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Review Article

MESENCHYMAL STEM CELLS: AN INNOVATIVE APPROACH IN PHARMACOKINETICS

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ABSTRACT

Multipotent mesenchymal stem cells (MSCs) are special kind of stem cells which originate from mesenchyme. These cells can be used as an imperative tool to study reproductive toxicity, carcinogenicity, mutagenicity, genotoxicity, and pharmacokinetics. This novel system may reveal toxicant-induced etiology, decipher detailed understanding on molecular mechanisms of toxicants induced pathways and also enumerate the safe dose of an investigational product. Hence, this could ultimately replace, improve or overtake current predictive models in toxicology. The particular review describes the utilization of MSCs in different field of toxicological and pharmacological research.

Keywords: Mesenchymal stem cells, Toxicant, Etiology, Pharmacokinetics.

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INTRODUCTION

Toxicity may result due to administration or exposure to drugs or chemicals or xenobiotic compounds or radiation or particulate matter or endogenous production of toxins by any microbial flora or transplanted cells. Such primary and auxiliary toxic agents may disrupt intracellular cell signaling and interrupt cell-to-cell interactions from intra- and extra-cellular communiqué and affect cellular architectures in biochemical, anatomical, cellular, psychological, and pathological level. This may be due to the complex interaction established between toxic agents and genes, proteins, RNA and cellular organelles [1,2]. These phenomena may alter biological cascades of circadian rhythm in human, affect the dynamic cellular function and metabolism, generate malignant tissues, change rhythmic beating passion of heart and can provoke psychological complications [3]. In the pharmaceutical settings, toxicological studies of new compounds or agents play the most key role to provide safety and accurate assessment of risk factors associated with novel drugs before administration to human beings. The US Food and Drug Administration states that it is essential to screen new molecules for pharmacological activity and toxicity potential in animals (21CFR Part 314) [4]. Traditionally, various animal models such as mice, rats, guinea pigs, and dogs are used to predict or anticipate toxicity of newly synthesized drugs or stringent chemicals or any suspected agents, as these agents might induce cardiotoxicity, hepatotoxicity, genotoxicity or epigenetic and reproductive toxicity in humans. However, there are several demerits of using animals in toxicological studies. The tests conducted in animal models are not yet well standardized. Apart from this, the animal models are not good representative of humans because inter-specific differences exist in accordance to pharmacokinetics and toxicokinetics background [5]. It often gives doubtful results during early (preclinical) or necessary late (clinical) assessment of newly synthesized drugs leading to termination of drug development programs (Fig. 1).

The ethical issues and cost of analysis add further constrain in the use of animals in toxicological studies. To reduce the risk and ambiguity that is often associated with testing, developing and screening of toxicity using experimental animals currently, suitable alternative *in vitro* models such as whole embryo cultures, primary cultures and permanent cell lines, cell lines established from specialized somatic cells are being used [7]. These biological systems may produce relevant and accurate information about toxic agents affecting the vital cellular functions. This may be due to the fact that common cellular sources are used to test the subject (suspected agents) and to see the effects [8]. Recently, the novel approach of using stem cell derived systems of embryonic origin such as embryonic carcinoma, embryonic stem (ES) and embryonic germ cells (GCs) and non-embryonic origin induce pluripotent cells (iPSc) and multipotent adult stem (AS) cells open new avenues in the field of pharmacokinetics and toxicological research [6]. These *in vitro* systems are now being used to extrapolate the correlation between toxic effects of toxicants and the doses causing organ toxicity, embryotoxicity and developmental toxicity (Fig. 2) so that a safe predetermined dose of chemicals can be prescribed before *in vivo* studies and clinical translation. This review describes the nontherapeutic potential applications of multipotent mesenchymal stem cells (MSCs), in particular, case pertaining to toxicological studies and pharmacological screening.

LETHALITY-INDUCED BY TOXIC AGENTS

The toxic agents target constituent of tissues by altering enzyme activity, interfering the binding of hormone to a specific receptor by creating structural homolog to alter its function, modulating the number of hormone receptors, changing the way of hormone synthesis and their affinities for specific molecules. Intercalation with nucleic acids, disturbances in electrolyte balance and the disorganization of cellular water and membrane lipids are illustrated as toxic processes involving ionic or van der Waals forces. The interaction results perturbation of biological pathways. When perturbations are sufficiently large or when the host is unable to adapt to the changed physiological conditions, it may contribute to nutritional, genetic, disease, or life-stage status factors. The biological function is compromised, and this leads to toxicity and disease. The circuitry affected by the chemical is expected to determine the shapes of curves of dose-response relationships for these perturbations. The responses are viewed as the results of an intersection of exposure and biological function. Hence, the determination of lethal doses that result progressive activation of toxicity pathways from perturbation of initial targets, through activation of stress controlling pathways, to overtly toxic responses (apical endpoints) is essential [9]. Traditionally, to determine toxicity, the LD50 assay has been used, which is a measure of the dose required to cause death in 50% of the animals under the study. An alternative to this assay was first suggested by the British Toxicology Society in 1984 and was based on administering a series of fixed doses and relying on the observation of clear signs of toxicity (altered morphology, biochemistry, and undesired by-products) rather than the endpoint of the assay being death. Recently, developed *in vitro* signatures and

computational models are being used to determine agents that induce toxicity, detect perturbation in cellular pathways that are expected to contribute to or result in adverse health effects. Pathway testing would require a suite of tests which could identify the range of significant perturbations of human biology. This might occur as a result of chemical exposure (Fig. 3).

STEM CELLS AND THEIR RESPONSE TO TOXICITY

The development of multicellular organism from egg to an adult is a complex series of interlocked events, which depends on precise coordination of time and space. From the time of implantation of totipotent zygote, the complex interaction between external environment and physiological stress results in a dynamic changeover from totipotent stem cells to pluripotent stem cells and multipotent stem cells that commit to form specific organ types. These mutually exclusive events are directed via differentiation, determination, and specification which ultimately result in the formation of an adult individual. Stem cells are uncommitted cells [10]. These cells are an important tool to study molecular mechanisms of different biological pathways, differentiation, and mechanisms of cellular commitment [11]. These are ideal experimental systems for the study of molecular events of development and analysis of agents that may alter these directed mechanisms. Stem cells can facultatively use both symmetric and asymmetric divisions to express constitutively the properties of self-renewal and differentiation (Fig. 4).

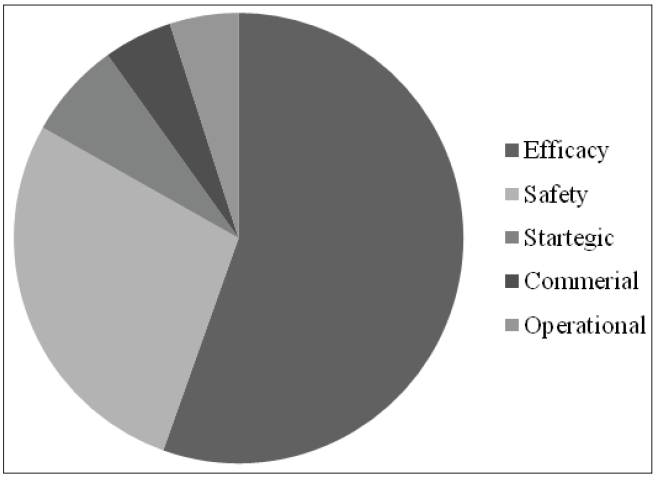

Fig. 1: Important causes of drugs failure (Data obtained from Suter-Dick *et al*. [6])

