Therapeutic potential of stem cells in auditory hair cell repair

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Published online on 04 Nov 2010

The prevalence of acquired hearing loss is very high. About 10% of the total population and more than one third of the population over 65 years suffer from debilitating hearing loss. The most common type of hearing loss in adults is idiopathic sudden sensorineural hearing loss (ISSHL). In the majority of cases, ISSHL is permanent and typically associated with loss of sensory hair cells in the organ of Corti. Following the loss of sensory hair cells, the auditory neurons undergo secondary degeneration. Sensory hair cells and auditory neurons do not regenerate throughout life, and loss of these cells is irreversible and cumulative.

However, recent advances in stem cell biology have gained hope that stem cell therapy comes closer to regenerating sensory hair cells in humans. A major advance in the prospects for the use of stem cells to restore normal hearing comes with the recent discovery that hair cells can be generated ex vivo from embryonic stem (ES) cells, adult inner ear stem cells and neural stem cells. Furthermore, there is increasing evidence that stem cells can promote damaged cell repair in part by secreting diffusible molecules such as growth factors. These results suggest that stem-cell-based treatment regimens can be applicable to the damaged inner ear as future clinical applications.

Previously we have established an animal model of cochlear ischemia in gerbils and showed progressive hair cell loss up to 4 days after ischemia. Auditory brain stem response (ABR) recordings have demonstrated that this gerbil model displays severe deafness just after cochlear ischemia and gradually recovers thereafter. These pathological findings and clinical manifestations are reminiscent of ISSHL in humans. In this study, we have shown the effectiveness of stem cell therapy by using this animal model of ISSHL.
Stem cells are undifferentiated cells that through replications have the capabilities of both self-renewal and differentiation into mature specialized cells. Broadly, there are two types of stem cells, embryonic stem cells and adult stem cells. Embryonic stem cell biology has been associated with ethical controversy and also their growth is difficult to control. Adult stem cells are located in tissues throughout the body and function as a reservoir to replace damaged or aging cells. Embryonic stem cells are by definitions, the master cells capable of differentiating into every type of cells either in-vitro or in-vivo. Several lines of evidence suggests, however, that adult stem cells and even terminally differentiated somatic cells under appropriate micro-environmental cues are able to be reprogrammed and contribute to a much wider spectrum of differentiated progeny than previously anticipated. Hematopoietic Stem Cells (HSCs), for example, from different sources have been shown to cross the tissue boundaries and give rise to the cells of the other germ layers.

In the past few years, the plasticity of adult cells in several post-natal tissues has attracted special attention in regenerative medicine. Stem cell therapies represent a new field of biomedical science which could provide in the future the cure for diseases until now considered incurable. The reconstitution of adult stem cells may be promising source for the regeneration of damaged tissues and for the resolution of organ dysfunction. However, there are two major limitations to the use of such cells:- (i) They are rare and (ii) Only a few types exist that can be isolated without harming the patient.

Due to the inability to efficiently and safely harvest or expand stem cells from most adult organs (e.g. liver, gastrointestinal tract, heart, brain), the majority of human stem cell trials have focused on clinical applications for HSCs, mesenchymal stem cells (MSCs), or both, which can be easily obtained in clinically sufficient numbers from peripheral blood, bone marrow, umbilical cord blood or placenta. HSCs can give rise to muscle, liver cells, astrocytes, etc, especially when co-cultured with the particular tissue progenitor cells and in presence of MSCs.

**Indications for HSC transplantation:**

1. **Malignant conditions:**
   A. **Allogeneic Stem Cell Transplantation (SCT):**
      1. Hematological Malignancies: CML, AML, ALL, NHL, HD, MDS.
2. Solid Tumors: Renal Cell Carcinoma, etc

B. Autologous Stem Cell Transplantation:

1. Hematological malignancies: AML, NHL, HD, MM
2. Solid Tumors: Neuroblastoma, EWS, RMS, etc

2. Non-Malignant conditions:

1. Hematological: Aplastic Anaemia, Fanconi Anaemia, Thalassemia, Sickle Cell Diseases, Myelofibrosis
2. Non Hematological: Osteopetrosis, Storage diseases (e.g. Gauchers)
3. Immune Disorders: SCID, HLH, etc.

