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CORRELATION OF PHYSICAL-CHEMICAL PROPERTIES OF HEALTHY AND PATHOLOGIC HUMAN BONES

*Marianna Utech**, *D. Vuono, P. De Luca and A. Nastro*

Department of Pianificazione Territoriale, University of Calabria, via P. Bucci, Arcavacata di Rende (CS), 87036, Italy

Human bone tissue is composed by an organic and mineral matrix. The organic matrix is formed by collagen I and II type and non-collagenous proteins, while mineral matrix is formed by calcium phosphates and hydroxyapatite. The morphology of bone is different. Internal part is spongy, while external part is compact. This study has determined the chemical-physical properties of healthy and pathologic bones.

Keywords: *bone, EDS analysis, pathology, SEM, thermal analysis*

Introduction

The bone is a connective tissue with a particular consistency. It presents an abundant extracellular substance, named osseous matrix, in which the collagen fibres are cemented in an organic substance, named osseine [1]. In the matrix there are cavities that contain the cells with their extensions, blood vessels, nerves and bone marrow. The inorganic salts (calcium phosphate, particularly) are present as hydroxyapatite crystals. They represent ca. 50 mass% of osseous matrix. The strength and hardness of bone is function of the interaction between the hydroxyapatite crystals and collagen fibres. The hydroxyapatite crystals represent the principal form of calcium salts deposit (and phosphorous salts) [2]. The osseous matrix is organized in plates. The plates have different names as function of their position: cancellous bone and massive bone. There is not a clean boundary between two types of bone. The cancellous bones have plaited plates, forming a three-dimensional network of trabecula. The massive bones the plates are tightly attached and layered, forming particular systems, arranged around blood vessels and external surface, named Harvers systems or osteons [3]. The cancellous bone has a high clinic importance, because it is lower than that of other bone. Therefore, the fractures are where the percentage of cancellous bone is higher [4]. The organic phase or extracellular matrix is composed (90–95%) by collagen type I and non-collagenous proteins, glycoproteins, sialoproteins and phosphoproteins [5]. Thermal studies about biomaterials were carried out by Budrugeac *et al*. [6] and by Tonin *et al*. [7]. The aim of this paper is to study the chemical-physical properties of healthy and pathologic bones. The different thermal behaviour can be use as method to test the seriousness of pathology. The research is not a statistical study.

Experimental

The samples of pathologic bone considered are those affected by arthritis (coxitis). They have been delivered by the Policlinico Universitario of Magna Graecia of Catanzaro (Italy). The permission of ethical committee has been issued by the Policlinico to orthopaedic division. The samples of bone (healthy and pathologic) are prepared washing with physiologic solution and treated in acetone for 20 days, to move lipid residues, cleared by tissues with a bistoury and crashed in powder. After the buffing step the bone are insert oven at ca. 100°C and homogenised using an agate mortar. In this conditions the samples are ready to be characterized. The samples are named with the initials of names of patients (Gentilor etc.). The thermal analysis was carried out on a Netzsch 429 instrument (TG-DSC). The temperature range was 20–800°C with a velocity of heating of 10 K min^{-1} in static air. The Ca/P ratio was determined by Link EDS ZAF-4/FLS. The morphology of the samples is studied by Cambridge SEM Stereoscan 360S scanning electron microscope.

Results and discussion

Healthy bone

Figure 1 shows the thermal curves (TG-DTG-DSC) of healthy bone. The TG curve presents mass loss of ca. 35%. The DTG curve shows three peaks due to three effects of mass loss. The DSC curve shows three

