Stem Cell Technique Literatures

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on stem cell research technique.

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Key words: stem cell; life; gene; DNA; protein; technique

Literatures

Aldave, A. J. and I. G. Wong (2002). "A novel technique for harvesting keratolimbal allografts from corneoscleral buttons." Am J Ophthalmol **134**(6): 929-31.

PURPOSE: To describe a method for obtaining partial-thickness keratolimbal allografts from corneoscleral buttons to be used in corneal limbal stem cell transplantation. DESIGN: Description of device design and technique for use. METHODS: Cyanoacrylate tissue adhesive, placed on the posterior side of a trephinated corneoscleral rim, is used to secure the allograft to a disposable acrylic sphere that is attached to a cylindrical base. RESULTS: After fixation of the corneoscleral rim to the acrylic sphere, keratolimbal allograft harvesting is performed as a continuous strip dissection with a 65 Beaver blade. This technique minimizes trauma to the epithelial stem cells, provides excellent stability of the corneoscleral rim during harvesting, and preserves the central corneal button. CONCLUSIONS: This method for obtaining keratolimbal allografts effectively provides tissue stabilization during harvesting, minimizing epithelial stem cell trauma and eliminating the need for whole donor globes.

Alegre, M., L. Puig, et al. (1997). "Primary neuroendocrine carcinoma of the skin expresses tyrosinase mRNA: detection by a specific nested PCR technique." Dermatology **194**(4): 334-7.

BACKGROUND: Tyrosinase transcripts have been identified in a wide range of normal organs and neoplasms such as cutaneous nevi, breast carcinomas, liposarcomas and schwannomas. The cells responsible for these positive results have not been accurately identified yet, but they might correspond to either fully differentiated melanocytes, melanocytic precursors or cells bearing potential for melanocytic differentiation. OBJECTIVE: To assess the expression of tyrosinase mRNA in surgical resection specimens and peripheral blood samples from 2 patients with primary neuroendocrine carcinomas of the skin (PNCS). METHODS: The expression of tyrosinase mRNA transcripts was tested by means of a reverse-transcriptase and nested PCR technique, using specific primers, in tissue samples from surgical specimens and heparinized blood samples from 2 patients with PNCS. RESULTS: The results of the test were positive in the 2 specimens of PCNS but negative in blood samples. CONCLUSION: Our findings provide further support for the hypothesis that PNCS originates from a pluripotent stem cell capable of neuroendocrine cell differentiation, keratin production, and, as reported herein, tyrosinase mRNA expression. It remains to be seen whether this technique can also be applied to patients with PNCS, as an adjunct to staging and treatment planning.

Aluigi, M., M. Fogli, et al. (2006). "Nucleofection is an efficient nonviral transfection technique for human bone marrow-derived mesenchymal stem cells." Stem Cells **24**(2): 454-61.

Viral-based techniques are the most efficient systems to deliver DNA into stem cells because they show high gene transduction and transgene expression in many cellular models. However, the use of viral vectors has several disadvantages mainly involving safety risks. Conversely, nonviral methods are rather inefficient for most primary cells. The Nucleofector technology, a new nonviral electroporation-based gene transfer technique, has proved to be an efficient tool for transfecting hard-to-transfect cell lines and primary cells. However, little is known about the

capacity of this technique to transfect adult stem cells. In this study, we applied the Nucleofector technology to engineer human bone marrow- derived mesenchymal stem cells (hMSCs). Using a green fluorescent protein reporter vector, we demonstrated a high transgene expression level using U-23 and C-17 pulsing programs: 73.7%+/-2.9% and 42.5%+/-3.4%, respectively. Cell recoveries and viabilities were 38.7%+/-2.9%, 44.5%+/-3.9% and 91.4%+/-1.3%, 94.31%+/-0.9% for U-23 and C-17, respectively. Overall, the transfection efficiencies were 27.4%+/-2.9% (U-23) and 16.6%+/-1.4% (C-17) compared with 3.6%+/-2.4% and 5.4%+/-3.4% of other nonviral transfection systems, such as FUGENE6 and DOTAP, respectively (p<.005 for all comparisons). Nucleofection did not affect the immunophenotype of hM-SCs, their normal differentiation potential, or ability to inhibit T-cell alloreactivity. Moreover, the interleukin-12 gene could be successfully transfected into hMSCs, and the immunomodulatory cytokine was produced in great amount for at least 3 weeks without impairment of its biological activity. In conclusion, nucleofection is an efficient nonviral transfection technique for hMSCs, which then may be used as cellular vehicles for the delivery of biological agents.

Apps, R., J. R. Trott, et al. (1991). "A study of branching in the projection from the inferior olive to the x and lateral c1 zones of the cat cerebellum using a combined electrophysiological and retrograde fluorescent double-labelling technique." Exp Brain Res **87**(1): 141-52.

The pattern of transverse branching in the olivocerebellar projection to the x zone in the vermis and the lateral c1 zone in the paravermis of the cat anterior lobe was studied using a combined electrophysiological and retrograde double-labelling tracer technique. Fluorochrome-tagged latex microspheres were well suited for this purpose. The results show that the region of olive that supplies climbing fibres to the two zones forms a continuous, rostrocaudally directed column about 2.25 mm in length, in a caudo-lateral to rostromedial part of the medial accessory olive (MAO), on average between A-P levels 12.50-10.50. This column may be divided into caudal and rostral halves that project respectively to the x and lateral c1 zones in the apical folia of lobules V/VIa. Partial overlap between these two territories occurs in an intermediate region (A-P levels 12.00-11.00) in middle MAO where olive cells that supply climbing fibres to either x or lateral c1 are intermingled with a smaller population of cells whose axons branch to provide climbing fibres to both zones. Quantitative analysis showed that, when different tracers were injected into each zone in the same animal, double-labeled cells represented only 5-7% of either single-labelled cell population within this area of overlap. It is concluded that, although some transverse branching is present within the olivocerebellar projection to the x and lateral c1 zones in the apical folia of lobule V, such branching is not extensive.

Ayello, J., C. Hesdorffer, et al. (1995). "A semiautomated technique for volume reduction of stem cell suspensions for autotransplantation." J Hematother **4**(6): 545-9.

Infusion of thawed cryopreserved autologous stem cells (SC) is associated with a variety of complications due to the presence of dimethyl sulfoxide (DMSO) and free hemoglobin and to volume overload. Commonly, the DMSO is not removed before infusion for fear that prolonged in vitro exposure of the cells to DMSO leads to loss of clonogenic activity. We describe a simple technique for the substantial reduction in volume and DMSO content of bone marrow (BM) and peripheral blood stem cell (PBSC) suspensions. Sixty-five patients were transplanted with thawed, volume-reduced SC cryopreserved according to Stiff et al. Semiautomated volume reduction was performed on a COBE 2991 cell processor. The median volumes of cryopreserved SC were 1152 ml and 933 ml for the pools of PBSC products and the mixed pools of BM and PBSC, respectively, whereas the volume of SC infused was 153 ml (78% reduction). There were no differences in cell recoveries between PBSC and BM (98%). Only 2 patients demonstrated minimal side effects during infusion. A cohort of 16 metastatic breast cancer patients underwent PBSC harvests following chemotherapy/G-CSF priming and subsequent autotransplantation. Median time to an absolute neutrophil count > 500/microliters was 8 days (range 6-14 days), and median time to a platelet count > 20,000/microliters was 11 days (range 6-18 days). Volume reduction of SC products without the risk of graft failure was performed simply and resulted in few complications during infusion.

Bakshi, A., C. Hunter, et al. (2004). "Minimally invasive delivery of stem cells for spinal cord injury: advantages of the lumbar puncture technique." J Neurosurg Spine **1**(3): 330-7.

OBJECT: Stem cell therapy has been shown to have considerable therapeutic potential for spinal cord injuries (SCIs); however, most experiments in animals have been performed by injecting cells directly into the injured parenchyma. This invasive technique compromises the injured spinal cord, although it delivers cells into the hostile environment of the acutely injured cord. In this study, the authors tested the possibility of delivering stem cells to injured

spinal cord by using three different minimally invasive techniques. METHODS: Bone marrow stromal cells (BMSCs) are clinically attractive because they have shown therapeutic potential in SCI and can be obtained in patients at the bedside, raising the possibility of autologous transplantation. In this study transgenically labeled cells were used for transplantation, facilitating posttransplantation tracking. Inbred Fisher-344 rats received partial cervical hemisection injury, and 2 x 10(6) BMSCs were intravenously, intraventricularly, or intrathecally transplanted 24 hours later via lumbar puncture (LP). The animals were killed 3, 10, or 14 days posttransplantation, and tissue samples were submitted to histochemical and immunofluorescence analyses. For additional comparison and validation, lineage restricted neural precursor (LRNP) cells obtained from E13.5 rat embryos were transplanted via LP, and these findings were also analyzed. CONCLUSIONS: Both BMSCs and LRNP cells home toward injured spinal cord tissues. The use of LP and intraventricular routes allows more efficient delivery of cells to the injured cord compared with the intravenous route. Stem cells delivered via LP for treatment of SCI may potentially be applicable in humans after optimal protocols and safety profiles are established in further studies.

Banerjee, M. and R. R. Bhonde (2006). "Application of hanging drop technique for stem cell differentiation and cytotoxicity studies." Cytotechnology **51**(1): 1-5.

The aim of our study is to explore the possibility of using an ancient method of culture technique- the hanging drop technique for stem cell differentiation and cytotoxicity testing. We demonstrate here a variety of novel applications of this age old technique not only to harness the differentiation potential of stem cells into specific lineages but also for cytotoxicity studies. Here we have prepared hanging drop cultures by placing 20 microl micro-drops of nutrient media and 10% Fetal Calf Serum (FCS) containing cells of interest on the lids of 60 mm dishes. Bottom plates of the dishes were filled with sterile Phosphate Buffer Saline (PBS) to avoid desiccation of samples. Lids were then placed on the bottom plates to achieve hanging drop cultures. We utilized this technique for cultivation of ciliated epithelia to study cytotoxicity and differentiation of bone marrow stromal cells. Most importantly the modified culture technique presented here is simple, economical and cost effective in terms of the time taken and the reagents required and are amenable to goal specific modification such as cytotoxicity testing. It is advantageous over the existing system in terms of retention of viability and functionality for longer duration and for providing three dimensional growth micro-environment making it useful for organotypic cultures and in vivo simulation.

Belecky-Adams, T., B. Cook, et al. (1996). "Correlations between terminal mitosis and differentiated fate of retinal precursor cells in vivo and in vitro: analysis with the "window-labeling" technique." Dev Biol **178**(2): 304-15.

We have investigated with high resolution the timing of retinal precursor cell commitment to specific differentiated fates, using an in ovo modification of the in vitro "window-labeling" technique (A. M. Repka and R. Adler, J. Histochem. Cytochem. 40, 947-953, 1992a). The method involves an initial injection of tritiated thymidine into chick embryos, followed a specific number of hours later by an injection of bromodeoxyuridine (BrDU); cells born during this period are identified by being labeled with thymidine but not with BrDU. We used this method to determine, in a narrow region adjacent to the choroid fissure, the fate of cells born during defined 5-hr intervals between Embryonic Days (ED) 4-8. All the cohorts gave rise to heterogenous differentiated populations, indicating that time of cell birth is not a major cell fate determinant. A progressive restriction in the developmental potential of precursor cells, however, was suggested by the observed decrease in the number of different populations generated during each 5-hr period from ED 4 to 8, and supported also by dissociated cell culture experiments investigating the fate of cells born at different developmental stages. Microenvironmental influences were tested in vitro using cells windowed-labeled in ovo for 5 hr on ED 5. After spending at least 72 hr within the retina before their isolation for culture, these cells mimicked their in vivo fate, giving rise predominantly to nonphotoreceptor neurons; a completely different behavior was observed when the cells were isolated after shorter exposures to the retinal microenvironment, when they gave rise predominantly to photoreceptors. Together with data demonstrating that differential cell death cannot account for these results, our results are consistent with the hypothesis that cell fate determination occurs after the time of terminal mitosis.

Benestad, H. B. and A. Reikvam (1975). "Diffusion chamber culturing of haematopoietic cells: methodological investigations and improvement of the technique." Exp Hematol **3**(4): 249-60.

Mouse bone marrow cells were cultured to determine some basic characteristics of the diffusion chamber (DC) technique. The main findings were as follows: (i) Incorporated 3H-thymidine was conveniently measured after deposition of a cell sample on glass fibre discs followed by methanol

washing. Varying the specific activity from approximately 2-approximately 20 Ci/mmole did not affect the relationship between DC cellularity and isotope incorporation. Isotope uptake was similar regardless of whether 3H-thymidine of high or low specific activity had been used. (ii) Higher cell yields were obtained when Millipore or Acropor filters were heat-sealed rather than glued to the plastic rings, when Millipore filters were moistened before DC filling, when we omitted gluing the closing plugs, and when we used an average pore size of 0.22 mum for the DC walls rather than one of 0.10 mum or 0.45 mum. Varying the ring thickness from 2.0-2.5 mm did not impair the cell growth, nor did removal of a softening agent from the plastic rings improve it. (iii) More cells were retrieved from DC carried by young rather than by older mice. Results were not influenced by sex or strain differences between the donor and the host or by the number of implants, whether single or double, within an individual host. (iv) Adding Ficoll to the pronase solution increased the yield of viable CFU-C. (V) Diffusion rate through the DC walls declined with increasing period of culturing, so that i.p. 3H-thymidine is not a flash label for 7-day cultures, for example. The great variability of 3H-thymidine diffusion into i.p. DC was markedly reduced by in vitro exposure to the unopened DC to the isotope. (vi) DC could be incubated in vitro in a medium devoid of protein for at least 6 hours without a fall in 3H-thymidine incorporation rate or CFU-C content, provided that pH was kept constant.

Bergan, R., F. Hakim, et al. (1996). "Electroporation of synthetic oligodeoxynucleotides: a novel technique for ex vivo bone marrow purging." Blood **88**(2): 731-41.

Recent data suggest that tumor cells contaminating reinfused bone marrow may contribute to relapse in patients undergoing autologous bone marrow transplantation. Purging strategies that are able to remove these contaminating tumor cells need to be developed. This study describes how electroporation (EP) can be used to improve intracellular delivery of synthetic antisense oligodeoxynucleotides (ODNs), thereby enhancing their ability to suppress a target protein. Antisense ODNs that were introduced into cells by EP led to immediate suppression of targeted c-myc protein; this was associated with rapid cell death in the diffuse histiocytic lymphoma, U937; Burkitt's lymphoma, ST486; breast carcinoma, MCF-7; and Ewing's sarcoma, CHP-100, cell lines. Electroporation was found to have little or no detrimental effect on cells responsible for murine hematopoietic long-term reconstitution as determined from in vivo competitive repopulation studies. Using human c-myc-directed antisense ODNs as a model for the application of this approach to bone marrow purging, selective killing of human lymphoma U937 cells relative to normal human bone marrow cells was shown in cell mixing studies. In vivo studies were performed in which a survival advantage was shown for athymic mice that were inoculated with antisense-treated U937 cells as opposed to control cells. These studies suggest that EP of bone marrow may be of use in enhancing intracellular delivery of a variety of molecular/pharmaceutical agents. Taken together, these data suggest that the use of electroporation to enhance delivery of antisense ODNs is a promising new approach towards ex vivo bone marrow purging.

Berthier, R., A. Kaufmann, et al. (1983). "Cryopreservation of human bone marrow cells by a modification of the two-step cooling technique." Cryobiology **20**(6): 637-43.

The first attempt to freeze human bone marrow cells with a two-step cooling method is reported. A simple and reliable way of obtaining stable first-step subzero freezing baths is described. One-milliliter samples each containing 20 X 10(6) bone marrow cells and 10% Me2SO were frozen in polypropylene cryotubes. Using these experimental conditions, the optimal freezing temperature was found to be in the range of -36 to 37.5 degrees C for BM progenitor cell (GM-CFC, CFUE, and BFUE) survival. Such temperatures were easily obtained in stable sludges of anisole or K2CO3 eutectic solution in water. The optimal holding time was 20 min before plunging tubes into liquid nitrogen. Similar or improved progenitor cell recoveries were observed compared with the conventional cooling technique. Adaptation of this two-step technique for the freezing of large volumes of BM cells for autografting is under investigation.

Berthold, F., R. Schumacher, et al. (1989). "Removal of neuroblastoma cells from bone marrow by a direct monoclonal antibody rosetting technique." Bone Marrow Transplant **4**(3): 273-8.

A one-step direct monoclonal antibody rosetting technique is described for removal of neuroblastoma cells from bone marrow. Two monoclonal antibodies (MoAbs) (BW 575, BW 625) were directly coupled to ox red blood cells by use of CrCl3. The IgG1 antibody BW 575 detects a 95-kD neuroblastoma cell-associated glycoprotein and the IgG3 antibody BW 625 recognizes the ganglioside GD 2. After coupling MoAbs to the erythrocytes, specific strong and stable rosettes were formed with neuroblastoma cells and effectively separated from mononuclear cells using density gradient centrifugation. A total of 1.5% IMR5 neuroblastoma

cells were reliably removed from mononuclear cells beyond the limit of detection (less than 0.01%) as judged by tetanus toxin labeling. No impairment of stem cell growth (CFU-GM, BFU-E, CFU-GEMM, CFU-M) was observed. Recovery rate of mononuclear cells ranged between 35 and 69%. A red blood cell/nucleated cell ratio more than 50:1 resulted in increased loss of mononuclear cells and a ratio less than 30:1 in incomplete neuroblastoma cell removal. Using indirect rosettes the purging efficacy was lower and the mononuclear cell loss higher. We conclude that the direct monoclonal antibody rosetting technique may be a technically simple and effective alternative purging method for neuroblastoma patients, which is applicable even in cases demonstrating weak expression of one antigen.

Bertram, H., H. Mayer, et al. (2005). "Effect of donor characteristics, technique of harvesting and in vitro processing on culturing of human marrow stroma cells for tissue engineered growth of bone." Clin Oral Implants Res **16**(5): 524-31.

The aim of this study was to assess the effect of donor characteristics and the technique of harvesting and in vitro processing on the efficacy of culturing of human mesenchymal stem cells (hMSCs) for tissue engineered growth of bone. Cultures of hMSCs were derived from iliac crest bone marrow aspirates (21 donors, age 11-76) and from cancellous bone grafting material (32 donors, age 13-84). Age had no significant effect on the ability to isolate and culture hMSCs, although the failure rate was 55.6% in donors beyond the age of 60, while it varied between 14.3% and 22.2% in donors under 60 years of age. Male and female donors had comparable failure rates (27.3% and 28.6%, respectively). Culturing of hMSCs was successful in 90.4% of marrow aspirates from 21 donors and in 62.5% of cancellous bone specimens from 35 donors. This difference was statistically significant (P=0.023). Regression analysis confirmed that at simultaneous testing of the three variables, only the source of cells significantly affected the result (P=0.043). Morphological evaluation of the unfractionated primary population showed a change in cell shape of the adherent cells from a triangular into thin spindle-shaped elongated form, which remains until confluence. When the cultures were exposed to osteoinductive medium, various morphotypes expressing different levels of alkaline phosphatase and secreting different amounts of mineral were evident. Morphology of marrow stroma cells (MSCs) from marrow aspirates was not different from MSCs derived from cancellous bone specimens. Expression of osteogenic markers in MSCs as shown by PCR as well, did not differ between the two sources. It is concluded that marrow aspirates and cancellous bone specimens produce comparable populations of MSCs. However, bone marrow aspirates from donors under the age of 60 years rather than cancellous bone chips are favourable for isolation and expansion of hMSCs for tissue engineered growth of bone.

Bitsch, R. G., C. Heisel, et al. (2007). "Femoral cementing technique for hip resurfacing arthroplasty." J Orthop Res **25**(4): 423-31.

The resurgence of metal-metal bearings has renewed interest in hip resurfacing, but a paucity of information exists regarding femoral cementing technique. We developed a laboratory model in which 72 open-cell foam specimens were used to simulate bone. Analyses of two cement viscosities, two foam porosities, and six cementing techniques were performed: manual cement application only, manual application and filling of one quarter of the component with cement, filling of half of the component, manual application and half component filling, full component filling, and manual application and full component filling. For manual application, cement was pressurized into the foam by rolling the finger tips. For component filling, a defined quantity of cement was poured into the component before pressing it onto the foam. Specimens were cut into quarters, and cement penetration was quantified in seven areas: top, chamfer, wall, interior area, and proximal, medial, and distal stem. The manual technique showed a 3-mm thick, even cement penetration of the outer fixation surface (top = 26 +/- 0 mm(2), chamfer = 14.9 +/- 0.2 mm(2), wall = 55.6 +/- 5.2 mm(2)). None of the other techniques showed a significantly higher penetration in these areas. Large differences were found between all techniques at the medial stem (27.7 +/- 17.5 mm(2), $p < 0.001$) and the interior area (128.5 +/- 69.6 mm(2), $p = 0.013$). An increasing degree of penetration occurred from manual cement application to manual application and full component filling. Sixteen specimens showed incomplete seating, which occurred with all techniques except the manual technique. The manual technique consistently gave an approximately 3-mm thick even cement penetration over the outer fixation area. Pouring any cement into the shell resulted in variable degrees of deeper penetration and a risk of incomplete seating, which have been associated with bone necrosis and early fracture.

Callera, F. and C. M. de Melo (2007). "Magnetic resonance tracking of magnetically labeled autologous bone marrow CD34+ cells transplanted into the spinal cord via lumbar puncture technique in patients with chronic spinal cord injury: CD34+ cells' migration into the injured site." Stem Cells Dev **16**(3): 461-6.

The purpose of this study was to demonstrate the possibility of delivering autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique (LP) in patients with spinal cord injury (SCI). Magnetic resonance imaging provides a noninvasive method for studying the fate of transplanted cells in vivo. Considering these propositions, we studied magnetic resonance tracking of autologous bone marrow CD34(+) cells labeled with magnetic nanoparticles delivered into the spinal cord via LP in patients with SCI. Sixteen patients with chronic SCI were enrolled and divided into two groups; one group got their own labeled-CD34(+) cells injected into the spinal cord via LP (n = 10); the others received an injection, but it contained magnetic beads without stem cells (controls, n = 6). CD34(+) cells were magnetically labeled with magnetic beads coated with a monoclonal antibody specific for the CD34 cell membrane antigen. Magnetic resonance images were obtained by a standard turbospin echo-T2 weighted sequences before and 20 and 35 days after post-transplantation. The median number of CD34(+) cells injected via LP was 0.7 x 10(6) (range 0.45 to 1.22 x 10(6)). Magnetically labeled CD34(+) cells were visible at the lesion site as hypointense signals in five patients of the labeled-CD34(+) group 20 and 35 days after transplantation; these signals were not visible in any patient of the control group. We suggested for the first time that autologous bone marrow CD34(+) cells labeled with magnetic nanoparticles delivered into the spinal cord via LP technique migrated into the injured site in patients with chronic SCI.

Cardoso, A. A., S. M. Watt, et al. (1995). "An improved panning technique for the selection of CD34+ human bone marrow hematopoietic cells with high recovery of early progenitors." Exp Hematol **23**(5): 407-12.

The human hematopoietic pluripotent repopulating "stem cell" is thought to be present within a minor subpopulation of CD34+ cells. This has not been definitively shown, although the more primitive CD34+ cell subset contains precursors for all lymphoid and nonlymphoid cell lineages. When purifying CD34+ cells, it is important to recover these early progenitors, which are more strongly immunoadsorbent to the separation devices. Using a commercialized panning system (AIS CELLector flasks), we observed that a high degree of purity requires a thorough washing procedure so that cells not binding or weakly binding to CD34 antibodies are removed. High recoveries can be obtained if the adherent cells are then efficiently detached by a 2-hour incubation in culture medium without added cytokines. In this way, we can routinely obtain 93.5 +/- 3.4% purity of CD34+ cells with a 74% yield of the multipotent colony-forming units (CFU-GEMM). Complete recovery of the putative "stem cell," or at least the early progenitor cell compartment (CD34+ CD38low/- CD34+ Thy-1+ cells), is also obtained. More than 30% of these cells can generate day-14 colonies in vitro. Comparable results were obtained when the separation was scaled up for clinical application using appropriate large-scale devices. The various incubation times of the procedure can be easily adjusted to the work schedule. This renders the procedure easy to handle, efficient, safe, and, because the cells can be observed under light microscopy, easy to control.

Carlsson, M. and M. Saeed (2008). "Intracoronary injection of contrast media maps the territory of the coronary artery: an MRI technique for assessing the effects of locally delivered angiogenic therapies." Acad Radiol **15**(11): 1354-9.

