Stem Cell Patent

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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 situuition 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 tipical and important topic of life science. This material collects some literatures on stem cell patent.
[Smith MH. **Stem Cell Patent**. *Stem Cell* 2013;4(3):142-160] (ISSN 1545-4570). http://www.sciencepub.net/stem. 8

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

Literatures

Assmus, B., J. Honold, et al. (2006). "Transcoronary transplantation of progenitor cells after myocardial infarction." N Engl J Med **355**(12): 1222-32.

BACKGROUND: Pilot studies suggest that intracoronary transplantation of progenitor cells derived from bone marrow (BMC) or circulating blood (CPC) may improve left ventricular function after acute myocardial infarction. The effects of cell transplantation in patients with healed myocardial infarction are unknown. METHODS: After an initial pilot trial involving 17 patients, we randomly assigned, in a controlled crossover study, 75 patients with stable ischemic heart disease who had had a myocardial infarction at least 3 months previously to receive either no cell infusion (23 patients) or infusion of CPC (24 patients) or BMC (28 patients) into the patent coronary artery supplying the most dyskinetic left ventricular area. The patients in the control group were subsequently randomly assigned to receive CPC or BMC, and the patients who initially received BMC or CPC crossed over to receive CPC or BMC, respectively, at 3 months' follow-up. RESULTS: The absolute change in left ventricular ejection fraction was significantly greater among patients receiving BMC (+2.9 percentage points) than among those receiving CPC (-0.4 percentage point, P=0.003) or no infusion (-1.2 percentage points, P<0.001). The increase in global cardiac function was related to significantly enhanced regional contractility in the area targeted by intracoronary infusion of BMC. The crossover phase of the study revealed that intracoronary infusion of BMC was associated with a significant increase in global and regional left ventricular function, regardless of whether patients crossed over from control to BMC or from CPC to BMC. CONCLUSIONS: Intracoronary infusion of progenitor cells is safe and feasible in patients with healed myocardial infarction. Transplantation of BMC is associated with moderate but significant improvement in the left ventricular ejection fraction after 3 months.

Butt, A. M. and J. Dinsdale (2005). "Fibroblast growth factor 2 induces loss of adult oligodendrocytes and myelin in vivo." Exp Neurol **192**(1): 125-33.

Oligodendrocytes are the myelin-forming cells of the CNS and are lost in demyelinating diseases such as multiple sclerosis (MS). A role for fibroblast growth factor 2 (FGF2) has been proposed in the pathogenesis of demyelination and the failure of remyelination in experimental models of MS. However, the in vivo effects of FGF2 on oligodendrocytes and oligodendrocyte progenitors (OPCs) in the adult CNS had not previously been determined. To address this, FGF2 was delivered into the cerebrospinal fluid (CSF) of the IVth ventricle and its actions were examined on the anterior medullary velum (AMV), a thin tissue that partly roofs the IVth ventricle and is bathed by CSF. FGF2 was administered twice daily for 3 days and AMV were analysed using immunohistochemical labelling; saline was administered in controls. The results show that raised FGF2 induces severe disruption of mature oligodendrocytes and a marked loss of myelin. At the same time, FGF2 treatment resulted in the aberrant accumulation of immature oligodendrocytes with a premyelinating phenotype, together with NG2-expressing OPCs. Axons are patent within demyelinated lesions, and they are contacted but not ensheathed by surviving oligodendrocytes, newly formed premyelinating oligodendrocytes and OPCs. These results demonstrate that raised FGF2 induces demyelination in the adult CNS, and support a role for FGF2 in the pathogenesis of demyelination and regulation of remyelination in MS.

Chan-Ling, T., D. S. McLeod, et al. (2004). "Astrocyte-endothelial cell relationships during human retinal vascular development." Invest Ophthalmol Vis Sci **45**(6): 2020-32.

PURPOSE: To evaluate evidence for the presence of vascular precursor cells (angioblasts) and astrocyte precursor cells (APCs) in the developing human retina and determine their relationship. METHODS: Pax-2/GFAP/CD-34 triple-label immunohistochemistry was applied to four retinas aged 12, 14, 16, and 20 weeks of gestation (WG) to label APCs, astrocytes, and patent blood vessels. APCs are Pax-2(+)/GFAP(-), whereas astrocytes are Pax-2(+)/GFAP(+). Adenosine diphosphatase (ADPase) enzyme histochemistry, which identifies endothelial cells and vascular precursors, was applied to human retinas aged 12, 16, 17, and 19 WG. Nissl stain, a nonspecific cell soma marker, was applied to 14.5-, 18-, and 21-WG retinas. Established blood vessels were visualized with CD34 and ADPase. RESULTS: Topographical analysis of the distribution of Nissl-stained spindle cells and ADPase(+) vascular cells showed that these two populations have similar distributions at corresponding ages. ADPase(+) vascular precursor cells preceded the leading edge of patent vessels by more than 1 millimeter. In contrast, Pax-2(+)/GFAP(-) APCs preceded the leading edge of CD34(+) blood vessels by a very small margin, and committed astrocytes (Pax-2(+)/GFAP(+)) were associated with formed vessels and nerve fiber bundles. Two populations of ADPase(+) cells were evident, a spindle-shaped population located superficially and a deeper spherical population. The outer limits of these populations remain static with maturation. CONCLUSIONS: A combination of Pax-2/GFAP/CD34 immunohistochemistry, Nissl staining, and ADPase histochemistry showed that the vascular precursor cells (angioblasts), identified using ADPase and Nissl, represent a population distinct from Pax-2(+)/GFAP(-) APCs in the human retina. These results lead to the conclusion that formation of the initial human retinal vasculature takes place through vasculogenesis from the prior invasion of vascular precursor cells.

Chapman, A. R. (2009). "The ethics of patenting human embryonic stem cells." Kennedy Inst Ethics J **19**(3): 261-88.

Just as human embryonic stem cell research has generated controversy about the uses of human embryos for research and therapeutic applications, human embryonic stem cell patents raise fundamental ethical issues. The United States Patent and Trademark Office has granted foundational patents, including a composition of matter (or product) patent to the Wisconsin Alumni Research Foundation (WARF), the University of Wisconsin-Madison's intellectual property office. In contrast, the European Patent Office rejected the same WARF patent application for ethical reasons. This article assesses the appropriateness of these patents placing the discussion in the context of the deontological and consequentialist ethical issues related to human embryonic stem cell patenting. It advocates for a patent system that explicitly takes ethical factors into account and explores options for new types of intellectual property arrangements consistent with ethical concerns.

Cho, S. W., O. Jeon, et al. (2006). "Preliminary experience with tissue engineering of a venous vascular patch by using bone marrow-derived cells and a hybrid biodegradable polymer scaffold." J Vasc Surg **44**(6): 1329-40.

OBJECTIVE: Currently available synthetic polymer vascular patches used in cardiovascular surgery have shown serious shortcomings, including thrombosis, calcification, infection, and lack of growth potential. These problems may be avoided by vascular patches tissue-engineered with autologous stem cells and biodegradable polymeric materials. The objective of this study was to develop a tissue-engineered vascular patch by using autologous bone marrow-derived cells (BMCs) and a hybrid biodegradable polymer scaffold. METHODS: Hybrid biodegradable polymer scaffolds were fabricated from poly(lactide-co-epsilon-caprolactone) (PLCL) copolymer reinforced with poly(glycolic acid) (PGA) fibers. Canine bone marrow mononuclear cells were induced in vitro to differentiate into vascular smooth muscle cells and endothelial cells. Tissue-engineered vascular patches (15 mm wide x 30 mm long) were fabricated by seeding vascular cells onto PGA/PLCL scaffolds and implanted into the inferior vena cava of bone marrow donor dogs. RESULTS: Compared with PLCL scaffolds, PGA/PLCL scaffolds exhibited tensile mechanical properties more similar to those of dog inferior vena cava. Eight weeks after implantation of vascular patches tissue-engineered with BMCs and PGA/PLCL scaffolds, the vascular patches remained patent with no sign of thrombosis, stenosis, or dilatation. Histological, immunohistochemical, and scanning electron microscopic analyses of the retrieved vascular patches revealed regeneration of endothelium and smooth muscle, as well as the presence of collagen. Calcium deposition on tissue-engineered vascular patches was not significantly different from that on native blood vessels. Immunofluorescent double staining confirmed that implanted BMCs survived after implantation and contributed to regeneration of endothelium and

vascular smooth muscle in the implanted vascular patches. CONCLUSIONS: This study demonstrates that vascular patches can be tissue-engineered with autologous BMCs and hybrid biodegradable polymer scaffolds.

Constantino, M., P. Christian, et al. (2001). "A comparison of techniques for detecting Invertebrate iridescent virus 6." J Virol Methods **98**(2): 109-18.

The aim of this study was to compare the sensitivity and precision of various methods for the detection and quantification of Invertebrate iridescent virus 6 (IIV-6) (Iridoviridae) isolated from a the stem-boring moth Chilo suppressalis, and to apply these techniques to the detection of covert infections in the wax moth, Galleria mellonella. The relationship between the virus concentration and absorbance at 260 nm was linear over the range of 1.6 x 10(9)-5.6 x 10(10) particles/ml. TCID(50) assays using 12 different cell lines indicated that two Drosophila lines, DL2 and DR1, had the highest susceptibility whereas cell lines from Aedes albopictus and Plutella xylostella were four orders of magnitude less sensitive. TCID(50) values for IIV-6 in Spodoptera frugiperda Sf9 cells gave the particle-infectivity ratios of 15-64 virus particles/IU. An insect bioassay involved injecting doses of 1-100 IIV-6 particles into the third instar G. mellonella larvae. The prevalence of patent infection was 20-26% at a dose of 1 particle per larva rising to 86-92% at 10 particles and 100% at doses of 50 or 100 particles. Of the insects that survived to adulthood, between 5.8 and 75% caused patent infections when injected into G. mellonella larvae, indicating that they were covertly infected. A PCR technique resulted in 95% detection at 1000 virus particles per insect. Of the insects that proved positive for covert infection by insect bioassay, 41% also proved positive by PCR analysis. It is concluded that the G. mellonella bioassay is highly reliable for detection of doses of 10 particles or more and for determining the relative activity of IIV-6 preparations at doses as low as 1 particle per insect. PCR had a slightly lower sensitivity followed by the insect cell culture assay.