* Author for correspondence: mariannautech@yahoo.it

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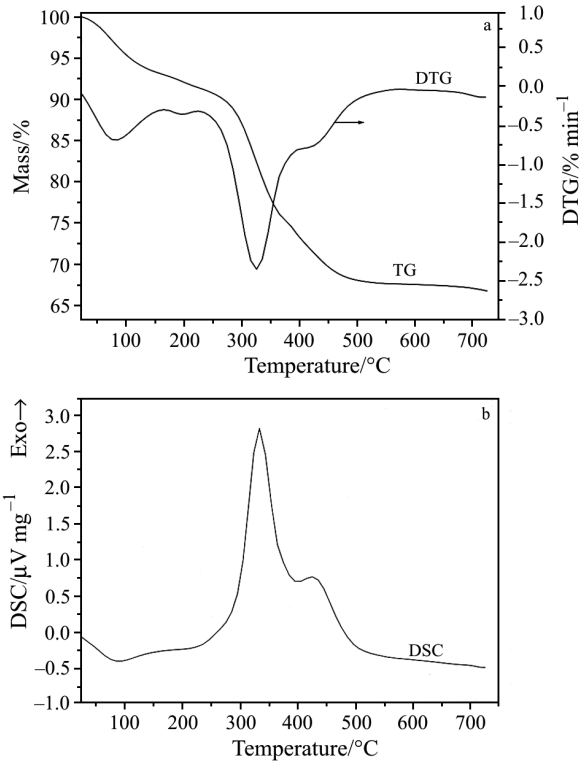

Fig. 1 a – TG-DTG and b – DSC curves of healthy bone

peaks. Between 75.1 and 98.0°C, endothermic peaks, there is the desorption of H_2O in bone. Between 319.1 and 338.1°C, exothermic peaks, there is the first decomposition of organic substances. Between 403.9 and 456.9°C, exothermal peaks, there is the second decomposition of organic substances in bone. This behaviour is characteristic of healthy bone. The peaks number never changed for healthy bone samples.

Pathologic bone

Figure 2 presents the TG curves of bones affected by primary coxitis (particular form of arthritis). Instead

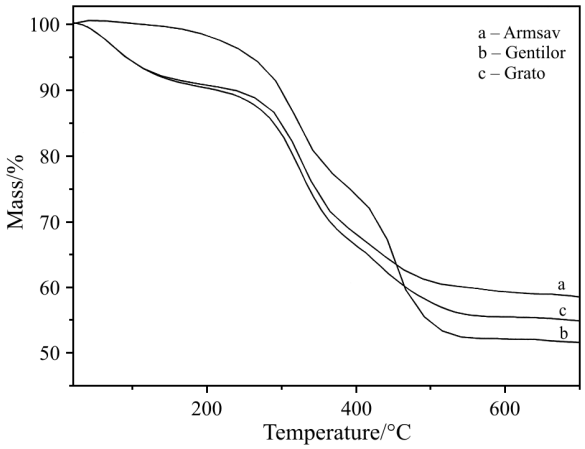

Fig. 2 TG curves of pathologic bone (primary coxitis)

Fig. 3 shows the DTG curves. In this case, the number of peaks is four for each sample. Figure 4 presents the DSC curves of these samples. The curves present a fourth peak between 462.0 and 529.6°C, probably due to the degradation of organic substance owing the pathology. Table 1 reports the DSC temperatures of samples affected by primary coxitis. DSC temperatures are in very little ranges. Table 2 presents the mass losses of samples affected by primary coxitis. The Gentilor sample presents a mass loss at ca. 501.6°C (IV DSC peak) higher than that of Armsav and Grato. This can be explained with a higher seriousness of pathology for Gentilor. This behaviour is due to a degeneration of organic matrix. The fourth DSC peak and its related mass loss is only present only in pathologic samples. The degeneration phenomenon of organic matrix is characteristic of gonitis and hernied disc too [8]. The mass losses increases with the seriousness of pathology. Figure 5 shows the DTG curves of bone affected by secondary coxitis. The number of peaks increases. This behaviour induces a dependence of pathology. There are four peaks for this form of arthritis too. In Fig. 6 is shown the DSC curves of these samples. The peak at

Table 1 Peak temperature of DSC curves of healthy and pathologic samples

Sample	Age	H_2O loss/°C endo	Org. I peak/°C exo	Org. II peak/°C exo	Org. III peak/°C exo
healthy bone	–	75.1	337.1	440.0	–
Gentilor	60	98.0	319.2	456.9	501.6
Armsav	65	84.4	336.9	436.2	541.9
Grato	72	90.8	338.1	403.9	436.4

Table 2 Mass losses of healthy and pathologic samples

Sample	Age	H_2O loss/%	Org. loss I peak/%	Org. loss II peak/%	Org. loss III peak/%	Total loss/%
healthy bone	–	7.56	27.14	–	–	34.70
Gentilor	60	3.00	21.29	11.56	11.56	47.41
Armsav	65	9.47	21.35	8.70	8.15	47.67
Grato	72	9.07	20.96	6.89	4.28	41.20