RATIONALE AND OBJECTIVES: The effects of locally delivered angiogenic factors or stem cells on the coronary artery perfusion territory are not well defined. Therefore, the aim of this study was to determine the ability of the selective injection of magnetic resonance contrast media (MR-CM) to map and quantify the territories of the major coronary arteries. MATERIALS AND METHODS: Selective coronary catheterization (n = 16 pigs) was performed under x-ray and magnetic resonance imaging (MRI) fluoroscopy in an x-ray and magnetic resonance suite. Catheters were placed in the left anterior descending (LAD), circumflex, or right coronary artery. The coronary perfusion territories were mapped by the intracoronary injection of MR-CM using first-pass perfusion (FPP) and early contrast-enhanced (CE) MRI. Cine MRI was used to quantify left ventricular (LV) mass. In 12 animals, the LAD coronary artery was occluded by microspheres to create infarctions. Infarct size was measured on delayed enhanced (DE) MRI after the intravenous injection of MR-CM. RESULTS: X-ray and magnetic resonance fluoroscopy were successfully used to catheterize the coronary arteries. The perfusion territories of the coronary arteries were defined as hyperenhanced regions on FPP and CE MRI. The LAD coronary artery territory was 33.7 +/- 2.2% of LV mass on FPP MRI and 33.0 +/- 2.3% on CE MRI (P = .63). Bland-Altman analysis showed close agreement between the two methods (0.7 +/- 5.0%). DE MRI demonstrated the infarcted myocardium as hyperenhanced subregions of the perfusion territory (7.5 +/- 1.2% of LV mass). CONCLUSIONS: Interventional cardiac x-ray and magnetic resonance fluoroscopy can be used to map and quantify the perfusion territory of each coronary artery. This experimental method can be

used before and after the local delivery of angiogenic factors and stem cell therapy to determine their efficacy.

Casey, T. T., J. B. Cousar, et al. (1988). "A simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues." Am J Pathol **131**(2): 183-9.

Routine fixation and paraffin embedding destroys many hematopoietic and lymphoid differentiation antigens detected by flow cytometry or frozen section immunohistochemistry. On the other hand, morphologic evaluation is difficult in flow cytometric or frozen section studies. A simplified three-step plastic embedding system using acetone-fixed tissues embedded in glycol-methacrylate (GMA) resin has been found to provide both excellent morphologic and antigenic preservation. With our system, a wide variety of antigens are detected in plastic sections without trypsinization or prolonged embedding procedures; pan-B (CD19, CD22), pan-T (CD7, CD5, CD3, CD2), T-subset (CD4, CD8, CD1, CD25) markers as well as surface immunoglobulin and markers for myeloid and mononuclear-phagocyte cells are preserved. In summary, modifications of plastic embedding techniques used in this study simplify the procedure, apparently achieve excellent antigenic preservation, and facilitate evaluation of morphologic details in relation to immunocytochemical markers.

Cedarbaum, J. M. and G. K. Aghajanian (1978). "Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique." J Comp Neurol **178**(1): 1-16.

Afferent connections to the rat locus coeruleus (LC), which contains exclusively noradrenergic neurons, have been traced using the technique of retrograde transport of horseradish peroxidase (HRP). In order to ensure accurate placement of adequate amounts of HRP in the LC, a microiontophoretic delivery technique coupled with single cell recording was employed. The use of electro physiological "landmarks" as aids in placing the injections is described. Following HRP injections into the LC, forebrain structures containing labelled neurons included the insular cortex, the central nucleus of the amygdala, the medial, lateral and magnocellular preoptic areas, the bed nucleus of the stria terminalis, and the dorsomedial, paraventricular and lateral hypothalamic areas. In the brainstem reactive neurons were observed in the central grey substance, the reticular formation, the raphe, vestibular, solitary tract and lateral reticular nuclei. In particular, the areas of catecholamine cell groups A1, A2 and A5 appeared to contain many reactive cells. Labelled neurons were also observed in the fastigial nuclei and in the marginal zones of the dorsal horns of the spinal cord. This pattern of afferent innervation supports suggestions for a role for the LC in behavioral arousal mechanisms and autonomic regulation.

Cortes, J. L., F. Cobo, et al. (2007). "Evaluation of a laser technique to isolate the inner cell mass of murine blastocysts." Biotechnol Appl Biochem **46**(Pt 4): 205-9.

hESCs (human embryonic stem cells) are pluripotent cells derived from the ICM (inner cell mass) of blastocysts that can be used to derive several kinds of cells of the human body for the treatment of some previously untreated diseases. In considering the future use of hESCs in regenerative medicine and cell-therapy programmes, several research centres have begun projects involving the derivation of hESC lines using spare human embryos from IVF (in vitro fertilization) cycles. In some stem-cell banks, such as ours, the law also permits us to obtain these cell lines. The low availability of spare IVF human embryos, and the low rate of success in the derivation of hESC lines, give these embryos a great research value that limits experiments with new techniques. The use of murine embryos would be a good model with which to do research to discover the best methodologies to use in order to derive new hESC lines. The aim of the present study was to evaluate a new method of isolation of the ICM and derivation of ESC lines in a murine blastocyst model using laser drilling to eliminate the trophectoderm cells and compare it with the usual control method consisting of culturing the whole murine blastocyst. We also tested the adhesion and growth of primary colonies of mESCs (murine ESCs) over two different growth surfaces, namely an MEF (inactive murine fibroblastic feeder layer) or gelatin-coated dishes, in order to achieve the best culture conditions for future derivation of human stem-cell lines for application in human transplantation.

Costantini, S., E. Di Capua, et al. (2006). "The management of severe vaginal obstruction from genital chronic graft-versus-host disease: diagnosis, surgical technique and follow-up." Minerva Ginecol **58**(1): 11-6.

AIM: Chronic graft-versus-host disease (GVHD) is one of the most important systemic late-onset complications of haematopoietic stem cell transplantation. Gynaecological manifestations are considered relatively rare, and involve lower genital tract skin and mucosa, causing vulvar scarring, vaginal stenosis, affecting the patients' sexual life, and

leading to more serious complications as haematocolpos, haematometra and abscesses. Genital GVHD can be treated with topical therapy when mild to moderate disease is present. Surgery is indicated in advanced and complicated cases to restore normal anatomy. The aim of this study is to propose a standard approach for the management of such condition when medical therapy is not effective. METHODS: From May 2000 to January 2002, 8 patients suffering from genital chronic GVHD were operated in our institution. We describe clinical and sonographic presurgical assessment, simple or ultrasonographic guided surgical technique, postsurgical treatment and follow-up. RESULTS: Surgery was completely successful in restoring genital anatomy in all the cases. Mean follow-up was of 17 months (6 to 38 months). Two patients early discontinued the postsurgical treatment. At 1 month complete vaginal patency was found in 6 cases, weak partial adhesions in 1 case, while 1 patient refused follow-up. Eventually, complete vaginal patency was maintained in 2 cases, and partial adhesions were found in 5 cases. CONCLUSIONS: The combined use of clinical examination and endosonography provides a precise assessment of the level of the obstruction. The surgical technique here described is feasible and successful in restoring normal anatomy, while long term results seem related to the compliance towards the postsurgical treatment.

Court, E. L., K. Davidson, et al. (2001). "C-kit mutation screening in patients with acute myeloid leukaemia: adaptation of a Giemsa-stained bone-marrow smear DNA extraction technique." Br J Biomed Sci **58**(2): 76-84.

The scarcity of viable tissue samples for leukaemia research is widely recognised and currently restrictive. Archival bone-marrow smears present a valuable resource that can be exploited easily for mutational analysis. Here, a modified technique to extract DNA is described, and used subsequently for mutation/polymorphism screening of the stem-cell factor receptor proto-oncogene c-kit in 23 patients with acute myeloid leukaemia (AML). The selected method was straightforward and used bone-marrow material scraped from periodic acid-Schiff, sudan black B and May-Grunwald/Giemsa-stained preparations, and treated initially with proteinase K prepared in digestion buffer to digest all proteinaceous matter. Following incubation, saturated sodium chloride was added and DNA extracted from the supernatant by phenol/chloroform/isoamyl alcohol treatment. Retrieved DNA was precipitated with ethanol at -20 degrees C overnight, washed with 95% ethanol, air-dried, resuspended using purite water and stored at -20 degrees C prior to use in mutational analysis. The extraction method described was compared with a commercial reagent for combined DNA, RNA and protein isolation using cryopreserved cells from 20 patients with AML. The quality of extracted DNA isolated by the two methods was comparable by polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) techniques. Bone-marrow biopsies are performed regularly on each AML patient to monitor the disease; therefore, an extraction method using this resource could liberate a valuable source of DNA for study (e.g. molecular investigations, including mutation/polymorphism screening etc.). This would allow fresh and programme-frozen cells to be reserved for those investigations requiring intact, viable cells. The use of archived bone-marrow smears would permit vast increase in the scope for retrospective testing and large-scale analyses.

Dal Cortivo, L., P. H. Cottu, et al. (2001). "Residual tumor cell contamination in peripheral blood stem cells collections of 117 breast cancer patients evaluated by immunocytochemical technique." J Hematother Stem Cell Res **10**(6): 855-62.

During the last years, high-dose chemotherapy and hematopoietic stem cell support have been thought to improve the treatment of poor-prognosis breast cancer. Nevertheless, the question remained as to whether the reinfusion of contaminating residual malignant cells could contribute to relapse. By using an immunocytochemical method, we have analyzed the tumor cell contamination of peripheral blood stem cells (PBSC) collected from advanced breast cancer patients. We studied 153 PBSC samples from 117 stage III and IV breast cancer patients and compared two screening methods-the usual microscopic observation and the automated cellular image analysis system (ACIS-assisted) screening. With manual observation, we found that 7 of 117 patients (5.9%) presented circulating epithelial tumor cells in 9 of 153 (5.8%) PBSC analyzed, whereas automated screening allowed positive detection in 15 of the same 117 patients (12.8%) and in 18 of the 153 PBSC (11.7%). No difference was found between presence or absence of circulating tumor cells and previous chemotherapy treatment (p = 0.5) or stage TNM (p = 0.13) in this group of poor-prognosis breast cancer. We did not find incidence of infusion of contaminated PBSC on overall survival or time to progression.

Dulic-Sills, A., M. J. Blunden, et al. (2006). "New flow cytometric technique for the evaluation of circulating endothelial progenitor cell levels in various disease groups." J Immunol Methods **316**(1-2): 107-15.

Circulating endothelial progenitor cells (EPC) localise to sites of ischaemia and play a role in vascular repair and re-endothelialisation of injured blood vessels. Low levels of EPCs are associated with cardiovascular disease (CVD) in the general population. It is not clear at present whether and how the numbers of circulating EPCs vary in diseases other than CVD. We have enumerated EPCs by the flow cytometric analysis of whole blood by using a novel cocktail of monoclonal antibodies. This consisted of CD2FITC, CD13FITC and CD22FITC to eliminate non-progenitor cells and VEGFR2PE and CD133-streptavidin-PeCy7 to include only EPCs. We analysed 250 patients with varying stages of uraemia, 36 patients with inflammatory bowel disease (IBD) and 9 patients with acute respiratory distress syndrome and compared this to 74 healthy controls. Using flow cytometry we were able to measure the circulating levels of EPCs, with a result available within hours of the sample being obtained. Circulating EPC numbers vary in different patient groups and healthy controls. In uraemic patients, irrespective of disease severity, there are lower numbers of circulating EPC numbers compared to normal controls (46.6+/-3.7 vs. 66.1+/-4.7; p=0.03). This new technique provides a means of monitoring patients and shows a reduction in circulating EPCs in uraemic patients; this abnormality may be a target of novel therapies.

Duncan, D., J. P. Rubin, et al. (2009). "Refinement of technique in injection lipolysis based on scientific studies and clinical evaluation." Clin Plast Surg **36**(2): 195-209, v-vi; discussion 211-3.

The unusual evolution of the practice of injection lipolysis has generated doubt regarding its safety and efficacy among many physicians. During the early years of this decade, mesotherapy was practiced by a few physicians, but the practice was not widespread. Paramedical practitioners and business developers saw the market potential for nonsurgical fat reduction, and the practice of injection lipolysis was packaged and sold before the mechanism of action was understood. Because of the early lack of scientific research and understanding of the limitations of injection lipolysis, many unsuitable patients were treated with this modality. To better understand the way injection lipolysis works, the inclusion and exclusion criteria for patients desiring treatment, and an accurate clinical evaluation format for potential treatment regions, a series of scientific studies was performed in 2007 and early 2008. These studies included a serial histopathology evaluation of treated patients over time, a stem cell study performed with the McGowan Research Institute in Pittsburgh, an animal study performed in conjunction with the Colorado State University veterinary school, and a prospective multicenter clinical trial using injection lipolysis in the back roll region. The purpose of these studies was to determine the way injection lipolysis works, how modifications of the formula and technique change the outcome, the role of each constituent component of various formulas, and the degree of fat reduction and skin retraction that is attainable with these treatments. The influence of depth of injection, distance between injection points, volume of injection, and ratios of constituent components was studied. The degree of topographic contour correction and the amount of volume reduction were evaluated. Following a review of these recent studies, an updated recommendation for the clinical practice of injection lipolysis was formulated.

Dyson, P. G., J. Q. Ho, et al. (1994). "The use of the APAAP technique as a rapid indicator of peripheral blood progenitor cell levels." Pathology **26**(3): 296-300.

Rapid and sustained engraftment following autotransplantation with peripheral blood stem cells (PBSC) depends on adequate numbers of stem cells and progenitor cells. In this study we have compared the number of myeloid progenitor cells quantitated using the colony forming units-granulocyte macrophage (CFU-GM) clonogenic assay with the number of CD34+ cells estimated both by flow cytometry and by the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. We have analysed 15 peripheral blood mononuclear cells (PBMNC) samples from 13 normal subjects and 179 PBMNC from 32 patients undergoing PBSC harvests during the recovery phase of high dose cyclophosphamide chemotherapy. The number of CD34+ cells measured by the APAAP technique correlated well with the number of CD34+ cells measured by flow cytometry (r = 0.727, p = 0.0001), and also with the number of CFU-GM measured in the clonogenic assay (r = 0.721, p = 0.0001). The APAAP method provides a rapid, reliable measure of progenitor cell levels that can be used to monitor the optimal time to harvest peripheral blood stem cells (PBSC), and to estimate the marrow repopulating ability (MRA) of stem cell preparations used for transplantation.

Eca, L. P., R. B. Ramalho, et al. (2009). "Comparative study of technique to obtain stem cells from bone marrow collection between the iliac crest and the femoral epiphysis in rabbits." Acta Cir Bras **24**(5): 400-4.

PURPOSE: To assess the technique for the collection of rabbit bone marrow stem cells from different regions to be used as an experimental model

in regenerative medicine. METHODS: Thirty rabbits were allocated into 2 groups: GROUP A, n=8, animals that underwent bone marrow blood (BMB) harvesting from the iliac crest; and GROUP B: including 22 rabbits that underwent BMB harvesting from the femur epiphysis. After harvesting, mononuclear cells were isolated by density gradient centrifugation (Ficoll - Histopaque). The number of mononuclear cells per ml was counted in a Neubauer chamber and cell viability was checked through Tripan Blue method. RESULTS: Harvesting from the iliac crest yielded an average of 1 ml of BMB and 3,6.10(6) cells/ml over 1 hour of surgery, whereas an average of 3ml of BMB and 11,79.10(6) cells./ml were obtained in 30 min from the femur epiphysis with a reduced animal death rate. CONCLUSION: The analysis for the obtention of a larger number of mononuclear cells/ml from rabbit bone marrow blood was more satisfactory in the femur epiphysis than in the iliac crest.

Erber, W. N., H. Asbahr, et al. (1992). "Peanut agglutinin (lectin from Arachis hypogaea) binding to hemopoietic cells: an immunophenotypic study using a biotin streptavidin technique." Pathology **24**(3): 173-6.

The lectin peanut agglutinin (PNA) was used to study the surface carbohydrate expression of galactose beta 1, 3, N-acetylgalactosamine by normal and malignant hemopoietic cells. Immunostaining was performed using biotinylated PNA and a streptavidin-alkaline phosphatase staining technique on 78 patients. The study was undertaken to enlarge on previous reports of lectin binding to cells of hemopoietic origin and to establish the potential role of biotinylated PNA as a component of an immunotoxin for in vitro purging of bone marrow in patients with multiple myeloma. In normals only monocytes, macrophages, centroblasts and plasma cells showed reactivity. Of the hematological malignancies, all cases of multiple myeloma were positive and non-Hodgkin's lymphoma cases with a large cell component had positive centroblasts. Two of 5 cases of acute myelomonocytic leukemia, one case of chronic myelomonocytic leukemia and one case of pleomorphic T cell non-Hodgkin's lymphoma showed PNA positive neoplastic cells. The reactivity of biotinylated PNA with centroblasts and plasma cells suggests that it may be of potential value when linked to a streptavidin-ricin conjugate in the in vitro purging of bone marrow of patients with multiple myeloma prior to autologous bone marrow transplantation.

Farnie, G., R. B. Clarke, et al. (2007). "Novel cell culture technique for primary ductal carcinoma in situ: role of Notch and epidermal growth factor receptor signaling pathways." J Natl Cancer Inst **99**(8): 616-27.

BACKGROUND: The epidermal growth factor receptor (EGFR) and Notch signaling pathways have been implicated in self-renewal of normal breast stem cells. We investigated the involvement of these signaling pathways in ductal carcinoma in situ (DCIS) of the breast. METHODS: Samples of normal breast tissue (n = 15), pure DCIS tissue of varying grades (n = 35), and DCIS tissue surrounding an invasive cancer (n = 7) were used for nonadherent (i.e., mammosphere) culture. Mammosphere cultures were treated at day 0 with gefitinib (an EGFR inhibitor), DAPT (N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester) (a gamma-secretase inhibitor), or Notch 4-neutralizing antibody. Mammosphere-forming efficiency (MFE) was calculated by dividing the number of mammospheres of 60 microm or more formed by the number of single cells seeded and is expressed as a percentage. The Notch 1 intracellular domain (NICD) was detected immunohistochemically in paraffin-embedded DCIS tissue from 50 patients with at least 60 months of follow-up. All statistical tests were two-sided. RESULTS: DCIS had a greater MFE than normal breast tissue (1.5% versus 0.5%, difference = 1%, 95% confidence interval [CI] = 0.62% to 1.25%, P<.001). High-grade DCIS had a greater MFE than low-grade DCIS (1.6% versus 1.09%, difference = 0.51%, 95% CI = 0.07% to 0.94%, P = .01). The MFE of high-grade DCIS treated with gefitinib in the absence of exogenous EGF was lower than that of high-grade DCIS treated with mammosphere medium lacking gefitinib and exogenous EGF (0.56% versus 1.36%, difference 0.8%, 95% CI = 0.33% to 1.4%, P = .004). Increased Notch signaling as detected by NICD staining was associated with recurrence at 5 years (P = .012). DCIS MFE was reduced when Notch signaling was inhibited using either DAPT (0.89% versus 0.51%, difference = 0.38%, 95% CI = 0.2% to 0.6%, P<.001) or a Notch 4-neutralizing antibody (0.97% versus 0.2%, difference = 0.77%, 95% CI = 0.52% to 1.0%, P<.001). CONCLUSION: We describe a novel primary culture technique for DCIS. Inhibition of the EGFR or Notch signaling pathways reduced DCIS MFE.

Fatima, A., V. S. Sangwan, et al. (2006). "Technique of cultivating limbal derived corneal epithelium on human amniotic membrane for clinical transplantation." J Postgrad Med **52**(4): 257-61.

BACKGROUND: The technique of transplantation of cultivated limbal epithelium rather than direct limbal tissue isa novel method of "cell therapy" involved in reconstructing the ocular surface in severe limbal stem cell deficiency [LSCD], caused

by chemical burns. AIM: To describe a simple feeder-cell free technique of cultivating limbal epithelium on human amniotic membrane[HAM]. MATERIALS AND METHODS: The limbal tissues (2 mm) were harvested from patients with LSCD. These tissues were proliferated in vitro on HAM supplemented by human corneal epithelial cell medium and autologous serum. Cultures covering more > or = 50% area of 2.5 x 5 cm HAM were considered adequate for clinical use. The cultured epithelium was characterized by histopathology and immunophenotyping. RESULTS: A total of 542 cultures out of 250 limbal tissues were cultivated in the laboratory from January 2001 through July 2005. The culture explants showed that clusters of cells emerging from the edge of the explants in one-three days formed a complete monolayer within 10-14 days. In 86% of cultures (464 of 542), the growth was observed within one-two days. Successful explant cultures were observed in 98.5% (534 of 542 cultures) with 91% explant cultures showing an area of > or = 6.25 cm2 (6.25 - 12.5 cm2 range). The cultivated epithelium was terminated between 10-14 days for clinical transplantation. The problems encountered were inadequate growth (2 of 542) and contamination (2 of 542). CONCLUSIONS: We demonstrate a simple technique of generating a sheet of corneal epithelium from a limbal biopsy. This new technique could pave the way for a novel form of cell therapy.

Figuerres, E., M. Olszewski, et al. (2001). "A flow cytometric technique using thiazole orange to detect platelet engraftment following pediatric stem-cell transplants." Cytotherapy **3**(4): 277-83.

BACKGROUND: Thiazole orange (TO) is a nucleic-acid-specific dye that enters cells without pretreatment. When it binds to either RNA or DNA, there is an increase in fluorescence emission. This property has been utilized to measure the amount of newly released platelets using flow cytometry. These newly released platelets differ from more mature platelets because they still contain residual amounts of RNA, and have become known as reticulated platelets. METHODS: Peripheral blood samples were collected at least 48 h following platelet infusion. For validation, manual reticulocyte counts obtained in the laboratory were compared with results obtained using TO and flow cytometry. Following validation, experiments using platelet-rich plasma were performed to evaluate the presence of reticulated platelets in the sample. RESULTS: Validation experiments comparing the manual and flow cytometric reticulocyte counts gave a strong relationship between the two values (r(2) = 0.92). Reticulated platelet studies performed on platelet-rich plasma samples yielded the following results. Patients who did not engraft within 4 days were significantly different from patients who did engraft within 4 days, idiopathic thrombocytoperic purpura (ITP) patients, and donor platelet segments (all P < 0.0008). Patients who engrafted within 4 days, ITP patients, and donor platelet segments were all statistically similar (all P > 0.08). DISCUSSION: The statistical difference between patients who did engraft within 4 days and those who did not suggests that this method could have an important clinical impact in determining those patients who are still in need of platelet support. However, great care must be taken when performing and analyzing the results.

Flores, M. G., B. Holm, et al. (2005). "A technique of bone marrow collection from vertebral bodies of cynomolgus macaques for transplant studies." J Surg Res **124**(2): 280-8.

BACKGROUND: Strategies to induce donor-specific allograft tolerance are best tested in preclinical models developed in nonhuman primates (NHPs). Most protocols prepare the recipient by infusing hematopoietic cells from the donor. We report here a procedure to isolate and characterize large numbers of bone marrow cells (BMCs) from cynomolgus monkeys (cynos) that can then successfully be transplanted into conditioned recipients. MATERIALS AND METHODS: Vertebral columns of five cynos were excised en bloc and separated into individual vertebrae. The cancelous bone was extracted with a core puncher, fractionated, filtered, centrifuged, and resuspended in transplantation media before being analyzed by flow cytometry. In two instances, the collected BMCs were reinfused into allogeneic recipients preconditioned with a nonmyeloablative regimen. Chimerism was monitored using short-tandem repeat analysis. RESULTS: The mean total BMCs yield was 25.5 x 10(9) (range of 4.00 x 10(9) to 59 x 10(9)) with mean cell viability of 93.4% (range: 90-96%). CD34+ cells and CD3+ cells averaged 0.34 and 3.91% of total BMCs, respectively. This resulted in absolute cell number yields of 1.02 x 10(8) and 1.15 x 10(9) for CD34+ and CD3+ cells, respectively. Graft-versus-host disease was absent in both bone marrow infused animals, and a maximum level of chimerism of 18% was detected at 3 weeks after BMCs infusion. CONCLUSION: We present here the first detailed report of a procedure to retrieve and characterize large numbers of BMCs from vertebral bodies of cynos and demonstrate that cells collected with this technique have the capability of engrafting in allogenic recipients.

Fukano, H., N. Hayatsu, et al. (2006). "A technique to enzymatically construct libraries which express short

hairpin RNA of arbitrary stem length." Biochem Biophys Res Commun **347**(3): 543-50.

Short interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) usually used for RNA interference (RNAi) are double-stranded RNAs (dsRNAs) of 21 base pairs. However, siRNAs and shRNAs of longer stem length have been reported to show more potent gene silencing. Here, we report a new technique to enzymatically construct shRNA libraries containing clones from firefly luciferase cDNA and Jurkat cDNA. The technique allowed the efficacious generation of shRNAs of arbitrary stem length as desired, providing the clones which potently silenced the specified gene expression and presenting a high efficiency rate of gene silencing. Our results indicate that the technique permits the rapid, efficient, and low-cost preparation of genomewide shRNA expression libraries not only for humans and mice but also for sorts of biological species and that the relevant libraries are applicable for the search of genes related to phenotype changes and of new targets for drug discovery.

Furuta, A., S. Miyoshi, et al. (2006). "Pulsatile cardiac tissue grafts using a novel three-dimensional cell sheet manipulation technique functionally integrates with the host heart, in vivo." Circ Res **98**(5): 705-12.

We devised a method of fabricating easily transplantable scaffoldless 3D heart tissue, made with a novel cell-sheet (CS) technology from cultured cardiomyocytes using a fibrin polymer coated dish. In the present study, we tested in vivo electrical communication which is essential for improving heart function between the host heart and the grafted CS. The epicardial surface of the ventricle of an anesthetized open-chest nude rat was ablated by applying a heated metal. Bilayered CS was obtained from neonatal rat primary culture. CS was transplanted onto the injured myocardial surface (sMI) (sMI+sheet group). The rats were allowed to recover for 1 to 4 weeks, to stabilize the grafts. Action potentials (APs) from the excised perfused heart were monitored by the fluorescence signal of di-4ANEPPS with a high speed charge-coupled device camera. The APs were observed under epicardial pacing of the host heart or the CS grafts. The pacing threshold of the current output was measured in the sMI+sheet group and in the nongrafted sMI group at the center of the sMI and in the normal zone (Nz). Bidirectional AP propagation between the sMI and Nz was observed in the sMI+sheet group (n=14), but was blocked at the marginal area of the sMI in the sMI group (n=9). The ratio of the pacing threshold (sMI/Nz) was significantly lower in the sMI+sheet than in the sMI group (3.0+/-0.7, 19.0+/-6.1 respectively P<0.05). There were neither spontaneous nor pacing-induced arrhythmias in these two groups. Bidirectional smooth AP propagation between the host heart and the grafted CS was observed. This finding suggested functional integration of this CS graft with the host heart without serious arrhythmia.

