DYNAMIC MECHANICAL COMPRESSION APPLIED IN A CONTINUOUS OR INTERMITTENT MANNER INFLUENCES CHONDROCTE METABOLISM.

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INTRODUCTION

The importance of mechanical stimulation in maintaining cartilage integrity is widely accepted. Accordingly, numerous *in vitro* studies have examined the effects of mechanical loading on chondrocyte metabolism. Generally, static compression suppresses proteoglycan synthesis and cell proliferation, while dynamic strain has a stimulatory effect [1]. Studies by the authors have utilised a cell-straining system for the application of physiological load to chondrocytes seeded in agarose gel [2-4]. The objectives of the current study was to further optimise mechanical conditioning regimes by investigating the stimulatory effects of dynamic compression using different numbers of cycles of compression, applied in a continuous or intermittent manner. Three markers of chondrocyte metabolism, namely, proteoglycan synthesis, cell proliferation and $^{\cdot}NO$ release have been measured.

MATERIALS AND METHODS

Preparation of chondrocyte / agarose constructs

Bovine chondrocytes were isolated from the metacarpalphalangeal joint by pronase and collagenase digestion and seeded in 3 % agarose type VII, at a cell concentration of 4 x 10^6 cells.ml^{-1}, as previously described [2]. Chondrocyte / agarose constructs were cultured in 1 ml of DMEM + 20 % FCS for 24 hours.

Application of mechanical compression

A well characterised cell-straining system (Zwick Testing Machines Ltd, Leominster, UK) was used to apply dynamic compression to the chondrocyte / agarose constructs [2]. Both continuous (C) and intermittent (I) compression regimes were employed (Fig. 1). In the former, the six durations involved the following:

- 1.5 hr continuous compression with 46.5 hr unstrained period,
- 3 hr continuous compression with 45 hr unstrained period,
- 6 hr continuous compression with 42 hr unstrained period,
- 12 hr continuous compression with 36 hr unstrained period,
- 24 hr continuous compression with 24 hr unstrained period or
- 48 hr continuous compression.

In addition, intermittent compression was applied for 1.5, 3, 6 or 12 hr compression with equivalent unstrained periods for a total of 48 hr. All strained constructs were subjected to 15 % dynamic compressive strain at 1 Hz, in 1 ml of medium + 1 µCi.ml^{-1} [^{3}H]-Tdr + 10 µCi.ml^{-1} $^{35}SO_4$ for the assessment of chondrocyte proliferation and proteoglycan synthesis, respectively. Control constructs were unstrained and maintained within the cell-straining apparatus.

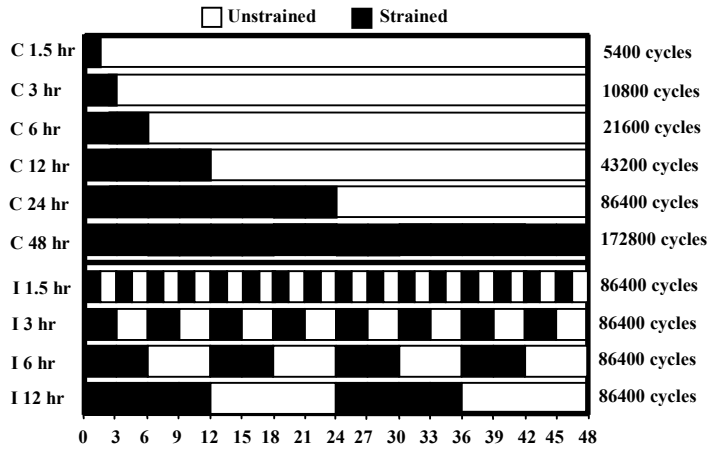

Fig. 1 Schematic diagram illustrating the experiments performed to optimise the dynamic stimulation of chondrocytes seeded in agarose constructs.

Biochemical analysis

Nitrite, an end product of $^{\cdot}NO$, was measured in the culture medium using the Griess reaction. [^{3}H]-Tdr and $^{35}SO_4$ incorporation was assessed by TCA and alcian blue precipitation, respectively [2-4].

Statistical analysis

All data represent the mean and SEM values of 24 replicates from two separate experiments. One way ANOVA with *post hoc* Bonferroni-

corrected *t*-tests were used to examine inter- and intra-group differences for absolute data. Unpaired Student's *t*-tests were used to examine normalised data between 1.5 hr and 3, 6, 12, 24 or 48 hr regimes. In all cases, 5 % was considered significant (*p<0.05).

RESULTS

Effects of continuous compression

Absolute and normalised values for $^{35}SO_4$ incorporation and [^{3}H]-Tdr incorporation by cells within unstrained constructs and constructs subjected to continuous compression, are presented in figure 2. Dynamic compression significantly enhanced $^{35}SO_4$ incorporation (0.01<p<0.001, Fig. 2A). The one exception involved dynamic strain applied with a minimum period (1.5 hr) of continuous compression, where there was a 20 % increase that was not statistically significant (p>0.05, Fig. 2A). For extended periods, there was a monotonic increase in stimulation (p<0.05, Fig. 2B), reaching a maximum value of approximately 60 % after 48 hr of dynamic loading (p<0.001, Fig. 2B). Continuous compression resulted in a significant increase of [^{3}H]-Tdr incorporation, such that cells subjected to 1.5 hr of dynamic strain stimulated the largest response (p<0.001, Fig. 2C). Further cycles of continuous compression decreased normalized [^{3}H]-Tdr incorporation values from approximately 73-38 % (p>0.05, Fig. 2D). By contrast, dynamic strain significantly inhibited nitrite release (0.01<p<0.001, data not shown).

