THE INFLUENCE OF CYCLIC STRAIN ON THE DIFFERENTIATION OF RAT BONE MARROW DERIVED PROGENITOR CELLS TO SMOOTH MUSCLE: IMPLICATIONS FOR VASCULAR TISSUE ENGINEERING

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I. INTRODUCTION

Two widely used therapies employed to alleviate cardiovascular disease are vascular grafting and stenting [1]. Vein grafting, widely used for arterial by-pass where the autogenous saphenous vein is commonly used, remains a significant clinical concern, with 10-30% of coronary artery grafts suffering thrombosis related occlusion within the first month and 95% requiring intervention within 10 years [2]. In recent years, attention has turned to tissue engineering, an approach whereby cells and/or tissues are combined, often with a biological or synthetic matrix to produce viable replacement tissues and organs. In order to be clinically viable, any vascular-tissue engineering technique should utilize a method by which autologous tissue could be easily harvested and used. It is possible that many of the problems noted with previously reported tissue engineered blood vessels are associated with use of terminally differentiated, autologous, SMC that are limited in their supply, and have only a limited capacity to repair or reconstitute tissues. An alternative source of autologous cells to use in vascular tissue engineering applications could be multi-potential stem cells that have been identified in adult tissues. Biochemical conditions have been identified that stimulate SMC differentiation from stem cells [3]], although it is likely that physiologically consistent biomechanical forces will also be essential in this process. In this paper, we report on the effect of 10% uniaxial cyclic strain on the morphology, proliferation and differentiation of bone marrow derived progenitor cells (BMPCs).

II. METHODOLOGY

Bone marrow aspirates were obtained from freshly euthanized, adult male Wistar rats from unrelated studies. The bone marrow was mixed in a 1:1 volume with Dulbecco's Modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum and 5% penicillin/streptomyocin and centrifuged at 1000 rpm for 5 minutes. The resulting cell pellet was resuspended and the cells were seeded on T175 flasks for 7 days at 37^{o}C, 5% CO_2. After 7 days, non-adherent cells were removed. The cells were then expanded to the required cell number and were used up to passage 2.

BMPC monolayers were washed 3 times in phosphate buffered saline and then incubated with 0.1% trypsin for 5 minutes to remove the cells. Following this, BMPCs were centrifuged at 1000rpm for 5 minutes to form a pellet. The BMPCs were then resuspended in DMEM and plated on flexible culture plates coated with type-I collagen (Flexcell Corp., McKeesport, PA) at 50,000 cells per well. BMPCs were subjected to 10% uniaxial cyclic strain at 1Hz for 7 days using the Flexcercell™ strain unit (Flexcell Corp., McKeesport, PA). Morphological analysis and antibody labelling for smooth muscle specific genes was then used to investigate cellular response.

ESULTS

After 14 days in culture, the cells grew in distinct multilayered colonies, a morphology associated with progenitor cells (Fig 1).

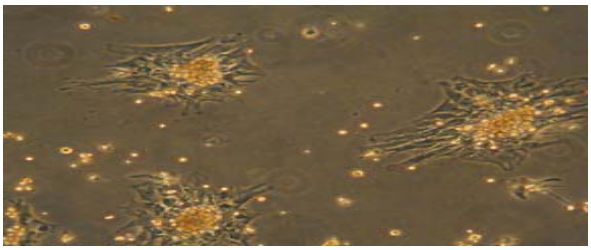
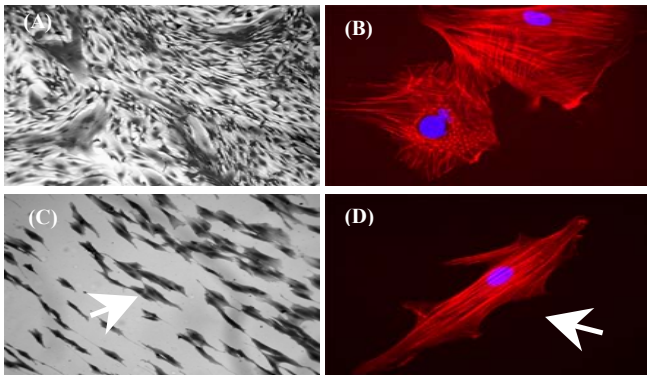


