Maturity of Human Bone Estimated by FTIR Spectroscopy Analysis: Implications for Ostheoporosis

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ABSTRACT

This work studies the possible variations of the properties of mineral and organic bone components with regard to the anatomical position and the patient's age. Autopsies of healthy human iliac crest have been analyzed within a wide range of ages (26-88), measuring different anatomical positions in trabecular bone by means of FT-IR spectroscopy. The study was focused on the analysis of ν1, ν3 phosphate, ν2 carbonate amida I and amida II bands. From the resulting spectra the cristallinity/maturity index, the collagen cross-links ratio and the carbonate/phosphate ratio were calculated. All of them provide information of bone mineral and collagen maturity. The results show a trend in the spatial distribution of mineral and collagen maturity in most of the samples. The most mature mineral and collagen of the bone were found to be located in the trabecular center, while the youngest were situated in the peripheral regions. However, this behavior has exceptions that seem to be related with the patient's age.

Keywords: Bone, hydroxyapatite, collagen type I, FT-IR spectroscopy.

1. INTRODUCTION

The bone matrix is a composite formed by both organic(mainly type I collagen) and mineral matter. The molecules of collagen in the organic matter form microfibrils that constitute bigger fibers through self-assembly. This fibrilar structure is completed with the deposition of small hydroxyapatite crystals which form the mineral component. The combination of both mineral and organic matter confer rigidity and resistance to the bone[1], which is essential to perform its biological supporting function.

From the mechanical point of view, the bone is made of an outer layer, known as cortical bone, and an inner spongy space conformed by trabecular bone and bone marrow. Both the trabecular and cortical bone are constantly renewed through the elimination of old and/or damaged bone and its substitution by new bone. This process is called "remodeling" and it is carried out by means of the combined action of osteoclasts and osteoblasts, specialized cells whose function is to reabsorb and to form bone tissue, respectively[2]. The remodeling process is responsible for the heterogeneity of the bone, in terms of the mineral and organic maturity, which presents different properties depending on the age of the bone tissue.

The crystal lattice that forms the mineral component is constantly undergoing ionic substitutions. The number and type of which vary with mineral age, modifying the stoichiometry. Among the existent substitutions that of carbonate ion CO_3^{2-} is important. It can occupy different places inside the lattice, leading to nonstoichiometric carbonated apatite, substituting to the group hidroxilo OH^- (type A) or the ion phosphate PO_4^{3-} (type B)[3].

The most important characteristic of collagen type I is the chemistry of its cross-linking and its molecular package, which plays an important role in the bone mineralization and gives the bone important mechanical properties. Seven main cross-links have been found in type collagen I: deH-DHLNL, deH-HLNL, deH-HHMD, pyr, d-pyr, pyrrole and HHL. The first three are $NaBH_4$-reducible and the rest non reducible[4]. The rate between both cross-link types varies with the age of the bone tissue.

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Several studies on the mineral and organic components have been carried out in homogenized bone, showing variations of their properties with regard to the patient's age in healthy bones[5] and with different pathologies such as osteopetrosis[6] or osteomalacia[7]. These studies do not consider the heterogeneity of the bone. However, the Fourier Transform Infrared spectroscopy (FT-IR) does. That is why it has been widely used to study the mineral and organic components of the human bone tissue. The variations of both components have been studied with the different anatomical positions in cortical and trabecular tissue in healthy bone[8-10] and osteoporotic bone with or without treatment[11, 12].

All these studies show the existence of modifications in the mineral and organic components of the homogenized bone tissue with the patient's age and pathology, as well as modifications with the anatomical position in healthy or pathological bone when the entire tissue is considered. However, there are few works that combine the modifications of both components with the age of the patient and, at the same time, consider bone heterogeneity. Bearing in mind that the remodeling process, intimately related with diverse pathologies, is modified with the patient's age and is the responsible for the heterogeneity of bone tissue, this type of works applied to healthy patient are very useful, since they provide some standard values of the mineral and collagen properties considering both of aspects. This gives way to a solid baseline for later studies of their possible variations with different bone pathologies. In this work autopsies of human iliac crest without pathology have been analyzed within a wide range of ages (26-88), measuring different anatomical positions in trabecular bone by means of FT-IR spectroscopy. The study was centered in the analysis of phosphate and carbonate ion bands, and amida I and amida II bands, characteristic of the collagen type. The materials and methods used in this work, as well as the discussion of the experimental results, are presented in the next sections.

