Stem Cells Preservation

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Review Article

ABSTRACT

Received: 10/12/2016 Revised: 15/12/2016 Accepted: 20/01/2017

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Keywords: Cryopreservation, Stem cells, Cell therapy, Preservation, Stabilization, Hematopoietic Embryonic stem cells are pluripotent stem cells which can give rise to all of the cell types that make up the body; embryonic stem cells derived from the inner cell mass of a blastocyst, an early-stage preimplantation embryo. Adult stem cells have demonstrated tremendous human therapeutic potential. Currently, human embryonic stem cells are used principally for understanding growth and disease development but also hold enormous medicinal potential. The capability to preserve stem cells is difficult for their use in medical applications. Preservation of cells allows the movement of cells between sites, as well as completion of safety and quality testing. Preservation allows the development of a 'manufacturing paradigm' for cell therapies, thereby maximizing the number of products that can be produced at a given facility.

Different modes of preservation and the current status of preservation of hematopoietic, mesenchymal and human embryonic stem cells can be studied in this article. Present and upcoming issues in the area of stem cell preservation will be discussed here.

INTRODUCTION

Stem cells are used to treat a large number of diseases and disorders. Hematopoietic stem cells is an example, which have traditionally been used to treat leukaemia are now being used to treat cardiac diseases, hereditary blood disorders and autoimmune diseases ^[1-7]. Preservation of stem cells is critical for stem cell research and medical application of stem-cell based therapies. Preservation allows development of cell banks with different major histocompatibility complex genetics and genetically modified clones. Collection of stem cells from different sources such as umbilical cord blood can be difficult to predict or control, the ability to preserve cells permits the storage of stem cells until later use in the research lab or medicinal application. The ability to preserve cells permits completion of quality and safety testing before use as well as movement of the cells inbetween the sites of collection, processing and medical administration ^[8-15]. Finally, the ability to preserve cells used therapeutically facilitates the development of a manufacturing paradigm for stem cell based therapies. The capacity to preserve the cells after production of the therapy facilitates coordination of therapy with a patient care regime and reduces staffing requirements of clinical cell production facilities ^[16-23].

Cryopreservation is one of the technique for cell preservation which involves the following steps: a) pre-freeze processing; b) introduction of a cryopreservation solution; c) freezing protocol; d) storage conditions; e) thawing conditions and f) post thaw assessment.

Hematopoietic Stem Cells preservation focused mainly on modification of the freezing medium and freezing and storage protocols. Dimethyl sulfoxide is used to cryopreserve HSCs, with a DMSO concentration of 10% being the most commonly used ^[24-31].

<u>Human Embryonic Stem Cells</u> are preserved in the following manner: Colonies of hESCs (100–400 cells) are placed in a vitrification solution composed of DMSO + EG + 0.5 mol/l sucrose. The colonies are loaded into straws and plunged into liquid nitrogen ^[32-38].

Many scientific <u>open access journals</u> on stem cell started the awareness in the public by its unrestricted access of articles to the society. The articles published in these <u>peer reviewed stem cells journals</u> will promote the steps to be

taken for the prevention of cancer. <u>Dr. Marek Malecki</u> from University of Wisconsin, USA is an Editor-in-Chief for the <u>Journal of Stem Cell Research & Therapy</u> and as an editor in chief his service for the journal's growth is extraordinary. The other journal like <u>Cell Biology: Research & Therapy</u> with an eminent <u>Editorial board</u> is striving for the betterment of cell biology research.

List of Major societies in USA for Stem Cell Research

Many <u>societies</u> in the USA committed to preserve and save the stem cells. The International Society for Stem Cell Research (ISSCR), an independent profitless organization and the voice of the stem cell research community, which was founded for the exchange of information on stem cell research. Few societies like <u>International Placenta Stem</u> <u>Cell Society</u>, <u>Stem Cell research Italy</u>, <u>European Biotechnology Thematic Network Association</u>, National Institute of Health – Stem Cell Lines, , <u>Egyptian Society of Stem Cell Therapy</u> etc. provides guidelines for human embryonic stem cell research as well as a compilation of frequently asked questions, National Academy of Sciences which helps in providing guidelines for Human Embryonic Stem Cell Research, Norwegian Center for Stem Cell Research, etc are involved in providing support to the stem cell preservation ^[39-45]. Some foundations and funding agencies like Christopher Reeve Foundation, Diamond Blackfan Anemia Foundation, Juvenile Diabetes Research Foundation, Multiple Sclerosis Foundation, Muscular Dystrophy Association, etc. helps in the stem cell research ^[46-53].

