Stem Cell Isolation Research Literatures

Ma Hongbao [1], Margaret Young [2], Yang Yan [1]

[1] Brookdale Hospital, Brooklyn, New York 11212, USA; [2] Cambridge, MA 02138, USA
ma8080@gmail.com

Abstract: The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the stem cell isolation related studies.
[Ma H, Young M, Yang Y. **Stem Cell Isolation Research Literatures**. Stem Cell. 2015;6(2):95-110] (ISSN 1545-4570). http://www.sciencepub.net/stem. 11

Key words: stem cell; isolation; life; research; literature

1. Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

The following introduces recent reports as references in the related studies.

Ahmadbeigi, N., M. Soleimani, et al. "Isolation, characterization, and transplantation of bone marrow-derived cell components with hematopoietic stem cell niche properties." Stem Cells Dev. 2013 Dec 1;22(23):3052-61. doi: 10.1089/scd.2013.0005. Epub 2013 Sep 14.

Although the unique role of hematopoietic stem cell (HSC) niche in hematopoiesis has long been recognized, unsuccessful isolation of intact niche units limited their in vitro study, manipulation, and therapeutic application. Here, we isolated cell complexes based on size fractionation from mouse bone marrow (BM), characterized the derived cells, and transplanted them to irradiated mice. These cell complexes were the origin of both BM mesenchymal stem cells and various hematopoietic lineages when kept in appropriate culture conditions. They also had the potential of recruiting circulating HSC. Intraperitoneal transplantation of these structures into irradiated mice not only showed long-lasting hematopoietic multilineage reconstitution, but also could recover the stromal cells of BM. In conclusion, this study for the first time provides evidences on the feasibility and efficacy of transplantation of HSC in association with their native specialized microenvironment. As the molecular cross-talk between HSC and niche is crucial for their proper function, the proposed method could be considered as a novel hematopoietic transplantation strategy.

Azari, H. "Isolation and enrichment of defined neural cell populations from heterogeneous neural stem cell progeny." Methods Mol Biol. 2013;1059:95-106. doi: 10.1007/978-1-62703-574-3_9.

The renewable source of neural stem cells (NSCs) with multi-lineage differentiation capability towards neurons, astrocytes, and oligodendrocytes represent an ideal supply for cell therapy of central nervous system (CNS) diseases. In spite of this, the clinical use of NSCs is hampered by heterogeneity, poor neuronal cell yield, predominant astrocytic differentiation of NSC progeny and possible uncontrolled proliferation, and tumor formation upon transplantation. The ability to generate highly enriched and defined neural cell populations from the renewable source of NSCs might overcome many of these impediments and pave the way towards their successful clinical applications. Here, we describe a simple method for NSC differentiation and subsequent purification of neuronal progenitor cells, taking advantage of size and granularity differences between neuronal cells and other NSC progeny. This highly enriched neuronal cell population provides an invaluable source of cells for both in vitro and in vivo studies.

Bellavia, M., R. Altomare, et al. "Towards an ideal source of mesenchymal stem cell isolation for possible therapeutic application in regenerative medicine." Biomed Pap Med Fac Univ Palacky Olomouc Czech

Repub. 2014 Sep;158(3):356-60. doi: 10.5507/bp.2013.051. Epub 2013 Jul 29.

BACKGROUND: The possibility of obtaining mesenchymal stem cells (MSCs) from fetal tissue such as amniotic fluid, chorionic villi and placenta is well-known and a comparison between MSCs originating in different sources such as fetal tissue and those from bone marrow in terms of yield and function is a topical issue. The mesenchymal stem cells isolated from bone marrow are well-characterized. Unfortunately the low quantitative yield during isolation is a major problem. For this reason, other tissue sources for MSCs are of paramount importance. CONCLUSION: In this review, starting from a description of the molecular and cellular biology of MSCs, we describe alternative sources of isolation other than bone marrow. Finally, we describe the potential therapeutic application of these cells.

Branch, M. J., W. Y. Yu, et al. "Isolation of adult stem cell populations from the human cornea." Methods Mol Biol. 2015;1235:165-77. doi: 10.1007/978-1-4939-1785-3_14.

Corneal blindness is a leading cause of vision loss globally. From a tissue engineering perspective, the cornea represents specific challenges in respect to isolating, stably expanding, banking, and effectively manipulating the various cell types required for effective corneal regeneration. The current research trend in this area focuses on a combined stem cell component with a biological or synthetic carrier or engineering scaffold. Corneal derived stem cells play an important role in such strategies as they represent an available supply of cells with specific abilities to further generate corneal cells in the long term. This chapter describes the isolation protocols of the epithelial stromal and endothelial stem cell populations.

Carattoli, A., D. Fortini, et al. "Isolation of NDM-1-producing Pseudomonas aeruginosa sequence type ST235 from a stem cell transplant patient in Italy, May 2013." Euro Surveill. 2013 Nov 14;18(46). pii: 20633.

We describe the first isolation of an NDM-1-producing Pseudomonas aeruginosa in Italy. In May 2013, a patient with acute lymphoblastic leukaemia and history of prior hospitalisation in Belgrad, Serbia, underwent stem cell transplantation at a tertiary care hospital in Rome, Italy. After transplantion, sepsis by NDM-1-producing P. aeruginosa occurred, leading to septic shock and fatal outcome.

Chan, T. M., H. J. Harn, et al. "Improved human mesenchymal stem cell isolation." Cell Transplant. 2014;23(4-5):399-406. doi: 10.3727/096368914X678292.

Human mesenchymal stem cells (hMSCs) are currently available for a range of applications and benefits and have become a good material for regenerative medicine, tissue engineering, and disease therapy. Before ex vivo expansion, isolation and characterization of primary hMSCs from peripheral tissues are key steps for obtaining adequate materials for clinical application. The proportion of peripheral stem cells is very low in surrounding tissues and organs; thus the recovery ratio will be a limiting factor. In this review, we summarized current common methods used to isolate peripheral stem cells, as well as the new insights revealed to improve the quantity of stem cells and their stemness. These strategies offer alternative ways to acquire hMSCs in a convenient and/or effective manner, which is important for clinical treatments. Improved isolation and mass amplification of the hMSCs while ensuring their stemness and quantity will be an important step for clinical use. Enlarged suitable hMSCs are more clinically applicable for therapeutic transplants and may help people live longer and better.

Chatzistamatiou, T. K., A. C. Papassavas, et al. "Optimizing isolation culture and freezing methods to preserve Wharton's jelly's mesenchymal stem cell (MSC) properties: an MSC banking protocol validation for the Hellenic Cord Blood Bank." Transfusion. 2014 Dec;54(12):3108-20. doi: 10.1111/trf.12743. Epub 2014 Jun 4.

BACKGROUND: Mesenchymal stem or stromal cells (MSCs) are a heterogeneous population that can be isolated from many tissues including umbilical cord Wharton's jelly (UC-WJ). Although initially limited in studies such as a hematopoietic stem cell transplantation adjuvant, an increasing number of clinical trials consider MSCs as a potential anti-inflammatory or a regenerative medicine agent. It has been proposed that creating a repository of MSCs would increase their availability for clinical applications. The aim of this study was to assess the optimal isolation and cryopreservation procedures to facilitate WJ MSC banking. STUDY DESIGN AND METHODS: Cells were isolated from UC-WJ using enzymatic digestion or plastic adhesion methods. Their isolation efficacy, growth kinetics, immunophenotype, and differentiation potential were studied, as well as the effects of freezing. Flow cytometry for common MSC markers was performed on all cases and differentiation was shown with histocytochemical staining. Finally, the isolation efficacy on cryopreserved WJ tissue fragments was tested. RESULTS: MSC isolation was successful using both isolation methods on fresh UC-WJ tissue. However, UC-WJ MSC isolation from frozen tissue fragments was impossible. Flow cytometry analysis revealed that

only MSC markers were expressed on the surface of the isolated cells while differentiation assays showed that they were capable of trilinear differentiation. All the above characteristics were also preserved in isolated UC-WJ MSCs over the cryopreservation study period. CONCLUSION: These data showed that viable MSCs can only be isolated from fresh UC-WJ tissue, setting the foundation for clinical-grade banking.

de Marval, P. L., S. H. Kim, et al. "Isolation and characterization of a stem cell side-population from mouse hair follicles." Methods Mol Biol. 2014;1195:259-68. doi: 10.1007/7651_2013_61.

The mouse skin is composed of at least three differentiating epithelial compartments: the epidermis, the hair follicle, and the associated glands such as the sebaceous glands. Proliferation of these epithelial cells takes place in the keratinocytes' layer or basal cell layer; in the periphery of the sebaceous gland (the basal layer of the gland) and in specific cell compartments around the hair follicle. In mouse skin, an epithelial stem cell population is thought to localize to the bulge region of the hair follicle, a segment that does not undergo regression during the hair cycle. In addition, several other putative stem cells and/or progenitors have been identified in different regions of the hair follicle. Using the Hoeschst exclusion technique, originally described in the hematopoietic system, it has been possible to isolate a mouse keratinocyte cell population with characteristics of stem cells (side-population, SP). One of the main features of these SP is their ability to efflux antimitotic drugs as well as some specific dyes. This characteristic allows for SP cells to be isolated based upon their capacity to efflux the dye Hoechst 33342, through a mechanism driven by a membrane transporter, the breast cancer resistance protein (BCRP1/ABCG2). In this chapter, we described the isolation of SP stem cells from adult mouse hair follicles utilizing the Hoeschst exclusion technique by flow cytometry analysis.

