Bioengineering of cultured epidermis from adult epidermal stem cells using Mebio gel suitable as autologous graft material

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Closure of burn wound is the primary requirement in order to reduce morbidity and mortality that are otherwise very high due to non-availability of permanent wound covering materials. Sheets of cultured epidermis grown from autologous epidermal keratinocyte stem cells are accepted world over as one of the best wound covering materials. In a largely populated country like ours where burn casualties occur more frequently due to inadequate safety practices, there is a need for indigenous research inputs to develop such methodologies. The technique to culturing epidermal sheets in vitro involves the basic Reheinwald-Green method with our own beneficial inputs. The technique employs attenuated 3T3 cells as feeders for propagating keratinocyte stem cells that are isolated from the epidermis of an initial skin biopsy of about 5 cm2 from the patient. The cultures are then maintained in Dulbecco's modified Eagle's medium strengthened with Ham's F12 formula, bovine fetal serum and various specific growth-promoting agents and factors in culture flasks under standard culture conditions. The primary cultures thus established would be serially passaged to achieve the required expansion. Our major inputs are into the establishment of (1) an efficient differential trypsinization protocol to isolate large number epidermal keratinocytes from the skin biopsy, (2) a highly specific, unique and foolproof attenuation protocol for 3T3 cells and (3) a specialized and significant decontamination protocol. The fully formed epidermal sheet as verified by immuno-histochemical and light & electron microscopic studies, is lifted on to paraffin gauze by incubating in a neutral protease. The graft is then ready to be transported to the operating theatre for autologous application. We have a capability of growing cultured epidermal sheets sufficient enough to cover 40 per cent burn wound in 28 days. The preliminary small area clinical applications undertaken so far revealed quicker healing proving the importance and usefulness of the method.

With this new approach a large number of moderate to severely burned patients could be saved in several burn centers across our country with reduced hospitalization period.
However, the cell based therapeutic option in burn-wound healing by the application of in vitro - cultivated sheets of epidermis from autologous epidermal keratinocyte stem cells uses no matrix. This technique is sufficient for burn wounds of 2nd & 3rd mixed degree. The burn wounds predominantly of 3rd?and 4th?mixed degree can not be healed by the thin cultured epidermis, thus requiring a cellular or cellular scaffold that more or less mimic for graft take in deeper burns.

With this aim, we are presently attempting to create such a scaffold using Mebiol gel, which could support the cultured epidermis for better transfer to the wound bed. Additionally, the usefulness of Mebiol gel in growing epidermal sheets without the necessity of FBS and/or animal origin feeder cells but using human feeder cells will also be tested.
Novel approach in the management of an oral premalignant condition - A case report

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Oral submucous fibrosis is a progressive oral disease first described by Pindborg and Sirsat 3 decades ago. It is a premalignant condition. The signs and symptoms depend on the involvement of the different sites in the oral cavity. The patient feels burning sensation for normal diet and trismus which may be so severe. If not properly treated the risk of malignant change in advanced cases of OSMF is relatively high. Wide ranges of treatment such as medical management, surgical therapy and physiotherapy have been attempted in the past, but none of them has proved to be a cure for this chronic fibrotic disease.

Histopathologically as the disease progresses, (i) change in the morphology of collagen, (ii) increased accumulation of amorphous collagen, and (iii) decreased collagen degradation results in decrease in number of blood vessels are observed in the affected area compared to the normal area. With an aim of bringing more blood supply to the affected area which is expected to bring more nutrients and help in collagen degradation, earlier application of vasodilators and studies with curcumin have been done, but still with no significant outcome.

As an alternative approach to improve the blood circulation, we have tried Autologous bone marrow stem cells which have been earlier applied in several diseases such as ischemic peripheral vascular diseases, ischemic heart diseases etc with proven improvement in angiogenesis.