Curley, D. and A. Sharples (2006). "Ethical questions to ponder in the European stem cell patent debate." J Biolaw Bus **9**(3): 12-6.

Patents may be refused in Europe on ethical grounds. Whereas in the past this issue has arisen only infrequently, recent developments in human embryonic stem cell research have given rise to conflicting opinions in Europe as to the approach that should be adopted in relation to patents. The United Kingdom Patent Office has adopted a positive policy towards inventions involving human embryonic stem cells, but the European Patent Office has to date refused to grant patent applications involving similar subject-matter. A series of legal questions on the role of ethics in granting European patents is now to be considered for clarification by the European Patent Office. The answers to these questions should eventually resolve the debate on the patenting of human embryonic stem cells throughout Europe.

Deregibus, M. C., V. Cantaluppi, et al. (2007). "Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA." Blood **110**(7): 2440-8.

Membrane-derived microvesicles (MVs) are released from the cell surface and are implicated in cell-to-cell communication. We evaluated whether MVs derived from endothelial progenitor cells (EPCs) are able to trigger angiogenesis. We found that EPC-derived MVs were incorporated in endothelial cells by interaction with alpha4 and beta1 integrins expressed on the MV surface. In vitro, MVs promoted endothelial cell survival, proliferation, and organization in capillary-like structures. In vivo, in severe combined immunodeficient (SCID) mice, MV-stimulated human endothelial cells organized in patent vessels. When incubated with RNase, despite their internalization into endothelial cells, MVs failed to induce in vitro and in vivo angiogenic effects. mRNA transfer was shown by transduction of GFP protein in endothelial cells by MVs containing GFP-mRNA and the biologic relevance by the angiogenic effect of MV-mRNA extract delivered by lipofectamine. Microarray ana-lysis and quantitative reverse transcription-polymerase chain reaction (RT-PCR) of MV-mRNA extract indicated that MVs were shuttling a specific subset of cellular mRNA, such as mRNA associated with the PI3K/AKT signaling pathway. Protein expression and functional studies showed that PI3K and eNOS play a critical role in the angiogenic effect of MVs. These results suggest that EPCs may activate angiogenesis in endothelial cells by releasing MVs able to trigger an angiogenic program.

Dunagan, T. T. and D. M. Miller (1986). "A review of protonephridial excretory systems in Acanthocephala." J Parasitol **72**(5): 621-32.

Our present understanding of the excretory system of Acanthocephala is largely the result of work done by 5 German scholars: Kaiser, Schepotieff, Meyer, Kilian, and von Haffner. Present studies indicate that a protonephridial system is restricted to the family Oligacanthorhynchidae. However, many members of this family have not had a protonephridial system described. Three nephridial designs have been described: 1) dendritic type, organized as branches of

a tree where each final branch terminates in a ciliated bulb; 2) capsular type, in which all ciliated bulbs empty directly into a common chamber; and 3) rudimentary type, consisting of a single cell with a patent ciliary pouch but no ducts to the outside. The first 2 types are a syncytia with 3 nuclei located in the capsule or stem wall and none in the flame bulbs. These excretory systems consist of 2 clusters of flame bulbs that empty separately into an expandable excretory bladder which in turn empties into ducts of the reproductive system. This urogenital system empties to the outside through a gonopore located at the tip of the penis in males and the posterior terminus of the vagina in females. Cilia occur in certain excretory tubes, depending on sex and species, but are unknown in the excretory bladder or ducts leading into it. The rudimentary type consists of a cell whose posterior extension terminates near the bursal lumen, but it is not known if this is significant for the discharge of material. There is no information on the physiology or biochemistry of the excretory system or its products.

Eberhard, Y., S. P. McDermott, et al. (2009). "Chelation of intracellular iron with the antifungal agent ciclopirox olamine induces cell death in leukemia and myeloma cells." Blood **114**(14): 3064-73.

Off-patent drugs with previously unrecognized anticancer activity could be rapidly repurposed for this new indication. To identify such compounds, we conducted 2 independent cell-based chemical screens and identified the antimicrobial ciclopirox olamine (CPX) in both screens. CPX decreased cell growth and viability of malignant leukemia, myeloma, and solid tumor cell lines as well as primary AML patient samples at low-micromolar concentrations that appear pharmacologically achievable. Furthermore, oral CPX decreased tumor weight and volume in 3 mouse models of leukemia by up to 65% compared with control without evidence of weight loss or gross organ toxicity. In addition, oral CPX prevented the engraftment of primary AML cells in nonobese diabetic/severe combined immunodeficiency mouse models, thereby establishing its ability to target leukemia stem cells. Mechanistically, CPX bound intracellular iron, and this intracellular iron chelation was functionally important for its cytotoxicity. By electron paramagnetic resonance, CPX inhibited the iron-dependent enzyme ribonucleotide reductase at concentrations associated with cell death. Thus, in summary, CPX has previously unrecognized anticancer activity at concentrations that are pharmacologically achievable. Therefore, CPX could be rapidly repurposed for the treatment of malignancies, including leukemia and myeloma.

Ellison, D. J., B. N. Nathwani, et al. (1989). "Interfollicular small lymphocytic lymphoma: the diagnostic significance of pseudofollicles." Hum Pathol **20**(11): 1108-18.

The pathologic, immunologic, and clinical features of 25 cases of interfollicular (IF) small lymphocytic lymphoma (SLL) characterized by pseudofollicles (PFs) in the IF region of the lymph nodes and by multiple reactive follicles (RFs) were examined. IFSLL is characterized morphologically by variable numbers and sizes of prolymphocytes (nuclei showing one centrally located prominent nucleolus) in the PFs and by small round lymphocytes in the IF region. The lymph nodes in our cases had multiple RFs (100%) and patent or partially patent sinuses (72%), with moderate expansion of the IF region (48%) and typically absent or minimal perinodal infiltration (48%). In 48% of the cases, the PFs surrounded the RFs, producing a pseudo-mantle zone pattern. Immunologic study showed the medium and large prolymphocytes to be mildly LN 1- and LN 2-positive, whereas the small prolymphocytes and lymphocytes were LN 1-negative and moderately LN 2-positive. Few cells in the IF region stained with UCHL-1 antibody. These data indicate the marked preponderance of the non-follicular center cell type of B cells in the IF areas. In all 11 cases tested, a monoclonal B cell population was found. The mean age of the patients was 62 years, with a male to female ratio of 1:1.7. B symptoms were present in 20% of the patients. Nineteen percent of the patients had clinical stage I or II disease, whereas 81% had stage IV disease. The median absolute lymphocyte count was 3,239 X 10(6), with a range of 767 to 13,770 X 10(6) cells/L. In six cases, the lymphocyte count was above 4,000 X 10(6), and in no case was it more than 15,000 X 10(6). It was difficult to distinguish these cases of IFSLL from lymphadenitis and other non-Hodgkin's lymphomas because it was difficult to recognize the subtle PF pattern in the presence of a partially preserved lymph node architecture. Because of the partially retained lymph node architecture and the expansion of the IF region by PFs, this lymphoma is thought to originate from the IF small B lymphocytes, which displayed an in situ growth pattern. Moreover, because of the predominant disease in the lymph nodes and the similarity of features in PFs and follicles, we conclude that IFSLL is a disease that is primary to the lymph nodes. IFSLL should be distinguished from mantle zone lymphoma and chronic lymphocytic leukemia.

Fischer, L. J., S. McIlhenny, et al. (2009). "Endothelial differentiation of adipose-derived stem cells: effects of endothelial cell growth supplement and shear force." J Surg Res **152**(1): 157-66.

BACKGROUND: Adipose tissue is a readily available source of multipotent adult stem cells for use in tissue engineering/regenerative medicine. Various growth factors have been used to stimulate acquisition of endothelial characteristics by adipose-derived stem cells (ASC). Herein we study the effects of endothelial cell growth supplement (ECGS) and physiological shear force on the differentiation of ASC into endothelial cells. MATERIALS AND METHODS: Human ASC (CD13(+)29(+)90(+)31(-)45(-)) were isolated from periumbilical fat, cultured in ECGS media (for up to 3 wk), and exposed to physiological shear force (12 dynes for up to 8 d) in vitro. Endothelial phenotype was defined by cord formation on Matrigel, acetylated-low density lipoprotein (acLDL) uptake, and expression of nitric oxide synthase (eNOS), von Willebrand factor (vWF), and CD31 (platelet endothelial cell adhesion molecule, PECAM). Additionally, cell thrombogenicity was evaluated by seeding canine autologous ASC onto vascular grafts implanted within the canine arterial circulation for 2 wk. RESULTS: We found that undifferentiated ASC did not display any of the noted endothelial characteristics. After culture in ECGS, ASC formed cords in Matrigel but failed to take up acLDL or express the molecular markers. Subsequent exposure to shear resulted in stem cell realignment, acLDL uptake, and expression of CD31; eNOS and vWF expression was still not observed. Grafts seeded with cells grown in ECGS (+/- shear) remained patent (six of seven) at 2 wk but had a thin coat of fibrin along the luminal surfaces. CONCLUSIONS: This study suggests that (1) ECGS and shear promote the expression of several endothelial characteristics in human adipose-derived stem cells, but not eNOS or vWF; (2) their combined effects appear synergistic; and (3) stem cells differentiated in ECGS appear mildly thrombogenic in vitro, possibly related, in part, to insufficient eNOS expression. Thus, while the acquisition of several endothelial characteristics by adult stem cells derived from adipose tissue suggests these cells are a viable source of autologous cells for cardiovascular regeneration, further stimulation/modifications are necessary prior to using them as a true endothelial cell replacement.