Devitt, S. M., C. M. Carter, et al. "Successful isolation of viable adipose-derived stem cells from human adipose tissue subject to long-term cryopreservation: positive implications for adult stem cell-based therapeutics in patients of advanced age." Stem Cells Int. 2015;2015:146421. doi: 10.1155/2015/146421. Epub 2015 Apr 5.

We examined cell isolation, viability, and growth in adipose-derived stem cells harvested from whole adipose tissue subject to different cryopreservation lengths (2-1159 days) from patients of varying ages (26-62 years). Subcutaneous abdominal adipose tissue was excised during abdominoplasties and was cryopreserved. The viability and number of adipose-derived stem cells isolated were measured after initial isolation and after 9, 18, and 28 days of growth. Data were analyzed with respect to cryopreservation duration and patient age. Significantly more viable cells were initially isolated from tissue cryopreserved <1 year than from tissue cryopreserved >2 years, irrespective of patient age. However, this difference did not persist with continued growth and there were no significant differences in cell viability or growth at subsequent time points with respect to cryopreservation duration or patient age. Mesenchymal stem cell markers were maintained in all cohorts tested throughout the duration of the study. Consequently, longer cryopreservation negatively impacts initial live adipose-derived stem cell isolation; however, this effect is neutralized with continued cell growth. Patient age does not significantly impact stem cell isolation, viability, or growth. Cryopreservation of adipose tissue is an effective long-term banking method for isolation of adipose-derived stem cells in patients of varying ages.

Doi, D., B. Samata, et al. "Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation." Stem Cell Reports. 2014 Mar 6;2(3):337-50. doi: 10.1016/j.stemcr.2014.01.013. eCollection 2014 Mar 11.

Human induced pluripotent stem cells (iPSCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy for Parkinson's disease. However, iPSC-derived donor cells inevitably contain tumorigenic or inappropriate cells. Here, we show that human iPSC-derived DA progenitor cells can be efficiently isolated by cell sorting using a floor plate marker, CORIN. We induced DA neurons using scalable culture conditions on human laminin fragment, and the sorted CORIN(+) cells expressed the midbrain DA progenitor markers, FOXA2 and LMX1A. When transplanted into 6-OHDA-lesioned rats, the CORIN(+) cells survived and differentiated into midbrain DA neurons in vivo, resulting in significant improvement of the motor behavior, without tumor formation. In particular, the CORIN(+) cells in a NURR1(+) cell-dominant stage exhibited the best survival and function as DA neurons. Our method is a favorable strategy in terms of scalability, safety, and efficiency and may be advantageous for clinical application.

Edri, R., Y. Yaffe, et al. "Analysing human neural stem cell ontogeny by consecutive isolation of Notch active neural progenitors." Nat Commun. 2015 Mar 23;6:6500. doi: 10.1038/ncomms7500.

Decoding heterogeneity of pluripotent stem cell (PSC)-derived neural progeny is fundamental for

revealing the origin of diverse progenitors, for defining their lineages, and for identifying fate determinants driving transition through distinct potencies. Here we have prospectively isolated consecutively appearing PSC-derived primary progenitors based on their Notch activation state. We first isolate early neuroepithelial cells and show their broad Notch-dependent developmental and proliferative potential. Neuroepithelial cells further yield successive Notch-dependent functional primary progenitors, from early and midneurogenic radial glia and their derived basal progenitors, to gliogenic radial glia and adult-like neural progenitors, together recapitulating hallmarks of neural stem cell (NSC) ontogeny. Gene expression profiling reveals dynamic stage-specific transcriptional patterns that may link development of distinct progenitor identities through Notch activation. Our observations provide a platform for characterization and manipulation of distinct progenitor cell types amenable for developing streamlined neural lineage specification paradigms for modelling development in health and disease.

Eubanks, E. J., S. A. Tarle, et al. "Tooth storage, dental pulp stem cell isolation, and clinical scale expansion without animal serum." J Endod. 2014 May;40(5):652-7. doi: 10.1016/j.joen.2014.01.005. Epub 2014 Mar 6.

INTRODUCTION: Dental pulp stem cells (DPSCs) have therapeutic potential for dentin and dental pulp regeneration. For regenerative approaches to gain clinical acceptance, protocols are needed to determine feasible ways to store teeth, isolate DPSCs, and expand them to clinical scale numbers. METHODS: In this study, 32 third molars were obtained from patients and immediately placed in saline or tissue culture medium followed by overnight storage at 4 degrees C or immediate isolation of DPSCs. Upon isolation, cells were expanded in medium containing either fetal bovine serum (FBS) or human serum (HS). Cell proliferation (population doubling time [PDT]), cell surface marker expression, and multipotency were compared between DPSCs in FBS and DPSCs in HS. RESULTS: The time frame of storage and storage medium did not affect the ability to isolate DPSCs. However, using HS instead of FBS in the initial isolation of DPSCs significantly decreased ($P < .01$) the isolation success rate from 89% (FBS) to 23% (HS). Yet, incorporating fibronectin in the DPSC initial isolation (using HS) significantly ($P < .01$) increased the isolation success rate to 83%. Interestingly, it was found that the proliferation rate was significantly ($P < .05$) higher for DPSCs in HS (PDT = 1.59 +/- 0.46) than that for DPSCs in FBS (PDT = 2.84 +/- 2.5). Finally, there was no difference in the expression of CD73, CD90, CD105, or multipotency (as measured by osteogenic, adipogenic, and chondrogenic differentiation) between DPSCs in FBS and DPSCs in HS. CONCLUSIONS: These findings show a clinically feasible method of storing third molars for the isolation of DPSCs. Additionally, DPSCs can be isolated and expanded to clinical scale numbers in media devoid of FBS and still maintain their phenotypic properties.

Ferro, F., R. Spelat, et al. "Dental pulp stem cell (DPSC) isolation, characterization, and differentiation." Methods Mol Biol. 2014;1210:91-115. doi: 10.1007/978-1-4939-1435-7_8.

Dental pulp stem cells (DPSC) have been proposed as an alternative to pluripotent stem cells to study multilineage differentiation in vitro and for therapeutic application. Standard culture media for isolation and expansion of stem cells includes animal sera or animal-derived matrix components (e.g., Matrigel((R))). However, animal-derived reagents raise significant concerns with respect to the translational ability of these cells due to the possibility of infection and/or severe immune reaction. For these reasons clinical grade substitutes to animal components are needed in order for stem cells to reach their full therapeutic potential. In this chapter we detail a method for isolation and proliferation of DPSC in a chemically defined medium containing a low percentage of human serum. We demonstrate that in this defined culture medium a 1.25 % human serum component sufficiently replaces fetal bovine serum. This method allows for isolation of a morphologically and phenotypically uniform population of DPSCs from dental pulp tissue. DPSCs represent a rapidly proliferating cell population that readily differentiates into the osteoblastic, neuronal, myocytic, and hepatocytic lineages. This multilineage capacity of these DPSCs suggests that they may have a more broad therapeutic application than lineage-restricted adult stem cell populations such as mesenchymal stem cells. Further the culture protocol presented here makes these cells more amenable to human application than current expansion techniques for other pluripotent stem cells (embryonic stem cell lines or induced pluripotent stem cells).

Fisher, C., T. L. Grahovac, et al. "Comparison of harvest and processing techniques for fat grafting and adipose stem cell isolation." Plast Reconstr Surg. 2013 Aug;132(2):351-61. doi: 10.1097/PRS.0b013e3182958796.

BACKGROUND: Variability in harvest and processing technique may impact the success of fat grafting. This study compared properties of fat grafts produced by differing methods and assessed volume retention of the grafted tissue in a nude mouse model.

METHODS: In phase I, fat was harvested by either suction-assisted lipoaspiration or ultrasound-assisted lipoaspiration and then filtered using two different pore sizes. Graft material was analyzed for average parcel size; relative oil, fat, and aqueous fractions; and stromal vascular fraction yield. Filtrands and filtrates were injected into athymic nude mice. In phase II, lipoaspirate harvested by suction-assisted lipoaspiration only was processed by centrifugation, cotton gauze rolling, or filtration, and then studied in a similar manner. RESULTS: Fat harvested by ultrasound- and suction-assisted lipoaspiration had comparable stromal vascular fraction counts and graft retention in vivo. Ultrasound-assisted lipoaspiration released only slightly more oil than suction-assisted lipoaspiration; filtering with either 500- or 800-microm pore size effectively removed fluid and oil. Centrifugation, cotton-gauze rolling, and filtration also effectively removed fluid and oil. In vivo graft retention and stromal vascular fraction yield was highest with the cotton gauze method. Histologic analysis of all explants showed intact adipose tissue. CONCLUSIONS: Ultrasound- and suction-assisted lipoaspiration yielded similar retention of fat grafts in a xenograft model. Processing with cotton gauze rolling may be best suited for grafting cosmetically sensitive areas of the body in which optimal retention is critical and lower total graft volumes are needed. Filtration and centrifugation both effectively removed fluid fractions and resulted in comparable graft retention, and are more feasible when larger volumes are required.

Gao, Y., X. Li, et al. "Isolation of a pluripotent neural stem cell from the embryonic bovine brain." Int J Mol Sci. 2015 Mar 13;16(3):5990-9. doi: 10.3390/ijms16035990.

We recently isolated stem cells derived from the brain of a bovine fetus, utilizing a particular mechanical separation method. After improving our experimental conditions, we obtained neural stem cells using an optimized culture medium system. The cells were expanded, established in continuous cell culture and used for immunofluorescence cytochemistry. RT-PCR showed that embryonic neural stem cells (NSCs) not only expresses the protein Sox2, Nestin but also Pax6, Musashi proteins and were differentiated into the three classical neuronal phenotypes (neurons, astrocytes, and oligodendrocytes).