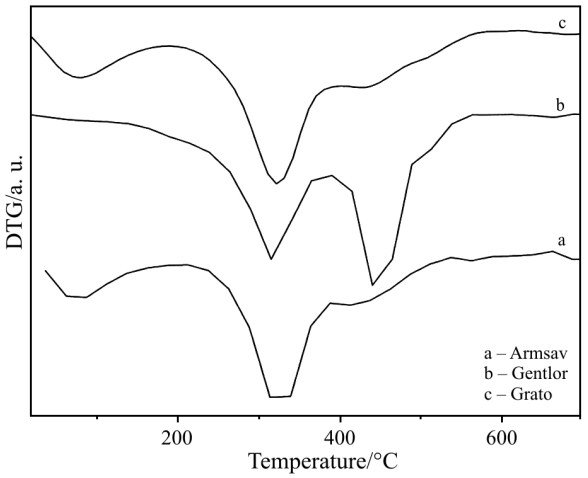

Fig. 3 DTG curves of pathologic bones (primary coxitis)

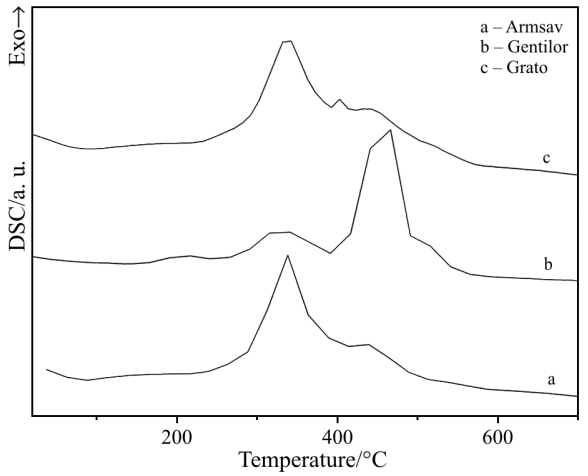

Fig. 4 DSC curves of pathologic bones (primary coxitis)

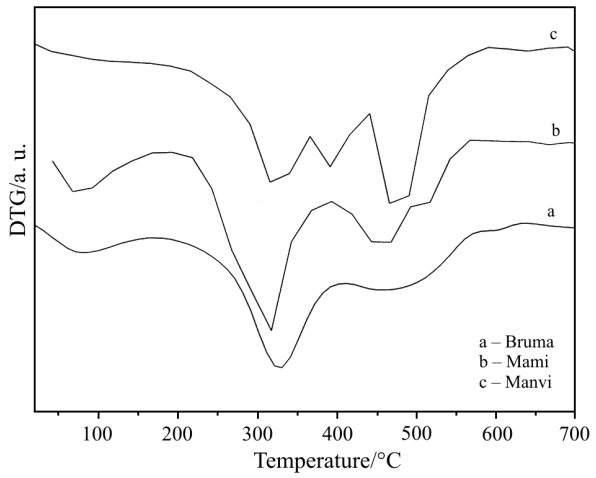

Fig. 5 DTG curves of pathologic bones (secondary coxitis)

ca. 520°C is function of pathology, as shown in primary coxitis. The mass losses are in Table 3. The sample MANVI has the mass loss higher than those of

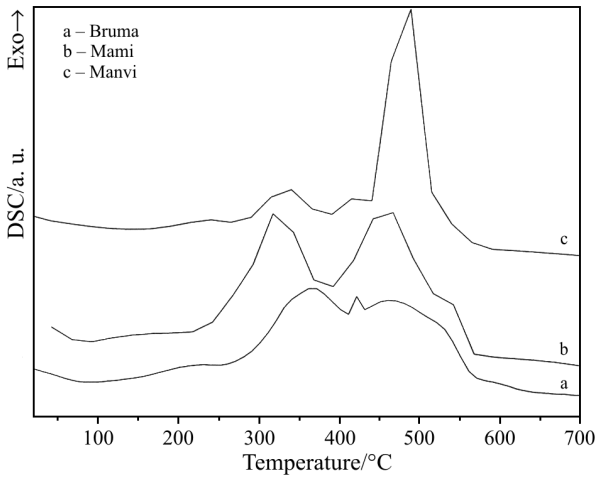

Fig. 6 DSC curves of pathologic bones (secondary coxitis)