George, J., I. Herz, et al. (2003). "Number and adhesive properties of circulating endothelial progenitor cells in patients with in-stent restenosis." Arterioscler Thromb Vasc Biol **23**(12): e57-60.

OBJECTIVE: Intact endothelialization machinery is essential to facilitate vessel healing after stent placement and to prevent restenosis. Circulating endothelial progenitor cells (EPC) have been demonstrated in the peripheral blood and shown to display endothelial functional properties, along with the ability to traffic to damaged vasculature. We reasoned that robust in-stent intimal growth could be partially related to impaired endothelialization resulting from reduced circulating EPC number or function. METHODS AND RESULTS: Sixteen patients with angiographically-demonstrated in-stent restenosis were compared with patients with a similar clinical presentation that exhibited patent stents (n=11). Groups were similar with respect to the use of drugs that could potentially influence EPC numbers. Circulating EPC numbers were determined by the colony-forming unit assay, and their phenotype was characterized by endothelial-cell markers. Adhesiveness of EPC from both groups to extracellular matrix and to endothelial cells was also assayed. Patients with in-stent restenosis and with patent stents displayed a similar number of circulating EPC. Fibronectin-binding was compromised in patients with in-stent restenosis as compared with their controls exhibiting patent stents. Patients with diffuse in-stent restenosis exhibited reduced numbers of EPC in comparison with subjects with focal in-stent lesions. CONCLUSIONS: Reduced numbers of circulating EPC in patients with diffuse in-stent restenosis and impaired adhesion of EPC from patients with restenosis provides a potential mechanism mediating the exuberant proliferative process. These markers, if further validated, could provide means of risk stratifying patients for likelihood of developing in-stent restenosis.

Gold, E. R. and T. A. Caulfield (2002). "The moral tollbooth: a method that makes use of the patent system to address ethical concerns in biotechnology." Lancet **359**(9325): 2268-70.

Patents granted for biotechnological innovations continue to cause social and ethical dilemmas. For example, much controversy surrounds the patenting of genes that predispose to breast-cancer, and in the USA the debate continues about whether or not stem-cell technology should be accessible to all. In this report, we argue that some of these concerns can be addressed within national patent systems. In particular, we examine the "order public or morality" clause that exists in most national patent procedures. Furthermore, we propose that patents for inventions that present social and ethical questions should be subject to suspension by an independent, transparent, and responsible tribunal made up of specialists in ethics, research, and economics. This suspension should be reversible so that, when the social or ethical concerns have been addressed in an appropriate

manner, the suspension can be lifted. Although controversial, such a flexible mechanism would assist governments and industry in enhancing public support for patents in the biotechnology area.

Haider, H. K., L. Ye, et al. (2007). "Skeletal muscle derived stem cells for myocardial repair." Recent Pat Cardiovasc Drug Discov **2**(3): 205-13.

Treatment of patients with heart failure secondary to myocardial infarction remains a therapeutic challenge. Extensive myocyte death in the heart and post-ischemic remodeling accentuate progressive expansion of the scar area and compromise left ventricular contractile function. The scarcity of resident stem cells in the heart and limited proliferative capacity of adult cardiomyocytes warrant novel strategies of outside intervention to supplement the inept intrinsic repair mechanism. Heart cell therapy using patient's own skeletal muscle derived myoblasts (SkMs) provides a relatively simple and inexpensive therapeutic option. Phase-I and II clinical trials supported by plethora of pre-clinical studies have shown the safety and effectiveness of SkMs engraftment in the treatment of infarcted heart. However, before widespread application of this approach in the clinical settings, there remain some fundamental issues including extensive donor cell death during acute phase after SkMs engraftment, failure of SkMs to adopt cardiac phenotype and transient ventricular arrhythmias subsequent to SkMs transplantation which require serious considerations. This review will provide profound analysis of merits and limitations of SkMs as the choice cells for heart cell therapy and will summarize the potential of genetic and pharmacological manipulation SkMs to enhance their therapeutic efficacy for myocardial repair. Present article also includes recent patent review coverage on this topic.

He, H., T. Shirota, et al. (2003). "Canine endothelial progenitor cell-lined hybrid vascular graft with nonthrombogenic potential." J Thorac Cardiovasc Surg **126**(2): 455-64.

OBJECTIVE: We sought to fabricate a compliant engineered vascular graft (inner diameter of approximately 4.5 mm and length of 6 cm) lined with endothelial progenitor cells derived from circulating peripheral canine blood and to verify its nonthrombogenicity potential in vivo. METHODS: Autologous circulating endothelial progenitor cells derived from the peripheral veins of 6 adult mongrel dogs were isolated by using a density gradient method. The cells were proliferated in vitro in EGM-2 culture medium, prelined on the luminal surface of in situ-formed collagen type I meshes as an extracellular matrix, and wrapped with a segmented polyurethane thin film with multiple micropores as a compliant scaffold. After canine carotid arteries were bilaterally implanted with these grafts for 1 and 3 months, microscopic observation, histologic staining, and immunochemical staining were performed to evaluate morphogenesis. RESULTS: After 33.3 +/- 10.5 days of culture in vitro, 4.2 +/- 1.2 x 10(6) endothelial progenitor cells were obtained. Eleven of the 12 engineered vascular grafts were patent. The grafts possessed smooth, glistening, and ivory-colored luminal surfaces at the predetermined observation period up to 3 months. The intimal layer was covered with confluent, cobblestone-like monolayered cells that were positively stained with factor VIIIB-related antigen. The thickness of the neoarterial walls was approximately 300 microm at 3 months after implantation. A few smooth muscle cells were observed in the medial tissue, and fibroblasts dominated the adventitial tissue. CONCLUSION: Circulating endothelial progenitor cells could be a substitute source of endothelial cells for endothelialization on small-diameter-vessel prostheses to ensure nonthrombogenicity.

Herzenberg, L. A., D. Parks, et al. (2002). "The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford." Clin Chem **48**(10): 1819-27.

The Fluorescence Activated Cell Sorter (FACS) was invented in the late 1960s by Bonner, Sweet, Hulett, Herzenberg, and others to do flow cytometry and cell sorting of viable cells. Becton Dickinson Immunocytometry Systems introduced the commercial machines in the early 1970s, using the Stanford patent and expertise supplied by the Herzenberg Laboratory and a Becton Dickinson engineering group under Bernie Shoor. Over the years, we have increased the number of measured FACS dimensions (parameters) and the speed of sorting to where we now simultaneously measure 12 fluorescent colors plus 2 scatter parameters. In this history, I illustrate the great utility of this state-of-the-art instrument, which allows us to simultaneously stain, analyze, and then sort cells from small samples of human blood cells from AIDS patients, infants, stem cell transplant patients, and others. I also illustrate analysis and sorting of multiple subpopulations of lymphocytes by use of 8-12 colors. In addition, I review single cell sorting used to clone and analyze hybridomas and discuss other applications of FACS developed over the past 30 years, as well as give our ideas on the future of FACS. These ideas are currently being implemented in new programs using the internet for data storage and analysis as well as developing new fluorochromes, e.g., green fluorescent protein and tandem dyes, with applications in such

areas as apoptosis, gene expression, cytokine expression, cell biochemistry, redox regulation, and AIDS. Finally, I describe new FACS methods for measuring activated kinases and phosphatases and redox active enzymes in individual cells simultaneously with cell surface phenotyping. Thus, key functions can be studied in various subsets of cells without the need for prior sorting.

Hibbert, B., Y. X. Chen, et al. (2004). "c-kit-immunopositive vascular progenitor cells populate human coronary in-stent restenosis but not primary atherosclerotic lesions." Am J Physiol Heart Circ Physiol **287**(2): H518-24.

Progress in the treatment of human in-stent restenosis (ISR) is hampered by an imprecise understanding of the nature of the cells that occlude vascular stents. Recent studies suggest that circulating vascular progenitor cells may mediate vascular repair and lesion formation. Moreover, functional endothelial progenitor cells appear to play a protective role in attenuating vascular lesion formation. Hence, we sought to answer two important questions: 1). Are primitive cells found in ISR lesions? 2). Is the abundance of cultured angiogenic cells (CACs) in patients with ISR different from that in patients with non-ISR lesions or normal controls? Human coronary atherectomy tissue from 13 ISR, 6 postangioplasty restenosis (RS), and 14 primary (PR) atherosclerotic lesions, as well as 15 postmortem coronary artery cross sections from young individuals without atherosclerosis, were studied. All 13 ISR and 4 of 6 RS tissue specimens contained cells that immunolabeled for the primitive cell marker c-kit and smooth muscle alpha-actin, whereas the intima and media of PR lesions and normal arteries were devoid of c-kit-immunopositive cells. The abundance of peripheral blood mononuclear cell-derived CACs was assessed in 10 patients with ISR, 6 patients with angiographically verified patent stents, and 6 individuals with no clinical evidence of coronary artery disease. CACs were less abundant in ISR patients than in non-ISR controls (13.9 +/- 3.1 vs. 22.3 +/- 6.7 cells/high-power field, $P < 0.05$), and both of these groups had fewer CACs than non-coronary artery disease patients (37.6 +/- 3.8, $P < 0.05$). These findings suggest a unique pathogenesis for ISR and RS lesions that involves c-kit-immunopositive smooth muscle cells. Moreover, the paucity of CACs in patients with ISR may contribute to the pathogenesis of ISR, perhaps because of attenuated reendothelialization.

