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Raman and surface-enhanced Raman scattering in the study of human rotator cuff tissues after shock wave treatment

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Important improvements of diseases of the rotator cuff supraspinatus tendons are seen after shock wave (SW) treatment. Neo-angiogenesis stimulation and hypercellularization result from short periods of treatment. The present work is an attempt to provide a first approach to these bioprocesses, most likely associated with structural aspects resulting from biochemical changes brought about by the SW. Immunohistochemical data indicate that collagen areas in the tissues are influenced the most by the SW. Presence of additional collagens I and III by the SW treatment is inferred from an observed increase of the tissue's tinctorial properties. The tools selected for our studies are Raman spectroscopy and the ultrasensitive surface-enhanced Raman scattering (SERS). Here we extract information from 1016 SERS spectra of 52 biopsies of human tendon tissues on Ag nanoparticles before and after the SW treatment. The spectral information is analyzed on the basis of Raman and SERS data of collagen types I and III and their most abundant amino acid components. SERS spectra of tissues reveal the presence of characteristic modes related mainly to amino acids. It has been found that the main differences between both tissue samples could be correlated with the structural conformational aspects of collagen.

Keywords: Raman; SERS; rotator cuff; collagens; shockwaves

Introduction

Shock waves (SW) are basically acoustic waves[1] transmitting pressure into tissues through their water component. Patients receive SW treatment in a single session by being subjected to 4000–6000 impulses at 0.3 mJ/mm^2 (flux energy density) percutaneously without anesthesia and in a recumbent position. The medical indications for this kind of treatment include chronic tendinopathies and delayed union or nonunion of bones.

In clinical studies, it is observed that the natural human repair mechanisms are stimulated or enhanced after the SW treatment; so this new therapeutic tool represents a novel opportunity to analyze the ontological healing capabilities using techniques such as spectroscopy and cytoimmunohistochemical techniques, which working together expand strongly our analysis capabilities and knowledge.

The main objective of the present large research area is to provide, to a first approximation, an explanation of the bioprocesses that could be associated with the structural aspects resulting from the biochemical changes brought about by SW. The biochemical and chemical nature of the tissues are extremely complicated (Fig. 1). The tools selected for tissue characterization in the present study are Raman spectroscopy and the analytical technique of surface-enhanced Raman scattering (SERS).

Raman spectroscopy is an optical technique that provides information on the molecular vibrations of any stable electronic state of a molecular system and is widely used for analytical studies in chemistry, geology, pharmacology and condensed-matter physics. It is a nondestructive analytical tool for the biochemical characterization of biological systems with several advantages, such as high sensitivity to small structural changes, noninvasive sampling capability, minimal sample preparation and high spatial resolution in the case of the new micro-Raman system.[2]

In addition, the noninvasive SERS technique displays several advantages that allow the study of biological materials at near-physiological conditions: low concentrations in aqueous solution, ultrasensitive analysis down to single-molecule detection (SMD)[3] using a low laser power (100 μW–10 mW) and fluorescence quenching. Therefore, the highly selective Raman spectroscopy, combined with the high sensitivity of SERS (enhancement factor up to 10^{10}),[4] provides an impressive set of tools to tackle the problem of identification of those changes at the molecular level induced by SW.

The enhancement of the Raman signal is directly related to the plasmonic waves in metal nanostructures.[5] In addition, factors due to metal–molecule interactions should be considered, such as the charge transfer (TC) phenomena.[6] The interpretation of the observed spectra will have to consider all these factors, and a case-by-case analysis will be required. For randomly oriented physisorbed molecules, where the enhancement is entirely due to plasmon enhancement, the SERS spectral interpretation may be facilitated by comparison with the normal Raman spectrum. For molecules chemisorbed to the metal surface, the spectral interpretation could be difficult; in fact, newly created surface–analyte bonds might modify the electronic structure of the adsorbate, and the complex formation on the surface may produce a strong modification of the reference Raman spectrum. Chemisorption takes place with the formation of a metal–analyte chemical bond.