Fig 1: Morphology of rat bone marrow derived cells in primary culture. Mag x10

10% cyclic strain markedly altered the morphology of BMPCs (Fig 2). Specifically, cells grown under static conditions were characterized by a radially flattened, appearance, which was consistent with the morphology the BMPCs show when grown on tissue culture polystyrene. BMPCs exposed to strain showed a tendency to elongate, and align perpendicular to the direction of strain.



actin arrangement of BMPCs in (A, B) static 2D culture and (C, D) 10% uniaxial cyclic strain for 7 days. Note the increased cell number evident in the control cultures. Arrow indicates direction of strain. Mag in (A, C) x10 and in (B,D) x40.

After 7 days of cyclic strain, the cells became strongly positive for smooth muscle α actin (Fig 3a, b) and h1-calponin (Fig 3c, d). Neither protein could be detected in control cultures, clearly demonstrating that the up-regulation of the genes was activated by the cyclic strain.

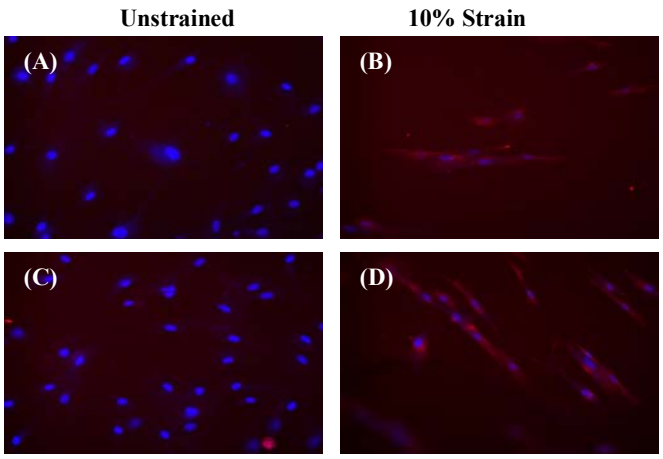


Fig 3: Detection of smooth muscle α actin in (A) unstrained and (B) 10% strained cultures and h1-calponin in (C) unstrained and (D) 10% strain. Note only strained cultures are positive for both proteins. Nuclei are labeled blue and stain is red. Arrow indicates direction of strain. Mag x20.

IV. DISCUSSION

Although bioactive molecules have been identified to stimulate differentiation to SMC [3], we believe that biomechanical forces will also play a prominent role. An understanding of such stimuli and how they influence differentiation will have great relevance in vascular tissue engineering and other therapies. In this paper we report on the response of bone marrow derived mesenchymal stem cells to 10% cyclic strain over a 7-day culture period. Our results suggest that BMPCs respond to cyclic strain by reducing proliferation, changing orientation and up-regulating expression of smooth muscle α actin and h1-calponin. Our results are consistent with findings from several other studies that have examined the effect of strain on cell types such as differentiated smooth muscle [4]. These previous studies have shown that cyclic strain causes cells to change their orientation and alter gene expression and proliferation kinetics [5]. In this study, we have also shown that cyclic strain stimulates expression of smooth muscle α- actin and h1-calponin in stem cells. It has been previously shown that vascular smooth muscle α-actin is upregulated in myocardium during cardiac hypertrophy provoked by load [6]. However, the exact mechanical stimuli that caused this expression were not identified.

In summary, we have demonstrated for the first time that cyclic strain alone will stimulate expression of smooth muscle genes in bone marrow derived progenitor cells, and this finding may have significant implications for all areas of vascular biology and tissue engineering

V. CONCLUSIONS

In conclusion, the results of this study demonstrate that primary bone marrow derived progenitor cells are load responsive and that cyclic strain can be used to stimulate expression of smooth muscle specific genes. Although the mechanisms by which this up-regulation occurs is yet to be elucidated, it clearly implicates proliferation and morphological changes as playing a part in the differentiation. To our knowledge, this study was the first to explore the effect of cyclic strain on BMPC differentiation. Future studies will examine the threshold at which the genes are activated and the effect of biochemicals and biomechanics as determinants of smooth muscle differentiation from stem cells.

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