Hu, H., Y. Chai, et al. (2009). "Pentagalloylglucose induces autophagy and caspase-independent programmed deaths in human PC-3 and mouse TRAMP-C2 prostate cancer cells." Mol Cancer Ther **8**(10): 2833-43.

Penta-1,2,3,4,6-O-galloyl-beta-d-glucose (PGG) suppresses the in vivo growth of human DU145 and PC-3 prostate cancer xenografts in nude mice, suggesting potential utility as a prostate cancer chemotherapeutic or chemopreventive agent. Our earlier work implicates caspase-mediated apoptosis in DU145 and LNCaP prostate cancer cells as one mechanism for the anticancer activity. We show here that, in the more aggressive PC-3 prostate cancer cell line, PGG induced programmed cell deaths lacking the typical caspase-mediated apoptotic morphology and biochemical changes. In contrast, PGG induced patent features of autophagy, including formation of autophagosomes and lipid modification of light chain 3 after 48 hours of PGG exposure. The "autophagic" responses were also observed in the murine TRAMP-C2 cells. Caspase inhibition exacerbated PGG-induced overall death. As for molecular changes, we observed a rapid inhibition of the phosphorylation of mammalian target of rapamycin-downstream targets S6K and 4EBP1 by PGG in PC-3 and TRAMP-C2 cells but not that of mammalian target of rapamycin itself, along with increased AKT phosphorylation. Whereas the inhibition of phosphatidylinositol 3-kinase increased PGG-induced apoptosis and autophagy, experiments with pharmacologic inducer or inhibitor of autophagy or by knocking down autophagy mediator Beclin-1 showed that autophagy provided survival signaling that suppressed caspase-mediated apoptosis. Knocking down of death receptor-interacting protein 1 kinase increased overall death without changing light chain 3-II or caspase activation, thus not supporting death receptor-interacting protein 1-necroptosis for PGG-induction of autophagy or other programmed cell death. Furthermore, PGG-treated PC-3 cells lost clonogenic ability. The induction by PGG of caspase-independent programmed cell death in aggressive prostate cancer cell lines supports testing its merit as a potential drug candidate for therapy of caspase-resistant recurrent prostate cancer.

Jakob, M. and T. Thum (2008). "Recent patents on cardiovascular stem cells." Recent Pat Cardiovasc Drug Discov **3**(1): 59-72.

Chronic heart failure has emerged as a leading cause of morbidity and mortality worldwide. Conceptually, replacement of akinetic scar tissue by viable myocardium and improvement of neovascularization should improve cardiac function and impede progressive left ventricular remodelling. Experimental and clinical studies suggest that transfer or mobilization of stem and progenitor cells can have a favourable impact on tissue perfusion and contractile

cardiac performance. The aim of the present review was to screen various available patent data bases to give an overview on different patent applications of cell-based and non-cell based therapies in regenerative cardiovascular medicine. The first part describes cell based methods and use of growth factors to improve cardiovascular function. Secondly, patents on methods to improve angiogenesis, re-endothelialization and vascular function are presented. Finally, we describe patents describing methods for improved differentiation of stem cells to cardiovascular cells, including the generation of cardiomyocytes from embryonic or adult stem cells. A systematic overview on the current patent situation about use of stem cells in cardiovascular medicine should facilitate future decision making in the development of novel therapeutic strategies in regenerative medicine.

Jin, S. W., D. Beis, et al. (2005). "Cellular and molecular analyses of vascular tube and lumen formation in zebrafish." Development **132**(23): 5199-209.

Tube and lumen formation are essential steps in forming a functional vasculature. Despite their significance, our understanding of these processes remains limited, especially at the cellular and molecular levels. In this study, we analyze mechanisms of angioblast coalescence in the zebrafish embryonic midline and subsequent vascular tube formation. To facilitate these studies, we generated a transgenic line where EGFP expression is controlled by the zebrafish flk1 promoter. We find that angioblasts migrate as individual cells to form a vascular cord at the midline. This transient structure is stabilized by endothelial cell-cell junctions, and subsequently undergoes lumen formation to form a fully patent vessel. Downregulating the VEGF signaling pathway, while affecting the number of angioblasts, does not appear to affect their migratory behavior. Our studies also indicate that the endoderm, a tissue previously implicated in vascular development, provides a substratum for endothelial cell migration and is involved in regulating the timing of this process, but that it is not essential for the direction of migration. In addition, the endothelial cells in endodermless embryos form properly lumenized vessels, contrary to what has been previously reported in Xenopus and avian embryos. These studies provide the tools and a cellular framework for the investigation of mutations affecting vasculogenesis in zebrafish.

Karlsson, U., J. Hyllner, et al. (2007). "Trends in the human embryonic stem cell patent field." Recent Pat Nanotechnol **1**(3): 233-7.

The successful derivation of human embryonic stem (hES) cell lines in late 1990s marks the birth of a new era in biomedical research. In the USA, this landmark invention is protected by granted composition-of-matter patents. In addition to these patents, several others have been granted on further development of hES cell research, such as on differentiated cell types and in vitro and in vivo use aspects. In Europe, there is presently no consensus pertaining to the patentability of hES cells, and all patent applications pending at the European patent office are therefore awaiting a principal decision by the Enlarged Board of Appeal. The authors argue that it will be of importance to the stem cell industry that patents are granted on inventions downstream in the value chain, e.g on specialised cell types derived from hES cells and different drug discovery applications. Patents and patent applications on such inventions for the three germ layers ectoderm/neuro, endoderm/hepato and mesoderm/cardio have been examined. The number of patents increased in the period 2001 to 2006 for all three lineages with ectoderm/neuro as the most patent intensive field. There where 9-13 times more US patent applications filed related to the three lineages compared to in Europe.

Kern, S. E. and D. Shibata (2007). "The fuzzy math of solid tumor stem cells: a perspective." Cancer Res **67**(19): 8985-8.

Apparently effective therapeutic agents very often fail to cure cancer patients. It is therefore attractive to wonder whether a specific resistant cell subset should be recognized and separately targeted. In solid tumors, such as carcinomas, a minor population of "cancer stem cells" has been proposed and sought experimentally in human tumors and isolated cell populations. It is often overlooked that the rationale and supportive data are essentially numerical and can be evaluated as such. A reevaluation of the published studies and related claims within awarded U.S. patents suggests that the mathematical support for the concept of therapeutically useful stem cells is weak and may even invalidate the foundations of these publications and patent claims. Mathematical arguments should be used more consistently, because they can serve as a guide for interpreting studies into cancer stem cells of solid tumors.

Kiatpongsan, S. (2006). "Intellectual property and patent in stem cell research era." J Med Assoc Thai **89**(11): 1984-6.

Stem cell therapy has obtained much attention, not only for its exceptional promise for curing many chronic disorders and degenerative

conditions but also for its great economic potential. Apart from expenses in research laboratories and ongoing clinical trials, intellectual properties, patent of stem cell differentiation protocols, and stem cell-derived medical products for cell and tissue therapy are of very high cost. Intellectual properties and patents are inevitably important issues for stem cell researchers. Stem cell researchers in most countries have a chance to develop affordable stem cell therapy, scientific progression, and innovations for their patients. However, for this to be done, appropriate solutions for international patent barrier must be created so that the owner of the original stem cell protocols and techniques can be acknowledge, build his reputation and reap reasonable financial benefits. International patent barriers will be a crucial step to move the whole stem cell research community forward.

Lim, S. H., S. W. Cho, et al. (2008). "Tissue-engineered blood vessels with endothelial nitric oxide synthase activity." J Biomed Mater Res B Appl Biomater **85**(2): 537-46.

Nondegradable synthetic polymer vascular grafts used in cardiovascular surgery have shown serious shortcomings, including thrombosis, calcification, infection, and lack of growth potential. Tissue engineering of vascular grafts with autologous stem cells and biodegradable polymeric materials could solve these problems. The present study is aimed to develop a tissue-engineered vascular graft (TEVG) with functional endothelium using autologous bone marrow-derived cells (BMCs) and a hybrid biodegradable polymer scaffold. Hybrid biodegradable polymer scaffolds were fabricated from poly(lactide-co-epsilon-caprolactone) (PLCL) copolymer reinforced with poly(glycolic acid) (PGA) fibers. Canine bone marrow mononuclear cells were induced in vitro to differentiate into vascular smooth muscle cells and endothelial cells. TEVGs (internal diameter: 10 mm, length: 40 mm) were fabricated by seeding vascular cells differentiated from BMCs onto PGA/PLCL scaffolds and implanted into the abdominal aorta of bone marrow donor dogs (n = 7). Eight weeks after implantation of the TEVGs, the vascular grafts remained patent. Histological and immunohistochemical analyses of the vascular grafts retrieved at 8 weeks revealed the regeneration of endothelium and smooth muscle and the presence of collagen. Western blot analysis showed that endothelial nitric oxide synthase (eNOS) was expressed in TEVGs comparable to native abdominal aortas. This study demonstrates that vascular grafts with significant eNOS activity can be tissue-engineered with autologous BMCs and hybrid biodegradable polymer scaffolds.