2. MATERIALS AND METHODS

Human iliac crest biopsies obtained at necropsy from seven patients with ages ranging from 26 to 88 (26, 30, 41, 55, 65, 78, 88) were used in the study. Biopsies were previously subjected to a histological study in which the morphometric parameters of the bone were measured. The results were compared to the characteristic average values for each age, which indicated that they were healthy bone samples.

The bone samples were fixed with ethanol, embedded in polymethyl methacrylate (PMMA) and cut into successive 6μm-sections using a Reichert-Jung microtome. Each section was mounted on a bristol board window. Finally, the samples were dried in the oven at 40ºC during 30 minutes and stored in a dryer until their measuring. (The drying avoids the appearance of water spectra bands, since they overlap with the amida I and amida II bands falsifying the measure).

Once prepared, the samples were measured in the transmission mode by means of a Nicolet spectrometer of simple beam whose measure range is from 11000 up to 375 cm^{-1} with a spectral resolution of 4 cm^{-1}. The spectrometer has a Nicolet Continuum microscope that enables the selection of the sample area we want to analyze.

Three random trabecular selections in each bone sample were measured in three regions. These regions were selected following a line from the trabecular center to the nearest surface. The regions were denoted C (region located at the trabecular geometric center), P (adjacent to the outer edge of the trabecula) and M (between the other two).

Nine regions were measured in the samples corresponding to 26, 30, 55, 65 and 78 years old, and 8 regions in the samples corresponding to 41 and 88 years old. The following notation was used: iX(n), where i varies from 1 to 3 and it represents the corresponding trabecula, X can be P, C or M depending on the anatomical position of the region inside each trabecula, and n indicates the patient's age in years. A region of PMMA without bone was also measured in order to remove its spectral contribution.

In all the studied samples, the spectra corresponding to trabecular surface adjacent regions show characteristic bands of PMMA in the region of interest. These bands overlap with those corresponding to the mineral and organic components of bone, hindering the qualitative and quantitative analysis of the last ones. The spectra were baseline corrected and the embedding media contribution spectrally subtracted using the PMMA peak at 1725 cm^{-1} that appeared in each one of the bone spectra and did not overlap with any characteristic band of bone.

Contribution of PMMA did not appear in the spectra of the other regions, so the subtraction was not necessary. Therefore, the PMMA does not penetrate into the trabecular bone, confirming the results obtained by other authors[13].

The phosphate $\nu 1$, $\nu 3$ (900-1200 cm^{-1}), carbonate $\nu 2$ (850-900 cm^{-1}) and collagen amide I (1600-1700 cm^{-1}) bands were deconvoluted in all the spectra. The peak positions from the second-derivate spectra were used as input into a curve-fitting program. The output was expressed as peak position, peak area, intensity and FWHM of the underlying bands.

From the resulting underlying peaks, several spectra indexes were calculated. The ratio of the relative areas of the 1020 and 1030 cm^{-1} peaks in phosphate band was used to assess the mineral cristallinity/maturity index. In the same way, the proportion of non reducible/ reducible collagen cross-links, defined as the ratio of the relative areas of the 1660 and 1690 cm^{-1} peaks in amida I band was also estimated. The integrated phosphate and carbonate areas were used to obtain the carbonate/phosphate ratio, which indicates the quantity of carbonate present in the apatite phase. Finally, in those cases in which the two types of carbonate substitutions appeared in the carbonate band, the ratio of the corresponding intensities peaks (located at 872 and 878 cm^{-1}) was calculated.

3. RESULTS

The assembly of the bone samples on bristol board windows improved the results obtained with KBr disks. In previous studies where the latter was used, it was necessary to consider bone regions of a minimum dimension of 50x50 microns, which implied a high gain. However, the use of bristol board windows has allowed us to study 20x20 microns bone regions using a smaller gain. Therefore, the bristol board use has improved the space resolution and the signal to noise ratio in the resulting spectra.