Every year many conferences on stem cell research and preservation are attracting lot of researchers in the field to expose their research and to know new researches and innovations in the field of stem cell preservation. <u>7th</u> <u>Annual Conference on Stem Cell and Regenerative Medicine</u> based on the theme "Advanced Approaches in Stem Cell and Regenerative Medicine" which included many research topics like Stem Cell Therapy, Stem cell & Regenerative Market, Tumour Cell Science, where highly affiliated Speakers like <u>Nilanjana Maulik</u>, <u>Barritault Denis</u>, Y James Kang, <u>Hazem Barmada</u>, etc. shared their valuable experiences in the field. With the success of 2016 conference, <u>8th Annual Conference on Stem Cell and Regenerative Medicine</u> which is going to be held during Sep 25-26, 2017 at Berlin, Germany for all the researchers worldwide.

2nd International Conference & Exhibition on Tissue preservation and Bio-banking which held on September 12-13, 2016 at Philadelphia, USA with a theme "Global Innovations in Tissue preservation and Biobanking Technologies". The <u>Biobanking-2016</u> Conference was carried out through various sessions with discussions on Cryopreservation Methods, Next Generation Biobanking, Germplasm Bank, Stem cell Biobanking, etc. Various sessions were chaired and co-chaired by: <u>Kelvin GM Brockbank</u> (Tissue Testing Technologies LLC, USA); <u>Simone Chevalier</u> (McGill Urology Director of Research, Canada), USA; Charles W Wang, (Shanghai Jiao Tong University, China); Yaffa Rubinstein (National Institute of Health, USA).

With the inspiration of the 2nd International Conference & Exhibition on Tissue preservation and Bio-banking, <u>Conference LLC</u> is inviting all the participants from all over the world to attend "<u>3rd International Conference &</u> <u>Exhibition on Tissue Preservation and Bio banking</u>" during august 23-24, 2017 in San Francisco, California, USA. Some of the upcoming Conferences on the stem cells preservation are <u>10th World Congress on Stem Cell and Bio</u> <u>banking</u> which is going to be held during October 23-24, 2017 in Osaka, Japan, <u>Bio banking Tissue 2017</u>

Procedure of collecting stem cells storage in a stem cell bank

The Stem cell's capacity to repair and <u>regenerate</u> is the basis for all the researches in the field of <u>stem cell</u> therapy. This unique characteristic of stem cell is very attractive. The research and findings in this field of stem cell therapy proved to create a revolution in the field of medicine ^[54-60]. In recent investigation, the knowledge on stem cells and its role in treatment of different diseases developed awareness among people and moved them to adopt stem cell collection and <u>preservation techniques</u>.

The stem cells are taken from the source from where the cells are harvested ^[61-68]. Based on its source stem cells are classified as <u>fetal stem cells</u>, embryonic stem cells, umbilical cord stem cells and adult stem cells ^[69-74]. Stem cells are utilized for treating diseases by altering its characteristics of undifferentiated to well differentiated specific cell type under controlled condition as shown in figure 1.

The cells formed from the zygotic division are called the <u>embryonic stem cells</u> because of its ability to develop into any type of cell ^[75-81]. The embryonic stem cells are removed from the embryos in vitro by adopting a technique called <u>in vitro fertilization</u> (IVF). In vitro fertilization is a method used for treatment of sterile couples. In this method the sperm is inserted into the egg under controlled conditions in a laboratory conditions ^[82-89]. The use of in vitro embryos for harvesting <u>stem cells</u> is a very accurate procedure which is done only by obtaining permission from the

couple whose egg and sperm are used in developing the embryo. The harvested embryonic stem cells are developed through cell culture technique. In this, the doctor decides the media and the required environmental and physiological parameters for the <u>cells</u> to grow into well defined, specific <u>cell type</u> ^[90-96]. The advantages of using embryonic stem cells in a research are that they are fresh and they are not obstructed by any condition and mostly devoid of any <u>chromosomal abnormality</u>. The main drawback of using these cells in the treatment of disease is its unsuppressed cell division which may leads to cancer ^[97-102].