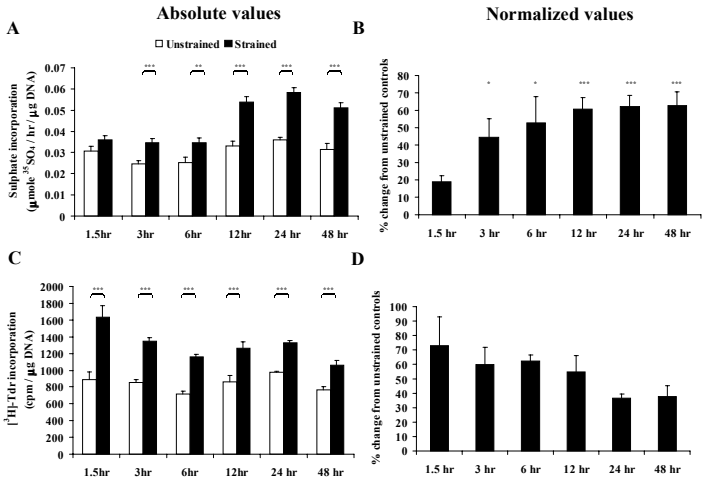

Fig. 2 The effects of continuous compression on $^{35}SO_4$ incorporation (A,B) and [^{3}H]-Tdr incorporation (C,D).

Effects of intermittent compression

The effects of intermittent compression on $^{35}SO_4$ incorporation, [^{3}H]-Tdr incorporation and nitrite release is presented in figure 3. Dynamic compression resulted in a significant increase of $^{35}SO_4$ incorporation for all conditions examined (0.01<p<0.001, Fig. 3A). It is evident that the magnitude of enhancement of $^{35}SO_4$ incorporation increased monotonically with successive durations of intermittent compression, such that a statistically significant difference was found between levels of stimulation for the 1.5 hr and 12 hr regimes (p<0.001, Fig. 3B). By contrast, optimum dynamic stimulation for [^{3}H]-Tdr incorporation was found when cells were subjected to a minimum period (1.5 hr) of intermittent compression (p<0.001, Fig. 3C). The level of stimulation decreased with extended periods, reaching a minimum value of approximately 28 %, which occurred after 12 hr intermittent compression (Fig. 2D). Dynamic strain significantly inhibited nitrite release for cells subjected to intermittent compression at all the durations investigated (0.01<p<0.001, Fig. 3E). A similar level of inhibition, ranging from 23-33 % was found for cells subjected to all regimes of intermittent compression (p>0.05, Fig. 3F).

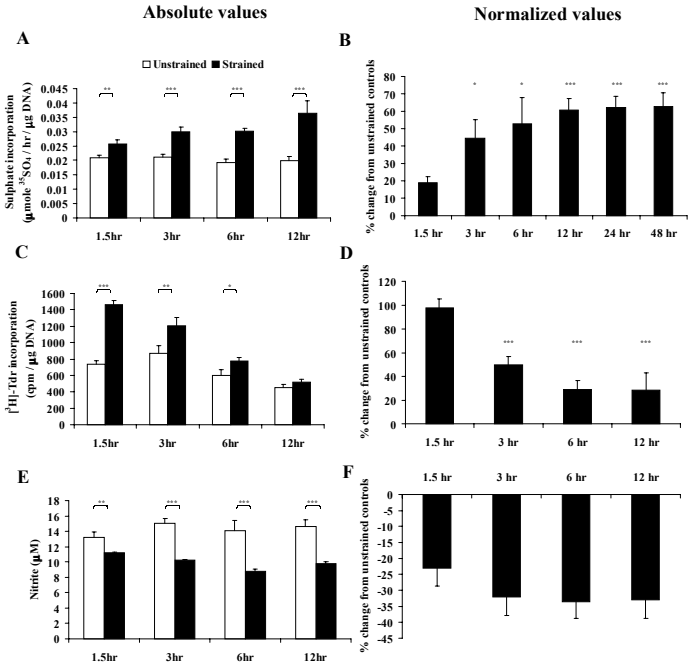

Fig. 3 The effects of intermittent compression on $^{35}SO_4$ (A,B), [^{3}H]-Tdr incorporation (C,D) and nitrite release (E,F).

DISCUSSION

The current study demonstrates that continuous or intermittent compression may differentially modulate chondrocyte metabolism. The stimulatory effects on proteoglycan synthesis, was dependent on the number of cycles of continuous compression, such that large numbers of continuous cycles enhanced the response (Fig. 2B). Furthermore, regular bursts of intermittent compression enhanced proteoglycan synthesis further (Fig. 3B). By contrast, only 5400 cycles was required for maximum stimulation of cell proliferation, with regular bursts inhibiting the proliferative response (Fig. 2D and 3D). The effects of continuous cycles of compression shows trends similar to constructs subjected to intermittent compression for NO release, where the early response is maintained during mechanical loading.

These data will have important implications for the *in vitro* conditioning of cell seeded tissue engineered scaffolds and will therefore facilitate the culture of functional tissues suitable for implantation.

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