3.1 Frequencies of the obtained underlying peaks and identification

The identification started from the underlying peaks obtained in the curve-fitting of phosphate $\nu 1$, $\nu 3$, carbonate $\nu 2$ and collagen amide I bands.

All the spectra show peaks corresponding to the $\nu 1$ and $\nu 3$ vibrational modes of the phosphate ion in the hydroxyapatite lattice. The peak corresponding to nondegenerate asymmetric stretching mode $\nu 1$ of phosphate group appears at approximately 960 cm^{-1}. The underlying bands are assigned to triply degenerated asymmetric stretching mode $\nu 3$ of phosphate at approximately 1030, 1060 and 1090 cm^{-1}. All the spectra also show two peaks at approximately 1020 and 1115 cm^{-1}, due to the presence of HPO_4^{2-} and /or CO_3^{2-} groups in nonstoichiometric hydroxyapatite. The underlying band located at 1078 cm^{-1} has the same origin, and appears in 3C(26), 1P(41), 1C(55), 1M(55), 2M(65), 2P(65) and 3M(65) bone regions. Lastly, many outlying bone regions and some others in the half area show a peak located at approximately 995 cm^{-1} indicating the presence of other phosphate phases.

The most intense peak on the carbonate band appears at approximately 872 cm^{-1} in all the bone regions. This peak represents type B carbonate in the hydroxyapatite lattice. In all regions, except for those located in the periphery, for 1M(26), 2M(26), 3M(26), 3M (41), 1M(65), 1M(78) and 3M (88), and in the regions 1C(26), 3C(26), 1C(55), 1M(55), 2C(55) and 2C(88). There is also another peak with smaller intensity at 878 cm^{-1} that represents type A carbonate in the hydroxyapatite.

Table1. Cristallinity/maturity index (1020/1030) and collagen cross-links ratio (1660/1690) obtained in trabecular bone regions.

	26		30		41		55		65		78		88	
	1020/ 1030	1660/ 1690	1020/ 1030	1660/ 1690	1020/ 1030	1660/ 1690	1020/ 1030	1660/ 1690	1020/ 1030	1660/ 1690	1020/ 1030	1660/ 1690	1020/ 1030	1660/ 1690
1C	1.4	1.2	2.0	1.8	1.8	3.0	3.1	2.4	1.4	2.0	2.7	2.3	2.0	4.1
1M	1.3	1.7	2.4	1.6	2.2	1.8	1.1	1.4	2.8	1.2	1.0	0.8	2.1	3.2
1P	0.4	0.8	0.7	0.6	1.3	1.2	0.5	1.0	1.2	0.8	0.5	0.6	2.7	3.4
2C	1.5	1.4	2.3	2.0	3.0	2.5	2.5	2.3	2.8	1.9	1.8	2.2	2.8	2.7
2M	0.8	0.7	2.1	2.6	1.3	2.0	1.5	1.3	1.1	1.4	1.6	2.2	3.2	3.4
2P	0.3	0.9	1.6	1.3	0.6	1.8	0.9	0.5	0.5	1.2	1.4	1.6	2.8	3.4
3C	1.1	1.0	2.7	2.2	-	-	-	-	2.4	2.6	3.3	2.4	-	-
3M	1.9	1.2	1.5	1.9	1.2	2.5	1.8	1.8	2.0	2.6	1.3	1.3	1.3	2.3
3P	0.7	1.0	1.2	1.5	0.5	0.6	1.7	1.7	2.4	2.2	1.0	0.9	1.0	1.0

In all cases there are three peaks on amida I band, at wave numbers of 1635, 1660 and 1690 cm^{-1}, and two peaks on amida II band, located at approximately 1555 and 1595 cm^{-1}. A third peak is found at approximately 1530 cm^{-1} appearing

in almost all the studied regions as well. The peaks belonging to the amida I band are components of stretching mode of carboxyl group C=O, and those belonging to the amida II band are assigned to a combination of stretching mode of C-N group and bending mode of N-H group. The spectral indexes were calculated in each one of trabecular regions. The obtained values are shown in Table 1.

