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Fatty Acid Composition in Bone Fluid from Knee Osteoarthritis Patients

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Abstract

Recently, the use of bone grafts increased in medical practice. Some bone grafts may have different mechanical and biological properties of natural bone. To improve bone grafts we need to record our knowledge about bone. In this work, we are interested in determining the chemical composition of bone fluid in the knee joint as it is a lipid-predominant fluid. Bone samples were obtained from knee cancellous bone. Samples were used of women aged 60 years old with osteoarthritis. Sample preparation is essential to guarantee the quality and dependability of the analysis results. Bone fluid was centrifuged for 15 minutes at 3000 rpm to eliminate the blood and debris. The supernatant was then gathered and filtered. Extraction of total lipids from the bone fluid according to the method of Bligh and Dyer (1959) with modification. The bone fluid of a fresh human cancellous bone was extracted by mechanical compression tests. It consists of 90% lipids. The fatty acid composition of the bone fluid was determined by gas chromatography. Methyl oleate is the most abundant.

Keywords: Bone grafting, Bone fluid, Fatty acid, Gas chromatography

Introduction

In recent years, the use of bone grafts has increased in medical practice (Miron & Zhang, 2018; Henry *et al*., 2019; Tu *et al*., 2019; Khoury, 2020; Tavares & Sheikh, 2022). It is used in trauma surgery to replace bone damage and repair bone fractures, defects, orthopedic disorders, and arthrodesis (Nandi *et al*., 2010; Zimmermann & Moghaddam, 2011; Lobb *et al*., 2019; Brink, 2021; Nazrul & Fareed, 2023). Some bone grafts may have different mechanical and biological properties of natural bone, which causes poor compatibility between bone and bone graft (Woodard *et al*., 2007; Schwarz & Herten, 2015; Kim *et al*., 2019; Chan *et al*., 2020; Böstman *et al*., 2021). In addition, immune reactions may occur in some individuals. Research continues to improve bone grafts by enhancing their biomechanical properties, biocompatibility, and ability to stimulate bone regeneration. There are many varieties of bone grafting, including autograft, allograft, and bone graft substitutes (Nazrul & Fareed, 2023). Cancellous autografts may be harvested from the proximal tibia, femur, calcaneum, olecranon, and distal radius (Schmidt, 2021). To improve bone grafts we need to record our knowledge about bone. In this study, we focus on characterizing the chemical composition of fluid inside the trabeculae of cancellous bone. This fluid participates in the response of the bone following mechanical loads. It is involved in the mechanical transduction of bone remodeling and transmitting information between bone cells (Jacobs *et al*., 1998; Qiu *et al*., 2002; Chen *et al*., 2003; Weatherholt *et al*., 2013; Lin *et al*., 2015; O'Carroll *et al*., 2018; Liu *et al*., 2019; Mi *et al*., 2019; Luo *et al*., 2020; Rubin & Rubin, 2020). The fluid movement and bone microcracks act as a stimulus to initiate bone remodeling and locally activate osteocytes (Smith *et al*., 2019), which transform the mechanical stimulus into a biochemical or electrical signal by secreting molecules (nitric oxide, osteopontin) or by increasing their concentration of intracellular calcium (Burr *et al*., 2002; Sato & Enomoto-Iwamoto, 2017; Vardakis *et al*., 2017). Authors found that the bone fluid has a composition similar to yellow bone marrow or blood plasma (Bakker *et al*., 2003; Ambrogini *et al*., 2010; Sansalone *et al*., 2013; Gómez-Barrena *et al*., 2015; Burger & Klein-Nulend, 2017; Lu & Qin, 2018; Allen & Burr, 2019; Senel *et al*., 2019; Garnero *et al*., 2020; Aghaloo & Moy, 2021; Matsuoka *et al*., 2021; Stewart *et al*., 2021; Zhang *et al*., 2021). In this work, we are interested in determining the chemical composition of the knee joint bone fluid as it is a lipid-predominant fluid.

Materials and Methods

Samples Collection and Preparation

Bone samples were obtained from knee cancellous bone. Samples were used of women aged 60 years old with osteoarthritis. Samples were recruited following the Guidelines of the Declaration of Helsinki following a protocol approved by the Ethics Committee of Rabta Hospital of Tunisia. The bone fluid inside the trabecular was obtained by mechanical compression test with the "LLOYd EZ50" machine. Then, the fluid was centrifuged at 3000 rpm for 15 minutes at 37°C. The supernatant was collected, filtered, and distributed in tubes. The samples are kept by freezing at -20°C until used.

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Extraction of total lipids from the bone fluid according to the method of Bligh and Dyer (1959) (Breil *et al*., 2017) with modification. The bone fluid was mixed with the extraction solvent consisting of a mixture of chloroform-methanol-distilled water (1: 1 :1, v /v/v). Then, the lipid extract was used to methylation the fatty acid.

Chemicals and Reagents

Chemical reagents were used in the total lipids' extraction (Chloroform CHCl3 and Methanol CH3OH). Chemical reagents were used for GC derivatization (CH3OH (0.5 M), 14% BF3, and petroleum ether (vapor pressure 7.99 psi at 20 °C).

GC Analysis

The GC used in this work was the Agilent 7890B gas chromatography. It consists of an Agilent CP6173 chromatography column (50 m * 250 µm * 0.2 µm). The flow rate was 1 ml/min. The initial oven temperature was 50 °C and held for 1 min. Then it rose to 210 °C for 10 min. The injector as standard. The injector volume was 1µl, and the temperature was 280°C. The detector temperature was 300°C, and the flow rate was 30 ml/min.

