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Assessment of Polarization on Raman Biomarker I_{1670}/I_{1640}

Methods

Four cortical bone samples from 4 separate donors were analyzed using the commercial Raman system (785nm, Xplora, Horiba Jobin Yvon, Edison, NJ, USA). Samples were scanned circumferentially along the grip region of the sample as per a prior study by Flanagan et al.¹ Ten scans were obtained in 4 orientations, each rotated 90° (Figure A1). There were a total of 20 scans that were oriented perpendicular to the laser and 20 scans that were oriented parallel to the laser per cortical bone sample.

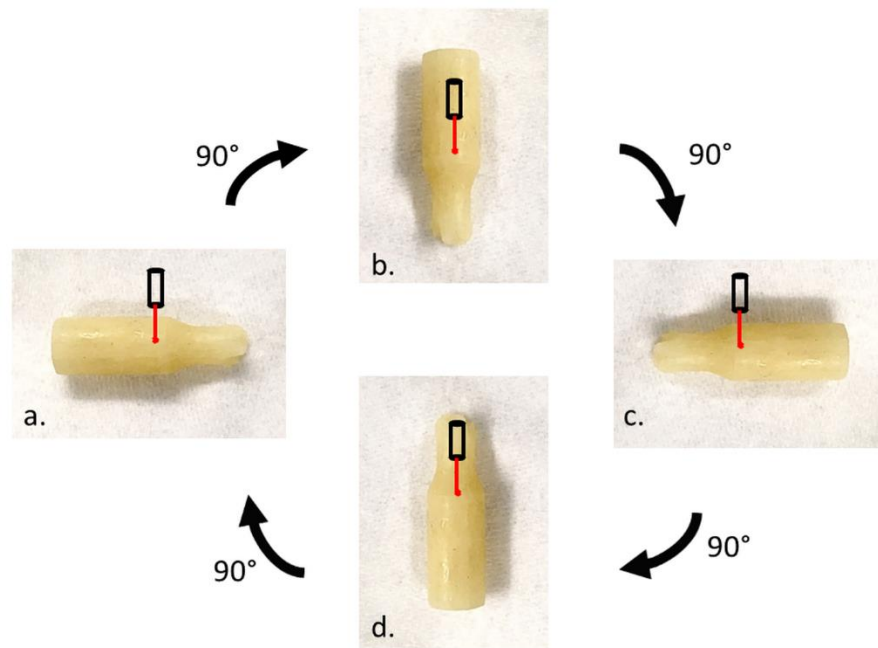
Statistics

Mann-Whitney U tests were used to compare the I_{1670}/I_{1640} collagen disorder biomarker between samples that were flipped 180° in both perpendicular (a. vs. c. in Figure A1) and parallel orientations (b. vs. d. in Figure A1). Mann-Whitney U tests were also used to compare the I_{1670}/I_{1640} collagen disorder biomarker between all perpendicular and parallel scans (a. and c. versus b. and d. in Figure A1). A multivariate linear regression model was created for the I_{1670}/I_{1640} collagen disorder biomarker with donor ID and orientation as independent variables. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS 20.0 (IBM, Armonk, NY).

Results

For each donor sample, there were no significant differences in the I_{1670}/I_{1640} collagen disorder biomarker between scans in either parallel (b. vs. d. in Figure A1) or perpendicular (a. vs. c. in Figure A1) orientations ($p>0.05$ for all samples, data not shown).

Mean values of the I_{1670}/I_{1640} collagen disorder biomarker based on parallel (b. and d. orientations in Figure A1) vs. perpendicular (a. and c. orientations in Figure A1) orientation are presented in table A1. There was no significant difference in the I_{1670}/I_{1640} biomarker based on sample orientation for any donor sample on univariate analysis ($p>0.05$). In the multivariate model, parallel vs. perpendicular orientation was not associated with the I_{1670}/I_{1640} biomarker ($p=0.438$) (table A2). However, donor ID was independently associated with the I_{1670}/I_{1640} biomarker ($p=0.001$). The multivariate model was significant ($p=0.003$), with an adjusted $R^2=0.060$.

**Figure A1.**

Ten Raman scans were obtained in four orientations rotated 90° with respect to the laser system. Raman scans were obtained in the grip region. Orientations a. and c. are flipped 180° with respect to each other and are both perpendicular to the laser. There was no significant difference in the I_{1670}/I_{1640} collagen disorder biomarker between orientations a. and c. in any donor ($p > 0.05$). Orientations b. and d. are flipped 180° with respect to each other and are both parallel to the laser. There was no significant difference in the I_{1670}/I_{1640} collagen disorder biomarker between orientations b. and d. in any donor ($p > 0.05$).

Table A1. Comparison of I₁₆₇₀/I₁₆₄₀ Biomarker in Parallel and Perpendicular Orientations*

Raman Biomarker	Description	Orientation	Donor 1	Donor 2	Donor 3	Donor 4	P-value***
I ₁₆₇₀ /I ₁₆₄₀	Collagen Quality	Perpendicular	1.41±0.22	1.30±0.17	1.36±0.14	1.26±0.17	0.051
		Parallel	1.39±0.27	1.32±0.12	1.29±.16	1.22±0.25	0.080
		P-value**	0.565	0.429	0.134	0.314	

*Presented as mean ± standard deviation

** P-value comparing biomarker values between different orientations for each donor by Mann-Whitney U-test

*** P-value comparing biomarker values among donors by analysis of variance

Table A2. Multivariate Linear Regression Model of I₁₆₇₀/I₁₆₄₀ Biomarker

Independent Variable	Slope Coefficient	95% Confidence Interval	P-value
Donor	-0.047	-0.074 - -0.020	0.001
Orientation	-0.024	-0.085 – 0.037	0.392

Table S1. Specimen Gauge Region Stress Equation

$$\sigma = \frac{k_t 32mg(L - \frac{t}{2})}{\pi d^3}$$

In this equation, k_t is the elastic stress concentration factor, m is the mass of weight for the desired stress level (kg), t is the bearing thickness (mm), d is the diameter of the gauge region (mm), and L is the length between the gauge region and the bearing end. An optical comparator at an accuracy of 25 μ m was used to measure the dimensions of L and d . A k_t of 1.04 was used.

References

1. Flanagan CD, Unal M, Akkus O, Rinnac CM. Raman spectral markers of collagen denaturation and hydration in human cortical bone tissue are affected by radiation sterilization and high cycle fatigue damage. *J Mech Behav Biomed Mater.* 2017;75:314-321.