance. For example, through large-scale approaches involving "omics", like transcriptomics or even toxicogenomics[8], contributing to the improvement of human health while reducing the use of animal models[47]. Although promising, microfluidic systems are costly and mostly experimental or under development, and there are no validated devices yet, although some validation studies of the devices are already under way[82].

There are no Brazilian regulations for the nontherapeutic use of stem cells or their bioproducts

It is now common sense that the potential use of stem cells goes far beyond therapeutic applications. The last decades were marked by the fast development of biotechnology techniques that brought along a deeper understanding of the physiology of stem cells. These techniques enabled the increasingly effective use of these cells in cellular therapies and opened up new possibilities of biotechnological applications. The growing understanding of how to modulate the activity and maintain these cells in ideal conditions has benefited



Source: Elaborated by the authors, based on literature review.

Figure 2. Nontherapeutic use of stem cells as an alternative method to animal testing. Human stem cells, based on their characteristics of self-replication or cell differentiation, or even the formation of organoids derived from stem cells, can be used in different nontherapeutic approaches for disease modeling, new drug studies and also toxicological assays. Several approaches can be used to evaluate the data, including large-scale screening trials, approaches using microfluidic devices or gene expression, protein or metabolite analyses, such as transcriptome, proteome and metabolomics analyses, among others. The main expected results with the use of stem cells in nontherapeutic approaches are the reduction of animal testing in laboratories and generation of data with greater relevance for the prediction of effect or toxicity in humans.

biotechnological areas of development of new drugs, pathophysiological and diagnostic research, in which stem cells have been shown to be promising tools with various potentialities for technical and scientific development[83]. This new look at stem cells follows a worldwide trend in which human materials like fluids, tissues and cells are increasingly seen as valuable sources of resources for the research and development of diagnostic and therapeutic methodologies[84].

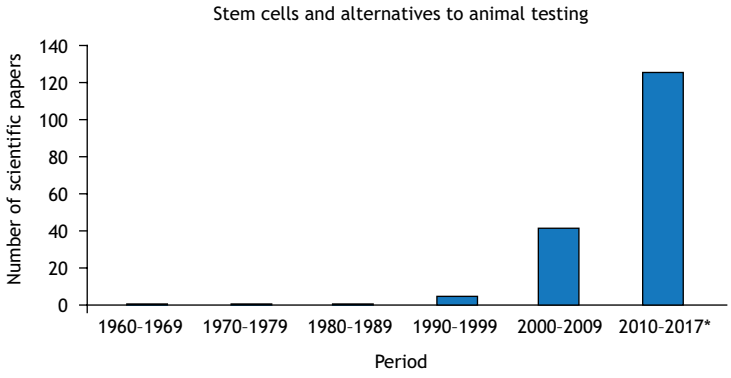
Although the benefits of using human material in the development of medical science are notorious, we cannot deny that this type of activity raises ethical, legal and technical concerns, since Brazil does not have specific legislation to regulate the collection, storage and use of human material for the purpose of technological development, diagnosis or even for use as an alternative method to animal testing[84]. The Brazilian legislation does not seem to have advanced at the same speed as the scientific-technological progress. There seems to be a gap between the regulatory framework and the scientific and technological progress in this area and there is no Brazilian legislation related to practices involving the application of human material, such as stem cells for diagnosis, drug development and toxicological testing. At the time of the promulgation of the Federal Constitution of 1988[85], for example, the application of stem cells as alternative methods to animal testing had not even been reported in the literature, however, interest in this approach has increased over time. In the last seven years, the number of publications about this topic has increased at least three times over the previous decade, indicating the increasing scientific interest in the use of stem cells in alternative methods to animal testing (Figure 3). It is therefore undeniable that the evolution of scientific knowledge should be considered for regulatory purposes.

Furthermore, lack of clarity and lack of specific regulation create barriers to the nontherapeutic application of stem cells and their acceptance for regulatory purposes. This question has gained importance and visibility and is currently the subject of discussion among the scientific community, academia, industry, regulatory agencies and legislators[86]. Consistent regulation that allows the evolution of medicine, science and technology while protecting human integrity is in the best interest of the community, healthcare professionals, academics, legislators, researchers, biotechnology companies, health sciences and other organizations, as it brings immeasurable benefits[87].