The umbilical cord blood is a rich source of stem cells; it is a connection between the mother and baby through which nutrients are transferred from placenta to the baby in the womb. In order to collect the cord blood for stem cells, the cord between the placenta and the baby is clamped and a trained person collects the blood from the umbilical cord by using a needle ^[103-109]. The collected blood is transferred to the sample vial and sent for storage. Sterile conditions are maintained during the process of collection to avoid contamination of the sample. People with a family history of genetic disorders or diseases can preserve their baby's <u>cord blood</u> which can be used effectively in future treatment of various diseases ^[110-117]. The collected blood can be stored. The complete family can be benefited by storing umbilical cord stem cells.

Adult stem cells are the existing cells in an adult. The cells of muscle tissue, bone marrow, skin cell and nerve cell are examples for adult stem cells ^[118-123]. The collection of stem cells from bone marrow requires a surgical procedure in which the donor is given anesthesia first and a needle is inserted into the bone marrow at a specific site to collect the stem cells ^[124-126]. Bone marrow transplantation is a widely used treatment method in treating various disorders. Collection of adult stem cells from blood is done by collecting the blood intravenously from one hand and passing it through a processor which separates the <u>stem cells</u> from the blood. Once the stem cells are separated, the blood is sent back to the body ^[127]. The drawback for adult stem cells is the present abnormalities or the cell damage due to various parameters. In comparison to the embryonic stem cells, the division of adult stem cells can be controlled and hence the risk of cancer is minimized to an extent ^[27].

Apart from these above mentioned stem cell types, the stem cells are discovered in <u>amniotic fluid</u> and menstrual blood. Stem cells from menstrual blood and its application in treating arthritis, cardiac disease has been proved in a research.



Figure 1. Stem cell preservation

CONCLUSION

Stem cell contribute to a natural healing and plays an important role for regenerative medicine. Stem cell banking through long-term storage of different stem cells represents a basic source to store original features of stem cells for patient-specific clinical applications ^[64]. Stem cells can heal the body, promote recovery, and offer an enormous amount of therapeutic potential. <u>Cord blood</u> holds promise for future medical procedures. Many scientists are still studying more ways to treat more diseases with cord blood. For example many researchers are using patients' own cord blood in trials for cerebral palsy and <u>Hypoxic ischemic encephalopathy</u>.

<u>Stem cell treatable diseases</u> continue to grow at a rapid pace. With the potential to become different cell types, researchers are searching for the possibility of using cord blood stem cells to treat some of the dangerous diseases such as heart diseases and stroke. Thus, saving the baby's cord blood now can ensure child's access to his/her own stem cells for such cellular therapy in the future.

