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Cell-biomaterial engineering report

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Introduction

Collagen is the most abundant protein in mammals, with collagen I found to represent around 90% of protein (by mass). Collagen II is less prevalent but is still very important to biological function, as it is the main component of cartilage, a key supportive tissue composed of chondrocytes (Di Lullo et al., 2002). It has been shown that decreasing ascorbate levels in chondrocytes decreases collagen II levels, which leads to de-differentiation. Brodkin et al. (2005) accomplished this by growing chondrocytes either in the presence of, or without ascorbate. We sought to reproduce their results by growing chondrocytes with ascorbate, but in the presence of dithriothreitol (DTT), an agent which reduces ascorbate to a form that is not easily taken up by cells, and therefore mimics a (-)ascorbate state (Maeng et al., 2009).

Results

We grew primary bovine chondrocytes with a cell density of $2.01 \cdot 10^6$ cells/mL in a Sigma-Aldirch 3D 1% alginate low viscosity bead culture with a viscosity of 250 cps at 2% and a G/M ratio with high M. The (+)DTT sample was incubated with 0.1 mM DTT. The cells were round and fragile, which lead to very low survival rates for both samples. The (+)DTT sample had particularly fragile cells - only 6 (+)DTT beads survived, though 12 (-)DTT beads also seemed meager. As a result, there were very few cells in both samples. Microscopy of the cells was extremely dim, and the images are not included in this report. cell lysates were collected for RNA and protein analysis.

RT-PCR analysis on RNA isolated from cell lysates

RNA samples were isolated with QIAshredder and RNeasy mini columns. Spectroscopy was performed on the isolated RNA samples: the (-)DTT sample had an A_{260} value of 0.006 and an A_{280} value of -0.001, and the (+)DTT sample had an A_{260} value of 0.006 and an A_{280} value of -0.004. The low absorbance values show that we had low purity RNA values, which we compensated for by not diluting the samples. RT-PCR was performed on $0.1ng$ of the RNA samples, then agarose gel electrophoresis was performed on the results of the RT-PCR, which is shown in Figure 1. Addition of DTT was found to have no effect on collagen I levels, but decreased collagen II levels, consistent with our hypothesis. Image processing was performed using ImageJ, and relative expression ratios were calculated (Abramoff et al., 2004). Background-subtracted values are shown in Table 1. After background subtraction, Relative expression ratios: CN I/GAPDH -DTT, 1.970; CN I/GAPDH +DTT, 2.388; CN II/GAPDH -DTT, 1.138; CN II/GAPDH +DTT, 0.513; CN I/II -DTT, 0.870; CN I/II +DTT, 0.367.

ELISA analysis on CN I and CN II proteins extracted from cell lysates

Protein samples were isolated using EDTA-citrate buffer (150 mM NaCl, 55 mM sodium citrate, and 30 mM EDTA), pepsin, and freezing. Enzyme-linked immunuosorbent assay (ELISA) analysis was performed on the isolated samples, though our samples were either marginally above the background or negative, meaning that we had no protein in our experiment.

Discussion

(Brodkin et al., 2005) had show that decreasing vitamin C levels causes de-differentiation of the chondrocytes and thus decreased collagen II levels. Our results verify this paper, as addition of DTT (which inhibits vitamin C uptake) decreases collagen II levels in comparison to the (-)DTT sample (three-fold difference, as shown in Table 1). This is also consistent with the findings of TM et al. (1994). However, our collagen I levels remained steady in the RT-PCR analysis shown in Figure 1. The reason behind the unchanged collagen I levels is unknown and requires further research to fully understand. Our ELISA analysis was not meaningful as there was not enough protein in the wells to produce enough detectable signal. This experiment could have been a success had we had enough alginate beads to perform all the analysis necessary. Larger bead size could be mediated by using a higher concentration of alginate for more stable beads. Also, there were a few steps in the protocol could be machine-automated in the future to ensure reproducibility. For example, pressing down on the syringe to extract the cells is not exactly the same from on person to the next. Testing DTT as a vitamin C inhibitor and therefore a collagen inhibitor in chondrocytes is still a viable question.

