Measurement Techniques for the Objective Diagnosis of Primary Gonarthrosis

F. Lamonaca, L.M. Caligiuri, M. Riccio, M.Vasile, and A.Nastro

***Abstract*—** The aim of the paper is to furnish a new methodology to (i) define chemical and physical parameters used as references to distinguish between a healthy human bone tissue and the one affected by primary gonarthrosis and (ii) to enrich the base of knowledge used in the biomaterials field. The methodology pointed out is based on measurement techniques used for civil engineering materials. Therefore, it is possible to assess that the obtained results depend only by the material under test and not by the specific measurement method.

Experimental results will be shown (i) to verify the suitability of the characterization method pointed out, and (ii) to allow the definition of parameters to evaluate the primary gonarthrosis diseases in human knees.

***Keywords*—** characterization, EDX analysis, measurement methods, primary gonarthrosis, SEM analysis, thermal analysis, XRD analysis,.

I. Introduction

GONARTHROSIS is a degenerative condition of knee joint with chronic and progressive course most frequently defined as changes involving articular cartilage damage (Fig.1), abnormal bone formation, reactive changes in synovial membrane and pathologic synovial fluid [1].

Knee arthrosis is the most common type of arthrosis [2]-[4]. Arthrosis can be primary and secondary. Pathogesis of primary degenerative changes in articular cartilage is based on loss of proteoglycans and decomposition of collagen skeleton of intracellular matter. In addition there is also "material fatigue" [5]-[6].

The site of initial damage remains unknown. Nowadays, it is known that in the process development, reparative reaction

"This work was partially supported by the Italian grant RIDITT, project "DI.TR.IM.MIS Diffusione e trasferimento di tecnologie ad imprese nel settore delle misure", funded by the Italian Ministry of Economic Development".

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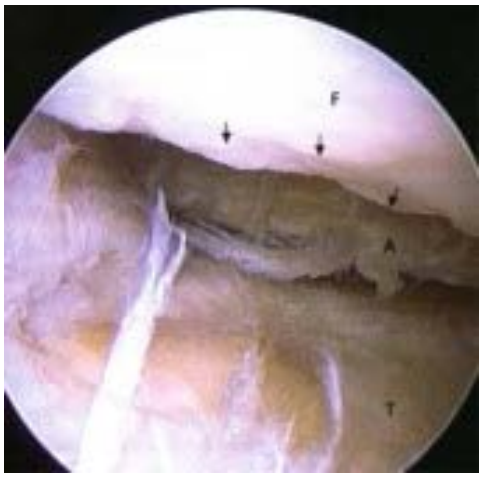

Fig.1 The loss of cartilage results in increasing stiffening and deformation of the joint [7]

of chondrocytes expressed by increase of synthesis of the main collagen types (type II, in lower degree types IX, VI, XI) and also of proteoglycans, is the primary event, at cell level [1]. The final result of the pathological process in gonarthrosis is imbalance between synthesis of articular cartilage and damage leading to its loss. Each cause or process inducing cartilage degradation exerts some effects on the occurrence and progression of gonarthrosis. Gonarthrosis is diagnosed on the basis of clinical and radiological examinations. Magnetic resonance, bone scintigraphy and arthroscopy, are also of importance. Some of these examinations depends on the pain level declared by the patients, some other from the expertise of the clinicians reading the radiological images.

In the paper objective measurement method to detect knee bone affected by gonarthrosis is presented [8]-[11]. It is based on the application of the Thermo Gravimetric (TG), Differential Thermo Gravimetric (DTG) and Differential Scanning Calorimetry (DSC) analysis to some milligram of knee bone. In order to validate the information retrieved by thermal analysis, the different crystallinity of the bone samples are evaluated by X-ray powder diffraction techniques (XRD) [12], while further information on their chemical composition is achieved by using scanning electron microscopy (SEM) [13], and X-Ray dispersive analysis (EDX)[14].

