1.0 INTRODUCTION

At present, the mobile phone usage is on the rise due to the unprecedented growth in the global communication industry, which has resulted in a dramatic increase in the number of wireless devices and mobile phone towers. In the last decade, there has been a massive proliferation of cell phone towers, which are placed close to human habitation.India has 97 crore mobile users and four lakh mobile towers to cater to them. This has led to calls getting disconnected during a conversation and so the plan is on by the telecom ministry to increase the number of mobile phone towers. People are not aware of radiofrequency electromagnetic field (RF-EMF) radiation and the ensuing health hazards, particularly on cells and DNA of animals and human and biosystem on the whole. There are special concerns regarding the safety of population and personnel exposed to radiofrequency (RF) radiation emitted by both the cellular phone terminals and the base-station antennas (BSA) **(Bergqvist, *et al*., 2001).**

The biological effects of radiofrequency electromagnetic fields and a possible potential relation to some health problems and cancer causation are controversial. There have been several experimental animal studies of the possible adverse health effects associated with environmental exposure to extremely low electromagnetic frequency (300MHz - 3GHz), which is a part of the non-ionizing radiation spectrum, such as that emitted by cell phone towers, electric transmitter and electric substations. Such exposures have been linked to disruption of bird migration, decreases of animal and other living organisms and their population. The GSM technology commonly used in this communication produces constant pulsed microwave radiation. The mobile phone towers are transmitting continuously even when nobody is using the mobile phone and so human and other organisms living near cell phone towers are in this radiation constantly. It is known from a variety of scientific studies and technologies that significant biological effects result from non-thermal, non-ionizing, pulsed RF-EMF radiation, which is utilized in most common modern digital cellular and cordless phone systems in India and around the world. The RF-EMF radiations are different from other electromagnetic radiation sources as FM radio, TV transmitters, high power density antennas and other microwave radiation. Basically, the radiation range of 800, 900, 1800 and 2450 MHz frequency of electromagnetic waves are used for the mobile phone towers and their communication. In India, commonly applied mobile communication technologies are the digital technologies GSM 900 and 1800. For the mobile phone signals to reach everywhere, the towers will have to be situated at every corner of the city and at every place of our country. These towers are an essential part of mobile communication network necessary to establish connection between the mobile telephone and the rest of the mobile network. Human beings are bioelectrical and RF-EMFs are known to influence the

electrical activity in the nervous system at their cell and molecular level to a large extent. Since the radiofrequency electromagnetic radiations also known as electro smog, cannot be seen, smelt or felt, no one would realize their potential harm over long periods of exposure until they manifest in some form of biological disorders. The International Agency for Research on Cancer (IARC), a part of World Health Organization (WHO), designated RF-EMF from cell phones as "possible human carcinogen" group 2B (WHO-2011).Tumor, diabetes mellitus, infertility, neurodegenerative disorders, and even suicides are on the rise in India. This is a type of invisible health hazard pollution (IHHP) and the modern technologically savvy society is exposed to it constantly **(Sivani, *et al*., 2014).**

There is a general concern about the possible hazardous health effects of exposure to electromagnetic frequency radiations (EMF) from mobile phone towers. In most Asian countries, towers have become ubiquitous to guarantee connectivity in large urban areas,and particularly in Chennai in the state of Tamil Nadu, India, around 18,000 base stations are found. Radiofrequency electromagnetic field radiations produce fields of varying intensity at various places and higher intensity fields exist near the cell phone towers. Earlier studies on the effects of high frequency EMF to animals, plants and human body have shown that long time high frequency exposure is very harmful for the animals, plants and human beings **(London, *et al*., 1999; Karunaratha MA. 2006).** High frequency electromagnetic waves (EMWs) radiate from the towers and interact with the biological factors. This interaction is a complex function of numerous parameters. EMWs in free space are characterized by the frequency, intensity of electric and magnetic fields, their direction of propagation and polarization. In the year 2010, a research agenda recommended by WHO for radiofrequency (RF) fields, were mainly on health effects. High priority areas were human studies, cell and stem cell studies (*in vitro*) and social science research on RF-EMF-related health concerns and perceived health risks.