References

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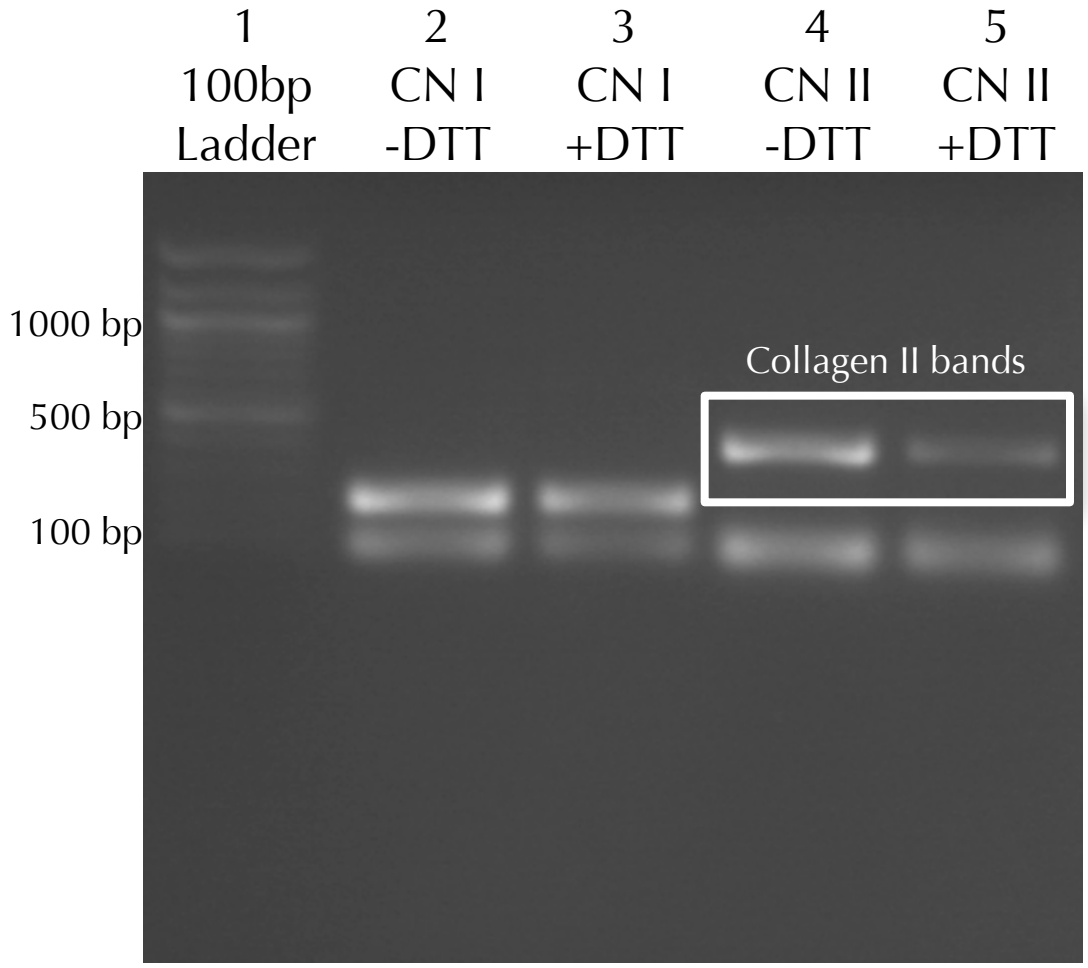
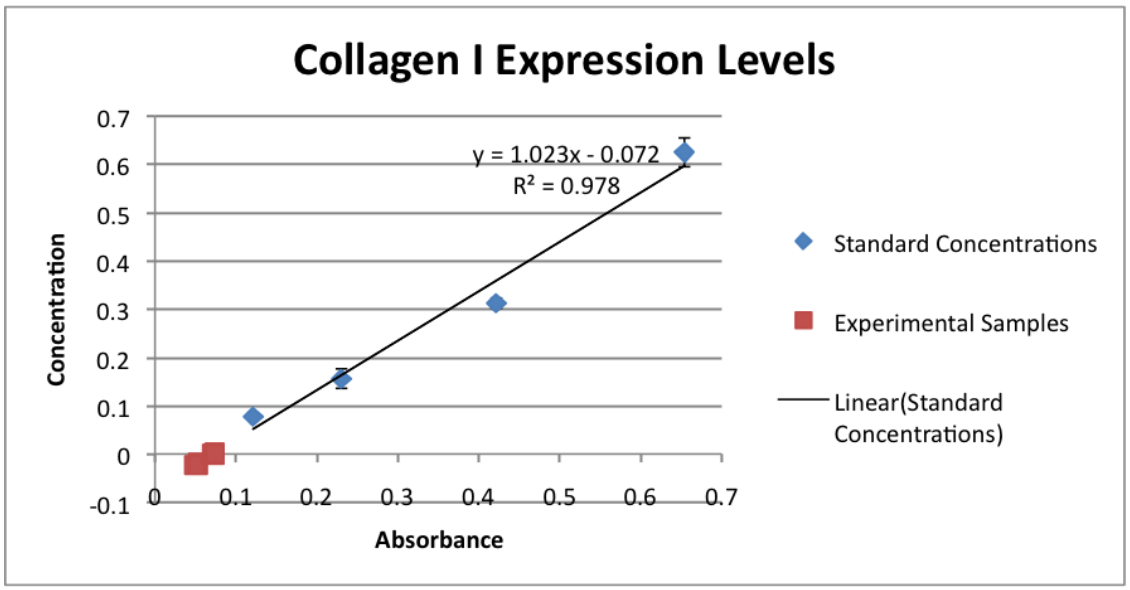
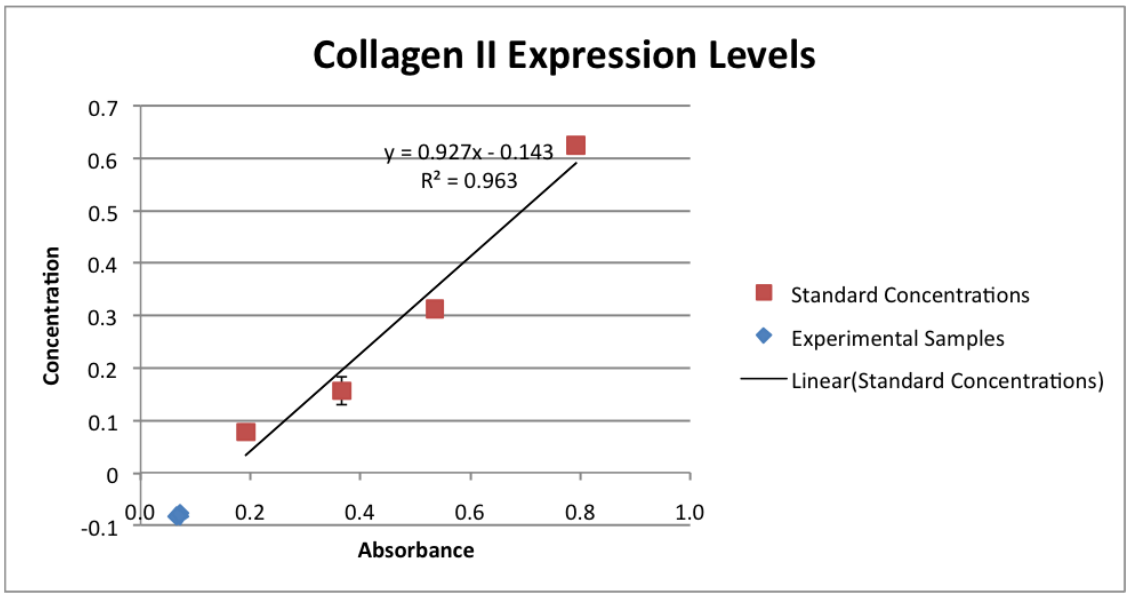