Symmetric divisions can expand stem-cell number by generating daughter cells that are destined to acquire the same fate thus, playing an important role in adult mammalian homeostasis. Asymmetric divisions result cells that are destined to acquire a different fate. It is noteworthy that asymmetric divisions can be governed by both intrinsic partitioning of fate regulators and asymmetric exposure to extrinsic cues [12]. ES cells derived from early embryonic cells represent an excellent *in vitro* model system to study the molecular mechanism associated with pluripotency, self-renewal and development. The remarkable capacity of ES cells to transform into variety of somatic and germinal cells reflects the dynamic events that occur *in vivo* [13]. ES cell lines were first derived from mice [14,15] but currently available from a variety of mammalian systems, including human [16]. They can propagate at undifferentiated state up to indefinite period under defined culture conditions [13,14,17]. The most reliable method for generating differentiated cells from ES cells is by induction of embryoid bodies (EBs). Many features of EBs resemble that of developing embryo. These EBs on attachment to permissive surface like gelatin, collagen, and treatment with appropriate inducer continue a programed differentiation into ectodermal, mesodermal, and endodermal lineages [18]. Hence, pluripotent ES cell lines recapitulate cellular developmental processes and gene expression patterns of early embryogenesis during *in vitro* differentiation which is hard to visualize in *in vivo* condition. Thus, ES cells hold great promise as an unlimited cell source for various clinical and biotechnological applications [19-21]. This disease-in-a-dish model is useful to understand molecular mechanisms of diseases and cellular responses to various therapeutic and toxic agents. Hence, *in vitro* culture of human ES (hES) cells is a suitable system to study etiology and prognosis of many degenerative diseases and a valuable tool for drug or toxicity screening and mechanistic studies including analysis of disease pathway and developmental toxicity [22]. Developmental toxicity is often associated with birth defects, low birth weight, and biological dysfunction. About 10% of birth defects are related with environmental factors including therapeutic agents and developmental toxicants [23]. Embryotoxicity can be assessed by ES cell test using differentiated cells of mouse ES cells. The test is successfully validated by the European Center for the validation of alternative methods (ECVAM) and considered as fundamental models to screen the unknown chemicals capable of causing embryotoxicity, such as cytotoxicity and differentiation and to know the altered physiological mechanisms caused due to toxicity from molecular level. In addition, differences in sensitivity between differentiated (adult) and embryonic cells are also taken into consideration. To predict the embryotoxic potential of a test substance, three endpoints are assessed such as the inhibition of differentiation into beating cardiomyocytes, the cytotoxic effects on stem cells, and the cytotoxic effects on 3T3 fibroblasts. A special feature of the EST is that it is

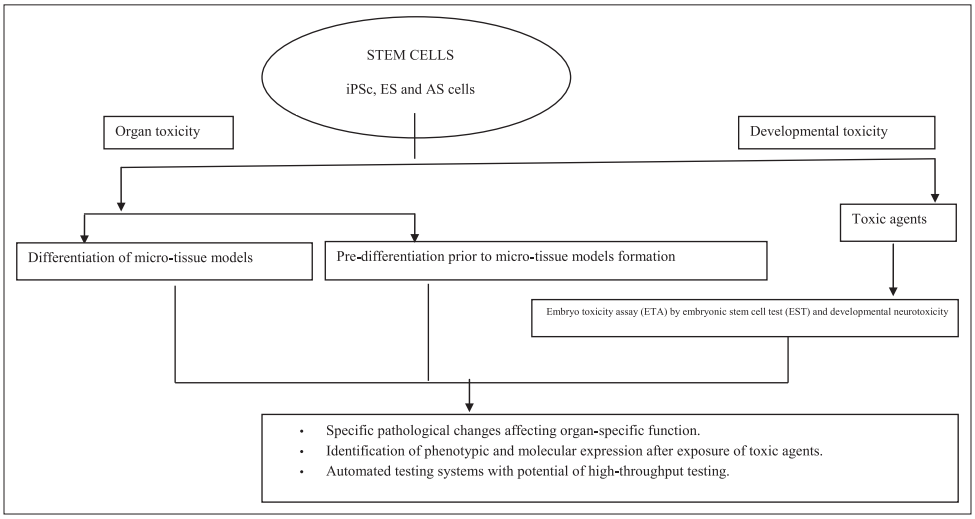

Fig. 2: Potential application of embryonic and adult stem cells to design toxicologically relevant organotypic microtissue models

