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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Stem cells and their clinical/therapeutic applications in biomedical and veterinary science – the perspectives

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Abstract

In recent years, stem cell research has got much importance due to its therapeutic potential in many incurable degenerative and chronic diseases. Stem cells are defined as unspecialized cells having the capacity of self-renewal by cell division, to proliferate extensively, and to differentiate into one or more cell/tissue types. Broadly stem cells can be categorized as embryonic stem cells and adult or tissue-specific stem cells or induced pluripotent stem cells. Therapeutic applications using adult stem cells (ASC) are promising for treating joint and bone diseases; cancer; haematological and bone marrow disorders; cardiac defects, non-healing wounds, spinal cord injuries, brain stroke and several other degenerative disorders. The application of embryonic stem cells (ESC) and induced pluripotent stem cells (iPS) is still in infancy. However, ESCs are used in generating transgenic animals, which contribute to the improvement of animal production traits. Mesenchymal stromal cells (MSC) show great promise as a biological therapeutic for a diverse range of unmet medical needs, but regulation of the stem cell based therapies is the need of the hour, mainly in the developing countries and other unregulated markets to avoid misuse of the novel therapeutic approaches. United States Food and Drug Administration (FDA) has put regulation in place for the clinical use of cellular therapy, and a few such FDA approved therapies are already available for limited clinical applications. Use of stem cells in combating stress; licensing of drugs and tissue engineering has gained attention as well. There must be a compliance with the privacy regulations in relation to the Health Insurance Portability and Accountability Act (HIPAA) to facilitate the transfer of cells and tissues. Apart from the regulatory concerns, the question of safety of stem cell therapy has to be answered. Thus protocols for thorough screening and testing of stem cells should be developed to preclude chances of contamination with biological and non-biological agents. The present paper describes the basics of stem cells, their characteristics, types, propagation, pluripotency and molecular control along with beneficial applications in biomedical, veterinary and pharmaceutical research. Potent clinical and therapeutic aspects discussed include the role of stem cells in regenerative medicine and treatment of several incurable ailments viz., spinal cord and brain injury, neurological and cardiac diseases, bone and cartilage repair, repair and regeneration of liver tissues, wound healing and sepsis treatment, combating deadly malady of cancer and leukemia. Apart from these, role of stem cells in generating transgenic animals, organ transplantation and gene therapy is also discussed in brief. Safety measures for stem cell based therapies have also been highlighted.

Keywords: Stem cell therapy, biomedicine, veterinary, clinical application, regenerative medicine, embryonic stem cell, adult stem cell, mesenchymal stem cell, induced pluripotent stem cells, transgenic animal, organ transplantation

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Introduction

Recent advancement in the field of biotechnology, molecular biology, genetics and immunology has revolutionized biomedical research and therapy in medical and veterinary fields. Sustained efforts have led to the development of many novel therapies and treatment modalities including cytokine therapy, gene silencing, siRNA, apoptin, phage therapy (bacteriophages, virophages, mycophages); yolk antibody therapy; herbal therapy, panchgavya and stem cell therapy (Amarpal, 2008; Gade et al., 2012 a, b; Dhama et al., 2013 a,b,c,d; Mahima et al., 2012; Tiwari et al., 2011; Tiwari et al., 2013). The subject of stem cell research has attracted tremendous interest of scientists and medical and veterinary professionals in the recent past due to its potential application of stem cells in numerous incurable diseases (Barry and Murphy, 2004; Flores-Figueroa et al., 2005 ; Pascale et al., 2007; Schöler, 2007; Amarpal, 2008; King and Miller, 2008; Brevini et al., 2008; Koch et al., 2008 and 2009; Kim and de Vellis, 2009; Wu et al., 2010; Brown et al., 2012; Gade et al., 2012 a, b). This paper describes the important development in the area of stem cell biology in general and its potential therapeutic applications in humans and companion animals.

What are stem cells?

Stem cells are defined as unspecialized cells having the capacity of self-renewal by cell division sometimes even after long periods of inactivity, to proliferate extensively, if not indefinitely and to differentiate into one or more cell/tissue types (Odorico et al., 2001; Pittenger et al., 1999; Koch et al., 2008; Gade et al., 2012 a,b).

Stem cells are present in all the multicellular organisms. Stem cells often get confused with progenitor cell but the difference lies in their self-renewal potential. The stem cells have unlimited self-renewal potential, whereas the progenitor cells have limited cycles of self-renewal. Stem cells are the foundation for every organ, tissue and cells in a multicellular organism in terms of tissue regeneration, repair and growth in adults. In a developing embryo, they differentiate in to pluripotent cells to generate organs and tissues. Stem cells also maintain the normal turnover of regenerative organs viz., blood, skin or tissues of the intestine (Schöler, 2007; Mitalipov and Wolf, 2009). Stem cells retain the ability to become some or all of the different types of cells in the body. This unique potential of these cells makes their application indispensable in the area of regenerative therapeutics. Stem cells are undoubtedly, most promising for the cell-based therapies that are currently tested in pre-clinical trials for a wide range of ailments (Gade et al. 2012 a,b).

