Guiding Migration and Differentiation of Rat Bone Marrow Stromal Cells using d.c. Electric Fields *in vitro* – Implications in Bone Tissue Engineering

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INTRODUCTION: The application of d.c. electric fields (EF) for the regeneration of new bone *in vivo* has been reported by a number of groups [1,2]. The use of electric fields as a guidance cue to direct cell behavior in tissue engineering applications has been strongly focused on cardiac myocytes [3], although d.c. electric fields have been used to study the directed migration of bone cells from rabbits *in vitro* [4]; the majority of studies relating to bone or bone cells use electromagnetic fields. In this study we report the directed migration of rat bone marrow stromal cells in response to d.c. EFs, their directed migration on medical grade PLLA substrates, and initial evidence that the d.c. EFs can affect cell differentiation.

METHODS: Rat bone marrow stromal cells were obtained from 6-7 week old rats as described by Maniatopoulos et al [5]. The BMSCs were cultured either without or with osteogenic supplements during the experiments (10mM β-glycerophosphate and 10^{-8}M dexamethasone). Cells were allowed to attach to tissue culture plastic, or to PLLA substrates produced by compression molding, for 24 hours. A d.c. EF of 200mV/mm was then applied through the culture medium for 6 hours using an experimental set-up described elsewhere. Live time-lapse microscopy was used to monitor the cell migration. The ALP activity of BMSCs subjected to a d.c. EF of 20mV/mm for 2, 5 and 10 days was determined using p-nitrophenol measurements, and were normalized for total DNA.

RESULTS: In response to the applied d.c. EF, cells migrated towards the cathode. BMSCs cultured on tissue culture plastic surfaces in non-osteogenic medium migrated at a mean velocity of 7.9 µm/hr (sem = 1.0 µm/hr, n=61). In contrast, BMSCs cultured in osteogenic medium migrated at a significantly ($p < 0.0001$) greater velocity towards the cathode, with a mean velocity of 19.4 µm/hr (sem = 1.0 µm/hr, n=107). The differences in directed cell migration is demonstrated in Figures 1, where each red dot corresponds to the final location of an individual cell after being exposed to the EF for 6 hours, relative to a common starting point of the origin; the cathode is to the left of the images. BMSCs attached to the surface of PLLA substrates also migrated towards the cathode when exposed to an EF in osteogenic medium, but the velocity was only 7.7 µm/hr; the decrease in migration speed compared to results on tissue culture plastic may be related to differences in cell attachment.

ALP activity, normalized for total DNA, from BMSCs exposed to a d.c. EF (20mV/mm) showed a significant increase after 5 days of culture compared to no field controls.

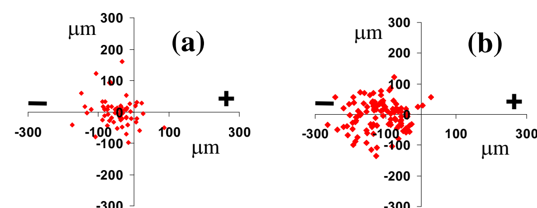

Fig. 1: Migration of BMSCs in a d.c. EF (200mV/mm) in (a) non-osteogenic and (b) osteogenic medium; cathode to the left.

DISCUSSION & CONCLUSIONS: The use of d.c. EFs (200mV/mm) can act as a strong guidance cue to the migration of BMSCs, although this response is affected by the cell phenotype and by the substrate surface chemistry. These results point towards d.c. EFs being a possible method for guiding cell migration through a porous scaffold.

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