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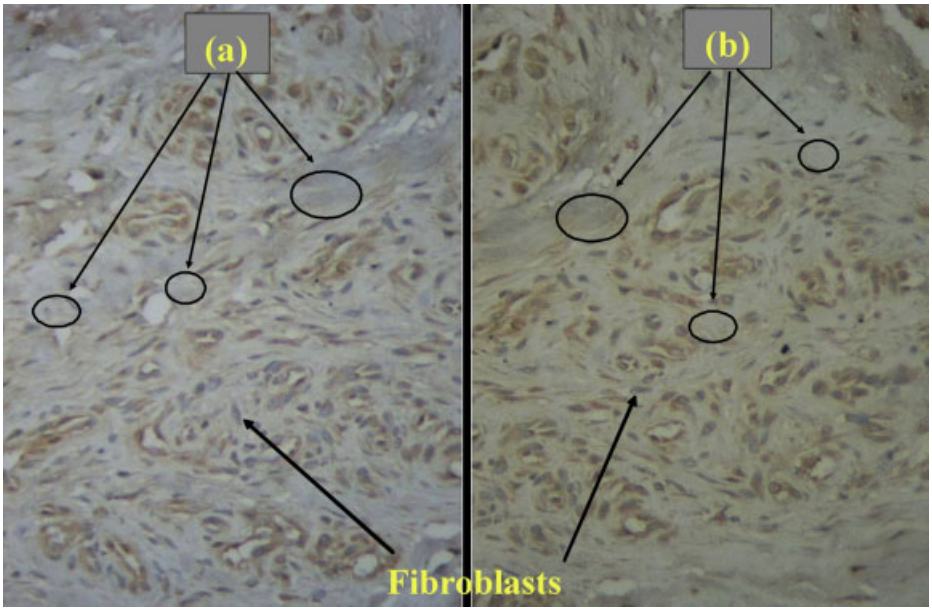

Figure 1. Optical microscope image (50×) of the tissue histological cut with specific markers. Circles indicate (a) collagen I and (b) collagen III areas.

Several metal nanostructures may be used for biomolecular spectral analysis. Preliminary work using colloidal metal particles obtained by reduction of the silver cation validated this particular approach of the SERS technique.[7] In the present work, we test a broad range of nanostructures to find the best protocol for our SERS experiments. SERS selection rules[8,9] also allow us to infer the organization and orientation of the molecules on the surface. Further, SERS substrates provide an innovative and powerful approach to protein and amino acid studies.[7]

It is encouraging to see that recent development in Raman spectroscopy are bringing to the forefront the possibility of using Raman and SERS as viable techniques for proteomics and medical diagnostics. In 2008, a publication offered a "protocol for label-free protein detection in proteomic research" based on SERS.[10] The present contribution represents one more step in that direction.

Selected bibliography indicates that FT-Raman spectroscopy has been already used to classify the degenerative grade of lesions of supraspinatus rotator cuff tendons.[11] The characteristic spectral variations in the 351 spectra of samples from 39 patients were identified, and the authors assigned several bands belonging to different components: proline (850–975 cm^{-1}); hydroxyproline (850–900 cm^{-1}); lipids, nucleic acids and carbohydrates (1050–1150 cm^{-1}); collagen (1300–1347, 1660, 1670 cm^{-1}); and elastin (1670 cm^{-1}).