Figure 1: **Gel electrophoresis performed on RT-PCR of RNA collected from CN I and CN II samples, each with a (-)DTT and a (+)DTT sample.** RNA samples were isolated with QIAshredder and RNeasy mini columns. Used 1.2% agarose gel. In all cases, the lower band is the GAPDH housekeeping gene. As the two bands in lanes 1 and 2 are of similar brightness, this showed that DTT has no effect on collagen I. However, as the band at 450 bp is dimmer in lane 4 than in lane 3, this showed that addition of DTT decreases the amount of collagen found in chondrocytes. Image processing was performed using ImageJ. Relative expression ratios: CN I/GAPDH -DTT, 1.970; CN I/GAPDH +DTT, 2.388; CN II/GAPDH -DTT, 1.138; CN II/GAPDH +DTT, 0.513; CN I/II -DTT, 0.870; CN I/II +DTT, 0.367.



(a) **ELISA analysis performed on collagen I samples.**



(b) **ELISA analysis performed on collagen II samples.**

Figure 2: **No appreciable protein was found in ELISA analysis.** Protein samples were isolated using EDTA-citrate buffer (150 mM NaCl, 55 mM sodium citrate, and 30 mM EDTA), pepsin, and freezing. Enzyme-linked immunuosorbent assay (ELISA) analysis was performed on the isolated samples, though our samples were either marginally above the background or negative, meaning that we had no protein in our experiment.

	Band 1 (Collagen I or II)	Band 2 (GAPDH)
CN I, -DTT	74.172	37.654
CN I, +DTT	55.452	23.225
CN II, -DTT	64.558	56.738
CN II, +DTT	20.36	39.705

Table 1: **Mean background-subtracted brightness values for RT-PCR performed on RNA collected from CN I and CN II samples.** Brightness was calculated using ImageJ analysis. The area of analysis was consistent across samples. In the gel, band 1 is the top band and band 2 is the bottom band.