These analysis are well known and deeply applied for engineering materials [15]-[17]. The main advantages of using

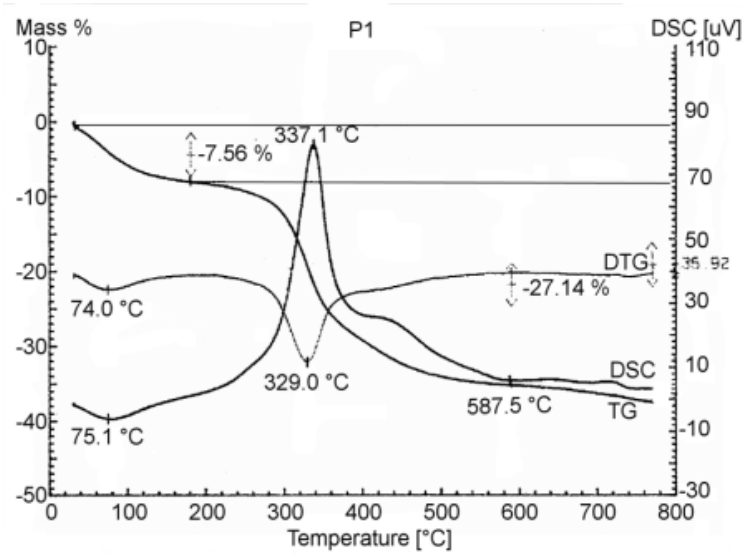

Fig.2 Thermogram of a healthy human knee bone sample (Patient#1) [19].

thermal analysis are: the use of well-known techniques permits to verify the traceability and the objectivity of the measurements; the use of reduced quantity of bone allows to be executed in ambulatory.

II. Materials and Methods

Experimental tests are executed on bone samples with weight in a range of 550-600 mg extracted by healthy knee belonging to Patient#1 and knees affected by primary gonarthrosis belonging to Patient#2, Patient#3 and Patient#4.

For the thermo analysis, the measurement system pointed out is composed by: NETZSCH STA 409 controlled by PC by using serial connection. The temperature of the curve peaks are collected automatically by PC. The weight of the used samples is 20 mg. The experimental conditions are the following: (i) reference: 20mg of Coalino Calcinato , (ii) flow speed: 15ml/min, (iii) environment: air, (iv) temperature step: 10°C/min, (v) initial temperature 20°C, (vi) final temperature 800°C. NETZSCH STA 409 allows to evaluate at the same time both TG, DTG and DSC. The advantage is the possibility to easily compare the three curves by assuming same environmental conditions. It is worth to remark that the value of the peaks depends on the balance that in the case of STA 409 has resolution 2μg. However, the peak values strongly depends from the experimental conditions and for this reason have no significance in the analysis. This justifies why the thermo-gravimetric curves can be plotted on the same figure with arbitrary scale on y axes. As concerning the temperature where the peak occurs, they are evaluated with a thermocouple with an accuracy of 0.2 °C [15].

The dried bone powders (~ 500 mg) were analyzed by XRD per phase identification and cristallinity evaluation. The XRD data were collected on Philips PW 1830 diffractometer using CuKα filtered radiation (α= 0.154050 nm), at 20 mA and 40kV, from 5° to 25°Θ degree. The Philips PW 1830 diffractometer is equipped with automatic sampler and controlled by PC.

The micrographs were taken on a Cambridge 360 Scanning Electron Microscope connected with link system EDX microanalysis.

III. Experimental Results

A. Thermal Analysis

Fig. 2 shows the TG the DTG and the DSC [8] of human healthy metatarsal head extracted by Patient#1. These curves are used as reference to compare the same curves obtained by samples extracted from bones affected by primary gonarthrosis disease.

Fig.3 shows the TG curves of bones affected by primary

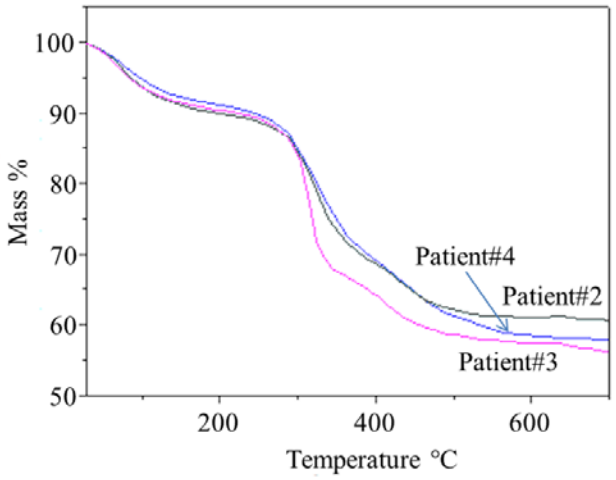

Fig.3 TG Curves of samples of bone affected by primary gonartrosis.

