SB³C2022
Summer Biomechanics, Bioengineering and Biotransport Conference
June 20-23, Eastern Shore, MD, USA

DOSE- AND TIME-DEPENDENT EFFECTS OF COLLAGENASE CLOSTRIDIUM HISTOLYTICUM INJECTION ON STIFFNESS AND THICKNESS OF IN VITRO TRANSVERSE CARPAL LIGAMENT

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INTRODUCTION

The transverse carpal ligament (TCL) is a band of tissue constituted by collagen fibers types I and III and is relevant to carpal tunnel syndrome due to its volar restriction of the median nerve. The most common surgical procedure for carpal tunnel syndrome is to transect the TCL, which can disrupt the important biomechanical functions of the TCL and result in side effects such as pillar pain and hand weakness [1]. A possible alternative to surgery is to decompress the median nerve by biochemically altering TCL stiffness and thickness.

Collagenase Clostridium Histolyticum (CCH) is an enzyme that breaks down collagen fibers, and has previously been used in injection to treat Dupuytren's contracture, a condition characterized by formation of collagen cords in the hand, causing finger contractures and impaired hand function [2]. Similar to its use for Dupuytren's contracture, CCH injection to the TCL could potentially be used as a treatment for carpal tunnel syndrome. In order to decrease TCL stiffness and thickness without disrupting the anatomy of the TCL, determining an effective and safe dose of CCH to deliver to the TCL is needed. Therefore, the objective of this study was to assess the time- and dose-dependent effects of CCH on the stiffness and thickness of the TCL in vitro.

METHODS

The TCLs of 9 fresh-frozen cadaveric hands were removed from the hand and each sutured to a fabric platform. On each TCL specimen, five injection points were marked on the volar surface of the TCL using tissue marking dye. These points were the center of the TCL (O), and four radial (A), ulnar (B), distal (C), and proximal (D) to the center by 5mm, respectively. The platform was then rigidly fixed in an incubator with the temperature set to 37ºC.

A coordinate system for each injection point was established by digitizing each of the five injection points using a MicroScribe digitizer (Figure 1). The x-axis was defined as the AB vector, the z-axis as the cross product of vectors AB and CD, and the y-axis as the cross product of the z-axis and x-axis.

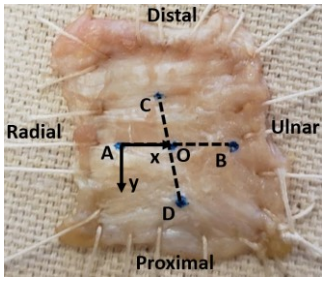

Figure 1. A TCL specimen and one of the five injection point coordinate systems

An 18L6 linear array transducer (SuperSonic MACH 30, Hologic Inc., Marlborough, MA, USA) was rigidly fixed to a 6-degree of freedom robot using a custom-made probe mount. A footprint coordinate system was established with its origin at the center of the probe footprint, its x-axis aligned with the horizontal axis of the image, its z-axis aligned with the vertical axis of the image, and its y-axis determined by crossing the z-axis with the x-axis.

CCH with a specific activity ≥125 Collagenase Digestion Units/mg solid (Sigma-Aldrich, St. Louis, MO) was dissolved in phosphate buffered saline to create five solutions of different concentrations such that the units of CCH per 5µL of each solution were 50U, 100U, 150U, 200U, and 250U, respectively. A microliter syringe with a 30G needle was used to inject 5µL of each solution into one of the five marked injection points at the middle of the TCL thickness.

A thin layer of ultrasound gel was applied to the surface of the TCL, and an ultrasound scan was done of each injection point immediately, 2 hours, 4 hours, 6 hours, 8 hours, and 24 hours after

injection (Figure 2). For each scan, the robot was programmed to move the probe to an initial position by superimposing the footprint coordinate system with each injection point coordinate system, except that the footprint coordinate system was offset 5mm in the negative-z direction. After the probe was moved to each initial position, the robot translated the probe 2mm in the positive and 2mm in the negative y-directions in the injection point coordinate system in 0.2mm steps. A shear wave elastography image and B-mode image were captured at each step.

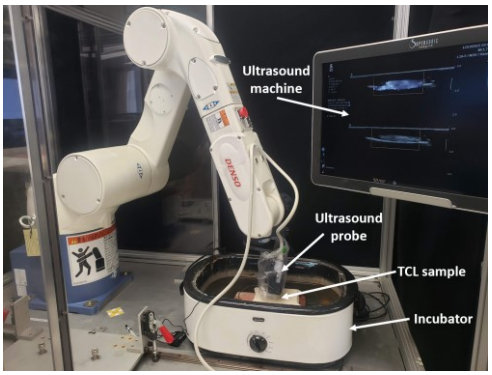

Figure 2. Experimental setup for scanning procedure

A region of interest for each injection point was defined as a rectangular prism centered on the injection point with a width of x=1mm, a length of y=1mm, and a height z that contained the entire TCL thickness. A custom LabVIEW program was used to determine the average shear wave speed of the TCL within the region of interest for each injection point from the shear-wave elastography images collected at each timepoint. TCL thickness was measured at each injection point from the B-mode image taken at that point using ImageJ.