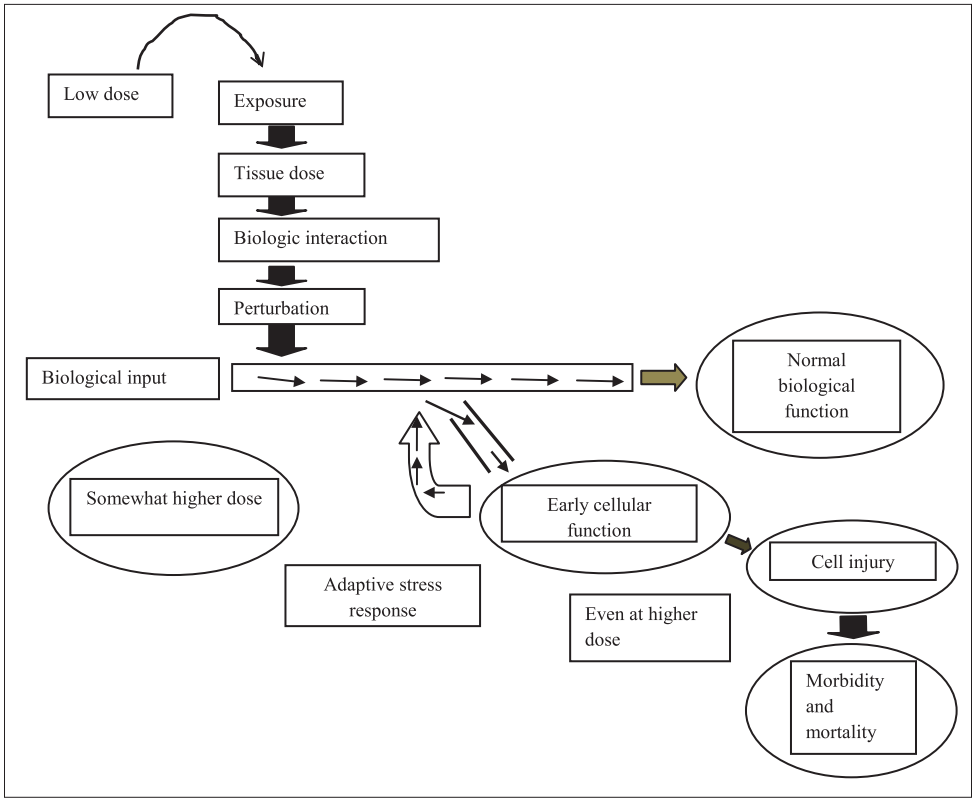

Fig. 3: Relationship between dose and toxicity

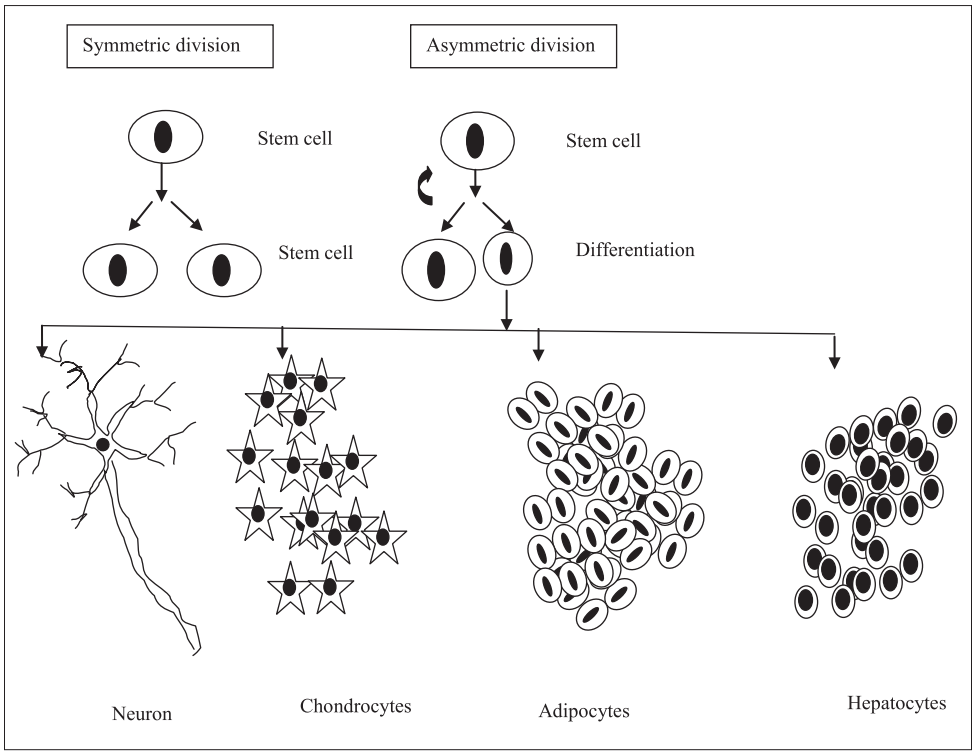

Fig. 4: Division and differentiation shown by stem cells

solely based on permanent cell lines so that primary embryonic cells and tissues from pregnant animals are not needed. This EST protocol is an ECVAM-validated method, in which the morphological assessment of contracting cardiomyocytes is used as endpoint for differentiation, and the molecular-based fluorescent-activated cell sorting-EST method, in which highly predictive protein markers specific for developing heart tissue are selected. With these methods, the embryotoxic potency of a compound can be assessed *in vitro* within 10 or 7 days, respectively [24]. Available literature suggests that this *in vitro* cultured ES cells model is currently considered as the best model to predict embryotoxicity, developmental toxicity, mutagenicity, and teratogenicity (Fig. 5).

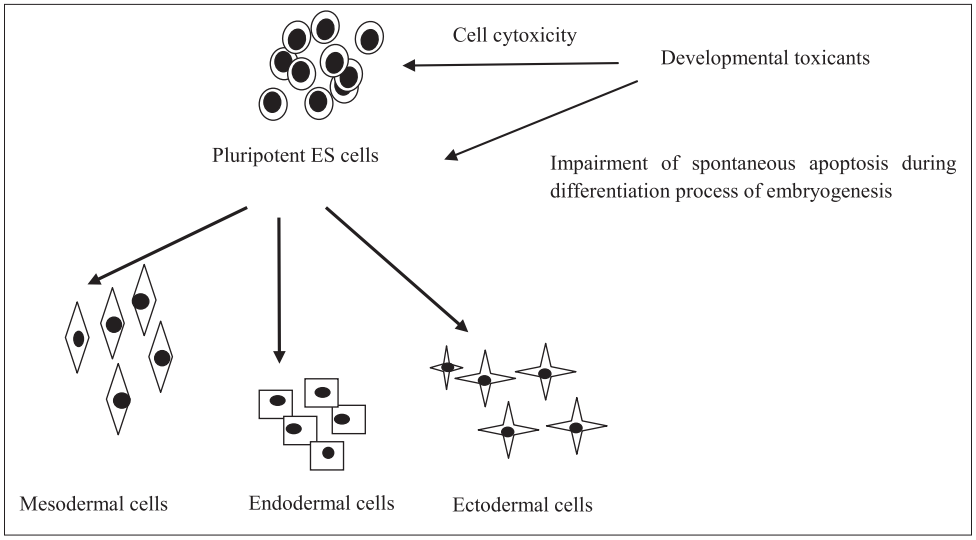

Fig. 5: ES cell derived differentiation-related endpoints using developmental toxicants

Recent studies demonstrate that endocrine disruptors (EDs) may be any estrogen-like and antiandrogenic chemicals, environmental agents [e.g., polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), dioxin, and some pesticides) can affect function of reproductive cells (GCs) and organs or tissues associated with reproduction. The compounds such as bisphenol-A, di-2-ethylhexyl phthalate (DEHP) and dibutyl phthalate, vinclozolin promote epigenetic transgenerational inheritance into subsequent generations [25]. Hence, extensive screening and multi-generation studies are required prior to registration of agrochemicals and before commercialization of pharmaceutical products. Currently, ES cells are not only used to test chemical compounds but also to identify the toxic effects of physical factors, such as electromagnetic fields emitted by digital mobile communication systems [26]. Despite several advantages, application of ES cells in biomedical research is often restricted due to ethical and social issues. To circumvent this problem, human-iPSCs are generated by reprograming the somatic cells and AS cells derived from several adult origin are currently being used. iPSCs are converted pluripotent stem cells. In this process, the adult somatic cells are "forced" to express five pivotal genes, namely Oct-3/4, SOX2, c-Myc, Klf4, and Nanog essential to maintain pluripotency [27] that are silenced in course of development. Currently, they play a key role in therapeutic and other biotechnological applications such as drug discovery, toxicology, disease modeling, and gene therapy [28]. It is demonstrated that iPSCs are currently being used for high-throughput screening and chronic toxicity assessment of cardiac, hepatic, and nervous tissues [29]. Recent report by Deshmukh *et al*. [30] describes that ES cells and iPSCs are efficiently used to screen cardiac and neurotoxic agents. iPSCs derived from different somatic cells may be used to produce unrepaired somatic cells for disease modeling or drug screening (Fig. 6).

However, sometimes iPSCs fail to reproduce the same effect successfully. This may be due to the fact that it is difficult to create pluripotent environment within a multipotent counterpart. During their lifetime, the adult humans are constantly being exposed to deleterious environmental impacts of a multiplicity of anthropogenic substances, number of drugs, chemicals, and pollutants which may induce toxic effects. So is it wise to use embryogenic cell-based models (ES cells and iPSCs cells) to predict the toxicity that is encountered by adult individuals? It is reported that neural stem cells or progenitor cells may be relevant models for alternative developmental neurotoxicity (DNT) testing [31]. When xenobiotic agents interfere with progressive development and growth, they alter cellular growth and differentiation, leading to permanent or temporary changes in tissue or organ structure and function, i.e., disease, disorder, and developmental malformations. All these mutually exclusive events are controlled by expression of a unique set of genes, i.e., influenced by many environmental (physical, chemical and biological) factors [32].

When stresses are intricated by any stressors (toxicants), this disrupts cell-to-cell communiqué and provokes an adaptive response and ends in non-adaptive consequences. All these phenomena influence stem cells niche and deregulate cell cycle progression, cellular function, intracellular communiqué, and signaling pathways. These pathways can activate or inactivate specific gene that regulates transcription of extant proteins or transcription factors (FTs) involved in proliferation, differentiation, apoptosis, stress responses, and senescence. But how do the toxic agents intricate teratogenesis, carcinogenesis and mutagenesis in targeted tissues? This may be due to the fact that toxicants involve initiation or inhibit intracellular signaling cascades that trigger acetylation, deacetylation, and methylation of histones leading to DNA damage by epigenetic modification of chromatin [33]. All these events may happen due to the fact that these toxicants disturb gap junction intercellular communication (GJIC) that maintains tissue homeostasis and controls cellular function, such as growth, differentiation, development, and apoptosis. Gap junctions are plaque-like protein structures that form contiguous channels between cells allowing for the passive diffusion of low molecular weight metabolites and second messengers between the molecular weight of 1-1200 Dalton (Da). Each channel is made up of two connexons, each residing in separate contiguous cells. The connexon is made up of six subunits that are termed as connexins [34]. Due to the central role of intercellular signaling through gap junctions, GJIC plays a significant role in coordinating signaling events that control gene expression. This property makes it an ideal biological endpoint to monitor the toxic effects of environmental agents, toxins and potential health hazards of pharmaceuticals. Gap junction communication through connexin-mediated junctions' connexin 43 (Cx43) plays a major role in development and differentiation of bone development. The extracellular communication or "stromal-epithelial-type" interactions trigger intracellular communication signals to modulate GJIC between either homologous or heterologous cells within tissues [32]. GJIC can be modulated either reversibly or irreversibly, by most, if not all, "tumor-promoting chemicals." Inhibition of GJIC has been postulated to mediate via non-genotoxic carcinogenic mechanism. This mechanism may relate to tumor promotion and progression. Recent studies demonstrate that inhibition of GJIC in rats and mice is well correlated with induction of both liver tumors and markers for peroxisomal proliferation which result from excess deposition of phthalate esters in the liver. However, species-specific differences exist in response to analog of phthalate esters as shown by studies with DEHP and diisononyl phthalates (DINP). However, GJIC was unaffected in some mammalian species hamsters

