ADMINISTRATION OF BONE MARROW Stromal CELLS FACILITATE AXONAL REGENERATION IN THE HEMISECTED ADULT RAT SPINAL CORD

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Background: Spinal Cord Injury causes a huge burden for patients, with a lack of effective surgical treatment & continuous cost of care. Bone Marrow Stromal Cells (BMSC) and artificial extracellular matrices have been shown to aid recovery in various models. We proposed the combined use of BMSCs with a Honeycomb Collagen (HC) matrix to aid axonal regeneration.

Materials & Methods: In vitro study of explanted rat Dorsal Root Ganglia onto BMSC infused HC scaffolds compared against the HC control were evaluated. Maximum neurite length after 10 days was calculated. The study continued to an in vivo study of spinal cord hemisection in rat models, with the injury site being surgically implanted with HC or BMSC+HC scaffolds. 4 weeks post injury the cords were evaluated for injury site volume compared to total cord volume. Basso Beattie Bresnahan score and sub score was used to analyse motor recovery.

Results: Explants showed a significant difference in neurite length, with BMSC+HC group producing 3x greater growth (p value 0.0004). The SCI injury model showed a tendency of BMSC+HC to have a smaller injury site volume. Motor recovery was significantly higher in the BMSC+HC group in both the BBB score and BBB subscore (p = 0.03 & p = 0.005 respectively).

Conclusion: We successfully showed that BMSCs have efficacy compared to HC controls in both in vitro and in vivo axonal regeneration, with a functional recovery being greater in the BMSC group. Therefore both structural and cellular support is needed for a surgical intervention to aid recovery.

THE ROLE OF FIBROBLAST GROWTH FACTOR SIGNALLING IN OSTEOSARCOMA

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Background: The mechanisms responsible for the development of osteosarcomas are unknown. Preliminary experiments using a mouse model of osteosarcoma (OS) suggest a role for fibroblast growth factor (FGF) signalling in oncogenic transformation of osteoblasts. We hypothesise a similar case in humans. We investigated the role of fibroblast growth factors and receptors (FGFRs) in the growth of human osteoblasts. Our aims: to compare FGFR expression between normal human osteoblasts (HOBs) and OS cells; compare effects of FGF on growth of HOBs and OS cells; and establish a 3-dimensional culture model representing an in vivo environment.

Methodology: Western blot analysis of FGFR expression was performed on HOBs and 3 human OS cell lines (MG-63, Saos2, U2OS). Using cell culture techniques, we investigated the effect of bFGF and SU5402 (FGFR inhibitor) on cell transformation and proliferation. In parallel to these in vitro experiments, we developed an in vivo model. Our system involves grafting tumours (explants from mouse model) onto chick chorioallantoic membrane (CAM).

Results: FGFR1 and FGFR4 were overexpressed in OS cell lines compared to HOBs. bFGF induced features of transformed cells in HOBS; this was reversed by SU5402. bFGF increased proliferation of OS cells in comparison to HOBS. All tumours grafted onto the CAM, especially those treated with bFGF, became vascularised.

Conclusion: This new evidence suggests a vital role for FGF signalling in the pathogenesis of OS. Furthermore, the first steps have been taken in developing an in vivo tumour culture system. This can be developed to study the effects of modulating exogenous factors on tumour growth.

ARTERIAL FUNCTION IN PATIENTS WITH DIABETES MELLITUS UNDERGOING CORONARY ARTERY BYPASS GRAFTING

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Objectives: Type-2 diabetes mellitus is a risk factor for morbidity/mortality following coronary artery bypass grafting (CABG), which may be attributable in part to arterial stiffening. We aimed to identify if patients with increased arterial stiffness pre-operatively had worse outcomes following CABG, whilst also investigating the potential molecular determinants of arterial stiffening within aortic biopsies.

Methods: Measurements of arterial stiffness, Pulse Wave Velocity, on 12 non-diabetics and 17 Type-2 diabetics were correlated with indirect predictors of poor outcome following CABG (peak post-operative troponin T, day 4 creatinine). Aortic biopsies were analysed by zymography for levels of the extracellular matrix enzyme MMP-2. Data are represented as means and percentages. Results were assessed using appropriate statistical tests and correlation coefficients were calculated. P<0.05 was considered significant.

Results: PWV was significantly increased in diabetics, 13.92 (11.78-16.46) m/s vs. non-diabetics 9.34 (8.70-10.02) m/s (p<0.001). In diabetics only, PWV was significantly and positively correlated with peak troponin T (r=0.65, pvalue=0.03) and day 4 creatinine (r=0.64, p value=0.02). Analyses of the aortic tissue indicated an increase in percentage MMP-2 activity (50% vs. 30%) in diabetics vs. non-diabetics (not statistically significant).