Hashem, S. I. and W. C. Claycomb "Genetic isolation of stem cell-derived pacemaker-nodal cardiac myocytes." Mol Cell Biochem. 2013 Nov;383(1-2):161-71. doi: 10.1007/s11010-013-1764-x. Epub 2013 Jul 23.

Dysfunction of the cardiac pacemaker tissues due to genetic defects, acquired diseases, or aging results in arrhythmias. When arrhythmias occur, artificial pacemaker implants are used for treatment. However, the numerous limitations of electronic implants have prompted studies of biological pacemakers that can integrate into the myocardium providing a permanent cure. Embryonic stem (ES) cells cultured as three-dimensional (3D) spheroid aggregates termed embryoid bodies possess the ability to generate all cardiac myocyte subtypes. Here, we report the use of a SHOX2 promoter and a Cx30.2 enhancer to genetically identify and isolate ES cell-derived sinoatrial node (SAN) and atrioventricular node (AVN) cells, respectively. The ES cell-derived Shox2 and Cx30.2 cardiac myocytes exhibit a spider cell morphology and high intracellular calcium loading characteristic of pacemaker-nodal myocytes. These cells express abundant levels of pacemaker genes such as endogenous HCN4, Cx45, Cx30.2, Tbx2, and Tbx3. These cells were passaged, frozen, and thawed multiple times while maintaining their pacemaker-nodal phenotype. When cultured as 3D aggregates in an attempt to create a critical mass that simulates in vivo architecture, these cell lines exhibited an increase in the expression level of key regulators of cardiovascular development, such as GATA4 and GATA6 transcription factors. In addition, the aggregate culture system resulted in an increase in the expression level of several ion channels that play a major role in the spontaneous diastolic depolarization characteristic of pacemaker cells. We have isolated pure populations of SAN and AVN cells that will be useful tools for generating biological pacemakers.

Heidari-Keshel, S., M. Rezaei-Tavirani, et al. "Tissue-specific somatic stem-cell isolation and characterization from human endometriosis. Key roles in the initiation of endometrial proliferative disorders." Minerva Med. 2015 Apr;106(2):95-108. Epub 2014 Dec 17.

AIM: The endometrial-proliferation related diseases leads to endometrial hyperplasia, i.e., endometriosis. Endometrial progenitor and stem cells play key roles in the beginning of endometrial proliferative disorders. The purpose of this study was the isolation of stem cells in the endometriosis lesion as well as the evaluation and comparison of the stemness-related target genes in endometriosis endometrial stem cells (EESCs), normal endometrial stem cell (ESCs), endometrial lesions stem cell (ELSCs) and bone marrow mesenchymal stem cells (MSCs). METHODS: EESCs, ESCs, ELSCs and MSCs were isolated. Flowcytometry and real-time PCR were utilized to detect the cell surface marker and expression pattern of 16 stemness genes. The

proliferation of all stem cells was observed by MTT assay. The differentiation potential was evaluated by alizarin red, oil red O and RT-PCR method. The karyotyping was performed on EESCs and ELSCs at passage 20. RESULTS: The unique patterns of gene expression were detected although EESCs, ESCs, ELSCs and MSCs have a background expression of stemness-related genes. Spindle-like morphology, normal karyotype, adipogenic and osteogenic potential, significantly expression of Oct4, SALL4, DPPA2, Sox2, Sox17 and also specific surface markers such as CD44, CD105, CD90, CD73 and CD146 in EESCs and ELSCs was observed. CONCLUSION: According to our data, stem cells in endometriosis endometrial and endometriosis are such a informative tools to study of pathogenesis of gynecological diseases. Furthermore, endometrial stem/progenitor cells which easily obtain from tissue may be valuable targets for early diagnosis of endometrial disorders in the future.

Hilkens, P., P. Gervois, et al. "Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells." Cell Tissue Res. 2013 Jul;353(1):65-78. doi: 10.1007/s00441-013-1630-x. Epub 2013 May 29.

Dental pulp stem cells (DPSCs) are an attractive alternative mesenchymal stem cell (MSC) source because of their isolation simplicity compared with the more invasive methods associated with harvesting other MSC sources. However, the isolation method to be favored for obtaining DPSC cultures remains under discussion. This study compares the stem cell properties and multilineage differentiation potential of DPSCs obtained by the two most widely adapted isolation procedures. DPSCs were isolated either by enzymatic digestion of the pulp tissue (DPSC-EZ) or by the explant method (DPSC-OG), while keeping the culture media constant throughout all experiments and in both isolation methods. Assessment of the stem cell properties of DPSC-EZ and DPSC-OG showed no significant differences between the two groups with regard to proliferation rate and colony formation. Phenotype analysis indicated that DPSC-EZ and DPSC-OG were positive for CD29, CD44, CD90, CD105, CD117 and CD146 expression without any significant differences. The multilineage differentiation potential of both stem cell types was confirmed by using standard immuno(histo/cyto)chemical staining together with an in-depth ultrastructural analysis by means of transmission electron microscopy. Our results indicate that both DPSC-EZ and DPSC-OG could be successfully differentiated into adipogenic, chrondrogenic and osteogenic cell types, although the adipogenic differentiation of both stem cell populations was incomplete. The data suggest that both the enzymatic digestion and outgrowth method can be applied to obtain a suitable autologous DPSC resource for tissue replacement therapies of both bone and cartilage.

Holmqvist, S., M. Brouwer, et al. "Generation of human pluripotent stem cell reporter lines for the isolation of and reporting on astrocytes generated from ventral midbrain and ventral spinal cord neural progenitors." Stem Cell Res. 2015 Jun 6;15(1):203-220. doi: 10.1016/j.scr.2015.05.014.

Astrocytes play a critical role during the development and the maintenance of the CNS in health and disease. Yet, their lack of accessibility from fetuses and from the brain of diseased patients has hindered our understanding of their full implication in developmental and pathogenic processes. Human pluripotent stem cells (PSCs) are an alternative source to obtain large quantities of astrocytes in vitro, for mechanistic studies of development and disease. However, these studies often require highly pure populations of astrocytes, which are not always achieved, depending on the PSC lines and protocols used. Here, we describe the generation and characterization of human PSC reporter lines expressing TagRFP driven by the ABC1D region of the human GFAP promoter, as new cellular model for generating homogenous population of astrocytes generated from CNS regionally defined PSC-derived neural progenitors. GFAABC1D::TagRFP-expressing astrocytes can be purified by fluorescent-activated cell sorting and maintain a bright expression for several additional weeks. These express canonical astrocyte markers NF1A, S100beta, CX43, GLAST, GS and CD44. These new cellular models, from which highly pure populations of fluorescence-expressing astrocytes can be obtained, provide a new platform for studies where pure or fluorescently labeled astrocyte populations are necessary, for example to assess pro-inflammatory cytokine and chemokine release in response to specific treatment, and uptake and degradation of fluorescently labeled pathogenic proteins, as reported in this study.

Hu, P., Y. Pu, et al. "Isolation, in vitro culture and identification of a new type of mesenchymal stem cell derived from fetal bovine lung tissues." Mol Med Rep. 2015 May 27. doi: 10.3892/mmr.2015.3854.

Lungderived mesenchymal stem cells (LMSCs) are considered to be important in lung tissue repair and regenerative processes. However, the biological characteristics and differentiation potential of LMSCs remain to be elucidated. In the present study, fetal lungderived mesenchymal stem cells (FLMSCs) were isolated from fetal bovine lung tissues

by collagenase digestion. The in vitro culture conditions were optimized and stabilized and the selfrenewal ability and differentiation potential were evaluated. The results demonstrated that the FLMSCs were morphologically consistent with fibroblasts, were able to be cultured and passaged for at least 33 passages and the cell morphology and proliferative ability were stable during the first 10 passages. In addition, FLMSCs were found to express CD29, CD44, CD73 and CD166, however, they did not express hematopoietic cell specific markers, including CD34, CD45 and BOLADRalpha. The growth kinetics of FLMSCs consisted of a lag phase, a logarithmic phase and a plateau phase, and as the passages increased, the proliferative ability of cells gradually decreased. The majority of FLMSCs were in G0/G1 phase. Following osteogenic induction, FLMSCs were positive for the expression of osteopontin and collagen type I alpha2. Following neurogenic differentiation, the cells were morphologically consistent with neuronal cells and positive for microtubuleassociated protein 2 and nestin expression. It was concluded that the isolated FLMSCs exhibited typical characteristics of mesenchymal stem cells and that the culture conditions were suitable for their proliferation and the maintenance of stemness. The present study illustrated the potential application of lung tissue as an adult stem cell source for regenerative therapies.

Iftimia-Mander, A., P. Hourd, et al. "Mesenchymal stem cell isolation from human umbilical cord tissue: understanding and minimizing variability in cell yield for process optimization." Biopreserv Biobank. 2013 Oct;11(5):291-8. doi: 10.1089/bio.2013.0027.

Human tissue banks are a potential source of cellular material for the nascent cell-based therapy industry; umbilical cord (UC) tissue is increasingly privately banked in such facilities as a source of mesenchymal stem cells for future therapeutic use. However, early handling of UC tissue is relatively uncontrolled due to the clinical demands of the birth environment and subsequent transport logistics. It is therefore necessary to develop extraction methods that are robust to real-world operating conditions, rather than idealized operation. Cell yield, growth, and differentiation potential of UC tissue extracted cells was analyzed from tissue processed by explant and enzymatic digestion. Variability of cell yield extracted with the digestion method was significantly greater than with the explant method. This was primarily due to location within the cord tissue (higher yield from placental end) and time delay before tissue processing (substantially reduced yield with time). In contrast, extraction of cells by explant culture was more robust to these processing variables. All cells isolated showed comparable proliferative and differentiation functionality. In conclusion, given the challenge of tightly controlled operating conditions associated with isolation and shipping of UC tissue to banking facilities, explant extraction of cells offers a more robust and lower-variability extraction method than enzymatic digestion.

