This is the first review to investigate the effects of Cu, Se and Zn supplements on the growth, proliferation, and differentiation of veterinary and human mesenchymal stem cells. Currently, mesenchymal stem cells are evaluated in clinical studies for their therapeutic potential to treat various diseases, disorders, injuries, in various tissue engineering approaches that could provide repair. To date, many reports have indicated several agents that could improve the proliferation and differentiation of mesenchymal stem cells. However, the efficacy of compounds such as growth factors or trace elements has not been validated by clinical trials.

Keywords: mesenchymal stem cells, Copper, Selenium, Zinc ion, trace element.

INTRODUCTION

Stem cells are pluripotent cells that are characterized by their self-renewal abilities through mitotic cell division, as well as by their potential for differentiation into multiple types of lineages such as: chondrogenic, osteogenic, adipogenic, myogenic, neurogenic, and hepatogenic (1-3). Two kinds of stem cells that isolated from animals and humans are embryonic stem cells which are derived from the inner cell mass of blastocyst, and adult stem cells known as multipotent marrow stromal cells, mesenchymal stem cells (MSCs) which under standard conditions (e.g., DMEM supplemented with 10% FBS) are a heterogeneous population of plastic-adherent, mostly spindle shaped, fibroblast-like cells present in most tissues, including adipose tissue, synovial membranes, bone, skin,
pancreas, blood, fetal liver, lung, and umbilical cord (2, 5). For the first time in the late 1960s, Friedenstein and colleagues carry out the study on bone marrow stromal cells (BMSC), establishing that single cell suspensions of BM were able to generate colonies of adherent fibroblast-like cells when cultured in vitro (4). Furthermore, in the 1970s functional characterization of the stromal cells in regulating the proliferation, differentiation and survival of hematopoietic stem cells was revealed by Dexter et al. (3). The morphology and cytochemical characterization of cultured stromal cells was further studied by Friedenstein and Castro-Malaspina. It has been indicated that MSCs include a heterogeneous population of cells with multilineage differentiation potential, with the ability to modulate oxidative stress, and to produce a variety of factors that are capable of regulating a broad range of biological functions (5). Among the most important are angiogenesis, secretion of neuroregulatory peptides, growth factors and cytokines that can have immunomodulatory, angiogenic, anti-inflammatory and anti-apoptotic effects (6).

The nutrients are divided into organic nutrients such as amino acids, carbohydrates, lipids and vitamins, inorganic salts and inorganic elements such as Co, Cu, Mg, Se, and Zn. Inorganic elements are known as trace elements that influence important chemical and biological processes. They possess important therapeutic properties for infectious and inflammatory diseases, central nervous system disorders and autoimmune diseases. Many studies demonstrated that trace elements existed in various forms, and kept a dynamic balance status in human body. Deficiency or excess of trace elements can induce body metabolic disorder and cellular growth disturbance, even mutation and cancerization (7). It has been demonstrated that many of these elements act as enzyme cofactors and are essential to the survival and growth of most cells. The aim of this review was to describe the state of the art on the role of Cu, Se and Zn as trace elements on stem cells. Like the cell seeding density, oxygen and carbon dioxide concentrations, temperature and pH, supplements that are used in culture medium, these elements affect proliferation, differentiation and aging of cells. Some trace elements are included in different types of media that are used for various types of cells. For example, DMEM/F-12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) (Gibco, Paisley, UK) is a widely used basal medium for supporting the growth of many different mammalian cells such as glial cells, fibroblasts, human endothelial cells, and rat fibroblasts. Compared to other basal media, F-12 contains a wider variety of components, including zinc sulfate, putrescine 2HCl, lipoic acid, Na hypoxanthine, and thymidine.
BIOMETICAL FUNCTIONS OF Cu, Se AND Zn AND THEIR EFFECTS ON MESENCHYMAL STEM CELLS

COPPER

Over the past few decades, the molecular mechanisms of Copper (Cu) on cells have been under intense investigation (8). Cu known as redox-active metal is one of the most important trace elements for the normal growth and development of the bone. Cu is recognized as part of the copper-binding proteins and enzymes such as Cu, Zn superoxide dismutase, cytochrome oxidase, ceruloplasmin, coagulation factors (8). Another role of Cu is in control of the striking metabolic changes that have long been known to occur in cancer cells (8). It is accepted that moderate and severe Cu deficiency cause osteoporosis and skeletal system abnormalities, respectively (9). In addition, the effects of Cu, calcium, manganese and zine supplements on improvement of spinal bone loss in postmenopausal women were clarified (10). Rodriguez et al. (2002) evaluated the effects of Cu on the functional characteristics of bone marrow mesenchymal stem cells (BMSCs) (11). Their results showed that the copper was able to modify the differentiation and the proliferative activity of MSCs obtained from postmenopausal women (9). This change is such that in a Cu supplemented medium cell proliferation rate decreased while osteogenic and adipogenic differentiation of MSCs increased (9).

