2.0 REVIEW OF LITERATURE

The impact of radiofrequency electromagnetic radiation (RF-EMF) from cell phone towers on living systems is a highly debated field of research worldwide. In 2011, the World Health Organization's International Agency for Research on Cancer (**WHO IARC**) classified RF-EMF as a possible human carcinogen, group 2B. Cell phones, cordless phones, cell phone towers and wireless devices in general emit these radiations. Some short time effects such as problems with cognition, memory, learning, behavior, reaction time, concentration and altered brainwave activity were reported in the scientific literature. The biophysical mechanisms that might account for such effects could be found in various articles and reviews **(Sage, 2012).** There is a serious limitation in not taking into account the nature of the pulsed RF-EMR signal, mainly the high intensity but intermittent, microsecond pulses, into account in the safety standards. This has proven to be a major flaw in the standards that govern RF-EMF guidelines. These types of signals are biologically active and can lead to alterations in the human systems over long periods of exposure **(Bezsaki, 2006; Strogatz, 2001, 2003).**

Hence, it is essential to re-think the safety standards to take into account the exquisite sensitivity of biological systems and tissue interactions where the exposures are pulsed and cumulatively insignificant over time-scale averaging, but highly relevant to body processes and functioning. If it is true that weak-field effects have control elements over synchronous activity of neurons in the brain, and other pacemaker cells and tissues in the heart and gut that drive essential metabolic pathways as a result, then this will go far in explaining why living tissues are apparently so reactive to very small inputs of pulsed RF-EMF, and lead to better understanding of what is required for new, biologically-based public exposure standards.

2.1 CELL PHONE TOWER STANDARDS AND THEIR IMPACTS

Very few people make use of the landline phones currently. Majority use the cell phones and in turn, there is a need to provide connectivity, which increases the number of cell phone tower installations very close to where people live and where the demand is more. All of the standards currently in place are based on RF-EMF ability to heat tissue, called thermal effects **(Levitt 1995)**. A longstanding criticism, going back to the 1950s, is that such acute heating effects do not take potentially more subtle non-thermal effects into consideration. Industry representatives try to reassure communities that facilities are many orders of magnitude

below what is allowed for exposure by standards-setting boards and studies **(Cooper *et al.,* 2006; Henderson and Bangay 2006; Bornkessel *et al.,* 2007).** These include standards by the International Commission on Non-Ionizing Radiation Protection (**ICNIRP**) used throughout Europe, Canada, and elsewhere **(ICNIRP 1998)**. This includes attaching antenna panels to the sides of buildings as well as roof-mountings, antennas hidden inside church steeples, barn silos, elevator shafts, and any number of other ‘‘stealth sites.’’ It also includes camouflaging towers to look like trees indigenous to areas where they are placed, e.g., pine trees in northern climates, cacti in deserts, and palm trees in temperate zones, or as chimneys, flagpoles, silos, or other tall structures **(Rinebold 2001)**. The standards currently adopted by the U.S. FCC uses a two-tiered system of recommendations put out by the National Council on Radiation Protection (NCRP) for civilian exposures (referred to as uncontrolled environments), and the International Electricians and Electronics Engineers (IEEE) for professional exposures (referred to as controlled environments) (**U.S. FCC 1997**). The trend away from landline phones is obviously increasing as wireless providers market their services specifically toward a mobile customer, particularly younger adults who readily embrace new technologies (**Silk *et al.,* 2010).**

The specific absorption rate (SAR) that is measured in (W/kg) of tissue is a reliable determinant and index for RF-EMF biological effects, as SARs reflect what is actually being absorbed rather than the energy quotient in space **(Lai, 2000).** Most of the short-term studies primarily looking into the thermal impacts of EMR exposure on biological systems have neither succeeded to detect any statistically significant changes in the biological processes nor could prove any acute change in health conditions at the present background levels of exposures **(Brent 1999; Hanowski Niemi and Blake 1996; Hoskote, Kapdi and Joshi 2008; Lönn *et al.,* 2005; Mixson *et al.* 2009; Zach and Mayoh 1984; Zach and Mayoh 1986).** On the other hand, long-term studies have reported alarming observations, detecting negative consequences on immunity, health, reproductive success, behaviour, communication, co-ordination, and niche breadth of species and communities **(Preece *et al.,*2007; Levitt and Lai 2010; Hardell *et al.,*2008; Hardell *et al.,*2007; Fernie and Bird 2001).**

Exposure to EMR field is shown to evoke diverse responses varying from aversive behavioural responses to developmental anomalies and mortality in many of the studied groups of animals such as bees, amphibians, mammals and birds **(Zach and Mayoh 1982; Zach and Mayoh 1982; Batellier *et al.,* 2008; Nicholls and Racey 2007; Bergeron 2008; Copplestone *et al.,* 2005; Sahib 2011).** Honeybees appear to be very sensitive to EMF (**Ho 2007; Sharma and Kumar 2010; Ho 2007**) and their behavioural responses, if scientifically documented,

could be used as an indicator of EMF pollution. Several investigations into environmental effects of EM fields are covered in some of the unpublished / grey literature and impact assessments submitted to various regulatory government agencies **(Bergeron 2008a; Bergeron 2008b; Cleveland, Fields, and Ulcek 1999; Copplestone *et al.*,2005; G. Kumar 2010; Hutter *et al.*,2006).** Such reports are either not in the public domain, or scattered and often difficult to access. Some earlier investigators also have contended that there is no measurable risk of reproductive failure and birth defects from EMF exposures in humans **(Brent *et al.*,1993)**, while several others do not agree with that conclusion **(Gandhi 2005; Kapdi, Hoskote and Joshi 2008; Pourlis 2009; G. Kumar 2010).** Studies carried out on the RF levels in North India, particularly at the mobile tower sites at Delhi have shown that people in Indian cities are exposed to dangerously high levels of EMF pollution **(Tanwar 2006).**

2.1.1 EFFECTS OF ELECTROMAGNETIC RADIATION ON LIVING SYSTEMS

Many biological effects have been reported at very low intensities comparable to the animal and human population experiences within 200 to 500 ft (60–150 m) of a cell phone tower, including effects that occurred in studies of animal and plants cell cultures and animals after exposures to low-intensity RF-EMF. Effects reported such as genetic disorders, growth problem, and in reproductive infertility, increases in permeability of the blood–brain barrier, behavioral changes, molecular and cellular interaction, metabolic rate changes and increases in cancer risk. There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. **Panagopoulous *et al.,* (2012),** reported decreased ovarian development and size of ovaries and premature cell death of ovarian follicles and nurse cells in *Drosophila melanogaster.* Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line **(Dasdag *et al*, 1999; Yan *et al.,* 2007; Otitoloju *et al.,* 2010; Salama *et al.,* 2008; Behari *et al.,* 2006; Kumar *et al.,* 2012). Gul *et al.,* (2009)** reported that rats exposed to stand by level RF-EMF had a decrease in the number of ovarian follicles in pups born for electromagnetic radiation-exposed female rats.

Extremely low frequency electromagnetic field was able to reduce the fertilization rate in swine animal model, and negatively affect early embryo development **(Bernabo *et al.,* 2010).** Some effects showed negative alterations of the nervous tissue in the brain and some sensory organs. Rapid cellular molecular alterations were seen in the rat brain after exposure to 900-MHz pulsed microwaves and power of 6 W/kg for 15-min **(Bonnefont *et al.,* 2004).** Also electromagnetic

waves emitted from mobile phones (900 MHz) reduced glutathione level in brain tissue and blood of exposed guinea pigs **(Meral *et al.,* 2007).** Electromagnetic field (EMF) emitted by a mobile phone with frequency 900 MHz caused derangement of chick embryo retinal differentiation **(Zareen *et al.,*2009).** Low-frequency electromagnetic fields caused chick embryos to have abnormal brain ventricles, spina bifida, eye malformation and growth retardation **(Lahijani *et al.,* 2007).**The thermal effects of mobile phones can cause eye-induced cataracts, corneal edema, endothelial cells loss and retinal degeneration **(Vignal *et al.,* 2008).** On the other hand electromagnetic waves were found to stimulate proliferation and differentiation of embryonic cells **(Parivar *et al.,* (2006).** This alteration might have its effect on cell proliferation in terms of increasing or reducing proliferation rate thus playing an important role during early embryonic development **(Panagopoulos, *et al.,* 2004, Zareen *et al.,* 2009).** A study attributed the effect to DNA damage in the insects and their reproductive cells to RF-EMF exposure **(Panagopoulos D. *et al.,* 2013).**

An experiment on Common Frog (*Rana temporalis,* new name *Hylarana temporalis*) indicated that radiation emitted by phone masts in a real-time situation may affect the development and may cause rise in mortality of exposed tadpoles. This research may have huge implications for the natural world, which is now exposed to high microwave radiation levels from a multitude of phone masts **(Balmori 2010).** However, it requires long-term monitoring studies for establishing any causative link between reproductive fitness and EMFs and such data was presently lacking. Moreover, available short-term studies are grossly inadequate.
For instance, a recent review that analysed the literature (**till 2001**) on the effects of EMF associated with mobile telephony on the prenatal and postnatal development of vertebrates reported that the majority of the studies examined indicated no strong impact on the animal reproduction and development **(Pourlis 2009).**

2.1.2 BIRDS AND BEES

The earliest reported study on impacts of microwave radiation on birds dates back to 1960s (**Tanner, Romero-Sierra, and Davie 1967**). In birds, their ability to fly exposes them to a greater risk of direct irradiation and hence they appear to be at greater risk as far as effects of EMRs are concerned (**Balmori 2005; Balmori and Hallberg 2007; Summers-Smith 2003; Zach and Mayoh 1982; Zach and Mayoh 1984; Zach and Mayoh 1982; Joris and Dirk 2007).** Observed effects of exposure to non-ionizing radiation in avian species are mostly from radiation-induced temperature increases **(Batellier *et al.,* 2008)**. The incubating avian egg

provides a model to study non-thermal effects of microwave exposure since ambient incubation temperature can be adjusted to compensate for absorbed thermal energy. Non-thermal levels of non-ionizing radiation can affect a bird's ability to recover from acute physiological stressors, apart from other potential physiological and behavioural repercussions.