1.1 ELECTROMAGNETIC RADIATION AND THEIR EFFECTS

1.1.1 MEASUREMENT OF ELECTROMAGNETIC RADIATION FROM CELL PHONE TOWERS

The radiofrequency electromagnetic radiation is measured by radiation detectors and they cover a range of radiations. They are either wideband survey meters or frequency-selective receivers/spectrum analyzers with calibrated receptor antennas for in situ measurements or compliance assessment. To verify compliance with frequency dependent limits and observe the

progression of radiofrequency emitters with their signals, technically suited and reproducible measurement methods and instruments have to be established. Compared to other radiation sources, such as FM radio or TV transmitters, the electromagnetic radiation has more effects. Therefore, very limited information is available on the exposure to cellular base station radiation in residential areas at different distances and directions of antenna sites. The objective of this field study was to collect measurement data, statistical evaluation, documentation and exposure assessment for cellular phone tower radiation in India. Measurements were conducted at different distances and directions, inside and outside of representative public and residential buildings. Frequency selective spectrum analysis was used to obtain GSM power densities following the current recommendations for GSM cellular phone radiation measurements.

Official international and national standards and safety guidelines (based on ICNIRP recommendations) are still only taking into account the risk of thermal effects of RF-EMF radiation. Most of the official electromagnetic radiation public exposure measurements are conducted to observe the percentage of the current standard with only broadband – not frequency selective - measurements. Only in very few cases, one or more percent of the (thermal) guideline value is reached or exceeded close to antenna sites. Exposure recommendations based on non-thermal effects are by far lower by many magnitudes. Frequency selective measurements are necessary to observe the cellular base station downlink frequencies. The use of cell phones has become widespread. The analogue NMT (Nordic Mobile Telephone) system introduced in the 1980s operated at an electromagnetic resonance of 900 MHz. A decade later, the GSM (General System for Mobile Communication) succeeded it with a radiofrequency of 900 MHz, pulsing at 217 Hz. The recent DCS (Digital Cellular System), which uses a radiofrequency of 1800 MHz has spread rapidly **(Roelandts, 2003)**. Cell phones have a wide range of specific absorption rate (SAR) depending on the model used and it is approximately 0.1-2 W/kg.

1.1.2 INSTRUMENT FOR RF-EMF RADIATION MEASUREMENT

It was measured by a detector, DETEX 189 (NESA Radiation Solutions Pvt. Ltd. 2002). Electromagnetic radiation comes from all the Electrical and Electronic Appliances, FM and TV towers, Cell Phones and Cell Towers and Wi-Fi. There is a growing concern about harmful hazards of cell phones and cell towers radiation as there are more than 900 million cell phone users and 5 lakhs cell phone towers in India. Large numbers of these towers are near residential buildings, schools, hospitals and office complexes. People living very close to the cell phone towers are in the lobe of radiation, which is being emitted continuously. They have

reported many health problems such as headaches, concentration problem, memory loss, sleep disturbance, joint pain, miscarriage, heart related problem and cancer. The measuring instrument depicts the electromagnetic radiation levels using color signals such as red for high, yellow for caution and green for safe.

Figure 1.1: Radiation measuring detector and its functional system

1.1.3 CELL PHONE TOWER BASICS AND STANDARDS IN INDIA

Particularly in India, a mobile tower, at a height of 20-55 m is placed on a building or is situated on the ground **(Fig.1.2).** The cell phone towers are made of iron and steel. The antenna installed on the cell phone tower, 20-30 cm of width, produces the RF-EMF radiation. There are different designs of antennae that vary in power density. Sector antennas for 2G and 3G transmission, broader sector antennas for 4G transmission and parabolic microwave antennas for point-to-point communications are used in urban and suburban areas. The cabin shelters and protects the network equipment, electrical supplies and the diesel generator. There are different types of cell phone towers used by cellular operators in India and they include the macro cell, micro cell or pico cell. Categorization is based on the purpose of the site rather than in terms of technical constraints, such as radiated power or antenna height.

