



**CALIFORNIA STATE SCIENCE FAIR  
2016 PROJECT SUMMARY**

<b>Name(s)</b> <b>Elisha D. Johnston</b>	<b>Project Number</b>  36551
<b>Project Title</b> <b>Investigating the Molecular Mechanisms of a Safe Promising Treatment for Chronic Joint Pain</b>	
<b>Objectives/Goals</b> My objective is to investigate prolotherapy. Prolotherapy is a localized injection-based procedure. Advocates claim that prolotherapy activates the body's natural healing mechanism to regrow cartilage. Researchers agree that the mechanism of action is poorly understood. I summarize results from the single published in vitro study into a testable hypothesis: Treatment with 15 microliters of a prolotherapy agent (P2G) is associated with increased proliferation of preosteoblast cells. <b>Abstract</b> <b>Methods/Materials</b> Following the single published in vitro study, I used standard mammalian cell culture techniques with preosteoblast cells and the phenol, glycerin, and dextrose (P2G) compound. To measure cell death and proliferation, I used an Olympus FSX100 Microscope (20X), a Beckman spectrophotometer (with colorimetric CellTiter 96 Aqueous One Solution Cell Proliferation Assay), and flow cytometry. <b>Results</b> To advance understanding about the mechanism of action, I developed a novel theoretical model of the way that prolotherapy regenerates cartilage. Through multiple experiments, I generated statistically significant evidence supporting the literature-derived hypothesis and key parts of my novel theoretical model. Importantly, I found that treatment with P2G induces moderate cell death ( $p < 0.05$ ) and that remaining cells proliferate faster than control ( $p < 0.05$ ). I leveraged my novel theoretical model, experimental results, and molecular biology and biochemistry concepts to propose original hypotheses, with the primary one as: Treatment with 15 microliters of P2G kills some cells, releasing IGF-1 growth factors that cause remaining cells to proliferate faster. <b>Conclusions/Discussion</b> In reproducing findings from the single published in vitro study, my project further validates the hypothesis that prolotherapy activates the body's healing mechanisms to regrow cartilage. Additionally, my novel theoretical model and original hypothesis further elucidate the molecular mechanisms. Molecular biology and biochemistry tools and techniques hold great promise to further investigate whether prolotherapy is an effective treatment for reducing chronic joint pain.	
<b>Summary Statement</b> My project aims to further validate the hypothesis that prolotherapy works by activating the body's natural healing mechanisms to help regenerate cartilage, thereby reducing chronic joint pain.	
<b>Help Received</b> I performed research at TheLab in Downtown LA. Dr. Andrali trained me in cell culture techniques and my father provided general safety supervision. As UCLA does not allow middle school students to actively participate in research, a tutor allowed me to observe while he acquired flow cytometry data.	