Two-way repeated measures ANOVAs were performed with factors of dose and time using SigmaPlot 14 (Systat Software Inc. Chicago, IL). Analyses were performed with an alpha level of 0.05.

RESULTS

Figure 3 shows shear wave speeds of each dose over time. There were no significant changes in shear wave speeds from time zero to any of the following timepoints within the doses of 50U, 100U, or 150U. For the dose of 200U, shear wave speeds decreased from time 0 by 12.05% and 18.70% at 8 and 24 hours, respectively. For the dose of 250U, shear wave speed decreased from time 0 by 11.96%, 11.46%, 11.20%, 13.92%, and 20.01% at 2, 4, 6, 8, and 24 hours.

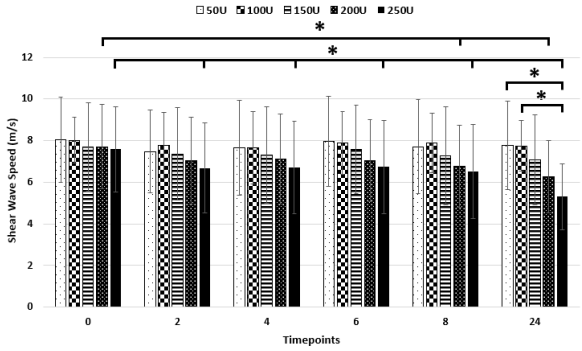

Figure 3. Shear wave speed of individual doses at different timepoints

Figure 4 shows the TCL thicknesses of each dose over time. TCL thickness for the 50U and 100U doses did not significantly change from time zero to any timepoint. For the 150U dose, TCL thickness significantly decreased after 8 hours and 24 hours by 4.74% and 7.28%, respectively. For the 200U dose, TCL thickness significantly decreased after 8 hours and 24 hours by 6.78% and 10.97%, respectively. For the 250U dose, TCL thickness significantly decreased after 6 hours, 8 hours, and 24 hours by 6.06%, 11.75%, and 14.92%, respectively.

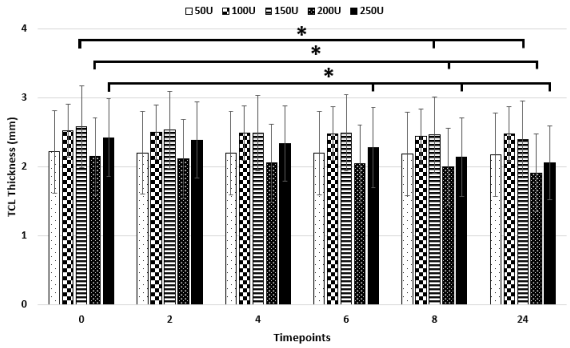

Figure 4. TCL thickness of individual doses at different timepoints

DISCUSSION

As the first steps in assessing CCH injection as a viable treatment for carpal tunnel syndrome, we examined the dose- and time-dependent effects of CCH on TCL stiffness and thickness using isolated TCLs from cadaveric hands. Robot-assistance allowed for precise and accurate probe positioning, and shear-wave elastography imaging was used to measure shear-wave speeds within the TCL. Because elastic modulus can be determined by multiplying shear-wave speed with tissue density, changes in TCL stiffness could be represented by changes in shear-wave speed.

For injection of doses less than 150U, the enzymatic activity is not apparent as there were no significant changes in shear wave speed at any timepoints. As the dose increases to 200U and 250U, the tissue breaks down as indicated by decrease in shear wave speed. None of the doses of CCH resulted in high enough degradation to visibly change the outer surface of the TCL or break through the entire TCL thickness and create holes, suggesting that the effects of the CCH were contained within the boundaries of the TCL. Too much degradation not contained within the boundaries of the TCL could affect the biomechanical function of the TCL or allow the CCH to leak out and affect the surrounding structures.

This study found that higher doses of CCH result in greater and faster degradation of the TCL. Furthermore, injecting 150U or less of CCH has no significant impact on the mechanical properties of the TCL. Injecting 200U of CCH decreased TCL stiffness and thickness after a prolonged time (greater than 8 hours). A dose of 250U was the most effective for TCL degradation, significantly decreasing stiffness after 2 hours and thickness after 6 hours, as well as having the greatest percent decrease in stiffness and thickness after 24 hours. Future studies can investigate the effect of multiple CCH injections to the TCL on carpal arch morphology in situ.

ACKNOWLEDGEMENTS

NIH R21AR075402A1

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