The available immunohistochemical data indicate that collagen areas in the tissues are influenced the most by SW.[12] Here we extract information from 1016 SERS spectra of 52 biopsies from different patients of tendon tissues treated with Ag nanoparticles before and after SW treatment. In this study, we scan and interpret the Raman and SERS spectra of type I and III collagens from rat and bovine tissues and their main amino acid components, namely, Pro, Gly and Ala, with the whole objective of analyzing the same spectra of the collagen areas in human tissues before and after SW treatment. Collagen type I is composed of two $\alpha 1$ chains and one $\alpha 2$ chain; collagen type III is composed of three identical $\alpha 1$ chains. Collagen type III helix is slightly longer than collagen I helix.[13] Collagen structure and stability have been studied by Shoulders and Raines,[14] and they have demonstrated that stereoelectronic effects and preorganization play a key role in their stability, revealing in detail the fibrillar structure of type I collagen, the prototypical collagen fibril. Collagen contains mainly the amino acids Pro 21%, Gly 33%, Ala 11%, the hydroxyl derivatives in position 3 and 4 for Pro, and 5-hydroxylysine. Thus, one may expect that the SERS spectrum should be dominated by characteristic bands of these amino acids. To our knowledge, no SERS spectrum of collagen has been reported. Animal models of tendinosis represent potentially efficient and effective means of furthering our understanding of human tendinopathy and its underlying pathology.[15]

Experimental

Clinical SW

The mechanisms for generating SW are based on the Dornier method,[16] which is similar to a lightning strike. A high-energy electrical discharge across a spark gap is ignited in a water bath. Patients with symptomatic rotator cuff tears received surgical indication with complete information and signed informed consents for the SW procedure and late surgery/biopsy study (8–10 weeks). The SW treatment was applied in a single session, percutaneously, releasing 4000 impulses 0.3 mJ/mm^2 over the affected areas in the shoulder. We used anOrthospec device (Medispec/Israel) and a Duolith SD1 device (Storz, Germany), both tuned for equal flux energy density.

Raman instrumentation

The Raman and SERS spectra were obtained by means of a Raman Renishaw Microscope System RM1000, equipped with the excitation laser wavelengths of 514, 633 and 785 nm, a Leica microscope and a thermoelectrically cooled CCD camera. Macro Raman measurements were obtained by using suitable macro accessories. The instrument was calibrated using the 520 cm^{-1} line of a Si wafer and a 50× objective. The resolution was set to 4 cm^{-1}, and 5–20 scans of 10 s each were averaged. Spectra

were recorded in the 200–4000 cm^{-1} region to observe both the Raman and SERS spectra. The spectral scanning conditions were chosen to avoid sample degradation.

Raman measurements

Raman spectra of collagens and amino acids were obtained directly from the samples deposited on gold foils by using the 785 nm laser line. Because of strong fluorescence, no Raman spectra for the tissues were obtained.

Collagens and amino acids

Collagen samples from type I and III rat tail and bovine tail tissues were purchased from Sigma and GenWay, and used without further purification. Ala, Gly, and Pro L-amino acids were obtained from Sigma and Gibco, their purity was checked by the routine infrared technique.

Tissue sample preparation

Human tendon tissue samples must be subjected to various physical and chemical steps before optical or electronic microscopy and vibrational spectroscopic studies. Samples for the immunohistochemical and SERS study were prepared following a described methodology consisting mainly in the following steps. The samples of tissues underwent a formalin 2% fixation treatment and were subjected to an automated process (Auto-Tech Leyka) that included alcoholic, xylolic and paraffin processes to obtain a sample ready for inclusion in solid paraffin. Using a microtome, tissue cuts of 1–10 μm were obtained. Only samples for the immunohistochemical study received hematoxilyn–eosin stain, Masson tri-chrome stain (collagens, light/polarized light) and toluidine blue stain (used for glycosaminoglycans and proteoglycans detection due to their important attraction to negative charges of sulfates abundantly present in these molecules).[17,18] According to histochemical results, increased tinctorial properties were observed by the SW treatment, suggesting additional presence of collagens I and III (Fig. 1). Samples for the SERS study were treated only by adding a Ag colloidal solution to the collagen areas. The positive clinical result suggests that the augmented collagens contribute to the final healing, but no patient has been operated after 1 year of SW treatment.