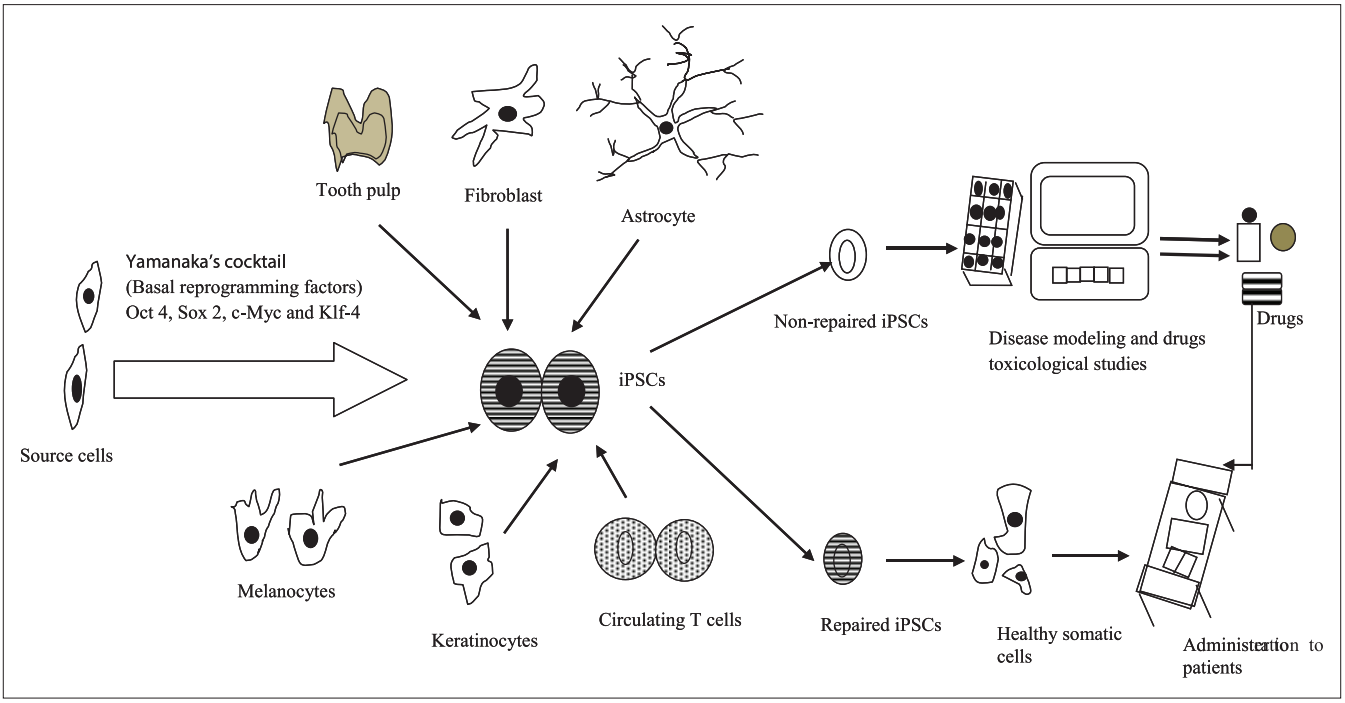

Fig. 6: Application of human-induced pluripotent stem cells in basic and applied research

and primates including humans in which phthalate treatment does not induce peroxisomal proliferation. I*n vitro* studies which extended the database to include human liver cells mirrored the *in vivo* situation. Peroxisomal proliferation has been characterized as a species-specific process essential for phthalate-induced rodent liver tumor induction. Thus, the GJIC data along with those from studies of peroxisomal proliferation support the view that the carcinogenic effects of DEHP and DINP in rodents are not relevant to humans [35]. These evaluated data on cells from other species cannot also explain the *in vivo* situation of human in the presence of any toxic agent. So currently, it is a prime concern for the researchers associated with pharmacokinetics and toxicological studies to design a reliable test system which sufficiently mirrors the *in vivo* situation of human.

But how to determine the toxic effects of any toxicant on cells, tissues or organs? The ability of chemicals with tumor-promoting or tumor-inhibiting activity to modulate gap junctional intercellular communication have been detected with two most extensively used assays, namely (1) metabolic cooperation assays and (2) dye-transfer assays [36]. In metabolic cooperation assays, a population of donor cells is incubated in the presence of an excess of a radiolabeled precursor (typically uridine) and then cocultured with unlabeled recipient cells. Under such conditions, quantitative autoradiography enables evaluation of the transfer of the resulting metabolites from loaded to unloaded cells as a function of time. In this type of experiment, coupling is demonstrated by the autoradiographic labeling of the cytoplasm of recipient cells due to the incorporation in their ribonucleic acid of radiolabeled nucleotide synthesized within donor cells and transferred across Cx channels. Radioactive nucleotide transfer allows for a direct evaluation of the permeability of Cx channels to endogenous molecules. The scrape loading/dye transfer technique is a simple functional assay for the simultaneous assessment of GJIC in a large population of cells [37]. Dye-transfer assays measure exchange of fluorescent dye from loaded cells to adjacent cells. It is demonstrated that a number of factors play an important role for routine screening of toxic agents. These include the requirement of biotransformation for some agents to exert effects on gap junctions. The tumor promoters and tumor inhibitors affect gap junctional permeability that influences many physiological mechanisms like protein kinase activation, changes in proton and Ca^{2+} intracellular concentration and oxyradical production [36]. Growth factors, hormones, extracellular matrix, and cytokines can also block GJIC [38]. In many AS cells, neither the connexin genes are expressed nor are the gap junctions functional [39]. The cancer cells completely lose expression of connexin gene. Hence, measurement of GJIC plays a significant role in determination of toxic level [40]. This involves the transfer of a low molecular weight fluorescent dye (<1000Da) between contiguous cells [41]. It is thought that chronic disruption of GJIC may release some factors that favor clonal expansion and ultimately tumor formation [42]. Reduced expression of GJs following treatment with nongenotoxic carcinogens appears to affect specific target organ [43,44]. Due to exposure to mutagens or carcinogens, xenobiotic compounds and EDs the immune response hikes to initiate intracellular signaling cascades and releases various bioactive secreting factors (Fig. 7).

Available literature suggests that [6,32,45] the different types of stem cells are involved in toxicity testing and screening of organ-specific toxicity (Fig. 8).