REFERENCES

- 1. Hewitt R and Watson P. Defining biobank. BiopreservBiobank. 2013;11:309-315.
- 2. Nietfeld JJ, et al. Life Probabilities of Hematopoietic Stem Cell transplantation in the U.S. Biology of Blood and Marrow Transplantation. 2008;14:316-322
- 3. Filocamo M, et al. Telethon Network of Genetic Biobanks a key service for diagnosis and research on rare diseases. Orphanet J Rare Dis. 2013;30:129.
- 4. McMurter B, et al. Parental views on tissue banking in pediatric oncology patients. Pediatr Blood Cancer. 2011;57:1217-1221.
- 5. Cheah S, et al. Permission to contact (PTC)--a strategy to enhance patient engagement in translational research. Biopreser Biobank. 2013;11:245-252.
- 6. Doherty KC and Burgess MM. Engaging the public on biobanks: outcomes of the BC biobank deliberation. Public Health Genomics. 2009;12:203-215.
- 7. Braun KL, et al. Cancer patient perceptions about biobanking and preferred timing of consent. BiopreservBiobank. 2014;12:106-112.
- 8. Master Z, et al. Cancer patient perceptions on the ethical and legal issues related to biobanking. BMC Med Genomics. 2013;6:8
- 9. Gaffney EF, at al. The human side of cancer biobanking. Methods Mol Biol. 2012; 823: 59-77.
- 10. Webster JD, et al. Quantifying histological features of cancer biospecimens for biobanking quality assurance using automated morphometric pattern recognition image analysis algorithms. J Biomol Tech. 2011;22:108-118.
- 11. Hamburg MA and Collins FS The path to personalized medicine. N Eng J Med. 2010;363:301-304.
- 12. Forsberg JS, et al. Changing perspectives in biobank research: from individual rights to concerns about public health regarding the return of results. Eur J Hum Genet. 2009;17:1544-1549.
- 13. Murtagh MJ, et al. Realizing the promise of population biobanks: a new model for translation. Hum Genet. 2011;130:333-345.
- 14. Riegman PH, et al. Biobanking for better healthcare. Mol Oncol. 2008;2:213-222.
- 15. Hewitt RE. Biobanking: the foundation of personalized medicine. Curr Opin Oncol 2011;23:112-119.
- 16. The Belmont Report (1979) Ethical Principles and Guidelines for the Protection of Human Subjects of Research. US, Department of Health and Human Services Washington DC
- 17. Declaration of Helsinki (1964) Ethical Principles for Medical Research Involving Human Subjects. World Medical Association
- 18. Tri Council Policy Statement 2nd edition (2010) Ethical Conduct for Research Involving Humans. Panel of Research Ethics, Government of Canada.
- 19. Presidential Bioethics Commission Issues Report on Clinical Trials Research in Developing Countries. 2001; National Bioethics advisory commission.
- 20. Hansson MG. For the safety and benefit of current and future patients. Pathobiology. 2007;74:198-205.
- 21. Vegvari A, et al. Biobank resources for future patient care: developments, principles and concepts. J Clin Bioinforma. 2011;1:24.
- 22. Asslaber M and Zatloukal K. Biobanks: transnational, European and global networks. Brief Funct Genomic Proteomic. 2007;6:193-201.
- 23. Asslaber M, et al. The Genome Austria Tissue Bank (GATiB). Pathobiology. 2007;74:251-258.
- 24. Vegvari A and Marko-Varga G. Clinical protein science and bioanalytical mass spectrometry with an emphasis on lung cancer. Chem Rev. 2010;110:3278-3298.
- 25. Sabel MS, et al. Proteomics in melanoma biomarker discovery: great potential, many obstacles. Int J Proteomics, 2011.

- 26. Waldman SA and Terzic A. Patient-centric clinical pharmacology advances the path to personalized medicine. Biomark Med. 2011;5:697-700.
- 27. Vaught JB, et al. Ethical, legal, and policy issues: dominating the biospecimen discussion. Cancer Epidemiol Biomarkers Prev. 2007;16:2521-2523.
- 28. Gaskell G and Gottweis H. Biobanks need publicity. Nature. 2011;471:159-160.
- 29. Goldman RE, et al. Rhode Islanders' attitudes towards the development of a statewide genetic biobank. Personalized Medicine. 2008;5:339-359.
- 30. Maschke KJ. Biobanks: DNA and Research. ed NY 2008.
- 31. Watts G. UK Biobank gets 10% response rate as it starts recruiting volunteers. BMJ. 2007;334:659.
- 32. Sheikh AA. Genetic research & human biological samples: some legal and ethical considerations. Med Law. 2004;23:897-912.
- 33. Auray-Blais C and Patenaude J. A biobank management model applicable to biomedical research. BMC Med Ethics. 2006;7:E4.
- 34. Beskow LM, et al. Developing a simplified consent form for biobanking. PloS One. 2010;5:e13302.
- 35. Pawlikowski J, at al. The analysis of the ethical, organizational and legal aspects of Polish biobanks activity. Eur J Public Health. 2010;20:707-710.
- 36. Murphy J, et al. Public perspectives on informed consent for biobanking. Am J Public Health. 2009;99: 2128-2134.
- 37. Hall MA, et al. Biobanking, consent, and commercialization in international genetics research: the Type 1 Diabetes Genetics Consortium. Clin Trials. 2010;7:S33-S45.
- 38. Knoppers BM. Biobanking: international norms. J Law Med Ethics. 2005;33:7-14.
- 39. Salvaterra E, et al. Banking together. A unified model of informed consent for biobanking. EMBO Rep. 2008;9:307-313.
- 40. Junghans C, et al. Recruiting patients to medical research: double blind randomised trial of "opt-in" versus "opt-out" strategies. BMJ. 2005;331:940.
- 41. Simon CM, et al. Active choice but not too active: public perspectives on biobank consent models. Genet Med. 2011;13:821-831.
- 42. Gottweis H, et al. Connecting the public with biobank research: reciprocity matters. Nat Rev Genet. 2011;12:738-739.
- 43. Morente MM, et al. TuBaFrost 2: Standardising tissue collection and quality control procedures for a European virtual frozen tissue bank network. Eur J Cancer. 2006; 42:2684-2691.
- 44. Hirtzlin I, et al. An empirical survey on biobanking of human genetic material and data in six EU countries. Eur J Hum Genet. 2003; 11:475-488.
- 45. Woods EJ and Thirumala S. Packaging Considerations for Biopreservation. Transfus Med Hemother. 2011;38:149-156.
- 46. Cardoso S, et al. Quality standards in Biobanking: authentication by genetic profiling of blood spots from donor's original sample. Eur J Hum Genet. (2010) 18:848-851.
- 47. Vaught J, et al. An NCI perspective on creating sustainable biospecimen resources. J Natl Cancer Inst Monogr. 2011;1-7.
- 48. Kronenthal C, et al. Broadening research consent in the era of genome-informed medicine. Genet Med. 2012;14:432-436.
- 49. Bunnik EM, et al. The new genetics and informed consent: differentiating choice to preserve autonomy. Bioethics. 2013;27:348-355.
- 50. Beauchamp TL and Childress JF. Principles of Biomedical ethics. Oxford University Press USA, 2013.
- 51. Beauchamp TL. Methods and principles in biomedical ethics. J Med Ethics. 2003;29:269-274.
- 52. Sotrup. Journal of Internal Medicine. 2011;269:370-382.
- 53. Wee R. Dynamic consent in the digital age of biology. J Prim Health Care. 2013;5:259-261.
- 54. Kaye J, et al. Ethical implications of the use of whole genome methods in medical research. Eur J Hum Genet. 2010;18:398-403.
- 55. Chadwick R. Personal genomes: no bad news? Bioethics. 2011;25:62-65.
- 56. Kegley JA. Challenges to informed consent. EMBO Rep. 2004;5:832-836.
- 57. Sheehan M. Can Broad Consent be Informed Consent? Public Health Ethics. 2011;4:226-235.