Although earlier research indicated that modulated radiofrequency radiation increased calcium-ion efflux in chick forebrain tissue, disagreement on experimental techniques and incongruous results among related studies have made final conclusions elusive. In an another study, which was carried out by National Research Centre of Canada on interaction of electromagnetic fields and living systems with special reference to birds, it was observed that following the onset of radiation, stabilizing period of the egg production in birds was affected **(Bigu, 1973)**. Birds have been shown to be able to reliably detect magnetic fields in both the field and laboratory. The rapidly increasing number of cell-phone subscribers was resulting in higher concentration levels of electromagnetic waves in the air, which clashes with the earth's electromagnetic field (Hyland, 2000). Some researchers have reported malformations in chicken embryos exposed to a sinusoidal bipolar oscillating magnetic field **(Balmori and Hallberg 2007)**. According to a thermal modeling study of a bird subjected to continuous wave (CW) microwave radiation (2.45 GHz), the model predicted that tolerance to microwave radiation for a bird was positively correlated with its mass and that ambient temperature is the environmental variable that has most influence on the level of tolerance for microwave radiation **(Byman *et al.*,1986).**

House Sparrow (*Passer domesticus)* is associated with human habitation and it is one of the indicator species of urban ecosystems. A declining population of the bird provides a warning that the urban ecosystem is experiencing some environmental changes unsuitable for living in the immediate future **(Kumar, 2010)**. London has witnessed a 75 per cent fall in House Sparrow population since 1994, which coincides with the emergence of the cell-phone **(Balmori, 2002)**. Electromagnetic radiation may be responsible, either by itself or in combination with other factors, for the observed decline of the sparrows in European cities **(Balmori, 2009, Balmori & Hallberg, 2007).** Research in Spain proved that the microwaves released from these towers are harmful to House Sparrows and the increase in the concentration of microwaves results into decrease in House Sparrow populations (**Everaert and Bauwen, 2007)**. Reproductive and co-ordination problems and aggressive behavior has also been observed in birds such as sparrows **(Balmori, 2005)**. General methodology used for such study was, from each area, all sparrows were counted in addition to the mean electric field strength **(Everaert & Bauwens, 2007)**. In similar studies in India, population of *Passer domesticus* was found fast disappearing from

areas contaminated with electromagnetic waves arising out of increased number of cell phones, in Bhopal, Nagpur, Jabalpur, Ujjain, Gwalior, Chindwara, Indore &Betul **(Dongre &Verma, 2009).** It was also observed that when 50 eggs of House Sparrow, exposed to electromagnetic radiation (EMR) for durations of five minutes to 30 minutes, all the 50 embryos were found damaged in a study carried out by the Centre for Environment and Vocational Studies of Punjab University **(Kumar 2010, Ram 2008)**.

The Male sparrows were seen at locations with relatively high electric field strength values of GSM base stations, providing evidence of how long-term exposure to higher levels of radiation negatively affects the abundance or behavior of House Sparrows in the wild. Thus, electromagnetic signals are associated with the observed decline in the sparrow population in urban areas. In monitoring a white stork (*Ciconia ciconia*) population in Valladolid (Spain) in vicinity of Cellular Phone Base Stations, the results indicated the possibility that microwaves are interfering with the reproduction of White Stork **(Balmori, 2010)**. In chicken, immune systems and reproductive systems are mainly damaged and increased embryonic mortality was seen due to RF-EMF radiation **(Youbicier-Simo *et al.,* 1999).**

Many recent studies have linked the electromagnetic radiations with an unusual phenomenon in bees known as 'Colony Collapse Disorder'. Colony Collapse Disorder (CCD) occurs in bees when a hive's inhabitants suddenly disappear, leaving only queens, eggs and a few immature workers. The vanished bees are never found, but thought to die solitarily far from home. The theory is that radiation from mobile phones interferes with bees' navigation systems, preventing them from finding their way back to their hives. Even the other animals, parasites and other bees, that normally would raid the honey and pollen left behind when a colony dies, refuse to go anywhere near the abandoned hives. Some scientists believe that CCD is the result of high electromagnetic radiation. As long back as early 1970s, **Wellenstein (1973)** had reported that the navigational skills of the honey bees were being impacted by high-tension lines. In a recent study **(Stefan *et al.,* 2010)** significant differences have been detected in returning of honeybees to their hives.

2.1.3 MAMMALS

Phone masts located in the living areas of mammals are continuously irradiating some species that could suffer long-term effects, like reduction of their natural defences, deterioration of their health, problems in reproduction and reduction of their useful territory through habitat

deterioration. Electromagnetic radiation can exert an aversive behavioural response in rats, bats and birds such as sparrows. Therefore microwave and radiofrequency pollution constitutes a potential cause for the decline of animal populations and deterioration of health of plants living near phone masts **(Balmori, 2005).** Arguably, the most serious concern about the impact of EMF on the living systems appears to be its long-term effects on genes and reproductive fitness of species. Today, there is evidence that electromagnetic radiation is genotoxic **(Blaasaas, Tynes, and Lie 2003; Joris and Dirk 2007).** Activity of bats seems to be much reduced in areas with Electro-magnetic fields with densities more than 2V/m **(Balmori, 2009).** Based on this fact it was recommended to use EMR to repel bats from wind farms **(Nicholls and Racey, 2007)**.

In another study in a free-tailed bat colony (*Tadarida teniotis*) the number of bats decreased when several phone masts were placed 80m from the colony **(Balmori *et al.,* 2007).** The electromagnetic radiations increase the permeability of blood-brain barrier in guinea pig and rats **(Persson *et al.,* 1997; Lai *et al.,* 1987).** These types of radiations are emitted from cell phones and their towers and may have potential adverse health reactions **Pourlis 2009; Cherry 2000).**There is a lot of evidence indicating non-thermal and non-ionizing influences of RF-EMF *in vivo*, such as epileptic encephalopathic activity in guinea pigs and rats in connection with particular radiations **(Sidorenko *et al.,* 1999).**

Some studies indicated that exposure to radiofrequencies promoted cancer in conventional mice and lymphoma in transgenic mice (Em-pim 1) that are prone to lymphoma **(Szmigielski *et al.,* 1982; Szudzinski *et al.,* 1982; Repacholi *et al.,* 1997).** However, **Sommer *et al.,* (2004)** showed that mobile phone exposure does not promote lymphoma in AKR/J mice. It has been firmly established that a significant level of DNA damage arises from endogenous products of cellular metabolism. For example, oxygen radicals generated *in vivo* during reduction of O2 were responsible for DNA damage, which was not associated with the exposure to environmental carcinogens. Oxygen radicals can attack DNA bases or the deoxyribosyl backbone to produce damaged bases or strand breaks.

They can also oxidize other cellular macromolecules such as lipids or proteins, to generate reactive electrophiles that bind covalently to DNA bases forming adduct. Endogenous DNA lesions are genotoxic and induce mutations.The level of DNA damage arising from endogenous cellular sources exceeds the level of lesions induced by exposure to exogenous chemical carcinogens or physical agents **(Pluskota-Karwatka *et al.,* 2006).** One of the biomarkers to measure the level of oxidative stress in an organism is alondialdehyde (MDA). MDA is one of

the many important parameters that cause toxic stress in living cells. MDA is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress mediated lipid peroxidation in tissues **(Dasdag *et al.,* 2008).** Most studies exploring the pathogenicity have used hamsters or guinea pigs as animal models. In these species, infection with virulent Leptospira causes a fatal acute disease similar to severe human **disease (Bharti *et al.,* 2003; Levett, 2001; McBride *et al.,* 2005).**

2.1.4 HUMANS

Since its inception, there have been concerns about the ill effects of the mobile towers and mobile phones. Despite being a relatively newly acknowledged form of pollution, EMRs and their negative impacts on biological systems and environment have already been reported by several studies. However most of the available scientific literature on the negative environmental effects of electromagnetic fields reports the results of experimental and epidemiological studies examining the impact on various aspects of human health **(Tanwar 2006; Savitz 2003; Preece *et al.,*2007; Oberfeld *et al.,* 2004; Navarro *et al.,* 2003; Lönn *et al.,* 2005; Kundi and Hutter 2009; Hardell *et al.,* 2007; Kapdi, S. Hoskote and Joshi 2008; Hallberg and Johansson 2002).** Studies have shown that usage of cell phones, exposure to cell phone radiation or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure **(Aitken et al, 2004; Agarwal *et al.,* 2007; Erogul *et al.,* 2006).**

The radiation from a cell phone penetrates deeper into the head of children **(Gandhi *et al.,* 1996; Wiart *et al.,* 2008)** and certain tissues of a child's head, e.g., the bone marrow and the eye, absorb significantly more energy than those in an adult head **(Christ *et al.,* 2010).** The same can be presumed for proximity to towers, even though exposure will be lower from towers under most circumstances than from cell phones. This is because of the distance from the source. The transmitter is placed directly against the head during cell phone use whereas proximity to a cell tower will be an ambient exposure at a distance. However, while SARs may be a more precise model, at least in theory, there were only a handful of animal studies that were used to determine the threshold values of SAR for the setting of human exposure guidelines **(de Lorge and Ezell 1980; de Lorge 1984).** Recent studies of whole body plane wave exposure of both adult and children phantoms demonstrated that when children and small persons are exposed to levels which are in compliance with reference levels, exceeding the basic restrictions cannot be excluded **(Dimbylow and Bloch 2007; Wang *et al.,* 2006; Kuhn *et al.,* 2007; Hadjem *et al.,* 2007).** While the whole frequency range has been investigated, such effects were found in the

frequency bands around 100 MHz and also around 2 GHz. For a model of a 5-year-old child it has been shown that when the phantom is exposed to electromagnetic fields at reference levels, the basic restrictions were exceeded by 40% **(Conil *et al.,* 2008)**. Moreover, a few studies demonstrated that multipath exposures could lead to higher exposure levels compared to plane wave exposure **(Neubauer *et al.* 2006; Vermeeren *et al.,* 2007**).