Kay, A. G., T. P. Dale, et al. "BMP2 repression and optimized culture conditions promote human bone marrow-derived mesenchymal stem cell isolation." Regen Med. 2015;10(2):109-25. doi: 10.2217/rme.14.67.

AIM: Human mesenchymal stem cells (hMSC) are multipotent progenitor cells. We propose the optimization of hMSC isolation and recovery using the application of a controlled hypoxic environment. MATERIALS & METHODS: We evaluated oxygen, glucose and serum in the recovery of hMSC from bone marrow (BMhMSC). Colony forming units-fibroblastic, cell numbers, tri-lineage differentiation, immunofluorescence and microarray were used to confirm and characterize BMhMSC. RESULTS: In an optimized (2% O(2), 4.5 g/l glucose and 5% serum) environment both colony forming units-fibroblastic ($p = 0.01$) and cell numbers ($p = 0.0001$) were enhanced over standard conditions. Transcriptional analysis identified differential expression of bone morphogenetic protein 2 (BMP2) and, putatively, chemokine (C-X-C motif) receptor 2 (CXCR2) signaling pathways. CONCLUSION: We have detailed a potential milestone in the process of refinement of the BMhMSC isolation process.

Khatami, S. M., S. Zahri, et al. "Stem Cell Isolation from Human Wharton's Jelly: A Study of Their Differentiation Ability into Lens Fiber Cells." Cell J. 2014 Winter;15(4):364-71. Epub 2013 Nov 20.

Recently, the use of stem cells has expanded into numerous areas including cell therapy. In this study, we investigated the differentiation capacity of human Wharton's jelly stem cells (hWJSCs) into lens fiber cells. Morphological changes and expressions of four crystallin genes (alphaA, alphaB, betaB1 and betaB3) were studied. The bovine vitreous body has been shown to induce expression of crystallin genes in hWJSCs. By using the vitreous as a lens fiber cell inducer, we showed that alphaB-, betaB1- and betaB3-crystallin genes expressed in hWJSCs.

Kong, B. H., J. H. Moon, et al. "Prognostic value of glioma cancer stem cell isolation in survival of primary glioblastoma patients." Stem Cells Int. 2014;2014:838950. doi: 10.1155/2014/838950. Epub 2014 Dec 11.

Cancer stem cells (CSCs) have been reported to be critical in the initiation, maintenance, and

progression of cancers. The expression of stem cell markers, such as podoplanin (PDPN), CD133, and nestin, may have been correlated with malignant progression. However, the effects of CSCs and stem cell markers on clinical outcomes in cancer patients remain unclear. In this study, we assessed the prognostic roles of glioma CSCs (gCSCs) isolation and stem cell markers in patients with primary glioblastoma (pGBM). A cohort of 39 patients with pGBM was separated into two groups, those positive or negative for gCSCs, and the correlation between gCSC and patient survival was evaluated. We observed significantly different cumulative survival (P = 0.045) when comparing patients positive for gCSCs patients and negative for gCSC. Among the patients positive for gCSCs, we observed no significant differences in survival between those whose gCSCs were each positive or negative for PDPN, CD133, or nestin. This study strongly supports the prognostic value of gCSCs isolation on the survival of patients with pGBM.

Kumar, S., S. Poojan, et al. "Rapid isolation of integrin rich multipotent stem cell pool and reconstruction of mouse epidermis equivalent." Am J Stem Cells. 2014 Mar 13;3(1):27-36. eCollection 2014.

We describe here epidermis reconstruction using multipotent mouse epidermal stem cells (EpSCs) enriched from keratinocyte isolates exploting exclusively the stem cell-adhesive property. This method excluded flowcytometry and was swift. Percent enrichment was measured by the uptake of Propidium iodide and Hoechst-33342 dye using flowcytometry to determine EpSCs yield. The sorted cells were characterized by analysis of stem cell markers using immunocytochemistry and immunoblotting techniques. Epidermis was reconstructed using the identified seeding density of EpSCs and the airlift tissue culture. Histology of natural vs reconstructed mammalian epidermis was also compared. Results showed a radical improvement of near 99% in the yield of integrin overexpressing EpSCs. The enriched EpSCs tested positive for biomarkers namely cytokeratin K-15 and, K-14, p63, beta-1-integrin, CD34 and could be passaged for longer durations. Adhesion sorted cells reconstructed the epidermis. The process of tissue reconstruction was faster using the adhesion sorted cells than the FACS sorted EpSCs. The product bioengineered using multipotent EpSCs was histologically similar to normal epidermis. Features like strata basalae, spinosum, granulosum, and corneum were alike real epidermis. The reconstructed epidermis displayed normal homeostasis, which can be considered an approximating actual product for investigative dermatology, toxicology, therapeutic research, regenerative medicine, and tissue engineering.

Lenihan, C., C. Rogers, et al. "The effect of isolation and culture methods on epithelial stem cell populations and their progeny-toward an improved cell expansion protocol for clinical application." Cytotherapy. 2014 Dec;16(12):1750-9. doi: 10.1016/j.jcyt.2014.06.005. Epub 2014 Oct 5.

BACKGROUND AIMS: The use of cultured epithelial keratinocytes in the treatment of burns and skin graft donor sites is well established in clinical practice. The most widely used culture method for clinical use was originally developed by Rheinwald and Green 40 years ago. This system uses irradiated mouse dermal fibroblasts as a feeder cell layer to promote keratinocyte growth, a process that is costly and labor-intensive for health care providers. The medium formulation contains several components of animal origin, which pose further safety risks for patients. Improvements and simplification in the culturing process would lead to clear advantages: improved safety through reduction of xenobiotic components and reduction in cost for health care providers by dispensing with feeder cells. METHODS: We compared the Rheinwald and Green method to culture in three commercially available, feeder-free media systems with defined/absent components of animal origin. RESULTS: During the isolation process, short incubation times in high-strength trypsin resulted in increased numbers of liberated keratinocyte stem cells compared with longer incubation times. All three commercially available media tested in this study could support the expansion of keratinocytes, with phenotypes comparable to cells expanded using the established Rheinwald and Green method. Growth rates varied, with two of the media displaying comparable growth rates, whereas the third was significantly slower. DISCUSSION: Our study demonstrates the suitability of such feeder-free media systems in clinical use. It further outlines a range of techniques to evaluate keratinocyte phenotype when assessing the suitability of cells for clinical application.

Leong, C., D. Zhai, et al. "Neural stem cell isolation from the whole mouse brain using the novel FABP7-binding fluorescent dye, CDr3." Stem Cell Res. 2013 Nov;11(3):1314-22. doi: 10.1016/j.scr.2013.09.002. Epub 2013 Sep 18.

Methods for the isolation of live neural stem cells from the brain are limited due to the lack of well-defined cell surface markers and tools to detect intracellular markers. To date most methods depend on the labeling of extracellular markers using antibodies, with intracellular markers remaining inaccessible in

live cells. Using a novel intracellular protein FABP7 (Fatty Acid Binding Protein-7) selective fluorescent chemical probe CDr3, we have successfully isolated high FABP7 expressing cells from the embryonic and adult mouse brains. These cells are capable of forming neurospheres in culture, express neural stem cell marker genes and differentiate into neurons, astrocytes and oligodendrocytes. Characterization of cells sorted with Aldefluor or antibodies against CD133 or SSEA-1 showed that the cells isolated by CDr3 exhibit a phenotype distinct from the cells sorted with conventional methods. FABP7 labeling with CDr3 represents a novel method for rapid isolation of neural stem cells based on the expression of a single intracellular marker.

Maass, K., A. Shekhar, et al. "Isolation and characterization of embryonic stem cell-derived cardiac Purkinje cells." Stem Cells. 2015 Apr;33(4):1102-12. doi: 10.1002/stem.1921.

The cardiac Purkinje fiber network is composed of highly specialized cardiomyocytes responsible for the synchronous excitation and contraction of the ventricles. Computational modeling, experimental animal studies, and intracardiac electrical recordings from patients with heritable and acquired forms of heart disease suggest that Purkinje cells (PCs) may also serve as critical triggers of life-threatening arrhythmias. Nonetheless, owing to the difficulty in isolating and studying this rare population of cells, the precise role of PC in arrhythmogenesis and the underlying molecular mechanisms responsible for their proarrhythmic behavior are not fully characterized. Conceptually, a stem cell-based model system might facilitate studies of PC-dependent arrhythmia mechanisms and serve as a platform to test novel therapeutics. Here, we describe the generation of murine embryonic stem cells (ESC) harboring pan-cardiomyocyte and PC-specific reporter genes. We demonstrate that the dual reporter gene strategy may be used to identify and isolate the rare ESC-derived PC (ESC-PC) from a mixed population of cardiogenic cells. ESC-PC display transcriptional signatures and functional properties, including action potentials, intracellular calcium cycling, and chronotropic behavior comparable to endogenous PC. Our results suggest that stem-cell derived PC are a feasible new platform for studies of developmental biology, disease pathogenesis, and screening for novel antiarrhythmic therapies.

Maria Cambuli, F., A. Rezza, et al. "Brief report: musashi1-eGFP mice, a new tool for differential isolation of the intestinal stem cell populations." Stem Cells. 2013 Oct;31(10):2273-8. doi: 10.1002/stem.1428.