- 58. Steinsbekk KS, et al. Broad consent versus dynamic consent in biobank research: is passive participation an ethical problem? Eur J Hum Genet. 2013;21:897-902.
- 59. Hofmann B. Broadening consent--and diluting ethics? J Med Ethics. 2009;35:125-129.
- 60. Caulfield. Medical Law International. 2009;10:85-100.
- 61. Howard HC, et al. Informed consent in the context of pharmacogenomic research: ethical considerations. Pharmacogenomics J. 2011;11:155-161.
- 62. Rotimi CN and Marshall PA. Genome Medicine. 2010;2:1-7.
- 63. Ebbesen M, et al. Further Development of Beauchamp and Childress' Theory Based on Empirical Ethics. J Clinic Res Bioeth. 2012;S6:1-7.
- 64. NBAC. Research Involving Human Biological Meterials. Ethical Issues and Policy Guidance. 1999;1:72.
- 65. Bethesda. NHLBI Working Group Results in Research Studies, Meeting Summary. National Heart, Lung, and Blood Institute, 2004.
- 66. Fabsitz RR, et al. Ethical and practical guidelines for reporting genetic research results to study participants: updated guidelines from a National Heart, Lung, and Blood Institute working group. Circ Cardiovasc Genet. 2010;3:574-580.
- 67. CoGfHESC, Council NR. Guidelines for Human Embryonic Stem Cell Research: The National Academies Press, 2005.
- 68. Behrman RE. Letter from AMP to the Secretary's Advisory Committee on Genetics, Health, and Society. 2007.
- 69. Lifton RP, et al. Molecular mechanisms of human hypertension. Cell. 2001;104:545-556.
- 70. Enright PL and Sherill DL. Reference equations for the six-minute walk in healthy adults. Am J RespirCrit Care Med. 1998;158:1384-1387.
- 71. Evans MJ and Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1982;292:154-156.
- 72. Kamihata H, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. Circulation. 2001;104:1046-1052.
- 73. Feigenbaum H. Echocardiography. 5th Ed. Philadelphia: Lea & Febigerp. 1994;675.
- 74. Cuspidi C, et al. Role of echocardiography and carotid ultrasonography in stratifying risk in patients with essential hypertension: the Assessment of Prognostic Risk Observational Survey. J Hypertens. 2002;20:1307-1314.
- 75. Jensen JS, et al. Arterial hypertension, microalbuminuria and risk of ischemic heart disease. Hypertension. 2000;35:898-903.
- 76. Menasche P, et al. Myoblast transplantation for heart failure. Lancet. 2001;357:279-280.
- 77. Le Blanc K and Ringdén O. Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2005;11:321-334.
- 78. Nichols WW and O'Rourke MF. Aging, high blood pressure and disease in humans. In: Arnold E., editor. McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles. 3rd ed. London/Melbourne/Auckland: Lea and Febigerpp: 1990;398-420.
- 79. Hansson L, et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. Lancet. 1998;351:1755-1762.
- 80. Subudhi BB, et al. Updates in Drug Development Strategies against Peptic ulcer. J Gastrointest Dig Syst. 2016;6:398.
- 81. Shimizu T and Nakagawa K. Novel Drug Development of the Next-Generation T790M Mutant Specific Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors for the Treatment of Advanced Non-Small Cell Lung Cancer. Biochem Anal Biochem. 2016;5:258.
- 82. Orlic D, et al. Bone marrow cells regenerate infarcted myocardium. Nature. 2001;410:701-705.
- 83. Petrenko AY, et al. Stem cells. Properties and clinical perspectives. Luhansk, Ukraine: Press Express. 2001;224-239.
- 84. Pittenger MF, et al. Multilineage potential of adult human mesenchimal stem cells. Science. 1999; 284:143-147.