Gerendai, I. and B. Halasz (2000). "Central nervous system structures connected with the endocrine glands. findings obtained with the viral transneuronal tracing technique." Exp Clin Endocrinol Diabetes **108**(6): 389-95.

This review is a summary of recent neuromorphological observations on the existence of multisynaptic neural pathways between the endocrine glands and the central nervous system (CNS) and its structures involved in this pathway. Introduction of the viral transneuronal tracing technique has made possible investigations of multisynaptic connections. The utility of this approach is based on the ability of the neurotropic virus to invade and replicate in neurons, and then gradually infect synaptically linked second-order, third-order. etc. neurons. Injecting the virus into the endocrine glands, this technique was used to identify cell groups in the spinal cord and in the brain which are connected with the adrenal gland, the gonads and the pancreas. Injection of the virus into these organs resulted in viral labeling of neurons in practically identical structures of the CNS including the intermediolateral cell column of the spinal cord, the vagal nuclei and certain other cell groups in the brain stem. In the hypothalamus the most intensive labeling was in the parvocellular part of the paraventricular nucleus and in the telencephalon labeled nerve cells were detected in the amygdala, the bed nucleus of the stria terminalis and in the preoptic area. It is known that the labeled CNS structures are members of descending pathways arising from the hypothalamic paraventricular nucleus or from other cell groups and terminating on neurons of the vagal nuclei and the intermediolateral cell column of the spinal cord. Experimental data support the view that the CNS structures and pathways connected with the endocrine glands are involved in the neural control of these organs.

Gerendai, I., I. E. Toth, et al. (2000). "Central nervous system structures labelled from the testis using the transsynaptic viral tracing technique." J Neuroendocrinol **12**(11): 1087-95.

In the present study, the transneuronal transport of neurotrophic virus technique was used to identify cell groups of the spinal cord and the brain that are transsynaptically connected with the testis. Pseudorabies virus was injected into the testis and after survival times of 3-6 days, the spinal cord and brain were processed immunocytochemically using a

polyclonal antibody against the virus. Virus-infected perikarya were detected in the preganglionic neurones of the spinal cord (T10-L1, L5-S1) and in certain cell groups and areas of the brain stem, the hypothalamus and the telencephalon. In the brain stem, the cell groups and areas in which labelled neurones were present included, among others, the nucleus of the solitary tract, the caudal raphe nuclei, the locus coeruleus and the periaqueductal grey of the mesencephalon. In the hypothalamus, virus infected perikarya were observed in the paraventricular nucleus and in certain other cell groups. Telencephalic structures containing labelled neurones included the preoptic area, the bed nucleus of the stria terminalis, the central amygdala and the insular cortex. These data identify a multisynaptic circuit of neurones in the spinal cord and in the brain which may be involved in the control of testicular functions.

Gerendai, I., I. E. Toth, et al. (2001). "Transneuronal labelling of nerve cells in the CNS of female rat from the mammary gland by viral tracing technique." Neuroscience **108**(1): 103-18.

Using the viral transneuronal tracing technique, the cell groups in the CNS transneuronally connected with the female mammary gland were detected. Lactating and non-lactating female rats were infected with pseudorabies virus injected into the mammary gland. The other group of animals was subjected to virus injection into the skin of the back. Four days after virus injection, infected neurons detected by immunocytochemistry, were present in the dorsal root ganglia ipsilateral to inoculation and in the intermediolateral cell column of the spinal cord. In addition, a few labelled cells could be detected in the dorsal horn and in the central autonomic nucleus (lamina X) of the spinal cord. At this survival time several brain stem nuclei including the A5 noradrenergic cell group, the caudal raphe nuclei (raphe obscurus, raphe pallidus, raphe magnus), the A1/C1 noradrenergic and adrenergic cell group, the nucleus of the solitary tract, the area postrema, the gigantocellular reticular nucleus, and the locus coeruleus contained virus-infected neurons. In some animals, additional cell groups, among others the periaqueductal gray and the red nucleus displayed labelling. In the diencephalon, a significant number of virus-infected neurons could be detected in the hypothalamic paraventricular nucleus. In most cases, virus-labelled neurons were present also in the lateral hypothalamus, in the retrochiasmatic area, and in the anterior hypothalamus. In the telencephalon, in some animals a few virus-infected neurons could be found in the preoptic area, in the bed nucleus of the stria terminalis, in the central amygdala, and in the somatosensory cortex. At the longer (5 days) survival time each cell group mentioned displayed immunopositive neurons, and the number of infected cells increased. The pattern of labelling was similar in animals subjected to virus inoculation into the mammary gland and into the skin. The distribution and density of labelling was similar in lactating and non-lactating rats. The present findings provide the first morphological data on the localization of CNS structures connected with the preganglionic neurons of the sympathetic motor system innervating the mammary gland. It may be assumed that the structures found virus-infected belong to the neuronal circuitry involved in the control of the sympathetic motor innervation of the mammary gland.

Gharaibeh, B., A. Lu, et al. (2008). "Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique." Nat Protoc **3**(9): 1501-9.

This protocol details a procedure, known as the modified preplate technique, which is currently used in our laboratory to isolate muscle cells on the basis of selective adhesion to collagen-coated tissue culture plates. By employing this technique to murine skeletal muscle, we have been able to isolate a rapidly adhering cell (RAC) fraction within the earlier stages of the process, whereas a slowly adhering cell (SAC) fraction containing muscle-derived stem cells is obtained from the later stages of the process. This protocol outlines the methods and materials needed to isolate RAC and SAC populations from murine skeletal muscle. The procedure involves mechanical and enzymatic digestion of skeletal muscle tissue with collagenase XI, dispase and trypsin followed by plating the resultant muscle slurry on collagen type I-coated flasks where the cells adhere at different rates. The entire preplate technique requires 5 d to obtain the final preplate SAC population. Two to three additional days are usually required before this population is properly established. We also detail additional methodologies designed to further enrich the resultant cell population by continuing the modified preplating process on the SAC population. This process is known as replating and requires further time.

Gibellini, D., G. Zauli, et al. (1995). "In situ polymerase chain reaction technique revealed by flow cytometry as a tool for gene detection." Anal Biochem **228**(2): 252-8.

We report a methodology for detecting specific DNA sequences directly inside cells, combining in situ PCR and flow cytometry. This technique is based on in situ PCR performed in the presence of digoxigenin-labeled dUTP to obtain a digoxigenin-labeled amplicon, which is then revealed by an anti-digoxigenin polyclonal antibody directly

conjugated to fluorescein. Fluorescence intensity is next evaluated by flow cytometry. Our experimental models were represented by the lymphoblastoid cell lines 8E5LAV, carrying an integrated HIV-1 DNA proviral copy per cell, and A.301, infected in vitro with HIV-1 (strain IIIB). The technique is described in detail with particular attention to the optimization of critical fixation and permeabilization steps. This method allows not only the detection but also an accurate quantification of the number of positive cells in a background of negative cells. Moreover, it has the potentiality to develop into a multiparametric method for the simultaneous study of specific DNA or RNA sequences and surface or intracellular markers.

Gidron, A., A. Verma, et al. (2005). "Can the stem cell mobilization technique influence CD34+ cell collection efficiency of leukapheresis procedures in patients with hematologic malignancies?" Bone Marrow Transplant **35**(3): 243-6.

A total of 415 leukaphereses in 201 patients stimulated with growth factor (GF; n = 119) or chemotherapy-GF (n = 296) were studied to determine CD34+ cell collection efficiency (CE). The pre-apheresis leukocyte count was 1-93 x 10(9)/l (median 20), and peripheral blood CD34 count (PBCD34) was 1-1104/microl (median 19). The total number of CD34+ cells collected was 4-6531 x 10(6) (median 151); corresponding to 0.1-111.4 x 10(6) (median 2.3) per kg. There was strong correlation between PBCD34 and the number of CD34+ cells collected (r = 0.9; P < 0.0001). CE was 7-145% (median 46). On multiple regression analysis, a higher leukocyte count (P < 0.0001) was the most important predictor of lower CE. CE with leukocytes < 20 was 7-145% (median 53%) compared to 10-132% (median 40%) with leukocyte > or = 20 (P < 0.0001). In all, 61% of the apheresis procedures performed after chemotherapy-GF occurred when leukocytes were < 20 compared to 21% of those performed after GF alone (P < 0.0001). We conclude that mobilizing patients with the combination of chemotherapy and GF rather than GF alone leads to leukapheresis being performed when the leukocyte count is low -- in a range that results in optimum CD34+ cell CE. Autologous stem cells should be mobilized with chemotherapy-GF rather than GF alone whenever possible.

Gilmore, M. J., H. G. Prentice, et al. (1982). "A technique for rapid isolation of bone marrow mononuclear cells using Ficoll-Metrizoate and the IBM 2991 blood cell processor." Br J Haematol **50**(4): 619-26.

Marrow from seven normal donors and patients has been layered onto Ficoll-Metrizoate (FM) under pressure in the IBM 2991 blood cell processor to isolate the mononuclear cell (MNC) population prior to allogeneic transplantation or cryopreservation. This separation method, which takes less than 90 min, is a further development since our previous report detailing the use of the IBM 2991 to produce a concentrated marrow 'buffy coat' for infusion (Gilmore & Prentice, 1981). By adding FM to the system, marrow stem cells are further concentrated in a small volume with removal of unwanted granulocytes and red blood cells. This facilitates in in vitro treatment of marrow with monoclonal antibodies (Granger et al. 1982) or drugs, for either the selective elimination of malignant cells prior to autologous bone marrow transplantation (BMT), or T lymphocytes in an attempt to prevent graft versus host disease (GvHD) in allogeneic BMT. Five of the seven marrows processed by this procedure have thus far been infused into lethally irradiated recipients with engraftment (allogeneic); the other two marrows have been cryopreserved.

Gollackner, B., F. J. Dor, et al. (2003). "Spleen transplantation in miniature swine: surgical technique and results in major histocompatibility complex-matched donor and recipient pairs." Transplantation **75**(11): 1799-806.

BACKGROUND: Spleen transplantation (Tx) between some strains of rodents can lead to donor-specific tolerance either spontaneously or after a short course of immunosuppression. This study developed a surgical technique for spleen Tx in miniature swine to investigate its immunologic impact in a large animal model. METHODS: The preferred surgical technique of spleen Tx (n=8) involved excision of the donor spleen with its vascular pedicle to the aorta and portal vein. Carrel patches of donor aorta and portal vein were anastomosed to the abdominal aorta and inferior vena cava, respectively, of the (splenectomized) recipient. The results in four major histocompatibility complex-matched pairs that were mismatched for the porcine allelic antigen are reported. Two recipients were untreated, one received a 12-day course of cyclosporine A (CsA) alone, and one received thymic irradiation (700 cGy) and CsA. Hematopoietic cell chimerism was followed by fluorescence-activated cell sorter, and graft survival was assessed by histology. RESULTS: Spleen Tx was technically successful. In two untreated pigs, chimerism was detected in the blood (maximum 5% for 17 and 25 days) and lymph nodes (maximum 6% for 28 and 56 days), but both grafts showed histologic rejection by day 28. In two treated pigs, chimerism was present in the blood for 47 and 57 days, and rejection was prevented, with follow-up for 57 and 217 days, respectively. CONCLUSION: Spleen Tx in major histocompatibility complex-matched pairs

treated with CsA+/-thymic irradiation results in prolonged chimerism and is associated with the development of in vivo unresponsiveness to the transplanted spleen.

Gorcs, T., M. Antal, et al. (1979). "An improved cobalt labeling technique with complex compounds." Acta Biol Acad Sci Hung **30**(1): 79-86.

Results of axonal labelings with CoCl2, cobaltous lysine and cobaltic lysine complexes are compared on dorsal roots and nerves of the spinal cord and brain stem in the living frog. The most satisfactory staining of fibres and terminals is given by CoCl2; its application however, is limited by its rather short (6--10 mm) axonal transport. Cobaltous lysine is transported somewhat better, but it gives a poor fibre staining in the spinal cord. The axonal transport of cobaltic lysine is the best, covering a distance of 40--50 mm. Combination of cobaltic lysine with 2--5% dimethyl sulphoxide greatly enhances the axonal uptake of cobalt and extends the distance of transport to 70--80 mm. It is assumed that better transport of cobalt complexes is achieved by their less toxic effect on the nerve cell.

Grossmann, J., J. M. Maxson, et al. (1998). "New isolation technique to study apoptosis in human intestinal epithelial cells." Am J Pathol **153**(1): 53-62.

Intestinal epithelial cells derive from stem cells at the base of the crypt and migrate along the crypt-lumen axis. Their life is terminated as they reach the luminal surface where they detach and are shed. Intestinal epithelial cells show evidence of apoptosis in the region of shedding, and cell death is thought to resemble a form of apoptosis called detachment-induced cell death, or anoikis. Human intestinal epithelial cells die rapidly in vitro due to loss of anchorage during isolation, making primary culture of these cells a goal that has not yet been reached. However, the molecular mechanisms underlying this process of anoikis are largely unknown. In this study, a novel protocol for the rapid, temperature-controlled isolation of highly purified human colonic epithelial cells from surgical specimens is described. Using this method, early molecular events of anoikis in nontransformed epithelial cells were studied. Intestinal epithelial cells were isolated at the beginning of the apoptotic cascade, before the activation of caspase 3 family members and cleavage of poly(ADP-ribose) polymerase and DNA fragmentation. Elucidating the molecular mechanisms of detachment-induced cell death may facilitate the establishment of long-term primary cultures of human intestinal epithelial cells and enhance our understanding of homeostasis in the intestinal epithelium.

Gu, C. M., Y. K. Zhu, et al. (2006). "Knockdown of survivin gene by vector-based short hairpin RNA technique induces apoptosis and growth inhibition in Burkitt's lymphoma Raji cell line." Neoplasma **53**(3): 206-12.

Survivin is the smallest member of mammalian IAP (inhibitor of apoptosis) family. It is ubiquitous during embryonic development but is not expressed in normal post-natal tissues, except the thymus, colonic epithelial cells and CD34+ hematopoietic stem cells. However, its expression is upregulated during neoplastic transformation in both solid organ and hematological malignancies, including leukemia and lymphoma. In this study, we used RNA interference with short hairpin RNA (shRNA) technique to inhibit survivin expression in a Burkitt's lymphoma cell line Raji and validated its effects on apoptosis and cell proliferation. A survivin-shRNA expression vector were constructed and introduced into Raji cells. Expression of survivin mRNA and protein was assessed by RT-PCR and western blot analysis. Apoptosis index of transfected cells was quantified by flow cytometry and cell proliferation was enumerated by trypan blue exclusion. In Raji cells treated with survivin-shRNA expression vector, survivin mRNA levels were significantly reduced by 67.14% (transient transfection) and 64.28% (stable transfection) respectively, compared with control-shRNA treated group and PBS treated group ($p<0.05$). The levels of survivin protein were significantly reduced by 62.50% (transient transfection) and 60.93% (stable transfection), compared with the two control groups ($p<0.05$). Apoptosis index was significantly increased during transient transfection and stable transfection, respectively 31.20+/-2.45% and 29.40+/-1.72% ($p<0.05$). Survivin-shRNA inhibited the proliferation of Raji cells of stable transfection. In conclusion, the vector-based survivin-shRNA can effectively reduce the expression of survivin gene and induce apoptosis and growth inhibition of transfected Raji cells. We suggest that survivin can be regarded as an ideal target for new anticancer intervention of NHL.

Guo, K. T., R. SchAfer, et al. (2006). "A new technique for the isolation and surface immobilization of mesenchymal stem cells from whole bone marrow using high-specific DNA aptamers." Stem Cells **24**(10): 2220-31.

Adult mesenchymal stem cells (aMSCs) are a stem cell population present in bone marrow, which can be isolated and expanded in culture and characterized. Due to the lack of specific surface markers, it is difficult to separate the MSCs from bone marrow directly. Here, we present a novel method using high-specific nucleic acids called aptamers.

Porcine MSCs were used as a target to generate aptamers by combinatorial chemistry out of a huge random library with in vitro technology called systematic evolution of ligands by exponential enrichment (SELEX). After cloning and sequencing, the binding affinity was detected using a cell-sorting assay with streptavidin-coated magnetic microbeads. We also used 12-well plates immobilized with aptamers to fish out MSCs from the cell solution and a fluorescein isothiocyanate-labeled aptamer to sort MSCs from bone marrow using high-speed fluorescence-activated cell sorting. The cells showed high potency to differentiate into osteogenic, as well as into adipogenic, lineages with typical morphological characteristics. Surface marker staining showed that the attached cells were CD29(+), CD44(+), CD45(-), CD90(+), SLA class I(+), SLA DQ(-), and SLA DR(-). Compared with existing methods, this study established a novel, rapid, and efficient method for direct isolation of aMSCs from porcine bone marrow by using an aptamer as a probe to fish out the aMSCs. This new application of aptamers can facilitate aMSC isolation and enrichment greatly, thereby enhancing the rate of aMSC-derived cells after in vitro differentiation for various applications in the emerging field of tissue engineering and regenerative medicine.

Hensel, N., V. Agarwala, et al. (1999). "A technique for dual determination of cytotoxic and helper lymphocyte precursor frequency by a miniaturized dye release method." Bone Marrow Transplant **23**(1): 71-8.

Helper (HTLPf) and cytotoxic (CTLPf) lymphocyte precursor frequency assays are increasingly used in bone marrow stem cell and organ transplant compatibility testing. Current techniques require large cell numbers and radioisotopes. To improve the technique, we developed a miniaturized fluorescent read-out combined HTLPf/CTLPf limiting dilution assay. The assay requires only 5 x 10(6) stimulators, 2 x 10(6) responders and 0.24 x 10(6) target cells in Terasaki plates (40 microl/well). For the HTLPf, culture supernatants from each well were assayed for IL-2 production. The IL-2-dependent proliferation of the mouse 9.12 cell line was detected by a semi-automated fluorescent dye technique. After addition of rhIL-2 (recombinant human IL-2) on days 3 and 7, CTLPs were detected on day 10 by measuring the lysis of dye-labeled targets. Results were comparable to standard radioisotope-based techniques. The assay had a coefficient of variation of approximately 30%. The assay detected helper CD4 cells, pure cytotoxic CD8, helper CD8 cells and helper/cytotoxic CD8 cells. Discrimination was demonstrated between HLA-matched related and non-related pairs. The ease of testing and small cell numbers required should facilitate further evaluation of HTLPf and CTLPf for compatibility testing in unrelated donor transplantation and monitoring immune responses following adoptive transfer of lymphocytes.

Hernigou, P., G. Mathieu, et al. (2006). "Percutaneous autologous bone-marrow grafting for nonunions. Surgical technique." J Bone Joint Surg Am **88 Suppl 1 Pt 2**: 322-7.

BACKGROUND: Bone marrow aspirated from the iliac crest contains progenitor cells that can be used to obtain bone-healing of nonunions. However, there is little available information regarding the number and concentration of these cells that are necessary to obtain bone repair. The purpose of this study was to evaluate the number and concentration of progenitor cells that were transplanted for the treatment of nonunion, the callus volume obtained after the transplantation, and the clinical healing rate. METHODS: Marrow was aspirated from both anterior iliac crests, concentrated on a cell separator, and then injected into sixty noninfected atrophic nonunions of the tibia. Each nonunion received a relatively constant volume of 20 cm(3) of concentrated bone marrow. The number of progenitor cells that was transplanted was estimated by counting the fibroblast colony-forming units. The volume of mineralized bone formation was determined by comparing preoperative computerized tomography scans with scans performed four months following the injection. RESULTS: The aspirates contained an average (and standard deviation) of 612 +/- 134 progenitors/cm(3) (range, 12 to 1224 progenitors/cm(3)) before concentration and an average of 2579 +/- 1121 progenitors/cm(3) (range, 60 to 6120 progenitors/cm(3)) after concentration. An average total of 51 x 10(3) fibroblast colony-forming units was injected into each nonunion. Bone union was obtained in fifty-three patients, and the bone marrow that had been injected into the nonunions of those patients contained >1500 progenitors/cm(3) and an average total of 54,962 +/- 17,431 progenitors. The concentration (634 +/- 187 progenitors/cm(3)) and the total number (19,324 +/- 6843) of progenitors injected into the nonunion sites of the seven patients in whom bone union was not obtained were both significantly lower (p = 0.001 and p < 0.01, respectively) than those in the patients who obtained bone union. The volume of the mineralized callus measured at four months on the computerized tomography scans of the patients who had union ranged from 0.8 to 5.3 cm(3) (mean, 3.1 cm(3)). There was a positive correlation between the volume of mineralized callus at four months and the number (p = 0.04) and concentration (p = 0.01) of

fibroblast colony-forming units in the graft. There was a negative correlation between the time needed to obtain union and the concentration of fibroblast colony-forming units in the graft (p = 0.04). CONCLUSIONS: Percutaneous autologous bone-marrow grafting is an effective and safe method for the treatment of an atrophic tibial diaphyseal nonunion. However, its efficacy appears to be related to the number of progenitors in the graft, and the number of progenitors available in bone marrow aspirated from the iliac crest appears to be less than optimal in the absence of concentration.

Holland, E. J., A. R. Djalilian, et al. (2003). "Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique." Ophthalmology **110**(1): 125-30.

OBJECTIVE: Aniridic keratopathy is a major cause of vision loss in patients with aniridia. Penetrating keratoplasty has been proven ineffective for the long-term treatment of this disorder because it does not address the stem cell deficiency that is the primary etiologic factor. We evaluated the role of keratolimbal allograft (KLAL), a stem cell transplantation technique, for the treatment of patients with aniridic keratopathy. DESIGN: Retrospective noncomparative interventional case series. PARTICIPANTS: Thirty-one eyes of 23 patients with aniridic keratopathy. INTERVENTION: KLAL. MAIN OUTCOME MEASURES: Ocular surface stability, visual acuity, and success of subsequent penetrating or lamellar keratoplasty. RESULTS: Thirty-one eyes of 23 patients were treated with KLAL and followed up for 12 to 117 months (mean, 35.7 months). Twenty-three eyes (74.2%) achieved a stable ocular surface. Overall, the mean visual acuity improved from 20/1000 to 20/165. Twenty eyes (64.5%) underwent subsequent penetrating keratoplasty. Fourteen corneal transplant grafts (70.0%) were successful, and six (30.0%) failed. Nineteen (90.5%) of 21 eyes receiving systemic immunosuppression obtained a stable ocular surface, whereas only 4 (40.0%) of 10 eyes not receiving systemic immunosuppression achieved ocular surface stability ($P < 0.01$). CONCLUSIONS: KLAL is effective in treating aniridic keratopathy. Patients receiving systemic immunosuppression have a greater likelihood of achieving ocular surface stability and improved visual acuity compared with those who receive only topical immunosuppression.

Hu, Y., K. Cai, et al. (2009). "Surface mediated in situ differentiation of mesenchymal stem cells on gene-functionalized titanium films fabricated by layer-by-layer technique." Biomaterials **30**(21): 3626-35.

In this work, multilayered and gene-functionalized titanium films composed of chitosan (Chi) and plasmid DNA (pEGFP-hBMP2, pGB) were employed to investigate the surface mediated in situ differentiation of mesenchymal stem cells (MSCs). The Chi/pGB multilayered structures were fabricated by layer-by-layer (LbL) assembly technique and degraded to release plasmid DNA complexes depending on bilayer numbers over 7 days. Therefore, the differentiation behaviors of MSCs cultured onto Chi/pGB multilayered titanium films surface were investigated. Chi/pGB LbL-modified titanium films show significant higher ($p<0.01$) transfection efficiency than those of other groups transfected by lipofectamine 2000 regarding the expression of green fluorescent protein (GFP). Reverse transcription-polymerase chain reaction (RT-PCR) assay revealed that MSCs adhered onto Chi/pGB LbL-modified titanium films could still express hBMP2 mRNA over 7 days culture. Compared with control groups, MSCs cultured onto Chi/pGB LbL-modified titanium films display significantly higher ($p<0.01$ or $p<0.05$) production levels of alkaline phosphatase (ALP) and osteocalcin over 7 days and 14 days culture, respectively. These results demonstrate that Chi/pGB LbL-modified titanium films are beneficial for sustained in situ inducing osteoprogenitor cells to differentiate into mature osteoblasts over long time. The approach presented here has potential applications in the development of gene-stimulating biomaterials and implant technology.

Huisman, A. M., H. G. Kuypers, et al. (1981). "Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with the multiple fluorescent retrograde tracer technique." Brain Res **209**(2): 271-86.