A 38 year old patient with oral submucosal fibrosis, proven by histopathology, and endothelial markers was injected 175 million BMMNCs into the area affected. The paramaters such as blanching, fibrous band have significantly improved, 4 weeks after the injection. We could observe positive changes clinically to prove the improvement. The mouth opening has improved to 35 mm from the previous 30.0 mm. Further histopathology and SEM studies with larger samples are done for establishing stem cell therapy’s safety and efficacy.
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Tissue Engineering Based Therapy for Articular Cartilage Defects - A New Approach

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Background:
Articular cartilage, the load-bearing tissue in diarthrodial joints, when damaged due to trauma could lead to osteoarthritis. At present, Autologous Cartilage Implantation is an established method in which patients own chondrocytes are isolated and then implanted after in vitro expansion over the affected area with bovine or porcine collagen matrix. This procedure results in more of Collagen Type I during in vitro expansion, which eventually becomes fibrocartilage. Also it requires growth factors. We have in this study tried growing human Chondrocytes without growth factors using synthetic scaffolds to grow more Collagen Type II

Materials and Methods:
Human cartilage specimens were harvested through arthroscopy from the non-weight bearing area of the knee joint from 13 patients who underwent surgical procedures of the knee joint after getting their informed consent. The tissues were transported in saline taking 1 hour to laboratory and subjected to digestion with Collagenase type II for 16~18 Hrs. The chondrocyte cells obtained after dissociation were divided into two groups for culture. Gr. I were embedded in a Thermogelation polymer (TGP) and Gr. II in basal culture media (DMEM + Ascorbic Acid) without using any growth factors. The Group II cells were viable only for 4 weeks and then started degenerating. The TGP-Chondrocytes scaffolds were grown for 16 weeks and the specimens were harvested at 4, 8, 12 and 16-week intervals and their morphology and molecular characteristics were studied by H&E staining, S-100 protein analysis and RT-PCR.

Results:
Human chondrocytes could be cultured in both TGP (group I) and Basal culture media (group II). The Gr. I cells were viable upto the 16th week while the Group II chondrocytes started degenerating after the 4 week. Both the groups were proven positive for S-100 protein, a Chondrocyte specific marker protein; Gr. II specimens after 4 weeks, and Gr. I specimens after 4, 8, 12 and 16 weeks. RT-PCR study of
the cells of group I were positive for TGF beta 3 (Proliferation, differentiation, and other functions), GR beta, GR alpha (Development, metabolism and immune Response) (glucocorticoid receptor alpha), AGGF (Apoptosis), VDR (Vitamin D3 Receptor), Col II (Type II Collagen).

**Conclusion:**

We have established a methodology by which Human chondrocytes could be cultured in vitro without any growth factors for a period of 16 weeks in a polymer-hydrogel scaffold. Upon further confirmation of their characteristics, the TGP grown chondrocytes can be used for autologous implantation to repair damaged cartilage area as the Collagen Type II which grows better without growth factors in the scaffold, eventually will become Hyaline cartilage is expected to give a longer disease free duration than the present method of ACI.
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Autologous Stem Cell Injection for Spinal Cord Injury - A Clinical Study from India

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Abstract:

We studied 100 patients with Spinal Cord injury (SCI) after Autologous Stem cell Injection in the Spinal fluid with a Follow up of 6 months post Stem cell injection. There were 69 males and 31 females; age ranging from 8 years to 55 years. Time after Spinal Injury ranged from 11 years - 3 months (Average: 4.5 years). The Level of Injury ranged from Upper Thoracic (T1-T7) - 34 pts, Lower thoracic (T7-T12) - 45 pts, Lumbar -12, Cervical-9 pts. All patients had an MRI Scan, urodynamic study and SSEP (somatosensory Evoked Potential) tests before and 3 months after Stem cell Injection.

80% of patients had Grade 0 power in the Lower limbs and rest had grade 1-2 power before stem cell injections. 70% of cases had complete lack of Bladder control and 95% had reduced detrusor function.