3.2 Cristallinity/maturity

The obtained values in the bone regions ranged from 0.5 to 3.2. These values show a clear tendency, which points out that the mineral with higher index is in the central regions, and decreases as the areas closer to the trabecular surface are considered. If the 30 year-old sample is considered, it is observed that the index is bigger in the central regions, taking values of 2.0, 2.3 and 2.7 in 1C(30), 2C(30) and 3C(30) respectively. The middle regions present a smaller cristallinity/maturity index, taking values of 2.4, 2.1 and 1.5 in 1M(30), 2M(30) and 3M(30). The youngest bone tissue is in the peripheral regions 1P(30), 2P(30) and 3P(30), where values of the index of 0.7, 1.6 and 1.2 are obtained. This behavior is also observed in the rest of the studied regions on samples corresponding to 26, 41, 55, 65 and 78 years old. The 88 year-old sample differs from this behavior, showing high values of cristallinity/maturity index that range from 2.0 to 2.8 in most of the regions.

In order to compare the obtained index values, the average values with their standard deviation were calculated in each anatomical position (C, M and P) for each one of the bone samples. The obtained results are shown in Figure 1. The average values obtained in different anatomical positions for each age were subjected to paired t-test. The results are summarized in Table 2.

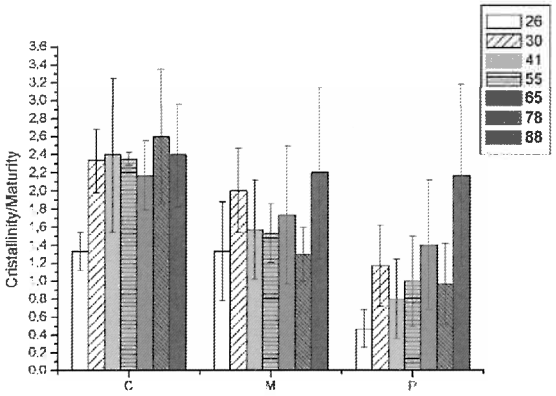

Figure 1. Obtained averaged cristallinity/maturity index values and standard deviation in C (center), M (middle) and P (peripheral) anatomical position, for each one of bone samples.

Table 2. Paired t-test between average cristallinity/maturity indexes in different anatomical positions for each sample.

	C-M	C-P	M-P
26	1.000	0.069	0.051
30	0.549	0.040	0.197
41	0.647	0.036	0.007
55	-	0.079	0.057
65	0.204	0.081	0.038
78	0.145	0.118	0.063
88	0.344	0.500	0.926

It is observed that the average values show the previously described trend. At the significant level of 0.20, there are differences between central and peripheral regions, and middle and peripheral regions in all samples, with the exception of the 88 year-old sample, which shows high values in all the anatomical positions.

It was observed that some peripheral regions showed crystallinity/maturity values lower than the corresponding average value. This happens in 1P(30), 2P(41), 3P(41), 2P(55) and 1P(78) regions. These regions are the same that present the highest intensity of the underlying peak at 995 cm-1, which represents non apatitic phosphate.

The obtained average values in the central regions do not show significant variations with age. They take, in all cases, values close to 2.4. However, the obtained average value in the 26 year-old sample was significantly smaller to that obtained in the rest of samples, with a value of 1.3. The average indexes obtained in middle regions do not show significant differences with age. They take values close to 1.7 in all cases. Peripheral regions show similar average cristallinity/maturity index with age, with the exception of the 88 year-old sample that shows a value of 2.3, significantly higher than the one obtained in the rest samples, close to 1.0.

3.3 Collagen cross-links ratio

The obtained results show a clear tendency, the same as in the crystallinity/maturity index, pointing out that the higher ratio occurs in the central regions, and decreases in the middle ones. Lower values are found in the peripheral regions. Considering the 30 year-old sample, it is observed that the ratio takes a value of 1.8, 2.0 and 2.2 in 1C(30), 2C(30) and 3C(30) respectively. A value that diminishes in the middle and peripheral regions, taking values of 1.6, 2.6 and 1.9 in 1M (30), 2M(30) and 3M(30), and of 0.6, 1.3 and 1.5 in 1P(30), 2P(30) and 3P(30) respectively. This same behavior is observed in the rest of the studied regions, corresponding to the 26, 41, 55 and 78 year-old samples.