In 8 rats 'True Blue' was injected into dorsal half of C5-C8 spinal grey, 5 days later 'Nuclear Yellow' was injected in midthoracic, upper lumbar, lumbosacral and sacral cord respectively. The animals were sacrificed about 43 hours after NY injections. The distribution of retrogradely labeled neurons was studied in Red Nucleus, in Ventrolateral Pontine Tegmentum and in Nucleus Raphe Magnus, all of which project to spinal dorsal grey. In Red Nucleus large populations of single TB-labeled neurons and single NY-labeled ones occurred in the dorsomedial and ventrolateral part, respectively. In addition, about 8% of the neurons labeled with TB from C5-C8 were double labeled with NY from L5-S1, and 35% from T7-8, which percentages resemble those of electrophysiological studies. However, in ipsilateral Nucleus Raphe Magnus about 40% of the TB-labeled neurons were double labeled from L5-S1. This

percentage resembles the 66% obtained in electrophysiological studies of reticulospinal collaterals. These findings in rat support electrophysiological findings in cat and show, that rubrospinal neurons distribute their fibers primarily to the grey matter of specific groups of spinal segments, while many of the raphe spinal neurons distribute fibers throughout the spinal cord.

Iliakis, G. E. and G. E. Pantelias (1990). "Production and repair of chromosome damage in an X-ray sensitive CHO mutant visualized and analysed in interphase using the technique of premature chromosome condensation." Int J Radiat Biol **57**(6): 1213-23.

Production and repair of chromosome damage were studied in interphase xrs-5 cells by means of premature chromosome condensation (PCC). The results obtained were compared with those previously reported for CHO cells. Production of chromosome damage per unit of absorbed radiation dose was in xrs-5 cells larger by a factor of 2.6 than in CHO cells (5.2 breaks per cell per Gy). Changes in chromatin structure, associated with the radiation-sensitive phenotype of xrs-5 cells, that increase the probability of conversion of a DNA double-stand break (dsb) to a chromosome break are involved to explain this effect. Repair of chromosome breaks as measured in plateau-phase G1 cells was deficient in xrs-5 cells and the number of residual chromosome breaks was practically identical to the number of lethal lesions calculated from survival data. This observation suggests that non-repaired chromosome breaks are likely to be manifestations of lethal events in the cell. The yield of ring chromosomes scored after a few hours of repair was higher by a factor of three in xrs-5 compared with CHO cells. This increase in ring formation suggests an increase in the probability of misrepair of chromosome damage that may stem either from the reduced ability of xrs-5 cells to repair dsb, or from the higher production of chromosome fragments observed per cell and per Gy.

Ivanovski, O., K. Kulkeaw, et al. (2009). "Characterization of kidney marrow in zebrafish (Danio rerio) by using a new surgical technique." Prilozi **30**(2): 71-80.

Zebrafish kidney marrow (ZKM), which is equivalent to the haematopoietic bone marrow of mammals, produces all major blood cell types, which morphologically resemble their mammalian counterparts. To be able to exploit the advantages of zebrafish genetics for analysis of the general mechanisms controlling self-renewal, proliferation and lineage decisions of vertebrate haematopoetic cell populations, it is essential to develop a simple surgical technique in order to identify, dissect and take out the ZKM without contamination with other surrounding tissues and cells. However, the size of adult zebrafish is small (average size: 2.5 cm) and the ZKM is an extremely protected organ and not easy to localize, which makes this procedure a great microsurgical challenge. Here we report a new microsurgical technique to identify, localize and dissect ZKM in adult zebrafish using a new approach. The potential advantages of this technique are summarized here: it allows purity of the sample, which is critical for performing flow cytometry analysis and/or cell number count; it enables visualization of the ZKM without a parenchimal incision, which simplifies the further dissection; the learning curve is short, requiring only basic microsurgical skills, and it is reliable and highly reproducible. To further characterize the kidney marrow cells obtained by this technique, we performed histology, flow cytometry, cytospin experiments and cell counts.

Jacobs, P., D. Dubovsky, et al. (1979). "Bone marrow culture in vitro. A technique for analysis and permanent recording of cellular composition." Exp Hematol **7**(4): 177-82.

The in vitro cloning of haematopoietic progenitor cells derived from blood or bone marrow is now an established technique for the study of normal and abnormal blood formation. In semi-solid agar the results are conventionally recorded as the number of clusters or colonies that grow on the plate under controlled culture conditions. However, the demonstration of detailed morphology within these cellular aggregates remains unsatisfactory. Aspiration techniques are cumbersome and invariably disturb cellular relationships within the supporting matrix while supravital staining is limited by variable uptake of dye by the agar. We describe a method in which the entire cell-containing layer is removed from the Petri dish, fixed, and after mounting on a glass-slide, is air-dried. This preparation stains well with a wide variety of biological dyes, is minimally influenced by background colouration of the culture medium and excellent demonstration of morphologic detail is possible. A permanent record of the cellular composition of the culture is easily obtained by mounting the stained agar disc.

Jankowski, R. J., C. Haluszczak, et al. (2001). "Flow cytometric characterization of myogenic cell populations obtained via the preplate technique: potential for rapid isolation of muscle-derived stem cells." Hum Gene Ther **12**(6): 619-28.

Myoblast transplantation has been investigated as a therapy for muscle-related diseases and as a gene delivery vehicle for therapeutic

recombinant proteins. Clinical successes involving muscle cell transplantation have been limited, in part because of poor donor cell survival, and the heterogeneous nature of myogenic donor cells has largely been ignored. We have previously reported an isolation technique, preplating, that results in purified myogenic cells that are capable of significantly higher rates of donor cell survival leading to enhanced gene transfer to skeletal muscle. Characterization of these purified cells revealed that they display markers common to stem cells and are capable of multilineage differentiation. This study was performed to phenotypically characterize, by flow cytometry, muscle-derived cell populations obtained by the preplate technique for the purpose of eventually developing a method to quickly identify and isolate viable muscle cells best suited for transplantation. Muscle cell cultures were analyzed for expression of the surface proteins Sca-1, c-Kit, and CD34. We found that the preplate technique purifies distinct myogenic cell subpopulations expressing CD34 alone (Sca-1 negative) and Sca-1 alone (CD34 negative), but that this expression is subject to change with time in culture. Isolation and transplantation of phenotypically pure Sca-1-positive myogenic cells, obtained by magnetic cell sorting, demonstrates the ability to quickly select viable myogenic cells capable of regenerating skeletal muscle and restoring dystrophin expression within dystrophic host skeletal muscle. Flow cytometric described phenotypes will aid in the rapid isolation of specific donor cell populations for muscle cell transplants and muscle cell-mediated gene therapies, thereby enhancing their future success.

Jedrzejczak, W. W. and Z. Pojda (1987). "Technique of preparation of hemopoietic cells of human fetal liver for transplantation." Arch Immunol Ther Exp (Warsz) **35**(1): 71-8.

The preparation of single cell suspension from human fetal liver is described. The technique is based on pressing the liver through wire mesh followed by repeated aspiration into a syringe through needles of decreasing internal diameter. Subsequently, the cell suspension is depleted of cell debris by a "triple medium sedimentation procedure" without net loss of hemopoietic cells. Following centrifugation and resuspension the preparation is ready for either transplantation or storage. About 2 and 11 X 10(9) cells were obtained per liver depending on the age of fetus in the range of 16 and 24 weeks of gestation in three representative preparations. The cell suspension contained comparable numbers of hemopoietic progenitors to the adult bone marrow suspension as assayed using plasma clot diffusion chamber technique.

Kalil, R. A., D. Ott, et al. (2008). "Autologous transplantation of bone marrow mononuclear stem cells by mini-thoracotomy in dilated cardiomyopathy: technique and early results." Sao Paulo Med J **126**(2): 75-81.

CONTEXT AND OBJECTIVES: There are few studies concerning bone marrow mononuclear cell (BMMC) transplantation in cases of nonischemic dilated cardiomyopathy. This study describes a novel technique of BMMC transplantation and the results up to one year after the procedure. DESIGN AND SETTING: This was a case series to evaluate the safety and viability of the procedure, at Instituto de Cardiologia do Rio Grande do Sul. METHODS: Nine patients with symptomatic dilated cardiomyopathy, functional class III/IV and left ventricular ejection fraction (LVEF) < 35% received BMMC (9.6 +/- 2.6 x 107 cells) at 20 sites in the ventricular wall, by means of thoracotomy of length 5 cm in the fifth left intercostal space. Echocardiograms and nuclear magnetic resonance (NMR) were performed. RESULTS: There were no major complications. The functional class results for the first six patients (preoperatively and at two, four, eight and twelve-month follow-ups, respectively) were: [IV-2, III-4] to [I-5, II-1] to [I-3, II-3] to [I-2, II-3] and [I-2, II-3]. Echocardiograms showed LVEF: 25.9 +/- 8.2; 32.9 +/- 10.4; 29.4 +/- 7.2; 25.1 +/- 7.9; 25.4 +/- 6.8% (p = 0.023); and % left ventricular (LV) fiber shortening: 12.6 +/- 4.4; 16.4 +/- 5.4; 14.3 +/- 3.7; 12.1 +/- 4.0; 12.2 +/- 3.4% (p = 0.021). LV performance variation seen on NMR was non-significant. CONCLUSION: Intramyocardial transplantation of BMMC in dilated cardiomyopathy cases is feasible and safe. There were early improvements in symptoms and LV performance. Medium-term evaluation revealed regression of LV function, although maintaining improved functional class.

Kamihira, M. and A. Kumar (2007). "Development of separation technique for stem cells." Adv Biochem Eng Biotechnol **106**: 173-93.

In recent years, human embryonic stem cells have been established, and somatic stem cells derived from various adult organs have been identified and characterized to differentiate into various kinds of functional cells. There have been attempts to use functional cells induced from such stem cells for tissue regeneration and cell therapy. The method is expected to become an important treatment for intractable diseases in the near future. Since tissues and organs generally contain only a small quantity of somatic stem cells, and since it is necessary to separate functional cells generated from stem cells for use in therapy, an effective method for specific cell separation is crucial to the practical application of

regenerative medicine. For the specific separation of cells, a fluorescence activated cell sorter using specific antibodies is a powerful tool, but the method is not suitable for large-scale processing and a special device is required. Although a magnetic cell separation system using immuno-magnetic fine particles is also commercially available, the system still needs special apparatus for large-scale processing. We developed a novel method for the separation of specific cells in an aqueous two-phase system using antibodies modified with a temperature-responsive polymer. The method enables the processing of a large quantity of cells without the requirement of a special device.

Kamondi, A., J. A. Williams, et al. (1992). "Membrane properties of mesopontine cholinergic neurons studied with the whole-cell patch-clamp technique: implications for behavioral state control." J Neurophysiol **68**(4): 1359-72.

1. The whole-cell patch-clamp technique was used to study the membrane properties of identified cholinergic and noncholinergic laterodorsal tegmental neurons in slices of rat brain maintained in vitro. 2. On the basis of their expression of the transient outward potassium current IA and the transient inward calcium current IT, three classes of neurons were observed: type I neurons exhibited a large IT; type II neurons exhibited a prominent IA; and type III neurons exhibited both IA and IT. 3. Combining intracellular deposition of biocytin with NADPH diaphorase histochemistry revealed that the vast majority of type III neurons were cholinergic, whereas only a minority of type I and type II neurons were cholinergic. Thus mesopontine cholinergic neurons possess intrinsic ionic currents capable of inducing burst firing. 4. Delineation of the intrinsic membrane properties of identified mesopontine cholinergic neurons, in concert with recent results regarding the responses of these neurons to neurotransmitter agents, has led us to present a unifying and mechanistic hypothesis of brain stem cholinergic function in the control of behavioral states.

Kim, M., J. Y. Kim, et al. (2002). "Evaluation of early post-transplant leukocyte recovery using the undiluted erythrocyte lysing technique." Ann Clin Lab Sci **32**(2): 159-63.

The undiluted erythrocyte lysing technique was evaluated to see if it provides more accurate total leukocyte counts and differential leukocyte counts of severely leukopenic blood samples, in order to detect the onset of hematopoietic recovery after stem cell transplantation. Leukocyte counts using the conventional automated cell counting technique were found to be inaccurate, especially in blood samples with total leukocyte counts < 500/microl. In cases where the difference between results by the two methods was >100/microl, a positive correlation was found between the difference value and the blood reticulocyte count ($r = 0.39$, $p = 0.002$). Hematopoietic recovery after stem cell transplantation in a group of patients with chronic myelogenous leukemia (CML) was different from that of non-CML groups. In the CML group, the initial leukocyte counts were higher and the number of days until neutrophil recovery was higher than in the non-CML groups. Also, the day on which the absolute neutrophil count (ANC) exceeds 100/microl could serve as an indicator of neutrophil recovery. This study shows that the undiluted erythrocyte lysing technique can be used to count leukocytes accurately, especially in severely leukopenic samples. This new method can detect neutrophil recovery at ANC > 100/microl, as well as at an earlier date than the conventional method.

Kim, S., S. E. Ahn, et al. (2007). "A novel culture technique for human embryonic stem cells using porous membranes." Stem Cells **25**(10): 2601-9.

We have developed a novel culture technique for human embryonic stem cells (hESCs) using a porous membrane with feeder cells. The feeder cells were seeded and attached to the bottom of a porous membrane and, subsequently, hESCs were cultured on the top of the membrane. This porous membrane technique (PMT) allowed hESCs to be successfully cultured and to be effectively and efficiently separated from the feeder cell layer without enzyme treatment. hESCs being cultured by PMT were observed to interact with feeder cells through pores of membrane, where the interaction was dependent on the pore size of the membrane used. It was also revealed that the number of attached hESC colonies depended on the concentration of feeder cells on the bottom of the membrane. On the other hand, hESC colonies did not attach to porous membrane, as feeder cells were in the presence of culture dish, not the porous membrane. The hESCs cultured on porous membranes not only exhibited expression of several undifferentiated markers and a normal karyotype, but they also formed teratomas consisting of three germ layers in in vivo study. Compared with the mechanical isolation technique conventionally used, PMT significantly decreased mouse vimentin gene expression in cultured hESCs. Thus, a PMT for hESC culture would be a useful tool to exclude enzyme treatment and to reduce contamination from feeder cells simultaneously. Disclosure of potential conflicts of interest is found at the end of this article.

Kishigami, S., E. Mizutani, et al. (2006). "Significant improvement of mouse cloning technique by treatment

with trichostatin A after somatic nuclear transfer." Biochem Biophys Res Commun **340**(1): 183-9.

The low success rate of animal cloning by somatic cell nuclear transfer (SCNT) is believed to be associated with epigenetic errors including abnormal DNA hypermethylation. Recently, we elucidated by using round spermatids that, after nuclear transfer, treatment of zygotes with trichostatin A (TSA), an inhibitor of histone deacetylase, can remarkably reduce abnormal DNA hypermethylation depending on the origins of transferred nuclei and their genomic regions [S. Kishigami, N. Van Thuan, T. Hikichi, H. Ohta, S. Wakayama. E. Mizutani, T. Wakayama, Epigenetic abnormalities of the mouse paternal zygotic genome associated with microinsemination of round spermatids, Dev. Biol. (2005) in press]. Here, we found that 5-50 nM TSA-treatment for 10 h following oocyte activation resulted in more efficient in vitro development of somatic cloned embryos to the blastocyst stage from 2- to 5-fold depending on the donor cells including tail tip cells, spleen cells, neural stem cells, and cumulus cells. This TSA-treatment also led to more than 5-fold increase in success rate of mouse cloning from cumulus cells without obvious abnormality but failed to improve ES cloning success. Further, we succeeded in establishment of nuclear transfer-embryonic stem (NT-ES) cells from TSA-treated cloned blastocyst at a rate three times higher than those from untreated cloned blastocysts. Thus, our data indicate that TSA-treatment after SCNT in mice can dramatically improve the practical application of current cloning techniques.

Koga, H., M. Shimaya, et al. (2008). "Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect." Arthritis Res Ther **10**(4): R84.

INTRODUCTION: Current cell therapy for cartilage regeneration requires invasive procedures, periosteal coverage and scaffold use. We have developed a novel transplantation method with synovial mesenchymal stem cells (MSCs) to adhere to the cartilage defect. METHODS: For ex vivo analysis in rabbits, the cartilage defect was faced upward, filled with synovial MSC suspension, and held stationary for 2.5 to 15 minutes. The number of attached cells was examined. For in vivo analysis in rabbits, an autologous synovial MSC suspension was placed on the cartilage defect, and the position was maintained for 10 minutes to adhere the cells to the defect. For the control, either the same cell suspension was injected intra-articularly or the defects were left empty. The three groups were compared macroscopically and histologically. For ex vivo analysis in humans, in addition to the similar experiment in rabbits, the expression and effects of neutralizing antibodies for adhesion molecules were examined. RESULTS: Ex vivo analysis in rabbits demonstrated that the number of attached cells increased in a time-dependent manner, and more than 60% of cells attached within 10 minutes. The in vivo study showed that a large number of transplanted synovial MSCs attached to the defect at 1 day, and the cartilage defect improved at 24 weeks. The histological score was consistently better than the scores of the two control groups (same cell suspension injected intra-articularly or defects left empty) at 4, 12, and 24 weeks. Ex vivo analysis in humans provided similar results to those in rabbits. Intercellular adhesion molecule 1-positive cells increased between 1 minute and 10 minutes, and neutralizing antibodies for intercellular adhesion molecule 1, vascular cell adhesion molecule 1 and activated leukocyte-cell adhesion molecule inhibited the attachment. CONCLUSION: Placing MSC suspension on the cartilage defect for 10 minutes resulted in adherence of >60% of synovial MSCs to the defect, and promoted cartilage regeneration. This adherent method makes it possible to adhere MSCs with low invasion, without periosteal coverage, and without a scaffold.

Koizumi, S., R. L. Fine, et al. (1985). "Enrichment of myeloid progenitor cells from normal human bone marrow using an immune-rosette technique." Exp Hematol **13**(6): 560-5.

In this study we have developed methods for purification of myeloid progenitor cells (CFU-Cs) from normal human bone marrow cells. Bone marrow aspirates were obtained from volunteers, and mononuclear cells (MNCs) were separated by Ficoll-Hypaque gradient centrifugation. T- and B-lymphocytes, monocytes, mature granulocytes, and erythroid precursors were eliminated by an immune-rosette technique using a panel of murine monoclonal antibodies and immunoglobulin (Ig)-coated sheep red blood cells (SRBCs). MNCs were treated with OKT3, B1, M3, Mo5, and EP1 monoclonal antibodies, which are reactive with T cells, B cells, monocytes, granulocytes, and erythroid precursors, respectively. Antibody-treated MNCs were incubated with SRBCs that had been coated with goat antirabbit IgG F(ab')2 and rabbit antimouse Ig for immune rosetting. Rosetted cells were then separated from nonrosetted cells in Ficoll-Hypaque. Nonrosetted cells were, in the second step, treated with an OKIa1 monoclonal antibody and again separated into an Ia+ and Ia- cell fraction by the same manner; 39% +/- 19.2% (mean +/- 1 SD, range 16.3%-75.4%) of CFU-Cs (colonies plus clusters) were recovered in the OKT3-, B1-, M3-, Mo5-, EP1- cell fraction, and the number of CFU-Cs grown in semisolid agar was 149.6 +/- 73.0 (64.0-309.0)/10(4) plated cells in this purified fraction,

representing an enrichment of 14.2 +/- 6.4 (6.0-27.3)-fold when compared with unseparated marrow cell fractions. CFU-Cs were enriched 17.7 +/- 8.6 (6.1-28.3)-fold in the Ia+ cell fraction. These purified myeloid precursors would be of value for in-depth studies of the interactions between hematopoietic progenitor cells and regulatory factors that influence their proliferation and differentiation and also of drug metabolism and determinants of cytotoxicity.

Koristek, Z., J. Sterba, et al. (2002). "Technique for PBSC harvesting in children of weight under 10 kg." Bone Marrow Transplant **29**(1): 57-61.

Peripheral blood stem cell (PBSC) harvesting in the smallest children (weight <10 kg) using separators is complicated by specific problems. The volume of the separation set exceeds 25% of the total blood volume and the vascular access is generally not sufficient. Therefore, a simple manual technique for PBSC harvesting was developed. Three children (6-9 kg), with newly diagnosed tumours were scheduled to be treated with three to six sequential courses of high-dose chemotherapy, each followed by PBSC support. PBSC harvests were started after mobilization using cyclophosphamide and G-CSF when the peripheral blood CD34+ cell count exceeded 50/microl. About 50 ml of blood was drawn from a venous catheter, injected into a transfer bag containing ACD-A, and centrifuged. The buffy coat obtained was pooled in a collection bag, remaining plasma and erythrocytes were immediately reinfused and a subsequent cycle started. From three to 13 cycles were performed in 1-3 days and 18.0-32.2 x 10(6) CD34+cells/kg were collected. We did not detect any bacterial contamination or any notable complications. Fifteen PBSC reinfusions have been performed to date, each with rapid engraftment taking between 7 and 13 days. Patients are in very good PR (18 months from diagnosis) or in CR (6 and 8 months). We can conclude that this procedure is feasible and safe.

Koutna, I., M. Klabusay, et al. (2006). "Evaluation of CD34+ - and Lin- -selected cells from peripheral blood stem cell grafts of patients with lymphoma during differentiation in culture ex vivo using a cDNA microarray technique." Exp Hematol **34**(7): 832-40.

OBJECTIVE: Hematopoietic stem cells (enriched in fraction of CD34+ cells) have the ability to regenerate hematopoiesis in all of its lineages, and this potential is clinically used in transplanting bone marrow or peripheral blood stem cells. Our objective was to assemble a suitable method for evaluating gene expression in enriched populations of hematopoietic stem cells. We compared biologic properties of cells cultured ex vivo obtained using two different ways of immunomagnetic separation (positive selection of CD34+ cells and negative selection of Lin- cells) by means of a cDNA microarray technique. METHODS: CD34+ and Lin- cells were enriched from peripheral blood stem cell (PBSCs) grafts of patients with non-Hodgkin's lymphoma. Isolated cells were in the presence of cytokine PBSCs, Flt-3 ligand, interleukin-3, interleukin-6, and granulocyte colony-stimulating factor. At days 0, 4, 6, 8, 10, 12, and 14 cells were harvested and analyzed by cDNA microarrays. Total cell expansion, CD34+, colony-forming unit for granulocyte-macrophage and megakaryocytes expansion, vitality, and phenotype of cells were also analyzed. RESULTS: cDNA microarray analysis of cultured hematopoietic cells proved equivalence of the two enrichment methods for PBSC samples and helped us characterize differentiating cells cultured ex vivo. CONCLUSION: Our methodologic approach is helpful in characterizing cultured hematopoietic cells cultured ex vivo, but it is also suitable for more general purposes. Equivalence of CD34+ and Lin- selection methods from PBSC samples proved by cDNA microarray may have an implication for graft manipulation in an experimental setting of hematopoietic transplantation. Total cell expansion and colony formation and phenotype from CD34+ selected and from Lin- samples were comparable.

Kuemmel, T. A., J. Thiele, et al. (1996). "Distribution of lectin binding sites in human bone marrow. Identification by use of an ultrastructural postembedding technique." J Submicrosc Cytol Pathol **28**(4): 537-51.

The purpose of this ultrastructural study was to detect various carbohydrate residues on mature elements of the major human haematopoietic cell lines (granulopoiesis, erythropoiesis and megakaryopoiesis), sinus endothelium and plasma cells under comparable experimental conditions. Marrow specimens were processed according to a modified postembedding technique with Unicryl as embedding resin. A broad panel of 10 digoxigenin (dig)-conjugated lectins was applied for staining and specificity was evaluated by incubation with their corresponding inhibitory sugars. Lectins under study were derived from Canavalia ensiformis (Con A), Triticum vulgare (WGA), Ulex europaeus-I (UEA-I), Baubinia purpurea (BPA), Erythrina cristagalli (ECA), Glycine max (SBA), Helix pomatia (HPA), Arachis hypogaea (PNA), Griffonia simplicifolia-I (GS-I) and its isotype GS-I-B4. As a common feature WGA was shown to be a prominent marker of cytoplasmic membranes, except for plasma cells. On the other hand, Con A turned out to be reactive with the nuclear envelopes in all haematopoietic cells and, additionally, exhibited a strong labelling of the rough endoplasmic reticulum in plasma cells. Granules of eosinophilic

granulocytes revealed staining of varying intensity with all lectins; however, inhibition was mostly incomplete. Several lectins (WGA, Con A, UEA, BPA, ECA, SBA and PNA) disclosed a clear cut differentiation of at least two subpopulations of granules in polymorphonuclear leukocytes. UEA-I (H type 2 specific) exhibited a high affinity to cytoplasmic membranes of erythropoietic precursor cells. In keeping with the blood group of our patient (O Rh+) membranes of red blood cells were completely negative with those lectins that are known to exhibit a group A or B specificity (HPA, GS-I-B4). As a remarkable finding the luminal and abluminal surfaces of sinusoidal endothelium revealed a specific reaction with UEA-I. Carbohydrate binding sites on the surface of endothelial cells may play a pivotal role in several functional processes such as cellular adhesion, traffic of mature cell elements across the marrow blood barrier and "homing' of haematopoietic stem cells.

Lahdetie, J., A. Suutari, et al. (1994). "The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of acrylamide in rat meiotic cells." Mutat Res **309**(2): 255-62.