Preparation of silver nanoparticles: SERS measurements

Metal surfaces of Ag nanoparticles for micro-SERS measurements were prepared by immobilizing the colloidal nanoparticles. A schematic picture on the sample preparation has been published.[7] An aqueous solution of the amino acid samples was obtained by dissolving the material in appropriate concentrations (10^{-4} to 10^{-5} M). Samples for SERS were prepared by adding aliquots of the sample solution to 1 ml of the silver colloid. The pH of the mixture was adjusted to a value close to 7, which corresponds to general human physiological conditions, by adding HCl and NaOH in aqueous solutions. Previous to the immobilization, 10 μl of a determined concentration of the amino acids was added to this mixture to find the lowest SERS active concentration. Afterwards, the colloid was activated by addition of 20 μl of 0.1 M aqueous potassium nitrate.[19] This activation was necessary to increase the nanoparticles' SERS activity by properly modifying their morphology. The aggregation is necessary for SERS experiments when working with large molecular systems such as tissues along with the 785 nm laser line. The size distribution of the nanoparticles is in the range 60–150 nm, with the most probable size around 80 nm. By adding the nitrate, the size distribution is maintained with a small increase in the number of nanoparticles of size higher than 100 nm. The extinction spectrum was asymmetric with a shoulder to the infrared. Then, about 20 μl of the final colloidal suspension was deposited onto the amino acid samples and dried at room temperature. The same colloidal suspension was also directly added to the solid tissue sample (1 cm^2 and 2 μm thick approx.). A similar procedure and sample size was used for the SERS study of the solid collagen samples. In the last two cases, the samples were dried at room temperature.

The spectral analysis indicates that a tissue sample displays multiple and different spectra arising from complex molecular systems. The immuhistochemical data allowed us to distinguish the collagen areas and to focus directly on them. In these areas, the thickness of the tissue samples corresponds to 1 μm, which is equal to the size of the laser spot. These conditions ensure that the highest proportion of components in the measurements corresponds to collagen. The spectral profile corresponds to collagen molecular systems. This drastically reduced the number of spectra to be scanned. In fact, we obtained high spectral reproducibility in a great percentage of the samples. The experimental procedure of sample preparation, along with the 785 nm laser line in the power range 100 μW–10 mW and the selected spectral scanning conditions, guaranteed nondestructive sample treatment. Thus, once the collagen area is identified, any statistical treatment under the present experimental conditions is unnecessary.

Results

The Raman and SERS spectra were registered for the Ag surface alone as well as for the surface with the amino acids, collagen and tissues samples. Spectral modifications in intensity and wavenumber of the analytes by the surface effect were evaluated from a structural viewpoint. The spectra of each chemical species coexisting in the biological sample before and after the SW treatment must be analyzed. To accomplish this task, the Raman and SERS spectra of type I and III collagens from rat and bovine tissues and their main amino acids components Ala, Gly and Pro were previously obtained.

Vibrational analysis: general information

The band assignment of the tissues, collagens and amino acids is based on our Raman and SERS information and that obtained from the published spectral study of the simplest amino acids such as Try,[20] Lyse[21], Cys[22], Pro and Val[23] and complex molecular systems such as collagen, gelatine and elastin[24]. In general amide I, II and III vibrations associated with the protein backbone and carboxylic stretches are expected in the 1220–1660 and 890–960 cm^{-1} regions.

Pinzaru *et al.*[25] in colon carcinoma and normal tissue assign the band of His at 1575 cm^{-1} and that of Phe and Try at about 1002 cm^{-1} which a band particularly enhanced in the SERS spectra.

The Raman spectra of collagens display evident peaks at 1669, 1452, 1269 cm^{-1} and a weak one at about 1000 cm^{-1}, the first corresponding to an amide I vibration.[26] Eleven bands were observed in the Raman spectrum of collagen by Lyng *et al.*[27] at 1655, 1447, 1299, 1243, 1082, 1060, 1032, 1002, 930, 854 and

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Table 1. Raman and SERS bands (cm^{-1}) in the range 1800–300 cm^{-1} of Ala[a], Gly, Pro and collagen[b] type I and III of rat tissue, type III collagen SERS, SERS of degenerated human tissue[a] (HT), SERS of HT after SW treatment (HTSW) and the most probable bands assignment[c]