- 85. Potapov IV, Effect of embryonic cardiomyocytes and mesenchymal cell transplantation on contractile function of the heart in experimental myocardial infarction. Bull TransplantolArtif Organ. 2002;3:88-89.
- 86. Taupin P. Stem cells and regenerative medicine. In: Pharmacology and therapy. Vol. III. New York: Nova Science Publishers (2008) 135.
- 87. Tomita S, et al. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation. 1999;100:247-256.
- 88. Wang JS, et al. Marrow stromal cells for cellular cardiomyoplasty: Feasibility and potential clinical advantages. J ThoracCardiovascSurg. 2000;120:999-1006.
- Klahr S, et al. The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. N Engl J Med. 1994;330:877-884.
- 90. Turnbull F, et al. Effects of different blood pressure-lowering regimens on major cardiovascular events in individuals with and without diabetes mellitus: results of prospectively designed overviews of randomized trials. Arch Intern Med. 2005;165:1410-1419.
- 91. Makino S, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest. 1999;103:697-705.
- 92. Asger A, et al. Effects of phosphodiesterase-5 inhibition by sildenafil in the pressure overloaded right heart. Eur J Heart Fail. 2008;10:1153-1157.
- 93. Ohno N, et al. Cell transplantation in non-ischemic dilated cardiomyopathy. A novel biological approach for ventricular restoration. Jpn J ThoracCardiovascSurg. 2002;50:457-460.
- 94. Stears AJ, et al. A double-blind, placebo-controlled, crossover trial comparing the effects of amiloride and hydrochlorothiazide on glucose tolerance in patients with essential hypertension. Hypertension. 2012;59:934-942.
- 95. Sakakibara Y, et al. Combined procedure of surgical repair and cell transplantation for left ventricular aneurysm: An experimental study. Circulation. 2002;106:193-197.
- 96. Strauer BE, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Nat Med. 2001;7:430-436.
- 97. Wang JS, et al. The coronary delivery of marrow stromal cells for myocardial regeneration: Pathophysiologic and therapeutic implications. J ThoracCardiovascSurg. 2001;122:699-705.
- 98. Tomita S, et al. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. J ThoracCardiovascSurg. 2002;123:1132-1140.
- 99. Boutouyrie P, et al. Amlodipine-valsartan combination decreases central systolic blood pressure more effectively than the amlodipine-atenolol combination: the EXPLOR study. Hypertension. 2010;55:1314-1322.
- 100. Gardner DK and Lane M. Amino acids and ammonium regulate mouse embryo development in culture. Biol Reprod. 1993;48:377-385.
- Lane M and Gardner DK. Ammonium induces aberrant blastocyst differentiation, metabolism, pH regulation, gene expression and subsequently alters fetal development in the mouse. Biol Reprod. 2003;69:1109-1117.
- 102. Vassiliev I, et al. Development of culture conditions for the isolation of pluripotent porcine embryonal outgrowths from in vitro produced and in vivo derived embryos. J Reprod Dev. 2010;56:546-551.
- 103. Wolff S. Aspects of the adaptive response to very low doses of radiation and other agents. Mutat Res. 1996;358:135–142.
- 104. Tang FR and Loke WK. Molecular mechanisms of low dose ionizing radiation-induced hormesis, adaptive responses, radio resistance, bystander effects, and genomic instability. Int J Radiat Biol. 2015;91:13–27.
- 105. Jong PA, et al. Estimation of cancer mortality associated with repetitive computed tomography scanning. Am J Respir Crit Care Med. 2006;173:199–203.
- 106. Najafi M, et al. The mechanisms of radiation-induced bystander effect. J Biomed Phys Eng. 2014;4:163–172.
- 107. Moskalev AA, et al. Radiation hormesis and radioadaptive response in Drosophila melanogaster flies with different genetic backgrounds: the role of cellular stress-resistance mechanisms. Bio gerontology. 2011;12:253–263.