MSCS AND TOXICOLOGICAL STUDIES

The mesenchyme is an embryonic connective tissue which is derived from the mesoderm (the middle embryonic layer) that harbors mesenchymatous cells which have a high rate of division and the ability to spread and migrate in early embryonic development between the ectodermal and endodermal layers. The MSCs are heterogeneous multipotent stem cells which play a pivotal role in the development of all evolving structures and organs from the mesenchyme during ontogeny [46]. MSCs lie at the top of the mesenchymal cell hierarchy and progress through discrete stages of differentiation in an orderly manner to give rise to functionally and phenotypically mature tissues, including bones, smooth muscles, tendons, and cartilages [47]. In general, these MSCs are considered to originate in the mesenchyme, but embryonic MSCs have recently been shown to be derived also from the neuroepithelium and neural crest. However, it remains unclear whether ontogenically distinct MSCs are endowed with specific functions. MSCs are multipotent stem cells residing in almost all postnatal organs and tissues. Like other stem cells, they have unique properties such as self-renewal, unlimited proliferation ability, and plasticity to generate various cell types. Earlier it was assumed that MSCs can differentiate only to mesodermal lineages (osteocytes, chondrocytes, and adipocytes)

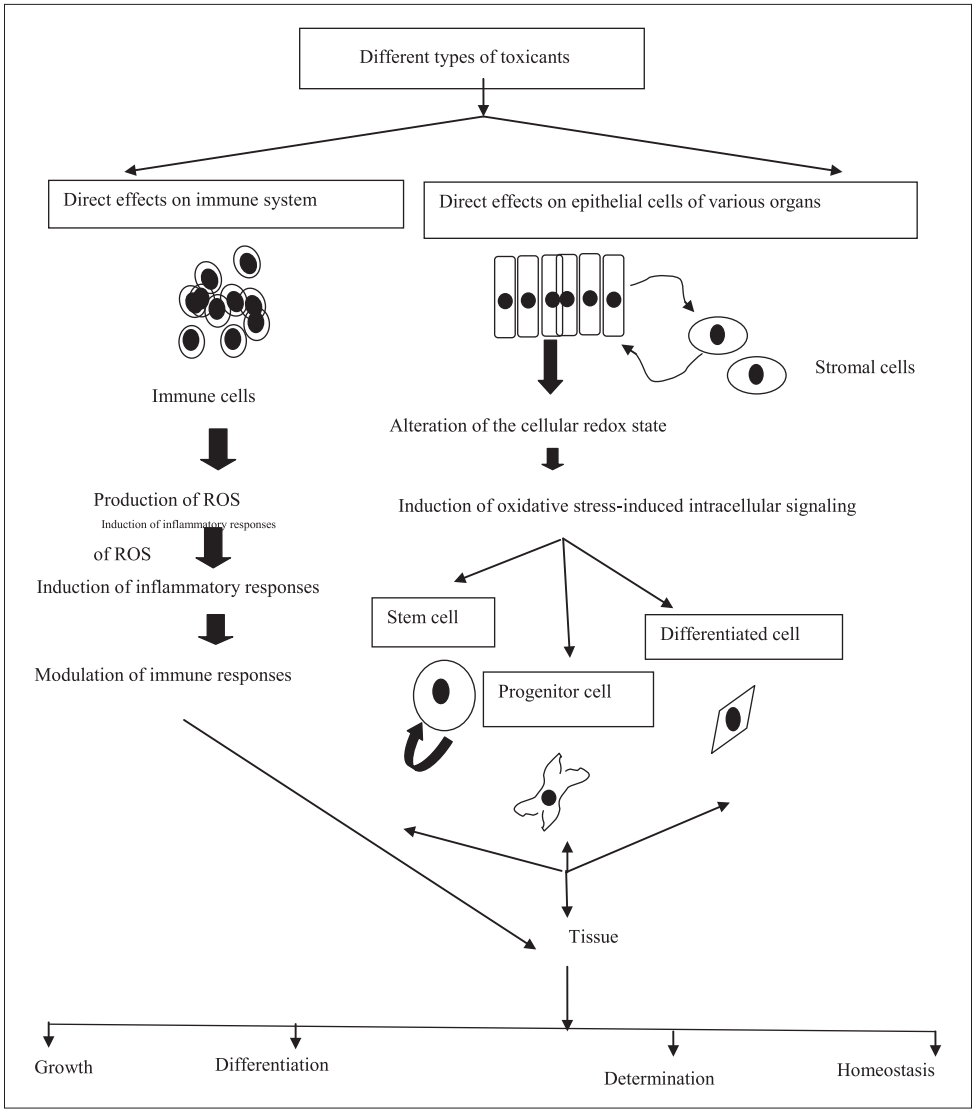

Fig. 7: Different physical, chemical, or biological agents affect normal cellular functions

but now it is well demonstrated that these can fabricate into distinct end-stage cell types of 3 primary germ layers such as mesodermal (bone, cartilage, muscle, bone marrow stroma, tendon/ligament, fat, dermis, and other connective tissues), ectodermal (epithelial, glial, and neural), and endodermal (hepatocyte and islet cell) lineages in *in vitro* and *in vivo* condition. Friedenstein *et al.* (1970) first reported the existence of non-hematopoietic multipotent precursor cells in bone marrow (BM) with skeletal and adipose potential [48]. This rare population also exists in adipose tissues, skeletal muscles, umbilical cord blood (UCB), placenta and Wharton's jelly tissues, UCB and several blood sources such as peripheral blood, menstrual blood of female other than BM. These cells can be derived, expanded, and manipulated *in vitro*. MSCs express the molecular markers which include CD90, CD105, and CD73 and fail to express the markers such as CD34, CD45, and CD14. Contemporary to other available cell lines, MSCs also express pluripotent genes that include the TFs Oct4, Sox2 and Nanog, which are normally express by hES cells [49]. Hence, this could be considered as safe alternative of ES cells and iPSCs. MSCs are special kind of stem cells that oscillate between pluripotency and multipotency. That is why MSCs are promising cell source for cellular therapy, tissue engineering, and regenerative medicine. This may be due to their inherent nature and plasticity to differentiate into multiple lineages. These have the ability to integrate into the host tissues without showing immune rejection by host immune system. This may be due to the fact that MSCs secrete a broad spectrum of bioactive macromolecules that are immune-regulatory and immune-suppressive in nature. This makes them most favorable choice for autogenic and allogenic transplantation [47]. These cells can be used to renew, repair and reform the vulnerable tissues, and therefore, widely applied to therapeutic cloning, tissue engineering, and regenerative medicine. The characteristic features of MSCs such as fibroblastic morphology, adherent nature, spheroid formation ability, easy genetic manipulation, and susceptibility to molecules that modify their natural behavior make them efficient candidate for non-therapeutic clinical research especially for pharmacological and toxicological studies. The spheroids may mimic three-dimensional (3D) organotypic models. These 3D *in vitro* culture models are expected to be relevant representative of the *in vivo* environment. Are the spheroids able to replace EBs needed for embryotoxicity and teratogenicity assay? Human MSCs can make cardiac connexins (Cx43, Cx 40 and Cx 45) and form gap junctional complex. It is recently reported that amniotic fluid-derived stem cells differentiate into cardiomyocyte-like cells and form gap junctions when directly mixed and cultured with neonatal rat ventricular myocytes [50]. Similar to other cells, toxic agents also affect viability, morphology, and function of MSCs. The effects of toxic agents on MSCs derived from different sources lead to undesirable consequences (Fig. 9).