Conclusions: PWV may be a useful tool for predicting a group of diabetic patients that have worse outcomes following CABG. Interventions to reduce arterial stiffness in the future could be focused on this high-risk subpopulation. A therapeutic target may include modulation of MMP-2 activity, a possible pathophysiology of aortic stiffening.

THE EFFECT OF AORTIC GRAFTS ON TONOMETRIC MEASUREMENT OF ARTERIAL COMPLIANCE

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Objectives: The aorta buffers pulsatile cardiac output into peripheral non-pulsatile flow. Tonometric measurements of arterial compliance can predict future cardiovascular events, but the segment of aorta responsible for the pathophysiological effects of arterial stiffening is unknown. We investigated this in patients with differing anatomical aortic segment prosthesis replacements.

Methods: We measured tonometric carotid-femoral Pulse Wave Velocity (PWV) using a SphygmoCor device in 4 patient groups: CONTROL subjects with low cardiovascular risk; THORACIC patients with aortic root grafts; ABDOMINAL patients with operated infra-renal aortic aneurysms;
UNOPERATED patients with unoperated infra-renal aortic aneurysms. Adjustments between groups were made for age, sex, mean arterial pressure, creatinine and the presence of diabetes using linear regression analysis. PWV was represented as the mean [95% CI lower-upper].

**Results:** A total of 105 studies were performed, CONTROL n=27, THORACIC n=30, ABDOMINAL n=25, UNOPERATED n=23. The ABDOMINAL group had an adjusted PWV of 11.9m/s [10.8-13.1], significantly higher than CONTROLS 8.8m/s [8.5-9.2] (p < 0.001), THORACIC 8.8m/s [8.0-9.6] (p < 0.001), and UNOPERATED 8.9m/s [8.0-9.8] (p < 0.001).

**Conclusions:** These data suggest that the replaced abdominal aorta reduces arterial compliance. Thoracic grafting does not have an effect on PWV indicating that the proximal aorta contributes less to tonometric measurements of arterial stiffness. We provide a basis for future work to investigate how prosthetic aortic replacement affects ventricular-arterial coupling and the impact of pharmacological manipulation of vascular function in such patients.

**ROLE OF POSITRON EMISSION TOMOGRAPHY (PET) IN PANCREATIC RESECTION FOR SUSPECTED PANCREATIC AND PERIAMPULLARY CANCER**

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**Background:** A significant proportion of patients undergo unnecessary laparotomy because of incorrect diagnosis and understaging of pancreatic and periampullary cancer.

**Methods:** A systematic review of studies assessing PET was performed. Medline, Embase, Cochrane trials register, and Science Citation Index were searched until November 2009. The gold standard test was laparotomy. Meta-analysis was performed using bivariate method and Littenberg-Moses method.

**Results:** Seven studies including 336 patients were included in the meta-analysis for distinguishing benign from malignant disease. The summary sensitivity and specificity were 0.892 and 0.745. This corresponds to a post-test probability of 0.56 for a positive PET (i.e., patient has a 44% probability of curative resection if PET was positive) and 0.02 for a negative PET (i.e., patient has a 98% probability of curative resection if PET was negative) compared to a baseline probability of 0.15 (i.e., patient has a 85% probability of curative resection if laparotomy was done without PET).

**Conclusions:** PET has no role in distinguishing benign and malignant periampullary disease. A positive PET scan is unreliable but a negative PET scan can confirm curative resectability with high accuracy.

**AN ANALYSIS OF TUMOURIGENESIS IN HUMAN MESENCHYMAL STEM CELLS EXPANDED IN VITRO**

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5-10% of fractures demonstrate significantly delayed healing or non-union. Although skeletal fixation has achieved limited success, no ideal treatment for non-union exists. One potential is the use of human mesenchymal stem cells (hMSCs) to enhance fracture healing. While hMSCs display immunosuppressive properties reducing the likelihood of rejection, side-effects could include tumourigenesis.

**Objectives:**
1. Do hMSCs become tumorigenic when expanded in vitro and grown on agar?
2. Do time, cell concentration and passage number affect tumourigenesis?

**Materials & Methods:** hMSCs were obtained from bone marrow aspirates from iliac crests of patients undergoing surgical treatment for non-union tibial fractures. Cells from 4 patients at 3 passages, positive (HCT cells) and negative control lines (AA/Cl cells), were seeded onto agar plates at different concentrations. Plates were incubated at 37°C, 5% CO2 for 4 weeks. Tumour colony numbers (image 1) and tumourigenicity were calculated weekly.

**CONCLUSIONS**
Tumourigenesis did not occur in hMSC’s expanded in vitro. These results may support existing studies confirming hMSC’s can be safely expanded in vitro for therapeutic use.

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