3. Non-Malignant conditions:

1. Autoimmune Disorders: systemic sclerosis, multiple sclerosis, systemic lupus erythematosus, juvenile idiopathic arthritis.
2. Heart Disease: HSCs expanded ex-vivo (especially after B-catenin treatment) have been found useful in the treatment of ischemic myocardial injury.
3. CNS: HSCs have been used in the treatment of ischemic stroke, spinal cord injury and neurological disease like Parkinson’s disease.
4. Diabetes Mellitus:-
5. Gastro-intestinal & liver diseases:-
   1. Cirrhosis end stage liver disease
   2. Acute Liver failure
3. Metabolic liver diseases
4. Severe Inflammatory bowel disease, Crohn’s disease.
5. Patients with refractory celiac disease
6. Kidney disease: following acute renal injury - tubular injury or glomerular injury.
7. Skin repair & regeneration

4. Generation of normal tissues: Potential therapeutic use of ex-vivo produced blood substitutes such as:

   1. RBCs
   2. Platelets

The outcome of HSCTs can be improved by various manipulations.

A. Before HSCT:

Expansion of HSCs using:
1. Culture with various cocktails: SCF + TPO + Flt-3l; IL-3 (promotes higher CD133 cell expansion) + IL-6 (maintains immature phenotype); G-CSF / GM-CSF + IL-3 + SCF, etc
2. cMPL agonist NR-101
3. Fetal liver stromal cells
4. MSCs: provide suitable cellular environment for in-vitro expansion of HSC & HPCs from umbilical cord blood.
5. Valproic acid; T-hoxb4-H, etc

Expansion should favor cell proliferation over cell differentiation.

B. During HSCT:

Enhance engraftment by using:
1. Mesenchymal stem cell infusions
2. Valproic acid
3. Double / Triple cord blood transplants

C. After HSCT:
Infusion of:
1. Dendritic Cells
2. T-Regs: for modulation autoimmune disease or for transplant tolerization; derived from CB-HPCs
3. NK-Cells

Uses of MSCs when used along with HSCs:

MSCs have low inherent immunogenicity. Sources: Bone marrow; Wharton’s Jelly umbilical cord.

1. Enhancement of hematopoietic engraftment provide the supportive micro-environmental niche for HSC
2. Prevention / suppression of GVHD: MSCs exert an immediate anti-inflammatory & immunomodulatory role; cause induction of transplant tolerance
3. MSCs home to damaged tissue to participate in regenerative process; rebuild diseased tissues along with HSCs
4. Foster engraftment of haploidentical HSCs
5. Cell therapy & tissue engineering

Though HSCs have been used in all the above indications under laboratory condition, in animal studies, or even in humans, there is lot that still needs to be done before HCTs become standard of care.

Status of HSCT in India:

A pubmed search for HSCT & India yielded only 87 publications. Though the first Allogenic BMT was done at TMH long back in 1983, in the last 25 years hardly 2000 transplants have been performed in a country of over a billion population. This is compared to nearly 4000 transplants done each year world over. As of now, there are approximately 20 units doing SCT in India, the main among them being CMC, Vellore; TMH, Mumbai & AIIMS, Delhi. Apart from these centers, two other centers which have consistently published are, Stem Cell Biology Laboratory of National Institute of Immunology, Delhi & Dr. H.L. Trivedi Institute of Transplantation Sciences, Ahmedabad. However, most of published work in this field from India is in the preliminary stages and it may take some time before the translational research reaches to the bedside.
Proceedings of the Annual Symposium on Regenerative Medicine (PASRM)

In vitro production of RBCs from ES, iPS & Stem cell banking in Japan
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Published online on 04 Nov 2010

The supply of transfusable red blood cells (RBCs) is not sufficient in many countries. If erythroid cell lines able to produce transfusable RBCs in vitro were established, they would be valuable resources. However, such cell lines have not been established.

