

## Dynamic compression influences the biochemical response of human mesenchymal stem cells cultured in agarose constructs

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**INTRODUCTION:** Mesenchymal stem cells have the potential to differentiate into the chondrogenic lineage and may therefore provide an alternative cell source for implantation in tissue engineered repair systems [1]. Mechanical compression is known to modulate the metabolic activity of chondrocytes [2]. Accordingly, the current study examines whether dynamic compression could influence the metabolism of human mesenchymal stem cells cultured in agarose constructs.

**METHODS:** Human mesenchymal stem cells (hMSC) were expanded in monolayer culture for up to 6 passages in high glucose DMEM + 10 % FBS. Cells were subsequently seeded in 3 % agarose type VII ( $4 \times 10^6$  cells.ml<sup>-1</sup>) and incubated under free-swelling conditions for a further 2, 4 or 6 days in 1 ml of a defined culture medium supplemented with 10 ng.ml<sup>-1</sup> TGF $\beta$ <sub>3</sub> (all from Cambrex Bioscience, Wokingham, UK).

In a separate experiment, a fully characterised cell-straining system (Zwick Testing Machines, Leominster, UK) was used to apply dynamic compression to constructs [2-3]. Constructs were subjected to 1.5 hour compression, with 4.5 hour unstrained period, for a total period of 48 hours. Intermittent compression was applied in a dynamic manner, with a compressive strain amplitude of 15 % at 1 Hz up to 2, 4 or 6 days of culture. All constructs were incubated in 1 ml of a defined medium supplemented with 10 ng.ml<sup>-1</sup> TGF $\beta$ <sub>3</sub>.

Constructs and medium were subsequently analysed using standard biochemical techniques [2-3]. Total DNA was determined using the Hoescht 33258 method and glycosaminoglycan (GAG) was determined using 1,9-dimethylmethylene blue (DMB) assay.

**RESULTS:** Figure 1 illustrates the effects of dynamic compression on DNA and GAG content in unstrained and strained constructs and constructs cultured under free-swelling conditions for up to 6 days. Total DNA and GAG levels were similar in free-swelling constructs cultured at each time period. No differences were found for DNA levels in unstrained and strained constructs cultured for 2 or 4 days. The application of dynamic compression resulted in a significant increase in DNA levels

after 6 days of culture ( $p < 0.001$ ; Fig. 1A). Dynamic compression inhibited GAG content at day 2 and 4 and significantly enhanced GAG levels after 6 days of culture ( $p < 0.001$ ; Fig. 1B).

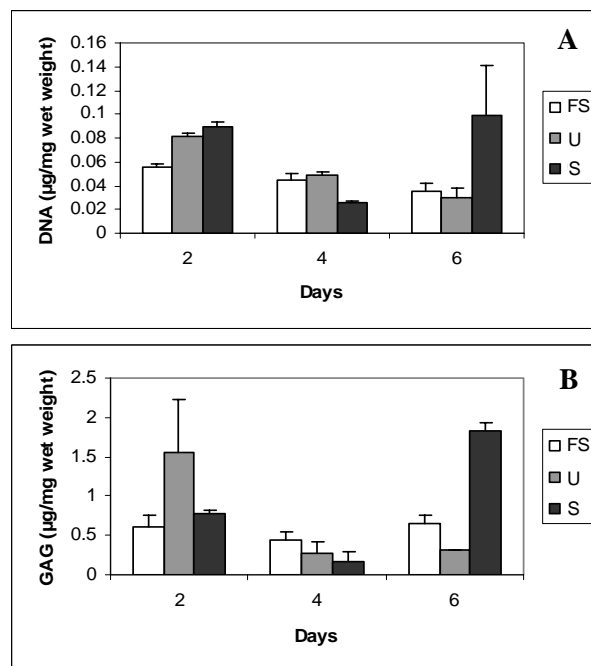


Fig 1: Total DNA (A) and GAG (B) content of constructs cultured under free-swelling (FS) conditions or in unstrained (U) and strained (S) constructs subjected to dynamic compression.

**DISCUSSION & CONCLUSIONS:** Preliminary results show that hMSC respond to dynamic compression by increasing DNA and GAG levels. This response is dependent on the culture period. Further study will determine if mechanical stimuli will influence the chondrogenic potential of mesenchymal stem cells.

**REFERENCES:** <sup>1</sup> F. Barry and J. Murphy (2004). <sup>2</sup> TT. Chowdhury *et al.* (2003). <sup>3</sup> DA Lee and DL Bader (1997).

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