Raman Ala	SERS Ala	Raman Gly	SERS Gly	Raman Pro	SERS Pro	Raman collagen I	Raman collagen III	SERS collagen III	SERS HT	SERS HTSW	Assignment
		1670 vw				1682 m	1674 s				Amide I
		1631 wb	1612 m	1622 mw					1631 m	1635 w	δNH_3^+, δNH_3^+
1595 m	1586 ms	1568 w	1567 ms	1550 m	1544 s			1541 mw	1545 sh	1544 ms	$\nu_{as}COO^-$, Lys
1497 w	1517 m	1513 m							1523 vs	1521 w	δNH, δNH_3^+ Lys
1481 s	1495 m							1495 vw		1502 w	δCH_3
1460 m	1452 w	1454 m	1473 mw	1450 md	1460 ml	1459 s	1451 s	1442 vw	1446 vw	1448 vs	δCH_3, δCH_2
	1422 w	1438 w									δCH_2
					1407 ms						νC-OH Pro
	1389 m								1387 w	1375 vs	δCH_3
1376 w	1351 m		1377 w	1373 m	1373 s			1323 ms	1365 wm	1348 m	ν_sCOO^-, Lys
1358 m		1324 s	1355 vs								ωCH_2 Gly
1304 m	1304 m		1305 ms	1319 vw	1322 w		1307 s			1316 w	δCH
			1282 w	1285 m	1304 w				1299 wm		δCH
	1262 m			1264 m		1278 sh	1276 md			1281 vw	ΔNH Amide III
1237 vw			1221 mt	1237 m		1250 vs	1251 m	1240 vw	1252 s	1227 w	δNH Amide III
1147 wb	1129 m	1138 w	1162 m	1174 md		1171 wm	1166 w	1164 dw	1161 mw	1153 vw	ωCH_2, tNH_2, rNH_3^+, OH-Lys.
1111 m		1105 vw		1117					1132 w	1127 vw	ΔNCH
			1071 m	1081 m	1088 w	1084 m			1074 vw		νCN Pro
1018 m	1031 s	1032 m		1033 m						1031 w	NCCN
	1006 w					1009 m	1008 w	1005s			Phe
			981 w	983 m	986 wt				997 m		νCCN, NCC
				951 m		942 sh	941 vw	942 m		964 ms	νCC skeletal Pro
918 w				919 s	916 w	925 s	924 vw		926 mb	931 w	νC-COO^-
850 vs		890 s		898 vs		879 sh	896 sh	883 vw		888 w	NCC Gly
				840 s	843 w	859 m	858 sd				NCC ring Pro
	805 w					822 m	819 vw			813 m	νCC skeletal
771 w	767 vw		766 vw	790 w	776 wm	763 wb	762 vwb			794 w	δCOO^-
		696 vw	671 w							668 ms	δ, ω COO^-
651 w	645 w	601 w		641 m	650 w	644 w		644 m	634 vw	631 wm	r,δ, ωCOO^-
530 s	523 vw				523 w	532 m		528 wm		534 w	δCCN, COO^- Ala
479 w		486 wb	453 w	447 m	471 md			498 wm		506 bw	Skeletal deformation
283 m				296 vw		297 vw	296 vw				Skeletal deformation

[a] SERS of amino acids and tissues from citrate-reduced Ag colloid.
[b] SERS of collagens from hydroxylamine-reduced Ag colloid.
[c] Band description: w, weak; vw, very weak; wb, weak broad; mw, medium weak; md, medium double; ms, medium strong; sh, shoulder; s, strong; wt, weak triplet.

813 cm^{-1}. Lines appearing at 1271 and 1248 cm^{-1} in the Raman spectra of collagen and elastin were assigned to the amide III mode; moreover, the Raman bands at 1668 and 1245 cm^{-1} and the weak one at 938 cm^{-1} suggest a mostly random structure for elastin.[24] Type I collagen has been identified in human skin through Raman micro spectroscopy[28]; the amide I (1655 cm^{-1}) and III (1246 cm^{-1}) bands of collagen were compared with the data on the isolated molecule.