Martin-Rendon, E. and D. J. Blake (2007). "Patenting human genes and stem cells." Recent Pat DNA Gene Seq **1**(1): 25-34.

Cell lines and genetically modified single cell organisms have been considered patentable subjects for the last two decades. However, despite the technical patentability of genes and stem cell lines, social and legal controversy concerning their 'ownership' has surrounded stem cell research in recent years. Some granted patents on stem cells with extremely broad claims are casting a shadow over the commercialization of these cells as therapeutics. However, in spite of those early patents, the number of patent applications related to stem cells is growing exponentially. Both embryonic and adult stem cells have the ability to differentiate into several cell lineages in an organism as a result of specific genetic programs that direct their commitment and cell fate. Genes that control the pluripotency of stem cells have been recently identified and the genetic manipulation of these cells is becoming more efficient with the advance of new technologies. This review summarizes some of the recent published patents on pluripotency genes, gene transfer into stem cells and genetic reprogramming and takes the hematopoietic and embryonic stem cell as model systems.

McCloskey, K. E., M. E. Gilroy, et al. (2005). "Use of embryonic stem cell-derived endothelial cells as a cell source to generate vessel structures in vitro." Tissue Eng **11**(3-4): 497-505.

Embryonic stem (ES) cells could potentially serve as an excellent cell source for various applications in regenerative medicine and tissue engineering. Our laboratory is particularly interested in generating a reproducible endothelial cell source for the development of prevascularized materials for tissue/organ reconstruction. After developing methods to isolate highly purified (>96%) proliferating populations of endothelial cells from mouse embryonic stem cells, we tested their ability to form three-dimensional (3-D) vascular structures in vitro. The ES cell-derived endothelial cells were embedded in 3-D collagen gel constructs with rat tail collagen type I (2 mg/mL) at a concentration of 10(6) cells/mL of gel. The gels were observed daily with a phase-contrast microscope to analyze the time course for endothelial cell assembly. The first vessels were observed between days 3 and 5 after gel construct formation. The number and complexity of structures steadily increased, reaching a maximum before beginning to regress. By 2 weeks, all vessel-like structures had regressed back to single cells. Histology and fluorescent images of the vessel-like structures verified that tube structures were multicellular and

could develop patent lumens. We have shown that endothelial cells derived, purified and expanded in vitro from ES cells sustain an important endothelial cell function, the ability to undergo vasculogenesis in collagen gels, indicating that endothelial products derived in vitro from stem cells could be useful in regenerative medicine applications.

Mei, Q. L., J. Y. Yang, et al. (2008). "Effects of granulocyte colony-stimulating factor on repair of injured canine arteries." Chin Med J (Engl) **121**(2): 143-6.

BACKGROUND: Endothelial progenitor cells (EPCs) derived from bone marrow may differentiate into endothelial cells and participate in endothelial repair. These cells can be mobilized into peripheral blood by cytokines, including granulocyte colony-stimulating factor (G-CSF). In the present study, we investigated the effects of G-CSF on neointimal formation and restenosis in a canine model of arterial balloon injury. METHODS: Sixteen male beagle dogs were injected subcutaneously with 20 microg x kg(-1) x d(-1) recombinant human G-CSF (n = 8) or normal saline (n = 8) for 1 week. On the fifth day of treatment, the dogs underwent renal arterial angioplasty. At 8 weeks after arterial balloon injury, angiographic observations were made and injured arteries were processed for morphometric analysis of neointimal formation. RESULTS: Peripheral white blood cell counts were increased by 3.34-fold compared to baseline on the fifth day of administration of G-CSF. Angiographies revealed that one stenosis had occurred among the eight injured renal arteries from dogs treated with G-CSF, whereas all injured renal arteries from dogs treated with normal saline remained patent. The mean extent of stenosis among injured arteries was 18.3% +/- 17.9% in the G-CSF treated group compared to 12.5% +/- 7.6% in the saline treated control group (P = 0.10). G-CSF treatment slightly increased neointimal thickness (0.42 +/- 0.15 mm vs 0.25 +/- 0.06 mm, P = 0.08) with an intima to media ratio of 0.83 +/- 0.49 vs 0.54 +/- 0.18 (P = 0.11). CONCLUSIONS: G-CSF treatment does not attenuate neointimal hyperplasia and restenosis formation in a canine model of renal arterial injury, suggesting that the therapeutic strategy for preventing restenosis by stem cell mobilization should be investigated further.

Meier, B. (2006). "The current and future state of interventional cardiology: a critical appraisal." Cardiology **106**(3): 174-89.

After 75 years of invasive and over 50 years of interventional cardiology, cardiac catheter-based procedures have become the most frequently used interventions of modern medicine. Patients undergoing a percutaneous coronary intervention (PCI) outnumber those with coronary artery bypass surgery by a factor of 2 to 4. The default approach to PCI is the implantation of a (drug-eluting) stent, in spite of the fact that it improves the results of balloon angioplasty only in about 25% of cases. The dominance of stenting over conservative therapy or balloon angioplasty on one hand and bypass surgery on the other hand is a flagrant example of how medical research is digested an applied in real life. Apart from electrophysiological interventions, closure ot the patent foramen ovale and percutaneous replacement of the aortic valve in the elderly have the potential of becoming daily routine procedures in catheterization laboratories around the world. Stem cell regeneration of vessels or heart muscle, on the other hand, may remain a dream never to come true.

Melero-Martin, J. M., M. E. De Obaldia, et al. (2008). "Engineering robust and functional vascular networks in vivo with human adult and cord blood-derived progenitor cells." Circ Res **103**(2): 194-202.

The success of therapeutic vascularization and tissue engineering will rely on our ability to create vascular networks using human cells that can be obtained readily, can be expanded safely ex vivo, and can produce robust vasculogenic activity in vivo. Here we describe the formation of functional microvascular beds in immunodeficient mice by coimplantation of human endothelial and mesenchymal progenitor cells isolated from blood and bone marrow. Evaluation of implants after 1 week revealed an extensive network of human blood vessels containing erythrocytes, indicating the rapid formation of functional anastomoses within the host vasculature. The implanted endothelial progenitor cells were restricted to the luminal aspect of the vessels; mesenchymal progenitor cells were adjacent to lumens, confirming their role as perivascular cells. Importantly, the engineered vascular networks remained patent at 4 weeks in vivo. This rapid formation of long-lasting microvascular networks by postnatal progenitor cells obtained from noninvasive sources constitutes an important step forward in the development of clinical strategies for tissue vascularization.

Meyburg, J., F. Hoerster, et al. (2008). "Use of the middle colic vein for liver cell transplantation in infants and small children." Transplant Proc **40**(4): 936-7.

INTRODUCTION: Because it is less invasive, intraportal liver cell transplantation (LCT) is an interesting alternative to whole organ transplantation. The inferior mesenteric vein is usually chosen for portal vein access. However, anatomical variations are common in children, so we investigated

catheter insertion into the middle colic vein. PATIENTS AND METHODS: Three children (3 weeks to 3 years; 3 to 14 kg) underwent LCT in our center for acute liver failure or severe neonatal urea cycle disorders. Small 4.2-French Hickman lines were surgically introduced into the middle colic vein and advanced to the portal vein stem. The patients received repetitive infusions of liver cells over a period of 4-11 days. RESULTS: Catheter insertion was feasible and tolerated well despite the poor clinical condition of 1 patient and the metabolic instability in the other 2 patients. Blood could be drawn from all catheters, and measurement of portal vein pressure was possible in 2 children. The patient with acute liver failure died after 11 days from complications of the underlying disease. In the other 2 children, portal vein catheters stayed patent for several months. CONCLUSIONS: The middle colic vein can be recommended for placement of intraportal LCT catheters even in small and critically ill infants.

Munoz-Sanjuan, I. (2009). "Glial progenitor cell transplantation and the generation of chimeric animal models with human brain cells: implications for novel therapeutics." Expert Opin Ther Pat **19**(12): 1639-46.

BACKGROUND: The potential of exogenous stem cell or progenitor cell transplantation as a novel therapeutic strategy to address unmet medical needs is a vast and important area of investigation. A recent US patent has been issued to Goldman from the University of Rochester based on pioneering studies with human fetal and adult-derived glial progenitor cells (GPCs), covering the generation of chimeric mouse/human animals. OBJECTIVE/METHOD: In this patent and associated manuscript, extensive chimerism due to grafting of human GPCs is associated with remyelination and functional rescue of mice congenitally deficient in oligodendrocyte survival and myelination, due to a deletion in the myelin basic protein gene (the shiverer mouse). This review highlights the implications of generating human/mouse chimeric animals for the study of human brain physiology, preclinical studies and the clinical application of progenitor cells towards the development of novel therapeutics for the treatment of demyelinating disorders. CONCLUSION: The use of GPCs offers promise for remyelination disorders, and the ability of these cells to repopulate the entire rodent nervous system should allow for the investigation of the physiological properties of human glial derivatives in an in vivo context, enhancing the understanding of mechanisms with a primary effect through the modulation of human glial cell biology.

Patel, A. N., L. Geffner, et al. (2005). "Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study." J Thorac Cardiovasc Surg **130**(6): 1631-8.