The sample corresponding to 88 years old moves away from this tendency, showing in most regions high ratio values ranging from 3.2 to 4.1. In this case the peripheral regions show high ratio values, which are comparable to those in the central and middle ones.

In order to compare the obtained ratio values, the average ratios and standard deviation were calculated in each anatomical position (C, M and P) for each of bone samples. The obtained results are shown in Figure 2. The data were subjected to paired t-test whose results are summarized in Table 3.

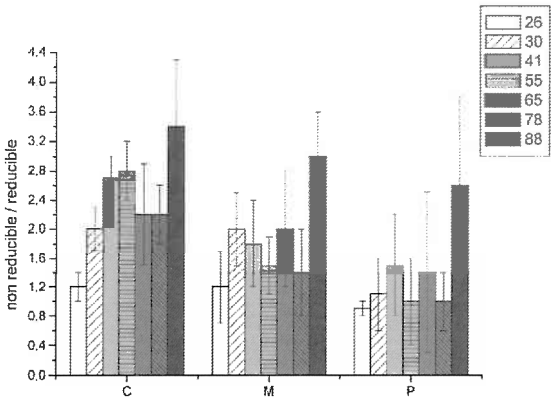

Figure 2. Obtained averaged collagen cross-links ratio values and standard deviation in C (center), M (middle) and P (peripheral) anatomical position, for each one of bone samples.

Table 3. Paired t-test between average collagen cross-links ratios in different anatomical positions for each sample.

	C-M	C-P	M-P
26	1.000	0.188	0.449
30	0.918	0.035	0.077
41	0.264	0.141	0.379
55	0.205	0.149	0.122
65	0.819	0.375	0.408
78	0.038	0.097	0.038
88	0.921	1.000	0.840

Again it is observed that the average values show the previously discussed trend. There are significant differences between central and peripheral regions in all samples, with the exception of the 65 and 88 year-old samples.

The obtained values in the central regions do not show significant variations with age, taking in all cases values around 2.6. However, the one obtained in 26 year-old sample was significantly smaller to those obtained in the rest of the samples, with a value of 1.2. In the middle and peripheral regions, the obtained average in the 88 year-old sample was significantly higher to the one obtained in the rest of samples, with a value of 3.0 versus 1.6 in the rest of the middle regions, and 2.6 versus 1.1 in the other peripheral regions.

3.4 Types of carbonate substitution

It was found in all cases that type B substitution was more common than type A. The latter was presented only in those regions with higher crystallinity/maturity and collagen ratio indexes. A ratio of 878 and 873 cm^{-1} peaks, corresponding to type A and type B carbonate substitution respectively was estimated. The obtained values are shown in Table 4. This ratio presents the highest values in those regions that show the biggest values of crystallinity/madurity index.

Table 4. Carbonate/phosphate ratio and type A/type B carbonate substitutions ratio (878/872) obtained in bone regions.

	26		30		41		55		65		78		88	
	CO32/ PO43-	872/ 878	CO32/ PO43-	872/ 878	CO32/ PO43-	872/ 878	CO32/ PO43-	872/ 878	CO32/ PO43-	872/ 878	CO32/ PO43-	872/ 878	CO32/ PO43-	872/ 878
1C	1.7	-	2.1	0.3	2.1	0.5	2.8	0.8	1.5	0.8	2.7	0.8	3.1	0.2
1M	2.2	-	2.3	0.3	1.6	0.5	2.0	0.5	1.9	0.3	2.4	-	3.4	0.2
1P	1.7	-	1.9	-	1.4	0.3	1.2	-	1.8	-	2.0	-	3.7	0.5
2C	1.2	0.2	2.1	0.4	2.0	0.5	2.6	0.5	2.1	0.6	3.7	0.4	2.7	0.2
2M	2.0	-	2.1	0.3	1.5	0.3	2.1	-	1.4	-	3.2	0.4	2.5	0.5
2P	0.3	-	1.9	0.3	0.9	-	0.5	-	2.0	-	1.4	0.3	1.8	0.6
3C	1.3	-	2.3	0.8	-	-	-	-	1.4	0.7	1.9	0.2	-	-
3M	1.8	-	1.8	0.3	1.6	-	2.3	-	1.2	0.8	1.7	0.2	2.3	-
3P	0.8	-	1.9	-	2.5	-	2.5	-	1.1	0.4	0.5	-	1.3	-