Some investigations have assessed effects of Cu on osteogenic differentiation of MSCs, angiogenesis promotion and stimulation of endothelial cell proliferation (12). Hence copper has been used clinically as a therapeutic agent to promote vascularization (13-15). Li et al. (2014) demonstrated that Cu may suppress osteogenic differentiation of MSCs and inhibit in vivo bone formation (12). In other words Cu can downregulate the expression of Runt-related transcription factor 2 (Runx2) and other osteogenic differentiation-related genes via the Runx2 signalling pathway.

SELENIUM

Selenium (Se) is an essential trace element required for the catalytic activity of mammalian selenoproteins such as thioredoxin reductase and glutathione peroxidase, a family of antioxidant enzymes that protect membrane lipids and macromolecules from oxidative damage by scavenging free radicals (16-20). These proteins have an anticancer effect and increase production of cytotoxic T-cells and natural killer cells (16). Moreover, protective effects of Se as an antioxidant in neurodegenerative diseases including stroke, cerebrovascular disease, Alzheimer's
disease, Parkinson's disease, familial amyotrophic lateral sclerosis, and Duchenne muscular dystrophy have been investigated (21). In a study, Yeo and Kang (2007) described the protective roles of selenite on both neurochemical and behavioral markers of H$_2$O$_2$-induced neurotoxicity in mouse brain-derived neural progenitor cells (22). They hypothesized that these protective effects might directly be related to thioredoxin reductase expression, optimum H$_2$O$_2$ removal, and a consequent inhibition of pro-apoptotic events such as caspase 3 and 9 activation (22).

Additionally, higher levels of Se have been associated with a lower risk of many types of neoplasia, including prostate, lung, colorectal, and possibly bladder, although the data are inconsistent (Rayman, 2005; Brinkman et al., 2006) (7). Se was thought to inhibit carcinogenesis through several different mechanisms, including reduction of oxidative stress and inflammation, enhancement of immune response, induction of apoptosis, cell cycle arrest, and transactivation of DNA repair genes (Smith et al., 2004; Rayman, 2005) (7). Cancer-protective effect of Se appears to be at a serum concentration > 10.6 µg/dL (16).

**ZINC**

Zinc (Zn) plays important roles in various biological functions such as metabolism of RNA and DNA, signal transduction, steroid receptor expression, gene expression and development and integrity of the immune system, but there is little accessible information regarding its effects on MSCs (16). Furthermore, it is believed that Zn as well as L-carnitine as antioxidant plays an important role in scavenging reactive oxygen species and protecting against accelerated aging of the skin and muscles (23, 24, 42). Zn is an essential trace element for the maintenance of germ cells, the progression of spermatogenesis, and the regulation of sperm motility (7). In some previous studies zinc was proposed as an additive to medical materials, including zinc-substituted hydroxyapatite powders, and coatings, tricalcium phosphate powders, and bioactive glass granules (25). In recent years, zinc oxide nanoparticles have been the subject of increasing research consideration, and are used in several products such as sunscreens, biosensors, food additives, pigments, and rubber manufacture. In the biomedical field, there have been few reports on zinc oxide nanoparticles cytotoxicity to mammalian cells (26). Some reports suggest that these nanoparticles are toxic against bacterial infections, neuroblastoma cells and vascular endothelial cells, and induce apoptosis in neural stem cells but do not have toxicity on cultured human dermal fibroblasts (25).

Zn deficiency can be accompanied by malabsorption, acrodermatitis enteropathica, chronic liver and renal disease, sickle cell disease, diabetes, malignancy, testicular atrophy, sperm abnormalities and other chronic illnesses.
Moreover, it has been clarified that Zn is present in sperm mitochondria and flagella (27), but there had been no reports to date concerning the effect of intracellular Zn upon sperm function and role of Zn during spermatogenesis in any detail (9, 27–29). Additionally, Zn is a significant catalytic component of over 100 different mammalian enzymes such as DNA and RNA polymerases, histone deacetylases and DNA ligase that are clearly needed for normal DNA replication and cellular proliferation (24). Zn supplementation provided in the drinking water (4 mmol/L) to pregnant mice resulted in an apparent increase in the number of proliferating cells. Zn deficiency appears to reduce stem cell proliferation during brain development (24). One of the mechanisms that may be responsible for reduced stem cell proliferation is the fact that Zn deficiency can elevate levels of glucocorticoid hormones (24).