Raman and SERS spectral analysis

In the following paragraphs we discuss the Raman and SERS bands assignment of type I and III collagens from rat and bovine tail tissues by using Raman and SERS data of their most abundant amino acid components. Next, we analyze the SERS spectra of the human rotator cuff tissue before and after the SW treatment on the basis of the spectral data of collagens and amino acids. The proposed bands assignment of the amino acids, collagens and tissues is displayed in Table 1.

Bands attributable to vibrations of the Ag–O, Ag–N or Ag–S bonds were not observed in the low wavenumber region. Thus, the SERS data interpretation was performed on the basis that only electrostatic interactions take place. In this optical arrangement and according to the SERS selection rules,[8,9] the spectral modifications mainly consist of band intensity variations. In general, it is expected that a chemical effect on the spectrum induces substantial wavenumber shifts, relative to the Raman spectrum. Unfortunately, due to fluorescence from the tissue

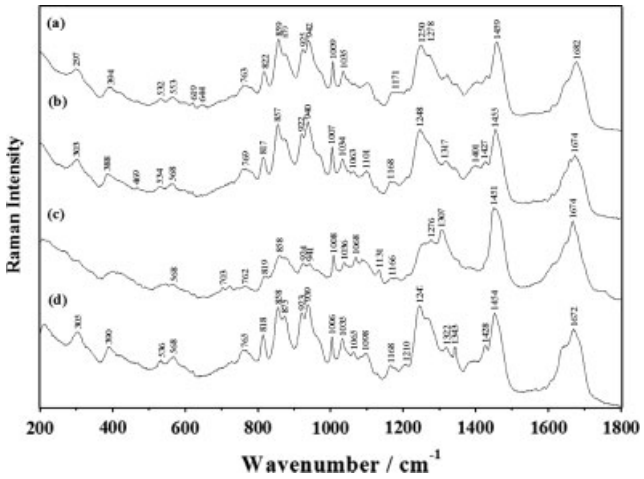

Figure 2. Raman spectra of collagens type I and III from rat and bovine tissue.

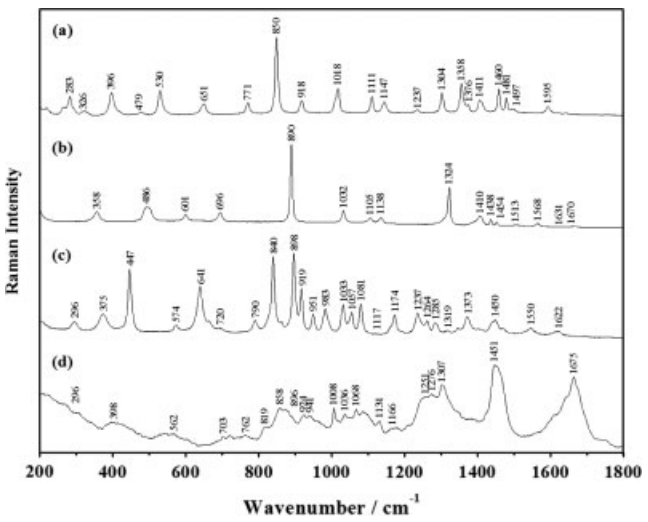

Figure 3. Raman spectra of (a) Ala, (b) Gly, (c) Pro and (d) collagen type III from rat tissue.

samples in the normal Raman spectrum, a comparison between Raman and SERS spectra could not be performed. On the other hand, chemical reactions between the metal surface and the analyte are normally verified in the case of Au surfaces which dramatically react with S, inducing even a bond cleavage. This is the reason why we have used Ag surfaces.

Raman and SERS spectra of collagens

The Raman spectral profiles of collagens I and III from rat and bovine tissue were nearly identical except that the medium band at 1307 cm^{-1} was only observed in rat type III collagen (Fig. 2). Minimal spectral differences were observed mainly in wavenumbers and some relative intensity variations could be ascribed to conformational aspects. The Raman spectra of collagen III from rat tissue along with those of Ala, Gly and Pro in the solid state are shown in Fig. 3. The spectral analysis is performed on the Raman spectrum only for type I and III collagens from rat tissue.