The results of the carbonate/phosphate ratio (%) obtained in each studied bone region are shown in Table 4. The average values and their standard deviation in each anatomical position for the different samples were also calculated and subjected to paired t-test. The obtained results are shown in Figure 3 and Table 5.

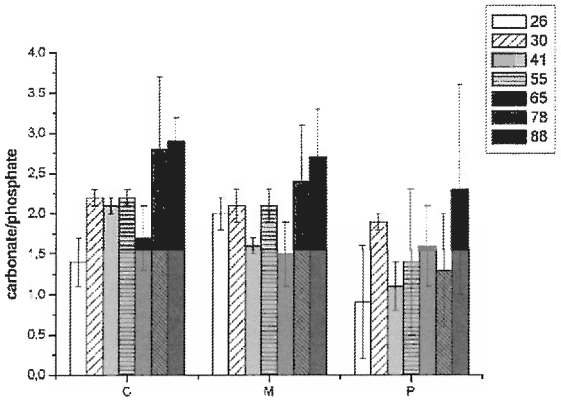

Figure 3. Obtained averaged carbonate/phosphate ratio values and standard deviation in C (center), M (middle) and P (peripheral) anatomical position, for each one of bone samples.

Table 5. Paired t-test between average carbonate/phosphate ratios in different anatomical positions for each sample.

	C-M	C-P	M-P
26	0.027	0.215	0.092
30	0.678	0.057	0.370
41	-	0.139	0.588
55	0.144	0.086	0.294
65	0.652	0.868	0.625
78	0.063	0.087	0.108
88	0.874	0.874	0.357

The averaged carbonate/phosphate ratio shows significance differences between central and peripheral regions in all cases, with the exception of 26, 65 and 88 year-old samples. The latter presents high ratio values in all anatomical positions.

The obtained values in the middle regions do not show significant variations with age, taking in all cases values around 2.1 %. In central regions the smaller ratios are founded in the 26 year-old sample, with a value of 1.4 %. The highest ones correspond to the 78 and 88 year-old samples, with values of 2.8 and 2.9 %, versus 2.0 % in the rest of samples. A Similar behavior is founded in peripheral regions, with values of 0.9 % and 2.3 % in the 26 and 88 year-old samples, versus 1.5 % in the rest.

4. DISCUSSION

The crystallinity/maturity index and collagen cross-links ratio allow the study of the maturity of bone tissue, being representative of the mineral and organic bone components respectively. The crystallinity/maturity index provides information of the group phosphate environment in the mineral lattice. Its value increases with the presence of more carbonate substitutions. Considering that the number of ionic substitutions grows with the age of the bone mineral, an increase of the crystallinity/maturity index indicates a higher mineral maturity. A collagen cross-links ratio increment is representative of a bigger collagen maturity, since reducible cross-links greatly diminish in concentration as the tissue matures.

The results strongly suggest that there is a difference in the spatial distribution of mineral and collagen maturity in all samples. More maturity bone mineral and collagen were found in the trabecular center. The youngest bone mineral and collagen were located in the peripheral regions. The samples corresponding to the 65 and 88 years old constituted an exception to this tendency. In this case the samples did not show any gradient. Both collagen and mineral of 88 year-old sample presented high values, indicating the maturity of the bone tissue in all sample regions. Carbonate/phosphate ratio shows a similar behavior. More carbonated mineral was located in the central regions. The Samples corresponding to 26, 65 and 88 years did not showed this tendency, in concordance with the results of cristallinity/maturity index and collagen cross-links ratio.