The public opinion and concerns about the potential human health hazards of short and long-term effect of exposure to radiofrequency (RF) radiation **(Dasdag *et al.,* 2009; Karadede *et al.,* 2009)** have also increased. There have been many studies to determine the health effects of radiation emitted from cellular phones. These include the possibility of initiation and/or promotion of carcinogenesis and the induction of genetic damage **(WHO, 1993).** The effects on sperm quality, motility and pathology in men, particularly for user of cell phones in pocket or belt, have been clearly established in various studies **(Agarwal *et al.,* 2009; De Iuliis *et al.,* 2009; Kumar, 2012).**

An important biomarker in the study of impacts of RF-EMF on living systems is Malondialdehyde (MDA), which is a naturally occurring product of lipid peroxidation and prostaglandin biosynthesis, two processes implicated in the pathogenesis of a number of cancers. Malonaldehyde-DNA lesions have been detected in a number of human tissues **(Pluskota-Karwatka *et al.,* 2006)**. Because of the importance of the oxidative stress in living systems, the number of studies that investigate effects of mobile phone exposure on oxidative stress has been increasing rapidly **(Dasdag *et al.,* 2004, 2008, and 2009).** Alzheimer's disease (AD) is one of the most common diseases in older people and it was recently reported that exposure to radiofrequency fields (RF) provides cognitive benefits for both normal and transgenic mice **(Arendash *et al.,* 2010). Arendash *et al.,* (2010)** stated that long-term exposure of radiofrequency field (RF, 918 MHz; 0.25 W/kg) provided cognitive benefits in an Alzheimer's diseases (AD) mice by means of reducing brain amyloidbeta (Abeta) deposition.

2.1.4.1 BRAIN

Exposure of the neural tissue to RF-EMF can cause electrophysiological changes in the nervous system **(Navakatikian and Tomashevskaya, 1994; Velizarov *et al.,* 1999)**. Some studies have suggested that RF-EMF induce tissue heating leading to tissue damage **(Gajsek *et al.,* 2002; Preece *et al.,* 1999).** Some effects are observed among mobile phone users at low intensity and after repeated exposure **(Hyland, 2000).** The efflux of calcium ions from brain

tissue is an important neurochemical effect of RF-EMF, as calcium ion plays an important role in the functions of the nervous system, such as the release of neurotransmitters **(Dutta *et al.,* 1989).** Moreover, RF-EMF activates endogenous opioids in the brain, which in turn cause a decrease in cholinergic activity leading to short-term memory deficit. The stress hormone ‘‘corticotropin releasing factor’’ is also involved **(Lai *et al.,* 1994).** The emissions of a mobile phone base station are usually described by its effective radiated power which is given in Watts (W) **(Nousir, 2002).** According to Tiwari and colleagues, who took blood samples from electromagnetic radiation-exposed and non-exposed people, noted that the exposed workers had higher concentration of the stress hormone (adrenalin) as well as DNA damage and oxidative stress (**Tiwari R. *et al.,* 2014**).

2.2 RF-EMF RADIATION EFFECTS ON STEM CELL

Stem cells obtained from guinea pig bone marrow (BMSCs) have been widely studied because of their relative easy access and differentiation to irradiated stem cell and normal stem cell of bone marrow **(Hung S., *et al.,* 2002, Sekiya I, *et al.,* 2002)**. Their multipotentiality and self-renewal has increased the attention to this stem cell model as a self-renewing cell source with applications in tissue culture, stem cell technology and regenerative medicine **(Grayson W.L. *et al.,* 2004, Kim H. *et al.,* 2005).** Particularly, animal bone marrow stem cells’ osteogenic potential has been extensively explored in the biological evaluation of bone tissue engineering scaffolding structures **(Meinel L., *et al.,* 2004, Meinel L., *et al.,* 2006).** In addition, their isolation based on the adherence to the culture substrates constitutes a straightforward strategy for elimination of non-mensenchymal lineages, reducing the dependency on complex cell isolation methods, which rely on the expression of specific surface markers. The MSCs are multipotent stem cells and have the fundamental capacity to differentiate into a limited range of cell lineages **(Gronthos *et al.,* 1996; Prockop, 1997).** The multilineage differentiation potentials of MSCs have been studied *in vitro* since their discovery, five decades ago. These studies, *in vitro* and *in vivo*, demonstrated that bone marrow MSCs, from guinea pig or mouse species, are able to differentiate into other cells, such as osteoblasts, chondroblasts, adipocytes, fibroblasts and skeletal myoblasts. MSCs can also acquire characteristics of non-mesodermal lineages, such as bone marrow cells, other tissue cells, *in vitro* and *in vivo* **(Pittenger *et al.,* 1999; Schwartz *et al.,* 2002; Verfaillie, 2002; Verfaillie *et al.,* 2002).** Finally, these cells, *in vivo*, have the capacities to generate a functional reconstitution of a given bone marrow in radiation-exposed animal stem cells and control stem cells when the damages are identified **(Friedenstein *et al.,* 1974; Latsinik *et al.,* 1986; Haynesworth *et al.,* 1992).**

There are at least several hundred published papers that report EMF (ELF/RF-EMF) can affect cellular oxidative processes (oxidative damage). Increased free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. Aging may make an individual more susceptible to the detrimental effects of ELF EMF from oxidative damage, since anti-oxidants may decline with age. Clearly, the preponderance of genetic studies report DNA damage and failure to repair DNA damage. Human bone marrow stem cells (hBMSCs) easily differentiate to osteogenic, adipogenic and chondrogenic lineages, and other kind of tissues or cells, including hepatocytes, cardiomyocytes and neurons **(Hung *et al.,* 2002; Sekiya *et al.,* 2002)**. Particularly, hBMSCsosteogenic potential has been extensively explored in the biological evaluation of bone tissue engineering scaffolding structures **(Meinel *et al.,* 2004; Meinel *et al.,* 2006)**. In addition, their isolation based on the adherence to the culture substrates constitutes a straightforward strategy for elimination of non-mensenchymal lineages, reducing the dependency on complex cell isolation methods, which rely on the expression of specific surface markers **(Hung *et al.,* 2002; Caterson *et al.,* 2002)**.Although autologous transplantation of *in vitro* expanded stem cells, via direct cell implantation on the site of the lesion or using cell-loaded engineered scaffolds, holds great promise, the common strategies for cell harvesting require somehow traumatic procedures for aspirating the bone marrow **(Hung *et al.,* 2002; Meinel *et al.,* 2004).** Therefore, in those studies where an autologous model is not required, it would be advantageous to work with a mesenchymal cell line displaying similar characteristics that could be isolated without inducing any trauma in the donor site or from tissues otherwise discarded in conventional surgical procedures. One example of such studies is the initial development and evaluation of new biomaterials or scaffolds, which may be tested and optimized *in vitro* prior to the *in vivo* studies that would require an autologous cell source **(De Oliveira and Nanci, 2004) (Pineda *et al.,* 2007; Catelas *et al.,* 2006).**

Stem cell biology has captured the imagination of biologists, tissue engineers, pharmaceutical company scientists, and indeed the general public, largely because of the prospect it seems to offer of manipulating cell fate to treat disorders for which there is no other effective therapy. The initial focus was on diseases like type 1 diabetes and Parkinson's disease (PD), in which attempts had already been made to treat patients with donor cells **(Langer 2010; Bjorklund *et al.,* 2003),** but it was quickly recognized that embryonic stem cell (ESC) behavior may not be easy to control, and developing cells as safe and effective products is not as straightforward as developing small molecule or protein-based drugs, for which a great deal of

experience has accumulated. The case of Geron, the biotechnology company that has been the first to initiate a clinical trial using ESC-derived cells, illustrates the hazards of developing a cell-based product (Struauss 2010). This led to efforts to use stem cell biology to identify and develop small molecule drugs to target endogenous stem cell populations, for example, to stimulate neurogenesis to treat stroke, traumatic brain damage, Alzheimer's disease (AD) or PD, or other disorders of mood or cognition **(Schmid and Duman 2007),** or to inhibit stem cell-like cells in solid tumors **(Gupta *et al.*,2009).**

It seems intuitively obvious that stem cells must respond to environmental and systemic signals to adjust cell production to varying demands. Several examples illustrate the responsiveness of stem cells to tissue extrinsic factors, although the underlying mechanisms remain poorly understood. A well-studied example is the response of ovarian stem cells to diet and insulin signals in Drosophila. On a protein-rich diet, GSCs and follicle stem cells (and their descendants) have high division rates, whereas on a protein-poor diet, these rates are reduced; this process requires insulin signaling **(Drummond-Barbosa and Spradling 2001)**. Brain-derived insulin-like peptides have been shown by genetic mosaic analysis to directly stimulate GSCs to control their proliferation **(Lafever and Drummond-Barbosa 2005)**. In contrast, daf-2/insulin receptor mutations in *C. elegans* have been reported not to affect proliferation of germ cells in a wild-type background, although this conclusion was based on comparisons of the total number of phosphohistone H3 (a mitotic marker)-positive cells per gonad arm **(Pinkston *et al*,.2006)**. Intriguingly, insulin signaling has an additional, separate role in controlling GSC maintenance via the niche in Drosophila (**H. J. Hsu and D. Drummond- Barbosa,** unpublished results). Notch signaling controls cap cell number **(Ward *et al.,* 2006; Song *et al.,* 2007)**. Insulin-like peptides regulate Notch signaling to maintain cap cell numbers, and they also promote cap cell–GSC association at least in part via E-cadherin. This illustrates the profound impact that the systemic environment can have on both stem cell activity and the niche that controls stem cell fate.

The neural stem cells (NSCs) also sense and respond to injury or physiological changes. For example, in adult rodents, focal cerebral ischemia leads to increased proliferation of neural progenitors in the SVZ and SGZ, including those far from the area of infarction **(Jin *et al.,* 2001; Zhang *et al.,* 2001; Arvidsson *et al.,* 2002; Parent *et al.,* 2002).** Insulin-like growth factor-1 (IGF-1) is a key diffusible factor mediating this response because anti- IGF-1 antibodies significantly inhibit ischemia-induced proliferation in the SVZ and SGZ in vivo **(Yan *et al.,* 2006)**. Low estradiol levels increase the number of newborn neurons in the dorsal region of the

SVZ following stroke injury, and this effect requires the estrogen receptors a and b **(Dubal *et al.,* 2006; Suzuki *et al.,* 2007).** It is unclear however, what cells are the direct IGF-1 or estradiol targets. Effects of androgens on normal adult neurogenesis in the dentate gyrus have also been reported, although this is due to increased cell survival **(Spritzer and Galea 2007).** Moderate estrogen levels stimulate cell proliferation and neurogenesis in the female rat hippocampus, and progesterone antagonizes this effect **(Tanapat *et al.,* 1999, 2005)**. Pregnancy stimulates the proliferation of SVZ NSCs via prolactin **(Shingo *et al.,* 2003)**. Pregnancy and postpartum also influence neurogenesis in the dentate gyrus, although specific effects on NSCs have not been directly examined. Low thyroid hormone levels lead to reduced proliferation of neural precursors in the SVZ **(Lemkine *et al.,* 2005)**. The location of NSCs near blood vessels is conducive to exposure to systemic signals. NSCs are also near axon terminals, and neuronal activity or signals from neuroblasts can influence NSC activity **(Riquelme *et al.,* 2008).** Nevertheless, dissecting the network of direct and indirect inputs into NSCs and their contributions to neurogenesis will require the manipulation of gene function in specific cell types followed by the analyses of individual steps of neurogenesis.