BACKGROUND: Autologous adult stem cell transplantation has been touted as the latest tool in regenerative medical therapy. Its potential for use in cardiovascular disease has only recently been recognized. A randomized study was conducted with a novel epicardial technique to deploy stem cells as an adjuvant to conventional revascularization therapy in patients with congestive heart failure. METHODS: After institutional review board and government approval, adult autologous stem cell transplantation (CD34+) was performed in patients with ischemic cardiomyopathy and an ejection fraction of less than 35% who were scheduled for primary off-pump coronary artery bypass grafting. Preoperatively, the patients underwent echocardiography, stress thallium imaging single photon emission computed tomography, and cardiac catheterization to identify ischemic regions of the heart and to guide in the selection of stem cell injection sites. The patients were prospectively randomized before the operative therapy was performed. Patient follow-up was 1, 3, and 6 months with echocardiography, single photon emission computed tomography, and angiography. RESULTS: There were 20 patients enrolled in the study. Ten patients had successful subepicardial transplantation of autologous stem cells into ischemic myocardium. The other 10 patients, the control group, only had off-pump coronary artery bypass grafting. There were 8 male and 2 female subjects in each group. The median number of grafts performed was 1 in both groups. On angiographic follow-up, all grafts were patent at 6 months. The ejection fractions of the off-pump coronary artery bypass grafting group versus the off-pump coronary artery bypass grafting plus stem cell transplantation group were as follows: preoperative, 30.7% +/- 2.5% versus 29.4% +/- 3.6%; 1 month, 36.4% +/- 2.6% versus 42.1% +/- 3.5%; 3 months, 36.5% +/- 3.0% versus 45.5% +/- 2.2%; and 6 months, 37.2% +/- 3.4% versus 46.1% +/- 1.9% ($P < .001$). There were no perioperative arrhythmias or neurologic or ischemic myocardial events in either group. CONCLUSIONS: Autologous stem cell transplantation led to significant improvement in cardiac function in patients undergoing off-pump coronary artery bypass grafting for ischemic cardiomyopathy. Further investigation is required to quantify the optimal timing and specific cellular effects of the therapy.

Rao, M. and M. L. Condic (2008). "Alternative sources of pluripotent stem cells: scientific solutions to an ethical dilemma." Stem Cells Dev **17**(1): 1-10.

Stem cell researchers in the United States have faced a quagmire of uncertainty due to multiple factors: the ethical divide over the use of embryos for research, the lack of clarity in federal guidelines governing this research, the restrictive patent situation surrounding the generation of new human embryonic stem (HES) cell lines; and the limits on types of research eligible for federal funding. In this commentary, we describe how recent advances in derivation of hES cell-like lines may allow at least some of these uncertainties to be resolved. More importantly, we suggest that the derivation of hES cell-like lines by morally acceptable methods would not only avoid the corrosive effects of a protracted ethical debate over stem cell research, but would also allow U.S. researchers to access federal funds and compete on a more level international playing field.

Rao, M. S. (2006). "Mired in the quagmire of uncertainty: The "catch-22" of embryonic stem cell research." Stem Cells Dev **15**(4): 492-6.

Pluripotent human embryonic stem (ES) cells hold remarkable therapeutic potential, but their use is fraught with moral, ethical, scientific, and political concerns. In this essay, I discuss how an odd combination of patent issues, presidential policy, market uncertainties, and evolving Food and Drug Administration regulations have together hindered the progress of ES cell research in the United States of America. This coalescence of issues is unique. I suggest that these factors explain why the United States has not been a dominant player in advancing ES research. I predict that small, noncontroversial changes would go far in ameliorating many of the roadblocks that now exist. Most of these changes would not require a change in policy or even action by the U.S. government; a simple clarification and definition would suffice. The reason these changes have met solid resistance is suggested to derive from financial rather than moral, ethical, or scientific issues.

Resnik, D. B. (2002). "The commercialization of human stem cells: ethical and policy issues." Health Care Anal **10**(2): 127-54.

The first stage of the human embryonic stem (ES) cell research debate revolved around fundamental questions, such as whether the research should be done at all, what types of research may be done, who should do the research, and how the research should be funded. Now that some of these questions are being answered, we are beginning to see the next stage of the debate: the battle for property rights relating to human ES cells. The reason why property rights will be a key issue in this debate is simple and easy to understand: it costs a great deal of money to do this research, to develop new products, and to implement therapies; and private companies, researchers, and health professionals require returns on investments and reimbursements for goods and services. This paper considers arguments for and against property rights relating to ES cells defends the following points: (1) It should be legal to buy and sell ES cells and products. (2) It should be legal to patent ES cells, products, and related technologies. (3) It should not be legal to buy, sell, or patent human embryos. (4) Patents on ES cells, products, and related technologies should not be excessively broad. (5) Patents on ES cells, products, and related technologies should be granted only when applicants state definite, plausible uses for their inventions. (6) There should be a research exemption in ES cell patenting to allow academic scientists to conduct research in regenerative medicine. (7) It may be appropriate to take steps to prevent companies from using patents in ES cells, products, and related technologies only to block competitors. (8) As the field of regenerative medicine continues to develop, societies should revisit issues relating to property rights on a continuing basis in order to develop policies and develop regulations to maximize the social, medical, economic, and scientific benefits of ES cell research and product development.

Ruch, R. J. and J. E. Trosko (1999). "The role of oval cells and gap junctional intercellular communication in hepatocarcinogenesis." Anticancer Res **19**(6A): 4831-8.

The role of oval cells, and Gap Junctional Intercellular Communication (GJIC) in hepatic differentiation and neoplasia is controversial. Oval cells accumulate in great number when hepatocyte regeneration is blocked following massive hepatotoxicity or after treatment with some hepatocarcinogens. This suggests oval cells are facultative stem cells or close progeny of liver stem cells that are activated only under specific conditions. Studies with oval cell lines clearly indicate that they can differentiate into hepatocytes and that neoplastic derivatives of oval cells can produce hepatocellular and biliary neoplasms. Because hepatocytes express Cx32 and biliary cells express Cx43, the differentiation of oval cells into hepatocytes or In addition, because Cx32 hemichannels and Cx43 hemichannels cannot form heterotypic patent channels, the type of connexin expressed by the differentiating oval cell will determine whether it communicates with hepatocytes or biliary epithelial cells, respectively. This communication may be necessary for the further differentiation and regulated growth of the differentiating oval cells and

impairment of this GJIC may contribute to the formation of hepatocellular and cholangiocellular neoplasms. The type of connexin expressed may also determine the susceptibility of the differentiating oval cells to the various types of rodent liver tumor promoters. Thus, three major points have been developed here. First, Cx32 or Cx43 expression and GJIC with hepatocytes or biliary epithelial cells, respectively, may determine the final differentiated fate of oval cells. Secondly, blocked GJIC may determine whether oval cells progress to hepatocellular or cholangiocellular carcinoma. Lastly, the ability of tumor promoters to block Cx32 or Cx43-mediated GJIC in differentiating oval cells may determine whether these agents promote the formation of hepatocellular or cholangiocellular carcinomas. Thus, GJIC may be the key factor in the differentiation of oval cells and blocked GJIC may promote their neoplastic transformation in a lineage-specific manner. In this chapter, we have outlined several new hypotheses on the role of oval cells and GJIC in hepatocarcinogenesis. We hope that other investigators will consider our ideas, but realize these views will be contentious to many. Our intent, however, was to stimulate discussion and debate, even argument, because truth often arises amidst controversy and may be found in the most peculiar places.

Seandel, M., J. M. Butler, et al. (2008). "Generation of a functional and durable vascular niche by the adenoviral E4ORF1 gene." Proc Natl Acad Sci U S A **105**(49): 19288-93.

Vascular cells contribute to organogenesis and tumorigenesis by producing unknown factors. Primary endothelial cells (PECs) provide an instructive platform for identifying factors that support stem cell and tumor homeostasis. However, long-term maintenance of PECs requires stimulation with cytokines and serum, resulting in loss of their angiogenic properties. To circumvent this hurdle, we have discovered that the adenoviral E4ORF1 gene product maintains long-term survival and facilitates organ-specific purification of PECs, while preserving their vascular repertoire for months, in serum/cytokine-free cultures. Lentiviral introduction of E4ORF1 into human PECs (E4ORF1(+) ECs) increased the long-term survival of these cells in serum/cytokine-free conditions, while preserving their in vivo angiogenic potential for tubulogenesis and sprouting. Although E4ORF1, in the absence of mitogenic signals, does not induce proliferation of ECs, stimulation with VEGF-A and/or FGF-2 induced expansion of E4ORF1(+) ECs in a contact-inhibited manner. Indeed, VEGF-A-induced phospho MAPK activation of E4ORF1(+) ECs is comparable with that of naive PECs, suggesting that the VEGF receptors remain functional upon E4ORF1 introduction. E4ORF1(+) ECs inoculated in implanted Matrigel plugs formed functional, patent, humanized microvessels that connected to the murine circulation. E4ORF1(+) ECs also incorporated into neo-vessels of human tumor xenotransplants and supported serum/cytokine-free expansion of leukemic and embryonal carcinoma cells. E4ORF1 augments survival of PECs in part by maintaining FGF-2/FGF-R1 signaling and through tonic Ser-473 phosphorylation of Akt, thereby activating the mTOR and NF-kappaB pathways. Therefore, E4ORF1(+) ECs establish an Akt-dependent durable vascular niche not only for expanding stem and tumor cells but also for interrogating the roles of vascular cells in regulating organ-specific vascularization and tumor neo-angiogenesis.

Sergi, C., M. Serpi, et al. (1999). "CATCH 22 syndrome: report of 7 infants with follow-up data and review of the recent advancements in the genetic knowledge of the locus 22q11." Pathologica **91**(3): 166-72.