As a part of the development and validation of the spermatid micronucleus test (SMNT) in the project 'Detection of Germ Cell Mutagens' sponsored by the CEC we studied the mutagenicity of acrylamide (AA) and mitomycin C (MMC). Of two alternative techniques, we used the 'dissection technique' based on microdissection of seminiferous tubules offering a narrow window for evaluation of cell stage sensitivity, and including DNA-specific staining and scoring. AA given as a single injection of 50 or 100 mg/kg did not significantly increase MN frequencies. When a subchronic treatment (4 x 50 mg/kg) was given, a significant increase over background was observed 18 and 19 days after the last injection, indicating genotoxic activity in preleptotene spermatocytes and late spermatogonial stages. MMC given as single injections of 0.5 or 1.0 mg/kg increased MN frequencies significantly 17, 18, 19 and 20 days after treatment as a result of clastogenicity in S phase cells. DNA flow cytometry did not show cytotoxicity of AA to preleptotene spermatocytes, but a small decrease in the numbers of stem cells. If spindle disturbances are caused by AA, as suggested, they were not detectable by induction of spermatid MN in vivo 1 or 3 days after treatment or by treatment with AA of cultured segments of seminiferous tubules undergoing meiotic divisions in vitro. In conclusion, the SMNT with the dissection technique is able to show the germ cell clastogenicity of AA and MMC. AA was observed to have a much weaker MN inducing potency than MMC.

Lee, I. C., J. H. Wang, et al. (2007). "Development of a useful technique to discriminate anterior cruciate ligament cells and mesenchymal stem cells--the application of cell electrophoresis." J Biomed Mater Res A **82**(1): 230-7.

Mesenchymal stem cells (MSCs) can differentiate into multiple nonhematopoietic cell lineages, including osteoblasts, chondrocytes, and ligament cells. The purpose of this study is to identify the difference between MSCs and anterior cruciate ligament (ACL) cells for the application of distinguishing these two cells during the process of MSCs differentiating into ACL cells. Although culture of MSCs and ACL cells have been studied extensively, it was found that these two cells could not be distinguished from their appearance, expression of surface antigens (including CD105, CD34, CD45, CD29, CD44, and CD71), alpha-smooth muscle actin, and mRNAs for type I collagen, type III collagen, and tenascin-C, based on a series of traditional methods for cell identification. Cell electrophoresis, measuring the electrophoretic mobility (EPM) of cells, was proposed to investigate the discrepancy in surface charge properties of MSCs and ACL cells. Surprisingly, the EPM value of MSCs is significantly greater than that of ACL cells ($p < 0.001$). Although cell electrophoresis cannot determine the specific surface protein, it can reflect the net surface charge density of cell membrane, which can be influenced by the dissociation of functional groups of peripheral membrane proteins. Therefore, it is suggested that cell electrophoresis, while simple and cheap in manipulation, can serve as a useful research tool to assist in identification of MSCs differentiating into ACL cells.

Lekhanont, K., L. Choubtum, et al. (2009). "A serum- and feeder-free technique of culturing human corneal epithelial stem cells on amniotic membrane." Mol Vis **15**: 1294-302.

PURPOSE: To describe a simple technique of cultivating human corneal epithelial stem cells using an Epilife culture medium under serum- and feeder-free conditions. METHODS: Cadaveric donor limbal corneal epithelial cells were cultured on denuded amniotic membranes using an explant technique that was free of serum and feeder cells in the Epilife medium containing a growth supplement of defined composition. These cells were assessed by phase contrast microscope. The expressions of the proposed corneal epithelial stem cell markers (p63, ATP-binding cassette member 2 (ABCG2), and cytokeratin 15 and 19) and differentiation markers (cytokeratin 3, 12, connexin 43, and p75) were analyzed using reverse transcription polymerase chain

reaction (RT-PCR) and immunocytochemical staining. RESULTS: Successful cultures were obtained, resulting in a monolayer to double layer cell sheets with a cobblestone-like morphology. RT-PCR and immunocytochemistry disclosed an expression of both putative limbal stem cell (LSC) markers and differentiation-associated markers in the cultured cells. Most of the cultured corneal epithelial cells that were immunopositive for putative LSC markers were smaller, more uniform, and closer to the limbal explant than cells positively stained with differentiation-associated markers. CONCLUSIONS: A serum- and feeder-free culture system using Epilife medium may grow human corneal epithelial equivalents, minimizing the risk of contamination during culture. The technique may also be useful for the clinical application of limbal stem cell culture.

Matouskova, E., D. Dudorkinova, et al. (1998). "Clonal expansion of epithelial cells from primary human breast carcinoma with 3T3 feeder layer technique." Folia Biol (Praha) **44**(2): 67-71.

3T3 feeder layer technique provided support for clonal growth and serial propagation of two apparently single epithelial cells isolated from a peroperative biopsy of a primary ductal breast carcinoma. The total culture lifetime was estimated to be more than 30 doublings, 21 of which took place during the primary culture. The two cells were the only survivors of two-week exposure to stressing conditions that resembled the microenvironment in a tumour (low pH, depleted nutrition and accumulation of metabolic waste). The epithelial character of the cells was proved by positive immunostaining for keratins 7/17. The majority of growing cells did not express keratin 19. Only quiescent cells in some colonies, which appeared to reach a more advanced stage of differentiation, expressed keratin 19. These features correspond with the characteristics of mammary luminal cells which in vivo undergo differentiation from the stem K19- to secretory K19+ cells. The luminal cells are supposed to be the target of malignant transformation in the mammary gland. The described technique opens a regular way for the in vitro clonal growth of individual primary cells from breast tumours. Such an approach can improve our understanding of the biology of breast cancer cell populations and also simplify the predictive chemosensitivity assay on breast cancer cells from individual patients.

Matsubara, T., S. Tsutsumi, et al. (2004). "A new technique to expand human mesenchymal stem cells using basement membrane extracellular matrix." Biochem Biophys Res Commun **313**(3): 503-8.

Mesenchymal stem cells (MSC) show a very short proliferative life span and readily lose the differentiation potential in culture. However, the growth rate and the proliferative life span of the stem cells markedly increased using tissue culture dishes coated with a basement membrane-like extracellular matrix, which was produced by PYS-2 cells or primary endothelial cells. Furthermore, the stem cells expanded on the extracellular matrix, but not those on plastic tissue culture dishes, retained the osteogenic, chondrogenic, and adipogenic potential throughout many mitotic divisions. The extracellular matrix had greater effects on the proliferation of MSC and the maintenance of the multi-lineage differentiation potential than basic fibroblast growth factor. Mesenchymal stem cells expanded on the extracellular matrix should be useful for regeneration of large tissue defects and repeated cell therapies, which require a large number of stem or progenitor cells.

Matsuda, T., Y. Saito, et al. (2007). "Cell sorting technique based on thermoresponsive differential cell adhesiveness." Biomacromolecules **8**(8): 2345-9.

Cell sorting of specific target cells from a mixture of different cell types is a prerequisite for development of functional engineered tissues based on stem-cell and tissue engineering. This paper presents a new method of cell sorting that uses a mixture of thermoresponsive cell-adhesive and non-cell-adhesive substances. The former substance is poly(N-isopropylacrylamide)-grafted gelatin (PNIPAM-gelatin) and the latter is PNIPAM. Graded cell adhesion, produced by mixed coating of these thermoresponsive substances at an appropriate mixing ratio, clearly differentiated the adhesive potentials of two bovine vascular cell types (endothelial cell and smooth muscle cell). The sequential procedures of detachment at room temperature and subsequent replating at 37 degrees C on dishes coated with a mixed coating with the same composition as that employed previously yielded remarkably pure target cells, as determined using confocal laser scanning fluorescence microscopy. This method, leading to harvesting of target cells, is characteristic of simple manipulation with no cell damage. Such advantages are expected to facilitate stem-cell and tissue engineering.

Matsuoka, T. and M. Tavassoli (1989). "Electron microscopic identification of hemopoietic progenitor cells by exploiting their sugar-recognizing receptors using a newly developed minibead technique." Exp Hematol **17**(4): 326-9.

To identify hemopoietic progenitor cells in electron microscopic preparations we exploited the presence of "homing receptors" with galactosyl,

mannosyl, and fucosyl specificities on their membrane surfaces. Amide-modified latex minibeads were covalently linked to the para-aminophenyl derivative of galactose, mannose, and fucose in pyranose form. Incubation of these probes with murine bone marrow cells led to specific binding of probes to a small proportion of marrow cells, comprising 0.5%, 0.6%, and 0.4% of the mononuclear marrow cell population, respectively, for galactosyl, mannosyl, and fucosyl probes. Specificity of the binding was demonstrated by inhibition in the presence of excess soluble sugars. The cells binding the probes were small, 4-5 microns, with characteristics similar to those described for progenitor cells. The implications of this technique for purification of hemopoietic progenitor cells are discussed.

Mayor, N. P., B. E. Shaw, et al. (2007). "A novel technique for NOD2/CARD15 genotyping using PCR-SSP." J Immunol Methods **327**(1-2): 82-7.

The Nucleotide-binding Oligomerisation Domain (NOD) 2 protein is encoded by the Caspase Recruitment Domain (CARD) 15 gene and has a critical role in innate immunity. Recent studies have implicated Single Nucleotide Polymorphisms (SNPs) of the NOD2/CARD15 gene with the onset of several Inflammatory Bowel Disorders (Crohn's Disease, Blau syndrome) and the progression of several malignant diseases. The identification of SNPs in the genotypes of donor and recipient pairs prior to haematopoietic stem cell transplantation have also been shown to predict for a worse outcome, specifically causing increases in the incidence and severity of acute Graft-versus-Host disease, disease relapse and mortality. In light of these widespread areas of interest, we have developed a Polymerase Chain Reaction assay using Sequence Specific Primers (PCR-SSP) to identify the three SNPs that have been implicated, (SNPs 8, 12 and 13). The assay has proven to be a rapid and accurate method of performing NOD2/CARD15 genotyping when compared to other techniques described to date.

Meijer, E., I. C. Slaper-Cortenbach, et al. (2002). "Increased incidence of EBV-associated lymphoproliferative disorders after allogeneic stem cell transplantation from matched unrelated donors due to a change of T cell depletion technique." Bone Marrow Transplant **29**(4): 335-9.

Here, the influence of T vs T and B cell depletion on the incidence of EBV-associated lymphoproliferative disorder (EBV-LPD) after bone marrow transplantation (BMT) from a matched unrelated donor (MUD) is analyzed. From 1982 to 1997 the soy bean agglutinin/sheep red blood cell (SBA/SRBC) method was used for T cell depletion. This technique is well established, but the use of SRBC has a risk of transmitting prions or viruses. Therefore, a new T cell depletion method was introduced, using CD2 and CD3 monoclonal antibodies (CD2/3 method) instead of SRBC. Unfortunately, this led to an unexpected high number of EBV-LPDs in patients receiving transplants from MUDs. SBA depletion was reintroduced and combined with the CD2/3 method (SBA/CD2/3) in this patient population, later replaced by B cell-specific (CD19 and CD22) antibodies (CD3/19/22 method). The number of T (x 10(5)/kg) and B (x 10(5)/kg) cells in the graft was 1.5 +/- 0.8 and 2 +/- 1 (T/B ratio 0.75), 2.2 +/- 2.0 and 41 +/- 21 (ratio 0.055), 5.0 +/- 0.0 and 2 +/- 1 (ratio 2.5), 2.5 +/- 1.2 and 10 +/- 6 (ratio 0.25) using the SBA/SRBC, CD2/3, SBA/CD2/3 and CD3/19/22 techniques, respectively. When B cell depletion was performed (SBA/SRBC, SBA/CD2/3, CD3/19/22) four out of 31 patients (13%) receiving a BMT from a MUD developed an EBV-LPD. Without B cell depletion (CD2/3) this occurred in five out of seven patients (71%) (P < 0.05). A T/B cell ratio in the graft of > or = 0.25 seems sufficient to significantly reduce the incidence of EBV-LPD after BMT from MUDs.

Menendez, P., O. Redondo, et al. (1998). "Comparison between a lyse-and-then-wash method and a lyse-non-wash technique for the enumeration of CD34+ hematopoietic progenitor cells." Cytometry **34**(6): 264-71.

The flow cytometric enumeration of CD34+ hemopoietic precursor cells (HPC) present in samples used for transplantation of HPC has proven to be the most powerful single parameter for prediction of engraftment. At present, several different methodological approaches are used for the flow cytometric enumeration of CD34+ HPC. In the present study we have compared two of these methods as regards enumeration of CD34+ HPC and their CD34+/CD19- and CD34+/CD19+ subsets: a lyse-non-wash procedure based on the use of a recently commercialized red cell lysing solution (Quicklysis, Cytognos, Salamanca, Spain) and a lyse-and-then-wash method in which the Becton Dickinson (San Jose, CA) FACS Lysing Solution was used. For that purpose a total of 52 samples corresponding to 20 G-CSF mobilized peripheral blood (PB) samples and 21 PB-derived leucapheresis products from patients undergoing autologous PB stem cell harvest, together with 11 bone marrow (BM) samples from healthy volunteers were analyzed. Our results show that for each of the three types of samples analyzed the use of the lyse-and-then-wash method is associated with significantly lower numbers of both total CD34+ HPC (P < or = 0.003) and its major CD34+/CD19- subset

(P < or = 0.01) while no significant changes are detected in the number of CD34+/CD19+ HPC in BM samples (P > 0.05). The use of an internal standard (reference beads) added just prior to data acquisition, showed that the differences between both methods are due to a selective loss of CD34+ HPC and its major CD34+/CD19- subset in BM (P=0.002 and P=0.003), PB (P < 0.0001 and P < 0.0001) and PB-derived leucapheresis products (P < 0.0001 and P=0.0001). Finally, addition of a centrifugation and washing step to a group of 11 leucapheresis samples lysed with Quicklysis showed that they did not significantly affect the overall number of total CD34+, CD34+/CD19- and CD34+/CD19+ HPC obtained. In line with these findings elimination of centrifugation and washing steps when FACS Lysing Solution was used to lyse mature red cells almost corrected for the selective loss of CD34+ HPC. In spite of these differences a significant degree of correlation (r > 0.83 in all cases) was found between both methods regarding the total number of CD34+, CD34+/CD19- and CD34+/CD19+ HPC present in the BM, PB and PB-derived leucapheresis samples analyzed in this study.

Mielke, S., R. Nunes, et al. (2008). "A clinical-scale selective allodepletion approach for the treatment of HLA-mismatched and matched donor-recipient pairs using expanded T lymphocytes as antigen-presenting cells and a TH9402-based photodepletion technique." Blood **111**(8): 4392-402.

Selective allodepletion is a strategy to eliminate host-reactive donor T cells from hematopoietic stem cell allografts to prevent graft-versus-host disease while conserving useful donor immune functions. To overcome fluctuations in activation-based surface marker expression and achieve a more consistent and effective allodepletion, we investigated a photodepletion process targeting activation-based changes in p-glycoprotein that result in an altered efflux of the photosensitizer TH9402. Expanded lymphocytes, generated using anti-CD3 and IL-2, were cocultured with responder cells from HLA-matched or -mismatched donors. Optimal results were achieved when cocultured cells were incubated with 7.5 muM TH9402, followed by dye extrusion and exposure to 5 Joule/cm(2) light energy at 5 x 10(6) cells/mL. In mismatched stimulator-responder pairs, the median reduction of alloreactivity was 474-fold (range, 43-fold to 864-fold) compared with the unmanipulated responder. Third-party responses were maintained with a median 1.4-fold (range, 0.9-fold to 3.3-fold) reduction. In matched pairs, alloreactive helper T-lymphocyte precursors were reduced to lower than 1:100 000, while third-party responses remained higher than 1:10 000. This establishes a clinical-scale process capable of highly efficient, reproducible, selective removal of alloreactive lymphocytes from lymphocyte transplant products performed under current Good Manufacturing Practice. This procedure is currently being investigated in a clinical trial of allotransplantation.

Musialek, P., W. Tracz, et al. (2006). "Transcoronary stem cell delivery using physiological endothelium-targeting perfusion technique: the rationale and a pilot study involving a comparison with conventional over-the-wire balloon coronary occlusions in patients after recent myocardial infarction." Kardiol Pol **64**(5): 489-98; discussion 499.

INTRODUCTION: Recent evidence shows poor efficacy of over-the-wire balloon catheter (OTW) coronary occlusive technique adopted widely for intracoronary bone marrow stem cell (BMSC) delivery. The waterfall effect of OTW-balloon inflation/deflation with reactive > or = 2-fold flow velocity increase might be partly responsible for poor BMSC retention. AIM: To evaluate the safety, feasibility and tolerability of perfusion-infusion BMSC delivery with the facilitation of cell rolling in contact with the coronary endothelium (a pre-requisite for downstream transmigration). METHODS: We randomly assigned 11 patients (age 41-72 years) with first anterior myocardial infarction treated with PTCA+stent and LVEF < or =45% at 6-9 days to OTW in-stent occlusive (3 x 3 min.) BMSC delivery or cell infusion via a perfusion catheter with multiple side holes (SH-PC). RESULTS: OTW and SH-PC patients had a similar infarct size (mean peak CK 4361 vs 4717 U/L), LVEF (41.2% vs 40.3%), infused mononuclear cell number (2.99 x 108 range 0.61-7.48 x 108 vs 3.28 x 108 range 1.64-4.39 x 108), CD 34(+) number (1.79 x 106 vs 1.62 x 106), cell viability (91.5% vs 91.8%) and clonogenicity (CFU assay). None of the SH-PC, but 67% of OTW patients, had ST-segment elevation with chest pain (and nsVT in one) that limited OTW occlusion tolerance to 50-110 sec. At 6 months DLVEF in the OTW vs SH-PC patients was +4.2% (2-6) vs +8.8% (5-16) by MRI and +4.8 (2-7) vs +13.8% (2-24) by SPECT. CONCLUSIONS: Our work indicates that the SH-PC technique can be used safely for intracoronary BMSC transplantation. Further research is needed to determine whether the putative advantages of physiological SH-PC delivery translate into enhanced BMSC homing.

Nafu, R., G. Lelkes, et al. (1997). "Ultrastructural study on the in vitro interaction between haemopoietic cells and stromal cells. A new method using gel technique." Haematologia (Budap) **28**(2): 97-107.

The ultrastructural study of the interaction between stroma and haemopoiesis is not an easy task because the loose attachment may be damaged during manipulation. This paper describes a technique by which the loose connection between preestablished stromal layer and attached haemopoietic cells (derived from blast colony forming cells) can be preserved and studied ultrastructurally. Stromal cultures were obtained from human bone marrow cells. Blast colony forming cells were studied by co-incubating the stroma with fetal calf serum supplemented McCoy's medium containing bovine plasma, thrombin and calcium to form a gel ('plasma clot'). Colony formers attached to the stroma formed myeloid colonies within 6 days. The semisolid plasma clot which solidifiers rapidly on the addition of glutaraldehyde or formaldehyde entraps the blastic colonies and haemopoietic cells in their position. Even the non-attached or mobile cells can be entrapped by this technique. The immature cells were found to be attached to the stromal surface and/or to the extracellular matrix, while the more mature cells migrated either to the surface of the colony or attached to the non-covered areas of the plastic surface. This method may offer a special technique to study dynamic interactions in other situations (e.g. chemotaxis etc.), too.

Nakahara, M., N. Nakamura, et al. (2009). "High-efficiency production of subculturable vascular endothelial cells from feeder-free human embryonic stem cells without cell-sorting technique." Cloning Stem Cells **11**(4): 509-22.

We previously reported a feeder-free culture method for pure production of subculturable vascular endothelial cells (VECs) from cynomolgus monkey embryonic stem cells (cmESCs) without as using cell-sorting technique. By this method, canonical vascular endothelial (VE)-cadherin/platelet-endothelial cell adhesion molecule 1 (PECAM1)-positive VECs (c-VECs) and atypical VE-cadherin/PECAM1-negative VECs (a-VECs) were generated without a contamination by pericytes, lymphatic endothelial cells, or immature ES cells. More recently, we established a unique culture technique to maintain human ESCs (hESCs) under a feeder-free and recombinant cytokine-free condition. Combining these two systems, we have successfully generated pure VECs from two lines of hESCs, khES-1 and khES-3, under a completely feeder-free condition. Our method is very simple: spheres generated from hESCs by floating culture using differentiation media supplemented with vascular endothelial growth factor, bone morphogenetic protein 4, stem cell factor, FMS-related tyrosine kinase-3 ligand, and interleukin 3 (IL3) and IL6 were cultured on gelatin-coated plates. Cell passage was performed by an ordinary enzymatic treatment. The hESC-derived differentiated cells demosntrated cord-forming activities and acetylated low-density lipoprotein-uptaking capacities. Moreover, they exclusively expressed von Willebrand factor and endothelial nitric oxide synthase. Flow cytometric analyses indicate that khES-3 generated both c-VECs and a-VECs as in the case of cmESCs. By contrast, khES-1 produced only a-VECs, which nonetheless demonstrated effective recruitment into neovascularity in vivo. Interestingly, a-VECs turned to express PECAM1 after transplantation into immunodeficient mice.

Nieponice, A., L. Soletti, et al. (2008). "Development of a tissue-engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique." Biomaterials **29**(7): 825-33.

There is a clinical need for a tissue-engineered vascular graft (TEVG), and combining stem cells with biodegradable tubular scaffolds appears to be a promising approach. The goal of this study was to characterize the incorporation of muscle-derived stem cells (MDSCs) within tubular poly(ester urethane) urea (PEUU) scaffolds in vitro to understand their interaction, and to evaluate the mechanical properties of the constructs for vascular applications. Porous PEUU scaffolds were seeded with MDSCs using our recently described rotational vacuum seeding device, and cultured inside a spinner flask for 3 or 7 days. Cell viability, number, distribution and phenotype were assessed along with the suture retention strength and uniaxial mechanical behavior of the TEVGs. The seeding device allowed rapid even distribution of cells within the scaffolds. After 3 days, the constructs appeared completely populated with cells that were spread within the polymer. Cells underwent a population doubling of 2.1-fold, with a population doubling time of 35 h. Stem cell antigen-1 (Sca-1) expression by the cells remained high after 7 days in culture (77+/-20% vs. 66+/-6% at day 0) while CD34 expression was reduced (19+/-12% vs. 61+/-10% at day 0) and myosin heavy chain expression was scarce (not quantified). The estimated burst strength of the TEVG constructs was 2127+/-900 mm Hg and suture retention strength was 1.3+/-0.3N. We conclude from this study that MDSCs can be rapidly seeded within porous biodegradable tubular scaffolds while maintaining cell viability and high proliferation rates and without losing stem cell phenotype for up to 7 days of in-vitro culture. The successful integration of these steps is thought necessary to provide rapid availability of TEVGs, which is essential for clinical translation.

Ogawa, T. (2000). "Spermatogonial transplantation technique in spermatogenesis research." Int J Androl **23 Suppl 2**: 57-9.

The spermatogonial stem cell is the foundation of spermatogenesis and it continues to divide throughout the lifetime of a male. It divides to renew itself as well as to produce daughter cells that finally differentiate to spermatozoa. Transplantation of spermatogonial stem cells from a fertile mouse to the testis of a sterile mouse results in the development of donor cell derived spermatogenesis. Furthermore, spermatogenesis of the rat and hamster occurs in immunodeficient mouse testes following spermatogonial transplantation. Cryopreservation of spermatogonial stem cells has been shown to be applicable in many species for later transplantation. In addition, certain forms of infertile donor germ cell can be transplanted to produce functional sperm in a recipient testis. These new techniques will be useful for basic research of spermatogenesis and for application to reproductive technology in the future.

Opitz, U. and H. J. Seidel (1978). "Studies on the target cell for the Friend virus (FV-P strain) using the CFU-E technique." Blut **37**(4): 183-92.

In order to characterize the target cell for the polycythemia inducing Friend virus (FV-P) in vivo, mice were treated by induction of plethorism, bleeding, Actinomycin D, and Busulfan before virus infection. The development of the Friend leukemia was then studied mainly using the CFUE technique for erythroid colony growth in vitro. This technique allows the quantification of a new cell type, an erythropoietin (Ep) independent colony forming cell. These Ep independent colonies were taken as marker for the disease. Their number with time after infection was correlated with the compartment size of pluripotent, granuloid committed and erythroid stem cells at the time of infection. The results indicate that the development of the Friend leukemia does not require the actual presence of CFUE, as seen using Actinomycin D, and is not correlated with the number of pluripotent or granuloid stem cells, as seen after Busulfan. It is, however, dependent on the erythropoietic state of the animal, as seen in plethoric mice and mice after bleeding. It is, therefore, concluded that the target cell for FV-P is located within the Ep-responsive cell compartment, between early (BFUE) and late (CFUE) erythroid precursor cells.

Park, D. J., J. H. Choi, et al. (2007). "Tissue-engineered bone formation with gene transfer and mesenchymal stem cells in a minimally invasive technique." Laryngoscope **117**(7): 1267-71.

BACKGROUND: The objective of this study was to use a chitosan-alginate gel to implant bone marrow-derived mesenchymal stem cells subcutaneously in a minimally invasive manner and promote bone formation by the simultaneously transferred osteogenic protein (OP)-1 (bone morphogenic protein-7) gene. The complex of polyethylenimine/luciferase plasmid DNA embedded in the gel was able to transfect HEK 293 cells on a culture dish or co-encapsulated in the gel. When injected into the subcutaneous space of mice, luciferase expression was two to three orders of magnitude increased above the background. To examine the efficacy of gene-, cell-, and combined gene- and cell-encapsulated gels in tissue generation, samples were injected into the subcutaneous space of 6-week-old athymic nude mice, and the OP-1 plasmid was studied. At 8 weeks after the injection, the gels only maintained their volumetric shape when human mesenchymal stem cells (hMSCs) were encapsulated, but otherwise the gels were partially dissolved. Transgene expression of OP-1 was clearly detected in the samples after 4 weeks but not after 8 weeks. Type II collagen was detected in all the gels containing the OP-1 plasmid, with or without hMSCs. The samples with the combination of OP-1 DNA and hMSCs revealed strong type II collagen expression as well as osteoid foci. CONCLUSION: These results suggest that combined gene and hMSC delivery within a chitosan-alginate gel could be an interesting approach for tissue engineering.