Pregnancy and lactation in mice influence the hair cycle because estrogens and prolactins inhibit anagen induction in telogen and catagen induction in anagen **(Paus *et al.,* 2008)**, although it is unclear whether stem cells are directly affected. Insulin acts as a major growth factor for human hair follicle by inducing IGF-1. IGF-1 and IGF-2 double knock-out and IGF-1 receptor knockout mice have epidermal hypoplasia and reduced hair follicle number **(Liu *et al.,* 1993)**, while transgenic mice expressing IGF-1 in the hair follicle have altered follicular proliferation and differentiation and abnormal hair growth cycle **(Weger and Schlake 2005).** It remains unclear, however, which cells are directly controlled by insulin or IGF-1 or how these signals are integrated with other local factors controlling hair follicle biology. Other stem cell systems also respond to systemic signals. Follicle-stimulating hormone promotes GDNF expression, which controls GSCs in the testis **(Tadokoro *et al.,* 2002).** Growth hormone stimulates NSC and mammary stem cell proliferation, and testosterone induces increased satellite cell numbers **(Sinha-Hikim *et al.,* 2003).** SVZ cell proliferation requires thyroid hormone and their a-receptor (Lemkine et al. 2005). Estrogen controls mammary stem cell proliferation via paracrine signals **(Lamarca and Rosen 2008).** In many of these examples, it remains to be demonstrated that stem cell numbers or activity are affected (as opposed to those of subsequent progenitors). Nevertheless, they likely represent the ‘‘tip of the iceberg’’ of systemic influences on stem cells. expression, which controls GSCs in the testis **(Tadokoro *et al.,* 2002).**

Growth hormone stimulates NSC and mammary stem cell proliferation, and testosterone induces increased satellite cell numbers **(Sinha-Hikim *et al.,* 2003).** SVZ cell proliferation requires thyroid hormone and their a-receptor **(Lemkine *et al.,* 2005).** Estrogen controls mammary stem cell proliferation via paracrine signals **(Lamarca and Rosen 2008).** In many of these examples, it remains to be demonstrated that stem cell numbers or activity are affected (as opposed to those of subsequent progenitors). Nevertheless, they likely represent the ‘‘tip of the iceberg’’ of systemic influences onstem cells. Many protocols have been published to isolate and expand mesenchymal stem cells, which have subtle and occasionallyquite significant differences. In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy established minimal criteria to define human MSCs, which include the following: adhesion to plastic, expression of specific surface antigens and capacity for in vitro multipotential (chondrogenic, adipogenic and osteogenic) differentiation, which is demonstrated by staining for cell type-specific markers. The immunophenotypic definition of MSCs requires that more than 95% of the population expresses the CD105, CD73 and CD90 surface antigens and that 2% of the population expresses the panleucocyte marker CD45, the primitive haematopoietic progenitor and endothelial cell marker CD34, the monocyte and macrophage markers CD14 and CD11, the B cell markers CD79 and CD19, or HLA class II **(Dominici *et al.,* 2006; Horwitz *et al.,* 2005)**.

2.3 ANIMAL-DERIVED BMSC AS A MODEL OF HUMAN BMSC

BMSCs are present in the bone marrow of humans as well as other animals such as mice, rats,rabbits, dogs, pigs, sheeps, horses, and cows **(Leboy *et al.,* 1991; Pereira *et al.,* 1998; Howlett *et al.,* 1986; Mankani *et al.,* 2006;Peterbauer-scherb *et al.,* 2010;Giannoni *et al.,*2008; Bourzac *et al.,* 2010; Kopesky *et al.,* 2010).** As BMSCs seem to be postnatal stem cells that are common among mammalian species, these animals have been used to investigate the origin and *in vivo* functions of BMSCs **(Takashima *et al.,* 2007; Mosca *et al.,* 2000).** In addition, these animal-derived BMSCs are considered useful as models of human BMSCs because it is not always easy to recruit a sufficient number of human BMSC donors for experimental use. Furthermore, more reliable results can be obtained by using animal-derived BMSCs because experimental animals have uniform genetic backgrounds and are housed under controlled conditions, eliminating behavioral and environmental variations that could influence BMSC properties. In fact, several studies have reported that the characteristics of human BMSCs varied significantly among donors **(Siddappa *et al.,* 2007; Agata *et al.,* 2010; Phinney *et al.,***

1999), while such variations are not observed in animal-derived BMSCs. Therefore, animal-derived BMSCs are considered to be useful alternatives to human BMSCs for laboratory experimentation. However, it remains to be known which animal's BMSCs offer the best model system to represent human BMSCs. In general, donor animals of BMSCs are chosen based on their costs and availabilities. However, it has been shown that there are a number of characteristic differences in the BMSCs among species (Kuznetsov and Robey, 1996). Therefore, it is important to consider species difference in addition to the costs and availabilities when selecting model systems for human BMSCs.

Considering their costs and availabilities, mice are more attractive candidates than other laboratory animals. However, rat BMSCs are used as a model of human BMSCs in our laboratory because mouse BMSC characteristics differ from those of human BMSCs. For example, mouse BMSCs need the support of feeder cells for their stable growth, while human BMSCs are able to grow in a feeder cell-independent manner **(Kuznetsov and Robey 1996).**Responses to differentiation stimuli are also different. While human BMSCs are readily induced to differentiate into theosteogenic lineage by dexamethasone, mouse BMSCs are less responsive to dexamethasone treatment 9 **Mizuno *et al.,* 2010**). Although the reasons why mouse BMSCs differ from human BMSCs remain unknown, it has been suggested that mouse BMSCs are very rare in the bone marrow and need support by other cells for their growth and differentiation **(Kuznetsov and Robey 1996).** On the contrary, rat BMSCs can be easily isolated from bone marrow and they are able to grow without feeder cells, as do human BMSCs **(Agata *et al.,* 2012)**. In addition, rat BMSCs are able to differentiate into multiple lineages under induction protocols used for human BMSCs **(Tan *et al.,* 2012**). Therefore, we believe that rat BMSCs offer a more appropriate model of human BMSCs, though fewer reagents and antibodies are available for rat cells than for mouse cells.

Bone marrow mesenchymal stem cells (BMMSCs) are hierarchical postnatal stem or progenitor cells capable of self-renewing and differentiating into osteoblasts, chondrocytes, adipocytes, and neural cells **(Friedenstein *et al.,* 1974; Prockop 1997).** BMMSCs express a unique surface molecule profile, including expression of STRO-1, CD29, CD73, CD90, CD105, CD146, Octamer-4 (Oct4), and stage-specific embryonic antigen-4 (SSEA4) (Gang *et al.,* 2007; Greco 2007). It is generally believed that BMMSCs are negative for hematopoietic cell markers such as CD14 and CD34 **(Covas *et al.,* 2008; Galmiche *et al.,*1993; Conget 1999; Haynesworth 1992; Martinez *et al.,* 2007; Sacchetti *et al.,* 2007).** BMMSCs have been widely used for tissue engineering **(Kwan *et al.,* 2002; Panetta *et al.,* 2009; Liu *et al.,* 2011).** Recently, a growing

body of evidence has indicated that BMMSCs produce a variety of cytokines and display profound immunomodulatory properties **(Nauta and Fibbe 2007; Uccelli *et al.,* 2007; Uccelli *et al.,* 2008),** perhaps by inhibiting the proliferation and function of several major immune cells, such as natural killer cells, dendritic cells, and T and B lymphocytes **(Aggarwal and Pittenger 2005).** These unique properties make BMMSCs of great interest for clinical applications in the treatment of different immune disorders **(Nauta and Fibbe 2007; Bernardo *et al.,* 2009; Le Blanc *et al.,* 2004; Chen 2006; Sun *et al.,* 2009).** BMMSCs were thought to be derived from the bone marrow stromal compartment, initially appearing as adherent, single colony clusters (colony-forming unitfibroblasts-CFU-F), and subsequently proliferating on culture dishes **(Friedenstein 1989)**. To date, the CFU-F assay has been considered one of the gold standards for determining the incidence of clonogenicBMMSC **(Clarke and McCann, 1989; Friedenstein *et al.,* 1970).** Since BMMSC are a heterogeneous population of stem cells, it is critical to identify whether BMMSC contain unique cell subsets with distinctive functions, analogous to the hematopoietic stem/progenitor cell system. In this study, we identified a subset of mouse BMMSCs in culture suspension and determined their immunomodulatory characteristics.