We developed a robust method to obtain differentiated cell lines following the induction of hematopoietic differentiation of mouse embryonic stem (ES) cells and established five independent hematopoietic cell lines using the method. Three of these lines exhibited characteristics of erythroid cells. Although their precise characteristics varied, each of these lines could differentiate in vitro into more mature erythroid cells, including enucleated RBCs. Following transplantation of these erythroid cells into mice suffering from acute anemia, the cells proliferated transiently, subsequently differentiated into functional RBCs, and significantly ameliorated the acute anemia.

Considering the number of human ES and induced pluripotent stem (iPS) cell lines that have been established so far, the intensive testing of a number of these lines for erythroid potential may allow the establishment of human erythroid cell lines similar to the mouse erythroid cell lines. In addition, our results strongly suggest the possibility of establishing useful cell lines committed to specific lineages other than hematopoietic progenitors from human ES and iPS cells.

The Cell Engineering Division of RIKEN BioResource Center is a not-for-profit public “Cell Bank” that accepts donation and deposit of human and animal cell materials developed by life science research community. We examine, standardize, amplify, preserve, and provide cell materials to the scientists around the world.

The stem cells such as ES cells and iPS cells are valuable in current biology and medical sciences. Thus, we are collecting such stem cell lines and aiming to contribute to the fields of developmental biology, transplantation medicine, regenerative medicine, and so on.
Ever since man invented fire he has been more frequently burning himself by this creation than by the naturally occurring bushfires. It is estimated that over 1.152 million people in India suffer from burn injuries requiring treatment every year and majority of them are women aged between 16-40 years and most of them occur in the kitchen.

The treatment for burns basically involves autologous skin grafting, which originated in India more than two thousand years ago (Sushruta Samhita), is still the gold standard for the wound resurfacing, although, autografting is difficult where graftable donor sites are limited. Although, Cadaver skin, porcine or bovine xenografts are used alternatively over the past thirty years, modern approaches like the Bioengineering of skin substitutes emerged during the past 20 years as advanced wound management technologies with no social impediment. They can be broadly categorized as Acellular and Cellular biotechnological products. The acellular products like Alloderm (LifeCell Corporation), Integra (Integra Life Sciences) act like template and depend on natural regeneration, while the cellular ones are either ‘Off-the-Shelf’ products like Apligraf (Organogenesis Inc) and Orcel (Ortec International) have allogenic elements and ‘home grown’ autologous cell products like Cultured Epithelial Autograft (CEA) and epidermal-dermal composite skin use synthetic or natural non-human matrices. The CEA is based on the ex-vivo epidermal stem cell-expansion and our laboratory has been engaged in CEA technique development with innovative cost-effective approach and yielded promising preliminary clinical success.

The basic methodological approach in CEA technique which is still clinically adopted by several developed countries involves the use of growth arrested mouse dermal fibroblasts as growth supportive matrix and is thus considered a drawback as a whole. Additionally, there is no superior enough method available to augment the growth of human keratinocyte stem cells capable of producing epithelia for large-scale grafting in burns and maintain long-term functionality as a self-renewing tissue. The normal functioning of such an in vitro constructed graft under long-term artificial growth conditions is limited by the difficulties of maintaining the epidermal stem cell compartment. An apparent answer to this problem of stem cell depletion during autograft preparation would be to start with a pure population of progenitor stem cells and derive sustainable autograft from them. We have been aiming to this solution and currently attempting to isolate a pool of epidermal progenitor cells using Mebiol gel, which is a Thermo-Reversible Gelation polymer and was shown by others to support the growth of multi-potent skin-derived epithelial progenitor-1 cells. Additionally, the usefulness of Mebiol gel in maintaining epidermal stem cell compartment without FBS and/or animal origin feeder cells is being investigated by our group.
Intralesional Application of Autologous Bone Marrow Stem Cells with Scaffold in Canine for Spinal Cord Injury