Most extensively studied human multipotent mesenchymal stromal cells are characterised by their ability to be plastic-adherent and expression of surface markers CD73, CD90, and CD105 and absence of CD45, CD34, CD14 [CD11b], CD79 [CD19], and HLA-DR. They should also possess tri-lineage differentiation potential towards osteoblasts, adipocytes, and chondroblasts (Horwitz et al., 2005, Gade et al., 2012 a, b). Widespread clinical use of human stem cells is largely restricted to the application of hematopoietic stem cells for the treatment of leukemias, lymphomas, solid tumors, and non-malignant disorders (Gratwohl et al., 2008; Koch et al., 2009). Multipotent mesenchymal stromal cells or mesenchymal stem cells (MSCs) have not yet reached the mainstream clinical practice, but the literature is full of reports on experimental work in animal models and sporadic reports of clinical trials, in which stem cell therapies have shown promising results.

Types of stem cells

Stem cells must have two basic defining properties, i.e. self renewal and differentiation potential. Self-renewal is the unique ability of stem cells to make an identical copy of itself while differentiation potential describes their potential to produce tissue specific cells. Stem cells can be categorized broadly as embryonic stem cells (ESC), adult or tissue-specific stem cells (ADC) or induced pluripotent stem cells (iPSCs). These can also be classified according to their potency as uni-, multi-, pluri-, and totipotent cells. Adult stem cells are derived from adult body organs whereas embryonic stem cells are drawn from embryos. Stem cells can also be classified as totipotent, pluripotent, multipotent and unipotent stem cells depending upon their differentiation potential. Embryonic stem cells obtained from an early stage embryo are totipotent, however, stem cells isolated from a hatched blastocyst are pluripotent. Pluripotent stem cells have inherent capacity of differentiation to cells of the three somatic germ layers (ectoderm, endoderm and mesoderm). ESCs may be derived from morulae (Strelchenko et al., 2004), intact blastocysts (Evans and Kaufman, 1981), inner cell mass (Martin, 1981), single blastomeres (Chung et al., 2006) or even from parthenogenetic embryos (Sritanaudomchai et al., 2007). However, their use is associated with ethical issues in humans as it involves destroying embryos to obtain the cells, and possibility of teratoma formation in animals.

Adult stem cells are found in various tissues of adults including bone marrow and contribute to tissue regeneration during adult life (Amarpal, 2008). Adult stem cells include hematopoeitic stem cells, mesenchymal stem cells, neural stem cells, skin stem cells, retinal stem cells etc. Mesenchymal stem cells have been most widely used for research and therapy in

experimental and preclinical, and clinical trials (Lee, 2008). Mesenchymal stem cell (MSCs) can be most abundantly isolated from bone marrow, fat, umbilical cord blood, amniotic fluid, dental pulp, tendons, synovial membrane, skeletal muscle and Warton's jelly (Friedenstein et al., 1970; De Bari et al., 2001; Shi and Gronthos, 2003; Igura et al., 2004; Tsai et al., 2004; Rogers and Casper, 2004; Xu et al., 2005; Bieback and Kluter, 2007; Bi et al., 2007; Crisan et al., 2008; Gade et al., 2012 a,b; Pratheesh et al., 2013). MSCs can differentiate into cells of mesodermal origin viz., adipocytes; osteoblasts and chondrocytes; tenocytes; skeletal myocytes and visceral stromal cells (Pittenger et al., 1999; Barry et al., 2004). Recent studies indicated that MSCs can also differentiate into cells of ectodermal origin such as neurons and endodermal origin, such as hepatocytes. Mesenchymal stem cells may be expanded without differentiating up to 40 generations.

Pluripotency – molecular control

Embryonic stem (ES) cells are pluripotent in nature that has the potential to generate all tissue types in a mammalian organism. Changes in the stem cell cycle depend on the tissue demand (He et al., 2009). Several transcription factors are involved in maintaining the stemness as well as their differentiation into progenitors of all three germ layers during embryogenesis. During mammalian embryogenesis, several factors are responsible for maintaining the pluripotent state in the mammalian embryo, which include: Oct -4 (transcription factor); Sox-2; Nanog (homeobox containing gene); and Fox- D3 (transcription factor winged helix in nature). Down-regulation of gene expression and onset of lineage specific transcription factor expression governs the loss of pluripotency (Cavaleri and Scholer, 2003; Chambers and Smith, 2004; Robson, 2004). Takahashi and Yamanaka (2006) described that four genes (Oct4, Sox2, Klf4, and c-Myc) were sufficient to reprogram adult somatic cells back to an ES cell-like pluripotent state, called induced pluripotent stem (iPS) cells. Recent studies also revealed that Sox-2 and Oct-4 could also play a role in germ layer fate determination during differentiation of stem cells. Oct4 suppresses neural ectodermal differentiation and promotes mesendodermal differentiation and Sox2 inhibits mesendodermal differentiation and promotes neural ectodermal differentiation (Thomson et al., 2011). Along with the transcription factors mentioned above, several signalling pathways including Wnt signalling and JAK-STAT pathways play a very important role in maintaining the stemness and pluripotent state (Kiger et al., 2001; Tulina et al., 2001; Murry and Keller, 2008; Nusse, 2008; Katoh, 2008; Katoh and Toh, 2008; Ling et al., 2009).

In recent years, newer mechanisms like niches or special tissue microenvironment have been studied well that has helped to understand the mechanism of regulation of stem cell. Candidate niches as well as regulatory molecules in several mammalian tissue viz., bone marrow and skin; gut and brain have been identified (Ryu et al., 2003; Ohlstein et al., 2004; Gokhale and Andrews, 2008).