The injector makes it possible to return the sample to the vapor state and drag it into the mobile phase at the entrance of the column. The gas phase passes through the column. The different molecules of the sample will separate according to the affinity of the stationary phase with these molecules and then pass through the detector that will measure the signal emitted by the sample compounds to be analyzed

Results and Discussion

Total lipid extraction allows us to determine the percentage of lipids in a bone fluid sample. It is constituted by 90% of lipids **(Figure 2)**. The result of a GC analysis is a chromatogram which is a diagram showing the evolution of the detector signal (with respect to the solute concentration) as a function of the election time. The chromatogram provides qualitative analysis by identifying compounds by peak position and quantitative analysis by determining compound concentration by measuring peak area **(Figure 1)**. The lipid composition of the human bone fluid shows the presence of saturated fatty acids such as myristic acid (C14:0), stearic acid (C18:0), palmitic acid (C16:0), arachidic acid (C20:0), and methyl heptadecanoid (C17:0). Unsaturated fatty acids such as the oleic acid (C18:1), gadoleic acid (C20:1), linoleic acid (C18:2), linolenic acid (C18:3) and palmitoleic acid (C16:1) **(Table 1)**.

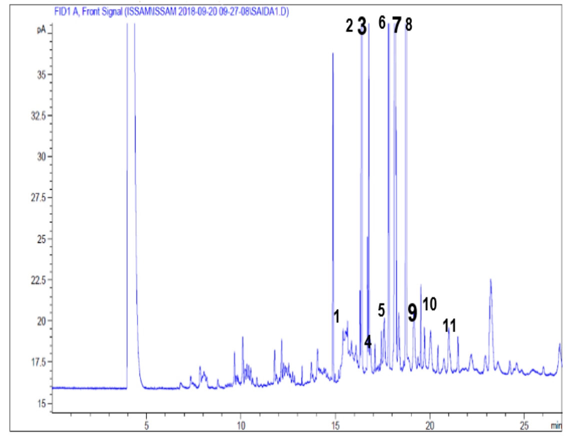
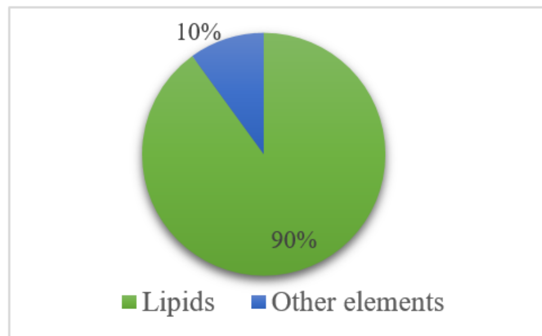
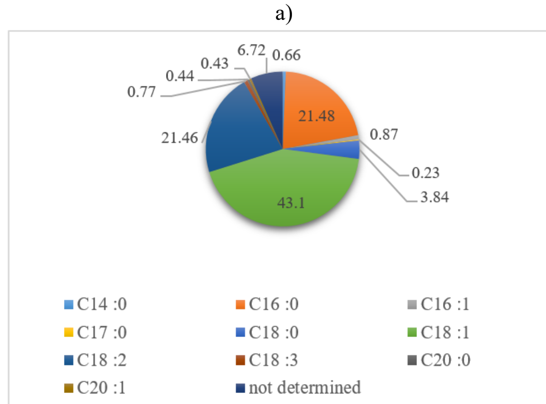


Figure 1. chromatogram of the knee bone fluid (60-year-old woman)



a)



b)

Figure 2. a) percentage of lipid in human bone fluid, b) fatty acid composition in osteoarthritis patient

Table 1. Lipid bone fluid composition by gas chromatography

No.	RT	Area	%
1	15.84	3.13854	0.19034
2	16.39	392.87674	23.82673
3	16.77	8.69672	0.52743
4	17.08	3.98513	0.24169
5	17.58	13.84467	0.83964
6	18.15	670.40521	40.65795
7	18.74	364.18152	22.08645

8	19.51	12.00959	0.72834
9	20.03	15.97711	0.96896
10	20.99	14.56668	0.88342

Table 2. Distribution of fatty acids in human bone fluid (Descriptive statistics)

No.	Name	Mean (%)	Standard deviation
1	Myristic acid C14:0	0.66	0.76
2	Palmitic acid C16:0	21.49	2.04
3	C16:1 palmetoleic acid	0.87	0.98
4	Heptadecanoic acid C17:0	0.23	0.01
5	C18 stearic acid: 0	3.85	2.75
6	C18 oleic acid: 1	43.11	5.72
7	Linoleic acid C18:2	21.47	1.13
8	Linolenic acid C18:3	0.77	0.05
9	Arachidic acid C20:0	0.44	0.46
10	11-eicosenoic acid C20:1	0.43	0.39

A descriptive statistical study was done using SPSS software to determine the average value of each fatty acid and the standard deviation between the different samples studied **(Table 2)**. The majority of compounds are oleic acid (43.1%), palmitic acid (21.48%), and linoleic acid (21.46%). Other compounds identified have lower percentages, citing stress acid (3.85%), acid myristic (0.66%), palmitoleic acid (0.87%), heptadecanoic acid (0.23%), acid arachidic acid (0.44%), 11-eicosanoid acid (0.43%) and -linoleic acid (0.77%).

Conclusion

The bone fluid of a fresh human cancellous bone was extracted by mechanical compression tests. It consists of 90% lipids. The fatty acid composition of the bone fluid was determined by gas chromatography. Methyl oleate is the most abundant. The future of bone grafting appears promising, with studies evaluating the composition of different bone tissues such as the fluid inside the trabeculae. To augment bony healing we use material similar to that of bone.

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Conflict of interest: None

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Ethics statement: Samples were recruited following the Guidelines of the Declaration of Helsinki following a protocol approved by the Ethics Committee of Rabta Hospital of Tunisia.

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