In India, macro cellular base station provide the main infrastructure for a mobile phone network and their antennas are mounted at sufficient height to give a clear view over the surrounding geographical area. The maximum power for individual macro cellular base station transmitter is 20 watt **(Kumar, 2010; CPCB 2010).** Recent surveys by World Health Organization (WHO) have shown that the RF exposures from base stations range from 0.002% to 2% of the levels of international exposure guidelines, depending on a variety of factors such as the proximity to the antenna and the surrounding environment. This is lower or comparable to RF exposures from radio or television broadcast transmitters. The National Council on Radiation Protection and Measurements (NCRP), USA, the Institute of Electrical and Electronics Engineers (IEEE) and

the International Commission on Non-ionizing Radiation Protection (ICNIRP) have adopted a whole-body SAR value of 4 watts per kilogram (4W/kg) as a threshold level of exposure.

The government of India adopted the guidelines of ICNIRP for electromagnetic radiation from cell phone towers in 2008. The cellular phone (GSM) services are being operated at 900 MHz and 1800 MHz frequency band in India. On 1st September 2012,the permissible power density was reduced to 4500 mW/sq.m for 900 MHz and 9200 mW/sq.m for 1800 MHz. The threshold zone for 900 MHz and the limit at which body starts feeling the radiation is 230,000 mW/sq.m and for 1800 MHz is 460,00 mW/sq.m for 900 MHz mW/sq.m. Therefore, the safety margin is 500 times that of ICNIRP limit of 450 mW/sq.m for 900 MHz and 920 mW/sq.m for 1800 MHz **(Kumar, 2010, 2013)(Table 1.1).**

The sources of RF-EMF from different antennas, such as AM towers to Wi-Fi also have some effects on animals and human health. The Wi-Fi radiation level is increasing every day in India because of the growing demand of wireless communication. Transmission power ranges between 100 KW to 100mW. Exposure threshold limits in terms of field strength and power density are then derived from this value. Under the circumstances, it is more practical and convenient to measure related radiated power density or E field or H field (averaged over a stipulated period for workers and general public) in the far-field region of interest at a cell site to ensure compliance with stipulated levels (rather than measuring SAR).

Table 1.1 Radio Frequency sources in India **(Kumar, 2010; DoT, 2012; TRAI 2014)**

RF sources	Operating Frequency	Transmission power	Number
AM Tower	540- 1600 KHz	100KW	197 Town
FM Tower	88-100MHz	10KW	503 Town
TV Tower	180-120 MHz	40KW	1201 Town
Cell phone tower	800-220 MHz	20W	5.4 lakh towers
Mobile phone	GSM- 1800 CDMA GSM- 900	1.2 W	800+ million
Wi-Fi	2.4-2.5 GHz	10-100 mW	Wi-Fi – Hot spot

The Indian government adopted ICNIRP guidelines of 1998 for safe power density. Hence, for GSM900 transmitting band (935-960 MHz), power density was 4.7W/m^2 and for GSM1800 transmitting band (1810-1880 MHz), it is 9.2 W/m^2. The ICNIRP guidelines clearly state that for simultaneous exposure to multiple frequency fields, the sum of all the radiation must be taken into consideration. However, in India, this limit is applied to individual carriers, so the radiation level exceeds by several times the norms prescribed by ICNIRP guidelines. Depending upon the total number of transmitters in that area, people (the elderly, housewives, small children) living near the towers are exposed to this radiation 24 hours a day. ICNIRP has considered only the thermal effects of radiation and not the non-thermal effects. The scientists all over the world have found non-thermal electromagnetic radiation to have significant health effects, which occur at levels much below the norms set by the government.

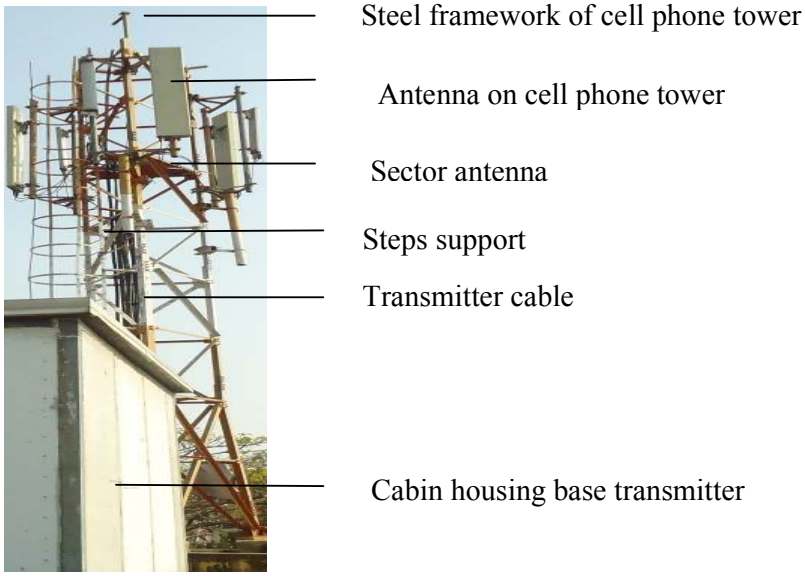
Table 1.2 International RF Exposure Standards and Guidelines