Historical background

Early botanical monographs documenting the regenerative competence of plant meristems provide evidence regarding the origin of the term 'stem cell'. In spontaneously occurring murine tumors, pluripotent embryonic cells were the first stem cells to be recognized (Brockes, 1997; Kiessling and Anderson, 2003). Embryonic stem (ES) cells were first derived from certain strains of mice (Evans and Kaufman, 1981). ES cell lines were later produced successfully for the rhesus monkey (Thomson et al., 1995) and human (Thomson et al., 1998). Irradiated mouse embryo fibroblasts as well as mitomycin-C treated murine embryonic fibroblasts (MEFs) were used for propagation of human embryonic stem cells (hES) which can spontaneously differentiate into neuronal cells (Reubinoff et al., 2001). Friedenstein group first described the MSC during 1960s, when they described that the bone marrow is a source of stem cells for mesenchymal tissues of adult life (Friedenstein et al., 1970). Friedenstein and his Co-workers observed a population of plastic-adherent, fibroblast-like cells that could differentiate into chondrocytes and osteoblasts and named them colony-forming unit fibroblasts following the culturing of bone marrow derived cells after the gradual removal of hematopoietic cells (Friedenstein et al., 1974). The ability of these cells to differentiate into cells of mesodermal origin led to name them as mesenchymal stem cells (Caplan, 1991).

Non-immunogenic characteristics of stem cells

Conventionally, use of autologous culture-derived cells seems to be logical as allogeneic cells could be associated with risk of rejection. However, use of autogenic mesenchymal stem cells for therapeutic application may be hampered by the time lag required for expansion of autogenic stem cells before application. Interestingly, marrow-derived stem cells appear to enjoy a degree of immune privilege and their use may require minimal need for immunosuppressive drugs (Jung et al., 2005). It has been demonstrated that MSCs may be immune-privileged cells that avoid allogeneic rejection as they are hypoimmunogenic, often lacking major histocompatibility complex (MHC)-II and costimulatory molecule expression, which prevents T-cell responses through modulation of dendritic cells and disrupting natural killer (NK). They

are also found to modulate CD8+, CD4+ T cell function (Di Nicola et al., 2002). Mesenchymal stromal cells (MSC) thus show great promise as a biological therapeutic agent for a diverse range of unmet medical needs (Ben-Hur et al., 2004).

Propagation of stem cells

Friedenstein et al. (1970) first time isolated stem cells as colony-forming unit fibroblasts from murine bone marrow. However, MSCs are not a unique feature of the bone marrow alone. They are also found in other tissues as mentioned earlier. MSCs have been isolated from a number of species like human, mouse, rat, mice, dog, cat, pig, horse, sheep, goat, cattle and buffaloes (Bosnakovski et al., 2006; Laura et al., 2008; Bosch et al., 2006; Eslaminejad et al., 2009; Nair et al., 2009; Gade et al., 2013; Pratheesh et al., 2013). The first successful report of mouse ESCs (Evans and Kaufman, 1981; Martin, 1981) was followed by ES cells isolation and characterization in other species like hamster, mink, rabbit, rat, monkey, marmoset, chicken, human, baboon, dog, cat, horse, pig, cow, sheep, goat and buffalo (Iannaccone et al., 1994; Thomson et al., 1998; Mitalipova et al., 2001; Notarianni et al., 1991; Saito et al., 2002; Dattena et al., 2006; Huang et al., 2010; Behboodi et al., 2011). Bone marrow is a rich source of mesenchymal stem cells, but even in the bone marrow aspirate they represent a minor fraction of mononuclear cells and thus they need to be expanded *ex-vivo* before application for therapeutic use. In animals as compared to ESCs, bone marrow derived MSCs can be isolated and cultured more easily with high *ex vivo* expansion rate and have a great potential for tissue repair and regeneration (Lin, 2011). This makes these cells as the most commonly used stem cells (Gade et al., 2012).

Applications of stem cells

As stem cells have got the potential in manipulating genes, it is assumed that research in this field can enormously help the field of agriculture as well as biomedical, veterinary and pharmaceutical research (Barry and Murphy, 2004; Flores-Figueroa et al., 2005 ; Pascale et al., 2007; Schöler, 2007; Amarpal, 2008; King and Miller, 2008; Brevini et al., 2008; Koch et al., 2008 and 2009; Kim and de Vellis, 2009; Wu et al., 2010; Brown et al., 2012; Gade et al., 2012 a, b). The stem cell research has opened a new area that has facilitated the understanding of cancer biology apart from genomic fingerpinting and development of the embryo at an early stage. Embryonic, adult and induced pluripotent stem cells are valuable for application in drug screening and tissue engineering (Amit et al., 2000; Gurtner et al., 2007). In Farm animals, embryonic stem cells (ESCs) may be helpful in generating transgenic animals (Rathjen et al., 1999; Zheng et al., 1999; Saito et al., 2003; Rolletschek et al., 2004; Brevini et al., 2008). However, the most promising use of stem cells has been in the area of regenerative medicine in the treatment of several incurable ailments (Amarpal, 2008; Gade et al., 2012 a, b).