MSCs can be isolated from many different common species. Among all these species, the human **(Pittenger Aveline *et al.,* 1999; Zvaifler *et al.,* 2000; Kuznetsov *et al.,* 2001; Covas *et al.,* 2005), the murine (Phinney *et al.,* 1999; Baddoo *et al.,* 2003)** and the rat **(Santa Maria *et al.,* 2004; Rochefort *et al.,* 2005; Rochefort *et al.,* 2006)** MSCs are the best characterized. Next to these three species, MSCs can also be isolated from guinea pigs, cats **(Martin *et al.,* 2002),** baboons **(Devine *et al.,* 2001),** sheep **(Airey *et al.,* 2004),** dogs **(Silva *et al.,* 2005),** pigs **(Moscoso *et al.,* 2005; Bosch *et al.,* 2006),** cows **(Bosnakovski *et al.,* 2005)** and horses **(Worster *et al.,* 2000; Ringe *et al.,* 2003)**.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-environement 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. These cells are obtained by flushing the marrow out of animal bones with culture medium or from human bone marrow aspirates and transferred into a culture dish **(Phinney *et al.,* 1999; Pittenger *et al.,*1999; Santa Maria *et al.,* 2004; Tropel *et al.,* 2004; Zhang *et al.,* 2004; Rochefort *et al.,* 2005; Miao *et al.,* 2006; Rochefort *et al.,* 2006).**

Cells with mesenchymal stem cells characteristics were isolated from several adult tissues including spleen, pancreas **(da Silva Meirelles *et al.,* 2006; Seeberger *et al.,* 2006),** liver **(Campagnoli *et al.,* 2001; Dan *et al.,* 2006),** kidney **(da Silva Meirelles *et al.,* 2006),** lung **(da Silva Meirelles *et al.,* 2006),** smooth muscle **(da Silva Meirelles *et al.,* 2006),** skeletal muscle **(Howell *et al.,* 2003; Barry *et al.,* 2004; Yoshimura *et al.,* 2007),** aorta (**da Silva Meirelles *et al.,* 2006),** vena cava **(da Silva Meirelles *et al.,* 2006)**, brain **(da Silva Meirelles *et al.,* 2006)**, thymus **(da Silva Meirelles *et al.,* 2006),** dental pulp **(Pierdomenico *et al.,* 2005),** deciduous teeth **(Barry *et al.,* 2004),** scalp tissue and hair follicle **(Shih *et al.,* 2005),** periosteum **(Barry *et al.,* 2004; Yoshimura *et al.,* 2007),** trabecular bone **(Barry *et al.,* 2004),** adipose tissue **(Barry *et al.,* 2004; Yoshimura *et al.,* 2007)** and synovium**(Barry *et al.,* 2004; Yoshimura *et al.,* 2007).** MSCs have also been isolated from fetal tissues similar to the adult tissues but also, with a variable portion, from several part of the placenta **(Igura *et al.,* 2004; Miao *et al.,* 2006)** including chorionic vili, amniotic fluid **(Tsai *et al.,* 2004),** fresh or cryopreserved umbilical cord blood (Erices *et al.,* 2000; Erices *et al.,* 2003) and umbilical cord vein **(Covas *et al.,* 2005).**

MSCs were also described in the peripheral blood of normal adult and women during and after the pregnancy, from a fetal origin and may persist for at least 60 years **(Zvaifler *et al.,* 2000; O'Donoghue *et al.,* 2004; Villaron *et al.,* 2004; Dazzi *et al.*,2006).** Blood samples represent aparticular important source of MSC, more accessible in Human than the bone marrow source.MSCs can be mobilized into the bloodstream after treatment (chemotherapy, cytokine injection) or physiopathological events inducing a physiological release of stem cells from their reservoirs in responses of stress signals such as cytokines like granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Recently, scientists show that several physiopathological circumstances, including hypoxia, myocardial infarction or encephalopathy can increase the release of MSCs in blood **(Erices *et al.,* 2000; Erices *et al.,* 2003; Romanov *et al.,* 2003; Lee *et al.,* 2004).** The types of stem cells are given in the figure below **(Figure 2.2).**

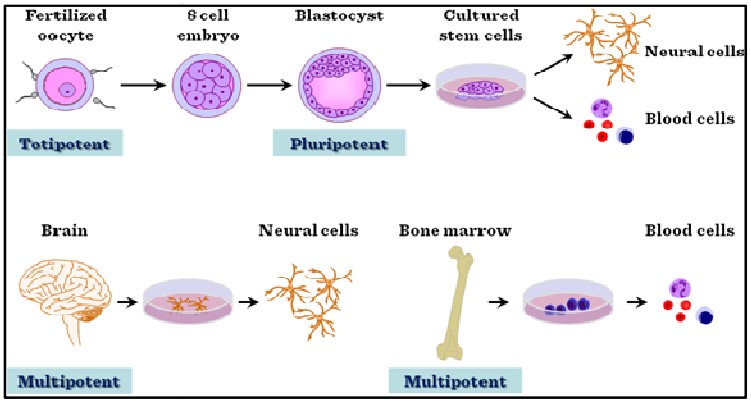


Figure 2.2: Types of stem cell

2.3.1 HISTORY OF MESENCHYMAL STEM CELL

In 1974, Alexander Friedenstein and colleagues were the first to identify mesenchymal stem cells (MSCs) (**Friedenstein *et al.*, 1974**). They were identified upon the other plastic adherent and non-adherent bone marrow cells suspension from rabbit and other rodent (rat, mouse, guinea pig) bones, non-phagocytic, enlarged and elongated cells that looked like fibroblasts that formed discrete colonies after passage. The specific colony was generated from a single cell and thus was called colony-forming unit-fibroblasts (CFU-F). Thereafter, *in vitro*, clonal cultures derived from individual CFU-F were introduced into diffusion chambers in experimental models where the formation of bone, cartilage and stromal elements were observed .According to Friedenstein was the first to consider the existence of stem cell niches within the bone marrow. Few years later, in 1980, Castro-Malaspina showed that the colonies described are of fibroblastic nature by immunologic studies **(Castro-Malaspina *et al.*, 1980; Dazzi *et al.*, 2006).** The Arnold Caplan, during the 1990s, defined MSCs as cells that could give rise to bone marrow and other tissues of human and animals, but also to cartilage, tendon and muscle **(Dennis *et al.*, 1999; Caplan *et al.*, 2006).**

In the year 1988, Maureen Owen used the term of "Stromal Stem Cells" to show that MSCs take part of the stromal layer of the bone marrow and did not belong to the haematopoietic stem cells **(Owen *et al.*, 1988)**. Darwin Prockop proposed the abbreviation of MSC for "Mesenchymal Stem Cell" or "Marrow stromal cells" because of their ability to differentiate into mesenchymal tissue and to serve also as a niche for other type of stem cells such as HSCs **(Prockop, 1997; Kopen *et al.*, 1999).** In 1999, James Dennis indicated that this cell type may not represent an authentic category of stem cell but in closer to progenitor cell situated downstream of stem cell

compartment and call these cells "Mesenchymal Progenitor Cell" (MPCs) **(Dennis *et al.,* 1999)**. Catherine Verfaillie described the culture-derived bone marrow-derived progenitor cells that may represent a more primitive cell type with different differentiation potential larger than that of MSCs, and they designated them as MAPCs for "Multipotent Adult Progenitor Cells" and "Mesodermal Progenitor Cells" **(Jiang *et al.,* 2002).**

2.3.2 SOURCES OF STEM CELLS

Recent work has examined the contribution of muscle to the blastema because myofibers/myotubes are readily identified and clearly represent a differentiated cell type. Muscle cell culture experiments are focused around Newt A1 and mouse C2C12 cells, both of which are mono-nucleated cells that can be induced to fuse and form myotubes in culture upon serum deprivation. These experiments uncovered a key difference between newt and mammalian *in vitro* derived myotubes. Newt A1 myotubes will enter S phase when exposed to high levels of serum or lower levels of thrombin-treated serum, while mouse C2C12 cells do not respond indicating the presence of an active signal in animal serum that leads to proliferation of newt A1 myotube nuclei **(Kumar *et al.,* 2000; Lo *et al.*,1993; Tanaka *et al.*,1999; Tanaka *et al.,* 1997; Velloso *et al.,* 2000; Velloso *et al.,* 2001).** However, it should be noted that the extent of differentiation and, therefore, relative developmental starting point for the two cell types is not clear. For example, newt A1 cells do not exhibit striation or peripheral alignment of nuclei **(Straube and Tanaka, 2006).** In addition, *in vivo* urodele amphibian muscles contain satellite-like cells that express Pax7 and are separated from the myofiber by a basement membrane (**Figure 2.3; Morrison *et al.,* 2006; Popiela, 1976).** It is doubtful that the cultured A1 myotubes contain the satellite-like cells that are present *in vivo.*

Therefore, whether or not *in vitro* generated myotubes represent *in vivo* myotube behavior needs to be fully determined. This is important because results from these cell lines have been interpreted to represent the urodele and mammalian condition. Because the cultured cells were serum starved to trigger myotube formation in the first place, an alternative interpretation of these experiments is that newt A1 cells are more flexible or "plastic" than mouse C2C12 cells. This may turn out to be the general case for urodele cells relative to mammalian cells, or it may be a feature unique to newt A1 cells, which may not be fully differentiated. In addition, while newt A1 cells do enter S phase after serum treatment, they do not go on to divide and the myotubes do not fragment (Straube and Tanaka, 2006). Forced expression of the *Msx1* gene (a homeodomain protein with known repressor functions) in C2C12 myotubes causes a small

fraction (5%) of cells to fragment into proliferating mononuclear cells **(Odelberg *et al.*,2000)**. Under the proper culture conditions, these cells can differentiate into adipocytes (fat), chondrocytes (cartilage), myocytes (muscle), or osteocytes (bone). Because C2C12 cells are multipotent to begin with, these results should be treated with caution. Nevertheless, this was a key demonstration that mammalian myofibers can be induced to reverse their differentiated state.

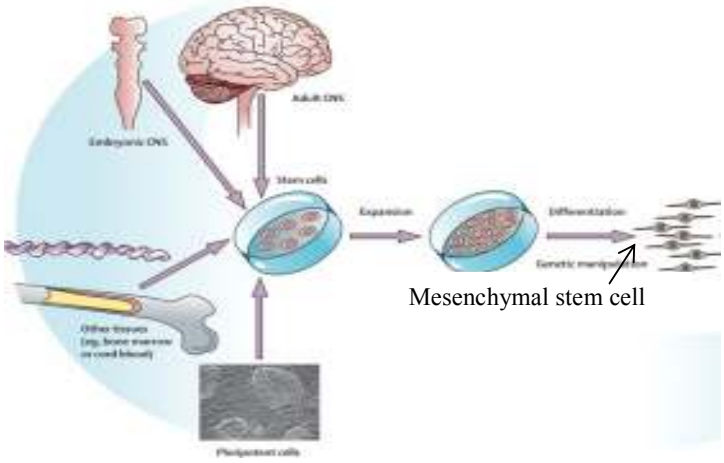
Complementary studies were also carried out in newts in which primary larval limb myofibers that normally fragment upon dissociation were inhibited from doing so via *Msx1* knockdown **(Kumar *et al.*, 2004).** Given the caveats mentioned above for cultured cells, the data argue that *Msx1* may be necessary and sufficient for differentiated muscle to fragment into proliferating mononuclear cells. To the contrary, *in vivo*morpholino-mediated knockdown of *Msx1* in individual larval axolotl tail muscle cells had no negative effect on the ability of these cells to fragment **(Schnapp and Tanaka, 2005).** This discrepancy has several potential explanations, including but not limited to the differences likely to exist between *in vitro* and *in vivo* conditions, and that the muscle cells of the limb and tail could exhibit a differential requirement for *Msx1* expression during fragmentation. To track dedifferentiation events, cultured cells can be labeled and implanted under the skin of regenerating limbs. *In vitro* differentiated myotubes, labeled with dye or viral insertion, remain stable in culture, but about 25% fragment upon implantation to generate proliferating mononuclear cells that contribute to the blastema **(Kumar *et al.*, 2000; Lo *et al.*, 1993)**. A few cells were eventually observed to form cartilage cells, suggesting a change in cell fate, but this was an extremely rare event **(Lo *et al.*, 1993).** On the other hand, reserve satellite-like cells also appear to contribute to the blastema **(Morrison *et al.*, 2006)**. Proliferating cells derived from satellite-like precursors were isolated in culture from adult newt myofiber explants. When tagged and implanted into regenerating adult limbs, these cells contributed to the blastema and many appeared to switch lineage into cartilage and even epidermal cells. To the contrary, another group found that implanted primary myofibers from juvenile axolotls can fragment and proliferate in the absence of satellite cells **(Kumar *et al.*,2004).**