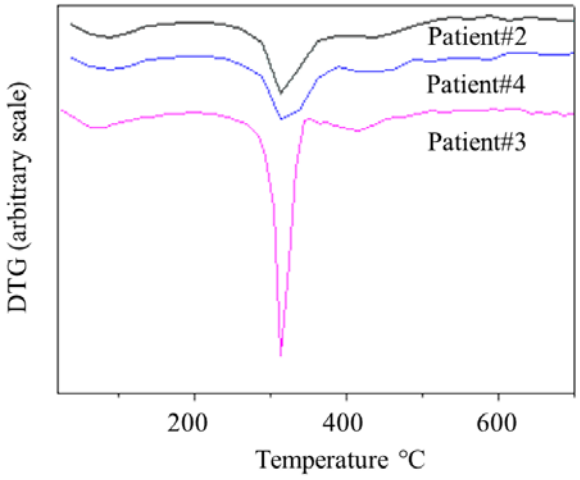

Fig.4 DTG Curves of samples of bone affected by primary gonartrosis.

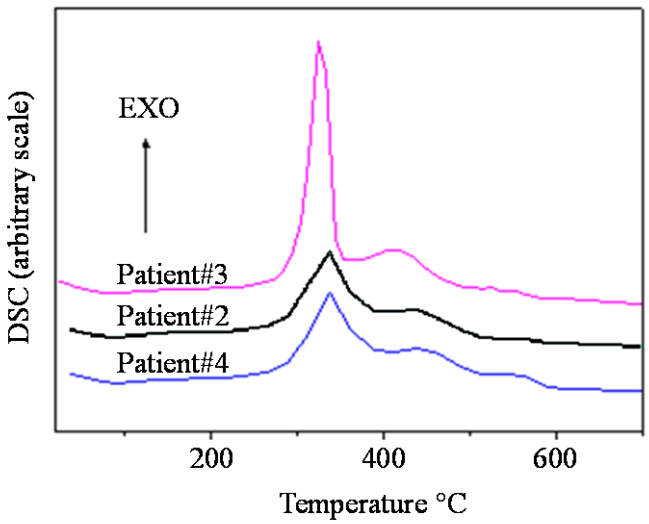

Fig.5 DSC Curves of samples of bone affected by primary gonartrosis.

gonarthrosis and belonging to Patient#2, Patient#3, Patient#4 respectively. TG curve allows to evaluate the percentage of sample weight loss respect to the temperature. Thermo-gravimetric profiles highlight that the Patient#3's sample loses more weight respect to the others.

Table I DSC peak temperatures

Sample	Age	Temp H2O loss ENDO (°C)	Temp I peak EXO (°C)	Temp II peak EXO (°C)	Temp III peak EXO (°C)
Patient #1	62	75,1	337,1	440,0	/
Patien t#2	60	87,9	333,6	432,6	540,3
Patien t#3	64	81,5	324,5	419,9	523,4
Patient #4	71	90,1	338,0	438,0	541,0

Fig.4 shows the DTG curves of the samples. In the curves there are 4 peaks corresponding in the TG at the temperature where there are weight loss.

Fig.5 shows DSC curves where can be highlighted the endothermic peak corresponding to the water loss (ranging between 75°C - 100°C), and exothermic peaks corresponding to the loss of organic matrix.

The water loss may provoke a first change in the protein molecules. After that, the organic components decomposition and burning start and it follows more than one step, according to the stage of the organic residual. Indeed, the second peak (at about 340°C) is generated by the decomposition of the organic matrix of the bone. The peak at about 420°C is generated by the degradation of residual organic material. The fourth exothermic peak at 520°C is generated by the degradation of the last organic part strictly linked to bone matrix.