RE-EMF LIMITS	**COUNTRIES**
9.2 W/m^2	ICNIRP and EU recommandation 1998 – adopted in India
3	Canada
2	Australia
1.2	Belgium
0.5	New zealand
0.24	Belgium
0.1	China
0.095	Italy
0.009	Germany
0.00001	New South Wales

1.1.4 MEASUREMENTS OF TEMPERATURE AND HUMIDITY

The temperature and humidity are taken into account for this RF-EMF radiation study. Reliable measuring instruments were used for the measurement from the commencement of the experiment to the end.

Figure 1.2: Cell Phone tower and antenna



1.2 IN VIVO PURSUIT USING ANIMAL MODEL

The RF-EMF radiation exposure studies previously have been linked to cancer, changes in gene and DNA or protein expression, cell signaling, oxidative studies, apoptosis, blood brain-barrier and genotoxicity. There were numerous studies on cell phones and cell phone tower RF-EMF exposures have demonstrated various biological effects, such as cell level damages, double- and single-strand DNA breaks, childhood leukemia, headaches, changes in sleep pattern, changes in cellular morphology and neural electrophysiology **(Bioinitiative Report, 2014)**. The standards which govern the electromagnetic field level are considered safe by many countries around the world.

1.2.1 GUINEA PIG AS AN EXPERIMENTAL ANIMAL MODEL FOR ELECTROMAGNETIC RADIATION IMPACT ASSESMENT

Figure 1.3: Guinea pig animal model

Scientific classification

Kingdom	**: Animalia**
Phylum	: Chordata
Class	: Mammalia
Order	: Rodentia
Suborder	: Rodentia
Infraorder	: Hystricognathi
Parvorder	: Caviomorpha
Family	: Caviidae
Subfamily	: Caviae
Genus	: Cavia
Species	: C. *porcellus*
Popular name	: Guinea pig

The guinea pig (*Cavia porcellus*) is also called as the cavy. The female guinea pigs are called as sows and the males as boars. Guinea pigs can live up to 8 years, although 5 years is the average lifespan. The weight of mature females ranges from 700-850 g and from 950-1200 g for adult males. Guinea pig is a species of gnawing animal (rodent), belonging to the family *Caviidae* and is the best known representative of caviomorph gnawing animals. **(Fig.1.5).**Guinea pigs are considered to be valuable research animals. For long, guinea pigs have been used in bacteriological and serological research, which have resulted in a sizeable accumulation of information on blood values, cell counts, elementary analyses and physical constants. Guinea pigs have been used frequently in nutritional research and in immunology experiments. The Duncan-Hartley strain guinea pigs are commonly used in biomedical and biotechnological research. The guinea pig is used as a model for many diseases such blood-related diseases, chromosomal damages, hair loss, corneal diseases, epilepsy, Alzheimer's, heart disease, leukemia, melanoma, arthritis, hearing, reproduction, asthma and allergies related to skin, among other disorders **(Schwenkee and Cragg, 2004; Suckow MA. *et al*., 2012).**

The guinea pig is especially an important model for the human immune system, as its immunological genes, more than that of the mouse genes, are similar to human. It is currently the best model for testing bio defense agents and is critical for vaccine testing. The guinea pig is

also useful for toxicological studies, since it is exquisitely sensitive to toxic effects of the environmental agents and has similar reactivity as humans **(Tambrallo and Fish, 2000).** Guinea pigs are also used by neurologists to study neurodevelopment disorders, where defects in the cerebellum lead to problems with coordination and behavioral traits **(SAFRI, 2013).** This animal has been used in scientific experimentation since the 17th century. The guinea pig is also a popular experimental animal for studying prevalent bacterial diseases such as tuberculosis and diphtheria **(Padilla-Carlin DJ. *et al.*, 2008).** The guinea pig is a potentially more relevant small animal species for predicting human safety. More recently human and guinea pig have been found to possess a similar oxido-reductase system, with the general antioxidant status of guinea pig and human being quite similar **(Buehler PW. *et al.*, 2007).**