Stem cells possess a significant potential for tissue generation to replace diseased or damaged areas in the body that also with risk of rejection and side effects at the minimum level. Thus, tissue regeneration using the stem cells is the only therapeutic option in certain degenerative diseases in human and animals. Local delivery or systemic infusion may be required for the transplantation of autologous or allogeneic stem cells into patients for stem cell therapy. Three basic mechanisms have been proposed to explain how MSCs could repair tissue injury: 1) creation of a milieu by secreting cytokines that enhance regeneration of endogenous cells, 2) transdifferentiation into the cells of host tissue, and 3) fusion with the host cells (Prockop et al., 2003; Spees et al., 2003; Mansilla et al., 2005). In case of diseases like Parkinosn's and heart disease as well as diabetes there is either dysfunction or death of specific types of cells. Stem cells can restore essential functions or losses by introducing new cells. Owing to these properties, stem cell therapy has gained enormous popularity in recent years (Forbes et al., 2002; Borge and Evers, 2003; McNeish, 2004; Fukuda and Takahashi, 2005; Yoon et al., 2005; Brederlay et al., 2006; Pascale et al., 2007; Brennand and Gage, 2011). The uses of stem cell based approaches in order to treat type I diabetes via islet transplantation is well documented in human medicine (Poulsom et al., 2002; Street et al., 2004). In the field of regenerative Veterinary medicine, autologous stem cell therapy involves harvesting tissue (viz., fat or bone marrow) followed by isolation and culture of the stem cells for administration. In veterinary medicine, autologous adipose tissue-derived mesenchymal stem cells (AD-MSC) therapy is available commercially since the year 2003.

Some striking examples of the therapeutic use of MSCs and ESCs have been reported recently (Carvalho et al., 2011; Lee et al., 2011). These address a broad spectrum of indications, including spinal cord injury, bone, cartilage and cardiovascular repair. Both mesenchymal and embryonic stem cells are utilized in therapeutics. The first successful allergenic stem cell graft in humans using donor bone marrow was undertaken in USA (Gatti et al., 1968; Bach et al., 1968). The bone marrow transplantation continues to provide the best example of the clinical use of adult stem cells. This application exploits the enormous *in-vivo* proliferative capabilities of the rare stem cell population present in bone marrow and peripheral and cord blood. Currently, there is an intense research effort is needed into *in vitro* expansion of these and other

types of bone marrow-derived stem cells for direct clinical application (Broxmeyer, 2005).

Wound healing

Krause et al. (2001) found that adult bone marrow cells give rise to epidermal keratinocytes, follicular epithelial cells, sebaceous gland cells, dendritic cells after their transplantation in mice. Autologous bone marrow derived nucleated cells have been transplanted in experimental rabbits and clinical cases to evaluate their tissue regeneration potential in full thickness wounds (Borena et al., 2009), burn wounds (Oloumi et al., 2008) and corneal alkali burn wounds (Ye et al., 2006). Several studies indicated that mesenchymal stem cells derived from the bone marrow could significantly impact wound healing in diabetic and non-diabetic animals, through cell differentiation and the release of paracrine factors. Culture expanded bone marrow-derived mesenchymal stem cells (BM-MSCs) have been shown to promote the healing of diabetic wounds (Wu et al., 2007; Wu et al., 2010; Gade et al., 2012 a, b).

More recently, allogeneic BM-MSCs exhibited similar survival, engraftment, and effect as syngeneic BM-MSCs in promoting wound healing. Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. Bone marrow derived mesenchymal stem cells were injected around wound and their application to the wound bed in an excisional wound model enhanced healing significantly in normal and diabetic mice (Wu et al., 2007). BM-MSC- treated wounds exhibited significantly faster wound closure, with increased re-epithelialization, cellularity, and angiogenesis. In addition to differentiating into keratinocytes and forming appendage-like structures, BM-MSCs in the wound enhance the proliferation of endogenous keratinocytes and increase the number of regenerating appendage-like structures (Wu et al., 2007; Wu et al., 2010).

Even xenogenic human MSCs were used for incisional wound healing and tissue regeneration in rabbit and sheep (Mackenzie and Flake, 2001; Stoff et al., 2009). In caprine, Wharton's jelly mesenchymal stem cells (WJMSCs) of umbilical cord were used to treat cutaneous wounds (Azari et al., 2011).