A plethora of experimental works unequivocally established that MSCs can directly differentiate into neurons, glial cells, cardiomyocytes, and liver-specific cells (hepatocytes, pancreatic ß cells). According to a

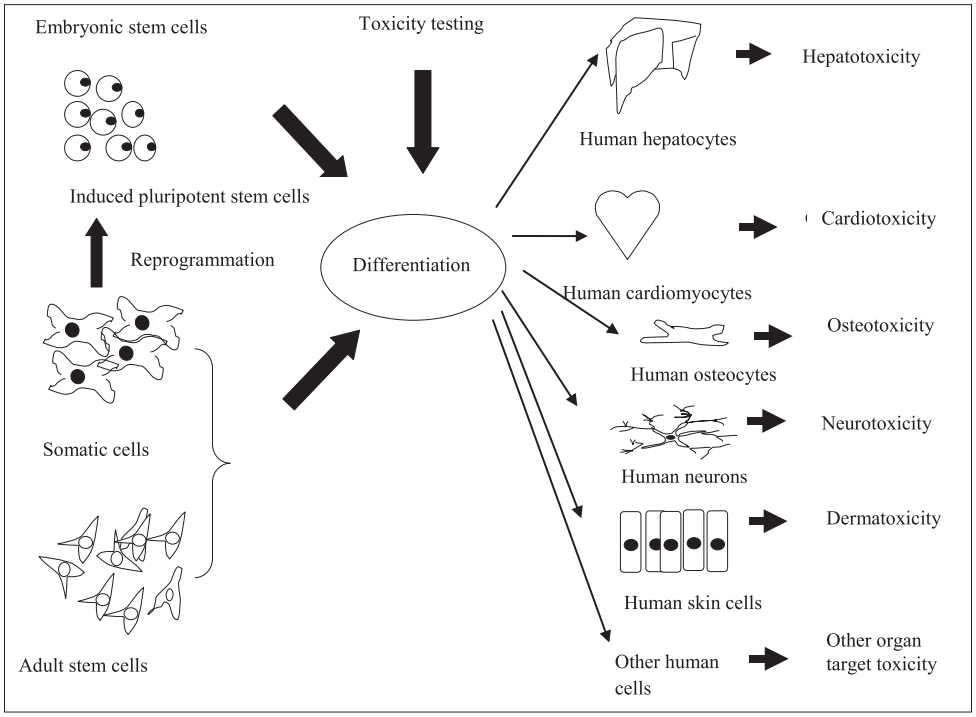

Fig. 8: Role of stem cells in screening of toxicity

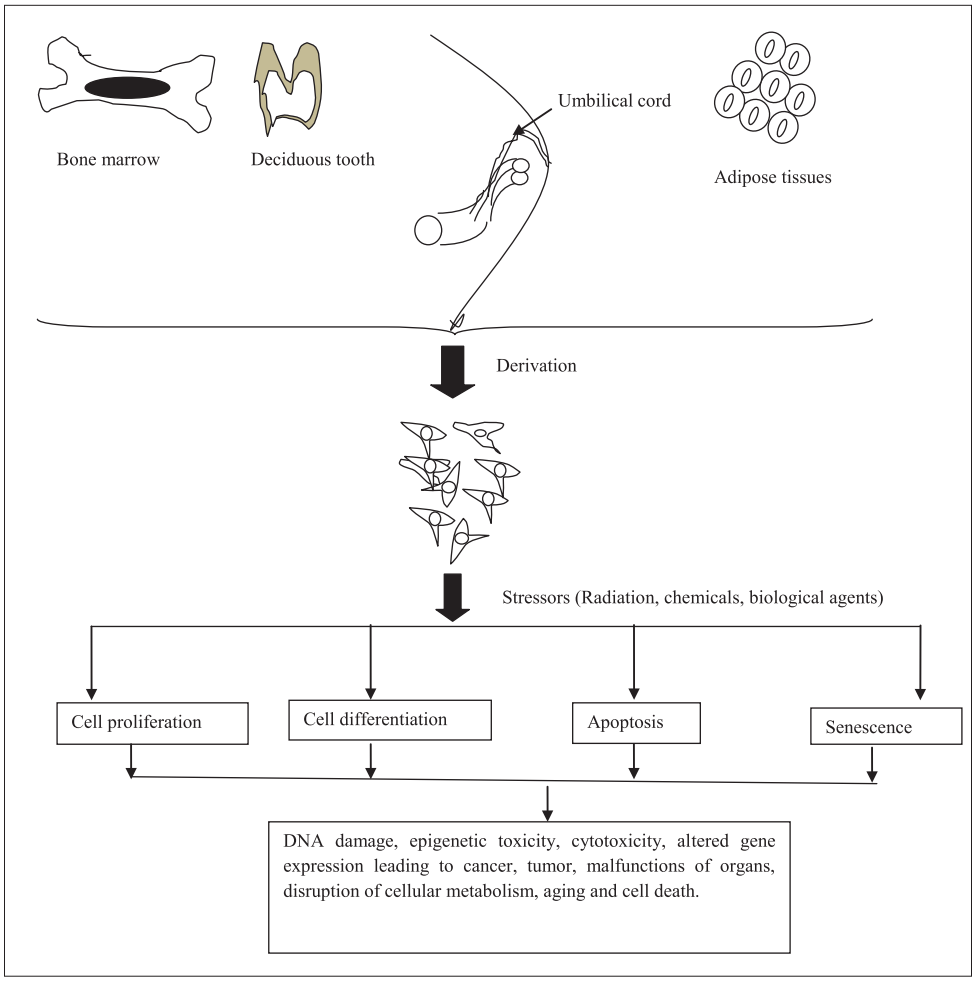

Fig. 9: Induced toxicity by different stressors on *in vitro* cultured mesenchymal stem cells

report that human bone marrow stem cells could able to differentiate to male GCs [51]. These bone marrow cell-derived GCs also express the known molecular markers of primordial GCs, such as fragilis, stella, Rnf17, Mvh and Oct4 as well as molecular markers of spermatogonial stem cells and spermatogonia including Rbm, c-Kit, Tex18, Stra8, Piwil2, Dazl, Hsp90 alpha, beta 1- and alpha 6-integrins. Recent study by Latifpour *et al*. [52] report that human umbilical cord (hUCMSCs) can trans-differentiate into primordial like GCs (PGCs) when *in vitro* culture was performed under specific condition (bone morphogenetic protein 4 followed by retinoic acid). Hence, MSCs can be efficiently utilized to study mechanisms and pathways of different toxicants provoking etiology of many diseases, toxicity testing, evaluation of cytotoxicity and genotoxicity of pharmaceuticals and toxicity screening of chemicals that induce neurotoxicity, cardiotoxicity, hepatotoxicity, reprotoxicity, and drug discovery [53]. For example, development of anti-obesity drugs requires adipocytes that can be used to test whether any chemicals and toxicants can affect this process. Scanu *et al*. [54] report that hMSCs can be used for acute toxicity testing of chemicals.

NEUROTOXICITY

The complexity of the developing human brain complicates efforts to assess DNT *in vitro*, as the underlying mechanisms may include selective cell death, delayed or aberrant differentiation, suppressed neurotransmission, disruption of the blood-brain barrier, or modulation of inflammatory signals by glial or microglial cells. Thus, an *in vitro* model to predict human neurotoxicity needs to recapitulate a diversity of cellular interactions during human brain development and should be reproducible both within an experiment and between experiments performed on different days or at different sites. The studies impressively demonstrate that capacity of neuronal precursor cells prepared from self-assemble 3D "organoids" and hMSCs offer a consistent, scalable source for diverse neural cell types including neuron, glial cells, and astrocytes in the presence of appropriate inducers. Recent report suggests that hUCMSCs differentiate into neural-like progenitor cells and matured oligodendroglial-like lineage when cultured in neurobasal medium supplemented with B27, 10 ng/ml of human recombinant bFGF, platelet-derived growth factor-AA and 100 ng/ml of Sonic Hedgehog [55,56]. Toxic effects of chemical compounds, environmental factors, naturally occurring substances, anthropogenic agents, and EDs can lead to neurotoxicity. Neurotoxicities may express as neuropathologic condition or as altered neurocthemical, electrophysiological or behavioral functions which, in turn, leads to temporary or permanent harm to the central or peripheral nervous system. Environmental toxicants or pharmaceutical agents can influence excitotoxic processes which exaggerate their deleterious effect. In case of excitotoxicity (a specific form of neurotoxicity), excessive stimulation of the neuron occurs due to spinal cord injury, stroke, or traumatic brain injury during which neurotransmitters like glutamate and similar substances are responsible for damage and death of nerve cells that can be measured by several assays (Fig. 10).