Peralta, M. and F. J. Denaro (2003). "The horseradish peroxidase technique for cell lineage studies." Cell Mol Biol (Noisy-le-grand) **49**(8): 1371-5.

The identification of cell lineage for a given cell type of a particular tissue is an important step in understanding how this process contributes to histogenesis. The importance in understanding cell lineage has relevance for both theoretical and practical reasons. For example, delineating and identifying histogenic principals is required to advance stem cell research and tissue regeneration. To document cell lineage in a given experimental preparation, a number of techniques have been developed. This typically requires the injection of a tracer compound into a founder cell. As this cell produces progeny, the tracer is passed on to the daughter cells. By detecting the tracer in the progeny or daughter cells the investigator can determine which cells originated from the cell that was originally injected with the tracer. By using such an approach it is possible to trace the developmental path from the first cells of the embryo to the specialized cells making the tissue of the adult. A number of tracer compounds have been used with good results in lineage tracing. One of these tracer

compounds is horseradish peroxidase (HRP). Several variations of the technique are available depending on what species are studied or what histological requirements are made by the study. A basic technique that can be adapted to individual needs is presented. Included in this protocol on lineage tracing are the procedures for injection, fixation, and the microscope evaluation of labelled cells in the Helobdella triseralis embryo. A brief discussion of the technique will note its advantages and disadvantages. Examples of labelled cell preparations are given to illustrate the technique.

Petersen, L. J., K. Brasso, et al. (1996). "Histamine release in intact human skin by monocyte chemoattractant factor-1, RANTES, macrophage inflammatory protein-1 alpha, stem cell factor, anti-IgE, and codeine as determined by an ex vivo skin microdialysis technique." J Allergy Clin Immunol **98**(4): 790-6.

BACKGROUND: The chemokines monocyte chemoattractant factor-1, RANTES, and macrophage inflammatory protein-1 alpha release histamine from human basophils, as well as rat and mouse mast cells. The purpose of this investigation was to determine whether these chemokines release histamine from human skin mast cells in situ. METHODS: A microdialysis technique was used to measure histamine release in skin. First, the model was validated by using anti-IgE, codeine, and stem cell factor (SCF); then the histamine-releasing effects of the chemokines were investigated. A total of 47 skin specimens from 41 donors were investigated. Hollow microdialysis fibers were inserted intradermally, and each fiber was slowly perfused (3 microliters/min). Anti-IgE, codeine, SCF, or chemokines were injected intradermally above individual fibers, and dialysate was collected at 2-minute intervals for 20 minutes. Each series of investigations comprised five to eight single experiments. RESULTS: Anti-IgE (4 to 4000 U/ml), codeine (0.001 to 1 mg/ml), and SCF (5.4 x (10(-11) to 10(-8) mol/L)) released histamine in a dose-dependent manner; maximum histamine release was 97.4, 116.3, and 9.5 pmol/20 min, respectively. Monocyte chemoattractant factor-1, RANTES, and macrophage inflammatory protein-1 alpha in concentrations of 10(-10) to 10(-6) mol/L did not release histamine; histamine release by 10(-6) mol/L chemokine was less than 0.2 pmol/20 min. None of the chemokines modulated anti-IgE-induced histamine release. In contrast, SCF significantly potentiated anti-IgE-induced histamine release by 33%. All chemokines, but not SCF, released histamine from human basophils. CONCLUSION: We conclude that the chemokines monocyte chemoattractant factor-1, RANTES, and macrophage inflammatory protein-1 alpha do not release histamine from human skin mast cells.

Pirruccello, S. J., C. J. Page, et al. (1999). "Comparison of ISHAGE protocol CD34 cell enumeration with a lineage negative backgating technique." Cytotherapy **1**(4): 279-86.

BACKGROUND: CD34(+) cell enumeration in PBSC apheresis products has become the standard for assessing graft hematopoietic potential. METHODS: An in-house, three color, lineage negative-gating technique [University of Nebraska Medical Center (UNMC) protocol] for CD34 cell enumeration was compared with the ISHAGE protocol over 100 apheresis products. Cell doses determined by both methods were compared with each other and to colony-forming units-granulocyte/macrophage (CFU-GM) assay results. RESULTS: Overall, the assays compared well with each other for samples with CD34 cell doses > 0.2 2 10(6)/kg (r values > 0.8). The ISHAGE method showed a constant negative bias, with a mean of 38% in comparison to the UNMC protocol, which was more linear at lower cell doses. Both assays showed similar correlation with CFU-GM doses after log conversion (UNMC, r = 0.915; ISHAGE, r = 0.917). When comparing integer values, however, the ISHAGE method correlated with CFU-GM only in the high dose range (CFU-GM > 2 2 10(4)/kg), while the UNMC method correlated across the entire measured range of CFU-GM doses. Finally, an inter-technologist gating reproducibility study (n = 6) yielded a 23% coefficient of variation (CV) for the ISHAGE method and a 7% CV for the UNMC method, when the same two sets of CD34 histograms were analyzed to calculate cell dose.

Polouckova, A., A. Vodvarkova, et al. (2001). "Comparison of two different methods for CD34+ selection and T cell depletion in peripheral blood stem cell grafts--our experiences with CellPro, E rosetting and CliniMACS technique." Neoplasma **48**(5): 374-81.

The aim of this study was to establish a suitable method for in vitro T cell depletion in peripheral blood stem cell grafts for mismatched/haploidentical transplantation in children and adults with severe hematological disorders and for autologous transplantation in patients with autoimmune diseases refractory to conventional immunosuppressive treatment. Two different selection techniques have been used: CD34+ selection using immunoaffinity columns (CellPro Ceprate) followed by T cell depletion by E-rosetting or CD34+ selection using submicroscopic paramagnetic beads (CliniMACS device) with T cell depletion in a one

step procedure. The mean purity and recovery of CD34+ cells and efficiency of T cell removal in the final product were compared. From March 1995 to December 1998 we prepared twelve allografts using Cell Pro system for eight children with high-risk hematological malignancies and six autografts for six patients with severe autoimmune diseases. From January 1999 to October 2000 we prepared fifteen allografts using CliniMACS system for ten children with high-risk hematological diseases and inborn metabolic disorders or primary immunodeficiences, five allografts for three adult patients with high-risk hematological malignancies and two autografts for two patients with autoimmune diseases. In allogeneic transplantation the median purity of CD34+ cells in the final products after CellPro and E-rosetting was 85.6% (55.3%-95.7%); median recovery was 24.8% (17%-35%), median transplanted doses of T cells per kilogram of body weight were 0.66x10(4) (0-2.8); in autologous transplantation the median purity of CD34+ was 92.6% (55.6%-96%), median recovery was 28% (22%-46.2%), median transplanted doses of T cells per kilogram of body weight were 0.39x10(4) (0.0-3.6). After CliniMACS technique the median purity of CD34+ cells was 94.87% (69.15%-99%),medianrecoverywas 58% (30%-79.6%), median transplanted doses of T cells per kg of body weight were 0.254x10(4) (0-14.15); in autologous transplantation the median purity of CD34+ was 94% (94%-94%, median recovery was 97.4% (95%-99.8%), median transplanted doses of T cells per kilogram of body weight were 0.87x10(4) (0.49-1.24).

Poot, M., J. R. Silber, et al. (2002). "A novel flow cytometric technique for drug cytotoxicity gives results comparable to colony-forming assays." Cytometry **48**(1): 1-5.

BACKGROUND: Drug sensitivity is commonly determined by assays that utilize colony formation to discriminate between surviving and lethally treated cells. These assays require cells with high plating efficiency that form discernible colonies, are time-consuming and laborious, and require manual counting of large numbers of colonies. To overcome these drawbacks, we developed a flow cytometric technique that assays survival of proliferative capacity in cultured cells. METHODS: Labeling with bromodeoxyuridine for 72 h followed by bivariate Hoechst 33258/ethidium bromide flow cytometry allows discrimination of nonproliferating cells from those that have undergone one to three divisions. Addition of an internal standard, chicken erythrocyte nuclei, permits determination of total cell number. To validate our assay, we used flow and colony-forming assays to determine the sensitivity of cell lines derived from Werner syndrome patients and unaffected individuals to 4-nitroquinoline-1-oxide (4NQO) and camptothecin. RESULTS: The flow and colony-forming assays yielded comparable sensitivity for each drug and essentially identical increases in drug sensitivity exhibited by Werner syndrome cells. CONCLUSION: Our results indicate that the flow assay is a less laborious surrogate for colony-forming assays. The flow technique will also facilitate the analysis of drug sensitivity in cells that are not amenable to colony-forming assays.

Qian, C., R. A. Tio, et al. (2007). "A promising technique for transplantation of bone marrow-derived endothelial progenitor cells into rat heart." Cardiovasc Pathol **16**(3): 127-35.

OBJECTIVE: To investigate the feasibility of intracoronary application of endothelial progenitor cells and the subsequent distribution within the heart. METHODS: Endothelial progenitors cells (EPCs) cultured from rat bone marrow were identified by double-positive staining with Dil-Ac-LDL and BS1-lectin. Twenty-four hours before cell transplantation, EPCs were labeled with 5-bromo-2'-deoxyuridine (BrdU). Cells (5 x 10(5) in 250-microl medium) were injected into healthy rats, either as intracoronary application (n=11) or as intramyocardial injection (n = 6). At 15 min or 3 days posttransplantation, hearts as well as other organs (lung, liver, kidney, and spleen) were collected and processed for subsequent BrdU immunohistochemistry. The number of BrdU-positive cells per tissue area was counted. RESULTS: Compared to intramyocardial injection, intracoronary administration resulted in more than twice as much positive cells in the heart ($P < .05$), with no local differences within the heart. Whereas after 15 min, EPCs were equally distributed in all examined organs (except for the spleen), cells that were still present after 3 days, approximately 10%, were selectively restricted to the heart.

Rallapalli, S., D. K. Bishi, et al. (2009). "A multiplex PCR technique to characterize human bone marrow derived mesenchymal stem cells." Biotechnol Lett **31**(12): 1843-50.

Human mesenchymal stem cells (MSCs), with capacity to differentiate into adipocytes, osteoblasts and chondrocytes, offer potential for the development of novel treatments. A critical question in MSCs biology is whether this cell population possesses a relatively uniform differentiation capability or is comprised of distinct subsets of progenitors committed to differentiate in particular pathways. To quantify the changes during growth of MSCs, we analyzed the mesenchymal phenotype and differentiation ability using a multi-marker PCR with six primer sets specific for CD73, CD90, CD105,

CD166, CD45 and beta-actin allowing a gel-based differential detection of the PCR products. To determine degree of variability of MSCs populations in terms of proliferation, cell proliferation assays were performed on expanded MSCs up to the sixth passage. At each passage, the osteogenic and adipogenic differentiation potentials of MSCs were verified by culture in inductive media. RT-PCR and cytochemical analysis revealed that, despite the loss of multipotentiality during expansion, certain markers remain expressed, indicating that these markers are unlikely to be reflective of the MSC's true 'stem cell' nature. Our results suggest that decrease in the expression of MSCs specific markers correlates with down-regulation of proliferation ability and differentiation efficiency of MSCs.

Rana, K., J. L. Liesveld, et al. (2009). "Delivery of apoptotic signal to rolling cancer cells: a novel biomimetic technique using immobilized TRAIL and E-selectin." Biotechnol Bioeng **102**(6): 1692-702.

The survival rate for patients with metastases versus localized cancer is dramatically reduced, with most deaths being associated with the formation of secondary tumors. Circulating cancer cells interact with the endothelial lining of the vasculature via a series of adhesive interactions that facilitate tethering and firm adhesion of cancer cells in the initial steps of metastasis. TNF-related apoptosis-inducing ligand (TRAIL) holds promise as a tumor-specific cancer therapeutic, by inducing a death signal by apoptosis via the caspase pathway. In this study, we exploit this phenomenon to deliver a receptor-mediated apoptosis signal to leukemic cells adhesively rolling along a TRAIL and selectin-bearing surface. Results show that cancer cells exhibit selectin-mediated rolling in capillary flow chambers, and that the rolling velocities can be controlled by varying the selectin and selectin surface density and the applied shear stress. It was determined that a 1 h rolling exposure to a functionalized TRAIL and E-selectin surface was sufficient to kill 30% of captured cells compared to static conditions in which 4 h exposure was necessary to kill 30% of the cells. Thus, we conclude that rolling delivery is more effective than static exposure to a TRAIL immobilized surface. We have also verified that there is no significant effect of TRAIL on hematopoietic stem cells and other normal blood cells. This represents the first demonstration of a novel biomimetic method to capture metastatic cells from circulation and deliver an apoptotic signal.

Rapallino, M. V. and A. Cupello (2001). "Holger Hyden's technique of preparation of single Deiters' neurons and study of permeability characteristics of their plasma membranes." Brain Res Brain Res Protoc **8**(1): 58-67.

The protocols described here refer to Hyden's technique of isolation and microdissection of vestibular Deiters' neurons from adult mammals. The isolation of Deiters' cells from bovine is described and an example is given of the immunocytochemical visualization of their GABA(A) receptors by monoclonal antibodies against the beta(2/3) subunit. In addition, the protocol of the method for isolation of Deiters' cells from adult rabbit brain stem, the preparation of their plasma membranes and the study of their permeability characteristics is presented. Also in this case, examples of its application to the determination of chloride permeability and its modulation by GABA are given.

Ravindranath, N. and M. Dym (1999). "Isolation of rat ventral prostate basal and luminal epithelial cells by the STAPUT technique." Prostate **41**(3): 173-80.

BACKGROUND: The prostatic epithelium consists principally of basal epithelial cells, luminal epithelial cells, and neuroendocrine cells. Several studies support the concept that among basal cells, a subpopulation of stem cells resides which is capable of giving rise to other stem cells, basal epithelial cells, and also luminal epithelial cells and neuroendocrine cells. Other investigators suggest that luminal epithelial cells can also regenerate prostatic epithelium. Availability of pure populations of basal and luminal epithelial cells will aid in studies on defining the cellular pathways of differentiation during normal and pathological conditions. This study was designed to isolate and characterize pure populations of basal and luminal epithelial cells from adult rat ventral prostates. METHODS: Sequential enzymatic digestion and differential plating permitted the separation of glandular epithelial cells from stromal cells. The glandular epithelial cells were subjected to the STAPUT technique. RESULTS: Two types of cell populations, a large single-cell population and a small single-cell population, were obtained and characterized as basal and luminal epithelial cells by immunostaining for cytokeratin 5 and cytokeratin 8, respectively. CONCLUSIONS: Our results indicate that purified populations of prostatic basal and luminal epithelial cells can be isolated by the STAPUT technique.

Riew, K. D., J. Lou, et al. (2003). "Thoracoscopic intradiscal spine fusion using a minimally invasive gene-therapy technique." J Bone Joint Surg Am **85-A**(5): 866-71.

BACKGROUND: Gene therapy has been utilized to achieve posterior intertransverse process fusion in rodents. To our knowledge, however, no one

has previously reported on the use of this technique to achieve anterior spinal fusion in mammals. The purpose of the present study was to determine if a gene-therapy technique can be utilized to achieve anterior intradiscal fusion in pigs with use of minimally invasive techniques. METHODS: Mesenchymal stem cells were isolated from each of three pigs, expanded in culture, and transduced with an adenovirus carrying either the gene for bone morphogenetic protein-2 (Adv-BMP2) or the control gene, beta-galactosidase (Adv-betagal). In vitro, assays were performed to detect BMP-2 expression as well as protein markers of bone formation. In vivo, four thoracic disc spaces in each of three pigs were injected thoracoscopically with cells after 1 cm (3) of the disc had been removed. In each of the three pigs, two discs were injected with autologous mesenchymal stem cells transduced with Adv-BMP2, the third disc was injected with cells transduced with Adv-betagal (control 1), and the fourth disc served as the sham-operated control (control 2). The three animals were killed six weeks after the implantation. Computerized tomographic scanning was performed on two of the specimens, and histological examination was performed on all specimens. The computerized tomographic scans and histological examinations were then interpreted in a blinded fashion. RESULTS: In the in vitro study, a human BMP-2 protein band was detected in the medium of Adv-BMP2-transduced stem cells but not in that of the control cells. The Adv-BMP2-transduced stem cells were associated with a fivefold increase in alkaline phosphatase activity compared with the controls as well as with matrix mineralization and increased protein expression of type-I collagen, osteopontin, and bone sialoprotein. In the in vivo study, radiographic examination demonstrated anterior spinal fusion in all six disc spaces that had been treated with implantation of Adv-BMP2-transduced stem cells. In contrast, the six control disc spaces had little or no intervening bone. Histological examination demonstrated bridging bone from end plate to end plate in all six disc spaces that had been treated with implantation of Adv-BMP2-transduced stem cells. The six control disc spaces had no bridging bone. CONCLUSION: The Adv-BMP2-transduced mesenchymal stem cells produced BMP-2 protein. Further, the cells differentiated into osteoblasts and induced anterior spinal fusion in six of six disc spaces in this pig model. Although many technical and practical challenges remain, the results of the present study suggest that it may eventually be possible to use similar techniques to achieve anterior spinal fusion in humans.

Sadiq, M. N., A. S. Arif, et al. (2009). "Use of supero-temporal free conjunctivo-limbal autograft in the surgical management of pterygium: our technique and results." J Ayub Med Coll Abbottabad **21**(4): 121-4.

BACKGROUND: A pterygium is a fibro-vascular, wing shaped encroachment of the conjunctiva on to the cornea. The prevalence rates ranges from 0.7 to 31% among different populations and also influenced by age, race, and exposure to solar radiations. Ultraviolet light-induced damage to the limbal stem cell barrier with subsequent conjunctivalisation of the cornea is the currently accepted aetiology of this condition. Indication of surgery include visual impairment, cosmetic disfigurement, motility restriction, recurrent inflammation and interference with contact lens wear. This Study was conducted to know the safety, efficacy and success with supero-temporal free conjunctivo-limbal auto graft transplantation in the surgical management of pterygium. METHODS: Prospective case series where supero temporal free conjunctivo-limbal auto grafting was performed on 72 eyes of 72 patients with vascular progressive pterygia. Pterygium recurrence was considered a surgical failure. Recurrence was defined as fibro vascular tissue crossing the corneo-scleral limbus on to the clear cornea in the area of previous pterygium excision before 6 months. RESULTS: Recurrence of pterygium was noted in three out of 72 eyes (4.16%), after a minimum follow up of 12 months to maximum of 25 months (Mean 14.6 months). No major intra-operative or postoperative complications were encountered. CONCLUSION: Supero temporal free conjunctivo-limbal auto graft appears to be a safe and effective technique in the surgical management of pterygium. The inclusion of limbal tissue in the conjunctival auto graft following pterygium excision appears to be essential to ensure low recurrence rate.

Sadraei, H., S. R. Abtahi, et al. (2009). "Assessment of potassium current in Royan B(1) stem cell derived cardiomyocytes by patch-clamp technique." Res Pharm Sci **4**(2): 85-97.

Embryonic stem cells are capable of differentiating to variety of cell tissues including cardiomyocytes. This developmental change is accompanied with a great deal of ion channel expression and functions. Mouse stem cell derived cardiomyocytes were prepared and separated to yield isolated single cell suspension for cell current recording. In the present study some properties of the K(+)-current in Royan B(1) stem cell derived cardiomyocytes were investigated using whole cell patch-clamp technique. When the holding potential was - 60 mV, in some cells a major outward current was elicited by square depolarizing pulses from -60 mV to +50 mV. This outward current was sustained for the duration of 300 ms test pulse. The sustained

outward K(+) current was inhibited by tetraethylammonium (10 mM) indicating the activity of Ca(2+) activated K(+) channel in these cells. In some of the cells with 0.2 mM 3,ethylene glycol-bis (beta-aminoethyl ether) N,N,N(`),N(`)-tetraacetic acid in the pipette, only a very small outward current was recorded which suggests that in these cells the voltage activated K(+) channels is either absent or if existed it is not fully functional. Other cells were in far between, indicating that voltage activated K(+) channels are developing in these cells but it is not yet fully functional. In conclusion, we have identified functional large conductance Ca(2+) activated K(+) channel in Royan B(1) stem cell derived cardiomyocytes.

Sawyer, B. M., N. B. Westwood, et al. (1994). "Circulating megakaryocytic progenitor cells in patients with primary thrombocythaemia and reactive thrombocytosis: results using a serum-deprived culture assay and a positive detection technique." Eur J Haematol **53**(2): 108-13.

A serum-free culture method was used to study the growth of megakaryocytic progenitor cells (CFU-Meg) from patients with elevated platelet counts. The culture technique was combined with immunocytochemistry (APAAP, CD61) for the identification of CFU-Meg derived cells in cytopreparations of cells eluted from the culture dishes. Twenty-six patients with primary thrombocythaemia (14 untreated patients, UPT, 12 treated patients, TPT), 14 patients with reactive thrombocytosis (RT) and 9 normal individuals were studied. Unstimulated growth of CD61-positive cells was detected in 8/14 UPT, 8/12 TPT, 12/14 RT and 5/9 normal subjects (with mean CD61-positive cell counts of 75, 579, 236 and 7 per cytopreparation respectively). Cultures supplemented with interleukin 3 contained CD61-positive cells in 11/14 UPT, 7/12 TPT, 14/14 RT and 5/9 normal subjects (with mean CD61-positive cell counts of 157, 589, 250 and 7 per cytopreparation respectively). Thus, this serum-free culture technique combined with sensitive positive identification of CFU-Meg derived cells failed to discriminate between PT and RT. These results cast doubt on the usefulness of serum-free culture assays for the detection of unstimulated CFU-Meg growth in the differential diagnosis of patients with elevated platelet counts.

Schlueter, A. J., S. K. Bhatia, et al. (2001). "Delineation among eight major hematopoietic subsets in murine bone marrow using a two-color flow cytometric technique." Cytometry **43**(4): 297-307.

BACKGROUND: Many methods have been developed specifically for identifying hematopoietic progenitor cells in murine bone marrow, but few methods allow rapid identification of multiple bone marrow populations. We describe a new, simple method for identifying simultaneously eight populations in murine bone marrow with two-color flow cytometry and phenotypically define these populations. METHODS: Bone marrow was stained with anti-Ly-6C and anti-B220 (CD45R) in one fluorochrome and wheat germ agglutinin (WGA) in another fluorochrome. The eight populations identified in this way were defined further primarily by four-color flow cytometry. RESULTS: Six of the eight populations were characterized phenotypically as containing erythroid, granulocytic, mast, early B, mature B, and stem cell populations. Two additional populations with phenotypic characteristics of partially differentiated precursor cells also were identified. One population was Ly-6C/B220+ and WGA-. It also expressed markers associated with early B, T, and/or dendritic cell differentiation. The second population was Ly-6C(hi)WGA(hi)Mac-1+ and was negative for numerous other lineage-specific and precursor markers. Its morphology suggested monocytic differentiative potential. CONCLUSIONS: A two-color flow cytometric assay profiles six bone marrow populations with identifiable phenotypes and two additional unique, putative hematopoietic precursor populations.

Sedlak, J. and B. Chorvath (1991). "Fluorescent double labeling of normal and malignant hematopoietic cells by monoclonal antibodies (FITC) and anthracycline cytostatic drug (Daunomycin): a cytometric technique for analysis of drug uptake in hematopoietic cell subpopulations." Neoplasma **38**(1): 13-20.

A technique of simultaneous double labeling of normal and neoplastic hematopoietic cells with FITC-conjugated monoclonal antibodies directed to selectively expressed hematopoietic cell surface antigens (green fluorescence) and the anthracycline cytostatic drug (Daunomycin, red fluorescence) was described. Flow cytometric analysis of double labeled cells permitted anthracycline cell content determination in peripheral blood lymphocytes, granulocytes, monocytes from healthy donors, T-(MOLT-4), non-T, non-B (REH) and myelomonocytic (U-937) leukemic cell lines. After mixing peripheral blood lymphocytes from healthy individuals with cultured leukemic cells labeled on a restrictively expressed hematopoietic cell differentiation antigen (CALLA-CD10-, MHC class II-DR-antigen, a myelomonocytic differentiation antigen) detected by corresponding monoclonal antibodies (DGH-10-1-A9,Bra30, BraC8), the described technique allowed separate measurements of anthracycline content in

leukemic cells vs. peripheral blood lymphocytes from healthy donors. Potential diagnostic aspects and research utilization of this technique are discussed.

See, E. Y., S. L. Toh, et al. (2008). "Technique to accurately quantify collagen content in hyperconfluent cell culture." J Mol Histol **39**(6): 643-7.

Tissue engineering aims to regenerate tissues that can successfully take over the functions of the native tissue when it is damaged or diseased. In most tissues, collagen makes up the bulk component of the extracellular matrix, thus, there is great emphasis on its accurate quantification in tissue engineering. It has already been reported that pepsin digestion is able to solubilize the collagen deposited within the cell layer for accurate quantification of collagen content in cultures, but this method has drawbacks when cultured cells are hyperconfluent. In this condition, Pepsin digestion will result in fragments of the cell layers that cannot be completely resolved. These fragments of the undigested cell sheet are visible to the naked eye, which can bias the final results. To the best of our knowledge, there has been no reported method to accurately quantify the collagen content in hyperconfluent cell sheet. Therefore, this study aims to illustrate that sonication is able to aid pepsin digestion of hyperconfluent cell layers of fibroblasts and bone marrow mesenchymal stem cells, to solubilize all the collagen for accurate quantification purposes.