It is possible that these disparate results can be explained by the difference in species and life cycle stages (adult versus juvenile), or by the different criteria used to assess whether satellite cells were present. It also remains possible that both myofiber fragmentation and satellite cell proliferation contribute to the blastema *in vivo* and their relative contribution may be age and/or species dependent. A definitive explanation for these discrepancies will be

important and most likely awaits *in vivo* cell tracking experiments. In another set of implantation experiments, cells isolated from the newt heart (cardiomyocytes, CMs) were isolated, tagged, and implanted into un-amputated or amputated limbs **(Laube *et al.*, 2006)**.

While CMs implanted into un-amputated limbs were stable and exhibited no special behavior, they were activated when implanted into day 5 regenerating limb blastemas and 65% gave rise to skeletal myotubes while a few expressed a cartilage cell marker. This is clear evidence of the plasticity of the differentiated state in adult urodele amphibians, but the magnitude of fate change is unclear because the transplanted CMs continued to express desmin, a marker found in many muscle cell types. While these experiments do not rule out a role for reserve stem/progenitor cells, they clearly illustrate that a large fraction of isolated newt CMs can at least switch muscle types. The different sources of stem cells from different organs in animals and human are as illustrated in the figure below **(Figure 2.3).**

Figure 2.3: Sources of stem cells



***In vivo* studies**

The *in vivo* data provide strong evidence to support the notion that differentiated cells can in fact change their functional state.Multinucleated myofibers were injected *in vivo* with a cell tracking dye and monitored during regeneration. Amputations that removed 50% or more of the muscle cell led to degradation, while amputations that "clipped" the muscle cell caused it to fragment into proliferating mononuclear cells. Only 15/58 (25%) clipped myofibers fragmented, which may indicate either an inefficient fragmentation process or point to a heterogeneous population of myofibers, some of which mononucleate more readily than others. On the other

hand, since fragmentation was observed in a small number of animals, this may be a rare event that does not play a major role in blastema formation. The authors calculate that muscle fragmentation contributes to roughly 17% of the blastema. In contrast, earlier work using triploid/diploid transplants suggested that dermal tissues contribute to roughly 43% of the blastema **(Muneoka *et al.,* 1986).**

To track individual neural progenitor cells (glial cells) during tail regeneration, spinal cords were electroporated immediately after tail amputation to force expression of GFP under the control of a glial-specific **(Echeverri and Tanaka, 2002).**While most of the cells gave rise to the expected neuronal and glial cell types, in 24% of the animal spinal cord cells migrated out of the regenerating spinal cord, contributed to the blastema, and gave rise to muscle. In 12% of animals glial cells gave rise to cartilage. These findings are significant because they clearly demonstrate that at least in the larval axolotl, neural progenitor cells of ectodermal origin can switch fate into mesodermally derived tissues during a regenerative response.

A series of transplants and single cell electroporations were recently performed to trace the lineage of spinal cord cells during tail regeneration **(McHedlishvili *et al.,* 2007)**. Spinal cord tissue transplants from GFP(+) to GFP(-) animals showed that the cellular precursors used to regenerate the spinal cord are recruited from within 500 μm of the amputation plane. The cells close to the amputation give rise to distal spinal cord cells, while cells farther from the amputation give rise to proximal cells. Single cell GFP electroporations and embryonic GFP(+) neural plate transplants revealed that in most cases, cells retain their regional identity during regeneration such that dorsal and ventral cells each give rise to cells of the same respective position **(McHedlishvili *et al.,* 2007).** However, in 8 of 21 electroporations and 3 of 5 transplants, cells changed dorso-ventral identities. In addition, a fraction of ventro-lateral cells near the terminal vesicle, a temporary structure that forms at the tip of the spinal cord during regeneration, migrated out of the spinal cord. These migratory cells apparently contributed to the tail blastema and gave rise to blood vessels, Schwann cells, and occasionally to muscle and cartilage cells. The results suggest that glial cells can serve as multipotential stem/progenitors during regeneration and that the terminal vesicle may represent an accumulation of de-dedifferentiated or reserve stem/progenitor cells. However, the exact nature of the transplanted cells remains in question and may include migratory neural crest cells, providing an intriguing line of questions for future investigation. These data add a layer of complexity to the regionalization and cellular make-up of the larval tail blastema and suggest that both lineages

restricted and multipotent cells exist in the regenerating urodele spinal cord. Whether this is also true during adult urodele tail regeneration is unknown.

In the mammalian skin, different types of stem cells reside in different microenvironments. Epidermal stem cells reside in the basal layer of the epidermis, while hair follicle stem cells reside in the bulge at the base of the permanent portion of the follicle **(Morrison and Spradling 2008).** Wnt and BMP signaling play critical roles in regulating bulge stem cells during hair follicle growth cycles **(Morrison and Spradling 2008).** Hair follicle stem cells reside in the bulge (bulge stem cells), and separate populations of stem cells reside in the basal layer of the epidermis and in the sebaceous gland. NSCs also sense and respond to injury or physiological changes. For example, in adult rodents, focal cerebral ischemia leads to increased proliferation of neural progenitors including those far from the area of infarction **(Jin *et al.,* 2001; Zhang *et al.,* 2001; Arvidsson *et al.,* 2002; Parent *et al.,* 2002).**The location of NSCs near blood vessels is conducive to exposure to systemic signals **(Liu *et al.,* 1993; Weger and Schlake 2005).**

Given the moral and ethical concerns surrounding the adult stem cell research other possible sources of multipotent cells have been sought. The Adult Stem Cell or tissue-specific stem cells do not elicit the same heated debate as embryonic stem cells and thus are gaining immense research momentum in the field of experimental research on stem cell. The bone marrow stem cell mostly using treatment and therapy in medical field and stem cell have two advantages; since the cells can be isolated from the experimental animal and identify the damages of stem cell and another one it is reduces the risk of tumor formation, which occurs with high frequency when mouse adult bone marrow stem cells (ASCs) were treated into histocompatible adult mice **(smith AG., 2001; Raff M. 2003**).

The unique biological properties and potential medical importance of stem cells the study of their underlying biology was the subject of intense investigations. The greatest advantage of using the patient's own stem cells treatment was due to the absence of immunogenicity of these cells. The Adult Stem cell from the experimental animal could be expanded in vitro, differentiated into the required cell type. There were however, several disadvantages with this approach, not the least being that adult stem cells are extremely rare and not approved for dissect the animal to isolate from mature tissues, and it was difficult to expand their numbers in vitro compared to embryonic stem cells.

2.4 HIGH-THROUGHPUT TECHNIQUES

Best among the high-throughput techniques used in RF-EMF studies include Flow Cytometry and RT-PCR analysis of genes of interest. The application of labeling fluorescence techniques and novel instrumentation has led to the development of quantitative real-time RT-PCR (qRT-PCR) methods that allow the real-time quantification of transcript levels **(Bustin SA, 2000).** Unlike traditional PCR, which detects amplification products at the end of the reaction, qRT-PCR allows amplification and detection to proceed simultaneously. It offers a rapid, automated method for the detection of multiple transcript levels with high sensitivity, reproducibility and a broad dynamic range **(Rongying *et al.,* 2007).** A common method for the normalization of qRT-PCR data is the simultaneous amplification of an endogenous reference, or a housekeeping gene **(Vandesompele J. *et al.,* 2002).**

It is essential to control for error between samples when measuring RNA expression. This error can be introduced at a number of stages throughout the experimental protocol (input sample, RNA extraction, reverse transcription, etc.). There are many methods to control for this error. One approach is to normalize to total RNA. However, this requires a reliable RNA quantification method and fails to take into account the variability of the reverse transcription and other steps. More recently, it has become clear that housekeeping genes like β-actin and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) may be inappropriate as internal references because of their variability. The quantitative real-time RT-PCR technique represents a sensitive and powerful method for the gel-free detection of mRNA with a tremendous potential for quantitative applications. Typically, the expression of the target gene is analyzed (in different reaction tubes or in the same reaction tube; i.e., multiplex) together with a reference gene to normalize the amount of the PCR template and, thus, to enable the calculation of the relative expression level of the target gene (i.e., normalized gene expression) Pfaffl, M.W. (2001). It is still a matter of debate whether the expressions of such housekeeping genes are in fact unaffected by a particular experimental setup or not **(Chang, T.J. *et al.,* 1998; Foss, D.L *et al.,* 1998; Thellin, O *et al.,* 1999).**

2.5 HISTOPATHOLOGY

A unifying hypothesis for a plausible biological mechanism to account for very weak field EMF bioeffects other than cancer may lie with weak field interactions of pulsed RF-EMF and ELF-modulated RF-EMF as disrupters of synchronized neural activity. Electrical rhythms in our brains can be influenced by external signals. This is consistent with established weak field

effects on coupled biological oscillators in living tissues. Biological systems of the heart, brain and gut are dependent on the cooperative actions of cells that function according to principles of non-linear, coupled biological oscillations for their synchrony, and are dependent on exquisitely timed cues from the environment at vanishingly small levels **(Buzsaki, 2006; Strogatz, 2003).** The key tosynchronization is the joint actions of cells that co-operate electrically and link populations of biological oscillators that couple together in large arrays and synchronize spontaneously. Synchronous biological oscillations in cells (pacemaker cells) can be disrupted by artificial, exogenous environmental signals, resulting in desynchronization of neural activity that regulates critical functions (including metabolism) in the brain, gut and heart and circadian rhythms governing sleep and hormone cycles **(Strogatz, 1987).** The brain contains a population of oscillators with distributed natural frequencies, which pull one another into synchrony (the circadian pacemaker cells). Strogatz has addressed the unifying mathematics of biological cycles and external factors disrupt these cycles **(Strogatz, 2001, 2003).**