The different trend and slopes of these curves respect to an healthy bone, may indicate the presence of the primary gonarthrosis. Indeed the last peak is not present in the healthy bone curves, then the degradation of the bone matrix may be related to the degeneration of the matrix, effect of the illness on the bone, confirming the recent literature [1],[2].

In table 1 are reported the temperature values where the DSC peaks occurs.

Table 2 shows the TG values. By comparing TG and DSC values, the samples affected by more severe intensity is Patient#4. This hypothesis could be validated by the fact that at 520°C there is a higher weight loss respect to the other samples.

However the main results of this preliminary analysis is that in the case of bone affected by illness there are 3 exothermic peaks instead of 2. Due to the fact that the third peak occurs after 100°C, this result can be easily observed also with measurement instruments less expensive respect to NETZSCH STA 409. Those instruments can equip ambulatories and hospitals and are able to furnish, in few minutes, information about the presence of primary gonarthrosis by analyzing few grams of bone obtained in local anaesthesia by a biopsy.

B. X-ray diffractometry (XRD)

X-rays are electromagnetic waves with a wavelength in the range of interatomic distances (0.1-10 Å). This match of length scales makes them suitable for crystalline materials study. For single-phase materials the crystal structure can be obtained directly using X-Ray powder diffraction (XRD). With the help of a database of known structures, XRD can be

Table II TG values

Sample	Age	**H2O loss (%)**	**Organic loss I peak (%)**	**Organic loss I peak (%)**	**Organic loss I peak (%)**	**Total organic loss (%)**	**Total loss (%)**
Patient#1	62	7,56	27,14	/	/	27,14	34,7
Patient#2	60	10,01	20,81	5,71	3,86	30,38	40,39
Patient#3	64	9,66	20,13	5,37	3,78	29,28	38,94
Patient#4	71	8,87	18,55	9,08	4,11	31,74	40,61

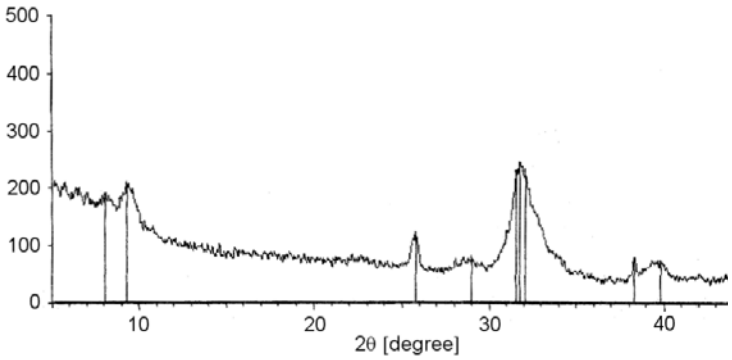

Fig.6 X-ray spectrum of a sample of healthy bone tissue[19].

Table III Number of Counts per Second (cps).

Sample	Age	2θ=26 (cps)	2θ=32 (cps)
Patient#1	62	122	229
Patient#2	60	50	119
Patient#3	64	69	282
Patient#4	71	79	289

used for phase identification [12].

For the experimental characterization of the samples, the XRD data were collected on Philips PW 1830 diffractometer using CuKα filtered radiation (α= 0.154050 nm), at 20 mA and 40kV, from 5° to 25°Θ degree. The Philips PW 1830 diffractometer is equipped with automatic sampler and controlled by PC. Moreover, the position of the peaks is automatically detected by PC. The position of the peaks depends on the crystallographic structures, while the peak intensity depends on the single crystallographic intensity axes .

It is worth to remark that the collagenic part is not detected by XRD analysis and it does not produce interferences with the definition of the pattern.

Fig.6 shows the X-ray pattern of a sample of healthy bone powder. The peaks of hydroxyapatite (mineral part) embedded in an amorphous matrix are highlighted. The low crystallinity of the bone mineral part is the normal characteristic of the healthy bones because the metastable condition, owing to the not very good crystallization of the hydroxyapatite, give to the bone the possibility of regeneration[8].

Fig. 7 shows the X-ray pattern of the samples extracted by Patient#2, Patient#3, and Patient#4 samples.