Shankaran, M., C. King, et al. (2006). "Discovery of novel hippocampal neurogenic agents by using an in vivo stable isotope labeling technique." J Pharmacol Exp Ther **319**(3): 1172-81.

Neurogenesis occurs in discrete regions of adult mammalian brain, including the subgranular zone of the hippocampus. Hippocampal neurogenesis is enhanced by different classes of antidepressants, but screening for neurogenic actions of novel antidepressants has been inefficient because of limitations of 5-bromo-2'-deoxyuridine labeling techniques. We describe an efficient in vivo method for measuring hippocampal neurogenesis involving incorporation of the stable isotope, (2)H, into genomic DNA during labeling with (2)H(2)O (heavy water). Male rodents received 8 to 10% (2)H(2)O in drinking water; DNA was isolated from hippocampal progenitor cells or neurons. Label incorporation into progenitor cells of Swiss-Webster mice revealed subpopulation kinetics: 16% divided with t(1/2) of 2.7 weeks; the remainder did not divide over 1 year. Progenitor cell proliferation rates in mice were strain-dependent. Chronic antidepressant treatment for 3 weeks, with (2)H(2)O administered during the final week, increased progenitor cell proliferation across all the strains tested. Fluoxetine treatment increased (2)H incorporation into DNA of gradient-enriched neurons or flow-sorted neuronal nuclei 4 weeks after (2)H(2)O labeling, representing the survival and differentiation of newly divided cells into neurons. By screening 11 approved drugs for effects on progenitor cell proliferation, we detected previously unrecognized, dose-dependent enhancement of hippocampal progenitor cell proliferation by two statins and the anticonvulsant topiramate. We also confirmed stimulatory activity of other anticonvulsants and showed inhibition of progenitor cell proliferation by isotretinoin and prednisolone. In conclusion, stable isotope labeling is an efficient, high-throughput in vivo method for measuring hippocampal progenitor cell proliferation that can be used to screen for novel neurogenic drugs.

Shen, S., L. Yuan, et al. (2009). "An effort to test the embryotoxicity of benzene, toluene, xylene, and formaldehyde to murine embryonic stem cells using airborne exposure technique." Inhal Toxicol **21**(12): 973-8.

Benzene, toluene, xylene, and formaldehyde are well-known indoor air pollutants, especially after house decoration. They are also common pollutants in the working places of the plastic industry, chemical industry, and leather industry. It has been reported that these pollutants cause people to be irritated, sick, experience a headache, and be dizzy. They also have the potential to induce asthma, aplastic anemia, and leukemia, even cause abortion or fetus malformation in humans. In this study, the airborne toxicity of benzene, toluene, xylene, and formaldehyde to murine embryonic stem cells (mES cells) were tested using airborne exposure technique to evaluate the mES cell airborne exposure model on embryotoxicity prediction. Briefly, mES cells were cultured on Transwell inserts and were exposed to an airborne surrounding of test chemicals in a chamber for 1 h at 37 degrees C. Cytotoxicity was determined using the MTT assay after further culture for 18 h at 37 degrees C in normal medium. The airborne IC(50) (50% inhibition concentration) of benzene, toluene, xylene, and formaldehyde derived from the fitted dose-response curves were 17,400 +/- 1290, 16,000 +/- 250, 4680 +/- 500, and 620 +/- 310 ppm, respectively. Formaldehyde was found to be the compound most toxic to mES cells compared to benzene homologues. The toxicity data had good correlation with the in vivo data. The results showed that the mES airborne exposure model may be used to predict embryotoxicity of volatile organic compounds.

Solchaga, L. A., E. Tognana, et al. (2006). "A rapid seeding technique for the assembly of large

cell/scaffold composite constructs." Tissue Eng **12**(7): 1851-63.

These studies address critical technical issues involved in creating human mesenchymal stem cell (hMSC)/ scaffold implants for cartilage repair. These issues include obtaining a high cell density and uniform spatial cell distribution within the scaffold, factors that are critical in the initiation and homogeneity of chondrogenic differentiation. For any given scaffold, the initial seeding influences cell density, retention, and spatial distribution within the scaffold, which eventually will affect the function of the construct. Here, we discuss the development of a vacuum-aided seeding technique for HYAFF -11 sponges which we compared to passive infiltration. Our results show that, under the conditions tested, hMSCs were quantitatively and homogeneously loaded into the scaffolds with 90+% retention rates after 24 h in perfusion culture with no negative effect on cell viability or chondrogenic potential. The retention rates of the vacuum-seeded constructs were at least 2 times greater than those of passively seeded constructs at 72 h. Histomorphometric analysis revealed that the core of the vacuum-seeded constructs contained 240% more cells than the core of passively infiltrated scaffolds. The vacuum seeding technique is safe, rapid, reproducible, and results in controlled quantitative cell loading, high retention, and uniform distribution.

Span, L. F., A. H. Pennings, et al. (2002). "The dynamic process of apoptosis analyzed by flow cytometry using Annexin-V/propidium iodide and a modified in situ end labeling technique." Cytometry **47**(1): 24-31.

BACKGROUND: To study the apoptotic process in time, we used the following flow cytometric (FCM) techniques: phosphatidylserine (PS) translocation by Annexin-V (AnV), DNA fragmentation by in situ end labeling (ISEL), and propidium iodide (PI) staining. Because PS translocation is assumed to be an early feature of programmed cell death (PCD), we questioned if AnV positivity implies inevitable cell death. METHODS: Apoptosis was induced in Jurkat cells by gamma-irradiation, incubation with camptothecin (CPT), or cytosine beta-D-arabinofuranoside (Ara-C). At different time intervals, PCD was quantified by AnV/PI and ISEL. To analyze the influence of cell handling procedures on PCD, we applied these three FCM techniques on CD34+ bone marrow (BM) stem cells after selection and after a freeze-thaw procedure. Various AnV/PI- CD34+ fractions were cultured in a single-cell single-well (SCSW) assay. RESULTS: Jurkat cells under three different detrimental conditions showed essentially the same pattern of apoptosis in time. Initially developed AnV+/PI- cells subsequently (within 1 h) showed ISEL positivity, after which they turned into AnV+/PI++ cells with even higher levels of ISEL positivity (80-90%). Eventually, they lost some of their PI and ISEL positivity and formed the AnV+/PI+ fraction. Cell handling of CD34+ cells caused high and variable AnV+/PI- fractions (overall range 23-62%). Within total AnV+ and AnV+/PI- populations, only a minority of CD34+ cells showed ISEL positivity (range 4-8% and 0.8-6%, respectively). Different fractions of AnV+/PI- CD34+ cells did have clonogenic capacity. CONCLUSIONS: PCD of cell suspensions in vitro can be followed accurately in time by these three FCM techniques. PS translocation is followed rapidly (within 1 h) by oligo-nucleosomal DNA fragmentation, after which cell (and nuclear) membrane leakage occurs. Detection of PS asymmetry by AnV-fluorescein isothiocyanate (FITC) is not always associated with (inevitable) apoptosis, as can be concluded from the proliferative capacity of AnV+ /PI- CD34+ cells in the SCSW assay.

Steadman, J. R., W. G. Rodkey, et al. (2001). "Microfracture: surgical technique and rehabilitation to treat chondral defects." Clin Orthop Relat Res(391 Suppl): S362-9.

Full-thickness articular cartilage defects rarely heal spontaneously. Some patients may not have clinically significant problems from chondral defects, but most eventually have degenerative changes. Techniques to treat chondral defects include abrasion, drilling, autografts, allografts, and cell transplantation. The senior author (JRS) developed the microfracture technique to enhance chondral resurfacing by providing a suitable environment for new tissue formation and taking advantage of the body's own healing potential. Microfracture has been done in more than 1800 patients. Specially designed awls are used to make multiple perforations, or microfractures, into the subchondral bone plate. Perforations are made as close together as possible, but not so close that one breaks into another. They usually are approximately 3 to 4 mm apart. The integrity of the subchondral bone plate must be maintained. The released marrow elements (including mesenchymal stem cells, growth factors, and other healing proteins) form a surgically induced super clot that provides an enriched environment for new tissue formation. The rehabilitation program is crucial to optimize the results of the surgery. It promotes the ideal physical environment for the marrow mesenchymal stem cells to differentiate into articular cartilagelike cells, ultimately leading to development of a durable repair cartilage that fills the original defect.

Takeuchi, T. and G. D. Palermo (2004). "Implications of cloning technique for reproductive medicine." Reprod Biomed Online **8**(5): 509-15.

The birth of Dolly following the transfer of mammary gland nuclei into enucleated eggs established cloning as a feasible technique in mammals, but the moral implications and high incidence of developmental abnormalities associated with cloning have induced the majority of countries to legislate against its use with human gametes. Because of such negative connotations, restrictive political reactions could jeopardize the therapeutic and scientific promise that certain types of cloning may present. For example, in addition to its proposed use as a way of generating stem cells, the basic technique of nuclear transplantation has proven useful in other ways, including its application to immature eggs as a new approach to the prevention of the aneuploidy common in older women, and for some recent advances in preimplantation genetic diagnosis. Thus, while attempts at reproductive cloning in man would seem premature and even dangerous at present, this field will require rational rather than emotional reactions as a basis for legislation if the therapeutic promise of stem cell research and the experimental potential of nuclear transplantation techniques are to be fully realized.

Thomander, L., J. Arvidsson, et al. (1982). "Distribution of sensory ganglion cells innervating facial muscles in the cat. An anatomical study with the horseradish peroxidase technique." Acta Otolaryngol **94**(1-2): 81-92.

The question of a possible sensory component in branches of the facial nerve innervating facial mimetic muscles in the cat was examined by the technique of retrograde axonal transport of horseradish peroxidase (HRP). HRP was applied to the proximal cut end of facial nerve branches innervating different facial muscle groups. Following survival periods of 71-75 h the animals were fixed by perfusion. Certain craniospinal sensory ganglia and the brain stem were processed histochemically for demonstration of HRP. HRP-labelled cell bodies, structurally resembling sensory neurons, were consistently observed ipsilaterally in the geniculate and proximal vagal ganglia and under certain conditions in the trigeminal ganglion. Measurements of HRP-labelled neurons in the geniculate and proximal vagal ganglia showed a wide size range but a unimodal size distribution with peaks in the small size range. These findings support the view that facial nerve branches innervating the mimetic muscles contain different types of sensory fibers.

Tsunoda, K., T. Baer, et al. (2001). "Autologous transplantation of fascia into the vocal fold: long-term results of a new phonosurgical technique for glottal incompetence." Laryngoscope **111**(3): 453-7.

OBJECTIVES: To study the long-term results of autologous transplantation of fascia into the vocal fold, and to evaluate our use of autologous transplantation instead of bovine collagen injection in cases of glottal incompetence. STUDY DESIGN: Retrospective study of the patients who have undergone autologous fascia transplantation using our new technique. METHODS: Follow-up studies were performed for at least 1 year (up to 3 y) on 9 autologous fascia transplant patients (6 cases with type 1 procedures and 3 cases with type 2 procedures). Clinical observations, including laryngeal stroboscopy, and measurement of maximum phonation time (MPT) were carried out. RESULTS: During 3 months after autologous fascia transplantation, MPT gradually increased and stroboscopy showed improved glottal closure. These improvements continued beyond 1 year in all cases of type 1 surgery and 2 of 3 cases of type 2 surgery. CONCLUSIONS: Autologous transplantation of fascia into the vocal folds as a phonosurgical treatment for glottal incompetence yields excellent long-term results. Temporal fascia appears to be a highly suitable tissue for transplantation in Reinke's space. However, the fascia is less suitable for transplantation in the muscle. We speculate that transplantation of temporal fascia leads to regeneration of vocal fold tissue, perhaps using a mechanism similar to stem cell transplantation in other organs.

Turner, C. W., J. Luzins, et al. (1992). "A modified harvest technique for cord blood hematopoietic stem cells." Bone Marrow Transplant **10**(1): 89-91.

Human umbilical cord blood is an excellent source of hematopoietic stem cells for research and bone marrow transplantation. We have developed a modified technique which effectively flushes and collects placental derived stem cells. Catheters were inserted into the umbilical artery and vein. Using this sterile and closed system technique, 10 saline diluted cord blood harvests had hematocrits of 16% to 25%, total mononuclear cell counts of 1.17 to 34.4 x 10(8) and volumes of 105-245 ml. The averaged extrapolated colony forming units from five cord blood harvests were as follows: CFU-GM 5.2 x 10(5) (9.96 x 10(4) to 1.24 x 10(6)), BFU-E 8.82 x 10(5) (2.40 x 10(4) to 1.61 x 10(6)) and CFU-GEMM 1.37 x 10(5) (7.33 x 10(3) to 3.18 x 10(5)). This quick technique avoids needle exposure and collects a significant volume of stem cells.

Tyer, C. L., J. J. Vredenburgh, et al. (1996). "Breast cancer cells are effectively purged from peripheral blood progenitor cells with an immunomagnetic technique." Clin Cancer Res **2**(1): 81-6.

Peripheral blood progenitor cells (PBPCs) are being used increasingly to provide hematopoietic support after intensive chemotherapy. However, many investigators have detected tumor cells contaminating PBPC collections. Methods that eliminate the tumor cells and spare the normal hematopoietic progenitor cells may improve the number of long-term, disease-free survivors after intensive chemotherapy. We developed an effective method using anti-breast cancer murine monoclonal antibodies (MoAbs) and immunomagnetic beads to eliminate a low percentage of breast cancer cells from PBPCs. We identified optimal anti-breast cancer MoAbs that react with membrane glycoproteins and conditions for selective removal of tumor cells. Using three anti-breast cancer MoAbs (260F9, 317G5, and 520C9) at 0.8 microgram/ml, a cell concentration of 2 x 10(8) cells/ml and a bead:total cell ratio of 0.75 beads:1 cell, we eliminated 3.3-4.8 (mean, 4.1) logs of tumor cells consistently from a model system with 1% breast cancer cells and 99% normal PBPCs. Similar levels of tumor cell elimination were obtained with three breast cancer cell lines. Colony-forming units were not affected adversely, with a mean recovery of 200% compared with the control. A clinical trial has begun that uses immunomagnetically purged, autologous bone marrow and PBPCs to support patients with metastatic breast cancer receiving high-dose chemotherapy.

Urbano-Ispizua, A., C. Solano, et al. (1998). "Allogeneic transplantation of selected CD34+ cells from peripheral blood: experience of 62 cases using immunoadsorption or immunomagnetic technique. Spanish Group of Allo-PBT." Bone Marrow Transplant **22**(6): 519-25.

The objective of this study was to analyze CD34+ cell recovery and T cell depletion (TCD) achieved in CD34+ cell grafts using either immunoadsorption or immunomagnetic methods applied to leukapheresis products from healthy donors. We also wanted to determine the kinetics of engraftment and incidence and severity of graft-versus-host disease (GVHD) after allogeneic transplantation of selected CD34+ cells. HLA-identical sibling donors received G-CSF. After leukapheresis, peripheral blood progenitor cells were selected using immunoadsorption (Ceprate SC) (n = 38) or immunomagnetic (Isolex 300) (n = 24) methods. Sixty-two patients, with a median age of 42 years (range 17-60) diagnosed with hematological malignancies were conditioned with either cyclophosphamide and total body irradiation (n = 43) or busulphan and cyclophosphamide (n = 19). GVHD prophylaxis consisted of cyclosporin A (CsA) and prednisone (n = 48), CsA alone (n = 11) and CsA and methotrexate (n = 3). The median yield and purity of CD34+ cells after the procedure was 65 and 66% with immunoadsorption, and 48 and 86% with immunomagnetic method, respectively. The median number (range) of CD34+ cells infused into the patients was 3.5 x 10(6)/kg (1-9.6). The median number (range) of CD3+ cells administered was 0.4 x 10(6)/kg (0.01-2) using immunoadsorption and 0.14 x 10(6)/kg (0.03-2.5) using immunomagnetic methods. Neutrophil recovery >500 and >1000/microl was achieved at a median (range) of 13 days (8-22) and 14 days (9-31), respectively. Platelets recovered to >20000 and >50000/microl at a median (range) of 13 days (0-128) and 18 days (0-180), respectively. Two patients developed graft failure. Acute GVHD in patients at risk was clinical grade 0 (n = 43), I (n = 8), II (n = 4) and III (n = 1). No patient developed acute GVHD grade IV. Chronic GVHD was limited in two cases and extensive in four cases. The actuarial probability of acute GVHD II-IV was 10% (95% CI, 1-19%), and of extensive chronic GVHD was 12% (95% CI, 11-13%). The cumulative incidence of transplant-related mortality was 12.6%, and this figure was 9% at 6 months. In conclusion, with the immunomagnetic procedure, a lower recovery and higher purity of CD34+ cells, and stronger TCD is obtained as compared to immunoadsorption (P = 0.008, P < 0.0001 and P = 0.0002, respectively). Our results also indicate that allogeneic transplantation of selected CD34+ cells is associated with a very rapid engraftment and with a low incidence of severe GVHD.

Vajpayee, R. B., S. Thomas, et al. (2000). "Large-diameter lamellar keratoplasty in severe ocular alkali burns: A technique of stem cell transplantation." Ophthalmology **107**(9): 1765-8.

PURPOSE: To evaluate the efficacy of large-diameter lamellar keratoplasty in cases of severe ocular alkali burns. DESIGN: Prospective, noncomparative, interventional case series. PARTICIPANTS: Nine eyes of nine patients with severe ocular alkali burns (grade III/IV) exhibiting corneal vascularization, conjunctivalization, and chronic inflammation were recruited from the Cornea Clinic of Dr. Rajendra Prasad Centre for Ophthalmic Sciences, New Delhi, a tertiary eye care center. INTERVENTION: Large-diameter lamellar keratoplasty was performed using McCarey-Kaufman media-preserved donor corneas. The patients were followed up for a minimum of 6 months. MAIN OUTCOME MEASURES: Symptomatic relief, time

to epithelialization, best-corrected visual acuity, Schirmer I, tear film break-up time, and central corneal clarity were the parameters evaluated. RESULTS: The mean duration between the injury and surgery was 29.5 +/- 19.4 months. No intraoperative complications were seen. Successful epithelialization of the ocular surface was achieved in all but one eye, and the mean time to epithelialization was 5.2 +/- 4.9 days. One eye had a persistent epithelial defect which was managed with a bandage soft contact lens. All patients achieved symptomatic relief. The preoperative best-corrected visual acuity was </=1/60 in all the patients. There was a significant improvement in vision in six eyes postoperatively (P = 0.013). The corneal clarity was grade 2+ or better in five eyes and 1+ in four eyes. No recurrence of corneal vascularization or signs of rejection were seen in any eye during the mean follow-up of 7.4 +/- 3.2 months. Causes of no improvement of vision included the presence of subepithelial nebulomacular haze in one eye caused by persistent epithelial defect and residual stromal haze. CONCLUSIONS: Large-diameter lamellar keratoplasty is a useful therapeutic modality in cases of severe alkali burns. It is a single-stage procedure that provides a stable ocular surface because of stem cell supplementation and may visually rehabilitate the patient.

Wilson, F. D., J. A. Dyck, et al. (1980). "A "whole-blood" technique for the quantitation of canine "T-lymphocyte" progenitors using a semisolid culture system." Exp Hematol **8**(8): 1031-9.

A whole blood technique is described for the growth of concanavalin A (Con A) stimulated canine lymphocyte colonies in semisolid medium. By eliminating the routine Ficoll-Paque (F-P) gradient lymphocyte isolation, this method avoids potential problems of growth modulation due to elimination of non-lymphoid accessory cells and the influences on colony formation associated with the selective effects of F-P on lymphocyte subpopulations. Thus, the technique more closely approximates the in vivo milieu. The whole-blood method also produces higher cloning efficiencies than methods using gradient isolation of lymphocytes. Studies over a wide range of blood concentration produced a linear response of in vitro colony formation although extrapolation of the cell-dose colony-response curve did not intersect zero. Mitogen titration data indicates that a relatively large dose of Con A is required for whole blood colony formation compared to the standard F-P method. The colonies ultrastructurally were composed of lymphoblastic and lymphocytic elements which were negative for non-specific esterase activity. Characterization of cells retrieved from the colonies using rosetting techniques indicates a high percentage of the colony cells relative to canine peripheral blood cells form rosettes with human erythrocytes.

Yamauchi, Y., K. Abe, et al. (1999). "A novel transgenic technique that allows specific marking of the neural crest cell lineage in mice." Dev Biol **212**(1): 191-203.

Neural crest cells are embryonic, multipotent stem cells that give rise to various cell/tissue types and thus serve as a good model system for the study of cell specification and mechanisms of cell differentiation. For analysis of neural crest cell lineage, an efficient method has been devised for manipulating the mouse genome through the Cre-loxP system. We generated transgenic mice harboring a Cre gene driven by a promoter of protein 0 (P0). To detect the Cre-mediated DNA recombination, we crossed P0-Cre transgenic mice with CAG-CAT-Z indicator transgenic mice. The CAG-CAT-Z Tg line carries a lacZ gene downstream of a chicken beta-actin promoter and a "stuffer" fragment flanked by two loxP sequences, so that lacZ is expressed only when the stuffer is removed by the action of Cre recombinase. In three different P0-Cre lines crossed with CAG-CAT-Z Tg, embryos carrying both transgenes showed lacZ expression in tissues derived from neural crest cells, such as spinal dorsal root ganglia, sympathetic nervous system, enteric nervous system, and ventral craniofacial mesenchyme at stages later than 9.0 dpc. These findings give some insights into neural crest cell differentiation in mammals. We believe that P0-Cre transgenic mice will facilitate many interesting experiments, including lineage analysis, purification, and genetic manipulation of the mammalian neural crest cells.

Yokoo, S., S. Yamagami, et al. (2008). "A novel isolation technique of progenitor cells in human corneal epithelium using non-tissue culture dishes." Stem Cells **26**(7): 1743-8.

The existence of adult stem cells or progenitor cells in the human corneal epithelium (i.e., self-renewing squamous cells) has long been suggested, but these cells have not yet been isolated. Here we describe a novel isolation technique using non-tissue culture dishes to enrich progenitor cells, which are able to reconstitute a three-dimensional human corneal epithelial equivalent from single cells in serum-, feeder-, and bovine pituitary extract-free medium. These cells showed original tissue-committed differentiation, a high proliferative capacity, and limited self-renewal. Laminin-5 was measured by mass spectrometric analysis. Pretreatment of cells with anti-laminin-5 antibody demonstrated that laminin-5 was important in allowing corneal epithelial progenitor cells to adhere

to non-tissue culture dishes. Hydrophilic tubes (used for cell collection throughout this study) are essential for efficient isolation of adherent corneal epithelial progenitor cells expressing laminin-5. These findings indicate that our new technique using non-tissue culture dishes allows the isolation of progenitor cells from human corneal limbal epithelium and that laminin-5 has a critical role in the adhesion of these cells.

Zannettino, A. C., J. R. Rayner, et al. (1996). "A powerful new technique for isolating genes encoding cell surface antigens using retroviral expression cloning." J Immunol **156**(2): 611-20.

cDNA expression cloning using retroviral vectors provides a means of stably introducing genes into target cells at efficiencies that surpass those achieved by transfection. Furthermore, retroviral vectors allow for the introduction and expression of complex cDNA libraries in a wide range of cell types, including cells of hemopoietic origin. Here we report a novel method for rapidly isolating genes encoding cell surface molecules (CSM) from a human bone marrow stromal cell cDNA library constructed in the retroviral vector, pRUFneo. With a newly described, highly efficient selection strategy using mAb and Ab-coated magnetic beads, we have successfully isolated six cDNA encoding previously defined CSM, including beta 1 integrin and endoglin. Moreover, we have used this approach to define the gene and hence the CSM identified by three previously unclustered mAb. These results confirm previous studies demonstrating the general utility of retroviral cDNA libraries and further extend their use to the expression cloning of cDNA encoding CSM.

Znojil, V. and E. Necas (1988). "The spleen colony technique. III. Comparison of the overlap effect and of errors in CFU-s determination and the [3H]-thymidine suicide data for several strains of mice." Cell Tissue Kinet **21**(3): 149-57.

A mathematical model of errors of the spleen colony technique is applied to data obtained from four mouse strains and F1 hybrids. The variance of the colony counts was close to the Poisson distribution in inbred mice and F1 hybrids. However, it should be checked regularly. The magnitude of the error in CFU-s determination and of the estimations of the S phase fraction was derived, and is presented relative to the mean colony counts for all mouse strains studied. The optimum spleen colony counts are generally higher than those which are commonly used. However, the utilization of the optimum spleen colony counts requires a correction for the effect of colony overlap.

