

THE TRIAXIAL COMPRESSION VESSEL: A NOVEL MECHANICALLY ACTIVE CULTURE SYSTEM FOR CARTILAGE EXPLANTS

A. D. Heiner and J. A. Martin

Department of Orthopaedic Surgery, University of Iowa, Iowa City, Iowa

Email: anneliесе-heiner@uiowa.edu

INTRODUCTION

Joint loading imposes complex stress states on articular cartilage, involving simultaneous solid matrix distortion, fluid flow, and hydrostatic compression. It is well established that loading regulates cartilage matrix biosynthesis (Steinmeyer et al., 1999; Quinn et al., 1998), suggesting that optimal stress conditions might promote cartilage stability and repair *in vivo*.

Unfortunately, most *in vitro* models used to investigate cartilage responses to stress have used simple devices that either exaggerate distortion and fluid flow (unconfined axial compression) or minimize these effects (hydrostatic compression), thus creating mechanical conditions unlike those found *in vivo*. To address this issue, we developed a culture device capable of more closely simulating complex *in vivo* stress states.

The device, termed the triaxial compression vessel (Figure 1), achieves this goal by permitting simultaneous and independent control of both axial and transverse compression. The cartilage explant is located in a cartilage housing, which holds the explant in position and keeps it chemically isolated. The lower chamber of the vessel applies transverse compression to the cartilage through a liquid pump. The upper chamber contains a piston which applies axial compression to the cartilage upon application of external air pressure. Culture nutrient medium is pumped to the cartilage through the platens, which are porous.

We are currently using this device to compare matrix synthesis in cartilage

explants exposed to axial compression alone, versus equal axial plus transverse compression. We hypothesize that while the high shear stress produced by axial compression alone suppresses synthesis (Wong et al., 1999), the minimal shear stress produced by equal axial plus transverse compression does not.

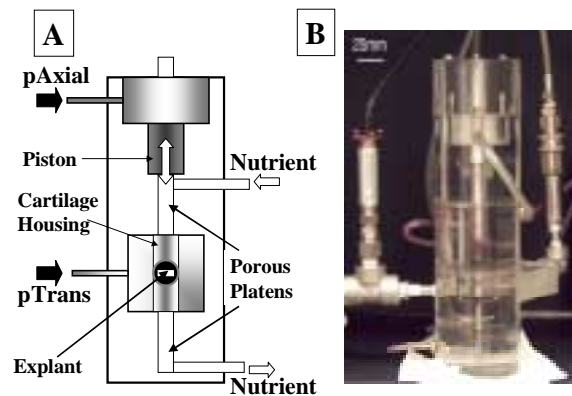


Figure 1. (A) Schematic and (B) physical appearance of triaxial compression vessel.

METHODS

Tibial plateau cartilage explants from three osteoarthritic human donors were cultured for 1 hour under one of three conditions: (a) 2 MPa axial compression at 1 Hz cyclic loading (axial alone), (b) 2 MPa axial plus 2 MPa transverse compression at 1 Hz cyclic loading (axial+transverse), or (c) not loaded (control). The explants were then incubated an additional 4 hours in growth medium containing 50 $\mu\text{Ci/ml}$ $^{35}\text{SO}_4$, to measure matrix (proteoglycan) synthesis. The $^{35}\text{SO}_4$ -labeled explants were washed, embedded and cryosectioned. Sections were collected and digested with papain. DNA content

(fluoroscopic assay) and $^{35}\text{SO}_4$ content (scintillation counter) were determined.

RESULTS AND DISCUSSION

The matrix synthesis data ($^{35}\text{SO}_4$ incorporation/ μg DNA) demonstrated high intrinsic donor-to-donor variability (Figure 2A). However, for all 3 donors, axial compression alone suppressed matrix synthesis. When the effects of donor variability were minimized by normalizing to controls within each experiment (CPM per μg for stressed explants/CPM per μg for controls), the axial alone group differed significantly from the axial+transverse group (2-tailed t-test, $p = 0.023$) (Fig. 2B).

The suppression of matrix synthesis by pure axial compression, which caused shear stress (equal to $\frac{1}{2}$ of the applied axial stress), solid matrix distortion, and fluid flow, contrasted sharply with the minimal effects of equal axial+transverse compression, which caused little or no shear stress (theoretically zero, for pure hydrostatic stress). Future studies will explore this relationship in greater detail by varying the balance between axial and transverse compression to achieve intermediate shear stress states. We expect these studies will help to determine which stress conditions are likely to optimize cartilage matrix synthesis. These data may then serve as a basis for manipulating stress conditions *in vivo* to promote cartilage repair.

SUMMARY

We have developed a triaxial compression vessel capable of applying simultaneous axial and transverse compression to cartilage explants. Axial compression alone suppressed matrix synthesis, while equal levels of axial and transverse compression had a much smaller effect.

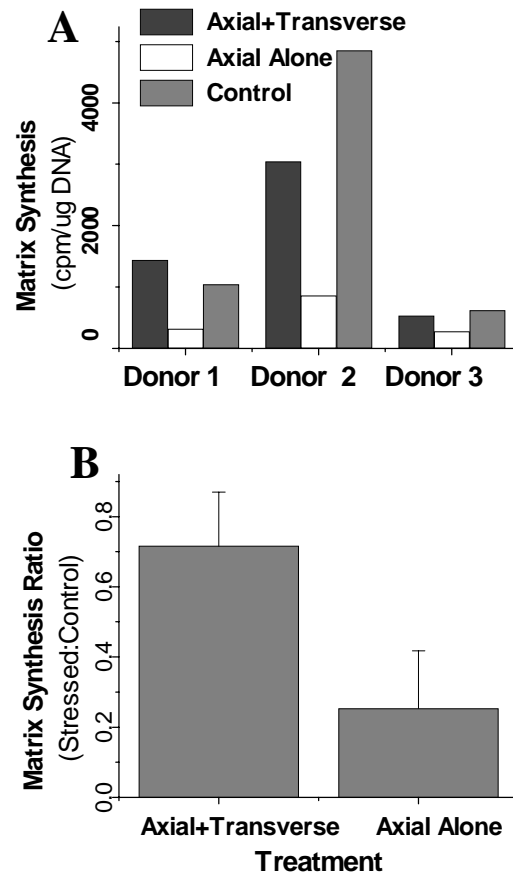


Figure 2. Stress effects on cartilage matrix synthesis. (A) $^{35}\text{SO}_4$ incorporation (CPM per μg DNA). (B) Ratio of stressed/control values. Bars indicate 1 std. dev. These groups are significantly different ($p=0.023$).

REFERENCES

- Quinn, T.M. et al. (1998) *J Cell Science* **111**, 573-583.
- Steinmeyer, J. et al. (1999) *Osteoarthritis Cartilage* **7**, 155-164.
- Wong, M. et al. (1999) *Matrix Biology* **18**, 391-399.

ACKNOWLEDGEMENTS

This research was funded by the University of Iowa Central Investment Fund for Research Enhancement.