CATCH 22 is a medical acronym for Cardiac defects, Abnormal facies, Thymic hypoplasia, Cleft palate, and Hypocalcemia, and a variable deletion on chromosome 22. The deletion within the chromosome region of 22q11 may occur in patients with three well-described dysmorphologic+ cardiological syndromes: DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), and conotruncal anomaly face syndrome (CTAFS). We report in detail seven infants with a deletion of the locus 22q11 showing overlapping clinical features of DGS and CTAFS with complex congenital heart defects (double outlet right ventricle, atresia or stenosis of the pulmonary valve, atrial and ventricular septal defects, patent ductus arteriosus, tetralogy of Fallot, major aortopulmonary collateral arteries, arcus aortae dexter, and persistence of the left superior vena cava). A homograft was implanted between the right ventricle and the main stem of the pulmonary artery in 2 patients, while a balloon valvuloplastic of the pulmonary valve was performed in one patient only. Pulmonary hemorrhage, acute hypoxia, and Aspergillus pneumonia were the complications. Death occurred in three out of seven patients. Recent advancements in the genetic knowledge of the locus 22q11 are described. Since the locus 22q11 is highly heterogeneous, the CATCH 22 acronym should be used and temporarily the old eponyms should be abandoned waiting for the identification of the different genes.

Shi, Q., V. Bhattacharya, et al. (2002). "Utilizing granulocyte colony-stimulating factor to enhance vascular graft endothelialization from circulating blood cells." Ann Vasc Surg **16**(3): 314-20.

Cells in the blood circulating through a vascular graft can contribute to endothelialization of its flow surface. We hypothesized that granulocyte colony-stimulating factor (G-CSF) could enhance this process by increasing circulating bone marrow progenitor cells. Ten dogs received composite grafts that were shielded from any source of endothelialization other than the circulating blood. On the seventh postoperative day and for 7 days thereafter, five dogs were injected subcutaneously with 10 mg/kg/day of human G-CSF. The additional five dogs, used as controls, received no G-CSF. Grafts were retrieved at 4 weeks. All dogs recovered promptly postoperatively. White cell counts in G-CSF dogs increased by an average of 9.5-fold at the end of treatment, and had returned to normal before retrieval. All grafts remained patent. G-CSF grafts had significantly higher endothelialization than the controls (82.2 +/- 9.2% vs. 23.7 +/- 4.4%, p = 0.0004), with extensive flow surface neointima, covered with a single layer of endothelium verified by FVIII/vWF and CD34 staining. Control grafts had virtually no neointima and were covered with a thin layer of fibrin coagulum. Significantly more endothelial-lined microvessels were also found in the G-CSF grafts than in the controls. Dogs treated with G-CSF have increased endothelialization of synthetic vascular grafts due to increased circulating bone marrow progenitor cells.

Shyntum, Y. and E. Kalkreuter (2009). "Stem cell patents--reexamination/litigation--the last 5 years." Tissue Eng Part B Rev **15**(1): 87-90.

U.S. patents directed to stem cell technologies have generated a high degree of interest and controversy. Many patents relating to stem cell technology have faced reexamination, litigation, or both. The U.S. Patent and Trademark Office (USPTO) recently upheld three Wisconsin Alumni Research Foundation (WARF) stem cell patents after reexamination requested by a third-party challenger in 2006. StemCells, Inc., and Neuralstem, Inc., both filed suits with respect to their patents related to neural stem cells. StemCells filed a suit on July 24, 2006, alleging infringement of its patents collectively referred to as "the neural stem cell patents," by Neuralstem, Inc. Neuralstem, Inc., filed a suit against StemCells, Inc., on May 7, 2008, alleging inequitable conduct during prosecution of StemCells' U.S. Patent No. 7,361,505. Both suits are yet to be decided. Pharmastem Therapeutics, Inc., had attempted to enforce its U.S. Patent Nos. 5,192,553 and 5,004,681, which resulted in invalidation of the patents in 2007. It remains to be seen what effect (if any) the recent increases in funding of stem research and the important U.S. Supreme Court decision on KSR v. Teleflex, Inc. (making it more difficult to establish nonobviousness of patentable subject matter) will have on challenges to stem cell patents.

Spranger, T. M. (2003). "Patent protection for stem cell procedures under the law of the European Union." Med Etika Bioet **10**(1-2): 4-8.

Stem cell research shows an immense diagnostic and therapeutic potential. The procedures based on human stem cells seem to allow new medical treatments for serious diseases like Parkinson's or Alzheimer's disease, leukaemia or diabetes. However, as no company or inventor would take the risk of immense investments without an adequate legal protection of the possible benefits arising out of their work, intellectual property law plays a pivotal role for the further development of stem cell techniques. Although international patent law knows protection of inventions using biological substances and living matter for about 160 years, patents on stem cells, DNA and other parts of the human body raise specific objections. Nevertheless, from a strictly legal angle, there are no barriers to patents on stem cell procedures. In particular, Art. 6 of the "Directive 98/44/EC of the European Parliament and of the Council of the European Union of July 6, 1998 on the legal protection of biotechnological inventions" - which qualifies inventions as unpatentable where their commercial exploitation would be contrary to ordre public or morality - does not hinder patent protection for stem cell research.

Taylor, P. L. (2005). "The gap between law and ethics in human embryonic stem cell research: overcoming the effect of U.S. federal policy on research advances and public benefit." Sci Eng Ethics **11**(4): 589-616.

Key ethical issues arise in association with the conduct of stem cell research by research institutions in the United States. These ethical issues, summarized in detail, receive no adequate translation into federal laws or regulations, also described in this article. U.S. Federal policy takes a passive approach to these ethical issues, translating them simply into limitations on taxpayer funding, and foregoes scientific and ethical leadership while protecting intellectual property interests through a laissez faire approach to stem cell patents and licenses. Those patents and licenses, far from being scientifically and ethically neutral in effect, virtually prohibit commercially sponsored research that could otherwise be a realistic alternative to the federal funding gap. The lack of federal funding and related data-sharing

principles, combined with the effect of U.S. patent policy, the lack of key agency guidance, and the proliferation of divergent state laws arising from the lack of Federal leadership, significantly impede ethical stem cell research in the United States, without coherently supporting any consensus ethical vision. Research institutions must themselves implement steps, described in the article, to integrate addressing ethical review with the many legal compliance issues U.S. federal and state laws create.

Then, S. N. (2004). "Stem cell technologies: regulation, patents and problems." J Law Med **12**(2): 188-204.

Human embryonic stem cell research promises to deliver in the future a whole range of therapeutic treatments, but currently governments in different jurisdictions must try to regulate this burgeoning area. Part of the problem has been, and continues to be, polarised community opinion on the use of human embryonic stem cells for research. This article compares the approaches of the Australian, United Kingdom and United States governments in regulating human embryonic stem cell research. To date, these governments have approached the issue through implementing legislation or policy to control research. Similarly, the three jurisdictions have viewed the patentability of human embryonic stem cell technologies in their own ways with different policies being adopted by the three patent offices. This article examines these different approaches and discusses the inevitable concerns that have been raised due to the lack of a universal approach in relation to the regulation of research; the patenting of stem cell technologies; and the effects patents granted are having on further human embryonic stem cell research.

Tong, H., Y. Ren, et al. (2009). "Clinicopathological study on peripheral T-cell non-Hodgkin lymphoma with bone marrow involvement: a retrospective analysis from China." Int J Hematol **90**(3): 303-10.

We reviewed 173 patients with an initial diagnosis of peripheral T-cell non-Hodgkin lymphoma (PTCL) and compared the patients with bone marrow involvement (BMI) to those without to have a better understanding of the clinical characteristics, treatments, survival and prognosis of PTCLs with BMI. We found that 40% (70/173) of the patients had BMI, and its frequency was 64% in angioimmunoblastic T-cell lymphoma (TCL), 46% in PTCL unspecified, 29% in anaplastic large T-cell lymphoma, 23% in extranodal NK/T-cell lymphoma and 13% in enteropathy-type TCL. In the BMI group, 36% of patients had lymphoma-associated hemophagocytic syndrome (LAHS), compared with 8% of the patients without BMI (8/103, $P < 0.001$). The estimated 1-year overall survival (OS) rates of patients with LAHS in the BMI and non-BMI groups were 5 and 49%, respectively. The increased levels of lactate dehydrogenase, fasting triglycerides and beta(2)-microglobulin between the BMI and non-BMI groups were not significantly different, but ferritin increased significantly and liver dysfunction-related diseases were seen more in the BMI group. As much as 51% of patients of the BMI group had anemia, compared with 27% of the patients without BMI ($P = 0.001$). The estimated 2-year OS rates in the two groups were 10 and 34%. The estimated 2-year OS rate of the 67 patients with BMI, who did not lose to follow-up, was 22%, compared with 38% in the non-BMI group. The median survival times of the 2 groups were 120 and 356 days. The estimated 2-year OS rate of patients treated by CHOP regimen was 9%, compared with 51% of those with intensive chemotherapy, with a significant difference (log rank $P = 0.0008$). The median survival time of the 14 patients subjected to chemotherapy combined with L: -asparaginase was 365 days and that of the 7 patients undergoing hemopoietic stem cell transplantation (HSCT) was 575 days. A total of 3 patients in a critical condition underwent plasmapheresis as initial therapy and achieved stable condition. We conclude that patients with PTCLs with BMI on initial diagnosis usually have hemaphagocytic syndrome and poor prognosis. BMI without lymphadenopathy is a patent clinical feature in most PTCLs. Patients with anemia on initial diagnosis in the BMI group usually have poor prognosis than those without. Intense chemotherapy, addition of L: -asparaginase in chemotherapy and HSCT are comparatively efficient treatments of PTCLs. For patients in critical conditions, plasmapheresis before chemotherapy would lower the risk and improve the tolerance to chemotherapy.