The band assignment of collagens was performed on the basis of the present results, data discussed in the previous section and related compounds.[29,30] The present proposed assignment

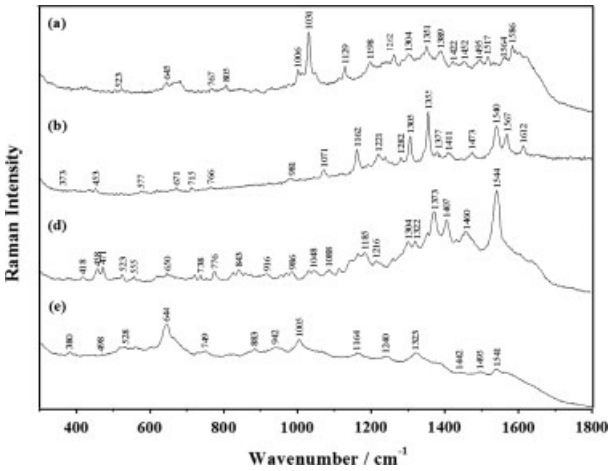

Figure 4. SERS spectra of (a) Ala, (b) Gly, (c) Pro and (d) collagen type III from rat tissue.

is shown in Table 1. The amide I bands observed at 1682 and 1674 cm^{-1}, the amide III bands in the range 1250–1280 cm^{-1} and the skeletal vibrations at 942 and 941 cm^{-1} for collagen I and III, respectively, suggest a random coil disordered conformation for both collagens in the solid state. Some amino acid bands are observed in the spectrum of collagens. This is the case for the bands of collagen type I from rat tissue at 1084, 942 and 859 cm^{-1} assigned to the νCN, νCC skeletal and ring modes of Pro, respectively. The band at 1009 cm^{-1} is attributed to Phe, while the weak band at 532 cm^{-1} corresponds to a δCCN, COO^- mode of Ala. The weak bands at 879 cm^{-1} in collagen type I and 896 cm^{-1} in collagen type III could be assigned to the amino acid Gly.

The SERS spectra of Ala, Gly, Pro and collagen III from rat tissue are displayed in Fig. 4 and the assignments of the main bands are shown in Table 1. Spectral modifications by the metal surface effect consist mainly of the disappearance of the conformational bands at 1674, 1276 and 924 cm^{-1}. The breathing Phe band at 1009 cm^{-1} in the collagens remains in SERS with the highest relative intensity and similar wavenumber; this suggests that the aromatic fragment is close to the surface, adopting a preferential orientation on the surface. The very weak Raman COO^- deformation band at 644 cm^{-1} increases in intensity by the surface effect but maintaining the same wavenumber. The Lys carboxylate stretching bands at 1323 and 1541 cm^{-1} are observed in the SERS, suggesting an approach of this group to the silver surface. The SERS selection rules indicate that a vibrational mode in which the oscillation is parallel to the incident laser will be enhanced.[8,9] On this basis, if the COO^- group is close enough to a hot spot, at least one of the stretching vibrations, symmetric or asymmetric depending on the group orientation, will be enhanced the most. In the SERS spectra of collagens, the $\nu_s COO^-$ mode is observed at 1323 cm^{-1} with a medium strong intensity, while the asymmetric mode is observed with medium to weak intensity at 1541 cm^{-1}. This suggests that this group is close to the surface but not exactly perpendicular to it.