The remodeling process could justify these results. Because of the remodeling there is a change in tissue age within the same sample. This process acts on the trabecular surface and as a result there is a higher probability to find younger tissue in the peripheral regions. This could explain the general trend found in the samples raging from 26 to 78 years old. The 88 year-old sample behaved in a different way. It showed a high mineral and collagen maturity comparable to the anatomic central, middle and peripheral regions. This result is coherent with the fact that in older bones, reabsorption has prevailed over formation in the remodeling process, being more unlikely to find recently formed tissue in the trabecular surface. It could justify the absence of a maturity gradient from the center to the trabecular surface in the sample corresponding to the age of 88.

The crystallinity/maturity average values obtained in the different samples were comparable to each other in central region, showing an apparent age independence. The 26 year-old sample constitutes an exception to this trend, with an average value smaller to the one obtained in the rest of the samples. In the middle regions and, basically, in the peripheral ones, mineral and collagen corresponding to the 88 year-old sample presented a significantly higher maturity than those corresponding to the rest of ages. The same happens with the collagen cross-links ratio. Averaged

carbonate/phosphate ratio showed differences with the age in the peripheral and central regions. The smaller values were founded in the 26 year-old sample whereas the highest ones were observed in 78 and 88 year-old samples. The Middle regions showed apparent age independence.

These results indicate that for each anatomical position, changes in mineral and collagen maturity can be observed when extreme-age groups are considered. Studies with more individuals would be necessary to achieve more conclusive results.

The crystallinity/maturity index and collagen cross-links ratio results obtained in the peripheral regions showed some values much smaller than the corresponding average value in the 26, 30, 41 and 55 year-old samples. These regions are those that present the highest intensity of the non apatitic phosphate underlying pick, located at 995 cm^{-1}. It is known that in early stages of the bone mineralization process, a deposit of nonapatitic calcium phosphates, denominated percusor phases, takes place [14]. These nonapatitic phases disappear gradually during maturation or bone aging. Therefore, the pick located at 995 cm^{-1} is indicative of young mineral. Its presence in some peripheral regions is a strong evidence that these surface regions are in the middle of the formation process, although they are not recognizable by conventional morphological procedures.

Comparing the crystallinity/maturity index and the collagen cross-links ratio values in each bone region or their average values for each anatomical position, it is observed that both of them show bigger or smaller maturity in the same regions and they vary in a similar way with the anatomical position or patient's age.

In all the studied bone regions it was found that type B carbonate substitution was more common than type A. Type A substitution only appeared in regions with the highest mineral maturity. In the early stages of bone mineral HPO_4^{2-} and CO_3^{2-} substitutions that occupy PO_4^{3-} place in the crystal lattice are common. As the bone crystal matures, these substitutions decrease and type A carbonate substitution increases. Therefore, the presence of type A carbonate substitution is indicative of the mineral's maturity and the obtained results are coherent with the values of the crystallinity/maturity index.

5. CONCLUSIONS

It has been presented a study of osseous pieces obtained from several autopsies of healthy human iliac crest in several patients aged between 26 and 88. FT-IR spectroscopy was used to measure the osseous samples spectra in $\nu 1$, $\nu 3$ phosphate, $\nu 2$ carbonate amida I and amida II bands in different trabecular bone regions.

From the resulting spectra, several coefficients were calculated. The cristallinity/maturity index, the collagen cross-links ratio and the carbonate/phosphate ratio provided information of bone mineral and collagen maturity. The results show a tendency in the spatial distribution of mineral and collagen maturity in most of the analyzed samples. The most mature mineral and collagen of the bone were found to be located in the trabecular center, while the youngest were situated in the peripheral regions. However, this behavior has exceptions that seem to be related with the patient's age.

This study has provided relevant information that proves the existence of modifications in the mineral and organic components of the healthy bone tissue with the patient's age and pathology, as well as modifications with the anatomical position. This type of work applied to healthy osseous tissue is very useful, since provides some standard values of the mineral and collagen properties considering both of aspects to establish a solid baseline in order to develop similar measurements of their possible variations with different bone pathologies, intimately related with the bone remodeling process related and diverse pathologies.

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