Currently, Brazilian legislation regulates the use of human hematopoietic stem cells for transplantation and cell therapy in a satisfactory manner, within what is now technologically available and possible. As reviewed by Narahashi et al., the legislation supports the use of hematopoietic stem cells for transplantation and treatment of a range of blood diseases and clinical research through a series of resolutions and technical standards, ensuring patient safety by regulating the collection, processing, packaging, storage, quality control, disposal and release tests for the use and transportation of these cells. However, these norms do not include MSCs nor the use of stem cells for technological development and diagnostic purposes[38].

When searching for information on the regulatory use of human stem cells for nontherapeutic purposes in Brazilian legislation, what we found is rather limited in relation to embryonic stem cells, as regulated by the Biosafety Law. It describes the limits and conditions for the use of embryonic stem cells in basic research. Nothing is described, however, regarding adult stem cells[12] or even the biotechnological application of stem cells.

In the absence of specific regulation for MSCs, we can consider these cells as biological material of human origin in general, and check what the legislation says regarding the nontherapeutic use of this type of material. Although Resolution n. 441, of May 12, 2011[88], regulates the establishment and operation of biobanks, it only provides for the use of material of human origin for therapeutic purposes and basic research on the pathophysiology of diseases that are not yet well understood. There is, however, no regulation that considers the use of this material in technological development or for diagnostic purposes, where



Source: Elaborated by the authors, based on literature review.

Figure 3. Timeline of the number of scientific papers available in the PubMed database with the terms: *células-tronco* (stem cells) and *métodos alternativos ao uso de animais* (alternative methods to animal testing). We considered the number of scientific papers published every decade from 1960 to September 30, 2017. We noted that there was no publication in the area until 1989. However, in the last seven years, the number of publications has increased at least three times over the previous decade, indicating the increasing scientific interest in the use of stem cells in alternative methods to animal testing.

this material will not generate knowledge, but rather will be a methodological tool used in medical-hospital routine or in the discovery or development of new drugs or products[84].

Resolution n. 466, of December 12, 2012, of the Brazilian Ministry of Health[89], which deals with research involving human beings, supported by Resolution n. 441/2011[88], which establishes rules for the creation of biobanks, bio-repositories and the use of biological material from these banks, regulates the use of biomaterials of human origin. According to these standards, the use must be restricted to what has been previously approved in a specific research project and always after consent of the material donor. Therefore, the donor and the National Commission for Research Ethics/Research Ethics Committee (Conep/CEP) must be notified and approve any use other than that previously established and approved in the initial project[88,89]. Unfortunately, these standards do not address the use of this material as a technological tool in diagnostic kits, toxicity assays or other technologies involved in the screening of new drugs for regulatory purposes. The legislation does not yet keep track of the regulatory need for new technological and scientific interventions, which means there are several regulatory challenges to the use of materials of human origin in research[83].

When considering the regulatory limitations involving stem cells, misconceptions may already be related to the "stem cell" term, which is currently considered rather generic and possibly confusing. The term is usually primarily linked to embryonic stem cells and to everything they imply in terms of ethical and legal issues, as reviewed by Diniz and Avelino[11]. However, we should consider other stem cells such as iPSCs, which do not appear to involve so many ethical issues, and adult stem cells such as hematopoietic stem cells, for example, that have long been used in cell therapy[90] or in the production of blood products in accordance with their own legislation, Law n. 10.205, of March 21, 2001[91]. Since these are different cells with distinct characteristics and applications, the means of regulating the use of these cells also need to be distinct and specific.