The intestinal epithelium self-renews rapidly and continuously throughout life, due to the presence of crypt stem cells. Two pools of these cells have been identified in the small intestine, which differ in position ("+4" or the bottom of the crypts), expression of specific markers (Bmi1/mTert or Lgr5/Ascl2), and cell cycle characteristics. Interestingly, the RNA-binding protein Musashi1 is expressed in both populations and therefore a potential marker for both stem cell types. In order to locate, isolate, and study Musashi1-expressing cells within the intestinal epithelium, we generated transgenic mice expressing GFP fluorescent protein under the control of a 7-kb Msi1 promoter. The expression pattern of GFP in the intestinal crypts of both small and large intestines completely overlapped that of Musashi1, validating our model. By using fluorescence-activated cell sorting, cellular, and molecular analyses, we showed that GFP-positive Msi1-expressing cells are divided into two major pools corresponding to the Lgr5- and mTert-expressing stem cells. Interestingly, monitoring the cell cycle activity of the two sorted populations reveals that they are both actively cycling, although differences in cell cycle length were confirmed. Altogether, our new reporter mouse model based upon Musashi1 expression is a useful tool to isolate and study stem cells of the intestinal epithelium. Moreover, these mice uniquely enable the concomitant study of two pools of intestinal stem cells within the same animal model.

Mason, S., S. A. Tarle, et al. "Standardization and safety of alveolar bone-derived stem cell isolation." J Dent Res. 2014 Jan;93(1):55-61. doi: 10.1177/0022034513510530. Epub 2013 Oct 29.

Cell therapies utilizing mesenchymal stem cells (MSCs) could overcome limitations of traditional treatments for reconstructing craniofacial tissues. This large-scale study explored a standardized methodology for the isolation and clinical-scale expansion of alveolar bone marrow-derived MSCs (aBMSCs). We harvested 103 alveolar bone marrow samples from 45 patients using 1 of 3 standardized methodologies. Following aBMSC isolation, cells were characterized through cell-surface marker expression and lineage-specific differentiation. Long-term cultures (> 50 population doublings [PDs]) were evaluated for transformational changes through senescence, gene expression, and karyotyping. Finally, aBMSC bone-forming potential was determined in vivo. More than 0.5 cc of bone marrow was needed to predictably isolate aBMSCs, and, regardless of methodology for harvest, cell-surface marker expression of CD73, CD90, CD105, and Stro-1 was similar for aBMSCs, being 89.8%, 98.8%, 93.8%, and 3.2%, respectively; all cells were negative for CD11b, CD19, and CD45.

aBMSCs exhibited multipotency, and karyotypes were normal up to 30 PDs, with significant cell senescence beginning following 35 PDs. Additionally, aBMSCs induced ectopic bone formation following subcutaneous transplantation into mice. These findings demonstrate a predictable approach for the isolation and safe clinical-scale expansion of aBMSCs, and thus, their clinical use could be considered for craniofacial regenerative therapies.

Mather, J. P., P. E. Roberts, et al. "Isolation of cancer stem like cells from human adenosquamous carcinoma of the lung supports a monoclonal origin from a multipotential tissue stem cell." PLoS One. 2013 Dec 4;8(12):e79456. doi: 10.1371/journal.pone.0079456. eCollection 2013.

There is increasing evidence that many solid tumors are hierarchically organized with the bulk tumor cells having limited replication potential, but are sustained by a stem-like cell that perpetuates the tumor. These cancer stem cells have been hypothesized to originate from transformation of adult tissue stem cells, or through re-acquisition of stem-like properties by progenitor cells. Adenosquamous carcinoma (ASC) is an aggressive type of lung cancer that contains a mixture of cells with squamous (cytokeratin 5+) and adenocarcinoma (cytokeratin 7+) phenotypes. The origin of these mixtures is unclear as squamous carcinomas are thought to arise from basal cells in the upper respiratory tract while adenocarcinomas are believed to form from stem cells in the bronchial alveolar junction. We have isolated and characterized cancer stem-like populations from ASC through application of selective defined culture medium initially used to grow human lung stem cells. Homogeneous cells selected from ASC tumor specimens were stably expanded in vitro. Primary xenografts and metastatic lesions derived from these cells in NSG mice fully recapitulate both the adenocarcinoma and squamous features of the patient tumor. Interestingly, while the CSLC all co-expressed cytokeratins 5 and 7, most xenograft cells expressed either one, or neither, with <10% remaining double positive. We also demonstrated the potential of the CSLC to differentiate to multi-lineage structures with branching lung morphology expressing bronchial, alveolar and neuroendocrine markers in vitro. Taken together the properties of these ASC-derived CSLC suggests that ASC may arise from a primitive lung stem cell distinct from the bronchial-alveolar or basal stem cells.

Moghbeli, M., F. Moghbeli, et al. "Cancer stem cell detection and isolation." Med Oncol. 2014 Sep;31(9):69. doi: 10.1007/s12032-014-0069-6. Epub 2014 Jul 27.

Only 10 % of cancer-related deaths result from primary tumors; most are caused by metastatic tumors. It is believed that the metastatic power of tumor cells is attributed to features of a stem cell-like subpopulation of tumor cells known as cancer stem cells (CSCs). Cancer stem cells are resistant to chemotherapeutic treatments and can induce dormancy in tumor cells for long periods. Detection, isolation, and characterization of CSCs in solid tumors are hallmarks of cancer-targeted therapies in recent years. There are inevitable similarities between normal and cancer stem cells; therefore, finding specific methods or markers to differentiate them is critical to cancer therapies. Considering CSCs involvement in tumor relapse and chemotherapeutic resistance, identification of such cells in tumors is imperative for effective targeted therapy. The present review introduces practical and specific protocols used to isolate CSCs from solid tumors from colon, esophagus, liver, breast, brain, and cervix.

Ovchinnikov, D. A., A. Hidalgo, et al. "Isolation of contractile cardiomyocytes from human pluripotent stem-cell-derived cardiomyogenic cultures using a human NCX1-EGFP reporter." Stem Cells Dev. 2015 Jan 1;24(1):11-20. doi: 10.1089/scd.2014.0195.

The prospective isolation of defined contractile human pluripotent stem cell (hPSC)-derived cardiomyocytes is advantageous for regenerative medicine and drug screening applications. Currently, enrichment of cardiomyocyte populations from such cultures can be achieved by combinations of cell surface markers or the labor-intensive genetic modification of cardiac developmental genes, such as NKX2.5 or MYH6, with fluorescent reporters. To create a facile, portable method for the isolation of contractile cardiomyocytes from cardiomyogenic hPSC cultures, we employed a highly conserved cardiac enhancer sequence in the SLC8A1 (NCX1) gene to generate a lentivirally deliverable, antibiotic-selectable NCX1cp-EGFP reporter. We show that human embryonic stem cells (and induced pluripotent stem cells) transduced with the NCX1cp-EGFP reporter cassette exhibit enhanced green fluorescent protein (EGFP) expression in cardiac progenitors from 5 days into the directed cardiac hPSC differentiation protocol, with all reporter-positive cells transitioning to spontaneously contracting foci 3 days later. In subsequent stages of cardiomyocyte maturation, NCX1cp-EGFP expression was exclusively limited to contractile cells expressing high levels of cardiac troponin T (CTNT), MLC2a/v, and alpha-actinin proteins, and was not present in CD90/THY1(+) cardiac stromal cells or CD31/PECAM(+) endothelial cells. Flow-assisted cytometrically sorted EGFP(+) fractions of differentiated cultures were highly

enriched in both early (NKX2.5 and TBX5) and late (CTNT/TNNI2, MYH6, MYH7, NPPA, and MYL2) cardiomyocyte markers, with a significant proportion of cells displaying a ventricular-like action potential pattern in patch-clamp recordings. We conclude that the use of the cardiac-specific promoter of the human SLC8A1(NCX1) gene is an effective strategy to isolate contractile cardiac cells and their progenitors from hPSC-derived cardiomyogenic cultures.

Patel, J., A. Shafiee, et al. "Novel isolation strategy to deliver pure fetal-origin and maternal-origin mesenchymal stem cell (MSC) populations from human term placenta." Placenta. 2014 Nov;35(11):969-71. doi: 10.1016/j.placenta.2014.09.001. Epub 2014 Sep 10.

The placenta is an abundant source of mesenchymal stem/stromal cells (MSC). Although presumed of translationally-advantageous fetal origin, the literature instead suggests a high incidence of either contaminating or pure maternal MSC. Despite definitional criteria that MSC are CD34-, increasing evidence suggests that fetal MSC may be CD34 positive in vivo. We flow sorted term placental digests based on CD34+ expression and exploited differential culture media to isolate separately pure fetal and maternal MSC populations. This method has considerable translational implications, in particular to clinical trials underway with "placental" MSC of uncertain or decidual origin.

Qureshi, A. T., C. Chen, et al. "Human adipose-derived stromal/stem cell isolation, culture, and osteogenic differentiation." Methods Enzymol. 2014;538:67-88. doi: 10.1016/B978-0-12-800280-3.00005-0.

Annually, more than 200,000 elective liposuction procedures are performed in the United States and over a million worldwide. The ease of harvest and abundance make human adipose-derived stromal/stem cells (hASCs) isolated from lipoaspirates an attractive, readily available source of adult stem cells that have become increasingly popular for use in many studies. Here, we describe common methods for hASC culture, preservation, and osteogenic differentiation. We introduce methods of ceramic, polymer, and composite scaffold synthesis with a description of morphological, chemical, and mechanical characterization techniques. Techniques for scaffold loading are compared, and methods for determining cell loading efficiency and proliferation are described. Finally, we provide both qualitative and quantitative techniques for in vitro assessment of hASC osteogenic differentiation.