Bone repair

The MSCs can undergo osteogenic differentiation, and exploration of the potential for using autologous stem cell therapy to augment bone repair and regeneration is well reported (Amarpal, 2008; Gade et al., 2012 a, b; Udehiya et al., 2013). MSCs stimulate new bone formation in areas of implant site, indicating that either these cells were infiltrating the adjacent host bone or stimulating the host bone to regenerate new bone (Tolley et al., 2004; Kraus and Kirker, 2006). MSCs are the most commonly used seed cells for bone repair, having the potential for *in vitro* expansion and osteogenic differentiation (Deans and Moseley, 2000; Barry and Murphy, 2004; Chao et al., 2007). Autologous MSCs are the optimal type of seed cell; both animal experiments and clinical trials indicate that bone constructed using autologous MSCs has strong osteogenic ability (Lucarelli et al., 2004; Quarto et al., 2001). The MSCs helps in bone healing in a critical-sized bone defect in rat (Kadiyala et al., 1997). The preclinical studies were carried out in laboratory animals like rat, rabbit. Canine segmental bone defects were treated with bone marrow derived MSCs loaded onto porous ceramic cylinders. The results obtained were encouraging showing significantly greater amount of bone as compared to control (Bruder et al., 1998). Among large-sized animals the use of sheep autologous BMSC in conjunction with hydroxyapatite ceramic (HAC)-based carriers results in faster bone repair compared to hydroxyapatite ceramic HAC alone (Kon et al., 2000). Goat bone marrow derived MSCs cultured with scaffolds could repair the segmental bone defect in the tibia by 8 weeks after surgery (Liu et al., 2010). These reports demonstrate the feasibility and efficiency of using MSCs to augment the repair of bone defects in animals (Frisbie and Smith, 2010). Autologous adipose derived stem cells (ADSCs) (3.2 × 107 cells) seeded on a composition scaffold made from hydroxyapatite (HA) and chitosan (CH) fibres has been successfully used for the treatment of nonunion of radius/ulna in a cross bred dog (Lee et al., 2009). In one of our studies, $2X10^6$ bone marrow derived stem cells were injected at the site of a non-union in a non-healing fracture of the humerus in a Doberman dog. One month later, the animals showed good bony union without the signs of lameness (Unpublished data). Ease of availability and capability of allogenic BM-MSCs to avoid immune rejection (Ryan et al., 2005) have made these cells an attractive alternative to autogenic marrow-derived cells (MDCs) for reconstructive surgery. Allogeneic mesenchymal stem cells loaded on hydroxyapatite–tricalcium phosphate implants enhanced the repair of the canine femur without the use of immunosuppressive therapy (Arinzeh et al., 2003). Recent studies suggested that there are minimal chances of rejection or immunogenic reaction when allogenic mesenchymal stem cells are used and they are as effective as autogenic stem cells in the repair of experimental bone defects (Udehiya et al., 2013).

Cartilage repair

Delivery of bone marrow concentrate to acute full-thickness cartilage defects has the clinical potential to improve cartilage healing in an equine model (Fortier et al., 2010). Similarly, mesenchymal stem cells may play a potentially important role in regenerating injured

joints (Murphy et al., 2003; Gade et al., 2012 a,b). MSCs can differentiate into chondrogenic lineage (Dennis et al., 2002) and can be utilized to treat cartilage defects. MSCs have been used *in vivo* to repair full-thickness, articular cartilage defects in animal models using various carrier matrices (Wakitani et al., 1994; Sellers et al., 2000). In rabbits, repair of full-thickness defects of articular cartilage was observed after transplantation of autologous MSCs dispersed in a type I collagen gel (Yan and Yu, 2007). Similarly, in the same animal model, encouraging results were obtained after injecting calcium phosphate and hyaluronan sponge, previously loaded with autologous bone-marrow derived MSCs, in knees with osteochondral defects (DeBari et al., 2003). Single intra-articular injection of AD-MSC has been found to be efficacious in chronic osteoarthritis of the coxo-femoral joint in dogs. Mesenchymal stem cells play an important role in regeneration of injured joint. Following induction of osteoarthritis in the knee joints of caprine, injection of autologous preparation of stem cells was found to be effective (Rodkey et al., 1999; Wakitani et al., 2002; Murphy et al., 2003). The articular cartilage defects were treated with MSCs with polymers (Solchaga et al., 1999), type I collagen (Wakitani et al., 1994), and polylactic acid (Dounchis et al., 2000). Infrapatellar fat pad derived mesenchymal stem cells were used in rabbits for treatment of osteoarthritis (Toghraie et al., 2011). In a caprine osteoarthritis model it was found that the local delivery of adult mesenchymal stem cells to injured joints stimulates regeneration of meniscal tissue and retards the progressive destruction (Murphy et al., 2003). Canine MSCs seeded in type I collagenglycosamino-glycan (CG) matrices were used in dogs for repair of cartilage defects of knee joints (Xiang et al., 2006). In large-animal models, sheep were treated with in vitro differentiated MSCs for repair of chronic osteochondral (Dattena et al., 2009; Zscharnack et al., 2010). MSC therapy provides a simple, arthroscopically applicable and clinically effective approach for cartilage repair. A lot of success has been achieved recently by injecting mesenchymal stem cells directly into the joint and is considered as a recent development. Such treatment in dogs has resulted in greater flexibility in joints and less pain (Csaki et al., 2007).

Tendon and ligament repair

Induction of MSC differentiation into connective tissues other than bone and cartilage, such as tendons and ligaments, has been investigated for a potential clinical application (Gade et al., 2012 a, b). Smith et al. (2003) reported therapeutic implantation of culture-expanded autologous bone marrow-derived MSCs into a spontaneously occurring core lesion of the superficial digital flexor tendon. This case demonstrated the feasibility of using culture-expanded MSCs for the repair of tendon injuries.

Race horses are especially prone to injuries of the tendon and ligaments and full functional recovery of the horses via conventional therapies is far from satisfactory. Equine suspensory ligament injuries are challenging because healing process is slow and re-injuries are common. Natural healing when continues for long periods ultimately results in scar tissue formation that reduces the flexibility and movement of joint rendering the horses useless. The mechanical properties of healing tendons and ligaments are, also not comparable to those of normal tissue. The quality of the tendon and ligament healing can be improved with altered therapeutic strategies including stem cell therapy. Ligament healing can also be enhanced by transplantation of mesenchymal stem cells (MSCs), which are demonstrated to differentiate into fibroblast-like cells in ligament injury sites in rats and survive up to 28 days (Watanabe et al., 2002; Carvalho et al., 2011).