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Published online on 04 Nov 2010

A three year old male non-descriptive companion dog was presented to the Small Animal Orthopedic Unit of Madras Veterinary College Teaching Hospital (MVC) with paraplegia of fourth degree neurological deficit of hind limbs due to automobile trauma. Radiographic views were suggestive of dislocation at T8-T9 vertebral segment with fracture of L2 vertebra. Myelography confirmed the signs of abrupt stoppage of the contrast column cranial to dislocated area and was interpretive of transected spinal cord at L2 level. Construct was prepared with bone marrow mononuclear cells (BMMNC) isolated from bone marrow aspirate of femur and the cells were seeded in Thermoreversible Gelatin Polymer (TGP) at the cell processing facility of Nichi-In Centre for Regenerative Medicine (NCRM) as per GMP protocols and was engrafted after hemilaminectomy and durotomy procedures in the MVC. Postoperatively the animal was clinically stable; however the animal died on the 7th day. Autopsy revealed co-morbid conditions like cystitis, nephritis and transmissible venereal tumor. Histopathology of the engrafted area revealed sustainability of aggregated stem cells that were transplanted revealing an ideal biocompatibility of the construct prepared with bone marrow mononuclear cells and polymer hydrogel for spinal cord regeneration in dogs. Further studies in similar cases will have to be undertaken to prove the long term efficacy.
Ex vivo expansion of Primate CD34+ Cells isolated from Bone Marrow and Human Bone Marrow Mononuclear Cells using a Novel Scaffold.

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Background: Bone marrow derived CD34+ cells have been in clinical application in patients with haematological malignancies. One of the major problems with this treatment is the non-availability of matched donors or the necessity of multiple transfusions depending upon the pathology. Recently evidences have been accumulating to prove the safety and efficacy of autologous CD34+ cells in diseases such as myocardial dysfunction, peripheral vascular diseases and neurological certain conditions. However there are only a few reports in the literature on ex vivo expansion of the bone marrow derived CD34+ cells. We have in two different studies proven that isolated CD34+ cells from baboon bone marrow and non-isolated BMMNCs from human bone marrow could be expanded with increase in percentage of CD34+ cells using a novel scaffold.

Methods:

Study 1: Bone marrow was derived from healthy baboons posterior iliac crest and mononuclear cells were isolated using density gradient method. Then the BMMNCs were subjected to magnetic bead (Miltenyi Biotech) separation of CD34+ cells, which were expanded ex vivo for one week.

Study 2: A portion of the bone marrow aspirated for clinical application from human patients was used after informed consent. The BMMNCs were subjected to ex vivo expansion without any further separation. Initial and post-expansion CD34+ percentage and quantity were evaluated at different intervals starting from 7 to 21 days. Both the studies used the same Thermo-Gelation Reversible Polymer Scaffold impregnated with same culture cocktails prepared in NCRM.

Results: Study 1 revealed increase in the quantity of CD34+ cells which formed colonies in the culture from the 5th day onwards. The study 2 revealed a significant increase in the total quantity of CD34+ cells percentage increased from 0.91% to 2.26% on day 7 (n=4), 1.53% to 2.93% on day 14 (n=1) and 1.32% to 11.71% (n=3) on day 21 on an average quantified using flow cytometry.

