ELECTRIC FIELD REGULATION OF CHONDROCYTE BIOSYNTHESIS IN AGAROSE GEL CONSTRUCTS

+*Szasz, N; *Hung, H; *Sen, S; and *Grodzinsky, A
+*Massachusetts Institute of Technology, Cambridge, MA

BACKGROUND: Physical factors can modulate chondrocyte biosynthesis of extracellular matrix (ECM) macromolecules important in normal cartilage, degeneration and repair[1,2]. Electric currents are coupled to mechanical deformation and fluid transport within cartilage due to the charged glycosaminoglycan (GAG) constituents of the ECM, and this coupling may play a role in cartilage homeostasis. Previous studies have shown that externally applied electric and electromagnetic fields applied to isolated chondrocytes[3] or chondrocytes in explant organ culture[4,5] can increase DNA synthesis[3], increase GAG content[4], and increase protein synthesis[5]. *We hypothesized that electric fields could modulate ECM biosynthesis by chondrocytes in agarose gels.* We used a 3-D chondrocyte-seeded agarose gel culture system well characterized[6,7] for chondrocyte culture and mechanotransduction. Our objectives were to (1) quantify the effects of applied electric fields on the magnitude and kinetics of proteoglycan (PG) and collagen synthesis by chondrocytes over a range of frequencies and time in culture, and (2) compare with other measures of cellular response including induction of stress response proteins and modulation of cell division.

METHODS: Chondrocytes were isolated from newborn calf femoral condyle cartilage by established methods[1,2]. Cells were cast at 1.5×10^7 cells/ml in 2% agarose and cultured at 37ºC in DMEM and 10% FBS medium. 5×5mm square, 1mm-thick samples were mounted in Teflon holders that fit within a rectangular channel in a 12"×2"×1" incubator-housed polysulphone chamber (Fig 1). Samples were separated from platinum electrodes by 1" agarose salt-bridges. 20μCi/ml ^{3}H-proline and 10μCi/ml ^{35}S-sulfate were added to the culture media to measure biosynthesis of proteins and PGs. In parallel studies, 20μCi/ml ^{3}H-thymidine was added to assess DNA synthesis as a measure of cell division. A 25 mA/cm^2 electric current density in the 0.01-10kHz frequency range was applied across test specimens for 20hrs at 37ºC using a feedback-controlled constant current source. Specimens were then washed, weighed wet, and digested. Aliquots were taken for scintillation counting, GAG and DNA measurements. Selected gel extracts were purified using Sephadex G-50 columns. Fractions containing ^{35}S-sulfate were pooled, concentrated, then run through a Toyopearl HW40S size exclusion FPLC column. Free ^{35}S-sulfate was added to one half of the samples to mark the free sulfate peak. Dowex 50XW8 ion-exchange chromatography was used to assess ^{3}H-hydroxyproline content. Data were analyzed using ANOVA and Tukey post-hoc analysis via SYSTAT 9 statistical software. For certain tests, days were pooled into five approximately equal sized groups (d1: days 5-6, d2: days 7-9, d3: days 10-12, d4: days 13-15, d5: days 16-25).

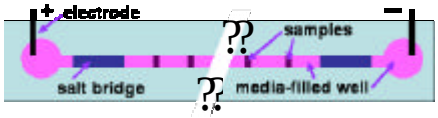
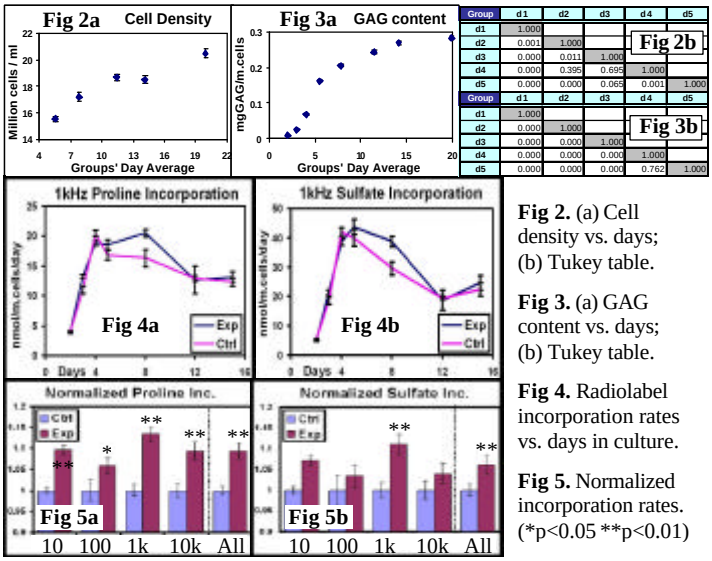


Fig 1. Experimental apparatus.