When a natural mechanical stimulus is combined with both bone marrow and adipose derived stem cells, regeneration of tendon tissue is promoted along with restoration of natural movement; thus re-injury rate in horses is greatly reduced due to stem cell therapy (Nixon et al., 2008). The bone marrow components were injected for recovery from ligament injuries in 100 horses and found effective (Herthel, 2001).

Bone marrow derived autologous MSCs along with collagen gel were used to repair the surgically induced patellar tendon defect in adult New Zealand White rabbits, the treated group showed significant improvement in its biomechanical properties after 4 weeks (Awad et al., 1999; Krampera et al., 2006). Similar combination was used for Achillies tendon repair in a rabbit model (Young et al., 1998; Butler and Awad, 1999). It was found that MSCs treated groups had better stress modulus, and strain energy density compared to the controls. In equine, autologous bone marrow derived MSCs after *in vitro* expansion were utilized and found effective for regeneration tendon matrix in superficial flexor tendon injury (Smith et al., 2003). The collagenase induced tendinitis in the superficial digital flexor tendon in 8 horses was treated with adipose derived nucleated cells (ADNC) injection. The treated group showed improved tendon organization as exhibited by cartilage oligomeric matrix protein (COMP) expression (Nixon et al., 2008). In race horses, the adipose derived MSCs were used to successfully treat experimental tendinitis (Carvalho et al., 2011).

Spinal cord injuries

Acute spinal injuries are common in canines and felines that lead to loss of tissue, including myelinated

fibre tracts responsible for carrying nerve impulses. The nervous tissue has limited regeneration capacity and complete restoration of locomotor activity is a challenge to modern therapeutics. The mesenchymal stem cells were found to have the ability to differentiate into oligodendrocytes and other cell types needed to restore neuronal function in injured spinal cord (Dobkin et al., 2006; Harris, 2008). Therefore transplantation of stem cells with the ability to differentiate into neurons and supporting cells may be a practical method for recovery in such cases (Gade et al., 2012 a, b). In addition to transdifferentiation, they may secrete growth factors that could support neuroprotection and/or axon regeneration. The potential of stem cells to support spinal cord repair has been studied extensively (Coutts and Keirstead, 2007; Harris, 2008). In order to repair spinal cord injuries, therapies based on stem cells have been found fruitful. The differentiation potential of stem cells was assessed and they not only differentiate but also integrate into axonal pathways and thus aid in regeneration of injured nerves (Dasari et al., 2007). Bone marrow derived MSCs were first used in Rhesus monkeys for nervous tissue regeneration which appeared promising (Deng et al., 2006). Xenogenic transplantation of human umbilical cord blood (UCB) stem cells in rats following spinal cord injury significantly enhanced locomotor function within 14 days after therapy as compared to the control group (Dasari et al., 2007). Intrathecal implantation of autologus bone marrow derived MSCs improved locomotor activity significantly in dogs within one week (Adel and Gabr, 2007). Similarly allogenic UCB derived MSC transplantation resulted in nerve regeneration in canine fetuses. In UCB, MSC treated group animal's gait improved in two weeks and the weight bearing of the pelvic limbs was also improved. The improved nerve conduction velocity and distinct structural consistency of the nerve cell bodies was observed in lesions treated with MSCs (Lindvall and Kokaia, 2006; Lim et al., 2007; Kramer et al., 2012).

Ischeamic brain injury

Neural stem cell therapy has raised the hopes in order to treat neurodegenerative diseases. In order to properly integrate in the brain cells that are injured, isolation as well as enrichment and propagation of neural stem cells are necessary. Use of compliant conditions of culture and differentiation of both embryonic as well as somatic stem cells in a directional manner the clinical application of such therapies is possible nowadays (Kim and de Vellis, 2009).

Damage caused by stroke injury to the central nervous system (CNS) is a major cause of death and disability in humans. Transplantation of MSC directly into adult rodent brain was found safe and it reduced functional deficits associated with the stroke (Harris, 2008). This supported the notion that MSCs can adopt neural cell fates and are feasible candidates for the treatment of stroke injury (Burns et al., 2009). Exogenous stem cells offer the complementary advantages of being available in unlimited numbers with additional control over fate, cell number, timing, and site of delivery. The fact that substantial functional gains have been observed in animal models after delivery of cells of both neural and non-neural origin in preclinical models of ischemic brain injury is encouraging (Gage, 2000).

MSCs were also found useful for treating cerebral infarction (Jeong et al., 2005) and ischemia (Chen et al., 2001), Myocardial infarction (Orlic et al., 2001), autoimmune disorders (Cristofanilli et al., 2011) in experimental models.

Myocardial infarcts

In dogs, cardiac disease causes significant morbidity and mortality, contributing to over 50% of mortalities in some breeds such as the Cavalier King Charles spaniel. The stem cell therapy would minimize loss of cardiomyocytes by reducing cell death, promote the return of a stunned and hibernating myocardium to normal function, stimulate revascularization of the damaged region by enhancing angiogenesis, and regenerate viable cardiomyocytes thereby preserving contractile function and reducing the opportunity for scarring (Caspi et al., 2007).