2.5.1 HISTOPATHOLOGY OF BRAIN

The cell phone towers are growing in number due to the increase in the use of mobile phone communications. Hence, there is an increasing concern about the interactions of electromagnetic radiation with the human organs and animal organs, in particular, with the brain. From histological point of view, research has shown controversial results. Repeated exposure to radio frequency electromagnetic radiation (RF-EMF) caused in the brain of guinea pigs **(Gordon, 1970; Baranski, 1972; Tolgskaya and Gordon, 1973).**No serious threat to the developing central nervous system of young male and female guinea pig from mobile phone radiation and cell tower radiation at intensities relevant to human exposure has been indicated **(Kumlin *et al.,* 2007)**. Additionally, other studies did not show any statistically significant histological changes or cell proliferation in the brain tissues due to exposure of animals to GSM **(Kim *et al.,* 2008; Moghimi *et al.,* 2009; Daniels *et al.,* 2009).** Also an increase in myelin degeneration of neurons in the brain of rats after repeated exposure to continuous 2450- MHz RFR has been demonstrated **(Switzer and Mitchell, 1977).** Moreover, minor changes in Golgi complexes and slight swelling of endoplasmic reticulum of snail neurons exposed to 2450 MHz microwave radiation have been also been reported **(Arber *et al.,* 1986).** Significant evidence for neuronal damage in the cortex, hippocampus and basal ganglia in the brains were found after 2 h exposure of GSM 900 MHz radiofrequency radiation (RFR) of different strengths **(Salford *et al.,* 2003).**

Damaged dark neurons in the hippocampus of rat offspring following exposure of pregnant rats to 0.9 GHz microwaves have been found **(Odaci *et al.,* 2008).** The RFR exposure has significantly induced marked morphological changes in the CA3 region of the hippocampus of the mobile phone-exposed in Wistar rats **(Narayanan *et al.,* 2010).** Male Wistar albino rats exposed to microwaves emitted by cellular phones has resulted in histological changes in the testes **(Dasdag *et al.,* 1999; Dasdag *et al.,* 1999; Ozguner *et al.*,(2005). However, Dasdag *et al.,* (2003)** could not reproduce the results of their previous work in Sprague-Dawley rats exposed to 890–915 MHz pulsed wave. Recently, **Salama *et al.,* (2010)** exposed adult rabbits to low intensity pulsed radio frequency emitted by a conventional mobile phone showed, significant decrease in the diameter of seminiferous tubules.

In developing chick embryos, radiation emitted from mobile phone caused damage to the kidneys **(Ingole and Ghosh, 2006).** Contrasting effects of RFR on the chick embryo retinal histomorphology have also been published **(Zareen *et al.,* 2009).** Due to the wide and growing use of mobile communication, particularly by children and teenagers, there is increasing concern about the interactions of electromagnetic radiation with the human organs, in particular, with the brain. From histological point of view, research has shown controversial results. Repeated exposure to radio frequency radiation (RF-EMF) caused edema and lesions in the brain of guinea pigs **(Gordon, 1970; Baranski, 1972; Tolgskaya and Gordon, 1973).** Also an increase in myelin degeneration of neurons in the brain of rats after repeated exposure to continuous 2450-MHz RFR has been demonstrated **(Switzer and Mitchell, 1977).** Moreover, minor changes in Golgi complexes and slight swelling of endoplasmic reticulum of snail neurons exposed to 2450 MHz microwave radiation have been also been reported **(Arber *et al.,* 1986).** Significant evidence for neuronal damage in the cortex, hippocampus and basal ganglia in the brains were found after 2 h exposure of GSM 900 MHz RFR of different strengths **(Salford *et al.,* 2003).** Damaged dark neurons in the hippocampus of rat offspring following exposure of pregnant rats to 0.9 GHz microwaves have been found **(Odaci *et al.,* 2008).**

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developing chick embryos, radiation emitted from mobile phone caused damage to the kidneys (**Ingole and Ghosh, 2006).** Contrasting effects of RFR on the chick embryo retinal histomorphology have also been published **(Zareen *et al.,* 2009).**The intensity of lipid peroxidation in the brain and liver tissue was spectrophotometrically measured, based on the thiobarbituric (TBA) response products from (Ohkawa*et al*. 1979). Homogenate absorption was measured at 532 nm. Malondialdehyde (MDA) – lipid peroxidation end product, concentration was expressed per mg/protein, using the molecular extinction coefficient of MDA (1.56 x 10-5 mol cm-1). Brain proteins were determined according to Lowry's method **(Lowry *et al.,* 1951),** using bovine serum albumin as standard.

A study showed that when people used a cell phone tower for 6 months, brain tissues on the same side of the head as the phone's antenna metabolized more glucose than did tissues on the opposite side of the brain. The researchers noted that the results are preliminary, and possible health outcomes from this increase in glucose metabolism are still unknown **(Volkow *et al.,* 2011). Laila *et al., (*2010)** reported histological changes in the different visceral organs including heart, lung, liver and kidney of guinea pigs exposed to RF-EMF radiation for 6 months (12h dark, 12h light/day). According to Autherobserved histopathological changes in the skin of guinea pig exposed to 900MHz radiation for six months **(Fehmi *et al.,* 2004).** Sarookhani *et al., (*2011)suggested that testosterone and FSH levels are disturbed as a result of mobile phone exposure and it possibly affects reproductive functions. Wistar rats (35 days old, male, six rats in each group) were exposed for 35 days. After the exposure period, single strand DNA breaks by microgel electrophoresis (comet assay) conducted. The study showed that the chronic exposure to these radiations cause statistically significant increase in DNA single strand breaks in brain cells of rats **(Paulraj & Behari, 2011).**Microwaves were used in histology laboratory more than twenty years ago. They have applied fixation, staining including immunochemistry but most of all to tissue processing.

The increased usage of electromagnetic (EM) principles for domestic and industrial purposes proves that EMF plays an important role in our daily life. The emergence of telecommunication services using EMF principles greatly enhanced the ability of individuals and groups to communicate with each other. Nowadays, phones are not only used for making and receiving calls but also for many other applications, such as banking transactions and web browsing. The number of mobile phones subscribers in the world was estimated to about 4.6billion in 2009, and it was expected to reach 5 billion by 2010**(Hamadoun, 2010).** This means that around one out of every two individuals in this world carries a mobile phone. The

increased usage and growing popularity of wireless technologies in RF EMF range represent one of the fast growing environmental influences. There are lot of controversies and adverse health effects associated with energies emitted by these technologies **(Schwan, 1980).**

2.5.2 HISTOPATHOLOGY OF KIDNEY

The animals and humans beings are unavoidably exposed to ambient electromagnetic fields (RF-EMF) generated from various electrical devices, particularly from cell phone tower and from power transmission lines. All of the electronic equipments that we use in our daily life, without thinking how much we use or how often we use would create RF-EMF **(Ongel *et al.,* 2009)**. In recent years histological and physiological studies have increased in the evaluation of the effects of electromagnetic fields on animal and human health **(Kang *et al.,* 1996; Shakawy, 2009).** It was reported that extremely low frequency EMF induced tissue damage in different organs of the experimental animals, kidney also damaged for effects of electromagnetic radiation (RF-EMF) **(Zare*et al.* 2007, Khayyat and Abou-Zaid 2009)**. Also, exposure to EMF adversely affects spermatogenesis, Sertoli and Leydig cells, kidney cell and neuron cells of experimental animals **(Forgacs *et al.,* 2004, Khaki *et al.,* 2006, Aydin *et al.,* 2007).** The present study investigates the possible histopathological effects of isothermal non ionizing electromagnetic fields (EMFs) on the kidney and testis of guinea pig and male mice **(Maryinz-Samano *et al.,* 2010).**

2.5.3 HISTOPATHOLOGY OF HEART

Two different group hearts were taken for histological study and done comparative observation in tissue level. However, concerns have been raised about the wide range of effects of electromagnetic radiation **(Bakker JM. *et al.,* 2001).** These included findings of abnormal blood vessels in heart region and brain development in newborn animals treated with radiation **(Kamphuis PJ. 2003).** Recent reports on follow in 6 months guinea pig treated with radiation confirm its adverse effects on brain growth and heart blood vessels damages **(Murphy BP. *et al.,* 2001)** and neuromotor developmental outcome **(Barrington KJ 2017).**

With regard to the cardiovascular system, shortterm side-effects, such as myocardial hypertrophy, manifested by increased ventricular septal and left ventricular wall thickness, and hypertension have been reported in animal and human studies (**Israel BA, 1993; Werner JC, 1992)**. Recent studies from our group suggested that RF-EMF radiation treatment may have

detrimental long-term effects on the heart, possibly initiated by temporary suppression of the proliferative capacity of cardiomyocytes during the exposure period of guinea pig and normal animal **(de Vries WB. *et al.*, 2006).**

2.5.4 HISTOPATHOLOGY OF LIVER

In the liver, the interlobular areas of guinea pig exposed to RF-EMF were rich in inflammatory cellular infiltration; the hepatocytes appeared vacuolated and contained denser nuclei (**Al-Glaib *et al.*, 2008).** Other studies are explained about liver cells damaged for effects of electromagnetic radiation on an experimental animal for example guinea pig and mice, described similar tissue changes using lower frequencies of electromagnetic radiation **(Attia *et al.*, 2002; Zsolt *et al.*, 2006).**Similarly, no harmful effects of chronic exposure of male and female mice (C57BL) to RFR on the kidney function, fertility and development of the animals have been reported **(Sommer *et al.*, 2009).**The objective of this study was to assess the effect of exposure to 900MHz, RFR on the histology and ultrastructure not only liver tissue mostly various tissues are damages of male and female young guinea pigs. Liver sections of group three showed more intensive inflammatory response around the central vein. Hepatocytes were swollen and their cytoplasm appeared to be highly vacuolated **(Al- Glaib *et al.*, 2007).** On histological examination by two board-certified head and neck pathologists who independently rated each slide in a blinded fashion. They came to a consensus grading scale after their initial independent examination. The histological parameters were semi-quantitatively defined by essential histological features, including the degree of fatty replacement, preservation of lobular architecture, preservation of ducts and acini, the presence of interstitial fibrosis, and the inflammatory component (**J. J. Sciubba and J. A. Bishop, 2006).**