Conclusion: This study has shown that ex vivo expansion of CD34+ cells with and without isolation is feasible. This technology may become a good tool to hematologists who may in need of a precious matched unit to be expanded multifold or may cryopreserve the donors’ bone marrow for subsequent application to same or similar patients. Patients and health adults who haven’t had an opportunity to store their cord blood also now have an option to store their own bone marrow cells in several small aliquots which could be thus expanded for future application when needed.
Proceedings of the Annual Symposium on Regenerative Medicine (PASRM)

Our Experience with Autologous Bone Marrow Stem Cell Application in Dilated Cardiomyopathy.

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Published online on 04 Nov 2010

Background - Use of autologous bone marrow stem cell is a newly evolving treatment modality for end stage cardiac failure as reported in the literature. We report our experience with two patients with dilated cardiomyopathy who underwent this treatment after failure of maximal conventional therapy.

Methods - A 29 year old Male patient with history of orthopnea and PND, with a diagnosis of dilated cardiomyopathy and echocardiographic evidence of severe LV dysfunction was referred for further treatment. His echo on admission showed EF of 17% and no other abnormal findings except elevated bilirubin levels. He was in NYHA functional class IV. He received intracoronary injection of autologous bone marrow stem cells in January 2009. 254X10^6 cells were injected with a CD34+ of 0.20%. His clinical condition stabilized and he was discharged home. He received a second injection of 22X10^6 in vitro expanded stem cells with a CD34+ of 0.72% in Aug 2009. He is now in NYHA class II-III with EF 24%.

A 31 year old Male patient with history of increasing shortness of breath, severe over the past 3-4 days was admitted for evaluation and treatment. His echo on admission showed EF of 20% and was in NYHA functional class IV. Coronary angiogram was normal and he was stabilized on maximal anti failure measures. He received intracoronary autologous bone marrow stem cell injection of 56X10^6 with a CD34+ of 0.53% in August 2009. His clinical condition stabilized over the next 10 days and he was discharged home.

Conclusions - In our experience of two cases of dilated cardiomyopathy, safety of intracoronary injection of autologous bone marrow stem cells both isolated and in vitro expanded has been proven in both the cases with efficacy proven in one of the cases. Long term follow-up of these two cases and inclusion of more number of similar cases where all available conventional therapies have not resulted in significant improvement for such studies are planned.
Two sittings of Autologous Bone Marrow Stem Cells within two years in a case of Ischemic Cardiomyopathy.

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Published online on 04 Nov 2010

**Background:** A 66yrs old Diabetic and Hypertensive female, who had Anterior Wall MI 5yrs ago and had undergone PTCA with Stent to LAD, was admitted for refractory CHF with Severe LVD 2yrs ago and the LVEF then was 25%. Coronary Angiogram was done which showed Total Occlusion of LAD and 50% Stenosis of RCA.

**Method:** 100ml of her bone marrow was harvested from posterior iliac crest and the BMMNCs were isolated as per cGMP protocols at NCRM, Chennai and 325X106 cells with a CD34+ count of 0.84% were injected the next day by transfemoral catheter into the coronary arteries. Post treatment she had clinical improvement. EF increased by 5%. She was in Class-II for 1 year. After 1 yr, she was admitted with severe CHF and EF had deteriorated to 20%.

This time BMMNCs isolated from the bone marrow were subjected to in vitro expansion by which the initial 0.15% CD34+ cells increased by nearly 30 fold to 4.62%. Totally 315X106 cells were injected into the coronaries. Post treatment there is clinical as well as Echo evidence of improvement and BNP level has come down by 30%.