Adipose as well as bone marrow derived stem cells have successfully been used to treat myocardial infarction in dog (Gade et al., 2012 a, b). Before injecting into the heart, stem cells derived from bone marrow and adipose tissue can be used for induction of a cardiac cell fate resulting in increased contractility of the heart along with reduction in the damaged area a few weeks after the application of stem cells (Beltrami et al., 2001; Black et al., 2007 and 2008). Stem cells seeded on to a patchy porous substance lead to induction of tissue regeneration (http://www.meih.org; http://www.ul.edu.lb).

Use of autologous bone marrow stem cells (a specific type) for treatment of myocardial infarction is a novel application of stem cell therapy that is gaining popularity nowadays (Zhang et al., 2001; Drukker and Benvenisty, 2003; Orlic et al., 2001 and 2003; Cai et al., 2004; Anderson, 2008). Clinical experiences based on animal studies at the early stages have shown that mesenchymal stem cells (MSCs) when therapeutically delivered improve function of heart after an acute myocardial infarction. This could be due to the fact that MSCs can generate various signalling molecules that are cardio-protective and can differentiate into a myocyte as well as into the lineage of the vascular system (Flores-Figueroa et al., 2005; Siepe et al., 2005; Schuleri et al., 2006; Coulter et al., 2008; Udehiya et al., 2013).

In a canine acute myocardial ischemia model, 100×10^6 MSCs were delivered 7 days after acute myocardial infarction (AMI) via intracoronary (IC) and transendocardial (TE) routes. This study suggested that MSC treatment is probably safe and effective after AMI. TE group showed higher cell retention (clusters even in the injury center of the infarct) with an increased vascularity and greater functional improvement than did the IC group (no clusters; cells at the border of the infarct). The higher local cell density in the TE group may be important for therapeutic effectiveness. Treatment of infarction in heart of rat with embryonic stem cells helps in generating new cardiomyocytes (embryonic origin) that get integrated within the infracted part of the myocardium of the host. Such therapy normalizes the architecture of the ventricles and reduces the signs of myocardial necrosis (Hodgson et al., 2004; Li et al., 2008).

Hepatic applications

The existence of liver stem cells within the adult bone marrow was first reported in 1999 and since then it had been confirmed in multiple further studies. MSC can be induced to a hepatic lineage by incubation with specific growth factors such as hepatocyte growth factor (HGF) and have not only been isolated from bone marrow, but can also be obtained from a number of other tissues such as umbilical cord blood and adipose tissue. The effectiveness of systemically administered MSC in the repair and regeneration of liver tissue has been most extensively studied in the carbon tetrachloride (CCl4) model of progressive liver fibrosis in mice (Rambhatle et al., 2003; Sakaida et al., 2004; Zhao et al., 2005; Oyagi et al., 2006). The studies documented only limited engraftment of donor MSC in the damaged liver.

Applications in solid organ transplantation

The use of MSC for preventing acute rejection following solid organ transplantation may have significant advantages, as immunosuppression is coupled with the ability to repair ischaemic damage and therefore MSC transplantation has the potential to target both inflammatory and alloimmune pathways. MSCs exert an immunomodulatory effect towards a large number of immune effector cells, including CD4+ and CD8+ T cells, NK cells, B cells, monocytes and dendritic cells (Peroni and Borjesson, 2011). However, results on prolongation of graft survival have been conflicting. Advancement in the field of stem cell research has led clinical trials to treat kidney diseases in cats and liver diseases in dogs (Togel et al., 2005; Quimby et al., 2013). Placement of adult stem cell seeds inside a tissue bed soil allowing the stem cells to differentiate into the tissue bed cells is a possible method of tissue regeneration in adults. Investigation concerning soil tissue conducive to regeneration is however still in its infancy (Young et al., 1998; Smith, 2001; Rossant, 2008; Cyranoski, 2009; Brown et al., 2012).

Control of infection

Sepsis is well recognized in people and dogs and is associated with high mortality rates. In dogs, for instance, reported mortality rates associated with septic peritonitis range from 21% to 68% (Butler and Campbell, 2010). In a study the bacteria from the gut were released into the abdomen, resulting in severe infection, inflammation and organ damage throughout the body. Six hours after inducing the infection, approximately half the mice were given an intravenous injection of mouse mesenchymal stem cells, while the other half received a control injection of a saline solution. Both groups of animals also received antibiotics, the standard treatment for sepsis. After five days, 50 per cent of the animals that received the cells were alive, compared to just 15 per cent of the control animals that did not receive the cells. MSCs may have potential application as adjuvant therapy in canines with severe bacterial infection and sepsis (Shirley et al., 2010).

Leukemia

Allogeneic blood as well as marrow transplantation (allogeneic BMT) in order to treat malignant hematologic disorders and genetic diseases requires myeloblative conditioning using the radiation and immunosuppressive drugs. Allogeneic BMT for treating leukemia type of cancers induce immune-mediated graft-versus-leukemia (GVL) reaction, which is beneficial for eliminating the residual pathological host cells and curing the cancer. For this reason, relatively non-myeloablative conditioning before allogeneic BMT has been introduced in recent years for establishing host-versus-graft tolerance. It helps in engraftment of donor immuno-hematopoietic cells for introducing GVL effects. Thus, allogeneic non-myeloablative stem cell transplantation is potentially a new approach in human medicine to successfully eradicate malignant cancer cells as well as genetically abnormal host hematopoietic cells (Lin et al., 1996; Slavin et al., 1998).