Studying the biological damage induced by non-ionizing radiation is necessary in assessment of maximum absorbed dose during radiotherapy or diagnosis. Moreover, development of protective agents presented new solutions for recovery of undesired tissue damage induced by ionizing radiation **(Borek, 2004).**The ionizing radiations cause damage of the cells directly by ionization of DNA and other cellular targets and indirectly through Reactive Oxygen Species (ROS) **(Borek, 2004).**It has been shown that radiotherapy causes killing of tumor cells but its usage causes threatening for the integrity and survival of surrounding normal cells **(Konopacka and Rogolinski, 2004).**The biohazards of irradiation appear from its cumulative levels in body that leads to cancer, cell death, genetic damage and tissue pathology **(Borek, 2004).** Irradiation causes DNA strand breaking **(Eric and Giaccia, 2012).** Lymphokines

are molecules that mediate molecular pathways common to ionizing irradiation induced apoptosis **(Epperly *et al.,* 2001; 2003).**Irradiation causes activation of caspase-3 by DNA fragmentation **(Epperly *et al.,* 2002).**As known exposure to ionizing radiation produces reactive oxygen species like hydroxyl radicals, superoxide anions and other oxidant as H2O2 which cause antioxidant/oxidant balance **(Thangasamy *et al.,* 2009).**Cytokines play important roles during inflammation and radiation **(Ao *et al.,* 2009).**

2.5.5 HISTOPATHOLOGY OF LUNG

The electromagnetic radiation from cell phone tower it damages in lung tissues particularly radiation effects to inside of the lungs. Radiations are not smelt and seen it is mixing with breathing air so easily entire inside. Screening electro-smokes have a variety of effects both for animal and human. One method of generating an electro-smoke is make very dangerous impacts like lung cancer, glioma this radiation exposing range is 3 to 300GHz **(Jarvis, A. *et al.,* 1997).** Evidence accumulated over the years indicates that, in certain circumstances, these smokes can produce morbidity. This is exemplified by numerous case reports **(Matarese, S. L., 1986).** In an attempt to elucidate the pathogenetic mechanism involved in radiation exposure area living peoples and animal, a number of animal models have been developed recently by several authors. Histological and immunohistological changes of the lung have been studied by experimental exposure to RF-EMF of either sensitized **(Kochman *et al.,* 1972; Wilkie, Pauli & Gygax, 1973)** or non-sensitized animals **(Salvaggio *et al.,* 1975; Stanford & Salvaggio, 1976).** These authors found evidence for the possible involvement of type I, II, III or IV allergic reactions or combinations of them.

The Guinea pigs (Caviaporcellus), which are not rodents based on phylogenetic analysis of amino acid sequences **(Graur *et al.,* 1991; D'Erchia *et al.,* 1996),** have recently been shown to be an excellent model for the study of IAV transmission **(Lowen *et al.,* 2006).** Earlier studies in guinea pigs were limited to the analysis of IAV-induced lung changes by histology and electron microscopy **(Azoulay- Dupuis *et al.,* 1984)** and immune responses such as delayed-type hypersensitivity **(Wetherbee, 1973; Phair *et al.,* 1979).** However, despite the new found importance of guinea pigs for the study of environmental factors that affect IAV transmission **(Lowen *et al.,* 2007, 2008),** very little is known about viral replication and histopathology in the respiratory tissues of this species. Here, we describe viral growth kinetics, target cells and histopathology in upper and lower airways of guinea pigs infected with the IAV strain A/Puerto

Rico/8/34 (H1N1), designated PR8 (ATCC VR-95) and A/Hong Kong/8/68 (H3N2), designated HK/68 (ATCC VR-544). These IAV strains were grown in embryonated eggs and titrated in Madin-Darby canine kidney (MDCK) cells as previously reported **(Chong *et al.,* 2008).**

In histological analysis, HK/68-infected lungs showed evidence of acute bronchiolitis, bronchointerstitial pneumonia and alveolitis. Early lesions following either PR8 or HK/68 infection consisted of massive immune cell infiltrates predominantly of neutrophils and mononuclear cells but also some eosinophils. By day 3 p.i., infected lungs showed severe bronchiolitis and alveolitis that were characterized by the presence of inflammatory cells within alveolar spaces. Our results showed that pathological lesions that developed in the upper and lower airways of guinea pigs corresponded with the time course of acute IAV infection. Since both HK/68 and PR8 have been widely used in animal model studies, our findings allow comparison of IAVinduced airway disease in guinea pigs with published results on airway lesions in mice and ferrets (**Mbawuike *et al.,* 2007; Sanford & Ramsay, 1987).**

For these IAV strains, infected guinea pigs showed somewhat more severe nasal and lung disease than have been previously reported for mice **(Iwasaki *et al.,* 1999; Chong *et al.,* 2008).** For instance, guinea pigs produced excessive amounts of mucus in the nasosinus tract by three days which were much greater than we previously observed in infected mice **(Chong *et al.,* 2008).** This suggested that infection in guinea pigs produced acute airway symptoms more similar to upper airway infection in humans.

2.5.6 HISTOPATHOLOGY OF MUSCLES

The histopathology of muscle has been studied since the latter part of the last century and it has been particularly profitable when correlated with clinical and other available data. By this method the specificity of the various clinical and histological changes may be determined. Complete uniformity about the value of muscle biopsy has not yet been achieved and **(Russell, 1957).** Nevertheless, increasing interest is being given to muscle pathology as recent publications demonstrate, such as that of **(Walton, 1964).** The success of a muscle biopsy is clearly dependent upon the correct choice of muscle for biopsy and the correct removal of a suitable piece for examination. In general it is often advisable to biopsy a muscle not too severely affected clinically especially if the procedure is to study early changes as in dystrophy research in addition to diagnostic purposes **(Pearson *et al.,* 1963).**

2.5.7 HISTOPATHOLOGY OF SKIN

The skin of male and female guinea pigs is exposed for 6 months showed not much difference, only mild histological changes **(Ozguner *et al.,* 2004).** Not only guinea pig skin damages, but also human skin fibroblast activity and morphological changes were reported after 6 months continuously exposure to cell tower and mobile phone radiation **(Pacini *et al.,* 2002; Irmak *et al.,* 2003).**Local exposure of hairless skin of rats to GSM-RFR did not demonstrated major histological variations in the skin **(Sanchez *et al.,* 2006).**Delayed hypersensitivity reactions are characterized histologically by perivascular infiltration of mononuclear cells. Recent investigations of these reactions in the skin of man and various laboratory animals **(Dvorak *et al.,* 1970)** have distinguished two forms of delayed hypersensitivity on clinical and histologic grounds. Classic delayed hypersensitivity (DH) is induced by sensitization with mycobacteria or with other antigens administered in mycobacteria-containing adjuvants. A second form of delayed hypersensitivity, cutaneous basophil hypersensitivity (CBH), is induced by a variety of immunizing procedures, which avoid the use of mycobacterial adjuvants. Both are affected by lymphocytes, but skin reactions. Two distinct histologic pictures of delayed hypersensitivity can be produced in the guinea pig cornea when animals are primed for CBH and DH **(Friedlander *et al.,* 1977).** In CBH-primed guinea pigs, large numbers of basophils and eosinophils infiltrate the cornea and limbus after intracorneal challenge. In DH-primed animals the infiltrate consists mainly of mononuclear cells and neutrophils. In order to determine the cellular components of DH reactions in the uveal tract, we induced **CBH** and DH in guinea pigs and studied the inflammatory response following intravitreal challenge.

Recently, in May 2011, the WHO's International Agency for Research on Cancer (IARC) has classified electromagnetic fields from mobile phones and other sources "possibly carcinogenic to human" and advised the public to adopt safety measures to reduce exposures, like use of hand-free devices or texting. Also, prolonged use of mobile phones affects the people because of field concentration and resonance in the vicinity of human brain. Earlier studies on the effects of high frequency EMF to human body have shown that long-time, high-frequency exposure is very harmful for the human body **(London *e. al.,* 1991; Karunaratha M. A. 2006).** Microwave fields have become a driving force of our civilization through their numerous applications in the scientific and the industrial as well as the military and civilian world. Today, due to the development of modern technology, the field of communication, radar astronomy, navigation and power etc. and widespread use of these waves among common generation causes the adverse health effect **(Lai & Singh 1997a; Altamura G 1997 and Black N, 2009).** Much

attention has been paid to health implications with high frequency electromagnetic field exposure since the last two decades. A large amount of work has been published on the biological effects of microwave radiation **(AnuKarinen, 2008, Hulter H. P. 2010, Panda *et al.*, 2010).**

A cautionary target level for cumulative, outdoor pulsed RF-EMR exposures for ambient wireless that could be applied to RF-EMR sources from cell tower antennas, Wi-Fi, WiMAX and other similar sources must be proposed. Research is needed to determine what is biologically damaging about intermittent pulses of RFR, and how to provide for protection in safety limits against it. With this knowledge it might be feasible to recommend a higher time-averaged number. A scientific benchmark of 0.003 uW/cm2 or three nanowatts per centimeter squared for 'lowest observed effect level' for RFR is based on mobile phone base station-level studies. Applying a ten-fold reduction to compensate for the lack of long-term exposure (to provide a safety buffer for chronic exposure, if needed) or for children as a sensitive subpopulation (if studies are on adults, not children) yields a 300 to 600 picowatts per square centimeter precautionary action level. This equates to a 0.3 nanowatts to 0.6 nanowatts per square centimeter as a reasonable, precautionary action level for chronic exposure to pulsed RF-EMF. Even so, these levels may need to change in the future, as new and better studies are completed. This is what the authors said in 2007 **(Carpenter and Sage, 2007, BioInitiative Report 2012)** and it remains true today in 2015. We leave room for future studies that may lower or raise today's observed 'effects levels' and should be prepared to accept new information as a guide for new precautionary action.