1.2.2 DEFINITION OF STEM CELL

Stem cells are generally described as clonogenic and undifferentiated cells that are able to self-renew and to differentiate into one or more types of differentiated and committed cells **(Reyes-Botella *et al*., 2000; Jiang *et al*., 2002).** To date, stem cells have been isolated and characterized from tissues and bone marrow of all ages, including embryonic, fetal and adult tissues and adult bone marrow. Among the basic term of adult (postnatal) stem cells, mesenchymal stem cells (MSCs) represent a population of multipotential cells, which are currently defined by a combination of morphologic, phenotypic and functional properties, and which are capable of giving rise to at least mesenchymal-derived tissues, including bone, cartilage, fat, tendon and muscle **(Friedenstein *et al*., 1974; Dazzi *et al*., 2006).** As a part of the stromal fraction, MSCs also regulate osteogenesis and are responsible, in part, for the regenerative capacity of bone tissue **(Friedenstein *et al*., 1974).**

Definition of mesenchymal stem cell (MSCs): The mesenchymal stem cells, as the other types of stem cells, own the same characteristics and meet the classic criteria that define a stem cell. One of the defining criteria of stem cells is the self-renewal ability or the ability to generate identical copy of themselves with the same capacities through mitotic division over extended time periods (clonality). In culture, after they have been passaged several times, MSCs have self-renewal activity, in *in vivo*cells collected from an experimental and control guinea pigs **(Reyes-Botella *et al*., 2000; Jiang *et al*., 2002).** Then, the basic characteristics of stem cells are their multipotenciality where a single cell can differentiate into a variety of lineage cells.

1.2.3 SOURCES OF STEM CELL

MSCs can be isolated from many different species. They include human stem cells **(Pittenger Aveline, *et al*., 1999; Zvaifler, *et al*., 2000; Kuznetsov, *et al*., 2001; Covas,*et al*., 2005),** murine stem cells isolated from bone marrow and tissues **(Phinney *et al*., 1999; Baddoo, *et al*., 2003),** rat MSCs isolated from the stromal bone marrow and at embryonic levels**(Santa Maria *et al*., 2004; Rochefort *et al*., 2005; Rochefort *et al*., 2006).**MSCs can also be isolated from adult guinea pigs and cats **(Martin *et al*., 2002).** MSC might be found with a variable proportion in different fetal and adult tissues, but these cells often represent a small portion of these tissues. Usually, they are isolated from the stromal fraction of adult bone marrow. Indeed, the bone marrow source is the most well studied and accessible, but MSCs form a rare population of the bone marrow micro environment and may represent only 0.01 - 0.0001% of the adult human bone marrow nucleated cells. This is considerably lower than the proportion of hematopoietic stem cells that represent about 1% of the marrow nucleated cells.

1.2.4 ADULT STEM CELL

Adult mesenchymal stem cells are mostly multipotent stem cells and are capable of self-renewal throughout the entire organism's life. The adult stem cells differentiate into other mature cell types. The adult stem cells are already committed and other cells, which are normally intermediate cellswith increased commitment, are called primordial cells. These types of stem cells reside within mature tissues or bone marrow and serve as limitless sources for new cells, enabling maintenance and repair of the tissue by continuously regenerating mature tissues either as part of normal physiology or as part of repair after injury. The adult bone marrow or tissues in higher organisms harbor cells, termed as adult stem cells, and these cells are reminiscent of un-programmed stem cells. Cells have many sources and the bone marrow is the most accessible source. Mesenchymal stem cells (MSCs), also known as marrow stromal cells, are defined as self-renewable, multipotent progenitor cells with the capacity to differentiate into tissues of the mesodermal lineage, including bone, cartilage, adipose, tendon and muscle **(Poutos &Giannoudis, 2005)**. MSCs have also shown to possess the capacity to differentiate into hepatic stem cells **(Peterson *et al*., 1999).**