Raoof, M., M. M. Yaghoobi, et al. "A modified efficient method for dental pulp stem cell isolation." Dent Res J (Isfahan). 2014 Mar;11(2):244-50.

BACKGROUND: Dental pulp stem cells can be used in regenerative endodontic therapy. The aim of this study was to introduce an efficient method for dental pulp stem cells isolation. MATERIALS AND METHODS: In this in-vitro study, 60 extracted human third molars were split and pulp tissue was extracted. Dental pulp stem cells were isolated by the following three different methods: (1) digestion of pulp by collagenase/dispase enzyme and culture of the released cells; (2) outgrowth of the cells by culture of undigested pulp pieces; (3) digestion of pulp tissue pieces and fixing them. The cells were cultured in minimum essential medium alpha modification (alphaMEM) medium supplemented with 20% fetal bovine serum(FBS) in humid 37 degrees C incubator with 5% CO 2. The markers of stem cells were studied by reverse transcriptase polymerase chain reaction (PCR). The student t-test was used for comparing the means of independent groups. P <0.05 was considered as significant. RESULTS: The results indicated that by the first method a few cell colonies with homogenous morphology were detectable after 4 days, while in the outgrowth method more time was needed (10-12 days) to allow sufficient numbers of heterogeneous phenotype stem cells to migrate out of tissue. Interestingly, with the improved third method, we obtained stem cells successfully with about 60% efficiency after 2 days. The results of RT-PCR suggested the expression of Nanog, Oct-4, and Nucleostemin markers in the isolated cells from dental pulps. CONCLUSION: This study proposes a new method with high efficacy to obtain dental pulp stem cells in a short time.

Raposio, E., G. Caruana, et al. "A novel and effective strategy for the isolation of adipose-derived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy." Plast Reconstr Surg. 2014 Jun;133(6):1406-9. doi: 10.1097/PRS.0000000000000170.

Adipose-derived stem cells are an ideal mesenchymal stem cell population for regenerative medical application. The isolation procedure is performed by mechanical isolation under a laminar air flow bench without using serum or animal-derived reagents; cells were characterized by flow cytometric analysis. Cell availability is improved compared with enzymatic digestion procedures. The adipose-derived stem cell mechanical isolating procedure presented here is easier, safer, cheaper, and faster than traditional currently performed enzymatic procedures.

Reinhardt, M., A. Bader, et al. "Devices for stem cell isolation and delivery: current need for drug discovery and cell therapy." Expert Rev Med Devices. 2015 May;12(3):353-64. doi: 10.1586/17434440.2015.995094. Epub 2014 Dec 25.

Isolation and purification of stem cells and their delivery into diseased or aged tissues or organs need special devices for proper transplantation of stem cells in order to achieve high cell retention at transplant site for repair or regeneration of tissues and organs. The clinical and preclinical importance of special devices such as Celution System, Isolex cell separation device, magnetic surface-enhanced Raman spectroscopic dots, microfluidic devices, immunomagnetic cell separation for stem cell separation and isolation are the main focus in this paper. Further, devices like trans-coronary delivery, trans-endocardial delivery, intracoronary delivery devices for stem cell application to the heart are described. Devices for stem cell application to the brain, the spinal cord and other tissues are also explained. We highlighted scaffolds with incorporated stem cells and other encapsulation devices used to transplant stem cells. Current needs of devices for stem cells isolation, purification and delivery for drug discovery and cell therapy are discussed.

Ringden, O., M. Remberger, et al. "Home care during neutropenia after allogeneic hematopoietic stem cell transplantation in children and adolescents is safe and may be more advantageous than isolation in hospital." Pediatr Transplant. 2014 Jun;18(4):398-404. doi: 10.1111/petr.12262.

After ASCT, children are isolated in hospital to prevent neutropenic infections. Patients living within two-h drive from the hospital were given the option of treatment at home after ASCT. Daily visits by an experienced nurse and phone calls from a physician from the unit were included in the protocol. We compared 29 children and adolescents treated at home with 58 matched hospital controls. The children spent a median time of 13 days at home (range 2-24 days) and 6 (0-35) days in hospital. The cumulative incidence of acute GVHD grades II-IV was 21% in the home-care children and 39% in the controls ($p = 0.1$). Chronic GVHD and probability of relapse were similar in the two groups. TRM at five yr was 11% in the home-care patients and 18% in the controls. Overall survival at three yr was 77% and 62%, respectively ($p = 0.33$). None of the patients died at home. Median costs were 38,748 euros in the home-care patients and 49,282 euros in those treated in the hospital ($p = 0.2$). We conclude that it is safe for children and adolescents to be treated at home during the pancytopenic phase after ASCT.

Samuel, E. R., L. Beloki, et al. "Isolation of highly suppressive CD25+FoxP3+ T regulatory cells from G-CSF-mobilized donors with retention of cytotoxic anti-viral CTLs: application for multi-functional immunotherapy post stem cell transplantation." PLoS One. 2014 Jan 17;9(1):e85911. doi: 10.1371/journal.pone.0085911. eCollection 2014.

Previous studies have demonstrated the effective control of cytomegalovirus (CMV) infections post haematopoietic stem cell transplant through the adoptive transfer of donor derived CMV-specific T cells (CMV-T). Strategies for manufacturing CMV immunotherapies has involved a second leukapheresis or blood draw from the donor, which in the unrelated donor setting is not always possible. We have investigated the feasibility of using an aliquot of the original G-CSF-mobilized graft as a starting material for manufacture of CMV-T and examined the activation marker CD25 as a targeted approach for identification and isolation following CMVpp65 peptide stimulation. CD25+ cells isolated from G-CSF-mobilized apheresis revealed a significant increase in the proportion of FoxP3 expression when compared with conventional non-mobilized CD25+ cells and showed a superior suppressive capacity in a T cell proliferation assay, demonstrating the emergence of a population of Tregs not present in non-mobilized apheresis collections. The expansion of CD25+ CMV-T in short-term culture resulted in a mixed population of CD4+ and CD8+ T cells with CMV-specificity that secreted cytotoxic effector molecules and lysed CMVpp65 peptide-loaded phytohaemagglutinin-stimulated blasts. Furthermore CD25 expanded cells retained their suppressive capacity but did not maintain FoxP3 expression or secrete IL-10. In summary our data indicates that CD25 enrichment post CMV stimulation in G-CSF-mobilized PBMCs results in the simultaneous generation of both a functional population of anti-viral T cells and Tregs thus illustrating a potential single therapeutic strategy for the treatment of both GvHD and CMV reactivation following allogeneic haematopoietic stem cell transplantation. The use of G-CSF-mobilized cells as a starting material for cell therapy manufacture represents a feasible approach to alleviating the many problems incurred with successive donations and procurement of cells from unrelated donors.

Shin, D. M., M. Suszynska, et al. "Very small embryonic-like stem-cell optimization of isolation protocols: an update of molecular signatures and a review of current in vivo applications." Exp Mol Med. 2013 Nov 15;45:e56. doi: 10.1038/emm.2013.117.

As the theory of stem cell plasticity was first proposed, we have explored an alternative hypothesis

for this phenomenon: namely that adult bone marrow (BM) and umbilical cord blood (UCB) contain more developmentally primitive cells than hematopoietic stem cells (HSCs). In support of this notion, using multiparameter sorting we were able to isolate small Sca1(+)Lin(-)CD45(-) cells and CD133(+)Lin(-)CD45(-) cells from murine BM and human UCB, respectively, which were further enriched for the detection of various early developmental markers such as the SSEA antigen on the surface and the Oct4 and Nanog transcription factors in the nucleus. Similar populations of cells have been found in various organs by our team and others, including the heart, brain and gonads. Owing to their primitive cellular features, such as the high nuclear/cytoplasm ratio and the presence of euchromatin, they are called very small embryonic-like stem cells (VSELs). In the appropriate in vivo models, VSELs differentiate into long-term repopulating HSCs, mesenchymal stem cells (MSCs), lung epithelial cells, cardiomyocytes and gametes. In this review, we discuss the most recent data from our laboratory and other groups regarding the optimal isolation procedures and describe the updated molecular characteristics of VSELs.

Shiozawa, Y., R. S. Taichman, et al. "Detection and isolation of human disseminated tumor cells in the murine bone marrow stem cell niche." Methods Mol Biol. 2013;1035:207-15. doi: 10.1007/978-1-62703-508-8_18.

The presence of disseminated tumor cells (DTCs) in the bone marrow is associated with poor prognosis of cancer patients. However, little is known about the biology of DTCs due to lack of relevant animal models. Here, we describe the methods for detecting and isolating human DTCs from the murine bone marrow niche by PCR using human Alu sequences and by fluorescence-activated cell sorting and immunohistochemistry using anti-HLA antibody. These strategies could be useful for exploring the biology of DTCs.

Spohn, G., E. Wiercinska, et al. "Automated CD34+ cell isolation of peripheral blood stem cell apheresis product." Cytotherapy. 2015 May 14. pii: S1465-3249(15)00858-0. doi: 10.1016/j.jcyt.2015.04.005.