One of the fields of investigation on Zn is osteoporosis. Zn is considered to be useful for the treatment of osteoporotic patients (25). Numerous evidences suggest that osteoporosis is the result of bilateral interaction between genetic susceptibility and environmental risk factors. Among them, definite essential trace elements were reported to be involved in osteoporosis. Zinc is evaluated as a chief element present in the bone and it has been shown to regulate osteoblastic function and bone formation. Previous studies demonstrated that administration of zinc ion prevented bone loss in rats (25, 26). Zinc ion has been shown to play a role in the preservation of bone mass by stimulating osteoblastic bone formation and inhibiting osteoclastic bone resorption in rat (30). The cellular mechanism of Zn ions has also been partially interpreted. Zn ions inhibited the formation of osteoclastic cells from bone marrow cells and could also reduce pit formation (31, 32). The stimulated effects of Zn ions on bone formation and mineralization process was clarified (33-34). The effects of different concentration (1×10^{-11}, 1×10^{-10}, 1×10^{-9}, 1×10^{-8} and 1×10^{-7} mol/L) of Zn ion on the osteogenic and adipogenic differentiation of bone marrow-derived mesenchymal stem cells (BMMSCs) and the adipogenic trans-differentiation of osteoblasts have been reported by Wang et al., (2007). They reported that except for 1×10^{-9} mol/L, other mentioned concentrations of zinc ions inhibited the osteogenic and adipogenic differentiation of MSCs (35). The effects of zinc ions on the expression of germ cell genes from BMMSCs was investigated by Ghasemzadeh-Hasankolai et al. (2012) (36). They reported that zinc sulphate (ZnSO4) can increase male fertility by regulation of the expression of testis germ cell-specific genes in the differentiation process and spermatogenesis (36).

In another investigation the effects of the small concentrations (2 and 5% by mol) of zinc added to the sol-gel bioactive glass on the osteogenic differentiation of MSCs were evaluated based on the alkaline phosphatase (ALP) activity of cells (25). The results demonstrated that ALP was significantly upregulated in the presence of Zn-containing bioactive glass granules, particularly with larger quantity of granules and at later culture times (over 14 days) (25).
Table 1
Relevant research on the effects of Cu, Se and Zn as trace elements on proliferation and differentiation of veterinary and human mesenchymal stem cells