- 108. Ahmed M and Rahman N. ATM and breast cancer susceptibility. Oncogene. 2006;25:5906-5911.
- 109. Wazer DE, et al. Loss of p53 protein during radiation transformation of primary human mammary epithelial cells. Mol Cellb Biol. 1994;14:2468–2478.
- 110. Worgul BV, et al. Atm heterozygous mice are more sensitive to radiation-induced cataracts than are their wild-type counterparts. Proc Natl Acad Sci U.S.A. 2002;99:9836–9839.
- 111. Kitahara CM, et al. A new era of low-dose radiation epidemiology. Curr Environ Health Rep. 2015; 2: 236-249.
- 112. Van Blitterswijk CA, et al. The biocompatibility of hydroxyapatite ceramic: a study of retrieved human middle ear plants. J Biomed Mater Res. 1990;24:433-453.
- 113. Suchanek W and Yoshimura M. Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. J Mater Res. 1998;13:94-117.
- 114. Abidi S and Murtaza Q. Synthesis and characterization of nano-hydroxyapatite powder usng wet chemical precipitation method. J Mater Sci Technol. 2014;30:307-31.
- 115. Sugiyama N, et al. Fabrication of Artificial Bone Materials using Bioceramics and Polymers having phosphoric acid groups. Arch BioCeramics Res. 2006;6:315-318.
- 116. Chu K, et al. Preparation and Characterization of Nano-Hydroxyapatite/Poly(ε-caprolactone)-Poly(ethylene glycol)-Poly(ε-caprolactone) Composite Fibers for Tissue Engineering. J Phys Chem. 2010;114: 18372-18378.
- 117. Takeoka Y, et al. IN-situ preparation of poly (L-lactic acid-co-glycolic acid)/hydroxyapatite composites as artificial bone materials. Polymer J. 2015;47:164-170.
- 118. Albertsson AC and Varma KI. Recent Developments in Ring Opening Polymerization of Lactones for Biomedical Applications. Biomacromolecules. 2003;4:1466-1686.
- 119. Yousefzadeh DK, et al. Internal barium shielding to minimize fetal irradiation in spiral chest CT: A phantom simulation experiment. Radiol. 2006;239.
- 120. Hopper KD, et al. Radioprotection to the eye during CT scanning. AJNR Am J Neuroradiol. 2001;22: 1194-1198.
- 121. Mahdavi M and Mozdarani H. Protective effects of famotidine and vitamin C against radiation induced cellular damage in mouse spermatogenesis process. Int J Radiat Res. 2011;8:223–230.
- 122. Linet MS, et al. Cancer risks associated with external radiation from diagnostic imaging procedures. CA Cancer J Clin. 2012; 62: 75–100.
- 123. Chang HK, et al. Clinical characteristics and management of benign transient non-organic ileus of neonates: A single-center experience. Yonse i Med J. 2014;55:157-161.
- 124. Yamauchi K, et al. Benign transient non-organic ileus of neonates. Eur J Pediatr Surg. 2002;12:168-174.
- 125. Edwards DK. Size of gas-filled bowel loops in infants. AJR Am J Roentgenol. 1980;135:331-334.
- 126. Hussain SZ and Di Lorenzo C. Motility disorders: Diagnosis and treatment for the pediatric patient. Pediatr Clin North Am. 2002;49:27-51.
- 127. Lacy BE, et al. Pathophysiology, evaluation and treatment of bloating: Hope, hype, or hot air? Gastroenterol Hepatol. 2011;7:729A-Â739.