BACKGROUND AIMS: Immunomagnetic enrichment of CD34+ hematopoietic "stem" cells (HSCs) using paramagnetic nanobead coupled CD34 antibody and immunomagnetic extraction with the CliniMACS plus system is the standard approach to generating T-cell-depleted stem cell grafts. Their clinical beneficence in selected indications is established. Even though CD34+ selected grafts are typically given in the context of a severely immunosuppressive conditioning with anti-thymocyte globulin or similar, the degree of T-cell depletion appears to affect clinical outcomes and thus in addition to CD34 cell recovery, the degree of T-cell depletion critically describes process quality. An automatic immunomagnetic cell processing system, CliniMACS Prodigy, including a protocol for fully automatic CD34+ cell selection from apheresis products, was recently developed. We performed a formal process validation to support submission of the protocol for CE release, a prerequisite for clinical use of Prodigy CD34+ products. METHODS: Granulocyte-colony stimulating factor-mobilized healthy-donor apheresis products were subjected to CD34+ cell selection using Prodigy with clinical reagents and consumables and advanced beta versions of the CD34 selection software. Target and non-target cells were enumerated using sensitive flow cytometry platforms. RESULTS: Nine successful clinical-scale CD34+ cell selections were performed. Beyond setup, no operator intervention was required. Prodigy recovered 74 +/- 13% of target cells with a viability of 99.9 +/- 0.05%. Per 5 x 10E6 CD34+ cells, which we consider a per-kilogram dose of HSCs, products contained 17 +/- 3 x 10E3 T cells and 78 +/- 22 x 10E3 B cells. CONCLUSIONS: The process for CD34 selection with Prodigy is robust and labor-saving but not time-saving. Compared with clinical CD34+ selected products concurrently generated with the predecessor technology, product properties, importantly including CD34+ cell recovery and T-cell contents, were not significantly different. The automatic system is suitable for routine clinical application.

Stasi, K., D. Goings, et al. "Optimal isolation and xeno-free culture conditions for limbal stem cell function." Invest Ophthalmol Vis Sci. 2014 Jan 20;55(1):375-86. doi: 10.1167/iovs.13-12517.

PURPOSE: To preserve limbal stem cell (LSC) function in vitro with xenobiotic-free culture conditions. METHODS: Limbal epithelial cells were isolated from 139 donors using 15 variations of three dissociation solutions. All culture conditions were compared to the baseline condition of murine 3T3-J3 feeders with xenobiotic (Xeno) keratinocyte growth medium at 20% O2. Five Xeno and Xeno-free media with increasing concentrations of calcium and epidermal growth factor (EGF) were evaluated at 5%, 14%, and 20% O2. Human MRC-5, dermal (fetal, neonatal, or adult), and limbal stromal fibroblasts were compared. Statistical analysis was performed on the number of maximum serial weekly passages, percentage of aborted colonies, colony-forming efficiency (CFE), p63alpha(bright) cells, and RT-PCR ratio of p63alpha/K12. Immunocytochemistry and RT-PCR for p63alpha, ABCG2, Bmi1, C/EBPdelta , K12, and MUC1 were performed to evaluate phenotype.

RESULTS: Dispase/TrypLE was the isolation method that consistently showed the best yield, viability, and CFE. On 3T3-J2 feeders, Xeno-free medium with calcium 0.1 mM and EGF 10 ng/mL at 20% O2 supported more passages with equivalent percentage of aborted colonies, p63alpha(bright) cells, and p63alpha/K12 RT-PCR ratio compared to baseline Xeno-media. With this Xeno-free medium, MRC-5 feeders showed the best performance, followed by fetal, neonatal, adult HDF, and limbal fibroblasts. MRC-5 feeders supported serial passages with sustained high expression of progenitor cell markers at levels as robust as the baseline condition without significant difference between 20% and 5% O2. CONCLUSIONS: The LSC function can be maintained in vitro under appropriate Xeno-free conditions.

Watson, L., S. J. Elliman, et al. "From isolation to implantation: a concise review of mesenchymal stem cell therapy in bone fracture repair." Stem Cell Res Ther. 2014 Apr 15;5(2):51. doi: 10.1186/scrt439.

Compromised bone-regenerating capability following a long bone fracture is often the result of reduced host bone marrow (BM) progenitor cell numbers and efficacy. Without surgical intervention, these malunions result in mobility restrictions, deformities, and disability. The clinical application of BM-derived mesenchymal stem cells (MSCs) is a feasible, minimally invasive therapeutic option to treat non-union fractures. This review focuses on novel, newly identified cell surface markers in both the mouse and human enabling the isolation and purification of osteogenic progenitor cells as well as their direct and indirect contributions to fracture repair upon administration. Furthermore, clinical success to date is summarized with commentary on autologous versus allogeneic cell sources and the methodology of cell administration. Given our clinical success to date in combination with recent advances in the identification, isolation, and mechanism of action of MSCs, there is a significant opportunity to develop improved technologies for defining therapeutic MSCs and potential to critically inform future clinical strategies for MSC-based bone regeneration.

Yoo, H. S., T. Yi, et al. "Mesenchymal Stem Cell Lines Isolated by Different Isolation Methods Show Variations in the Regulation of Graft-versus-host Disease." Immune Netw. 2013 Aug;13(4):133-40. doi: 10.4110/in.2013.13.4.133. Epub 2013 Aug 26.

Since the discovery of the immunomodulation property of mesenchymal stem cells (MSCs) about a decade ago, it has been extensively investigated whether MSCs can be used for the treatment of immune-related diseases, such as graft-versus-host disease (GvHD). However, how to evaluate the efficacy of human MSCs for the clinical trial is still unclear. We used an MHC-mismatched model of GvHD (B6 into BALB/c). Surprisingly, the administration of the human MSCs (hMSCs) could reduce the GvHD-related mortality of the mouse recipients and xenogeneically inhibit mouse T-cell proliferation and IFN-gamma production in vitro. We recently established a new protocol for the isolation of a homogeneous population of MSCs called subfractionation culturing methods (SCM), and established a library of clonal MSC lines. Therefore, we also investigated whether MSCs isolated by the conventional gradient centrifugation method (GCM) and SCM show different efficacy in vivo. Intriguingly, clonal hMSCs (hcMSCs) isolated by SCM showed better efficacy than hMSCs isolated by GCM. Based on these results, the MHC-mismatched model of GvHD may be useful for evaluating the efficacy of human MSCs before the clinical trial. The results of this study suggest that different MSC lines may show different efficacy in vivo and in vitro.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

References

1. Ahmadbeigi, N., M. Soleimani, et al. "Isolation, characterization, and transplantation of bone marrow-derived cell components with hematopoietic stem cell niche properties." Stem Cells Dev. 2013 Dec 1;22(23):3052-61. doi: 10.1089/scd.2013.0005. Epub 2013 Sep 14.
2. Azari, H. "Isolation and enrichment of defined neural cell populations from heterogeneous neural stem cell progeny." Methods Mol Biol. 2013;1059:95-106. doi: 10.1007/978-1-62703-574-3_9.
3. Bellavia, M., R. Altomare, et al. "Towards an ideal source of mesenchymal stem cell isolation for possible therapeutic application in regenerative medicine." Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2014 Sep;158(3):356-60. doi: 10.5507/bp.2013.051. Epub 2013 Jul 29.
4. Branch, M. J., W. Y. Yu, et al. "Isolation of adult stem cell populations from the human cornea." Methods Mol Biol. 2015;1235:165-77. doi: 10.1007/978-1-4939-1785-3_14.
5. Chan, T. M., H. J. Harn, et al. "Improved human mesenchymal stem cell isolation." Cell Transplant. 2014;23(4-5):399-406. doi: 10.3727/096368914X678292.
6. Chatzistamatiou, T. K., A. C. Papassavas, et al. "Optimizing isolation culture and freezing methods to preserve Wharton's jelly's mesenchymal stem cell