The neurotoxicity test model allows studying the adverse effect of drug candidates on neuronal cells with the help of neurotoxicity assays for screening of compounds. This test system allows the reduction in production cost of preclinical development of drugs. Impaired calcium signaling and calcium measurements may be important criteria to know about neurotoxic agents. There is a decrease in depolarization, elicited by calcium elevation that accompanies the release of the specific neurotransmitter synthesized by a given neuron. Thus, this outcome provides valuable information about the toxicity of an uncharacterized compound to assess the toxicity of various compounds. Comparing cytotoxic or apoptosis-triggering effects of a given compound at different stages of neuronal differentiation also provide useful information about the severity of that particular agent. The toxicity of corticosteroid used in ophthalmological therapy of treatment of age-related macular degeneration (AMD) has been tested using MSCs. For this, MSCs were seeded with triamcinolone acetonide (9a-fluoro-16a-hydroxyprednisolone, TA), intravitreal triamcinolone, and dexamethasone at different concentration (0.01 mg/mL, 0.1 mg/mL, and 1.0 mg/mL) [57]. The study of evaluation of drug toxicity reveals that a strong relationship exists between concentration of drugs and time with viability of MSCs. As the concentration of drugs and duration increases the viability decreases. This study demonstrates the need to use low concentration corticosteroid in pharmaceutical formulation of AMD. The developing brain is particularly vulnerable to toxic agents, even at exposure levels that have no lasting effects in the adult nervous system. Therefore, DNT assessment is a serious concern for environmental chemicals, drugs and new chemical entities. By using adipose tissue-derived MSCs (AT-MSCs) Qasemian Lemraski *et al*. [58] demonstrated that lead (Pb) is a potent DNT. This study further explains how MSCs could be utilized to determine toxic potential of chemicals. *In vitro* cytotoxicity tests are also typically carried out with transformed, immortalized cell lines, or primary cells. Immortalized cells are readily available and easily maintained, although they usually show anomalous behavior and phenotypes, which do not reflect the mechanisms observed in their normal homologous cells. Primary cells are indeed considered a better option as model systems for predicting toxicological behavior, although they are limited in quantity and suffer from batch-to-batch variation due to the need to isolate them freshly for each study. In particular, hMSCs have never been adopted to develop *in vitro* model systems for acute toxicity tests of chemicals. Therefore, the main aim of the study was to verify the possibility of using hMSCs as an alternative method to estimate *in vivo* starting dose for acute toxicity. As suggested by ICCVAM, 12 reference chemicals were assessed in the present study and a Neutral Red Uptake assay was performed. It is reported for the first time that MSCs isolated from human bone marrow can undoubtedly be used to test acute neurotoxicity [55].

CARDIOTOXICITY

Cardiovascular disease is the lead cause of death worldwide. Every year 17 million people die of cardiovascular disease. Out of this 11 million die as a result of cardiac disease and 5.5 million deaths are related to stroke. It is estimated that myocardial infarction carries a mortality rate 7% (with aggressive therapy). Even more distressing condition record due to congestive heart failure causing mortality 20% in 1 year. The major agents responsible for cardiovascular diseases are hypertension, diabetes, stress, the chronic use of few drugs, cigarette smoking, elevated cholesterol, obesity, physical inactivity, and aging [59]. The spectra of cardiovascular diseases are amenable to therapeutic intervention via cell engraftment, organ transplants, and angioplasty. However, these treatments are far from the reach of common people. These costly treatments may be due to the fact that adult cardiomyocytes have a limited regenerative capacity, and their loss permanently results in impaired myocardial contractile function leading to loss of cardiac function and heart failure. In fact, some class of drugs (cardiotoxic agents) are responsible for cardiac damage (Table 1).

Efforts are being made to develop different ways of treating cardiovascular diseases that involve not only producing immunocompatible cardiomyocytes but also establishing more sophisticated cellular drug discovery and test systems. Much faster heart rate in humans makes the rodent model unsuitable to mimic the basic physiological functions of heart of human. The contractile nature and function of *in vitro* differentiated cardiomyocytes have additional advantage as these respond in similar manner as fetal cardiomyocytes to the drugs. Hence,, these ultimately provide an optimum homogenous cell culture system for screening of cardiotoxic agents and improved high-throughput drug discovery process. Several protocols and strategies have been reported for *in vitro* differentiation of cardiomyocytes from MSCs. Cultured MSCs differentiate into beating cardiomyocytes in the presence of 5-azacytidine [60]. It is well documented that the compounds that do not interfere with ion channel functionality also causes cardiotoxic insults. In many cases of cardiotoxicity, a direct interaction of drugs with specific ion channels expressed by the cardiomyocytes leads to alteration in ion conduction through these specific channels. Effects of the agents like drugs and chemicals on potassium currents could lead to QT-prolongation,

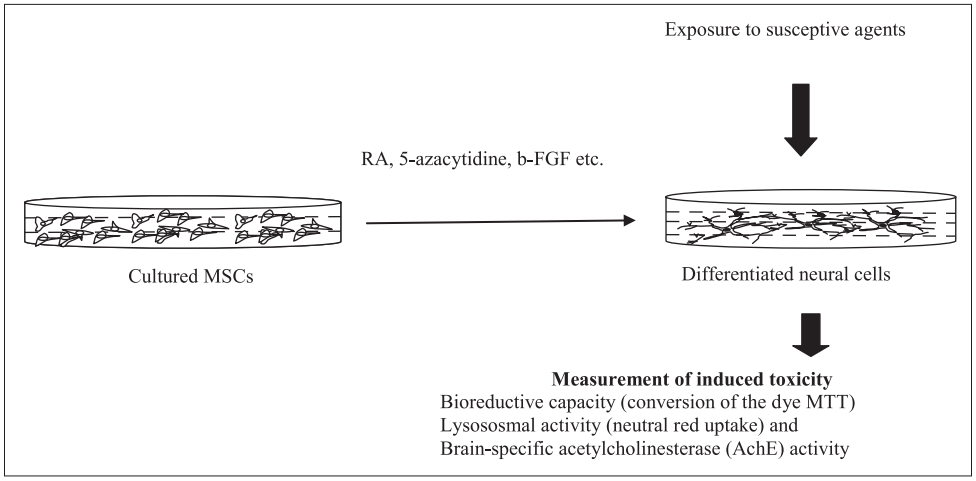

Fig. 10: Neuronal differentiation of mesenchymal stem cells and measurement of neurotoxicity by different assays

Table 1: Some cardiotoxic agents

Class of drugs	Drugs	Induced toxicity	Degree of incidence
Anthracyclines	Doxorubicin	Acute - arrhythmias	Very frequent
Alkylating agents	Cyclophosphamide	Myocarditis, CHF	Common
	Ifosfamide	Myocarditis, CHF	
	Cisplatin	Ischemia, CHF	
Antimetabolites	5-Fluorouracil	Ischemia	Common
	Cepecitabine	CHF	Rare
	Cytarabine	Ischemia	Rare
Other	Coccain	Ischemia, CHF	Very frequent in Europian country and USA

CHF: Congestive heart failure

potentially fatal arrhythmias and sometimes cardiomuscular damage without affecting ion channels [61].