**Conclusion:** Isolated and expanded CD34+ cells from bone marrow mononuclear cells of autologous origin, administered into the coronaries in an Ischemic Cardiomyopathy patient has been proven to be safe. The clinical and Echo cardiographic improvement that has sustained for long-term, proves the feasibility and efficacy of two consecutive autologous bone marrow stem cell applications, one isolated and the second ex vivo expanded. More case studies may be undertaken to further evaluate the results.
The ocular surface is exceptionally rich in complexity and functionality. Severe ocular surface disorders/conditions, such as chemical or thermal injuries, Stevens-Johnson syndrome (SJS), ocular cicatrical pemphigoid, neurotrophic keratopathy, chronic limbitis, and severe microbial keratitis cause significant morbidities and even corneal blindness. Hypofunction may be caused by Aniridia, Neurotroph, Endocrine, Pterygium and Chronic limbitis. Approximately 6000 patients are seen in Ocular Surface Clinic every year; almost 80% have some form of dry eyes. About 125 new patients of Stevens Johnson Syndrome are seen in a year of which approximately 25% may benefit from Cultured Epithelial Transplant and 75 new patients of thermal/chemical injury in a year of which almost 80% will benefit from Stem Cell Transplantation. Of the 128 severe vernal keratoconjunctivitis which were seen in the ocular surface clinic, 10% require stem cell transplantation. Nearly 30 new cases of Ocular cicatrical pemphigoid every year are seen and they may need stem cell transplantation. In addition, several patients with persistent epithelial defects may benefit from limbal stem cell transplantation to alleviate, maintain conjunctivalization regression and corneal avascularity limbal deficiency, and restore vision. Even if granted that this statistics is for a single large ophthalmic hospital, for a large country as India with 1.1 billion populations, the number of patients requiring corneal stem cell transplantation is enormous. Stem cells in the palisades of Vogt participate in regeneration and preservation of corneal transparency and avascularity. The diminished regenerative capacity seen in LSCD is characterized by persistent epithelial defects, erosion and ulceration, conjunctivalization and neovascularization, and chronic inflammation. Standard corneal transplantation for restoration of corneal clarity and avascularity is a contraindication in the surgical management protocol of LSCD patients. Autologous limbal transplantation Despite its success, its utility is limited. the requirement for a sizable limbal donation; up to 30-40% must be harvested from the contralateral donor eye and its harvest may theoretically harm the structural integrity, cause subclinical LSCD or cryptogenic changes in the donor eye. Ex vivo expansion and cultivation techniques for autologous limbal stem cells are being actively investigated. , the use of human AM for ocular surface regeneration (OSR) and as a growth support substrate for ex vivo expansion of autologous corneal equivalent epithelial cells and their successful OSR in animal cornea model, as well as human, was reported. The conventional cultivation methods for corneal epithelial tissues for clinical transplantation applications involve utilization of xenobiotic materials such as fetal bovine serum (FBS) and murine-derived feeder cells. FBS-free culture systems have been developed to reduce the risk of zoonotic infection, but these have the disadvantage of reduced efficacy for cell propagation. it must be emphasized that AMT is not a substitution for LSCT and AMT
should not be performed when true LSCD exists because AM only provides a supportive matrix for the limbal stem cells to migrate, proliferate and restore the corneal surface. There are several disadvantages of AMT and LSCT technique. This delicate procedure requires technical skill for the preparation of AM with attached corneal epithelial cells and surgical dexterity to manipulate the AM onto the ocular surface.

A rabbit model for transplantation of cultivated corneal limbal stem cells onto corneal stem cell deficient animals was developed & its results are very encouraging for similar studies in human corneal surface reconstruction. Our investigations indicated that Ex vivo cultivation of human corneal limbal stem cells (HCLSC) occurred with ease in the thermoresponsive biodegradable gel – “Mebiol Gel”. The growth rate within Mebiol Gel varied from 8 - 10 days to develop a monolayer formation with all the HCLT from different eye donors five - six times more than HCLSC multiplying on human amniotic membrane & the cultivated cells were identifiable as Corneal stem cells, Transient amplifying cells, Pluripotent cells and Mature corneal epithelial cells. Cells cultivated within Mebiol Gel can be used for Therapeutic purposes with a distinct possibility of avoiding a human tissue in the form of human amniotic membrane. The rabbit model study can be replicated in human corneal surface reconstruction with corneal limbal stem cells cultivated in Mebiol Gel to CLCD patients.