We Extracted CD34 and CD 133 marked Stem cells from 100 ml of Bone marrow Aspirate using Ficoll Gradient method with Cell counting done using flowcytometry.15 ml of the Stem cell concentrate was injected into the Lumbar spinal fluid in aseptic conditions. The CD 34/CD45 counts ranged from 120-400 million cells in the total volume.

6 months after Injection, 8 patients had more than 2 grades of Motor power improvement, 3 are able to walk with support. 1 patient with T12/L1 injury was able to walk without support. 12 had sensory tactile and Pain perception improvement and 8 had objective improvement in bladder control and Bladder Muscle contractility. A total of 18 patients had reported or observed improvement in Neurological status. 85% of patients who had motor Improvement had Lesions below T8. MRI, SSEP and Urodynamic Study data are gathered at regular intervals.

Conclusion:

This study shows that Quantitative and qualitative Improvement in the Neurological status of paralyzed patients after Spinal cord injury is possible after autologous bone marrow Stem cell Injections in select patients.
There was no report of Allodynia indicating the safety of the procedure. Further studies to (i) quantify the neurological and vascular damage and to standardize the dosage, (ii) Identify Mechanism of action of each group of Stem cells (HSCs, MSCs, Naive cells, Purified subsets etc) on Nerve Tissue both In-vivo and In-vitro will be necessary to Confirm Above results.
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Autologous Bone Marrow stem cell Infusion (AMBI) therapy for Chronic Liver Diseases

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Background:
Liver Cirrhosis is the end stage of chronic liver disease which may happen due to alcoholism, viral infections due to Hepatitis B, Hepatitis C viruses and is difficult to treat. Liver transplantation is the only available definitive treatment which is marred by lack of donors, post operative complications such as rejection and high cost. Autologous bone marrow stem cells have shown a lot of promise in earlier reported animal studies and clinical trials. We have in this study administered in 22 patients with chronic liver disease, autologous bone marrow stem cell whose results are presented herewith.

Materials and Methods:

In 22 patients with chronic liver diseases of Child B-C category, Autologous Stem Cell has been transfused, upon getting approval from the ethics committee and informed consent. Under short GA, 200-300ml of bone marrow was tapped. The bone marrow stem cells were isolated using density gradient fractionation method and processed, suspended as per protocols earlier published (Terai et al., doi:10.1634/stemcells.2005-0542). The processing was done in cGMP facility under stringent aseptic precautions. Endotoxin test clearance was obtained (<0.25EU) and CD34+ analysis was performed using FACS. A cell count of 240 to 1068 X 10\textsuperscript{6} was administered intravenously through the median cubital vein. Liver function tests, ultrasound and CT were performed before the administration, thereafter at 4 and 8 weeks of infusion. The protocols used were the same as used by Terai et al., Yamaguchi University, Japan. Acites, albumin, bilirubin, radio lucency of liver and overall quality of life were studied in all these patients. Liver biopsies were not done due to lack of patient compliance. Standard work up of chronic liver disease by viral marker, copper, Alfa Feto Protein etc., were performed.

Results:

The administration of the transfusion did not have any adverse reactions in the patients. 67\% of patients showed an increase of serum
albumin that was significant, 73% showed significant reduction of ascites, 32% showed drop in bilirubin. The overall quality of life of index (QULI) was significantly improved in 82% of the patients.

Conclusion:

Autologous bone marrow stem cells administered in chronic liver disease patients has yielded significantly good results and has been safe. Although a pilot clinical trial, the study shows promises for newer exciting cell based therapies for chronic liver disease.
Use of Bone Marrow derived Stem Cells in Patients with Cardiovascular Disorders

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Patients with end stage heart failure have very few treatment options. The long waiting times for transplant and the complications associated with immunosuppression has led to the search for alternatives. Subsequent to the isolation and characterization of stem cells, tremendous advances have been made and the safety and feasibility of autologous bone marrow derived stem cells has been proven in preclinical studies. Clinical studies have also shown mobilized cells repair the infarcted heart, improving function and survival. We have started a clinical study to evaluate the efficacy of bone marrow derived stem cells. Bone marrow was aspirated from the right iliac crest and the stem cells were isolated by density gradient method and suspended according to the mode of delivery.