Torre, M. L., M. Faustini, et al. (2007). "Cell encapsulation in mammal reproduction." Recent Pat Drug Deliv Formul **1**(1): 81-5.

Cell encapsulation is an evolving branch of biotechnology with numerous applications including the enhancing of reproductive performance both in humans and other mammal species. Over the last twenty years male and female gametes and embryos have been encapsulated with or without somatic cells, for different purposes, such as semen controlled release, in vitro gametogenesis, embryo culture after in vitro fertilization and cell preservation. In this paper the state-of-the-art of this field (leaving aside that involving embryonic stem cells) is reviewed in terms of scientific literature and patent production. The patents and papers underline a widespread use of

alginate which is a natural anionic, biocompatible, biodegradable polymer that mimics the extracellular matrix or the basal membrane and supports cell functions and metabolism. Gamete and embryo encapsulation techniques tend to fall into two main groupings: the "classical" three-step method, and the more recent one-step method. However, all of these encapsulation techniques are moving towards new, interesting applications since they can be easily tailor-made to fit a variety of cell lines.

Witek, R. (2005). "Ethics and patentability in biotechnology." Sci Eng Ethics **11**(1): 105-11.

The systems of patent rights in force in Europe today, both at the level of national law and on the regional level, contain general clauses prohibiting the patenting of inventions whose publication and exploitation would be contrary to "ordre public" or morality. Recent years have brought frequent discussion about limiting the possibility of patent protection for biotechnological inventions for ethical reasons. This is undoubtedly a result of the dynamic development in this field in the last several years. Human genome sequencing, the first successful cloning of mammals, and the progress in human stem cell research present humanity with many new questions of an ethical nature. Directive 98/44 of the European Parliament and of the Council of July 6, 1998, on the Legal Protection of Biotechnological Inventions created a new basis for patent protection in this field of technology. Based on the European experience to now, however, it must be said that patent law is not the right place to legislate the consequences of the morality of an invention.

Yang, Z., J. Tao, et al. (2006). "Shear stress contributes to t-PA mRNA expression in human endothelial progenitor cells and nonthrombogenic potential of small diameter artificial vessels." Biochem Biophys Res Commun **342**(2): 577-84.

Seeding endothelial progenitor cells (EPCs) onto the surface of vascular grafts has been proved to be a promising strategy to improve nonthrombogenic potentials of small diameter artificial vessels. Here, we investigated whether in vitro shear stress modulates the tissue-type plasminogen activator (t-PA) secretion and mRNA expression in human EPCs and improves patency of the EPC-seeded polyurethane small diameter vascular grafts implanted in the canine carotid artery in vivo. In vitro shear stress, in a dose-dependent manner, increased t-PA secretion and mRNA expression of human EPCs. The in vivo implantation of EPC-seeded vascular grafts remained highly patent in shear stress pretreatment compared with stationary condition. The present findings demonstrate for the first time that in vitro shear stress can enhance t-PA secretion and gene expression in human EPCs, which contributes to improvement in nonthrombogenic potentials of EPC-seeded small diameter artificial vessels with maintenance of in vivo highly patency rate.

Yelda, T., U. Berrin, et al. (2007). "Intracoronary stem cell infusion in heart transplant candidates." Tohoku J Exp Med **213**(2): 113-20.

The stem cell transplantation is emerging as a potential therapeutic modality for patients with heart failure. It has been demonstrated that intracoronary stem cell transplantation had beneficial effects on left ventricular perfusion and contractile functions. We hypothesized that patients with end-stage ischemic cardiomyopathy, who are candidates for heart transplantation, could also benefit from autologous intracoronary stem cell transplantation. We performed a prospective, open-labeled study in 10 patients with end-stage ischemic cardiomyopathy, who were on the waiting list for heart transplantation. Each patient received bone marrow-derived mononuclear cell infusion via balloon catheter in the target vessel, which had been revascularized by percutaneous intervention and was patent before the procedure. Clinical and laboratory evaluations, a treadmill exercise test, echocardiography, and single photon emission tomography (SPECT) were performed to the patients at baseline and 6 months after stem cell infusion. At 6-month follow-up of the eight patients who were able to complete the study, we revealed a significant increase in ejection fraction (from 30.0 +/- 6.6% to 36.2 +/- 7.3%; p = 0.001) in echocardiographic evaluation. SPECT evaluation also displayed a reduction in infarct area (50.4 +/- 16.1% to 44.1 +/- 12.5%; p = 0.003). Both myocardial oxygen consumption (p = 0.001) and metabolic equivalents (p = 0.001) were significantly increased at 6-month follow-up. These results demonstrate that intracoronary stem cell transplantation ameliorates heart failure symptoms and improves left ventricular function and perfusion. Therefore intracoronary stem cell transplantation may be used as an alternative treatment option for heart transplant candidates.

Yokota, T., H. Ichikawa, et al. (2008). "In situ tissue regeneration using a novel tissue-engineered, small-caliber vascular graft without cell seeding." J Thorac Cardiovasc Surg **136**(4): 900-7.

OBJECTIVE: Various types of natural and synthetic scaffolds with arterial tissue cells or differentiated stem cells have recently attracted interest as potential small-caliber vascular grafts. It was thought that the synthetic graft with the potential to promote autologous tissue regeneration without any seeding would be more practical than a seeded graft.

In this study, we investigated in situ tissue regeneration in small-diameter arteries using a novel tissue-engineered biodegradable vascular graft that did not require ex vivo cell seeding. METHODS: Small-caliber vascular grafts (4 mm in diameter) were fabricated by compounding a collagen microsponge with a biodegradable woven polymer tube that was constructed in a plain weave pattern with a double layer of polyglycolic acid (core) and poly-L-lactic acid (sheath) fibers. We implanted these tissue-engineered vascular grafts bilaterally into the carotid arteries of mongrel dogs (body weight, 20-25 kg). No anticoagulation regimen was used after implantation. We sacrificed the dogs 2, 4, 6, and 12 months (n = 4 in each group) after implantation and evaluated the explants histologically and biochemically. RESULTS: All of the tissue-engineered vascular grafts were patent with no signs of thrombosis or aneurysm at any time. Histologic and biochemical examinations showed excellent in situ tissue regeneration with an endothelial cell monolayer, smooth muscle cells, and a reconstructed vessel wall with elastin and collagen fibers. CONCLUSION: Our study indicated that this novel tissue-engineered vascular graft promoted in situ tissue regeneration and did not require ex vivo cell seeding, thereby conferring better patency on small-caliber vascular prostheses.

Zhang, L., J. Zhou, et al. (2008). "A novel small-diameter vascular graft: in vivo behavior of biodegradable three-layered tubular scaffolds." Biotechnol Bioeng **99**(4): 1007-15.

Small-diameter vascular grafts are potential substitutes for damaged vessels in patients, but most biodegradable grafts available now are not strong enough. The present study examined the burst strength, radial compliance, suture retention strength for a novel biodegradable tubular scaffold and investigated its behavior in vivo. The tubular scaffold (6-mm i.d., 4 cm long) has three layers including porous polylacticglycolic- acid in both inner and outer layers, a compact polyurethanes layer in midst. Bone marrow stromal cells (bMSCs) were seeded on the scaffolds and cultured for 7 days in vitro to construct tissue engineered vascular grafts which were then implanted in canine abdominal aorta. After 1, 3, 6, 12 and 24 weeks, the grafts were retrieved and evaluated histologically, angiographically and immunohistochemically. The biodegradable tubular scaffolds showed wall thickness of 0.295 mm to 0.432 mm; radial compliance of 3.80%/100 mmHg approximately 0.57%/100 mmHg, burst strength of 160 kPa approximately 183 kPa, and suture retention strength of 1959 N/cm(2) approximately 3228N/cm(2). The implanted grafts were fully patent without any signs of dilation or obstruction after 3 months' implantation. Scanning electron microscopy revealed a confluence endothelial cell layer spreading on the inner surface of the grafts. Immunohistochemistry of the retrieved grafts showed that vWF-stainin, alphaSMA-staining were positive in the inner and medium layer respectively. Masson's trichrome staining showed that amount of collagen fibers existed in the grafts wall. Overall, these novel three-layered scaffolds exhibited favourable mechanical strength, long term patency and good remodeling in vivo.

Zhu, C., D. Ying, et al. (2008). "Development of anti-atherosclerotic tissue-engineered blood vessel by A20-regulated endothelial progenitor cells seeding decellularized vascular matrix." Biomaterials **29**(17): 2628-36.

To investigate whether decellularized vascular tissues and A20-regulated endothelial progenitor cells can be used for constructing a transgenic tissue-engineered blood vessel with anti-atherosclerotic vascular stenotic properties. A20 gene-transfected endothelial progenitor cells differentiated endothelial cells and smooth muscle cells attached to and migrated into the decellularized porcine vascular scaffolding in a bioreactor. The histology of the conduits revealed viable and layered tissue. Scanning electron microscopy showed confluent, homogeneous tissue surfaces. The mechanical strength of the pulsed constructs was similar to that of the human artery. In vivo, the A20 gene-transfected tissue-engineered blood vessels were transplanted into the carotid artery of a rat for 6 months. Blood vessel xenotransplantation caused hyperacute rejection; all transplanted control blood vessels were completely rejected, but A20-transfected tissue-engineered blood vessels demonstrated good flow on implantation, and remained open for 6 months postoperatively, as assessed by Doppler. The HE stain demonstrated that the vessels were patent, without evidence of stenosis or dilatation after 6 months. These results demonstrate that transgenic tissue-engineered blood vessels have long-term patency and unique anti-stenotic properties.

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7/8/2013