International examples of regulatory approaches for the nontherapeutic use of stem cells

Although international legislation is several steps ahead of the Brazilian legislation in terms of "use of material of human origin", reflecting a smaller distance between the state of the art and the regulatory framework, stem cell research and its application in the modeling of diseases or in drug discovery still raises ethical and legal discussions around the world. Issues that are not yet clear include, for example, iPSCs and adult stem cells that may have applications that go far beyond cell research and therapy, a fact that seems to be neglected in current legislations[90]. The ethical and legal issues surrounding stem cell research are not limited to the destruction of the embryo[92], which appears to be the main concern of the population and perhaps one of the main aspects addressed by the current legislation. For example, iPSCs are derived from adult somatic cells that can be donated legally for research and do not involve the use of embryos. However, once transformed into cell lineages that will be stored in libraries and possibly used as biomaterials for nontherapeutic purposes, issues related to donor privacy, consent and ownership are becoming more intricate and unprecedented[90,93].

Although the marketing of human organs and tissues is banned in most parts of the world, several countries already permit the marketing of human material for nontherapeutic purposes as a result of updated legislation in response to biotechnological development[94]. In Europe, the use of stem cells for nontherapeutic purposes is permitted and supported by the European Medicines Agency (EMA), which understands that these products are classified as Advanced Therapy Drugs for human use, a category which includes gene therapy products, tissue engineering and somatic cell therapy[38]. Even so, there are still some obscure issues in the regulation of this type of application. The main uncertainties concern the amount and importance of the intellectual property added to the human material needed for that material to become a marketable good. Some European countries understand that the expenditure associated with the collection, processing and storage of the material constitutes substantial intellectual property that justifies the treatment of the material as a product. This is not to say that there is no concern about the secrecy, privacy and consent of the donors. These items are seriously considered and regulated by codes of ethics that seek the protection and integrity of the volunteers[95].

At first, European regulation was more restrictive, allowing only biobanks connected to hospitals, which meant the use of materials of human origin for therapeutic purposes only. However, growing biotechnology development and industry pressure have forced legislators to push for changes in laws. In 2008, new regulations (EC n. 1.394/2007, of November 13, 2007)[96] entered into force to facilitate the marketing of tissue engineering products of human origin. One of the major changes that this new regulation brought about was the discrimination between human organs and tissues, by establishing that human tissues, unlike organs, could be marketable goods. However, this legislation leaves it to each member country of the European Union to regulate the ethical and legal issues involved in the use of human material for nontherapeutic purposes. Thus, each country has established its own rules, and although the sale of human material is generally prohibited, this does not prevent materials that have been isolated, purified and minimally altered from being patented and marketed[94].

The United States, albeit differently from Europe, also has legislation that accompanies industrial and technological development and allows the marketing of therapeutic and biotechnological products derived from bioengineering of human tissues and cells[97]. The Public Health Service Act (PHSA) distinguishes the use of stem cells according to two classifications: cells that are widely handled are considered to be biological products, drugs and medical devices and are regulated as such. Non-handled cells, on the other hand, are considered as "minimally-handled products" and therefore less risky technologies that do not require approval to be marketed[38]. Furthermore, in the United States, tissue banks can repay money by recruiting donors (including corpses), collecting, processing and storing materials

of human origin, including blood and gametes. As pointed out by Pirnay et al., although materials are always donation products, today the legitimacy of a process that makes voluntary donations a profitable business is being discussed[94].

Although American legislation may seem very open and permissive while its European counterpart is still not homogeneous and needs adjustment, international measures, even imperfect ones, ensure that European and American citizens have access to innovative therapies with greater agility[97] or, ultimately, do not impose so many barriers to the biotechnological use of human material or development of alternative methods to animal testing.

Example of regulation of blood and blood products - prospects for the use of stem cells for nontherapeutic purposes in Brazil

Blood donation is a widespread practice in Brazil. In addition to direct therapeutic use, blood, a human material from voluntary donors, is also used to obtain blood derivatives[98]. Perhaps a careful analysis and understanding of how this material is often seen as an input can guide discussions and open new prospects to the nontherapeutic use of stem cells, especially those cells that can be obtained from disposable material or through non-invasive methods like urine collection.