From Jan 2007 till date 10 patients (8 adults, 2 children, age) with end stage cardiovascular disorder of varied etiology (Ischemic left ventricular dysfunction - 6 patients, Primary pulmonary hypertension - 2 patients, Dilated cardiomyopathy -1 patient, Biventricular non-compaction -1 patient) underwent stem cell therapy. All patients were evaluated and cardiac function was measured by using echocardiography and thallium scintigraphy. There were no procedure related complications. These patients are being regularly followed-up and one patient who has completed 6-month follow-up has shown improvement in perfusion as well as increase in ejection fraction of 10%. Stem cell therapy in patients with end-stage cardiovascular disorder might be a promising tool by means of angiogenesis and other paracrine mechanisms.

Image:

Nuclear study images of one of the patients included in the study. The upper one before and lower one six months after the injection.
The Ejection fraction was 16% (Pre-treatment image on top) which increased to 22% after Autologous Bone Marrow Stem Cell Therapy (Post-treatment-bottom image) according to the author.
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Our Experience in treating Ischemic Ulcer of a Lower Limb in 4 diabetic patients with Autologous Bone Marrow Stem Cells

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Background:

Chronic limb ischemia is an outcome of peripheral arterial occlusive disease. When conventional medical and surgical treatments are not feasible, amputation is the only option left. Recent studies report that the injection of bone marrow mononuclear cells and Peripheral blood mononuclear cells rich in CD34+ cells have resulted in symptomatic recovery, improved functional activity of the ischemic limb as well as healing of the ulcers. Here we report our experience with 4 patients of such case where autologous bone marrow mononuclear cells were injected and the patient followed up for 6 months.

Materials and Methods: Four patients with critical limb ischemia with ulcers were referred for amputation of their limb. A 68-year-old female with critical limb ischemia with an ulcer in the left leg measuring 30X12 cm over the posterior portion of the leg and extending to the medial aspect of the foot measuring 14X10 cm, a 65-year-old male with necrotic wound in his lower foot, a 69-year-old male with a deep wound in his lower foot and a 61-year-old male with ulcer in his toe amputated with all the toe fingers. The first two patients were given injections for more than one sitting at appropriate intervals specified by the clinician. Under short general anesthesia, 110 ml of Bone marrow was aspirated each time, transported in Acid Citrate Dextrose and was processed for mononuclear cells (MNC) by Ficoll density gradient centrifugation, following the cGMP protocols. The MNC concentrate was injected at various sites in the Gastrocnemius muscle and the surrounding area after necessary debridement. Skin grafting was performed in the first two patients and followed up for a period of at regular intervals of 6 to 9 months. The patients have been followed up at regular intervals for six months after the treatment with investigations such as Ankle-Brachial Index, Doppler and Angiogram.

Results:

All the patients showed improvements with healthy granulation gradually started appearing in the areas which were previously
unhealthy and ischemic. Slow granulation was found in-patient 3 and but the patient 4 died because of other factor such as renal failure, peritoneal dialysis and cardiac failure. Patients 1 and 2 had healthy granulation, uniform revascularization and after a period of 9 months, healing was completely possible.

**Conclusion:**

Stem cell therapy is definitely useful where, revascularization is not feasible at the same time, renal failure, cardiac failure, etc do present some difficulties. All the parameters need to be taken care. Growth factors or plastic surgery need not be used for stem cell therapy thus considering only the appropriate time of injections. As Autologous Bone Marrow stem cell therapy helps in neoangiogenesis and wound healing process in case of chronic ischemic wounds it can be applied in cases as reported herewith.