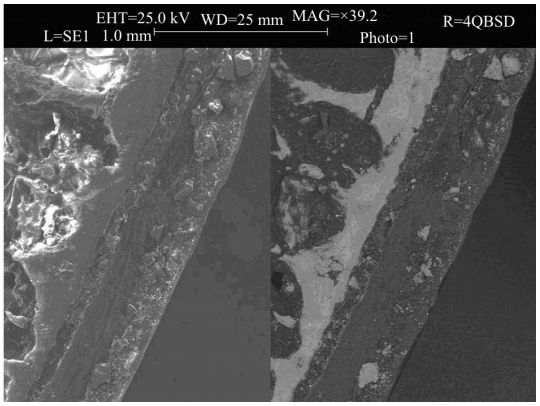

Fig. 7 SEM image of healthy bone

MAMI and BRUMA. This is function of the seriousness of pathology. Table 4 shows the DSC temperatures of samples affected by secondary coxitis.

Figure 7 shows the SEM image of healthy tissue of bone. The right side of picture is a backscattered image of sample. It can be seen the characteristic layers of this part of bone (head of femur). The different colours of layers correspond to different concentrations of elements in bone, Ca and P particularly. The EDS analysis of pathologic causes a degradation of mineral matrix. Table 5 presents the chemical analysis by EDS. The Ca/P ratio of healthy sample is very close to that of hydroxyapatite, while the pathologic sample present a different value. decreases as function of position (for light part). The internal part has a Ca/P ratio of ca. 1.69.

Conclusions

The pathologic bones present a different behaviour compared to healthy bones. The thermal analysis can be used as qualitative method to measure the seriousness of pathology. The chemical analysis determined by EDS is not function of pathology and age of patients.

Table 3 Mass losses of healthy and pathologic samples

Sample	Age	H_2O loss/%	Org. loss I peak/%	Org. loss II peak/%	Org. loss III peak/%	Total loss/%
healthy bone	–	7.56	27.14	–	–	34.70
Bruma	65	6.20	26.88	15.75	–	48.83
Manvi	71	3.96	17.49	8.04	16.93	46.42
Mami	73	10.09	28.13	17.70	6.95	62.87

Table 4 Peak temperature of DSC curves of healthy and pathologic samples

Sample	Age	H_2O loss/°C endo	Temp. I peak/°C exo	Temp. II peak/°C exo	Temp. III peak/°C exo
healthy bone	–	75.1	337.1	440.0	–
Bruma	65	92.3	365.8	419.9	462.0
Manvi	71	110.0	332.1	407.5	481.1
Mami	73	84.0	327.1	457.1	529.6

Table 5 Chemical analysis by EDS of healthy bone

	Layer of bone				
	external dark part	external light part	light medium part	dark internal part	light internal part
Na/%	0.00	0.92	1.50	0.00	0.55
σ_{Na}	0.048	0.085	0.103	0.055	0.102
S/%	4.93	1.45	0.41	0.00	0.22
σ_S	0.031	0.035	0.040	0.030	0.043
K/%	1.96	0.32	0.05	0.00	0.00
σ_K	0.033	0.034	0.032	0.027	0.041
Al/%	0.23	1.22	0.11	0.76	0.00
σ_{Al}	0.025	0.043	0.043	0.031	0.045
Si/%	0.49	1.80	0.03	1.24	0.05
σ_{Si}	0.022	0.035	0.041	0.024	0.046
Ca/%	22.05	26.68	18.30	7.52	20.10
σ_{Ca}	0.061	0.088	0.112	0.050	0.162
Mg/%	0.00	0.13	0.80	0.00	0.44
σ_{Mg}	0.021	0.058	0.072	0.034	0.072
P/%	0.57	4.43	15.84	0.64	11.87
σ_P	0.038	0.050	0.092	0.028	0.109
O/%	69.77	63.06	62.96	89.85	66.77
Sum/%	100.00	100.00	100.00	100.00	100.00
Ca/P	38.44	6.03	1.16	11.78	1.69

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