CONTROLS: Radiolabel incorporation in samples in the stimulation chamber with the electric field set to zero was statistically equivalent to that in the control chamber. No change in pH was observed for AC experiments using a pH electrode. We measured the temperature directly and tested for heating indirectly via the induction of heat shock protein 70 (hsp70). Neither measurement revealed any changes in temperature. A statistical test for variations between the 7 locations was performed to determine if the samples near the two electrodes were affected by electrode products that passed through the salt bridges. ANOVA and student t-tests showed no statistical variation ($p>0.1$).

RESULTS: DNA content showed an increase in cell density (day-pooled) with time in culture (ANOVA, $p<0.001$, Tukey post-hoc, Fig 2). Statistical tests showed no difference in the DNA content of stimulated and non-stimulated control samples. GAG content of specimens also increased with time in culture (ANOVA $p<0.001$, Tukey post-hoc, Fig 3). Statistical tests showed no difference in GAG content of stimulated and non-stimulated samples (ANOVA $p=0.619$). Proline and sulfate incorporation in non-stimulated samples initially increased, reaching a plateau by day 5, and then slowly declined to steady state by day 12 (ANOVA $p<0.001$, Fig 4a,b). In stimulated samples, radiolabel incorporation was similar to non-stimulated controls up to day 5, but then increased significantly compared to controls from days 5-12 (Fig 4a,b). Incorporation in stimulated samples normalized to controls showed that AC fields increased proline incorporation overall ($p<0.001$) and at each frequency ($p_{10}=0.004$, $p_{100}=0.016$, $p_{1k}<0.001$, $p_{10k}<0.001$, Fig 5). No significant differences were found between the effects at various frequencies (ANOVA $p=0.144$). Similarly, AC fields increased sulfate incorporation overall ($p<0.001$) and at 1kHz ($p_{1k}=0.001$), but no increase was found at other frequencies ($p_{10,100,10k}>0.1$) (Fig 5). Nevertheless, no significant difference was found between the effects of the various frequencies. Biochemical composition of the radiolabeled constituents was assessed using gel-filtration and ion-exchange chromatography. DMB binding and scintillation counting of G50, Dowex, and FPLC fractions showed that essentially all of the ^{35}S-sulfate was incorporated into GAG and 85% of proline was incorporated into collagen, similar to that found in previous studies using newborn calf cells[1]. In preliminary studies, ^{3}H-thymidine incorporation indicated a significant decrease in chondrocyte cell division in stimulated samples compared to controls on day 5.



Group	d1	d2	d3	d4	d5
d1	1.000				
d2	0.001	1.000			
d3	0.000	0.011	1.000		
d4	0.000	0.395	0.695	1.000	
d5	0.000	0.000	0.065	0.001	1.000

Group	d1	d2	d3	d4	d5
d1	1.000				
d2	0.000	1.000			
d3	0.000	0.000	1.000		
d4	0.000	0.000	0.000	1.000	
d5	0.000	0.000	0.000	0.762	1.000

Fig 2. (a) Cell density vs. days; (b) Tukey table.

Fig 3. (a) GAG content vs. days; (b) Tukey table.

Fig 4. Radiolabel incorporation rates vs. days in culture.

Fig 5. Normalized incorporation rates. (*p<0.05 **p<0.01)

DISCUSSION: *Electric field stimulation in the 10Hz-10kHz frequency range significantly increased the synthesis of collagen and GAG in a chondrocyte-seeded agarose culture system.* The increase in DNA content and GAG content with time in our agarose model system (Fig 2,3) is consistent with previous findings[6]. Western analysis showed no difference in hsp70 levels between stimulated and non-stimulated samples, suggesting that this stress response pathway is not associated with the changes in biosynthesis. The electric current amplitude used in these studies is an order of magnitude greater than that estimated to be associated with compression-induced streaming currents in vivo[5]. It remains to be shown whether endogenous streaming currents caused by compression of cartilage are related to these observations. Nevertheless, the results suggest that local electric fields may play a role in regulation of chondrocyte metabolism. The possibility that electric fields can downregulate cell division while simultaneously upregulating ECM biosynthesis (day 5) suggests an example of a coordinated signaling role of the electric field. Ongoing studies focus on upstream signaling and transcriptional events that could lead to the changes observed by probing calcium signaling, MAPK pathways, and gene transcription.

REFERENCES: [1]Sah+, J Orthop Res, 1989, 7:619-36. [2]Buschmann+, ABB, 1999, 366:1-7. [3]Rodan+, Science, 1978, 199:690-2. [4]Liu+, Osteoarthritis Cartilage, 1996, 4:63-76. [5]MacGinitie+, J Orthop Res, 1994, 12:151-60. [6]Buschmann+, J Cell Science, 1995, 108:1497-1508. [7]Lee+, J Orthop Res, 1998, 16:726-33.

ACKNOWLEDGMENTS: We thank Dr. E H Frank for his help with the experimental setup. Supported by NIH Grant AR33236 and the John Reed fund.