References

1. Aldave, A. J. and I. G. Wong (2002). "A novel technique for harvesting keratolimbal allografts from corneoscleral buttons." Am J Ophthalmol **134**(6): 929-31.
2. Alegre, M., L. Puig, et al. (1997). "Primary neuroendocrine carcinoma of the skin expresses tyrosinase mRNA: detection by a specific nested PCR technique." Dermatology **194**(4): 334-7.
3. Aluigi, M., M. Fogli, et al. (2006). "Nucleofection is an efficient nonviral transfection technique for human bone marrow-derived mesenchymal stem cells." Stem Cells **24**(2): 454-61.
4. Apps, R., J. R. Trott, et al. (1991). "A study of branching in the projection from the inferior olive to the x and lateral c1 zones of the cat cerebellum using a combined electrophysiological and retrograde fluorescent double-labelling technique." Exp Brain Res **87**(1): 141-52.
5. Ayello, J., C. Hesdorffer, et al. (1995). "A semiautomated technique for volume reduction of stem cell suspensions for autotransplantation." J Hematother **4**(6): 545-9.
6. Bakshi, A., C. Hunter, et al. (2004). "Minimally invasive delivery of stem cells for spinal cord injury: advantages of the lumbar puncture technique." J Neurosurg Spine **1**(3): 330-7.
7. Banerjee, M. and R. R. Bhonde (2006). "Application of hanging drop technique for stem cell differentiation and cytotoxicity studies." Cytotechnology **51**(1): 1-5.
8. Belecky-Adams, T., B. Cook, et al. (1996). "Correlations between terminal mitosis and differentiated fate of retinal precursor cells in vivo and in vitro: analysis with the "window-labeling" technique." Dev Biol **178**(2): 304-15.
9. Benestad, H. B. and A. Reikvam (1975). "Diffusion chamber culturing of haematopoietic cells: methodological investigations and improvement of the technique." Exp Hematol **3**(4): 249-60.
10. Bergan, R., F. Hakim, et al. (1996). "Electroporation of synthetic oligodeoxynucleotides: a novel technique for ex vivo bone marrow purging." Blood **88**(2): 731-41.
11. Berthier, R., A. Kaufmann, et al. (1983). "Cryopreservation of human bone marrow cells by a modification of the two-step cooling technique." Cryobiology **20**(6): 637-43.
12. Berthold, F., R. Schumacher, et al. (1989). "Removal of neuroblastoma cells from bone marrow by a direct monoclonal antibody rosetting technique." Bone Marrow Transplant **4**(3): 273-8.
13. Bertram, H., H. Mayer, et al. (2005). "Effect of donor characteristics, technique of harvesting and in vitro processing on culturing of human marrow stroma cells for tissue engineered growth of bone." Clin Oral Implants Res **16**(5): 524-31.
14. Bitsch, R. G., C. Heisel, et al. (2007). "Femoral cementing technique for hip resurfacing arthroplasty." J Orthop Res **25**(4): 423-31.
15. Callera, F. and C. M. de Melo (2007). "Magnetic resonance tracking of magnetically labeled autologous bone marrow CD34+ cells transplanted into the spinal cord via lumbar puncture technique in patients with chronic spinal cord injury: CD34+ cells' migration into the injured site." Stem Cells Dev **16**(3): 461-6.
16. Cardoso, A. A., S. M. Watt, et al. (1995). "An improved panning technique for the selection of CD34+ human bone marrow hematopoietic cells with high recovery of early progenitors." Exp Hematol **23**(5): 407-12.
17. Carlsson, M. and M. Saeed (2008). "Intracoronary injection of contrast media maps the territory of the coronary artery: an MRI technique for assessing the effects of locally delivered angiogenic therapies." Acad Radiol **15**(11): 1354-9.
18. Casey, T. T., J. B. Cousar, et al. (1988). "A simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues." Am J Pathol **131**(2): 183-9.

19. Cedarbaum, J. M. and G. K. Aghajanian (1978). "Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique." J Comp Neurol **178**(1): 1-16.
20. Cortes, J. L., F. Cobo, et al. (2007). "Evaluation of a laser technique to isolate the inner cell mass of murine blastocysts." Biotechnol Appl Biochem **46**(Pt 4): 205-9.
21. Costantini, S., E. Di Capua, et al. (2006). "The management of severe vaginal obstruction from genital chronic graft-versus-host disease: diagnosis, surgical technique and follow-up." Minerva Ginecol **58**(1): 11-6.
22. Court, E. L., K. Davidson, et al. (2001). "C-kit mutation screening in patients with acute myeloid leukaemia: adaptation of a Giemsa-stained bone-marrow smear DNA extraction technique." Br J Biomed Sci **58**(2): 76-84.
23. Dal Cortivo, L., P. H. Cottu, et al. (2001). "Residual tumor cell contamination in peripheral blood stem cells collections of 117 breast cancer patients evaluated by immunocytochemical technique." J Hematother Stem Cell Res **10**(6): 855-62.
24. Dulic-Sills, A., M. J. Blunden, et al. (2006). "New flow cytometric technique for the evaluation of circulating endothelial progenitor cell levels in various disease groups." J Immunol Methods **316**(1-2): 107-15.
25. Duncan, D., J. P. Rubin, et al. (2009). "Refinement of technique in injection lipolysis based on scientific studies and clinical evaluation." Clin Plast Surg **36**(2): 195-209, v-vi; discussion 211-3.
26. Dyson, P. G., J. Q. Ho, et al. (1994). "The use of the APAAP technique as a rapid indicator of peripheral blood progenitor cell levels." Pathology **26**(3): 296-300.
27. Eca, L. P., R. B. Ramalho, et al. (2009). "Comparative study of technique to obtain stem cells from bone marrow collection between the iliac crest and the femoral epiphysis in rabbits." Acta Cir Bras **24**(5): 400-4.
28. Erber, W. N., H. Asbahr, et al. (1992). "Peanut agglutinin (lectin from Arachis hypogaea) binding to hemopoietic cells: an immunophenotypic study using a biotin streptavidin technique." Pathology **24**(3): 173-6.
29. Farnie, G., R. B. Clarke, et al. (2007). "Novel cell culture technique for primary ductal carcinoma in situ: role of Notch and epidermal growth factor receptor signaling pathways." J Natl Cancer Inst **99**(8): 616-27.
30. Fatima, A., V. S. Sangwan, et al. (2006). "Technique of cultivating limbal derived corneal epithelium on human amniotic membrane for clinical transplantation." J Postgrad Med **52**(4): 257-61.
31. Figuerres, E., M. Olszewski, et al. (2001). "A flow cytometric technique using thiazole orange to detect platelet engraftment following pediatric stem-cell transplants." Cytotherapy **3**(4): 277-83.
32. Flores, M. G., B. Holm, et al. (2005). "A technique of bone marrow collection from vertebral bodies of cynomolgus macaques for transplant studies." J Surg Res **124**(2): 280-8.
33. Fukano, H., N. Hayatsu, et al. (2006). "A technique to enzymatically construct libraries which express short hairpin RNA of arbitrary stem length." Biochem Biophys Res Commun **347**(3): 543-50.
34. Furuta, A., S. Miyoshi, et al. (2006). "Pulsatile cardiac tissue grafts using a novel three-dimensional cell sheet manipulation technique functionally integrates with the host heart, in vivo." Circ Res **98**(5): 705-12.
35. Gerendai, I. and B. Halasz (2000). "Central nervous system structures connected with the endocrine glands. findings obtained with the viral transneuronal tracing technique." Exp Clin Endocrinol Diabetes **108**(6): 389-95.
36. Gerendai, I., I. E. Toth, et al. (2000). "Central nervous system structures labelled from the testis using the transsynaptic viral tracing technique." J Neuroendocrinol **12**(11): 1087-95.
37. Gerendai, I., I. E. Toth, et al. (2001). "Transneuronal labelling of nerve cells in the CNS of female rat from the mammary gland by viral tracing technique." Neuroscience **108**(1): 103-18.
38. Gharaibeh, B., A. Lu, et al. (2008). "Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique." Nat Protoc **3**(9): 1501-9.
39. Gibellini, D., G. Zauli, et al. (1995). "In situ polymerase chain reaction technique revealed by flow cytometry as a tool for gene detection." Anal Biochem **228**(2): 252-8.
40. Gidron, A., A. Verma, et al. (2005). "Can the stem cell mobilization technique influence CD34+ cell collection efficiency of leukapheresis procedures in patients with hematologic malignancies?" Bone Marrow Transplant **35**(3): 243-6.
41. Gilmore, M. J., H. G. Prentice, et al. (1982). "A technique for rapid isolation of bone marrow mononuclear cells using Ficoll-Metrizoate and the IBM 2991 blood cell processor." Br J Haematol **50**(4): 619-26.
42. Gollackner, B., F. J. Dor, et al. (2003). "Spleen transplantation in miniature swine: surgical technique and results in major histocompatibility complex-matched donor and recipient pairs." Transplantation **75**(11): 1799-806.
43. Gorcs, T., M. Antal, et al. (1979). "An improved cobalt labeling technique with complex compounds." Acta Biol Acad Sci Hung **30**(1): 79-86.
44. Grossmann, J., J. M. Maxson, et al. (1998). "New isolation technique to study apoptosis in human intestinal epithelial cells." Am J Pathol **153**(1): 53-62.
45. Gu, C. M., Y. K. Zhu, et al. (2006). "Knockdown of survivin gene by vector-based short hairpin RNA technique induces apoptosis and growth inhibition in Burkitt's lymphoma Raji cell line." Neoplasma **53**(3): 206-12.
46. Guo, K. T., R. SchAfer, et al. (2006). "A new technique for the isolation and surface immobilization of mesenchymal stem cells from whole bone marrow using high-specific DNA aptamers." Stem Cells **24**(10): 2220-31.
47. Hensel, N., V. Agarwala, et al. (1999). "A technique for dual determination of cytotoxic and helper lymphocyte precursor frequency by a miniaturized dye release method." Bone Marrow Transplant **23**(1): 71-8.
48. Hernigou, P., G. Mathieu, et al. (2006). "Percutaneous autologous bone-marrow grafting for nonunions. Surgical technique." J Bone Joint Surg Am **88 Suppl 1 Pt 2**: 322-7.
49. Holland, E. J., A. R. Djalilian, et al. (2003). "Management of aniridic keratopathy with keratolimbal allograft: a limbal stem cell transplantation technique." Ophthalmology **110**(1): 125-30.
50. Hu, Y., K. Cai, et al. (2009). "Surface mediated in situ differentiation of mesenchymal stem cells on gene-functionalized titanium films fabricated by layer-by-layer technique." Biomaterials **30**(21): 3626-35.
51. Huisman, A. M., H. G. Kuypers, et al. (1981). "Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with the multiple fluorescent retrograde tracer technique." Brain Res **209**(2): 271-86.
52. Iliakis, G. E. and G. E. Pantelias (1990). "Production and repair of chromosome damage in an X-ray sensitive CHO mutant visualized and analysed in interphase using the technique of premature chromosome condensation." Int J Radiat Biol **57**(6): 1213-23.
53. Ivanovski, O., K. Kulkeaw, et al. (2009). "Characterization of kidney marrow in zebrafish (Danio rerio) by using a new surgical technique." Prilozi **30**(2): 71-80.
54. Jacobs, P., D. Dubovsky, et al. (1979). "Bone marrow culture in vitro. A technique for analysis and permanent recording of cellular composition." Exp Hematol **7**(4): 177-82.
55. Jankowski, R. J., C. Haluszczak, et al. (2001). "Flow cytometric characterization of myogenic cell populations obtained via the preplate technique: potential for rapid

isolation of muscle-derived stem cells." Hum Gene Ther **12**(6): 619-28.
56. Jedrzejczak, W. W. and Z. Pojda (1987). "Technique of preparation of hemopoietic cells of human fetal liver for transplantation." Arch Immunol Ther Exp (Warsz) **35**(1): 71-8.
57. Kalil, R. A., D. Ott, et al. (2008). "Autologous transplantation of bone marrow mononuclear stem cells by mini-thoracotomy in dilated cardiomyopathy: technique and early results." Sao Paulo Med J **126**(2): 75-81.
58. Kamihira, M. and A. Kumar (2007). "Development of separation technique for stem cells." Adv Biochem Eng Biotechnol **106**: 173-93.
59. Kamondi, A., J. A. Williams, et al. (1992). "Membrane properties of mesopontine cholinergic neurons studied with the whole-cell patch-clamp technique: implications for behavioral state control." J Neurophysiol **68**(4): 1359-72.
60. Kim, M., J. Y. Kim, et al. (2002). "Evaluation of early post-transplant leukocyte recovery using the undiluted erythrocyte lysing technique." Ann Clin Lab Sci **32**(2): 159-63.
61. Kim, S., S. E. Ahn, et al. (2007). "A novel culture technique for human embryonic stem cells using porous membranes." Stem Cells **25**(10): 2601-9.
62. Kishigami, S., E. Mizutani, et al. (2006). "Significant improvement of mouse cloning technique by treatment with trichostatin A after somatic nuclear transfer." Biochem Biophys Res Commun **340**(1): 183-9.
63. Koga, H., M. Shimaya, et al. (2008). "Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect." Arthritis Res Ther **10**(4): R84.
64. Koizumi, S., R. L. Fine, et al. (1985). "Enrichment of myeloid progenitor cells from normal human bone marrow using an immune-rosette technique." Exp Hematol **13**(6): 560-5.
65. Koristek, Z., J. Sterba, et al. (2002). "Technique for PBSC harvesting in children of weight under 10 kg." Bone Marrow Transplant **29**(1): 57-61.
66. Koutna, I., M. Klabusay, et al. (2006). "Evaluation of CD34+ - and Lin- -selected cells from peripheral blood stem cell grafts of patients with lymphoma during differentiation in culture ex vivo using a cDNA microarray technique." Exp Hematol **34**(7): 832-40.
67. Kuemmel, T. A., J. Thiele, et al. (1996). "Distribution of lectin binding sites in human bone marrow. Identification by use of an ultrastructural postembedding technique." J Submicrosc Cytol Pathol **28**(4): 537-51.
68. Lahdetie, J., A. Suutari, et al. (1994). "The spermatid micronucleus test with the dissection technique detects the germ cell mutagenicity of acrylamide in rat meiotic cells." Mutat Res **309**(2): 255-62.
69. Lee, I. C., J. H. Wang, et al. (2007). "Development of a useful technique to discriminate anterior cruciate ligament cells and mesenchymal stem cells--the application of cell electrophoresis." J Biomed Mater Res A **82**(1): 230-7.
70. Lekhanont, K., L. Choubtum, et al. (2009). "A serum- and feeder-free technique of culturing human corneal epithelial stem cells on amniotic membrane." Mol Vis **15**: 1294-302.
71. Matouskova, E., D. Dudorkinova, et al. (1998). "Clonal expansion of epithelial cells from primary human breast carcinoma with 3T3 feeder layer technique." Folia Biol (Praha) **44**(2): 67-71.
72. Matsubara, T., S. Tsutsumi, et al. (2004). "A new technique to expand human mesenchymal stem cells using basement membrane extracellular matrix." Biochem Biophys Res Commun **313**(3): 503-8.
73. Matsuda, T., Y. Saito, et al. (2007). "Cell sorting technique based on thermoresponsive differential cell adhesiveness." Biomacromolecules **8**(8): 2345-9.
74. Matsuoka, T. and M. Tavassoli (1989). "Electron microscopic identification of hemopoietic progenitor cells by exploiting their sugar-recognizing receptors using a newly developed minibead technique." Exp Hematol **17**(4): 326-9.
75. Mayor, N. P., B. E. Shaw, et al. (2007). "A novel technique for NOD2/CARD15 genotyping using PCR-SSP." J Immunol Methods **327**(1-2): 82-7.
76. Meijer, E., I. C. Slaper-Cortenbach, et al. (2002). "Increased incidence of EBV-associated lymphoproliferative disorders after allogeneic stem cell transplantation from matched unrelated donors due to a change of T cell depletion technique." Bone Marrow Transplant **29**(4): 335-9.
77. Menendez, P., O. Redondo, et al. (1998). "Comparison between a lyse-and-then-wash method and a lyse-non-wash technique for the enumeration of CD34+ hematopoietic progenitor cells." Cytometry **34**(6): 264-71.
78. Mielke, S., R. Nunes, et al. (2008). "A clinical-scale selective allodepletion approach for the treatment of HLA-mismatched and matched donor-recipient pairs using expanded T lymphocytes as antigen-presenting cells and a TH9402-based photodepletion technique." Blood **111**(8): 4392-402.
79. Musialek, P., W. Tracz, et al. (2006). "Transcoronary stem cell delivery using physiological endothelium-targeting perfusion technique: the rationale and a pilot study involving a comparison with conventional over-the-wire balloon coronary occlusions in patients after recent myocardial infarction." Kardiol Pol **64**(5): 489-98; discussion 499.
80. Nafu, R., G. Lelkes, et al. (1997). "Ultrastructural study on the in vitro interaction between haemopoietic cells and stromal cells. A new method using gel technique." Haematologia (Budap) **28**(2): 97-107.
81. Nakahara, M., N. Nakamura, et al. (2009). "High-efficiency production of subculturable vascular endothelial cells from feeder-free human embryonic stem cells without cell-sorting technique." Cloning Stem Cells **11**(4): 509-22.
82. Nieponice, A., L. Soletti, et al. (2008). "Development of a tissue-engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique." Biomaterials **29**(7): 825-33.
83. Ogawa, T. (2000). "Spermatogonial transplantation technique in spermatogenesis research." Int J Androl **23 Suppl 2**: 57-9.
84. Opitz, U. and H. J. Seidel (1978). "Studies on the target cell for the Friend virus (FV-P strain) using the CFU-E technique." Blut **37**(4): 183-92.
85. Park, D. J., J. H. Choi, et al. (2007). "Tissue-engineered bone formation with gene transfer and mesenchymal stem cells in a minimally invasive technique." Laryngoscope **117**(7): 1267-71.
86. Peralta, M. and F. J. Denaro (2003). "The horseradish peroxidase technique for cell lineage studies." Cell Mol Biol (Noisy-le-grand) **49**(8): 1371-5.
87. Petersen, L. J., K. Brasso, et al. (1996). "Histamine release in intact human skin by monocyte chemoattractant factor-1, RANTES, macrophage inflammatory protein-1 alpha, stem cell factor, anti-IgE, and codeine as determined by an ex vivo skin microdialysis technique." J Allergy Clin Immunol **98**(4): 790-6.
88. Pirruccello, S. J., C. J. Page, et al. (1999). "Comparison of ISHAGE protocol CD34 cell enumeration with a lineage negative backgating technique." Cytotherapy **1**(4): 279-86.
89. Polouckova, A., A. Vodvarkova, et al. (2001). "Comparison of two different methods for CD34+ selection and T cell depletion in peripheral blood stem cell grafts--our experiences with CellPro, E rosetting and CliniMACS technique." Neoplasma **48**(5): 374-81.
90. Poot, M., J. R. Silber, et al. (2002). "A novel flow cytometric technique for drug cytotoxicity gives results comparable to colony-forming assays." Cytometry **48**(1): 1-5.
91. Qian, C., R. A. Tio, et al. (2007). "A promising technique for transplantation of bone marrow-derived endothelial progenitor cells into rat heart." Cardiovasc Pathol **16**(3): 127-35.

92. Rallapalli, S., D. K. Bishi, et al. (2009). "A multiplex PCR technique to characterize human bone marrow derived mesenchymal stem cells." Biotechnol Lett **31**(12): 1843-50.
93. Rana, K., J. L. Liesveld, et al. (2009). "Delivery of apoptotic signal to rolling cancer cells: a novel biomimetic technique using immobilized TRAIL and E-selectin." Biotechnol Bioeng **102**(6): 1692-702.
94. Rapallino, M. V. and A. Cupello (2001). "Holger Hyden's technique of preparation of single Deiters' neurons and study of permeability characteristics of their plasma membranes." Brain Res Brain Res Protoc **8**(1): 58-67.
95. Ravindranath, N. and M. Dym (1999). "Isolation of rat ventral prostate basal and luminal epithelial cells by the STAPUT technique." Prostate **41**(3): 173-80.
96. Riew, K. D., J. Lou, et al. (2003). "Thoracoscopic intradiscal spine fusion using a minimally invasive gene-therapy technique." J Bone Joint Surg Am **85-A**(5): 866-71.
97. Sadiq, M. N., A. S. Arif, et al. (2009). "Use of supero-temporal free conjunctivo-limbal autograft in the surgical management of pterygium: our technique and results." J Ayub Med Coll Abbottabad **21**(4): 121-4.
98. Sadraei, H., S. R. Abtahi, et al. (2009). "Assessment of potassium current in Royan B(1) stem cell derived cardiomyocytes by patch-clamp technique." Res Pharm Sci **4**(2): 85-97.
99. Sawyer, B. M., N. B. Westwood, et al. (1994). "Circulating megakaryocytic progenitor cells in patients with primary thrombocythaemia and reactive thrombocytosis: results using a serum-deprived culture assay and a positive detection technique." Eur J Haematol **53**(2): 108-13.
100. Schlueter, A. J., S. K. Bhatia, et al. (2001). "Delineation among eight major hematopoietic subsets in murine bone marrow using a two-color flow cytometric technique." Cytometry **43**(4): 297-307.
101. Sedlak, J. and B. Chorvath (1991). "Fluorescent double labeling of normal and malignant hematopoietic cells by monoclonal antibodies (FITC) and anthracycline cytostatic drug (Daunomycin): a cytometric technique for analysis of drug uptake in hematopoietic cell subpopulations." Neoplasma **38**(1): 13-20.
102. See, E. Y., S. L. Toh, et al. (2008). "Technique to accurately quantify collagen content in hyperconfluent cell culture." J Mol Histol **39**(6): 643-7.
103. Shankaran, M., C. King, et al. (2006). "Discovery of novel hippocampal neurogenic agents by using an in vivo stable isotope labeling technique." J Pharmacol Exp Ther **319**(3): 1172-81.
104. Shen, S., L. Yuan, et al. (2009). "An effort to test the embryotoxicity of benzene, toluene, xylene, and formaldehyde to murine embryonic stem cells using airborne exposure technique." Inhal Toxicol **21**(12): 973-8.
105. Solchaga, L. A., E. Tognana, et al. (2006). "A rapid seeding technique for the assembly of large cell/scaffold composite constructs." Tissue Eng **12**(7): 1851-63.
106. Span, L. F., A. H. Pennings, et al. (2002). "The dynamic process of apoptosis analyzed by flow cytometry using Annexin-V/propidium iodide and a modified in situ end labeling technique." Cytometry **47**(1): 24-31.
107. Steadman, J. R., W. G. Rodkey, et al. (2001). "Microfracture: surgical technique and rehabilitation to treat chondral defects." Clin Orthop Relat Res(391 Suppl): S362-9.
108. Takeuchi, T. and G. D. Palermo (2004). "Implications of cloning technique for reproductive medicine." Reprod Biomed Online **8**(5): 509-15.
109. Tsunoda, K., T. Baer, et al. (2001). "Autologous transplantation of fascia into the vocal fold: long-term results of a new phonosurgical technique for glottal incompetence." Laryngoscope **111**(3): 453-7.
110. Turner, C. W., J. Luzins, et al. (1992). "A modified harvest technique for cord blood hematopoietic stem cells." Bone Marrow Transplant **10**(1): 89-91.
111. Tyer, C. L., J. J. Vredenburgh, et al. (1996). "Breast cancer cells are effectively purged from peripheral blood progenitor cells with an immunomagnetic technique." Clin Cancer Res **2**(1): 81-6.
112. Urbano-Ispizua, A., C. Solano, et al. (1998). "Allogeneic transplantation of selected CD34+ cells from peripheral blood: experience of 62 cases using immunoadsorption or immunomagnetic technique. Spanish Group of Allo-PBT." Bone Marrow Transplant **22**(6): 519-25.
113. Vajpayee, R. B., S. Thomas, et al. (2000). "Large-diameter lamellar keratoplasty in severe ocular alkali burns: A technique of stem cell transplantation." Ophthalmology **107**(9): 1765-8.
114. Wilson, F. D., J. A. Dyck, et al. (1980). "A "whole-blood" technique for the quantitation of canine "T-lymphocyte" progenitors using a semisolid culture system." Exp Hematol **8**(8): 1031-9.
115. Yamauchi, Y., K. Abe, et al. (1999). "A novel transgenic technique that allows specific marking of the neural crest cell lineage in mice." Dev Biol **212**(1): 191-203.
116. Yokoo, S., S. Yamagami, et al. (2008). "A novel isolation technique of progenitor cells in human corneal epithelium using non-tissue culture dishes." Stem Cells **26**(7): 1743-8.
117. Zannettino, A. C., J. R. Rayner, et al. (1996). "A powerful new technique for isolating genes encoding cell surface antigens using retroviral expression cloning." J Immunol **156**(2): 611-20.
118. Znojil, V. and E. Necas (1988). "The spleen colony technique. III. Comparison of the overlap effect and of errors in CFU-s determination and the [3H]-thymidine suicide data for several strains of mice." Cell Tissue Kinet **21**(3): 149-57.
119. Ma H, Chen G (2005). Stem Cell. J Am Sci. 1(2):90-92. http://www.sciencepub.net/american/0102/14-mahongbao.pdf.
120. Ma H, Chenrg S (2007). Eternal Life and Stem Cell. Nat Sci. 5(1):81-96. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
121. Ma H, Chenrg S (2007). Review of Stem Cell Studies. Nat Sci. 5(2):45-65. http://www.sciencepub.net/nature/0502/09-0247-mahongbao-stem-ns.pdf.
122. Yang Y, Ma H (2010). Germ Stem Cell. Stem Cell. 1(2):38-60]. http://www.sciencepub.net/stem/stem0102/07_1348stem0102_38_60.pdf.
123. Pubmed. Stem Cell. http://www.ncbi.nlm.nih.gov/pubmed/?term=stem+cell.
124. Wikipedia. Stem Cell. http://en.wikipedia.org/wiki/Stem_cell.

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