1.2.5 ISOLATION OF BONE MARROW

There has been continuing interest in both the biology and experimental applications of the adult stem like cells from bone marrow, referred to as either mesenchymal stem cells or marrow stromal cells (MSCs) **(Prockop DJ. *et al., 2003)***. MSCs from guinea pig and rat bone marrow have been the most extensively characterized, in part because they are relatively easy to isolate by their adherence to plastic and can be extensively expanded in culture. Also, the guinea pig and rat MSCs can differentiate into multiple cell phenotypes, including bone, fat, cartilage, muscle, epithelium and early neural progenitors. The guinea pigs MSCs are far more difficult both to isolate from bone marrow and to expand in culture than guinea pig or rat MSCs. In contrast to both type of MSCs, the cultures of guinea pig MSCs (gMSCs) are frequently contaminated by hematopoietic progenitors that overgrow the cultures. These features of gMSCs greatly limited the ability to test the cells in the large number of interesting genetic models that are now available as transgenic mice **(Phinney DG.*et al., 1999)***. The bone marrow cells are studied for application in regenerative veterinary medicine and experimental analysis because of their diversity, cellular plasticity and low tumorigenesis. Recent studies demonstrate that bone marrow cells not only reconstitute blood cells, but also contribute in muscle, brain, liver, heart and vascular endothelium formation. The isolation, quantification and expansion of these cells identify that cell damages can be used as pathologies that affect humans and animals **(Barker.*et al*. 2003).**

1.2.6 ISOLATION OF MONONUCLEAR CELLS FROM BONE MARROW

Adult bone marrow contains at least two types of stem cells: hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), sometimes also referred to as marrow stromal cells **(Majumdar MK.*et al*., 1998).** The isolation of mononuclear cells (MNCs), although do not belong to the set of molecular procedures, is the first step of cell sample processing used in many molecular diagnostic test in stem cell culture. Erythrocyte, plasma is often performed prior to nucleic acid extraction or before the separation of specific cell subsets to increase the yield and purity of these procedures.

1.2.7 CRYOPRESERVATION OF STEM CELL

In recent decade stem cells have been the objects of thorough attention of researches in cell biology, experimental and clinical medicine. The interest on stem cells is stipulated by the ability of these cells for unlimited self-renewal and formation for all types of differentiated cells in an organism. The prospects and other diseases are pinned on their therapeutic application. Stem cells use opens huge perspectives of transplantology up to the creation of new organs. For more than 20 years, the object of multiple studies by the collaborators from various institutes, such as the National Academy of Sciences of Ukraine,for problems related to cryobiology and cryomedicine, is stem cells derived from different sources. The two main types or sources of stem cells were adult stem cells and embryonic stem cells, as well as fetal tissues and the fetal stem cell as sources. Mandatory condition of effective clinical uses of stem cells is the establishment of low temperature banks for the material enriched by the cells.

1.3 FLOW CYTOMETRY ANALYSIS

Flow cytometry is a useful technique due to the fact that the cells can be monitored, providing sensitive and specific information about each single cell. In relation to the optics of flow cytometry, when the light source hits a cell, amount of light scattered to the side is detected by the size and shape of the cell. Flow cytometers use lasers as their source to excite cells. The excitation from the lasers must be equivalent to the absorption wavelengths of flurochromes used. The argon laser is most commonly used since it produces several lines in the UV and can excite fluoresce in, which is a common fluorochrome. The other parameter detected is forward scatter and it provides information about the surface properties, complexity of the cells and can determine how granulated the cells are. Various populations of cells can be distinguished from the information provided by side and forward scatter following acquisition of samples. In addition, antibodies have fluorescence attached enabling the surface expression of specific cell markers.

1.3.1 DIFFERENTIATION OF MESENCHYMAL STEM CELL

Mesenchymal stem cells (MSCs) can make several types of cells belonging to our skeletal tissues, such as cartilage, bone and fat. Scientists are investigating how MSCs might be used to treat bone and cartilage diseases. Bone marrow stromal cells (BMSC) normally give rise to bone, cartilage, and mesenchymal cells. Recently, bone marrow cells have been shown to

have the capacity to differentiate into myocytes, hepatocytes and glial cells. We now demonstrate that human and guinea pig BMSC can be induced to differentiate into neural cells under experimental cell culture conditions.