(MSC) properties: an MSC banking protocol validation for the Hellenic Cord Blood Bank." Transfusion. 2014 Dec;54(12):3108-20. doi: 10.1111/trf.12743. Epub 2014 Jun 4.
7. de Marval, P. L., S. H. Kim, et al. "Isolation and characterization of a stem cell side-population from mouse hair follicles." Methods Mol Biol. 2014;1195:259-68. doi: 10.1007/7651_2013_61.
8. Devitt, S. M., C. M. Carter, et al. "Successful isolation of viable adipose-derived stem cells from human adipose tissue subject to long-term cryopreservation: positive implications for adult stem cell-based therapeutics in patients of advanced age." Stem Cells Int. 2015;2015:146421. doi: 10.1155/2015/146421. Epub 2015 Apr 5.
9. Doi, D., B. Samata, et al. "Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation." Stem Cell Reports. 2014 Mar 6;2(3):337-50. doi: 10.1016/j.stemcr.2014.01.013. eCollection 2014 Mar 11.
10. Edri, R., Y. Yaffe, et al. "Analysing human neural stem cell ontogeny by consecutive isolation of Notch active neural progenitors." Nat Commun. 2015 Mar 23;6:6500. doi: 10.1038/ncomms7500.
11. Eubanks, E. J., S. A. Tarle, et al. "Tooth storage, dental pulp stem cell isolation, and clinical scale expansion without animal serum." J Endod. 2014 May;40(5):652-7. doi: 10.1016/j.joen.2014.01.005. Epub 2014 Mar 6.
12. Ferro, F., R. Spelat, et al. "Dental pulp stem cell (DPSC) isolation, characterization, and differentiation." Methods Mol Biol. 2014;1210:91-115. doi: 10.1007/978-1-4939-1435-7_8.
13. Fisher, C., T. L. Grahovac, et al. "Comparison of harvest and processing techniques for fat grafting and adipose stem cell isolation." Plast Reconstr Surg. 2013 Aug;132(2):351-61. doi: 10.1097/PRS.0b013e3182958796.
14. Gao, Y., X. Li, et al. "Isolation of a pluripotent neural stem cell from the embryonic bovine brain." Int J Mol Sci. 2015 Mar 13;16(3):5990-9. doi: 10.3390/ijms16035990.
15. Hashem, S. I. and W. C. Claycomb "Genetic isolation of stem cell-derived pacemaker-nodal cardiac myocytes." Mol Cell Biochem. 2013 Nov;383(1-2):161-71. doi: 10.1007/s11010-013-1764-x. Epub 2013 Jul 23.
16. Heidari-Keshel, S., M. Rezaei-Tavirani, et al. "Tissue-specific somatic stem-cell isolation and characterization from human endometriosis. Key roles in the initiation of endometrial proliferative disorders." Minerva Med. 2015 Apr;106(2):95-108. Epub 2014 Dec 17.
17. Hilkens, P., P. Gervois, et al. "Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells." Cell Tissue Res. 2013 Jul;353(1):65-78. doi: 10.1007/s00441-013-1630-x. Epub 2013 May 29.
18. Holmqvist, S., M. Brouwer, et al. "Generation of human pluripotent stem cell reporter lines for the isolation of and reporting on astrocytes generated from ventral midbrain and ventral spinal cord neural progenitors." Stem Cell Res. 2015 Jun 6;15(1):203-220. doi: 10.1016/j.scr.2015.05.014.
19. Hu, P., Y. Pu, et al. "Isolation, in vitro culture and identification of a new type of mesenchymal stem cell derived from fetal bovine lung tissues." Mol Med Rep. 2015 May 27. doi: 10.3892/mmr.2015.3854.
20. Iftimia-Mander, A., P. Hourd, et al. "Mesenchymal stem cell isolation from human umbilical cord tissue: understanding and minimizing variability in cell yield for process optimization." Biopreserv Biobank. 2013 Oct;11(5):291-8. doi: 10.1089/bio.2013.0027.
21. Kay, A. G., T. P. Dale, et al. "BMP2 repression and optimized culture conditions promote human bone marrow-derived mesenchymal stem cell isolation." Regen Med. 2015;10(2):109-25. doi: 10.2217/rme.14.67.
22. Khatami, S. M., S. Zahri, et al. "Stem Cell Isolation from Human Wharton's Jelly: A Study of Their Differentiation Ability into Lens Fiber Cells." Cell J. 2014 Winter;15(4):364-71. Epub 2013 Nov 20.
23. Kong, B. H., J. H. Moon, et al. "Prognostic value of glioma cancer stem cell isolation in survival of primary glioblastoma patients." Stem Cells Int. 2014;2014:838950. doi: 10.1155/2014/838950. Epub 2014 Dec 11.
24. Kumar, S., S. Poojan, et al. "Rapid isolation of integrin rich multipotent stem cell pool and reconstruction of mouse epidermis equivalent." Am J Stem Cells. 2014 Mar 13;3(1):27-36. eCollection 2014.
25. Lenihan, C., C. Rogers, et al. "The effect of isolation and culture methods on epithelial stem cell populations and their progeny-toward an improved cell expansion protocol for clinical application." Cytotherapy. 2014 Dec;16(12):1750-9. doi: 10.1016/j.jcyt.2014.06.005. Epub 2014 Oct 5.
26. Leong, C., D. Zhai, et al. "Neural stem cell isolation from the whole mouse brain using the novel FABP7-binding fluorescent dye, CDr3." Stem Cell Res. 2013 Nov;11(3):1314-22. doi: 10.1016/j.scr.2013.09.002. Epub 2013 Sep 18.
27. Ma H, Chen G. Stem cell. The Journal of American Science 2005;1(2):90-92.
28. Ma H, Cherng S. Eternal Life and Stem Cell. Nature and Science. 2007;5(1):81-96.
29. Ma H, Cherng S. Nature of Life. Life Science Journal 2005;2(1):7 - 15.
30. Ma H, Yang Y. Turritopsis nutricula. Nature and Science 2010;8(2):15-20. http://www.sciencepub.net/nature/ns0802/03_1279_hongbao_turritopsis_ns0802_15_20.pdf.

31. Ma H. The Nature of Time and Space. Nature and science 2003;1(1):1-11.Nature and science 2007;5(1):81-96.
32. Maass, K., A. Shekhar, et al. "Isolation and characterization of embryonic stem cell-derived cardiac Purkinje cells." Stem Cells. 2015 Apr;33(4):1102-12. doi: 10.1002/stem.1921.
33. Maria Cambuli, F., A. Rezza, et al. "Brief report: musashi1-eGFP mice, a new tool for differential isolation of the intestinal stem cell populations." Stem Cells. 2013 Oct;31(10):2273-8. doi: 10.1002/stem.1428.
34. Mason, S., S. A. Tarle, et al. "Standardization and safety of alveolar bone-derived stem cell isolation." J Dent Res. 2014 Jan;93(1):55-61. doi: 10.1177/0022034513510530. Epub 2013 Oct 29.
35. Mather, J. P., P. E. Roberts, et al. "Isolation of cancer stem like cells from human adenosquamous carcinoma of the lung supports a monoclonal origin from a multipotential tissue stem cell." PLoS One. 2013 Dec 4;8(12):e79456. doi: 10.1371/journal.pone.0079456. eCollection 2013.
36. Moghbeli, M., F. Moghbeli, et al. "Cancer stem cell detection and isolation." Med Oncol. 2014 Sep;31(9):69. doi: 10.1007/s12032-014-0069-6. Epub 2014 Jul 27.
37. National Center for Biotechnology Information, U.S. National Library of Medicine**.** http://www.ncbi.nlm.nih.gov/pubmed. 2015.
38. Ovchinnikov, D. A., A. Hidalgo, et al. "Isolation of contractile cardiomyocytes from human pluripotent stem-cell-derived cardiomyogenic cultures using a human NCX1-EGFP reporter." Stem Cells Dev. 2015 Jan 1;24(1):11-20. doi: 10.1089/scd.2014.0195.
39. Patel, J., A. Shafiee, et al. "Novel isolation strategy to deliver pure fetal-origin and maternal-origin mesenchymal stem cell (MSC) populations from human term placenta." Placenta. 2014 Nov;35(11):969-71. doi: 10.1016/j.placenta.2014.09.001. Epub 2014 Sep 10.
40. Qureshi, A. T., C. Chen, et al. "Human adipose-derived stromal/stem cell isolation, culture, and osteogenic differentiation." Methods Enzymol. 2014;538:67-88. doi: 10.1016/B978-0-12-800280-3.00005-0.
41. Raoof, M., M. M. Yaghoobi, et al. "A modified efficient method for dental pulp stem cell isolation." Dent Res J (Isfahan). 2014 Mar;11(2):244-50.
42. Raposio, E., G. Caruana, et al. "A novel and effective strategy for the isolation of adipose-derived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy." Plast Reconstr Surg. 2014 Jun;133(6):1406-9. doi: 10.1097/PRS.0000000000000170.
43. Reinhardt, M., A. Bader, et al. "Devices for stem cell isolation and delivery: current need for drug discovery and cell therapy." Expert Rev Med Devices. 2015 May;12(3):353-64. doi: 10.1586/17434440.2015.995094. Epub 2014 Dec 25.
44. Ringden, O., M. Remberger, et al. "Home care during neutropenia after allogeneic hematopoietic stem cell transplantation in children and adolescents is safe and may be more advantageous than isolation in hospital." Pediatr Transplant. 2014 Jun;18(4):398-404. doi: 10.1111/petr.12262.
45. Samuel, E. R., L. Beloki, et al. "Isolation of highly suppressive CD25+FoxP3+ T regulatory cells from G-CSF-mobilized donors with retention of cytotoxic anti-viral CTLs: application for multi-functional immunotherapy post stem cell transplantation." PLoS One. 2014 Jan 17;9(1):e85911. doi: 10.1371/journal.pone.0085911. eCollection 2014.
46. Shin, D. M., M. Suszynska, et al. "Very small embryonic-like stem-cell optimization of isolation protocols: an update of molecular signatures and a review of current in vivo applications." Exp Mol Med. 2013 Nov 15;45:e56. doi: 10.1038/emm.2013.117.
47. Shiozawa, Y., R. S. Taichman, et al. "Detection and isolation of human disseminated tumor cells in the murine bone marrow stem cell niche." Methods Mol Biol. 2013;1035:207-15. doi: 10.1007/978-1-62703-508-8_18.
48. Spohn, G., E. Wiercinska, et al. "Automated CD34+ cell isolation of peripheral blood stem cell apheresis product." Cytotherapy. 2015 May 14. pii: S1465-3249(15)00858-0. doi: 10.1016/j.jcyt.2015.04.005.
49. Stasi, K., D. Goings, et al. "Optimal isolation and xeno-free culture conditions for limbal stem cell function." Invest Ophthalmol Vis Sci. 2014 Jan 20;55(1):375-86. doi: 10.1167/iovs.13-12517.
50. Watson, L., S. J. Elliman, et al. "From isolation to implantation: a concise review of mesenchymal stem cell therapy in bone fracture repair." Stem Cell Res Ther. 2014 Apr 15;5(2):51. doi: 10.1186/scrt439.
51. Wikipedia. The free encyclopedia. http://en.wikipedia.org. 2015.
52. Yoo, H. S., T. Yi, et al. "Mesenchymal Stem Cell Lines Isolated by Different Isolation Methods Show Variations in the Regulation of Graft-versus-host Disease." Immune Netw. 2013 Aug;13(4):133-40. doi: 10.4110/in.2013.13.4.133. Epub 2013 Aug 26.