Generation of transgenic animals

Isolation of totipotent stem cell from embryos and subsequent incorporation of the desired DNA into the embryo of the host results in generation of chimeric animals (Emerging Technology Series, 1998; http://www.fao.org). A type of adult stem cells, spermatogonial stem cells (SSCs) can differentiate in the niche of a testis, which are used for the generation of transgenic animals by either transplantation or by

directly injecting in the seminiferous tubules (Brinster, 2002; Miao, 2011). Blastocyst injection using transgenic pluripotent stem cells is also an alternative approach for the generation of transgenic animals.

Stem cell oriented products and application of gene therapy

There is every possible chance of curing a disease hypothetically if an individual is having a disease caused by single gene mutation using either their own stem cells following *in vitro* gene modification or allogeneic adult stem cells. Previously, somatic cell therapy and gene therapy were synonymous, but now they are well distinguished and the stem cell therapy without any genetic manipulation is more widely acceptable than the gene therapy approaches. Food and Drug Administration (FDA) gives approval to treatment of somatic cells manipulated *ex vivo* with a gene therapy vector and such a strategy find its relevance at present due to greater possibility of manipulating isolated population of stem cells in culture. The products that come under biological products are those containing genetically modified cells for transplantation as well as viral vectors (Food and Drug Administration, 1993; Kessler et al., 1993; Weissman, 2000; Lawrenz et al., 2004; Maitra et al., 2005). Gene therapy strategies have also aided to the treatment of intracranial tumors in dogs (Krishnamurthy et al., 2009).

Safety measures for stem cell based therapies

The safety, both short-term and, in particular, long-term, of stem cell technologies is largely unknown. To date, there have not been any reports of significant adverse reactions to transplantation of BMSCs. However, this could be due to reporting bias. Until clinical efficacy and safety of stem cell therapy are proven, at least, we can assume that these procedures do no harm. The Food and Drug Administration (FDA) has jurisdiction over the production as well as marketing of any stem-cell–based therapy in the interest of public safety. The recently promulgated regulations regarding cells and tissues; and cellular and tissue-based products provide an appropriate regulatory structure for the development of wide range of stem-cell–based products in order to replace or repair damaged tissue (Halme and Kessler, 2006). It is not required to screen and test self cells for communicable diseases but certainly is required for transplantation between two individuals. Screenings as well as testing is required additionally for tissues possessing specific risk of transmitting disease viz., viable leukocyte-rich or reproductive cells (or tissues). There is however no need of screening while transferring gamete from a closely related (sexually) donor. An important concern is the use of fetal calf serum for culturing of cells which as per FDA specification must come from a country which is certified to be disease free, especially the bovine spongiform encephalitis (BSE). The guidelines of xenotransplantation must be strictly adhered to while using xenogenic feeder cells. Regulations will however remain the same (as is applicable to several other stem cell based products involving genetic modification of cells) if there is involvement of embryos generated by somatic cell nuclear transfer. A donation of stem cell or embryo in every single case is required to be archived in order to identify additional infectious agents and genetic disease markers. Current knowledge of the donor contact information is of utmost importance as there may be year gaps between donation and subsequent clinical use of product. There must be a compliance with the privacy regulations in relation to the Health Insurance Portability and Accountability Act (HIPAA) to facilitate the transfer of cells and tissues; gametes or embryos (Holden, 2002; Lo et al., 2005; Greely, 2006; http://stemcells.nih.gov/info/scireport).

Other applications of stem cells

The stem cells in the hair follicle can help to combat stress conditions such as: physical injury (Jaks et al., 2010). In order to conduct screening as well as toxicity assays in animal models *in vitro*; and the corresponding *in vivo* models, stem cell based disease models developed by various scientists have drawn attention of the clinicians. This has made the licensing of various drugs justified (Pouton and Haynes, 2005; Ebert and Svendsen, 2010). In the process of tissue engineering, understanding the mechanism of differentiation and expansion of stem cells has helped to make advancement in the field of regenerative medicine (Guillot et al., 2007).

Conclusion and future perspectives

In order to determine the safety and efficacy of stem cell based products, much has to be learned. The research and development should continue in this area to understand the several unknown molecular control and regulation in the life cycle of stem cells. The risk assessment of inappropriate cell functioning can be hastened provided the biology of self-renewal as well as differentiation is understood properly. Risk of carcinogenesis may be associated particularly with the induced pluripotent stem cells and embryonic stem cells. In order to ensure safety of stem cell therapy, non-invasive tracing of transplanted cells *in vivo* and development of techniques for identification of mixed population of cells in culture are critical factors. Ethical issues associated with harming of animal embryos and animal welfare also need to be given due consideration in the area of stem cell based disease modelling and therapeutic application. It is likely that there will be evolution of new regulatory frameworks to control

development of new stem cell based therapies and generation of transgenic animals. The safety and efficacy of the next generation of stem cell based products must be ensured by providing an appropriate structure through the existing regulations pertaining to products (biological) as well as tissues. The field of tissue engineering must be given special attention. Above all, it is mandatory for the clinicians, researchers and scientists to be aware of the regulations concerning stem-cell based therapies and at the same time their application to ensure safety to animal and human population. Much is needed to be understood and explored to propagate, popularize and take advantages of stem cell therapy and its other useful applications in biomedical research that include both veterinary and medical field for safeguarding health of humans and animals.

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