The mesenchymal stem cells were differentiated into various cell lineages such as adipocytes, bone tissues and osteogenic cells. Adipocytes are a reserve of energy as they stock lipids and when energy is required, the lipids are broken down into free fatty acids. They may also have other functions that are important in controlling metabolic activity by the secretion **(Kershaw EE. *et al*., 2004).** Adipoctye differentiation is characterized by sequential changes in the expression of specific genes that determine the specific adipocyte phenotype of the cells. This is reflected by the appearance of various early, intermediate and late mRNA/protein markers and triglyceride accumulation. The regulation of adipocyte genes occurs primarily at the transcriptional level. Several transcription factors that play a central role in the control of adipogenesis were identified. Indeed under *in vivo*, formation of fat cells is stimulated by hormonal inducers. The subsequent transcriptional regulation of adipogenesis has been studied by several *in vitro* models employing human and guinea pig MSCs or pre-adipocytes and in some knockout mice. For guinea pig MSCs, a differentiation cocktail containing dexamethasone, indomethacin, 3 isobutyl l-methylxanthine, and insulin has commonly been used to obtain adipocytes **(Pittenger MF. *et al.,* 1999; Noth U. *et al.,* 2002; Nuttal ME. *et al.,* 2004).** The induction of adipogenesis*in vitro* is considered to involve one or two rounds of clonal expansion followed by the expression of adipogenesis specific genes and attainment of the adipogenic phenotype **(Rosen ED. *et al.,* 2000; MacDougald OA. & Mandrup S. *et al.,* 2002).**

1.3.2 CHARACTERIZATION OF MESENCHYMAL STEM CELL

The mesenchymal stem cells have been successfully used in modern research. They must be isolated from their sources initially. In the adult bone marrow stem cells, very small amount of somatic stem cells (ASCs) are present and it is not quite difficult to locate and extract them. The somatic stem cells are only around 1 in every 10,000 bone marrow stem cells. Adult stem cells have now been identified in most tissues and organs of the body. The best characterization are those found in organs undergoing rapid cellular turnover, such as mesechymal stem cell found in bone marrow and other tissues of animals. Even though there is interest and huge potential associated with MSCs, cell characterization technique is yet to be achieved. Both cell types, such as mesenchymal and hematopoietic stem cells, can be isolated from the mononuclear cell fraction of bone marrow aspirates, and HSCs can be further enriched by immune magnetic isolation based on specific surface antigens like CD34 or CD45. MSCs

lack a unique surface antigen that could be used for positive selection, and therefore the general strategy for the enrichment of MSCs is based on the adherence of cells to plastic dishes in medium with low serum **(Pittenger MF. *et al.*, 1999)**.

In vitro MSCs grow as a homogenous population of adherent cells and express a set of marker proteins on their surface, including CD105 and CD73, sometimes also referred to as SH2 and SH3, CD44, CD90, and CD29. Since these markers are not specific for MSCs, they are mainly characterized by their ability to differentiate into multiple mesenchymal lineages, including osteocytes, chondrocytes, adipocytes, and skeletal muscle cells under controlled *in vitro* conditions.

1.4 RT-PCR ANALYSIS

The Real Time Polymerase Chain Reaction (RT-PCR) is the ability to monitor the progress of the PCR as it occurs. Data is collected throughout the PCR analysis and it has completely revolutionized the way one approaches PCR-based quantitation of DNA and RNA. The reactions are characterized by the point in time during cycling amplification of a target, which is first detected, rather than the amount of target accumulated after a fixed number of cycles. There are three major steps that make up each cycle in a real-time PCR reaction. Reactions are generally run for 40 cycles.

Denaturation: High temperature incubation is used to "melt" double-stranded DNA into single strands and loosen secondary structure in single-stranded DNA. The highest temperature that the DNA polymerase can withstand is typically used (usually 95°C). The denaturation time can be increased if template GC content is high.

Annealing: During annealing, complementary sequences have an opportunity to hybridize, so an appropriate temperature is used that is based on the calculated melting temperature (Tm) of the primers (5°C below the Tm of the primer).