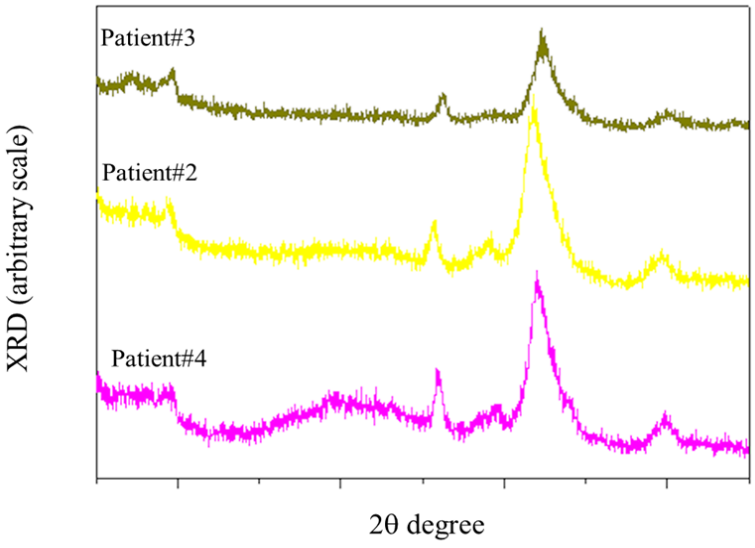

Fig.7. X-ray spectrum of the samples of bones affected by primary gonarthrosis-

By comparing Fig.6 with Fig.7, it is highlighted that the shape of the spectra is quite similar to that of a healthy bone. The data reported in Tab.3 show the intensity values of the Count per Second (cps) detected during the X-ray spectrum analysis. The three samples of bones affected by primary gonartrosis show: (i) quite similar behavior to that of the healthy bone as concerning the position of the peaks, (ii) higher crystallinity of the mineral component with respect to the healthy bone highlighted by different intensities of peaks (cps number).

It is worth to highlight that the XRD analysis is qualitative and then it does not furnish enough information to detect the degree of the diseases.

C. Scanning Electron Microscopy analysis

The Scanning Electron Microscopy (SEM) technique uses an electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected. Those signals contain information about the sample surface topography and composition. SEM can achieve resolution better than 1 nanometer according to the microscope type. [14].

Moreover, the local elemental analysis or chemical characterization is obtained by exciting the electrons of the sample with high-energy beam of charged particles (electrons or protons), or a beam of X-rays [20]. The excited electrons left their energy shells that will be occupied by electron coming from higher energy levels. When the excitation is stopped the electrons return to their initial shells giving back energy in the form of X-ray. The number and energy of the X-rays emitted by a specimen can be measured by an energy-dispersive spectrometer. The characterization is possible because each element has a unique atomic structure allowing a unique set of peaks on its X-ray spectrum [12].