The presence of a medium double band at 1164 cm^{-1} also suggests the possibility of the approach of hydroxylysine to the Ag surface. This amino acid is also present in the structure of collagen and is typical of this protein. Other Raman bands, mainly those corresponding to CH deformations near 1450 and 1307 cm^{-1}, are weakly observed in the SERS spectrum. Most Raman/SERS spectral changes suggest that the collagen interacts with the metal surface

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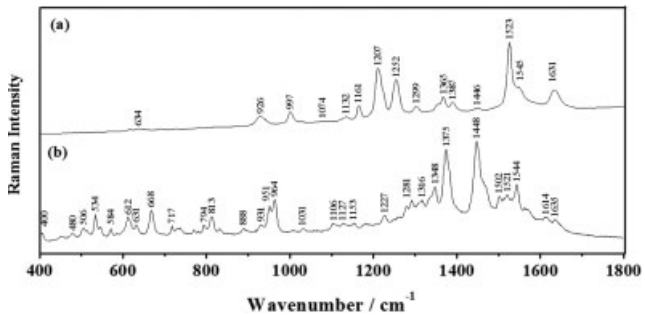

Figure 5. SERS spectra of human tissue (a) before and (b) after SW treatment.

and as a consequence modifies its conformation. The spectral behavior of the COO^- group is consistent with a net COO^- surface interaction. The above general interpretation is well supported by similar studies in the amino acids Trp,[20] Lys,[21] Cys,[22] His,[31], as well as in several others[32] including Phe and Tyr.[33] Other bands corresponding to the most abundant amino acids Pro, Gly and Ala are observed in the SERS spectrum at 942, 883 and 528 cm^{-1}, respectively. The corresponding molecular residues are therefore exposed to the metal surface.

SERS spectra of tissues

On the basis of immunohistochemical data, we focused on the SERS spectra of the collagen areas (Fig. 1). In this area, a similar spectral profile was observed for most of the tissue samples. Most of the results were difficult to analyze; however, some of them displayed reproducible signals and were finally selected for interpretation. The selected SERS spectra of tissues before (HT) and after the SW treatment (HTSW) are displayed in Fig. 5. Their wavenumbers are listed in Table 1. The SERS spectra contain bands ascribed to different vibrations of the amino acid components. In the HT, signals correspond mainly to NH vibrations at 1631, 1252, 1207 and 1161 cm^{-1} and NH and carboxylate deformations and Lys modes at 1523 and 1365 cm^{-1}. In HTSW, the most intense bands are ascribed to the aliphatic and skeletal modes at 1448, 1375, 964 and 813 cm^{-1}; medium carboxylate bands are observed at 1544, 1348 and 668 cm^{-1}. The most relevant spectral modifications (wavenumbers shift and intensity) by the SW treatment are observed for the amino, carboxylate and aliphatic as well as the skeletal bands. The spectral modifications of the molecular carboxylate and amino fragments in the SERS spectra in both tissues suggest that these groups in collagens are exposed to the metal surface in different ways. Thus, the structural changes could be associated with the conformational changes in the collagen structure. In fact, most bands attributable to the vibrations of amino acids and collagens, as indicated in Table 1, keep their wavenumbers but change the relative intensities in some cases. Moreover, in SERS, the way a molecular system interacts with the metal surface defines the spectral profile. The differences could be also related to an immature state of both collagens, considering that all biopsies were taken 8–10 weeks after the SW procedure. In normal conditions, according turnover/remodeling of collagens in tendons occurs, they must suffer conformational/structural changes also due to their relationship with other collagens and proteoglycans during maturation.

Conclusions

Immunohistochemical data of human rotator cuff samples in degenerated and SW-treated tissues directed the present spectral study to the collagen areas of the tissues. The additional presence of collagens I and III by SW treatment is concluded from an increase of the tissue's tinctorial properties. The data analyses from 1016 SERS spectra of 52 biopsies of tendon tissues coated by Ag nanoparticles before and after the SW treatment and performed on the basis of vibrational data of collagens type I and III of rat and bovine tissues and their most abundant amino acid components indicate that the spectra are mainly dominated by the vibrational modes of the amino acids in the collagen protein. A comparison of the tissues indicates that the main observed differences between both tissue samples before and after the SW treatment are related to structural modifications in collagen, probably with respect to conformational aspects. The minimal spectral differences observed in the Raman spectra of collagens were related to differences in conformation. Changes in the Raman/SERS spectra suggest that he collagen interacts with the metal surface, which modifies its conformation. A spectral study on the effect the SW has on isolated collagen systems is in progress in our laboratory.

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