<table>
<thead>
<tr>
<th>Trace element</th>
<th>Author names</th>
<th>Year of publication</th>
<th>Title of publication</th>
<th>Modeling</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Copper</strong></td>
<td>Rodriguez et al.</td>
<td>2002</td>
<td>Modulation of the proliferation and differentiation of human mesenchymal stem cells by copper</td>
<td>Human</td>
<td>Cu increases the osteogenic and adipogenic differentiation potential of MSCs and decreases proliferative activity of cells (9).</td>
</tr>
<tr>
<td><strong>Selenium</strong></td>
<td>Yeo &amp; Kang</td>
<td>2007</td>
<td>Selenium effectively inhibits ROS-mediated apoptotic neural precursor cell death <em>in vitro</em> and <em>in vivo</em> in traumatic brain injury</td>
<td>Mice</td>
<td>Protective function of selenite through attenuation of secondary pathological events most likely results from its comprehensive effects that block apoptotic cell death, ensuring maintenance of functional neurons and inhibition of astroglialosis (22).</td>
</tr>
<tr>
<td><strong>Selenium</strong></td>
<td>Park et al.</td>
<td>2014</td>
<td>Selenium improves stem cell potency by stimulating the proliferation and active migration of 3T3-L1 preadipocytes</td>
<td></td>
<td>Selenium stimulates stem cell potency by increasing the proliferation and active migration of 3T3-L1 cells (38).</td>
</tr>
<tr>
<td><strong>Yin L.M. et al.</strong></td>
<td></td>
<td>2013</td>
<td>Effects of sodium copper chlorophyllin on mesenchymal stem cell function in aplastic anemia mice</td>
<td>Male and female BALB/c and DBA/2 mice</td>
<td>Sodium copper chlorophyllin promotes the proliferation, differentiation and immunoregulatory capacity of MSCs in mice with aplastic anemia (37).</td>
</tr>
<tr>
<td><strong>Wang et al.</strong></td>
<td></td>
<td>2014</td>
<td>Inhibition of osteogenic differentiation of mesenchymal stem cells by copper supplementation</td>
<td>Sprague Dawley rats</td>
<td>Cu inhibits osteogenic differentiation of Rat bone marrow MSCs (rBMCs) (12).</td>
</tr>
<tr>
<td>Author(s)</td>
<td>Year</td>
<td>Study Description</td>
<td>Species</td>
<td>Findings</td>
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<tr>
<td>Daeian et al.</td>
<td>2014</td>
<td>Selenium supplementation in patients undergoing hematopoietic stem cell transplantation: effects on pro-inflammatory cytokines levels</td>
<td>Human</td>
<td>Selenium has no effect on pro-inflammatory cytokines levels in patients undergoing hematopoietic stem cell transplantation (39).</td>
<td></td>
</tr>
<tr>
<td>Song et al.</td>
<td>2014</td>
<td>Reactive oxygen species regulate the quiescence of CD34-positive cells derived from human embryonic stem cells</td>
<td>Human</td>
<td>Selenium enhances vascular differentiation and promotes proliferation of human embryonic stem cells-derived angioblasts (28).</td>
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<tr>
<td>Zinc</td>
<td></td>
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<tr>
<td>Tassabehji et al.</td>
<td>2006</td>
<td>Zinc regulation of adult stem cell proliferation and neurogenesis in the rat dentate gyrus</td>
<td>Adult rats</td>
<td>Zinc deficiency impairs neurogenesis and neuroplasticity in the dentate gyrus leading to behaviors consistent with depression in adult rats (40).</td>
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<tr>
<td>Wang et al.</td>
<td>2007</td>
<td>Effect of zinc ion on the osteogenic and adipogenic differentiation of mouse primary bone marrow stromal cells and the adipocytic trans-differentiation of mouse primary osteoblasts</td>
<td>Female KM mice</td>
<td>Cell growth is almost unaffected by zinc supplements. Zinc ion inhibits osteogenic and adipogenic differentiation of mesenchymal stem cells (35).</td>
<td></td>
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<tr>
<td>Yamaguchi et al.</td>
<td>2009</td>
<td>Zinc is an essential trace element for spermatogenesis</td>
<td>Cultivated male Japanese eels</td>
<td>Zinc is an essential trace element for the maintenance of germ cells, the progression of spermatogenesis, and the regulation of sperm motility (27).</td>
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<tr>
<td>Levenson &amp; Morris</td>
<td>2011</td>
<td>Zinc and neurogenesis: making new neurons from development to adulthood</td>
<td>---------</td>
<td>Zinc deficiency, both during development and adulthood, reduces neurogenesis by limiting the number of proliferating neuronal precursor cells in the central nervous system (24).</td>
<td></td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Study Description</td>
<td>Animal Model</td>
<td>Key Findings</td>
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<tr>
<td>Taccola et al.</td>
<td>2011</td>
<td>Zinc oxide nanoparticles as selective killers of proliferating cells</td>
<td>Wistar furth rats</td>
<td>Zinc oxide nanoparticles have the potential to function as natural selective killers of all highly proliferating cancerous and non-cancerous cells, (26).</td>
<td></td>
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<tr>
<td>Ghasemzadeh-Hasankolai et al.</td>
<td>2012</td>
<td>Effect of zinc ions on differentiation of bone marrow-derived mesenchymal stem cells to male germ cells and germ cell-specific gene expression in rams</td>
<td>Shal strain ram</td>
<td>Zn ions can increase male fertility by regulation of the expression of testis GC-specific genes during the differentiation process and spermatogenesis (36).</td>
<td></td>
</tr>
<tr>
<td>Liu et al.</td>
<td>2013</td>
<td>Effects of zinc transporter on differentiation of bone marrow mesenchymal stem cells to osteoblasts</td>
<td>Sprague Dawley female rats</td>
<td>ZnT7 is involved in the switch from the undifferentiated state of MSC to an osteogenic program, and marking the expression level of ZnT7 may be useful in the detection of early osteogenic differentiation (41).</td>
<td></td>
</tr>
<tr>
<td>Sahin et al.</td>
<td>2014</td>
<td>Increased stem cell marker expressions during the peri-implantation period in the rat endometrium: constructive role of exogenous zinc and/or progesterone</td>
<td>Female Wistar Albino rat</td>
<td>Zinc could be useful in the induction of implantation rates and expression of αvβ5 integrin, vitronectin, and embryonic stem cell markers might be increased in the presence of zinc (7).</td>
<td></td>
</tr>
<tr>
<td>Oh et al.</td>
<td>2014</td>
<td>Effects on growth and osteogenic differentiation of mesenchymal stem cells by the zinc-added sol-gel bioactive glass granules</td>
<td>Adult rats</td>
<td>Zinc addition to bioactive glass may be useful in development of biomaterials for the stimulation of adult stem cell in bone tissue engineering (25).</td>
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</tbody>
</table>
CONCLUSION

Although studies (Table 1) showed that trace elements could affect proliferation, cell viability, bone metabolism and differentiation, further investigations on the impact of these elements on growth and development of the mesenchymal stem cells is required.

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