In a more detailed look at the legislation, the Brazilian Federal Constitution of 1988[85] is the guide of all the legislation of the country. In Section II - Health, Article 199 § 4, it says:

> The law shall provide for conditions and requirements that facilitate the removal of organs, tissues and human substances for the purpose of transplantation, research and treatment, as well as the collection, processing and transfusion of blood and its derivatives, and any type of marketing is prohibited.

The regulation of this item of the Federal Constitution with regard to blood and blood products was later enforced by the introduction of Law n. 10.205/2001. Among other issues, article 14 of Chapter II on Principles and Guidelines addresses voluntary blood donation, marketing prohibition, permission for the remuneration of expenses with supplies, reagents, disposables, among others, used in the processing of samples, as well as questions regarding both donor safety and sample quality[91]. Perhaps the national blood and blood derivatives guidelines can be analyzed at least in part as examples, in order to serve as a starting point for the creation of legislation and regulation on the nontherapeutic use of stem cells, for example in the development of alternative methods to animal testing. This approach was also suggested by Carias et al. when they reviewed the Brazilian legislation at the time related to the regulation of article 199, paragraph 4, of the Federal Constitution. They proposed the discussion of a model that was similar to the management of surplus blood and blood derivatives for biological materials of human origin, citing its use in toxicological tests in addition to the application in research or therapy[98].

The determination of the types of human material that can be considered for the biotechnological use of stem cells is also a favorable argument for its use. For adult MSCs, these can be obtained from disposal materials, which are usually waste from healthcare services. The final destination of this waste is usually its destruction or disposal. Among these materials we can cite adipose tissue[28] from liposuction or dermolipectomy, dental pulp[99], umbilical cord[31,32], for which no other invasive procedure for obtaining the cells is performed other than the one the donor was already willing to undergo. Rather than being destined for destruction and disposal, such disposal materials could be donated and destined for laboratories where stem cells could be isolated. Easy access is also an advantage of the tissues that can originate pluripotent cells, as is the case of blood[100] and even urine[101]. These surplus human tissues or fluids with no therapeutic value should be considered in a different manner, considering their source and form of production for the purpose of isolation or induction of pluripotent stem cells.

The information above can guide legislators about the progress of science and the need for regulatory change. It is vital that science and legislation move at the same pace so that progress in science and technological development can benefit society with their advance. However, there is no national regulation on the use of stem cells or bioproducts of human origin for diagnostic purposes, biotechnological processes or even animal replacement, although the legislation is quite comprehensive and mature in terms of use of blood and blood products[98], and can therefore serve as a model for questions not yet covered.

In recent years, scientific research focusing on alternative methods to animal testing has sought not only to foster a more rational use of animal models, but also to provide data of greater relevance to human health[8]. Human stem cells stand out as promising tools to address these two main matters. Many *in vitro* models using stem cells have been developed and are of scientific relevance[8]. It is therefore necessary and urgent to discuss the possible application and regulation of the use of these models in Brazil, at the risk of not following global efforts to reduce animal testing. These models can also improve the provision of methods with high potential for predicting toxicity in humans.

CONCLUSIONS

There is a consensus that the marketing of human tissues is prohibited in order to maintain the integrity of the individual, as ruled by the Brazilian Federal Constitution. Nevertheless, most countries have already established laws to allow bioengineered products of human origin to be used for nontherapeutic purposes. They even allow that they be considered marketable goods in certain situations where they have been modified enough to warrant intellectual property. Another alternative is the use of regulations for the use of blood and blood products as the basis for legislation on the application of stem cells for nontherapeutic purposes, in which there is no marketing, but rather a reimbursement of the costs associated with the procedures of collection, processing, storage and distribution. In

order for Brazil not to distance itself from global trends in the development of science and technology, it is important that a broad debate be encouraged with all the stakeholders. This debate should define what measures are to be adopted so that the regulatory practice of use of human material, such as stem cells, especially for the application of alternative methods to animal testing, allows the country to achieve international levels of reduction, refinement and replacement of animal testing. Moreover, this should encourage the use of assays with greater relevance for prediction of toxicity in humans.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.