Unfortunately, modern training in toxicology and pharmacokinetics is directed primarily at specific ligand-receptor interactions at the expense of system physiology. Current physiological testing strategies may potentially miss cardiac effects that manifest chronically, vascular and microvascular effects. It results from toxicity initiated in other tissues and microvascular physiology and toxicology in the context of model development, application, and underlying pathology. Cancer chemotherapies might cause cardiomyocyte apoptosis and dysfunction. However, different chemotherapeutics might have different toxicity induction mechanisms. This new approach utilizes a dual-channel automated image cytometer that allows for simultaneous measurement of the cardiomyocyte action potential and calcium transient using voltage and calcium-sensitive dyes. ESC- and iPSC-derived cardiomyocytes have recently been used to study doxorubicin-triggered toxicity [62]. MSCs-derived cardiomyocytes are suitable to study the effects of compounds which do not interfere with the ion channel functions but still cause cardiotoxicity. Doxorubicin, which belongs to the anthracycline family, has been proven to be effective in different tissue-derived cancer diseases, including cancer of the breast, lung, stomach, bladder, and skin. Despite the anti-tumoral properties of doxorubicin, myelosuppression, and particularly cardiotoxicity restrict its clinical use [63]. Doxorubicin also induces toxic effect in endogenous MSCs [64]. It is reported that BMMSCs, isolated from rats that receive doxorubicin, show slower proliferation rate and lower differentiation capacity, decreased connexin-43 production and hindered MSCs capacity to respond to cardiomyogenic differentiation stimuli [65]. So considering above cases, the conclusion can be drawn that MSCs could be a better option to detect cardiotoxic agents. Two clinically decisive biomarkers of cardiac damage that are sensitive indicators for doxorubicin-induced toxicity have been studied. Troponin T (TnT) is a useful biomarker for studying drug-induced toxicity effects on cardiac cells. After induction of doxorubicin, MSCs-derived cardiomyocytes released detectable levels of cardiac TnT and fatty acid-binding protein 3 in a dose-dependent manner [62]. Based on the availability of very sensitive and rapid analytical tools for these biomarkers, the assay lends itself well to miniaturization and high-throughput formats.

HEPATOTOXICITY

Culture of 3D tissue (*in vitro*) models can capture cell-cell and cell-matrix interactions that happen in *in vivo* counterpart [66]. Hepatocytes, the major cells of the liver, metabolize most compounds and thereby can be used to predict many pharmacological characteristics of a drug. It is still to improve and develop new models, in some areas such as hepatotoxicity. Presently, only hepatocyte-like cells (HLCs) expressing low levels of liver-specific markers, especially drug metabolizing and detoxifying enzymes are usually obtained, making them still unsuitable as metabolically competent cells for toxicity studies. The only exceptions are some hepatoma cell lines, particularly the HepaRG cell line that can differentiate from a bipotent progenitor stage to attain the functional capacity of normal adult hepatocytes in primary culture without losing the indefinite growth property of transformed cells [67]. Recently, Kwon *et al*. [68] report that differentiated HLCs from human AT-MSCs may be used as *in vitro* hepatotoxicity screening system. The toxic effect of arsanilic acid (Ars) and acetaminophen (AAP) on the hepatic development were determined. The hepatic differentiation from AT-MSCs was confirmed by an increase in hepatic proteins or genes, the cytochrome P450 (CYP) activities, albumin secretion, and glycogen storage. The toxic effects of these hepatotoxicants on enzymatic activities of lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase do not significantly differ in response to Ars treatment. AAP treatment increases the activities of all enzymes in a dose-dependent manner, significantly at concentration of 2.5 and 5 mM of AAP. The activities of CYP3A4 were not changed by AAP and Ars treatments. The activities of CYP1A2 were increased by AAP, whereas it was decreased by Ars treatment. This study demonstrates that AAP is more serious hepatotoxicant compared to Ars. Ionizing radiation is

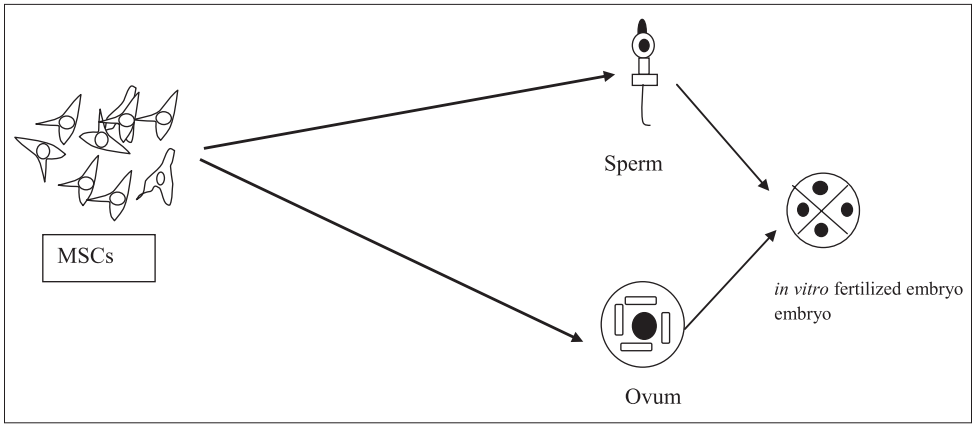

Fig. 11: Differentiation of mesenchymal stem cells into different reproductive cells

often used to treat malignancies such as breast cancer, lymphoma, Ewing sarcoma, soft tissue sarcomas and rectal and anorectal carcinomas. At the same time, radiation can induce chromosomal and gene mutation. This can cause epigenetic changes of genome leading to many diseases like skin cancer and induce oxidative stress which in turn alters gene expression. The experimental data put forth by Cao *et al*. [69] indicate that irradiation produces free radicals that adversely affect the survival of MSCs in both distal and proximal femora of mouse. Irradiation injury to the vasculatures and the microenvironment affects the niches for stem cells during the recovery period. This study is further supported by the investigation undertaken by Cruet-Hennequart *et al*. [70] on human bone marrow-derived MSCs.

MSCS AND REPRODUCTIVE TOXICITY

EDs are naturally occurring compounds or man-made substances that interfere with the function of endocrine system of the body. EDs may be any estrogen-like and anti-androgenic chemicals, environmental agents (e.g., PCBs, DDT, dioxin, and some pesticides) or biological stressors like oxidative stress or pharmacological agents like radiation and drugs. Exposure to such toxic agents at any stage of embryonic development right from maturation of gonads to post-natal development may alter the transporter expression and activity that maintain fetal growth [9]. These agents may bring epigenetic modification of placental gene expression and cause disability, neuronal disorders and abnormal behavior in offspring. MSCs represent a good model to assess reproductive toxicants as these can be used to recapitulate the different stages of development by differentiating into the cells associated with reproduction (Fig. 11).

Recent studies have shown that MSCs under appropriate conditions can differentiate into various cell types including GCs. These studies also show that MSCs without any induction express some GC-specific genes innately. Moreover, one report suggests that female MSCs have a greater tendency to differentiate into female instead of male GCs and male BM-MSCs appeared more prone to differentiate into male rather than female GC. Recently, it is investigated that a rat model with damaged ovaries caused by using an anticancer agent, cyclophosphamide was able to restore ovarian function after transplantation of A-MSCs. So, MSCs were shown to be capable of inducing angiogenesis and restoring the number of ovarian follicles and corpora lutea in ovaries [71]. hUCMSCs can differentiate into PGC under specific *in vitro* condition [53]. Thus, MSCs represent a good model to assess reproductive toxicants as these can differentiate into different reproductive cells recapitulating different stages of development of GCs. But do MSCs establish all *in vivo* developmental patterns and phases in *in vitro* environment?

CONCLUSION

Today, modern society is more dependent on the use of a wide range of different chemicals that provide substantial and highly appreciated benefits, but at the same time, they have the potential to cause damages to the environment and human health. Toxicogenomic technologies can facilitate the screening of chemical compounds for their ability to cause toxicity. Recently pharmaceutical industries develop the most advanced toxicogenomic screening applications. This reflects incentives to screen out undesirable properties and more efficiently identify drug candidates with the safest and the most efficacious profiles. Further research is suggested to develop new assay for screening chemical toxicity using MSCs that would help the public about the risk factors associated with that chemicals.

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