Fig. 8 shows the images obtained by the SEM

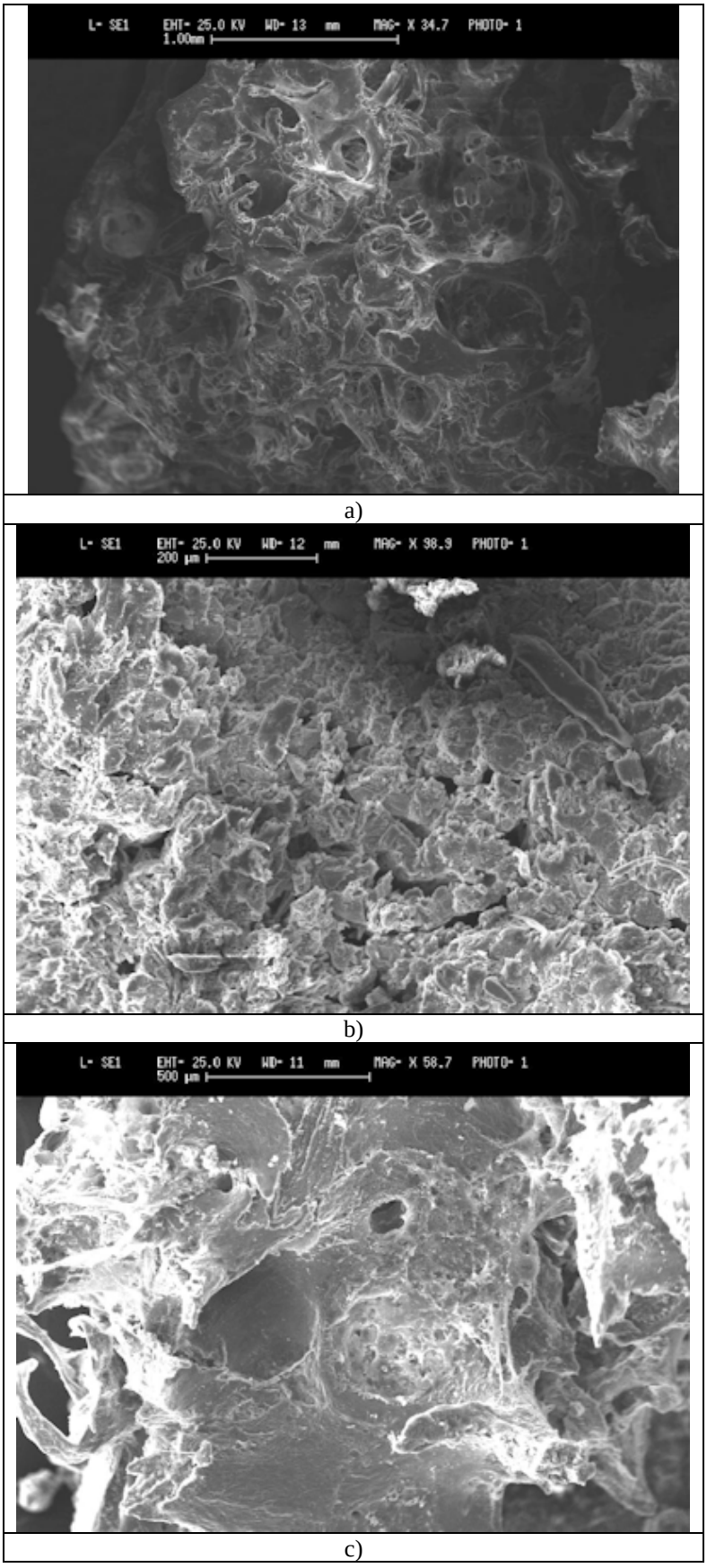

Fig.8. SEM image of the samples extracted by a) Patient#2, b) Patient#3, and c)Patient#4.

Patient#2 (Fig.8a)), Patient#3 (Fig.8b)), and Patient#4 (Fig.8c).

According to the results obtained with the thermo analysis, the SEM analysis may confirm the hypothesis that a higher intensity of primary gonartrosis disease may be due to a higher Collagen/HPA ratio as reveled by using the TG. Indeed, the Chemical analysis of the pathologic bones performed with electron microscope with energy dispersive detector (EDX) showed Ca/P ratio equal to 2.13 for the samples extracted from Patient#2, 2.68 for Patient#3, 2.10 from Patient#4. This values are higher with respect to the typical stechiometric value of the pure hydroxyapatite (1.67) [19] and different from the ratio of healthy bone (normally less than 1.67 [21]). More investigations are in progress to well define these values and the role of the Ca/P ratio.

IV. Conclusion

In the paper a new possible diagnostic measurement methods to characterize bone tissues extracted by human knees is presented. The proposed methodology is based on measurement methods and instrumentations typically applied for civil and industrial engineering materials analysis. The proper combination of well-known measurement techniques and commercial measurement instruments allow assessing the traceability of the measurement. Experimental tests are executing by performing the TA, XRD, SEM and EDX of samples extracted by healthy knee bone, and the ones affected by primary gonarthrosis furnished by Patient#2, Patient#3 and Patient#4. The experimental results highlight a different behavior of the healthy human bone tissue respect to the one affected by primary gonarthrosis disease. In particular, the exothermic peak at about 520 °C could be an intrinsic characteristic of the disease, since in the sample of healthy bone tissue, this peak is not present.

The presence of this third exothermic peak can be easily detected also with measurement instruments less accurate than the NETZSCH STA 409, and can be used as discriminant to distinguish between healthy bones and the ones affected by primary gonarthrosis.

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