Extension: At 70-72°C, the activity of the DNA polymerase is optimal, and primer extension occurs at rates of up to 100 base pairs per second. When an amplicon in real-time PCR is small, this step is often combined with the annealing step using 60°C as the temperature. The real time PCR has two steps including quantitative reverse transcriptase PCR (qRT-PCR) and reverse transcription of cDNA using a reverse transcriptase. Currently, the most important technique for the accurate quantitation of gene expression is the fluorescent quantitative real-time PCR

(**Freeman WM. *et al*., 1999**). Various variants of this technique are currently applied. The fluorescent signal is generated by dyes intercalating into dsDNA (**Schneeberger C. *et al*., 1995).**

1.4.1 SAMPLE PREPARATION

The integrity of purified RNA is critical to all gene expression analysis techniques. The preparation of intact cellular total RNA is the first marker in gene quantification. For successful and reliable diagnostic use, real-time PCR needs high quality, DNA-free, and un-degraded RNA **(Swift GH. *et al*., 2000; Mannhalter C.*et al*., 2000).** The source of RNA, sampling techniques, experimental animal tissue, biopsy material, single cell sampling and laser micro dissection **(Lockey C. *et al*., 1998).** However, in animal reservoirs, such as the domestic and wild rat, guinea pigs, infection produces chronic and persistent asymptomatic carriage in the renal tubules (**Bharti *et al*., 2003; Levett 2001; McBride *et al*., 2005).**

1.4.2 RNA ISOLATION AND GENE AMPLIFICATION PROCESS

A common method for the normalization of qRT-PCR data is the simultaneous amplification of an endogenous reference or a housekeeping gene **(Bustin, SA. 2000; Vandesompele J. *et al*., 2002).** Hence, the transcript level of β-actin appears to vary widely in response to experimental treatments and *GAPDH* gene expression also varies during development (**Bustin SA. 2000; Thellin O. *et al*., 1999).** As a consequence, it has been suggested that a set of reference genes should be used for normalization and the genes comprising the set should be validated for each type of experiment **(Bustin SA., 2000; Vandesompele J. *et al*., 2002).**

However, neutrophils have frequently been implicated in the pathogenesis of many diseases because they can produce various cytokines, chemokines and other proinflammatory mediators **(Cassatella MA. *et al*., 1995).** The genes used as references are often referred to as housekeeping genes, assuming that those genes are constitutively expressed in certain tissues and under certain circumstances. However, the literature shows that the expression levels of the so-called "housekeeping genes" may vary in different tissues, different cell types, and different disease stages **(Warrington JA.*et al*., 2000).**

1.5 HISTOPATHOLOGY

The histopathology is a branch of pathology, which deals with the study of diseases and identifies cell and tissue damage in animal, plant and human tissue, such as blood or bone marrow and tissue of organs. The tissue undergoes some process during experimentation and after the completion of this process; it is examined microscopically to arrive at a particular diagnosis. To identify this, it is important that the tissue must be prepared in such a manner that it is sufficiently thick or thin to be examined microscopically and all the structures in a tissue may be differentiated. This study basically examines the tissues and other parts of the organs, such as tissue handling, processing and staining.

The animal tissue is collected after the animal has been sacrificed and dissected at the end of the experimental study. The human tissue is taken during the surgery, as well as autopsy tissue or infected tissue, to identify any tumor tissues or cell or blood vessel damages, which can be easily identified. The histological sample preparation includes specimen preparation, tissue-mounting process, sectioning process, and finally staining for identifying the tissue damages. Whole-mounting process is prepared with the entire animal, such as parasites, mosquitoes and other micro-organisms. The majority of preparations in histology are related to sectioning. The tissues are cut to about 3-5 mm pieces, processed to a thickness of 5 microns and sliced by the microtome. The embedding process is preparing by paraffin wax, and finally these are immediately fixed in alcohol to present the cellular structures and stained.

1.6 SIGNIFICANCE OF RESEARCH ON NON-THERMAL, NON-IONIZING, LOW LEVEL RF-EMF RADIATION EFFECTS ON STEM CELLS

There have not been many studies of cell phone tower exposure in real-life situations, especially in a place like India, Chennai in particular, where large number of cell phone towers is placed close to where people live. Hence, histopathological studies and stem cell studies, to decipher the effects of RF-EMF at a cellular level and to particular organs of exposed animals are undertaken. This study on the molecular and genetic effects of long-term, cumulative, whole body RF-EMF exposure from living in the vicinity of a cell phone tower will shed light on this very important global issue.