A NEW INSTRUMENTED BIOLOGICAL DEVICE DESIGNED TO APPLY MECHANICAL SHOCKS TO BONE CELLS

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Abstract: A new device called biomechanical stimulation device (BSD) has been recently developped and is under patenting process. This BSD allows to apply shocks to a biomaterial disc, on which bone cells have been seeded. To observe the real behaviour of the biomaterial under shock loading, the BSD is instrumented with an impact hammer and an accelerometer. Force and acceleration signals are recorded, and signal analysis can be performed, in particular Fourier analysis. The results obtained lead to a better understanding of the stimulus that the cells can perceive at the top surface of the biomaterial disc. It appears that mechanical shocks applied at 1 Hps (Hit per second) or 10 Hps generate a frequency content up to 35 kHz. The main further objective will be to characterize the influence of mechanical shocks on bone cells proliferation.

1 INTRODUCTION

Bone cells activity deals with several medical stakes like osteoporosis and osteogenesis imperfectae, but also with biocompatibility in the case of bone prosthesis implantation. Bone cells activity is related to mechanical stimulation. Actually, the right term for ”bone cells activity” is osteogenesis. Osteogenesis consists in a balance between bone synthesis (ensured by osteoblastic cells) and bone resorption (ensured by osteoclastic cells) (Bilezikian JP, 2002). Osteoblasts and osteoclasts do not act at the same time : it is a continuous looped process. First, the osteoclasts destroy the bone and make holes. Then, the osteoclasts withdraw and the osteoblasts take their place to form the bone. After bone creation, osteoblasts leave and osteoclasts come again to destroy the bone.

H. M. Frost showed with his Mechanostat (Frost, 1987) that the osteogenic process is strongly influenced by mechanical stimuli. Previous studies have reported different kinds of mechanical stimuli that are efficient for osteogenesis: ultrasounds, hydrostatic pressure, fluid shear stress, biaxial and uniaxial stretch, bending, nanostimulation with atomic force microscopy and acceleration forces (Tjandrawinata et al., 1997; Kacena et al., 2003; Hatton et al., 2003). The effect of acceleration forces on osteogenesis has already been analysed, but the exact stimulation that the cells could perceive remains not entirely known.

Recently, a new biomechanical stimulation device (BSD) has been developed and patented. It has been built in the Ecole Nationale Superieure des Mines de Saint Etienne (ENSMSE). This device aims at applying shocks to cells without direct contact (acceleration forces) between the cells and a mechanical impactor. Shocks are applied to a biomaterial disc on which cells are seeded. This allows to use different biomaterials in order to test their biocompatibility. Adhesion of the cells on the biomaterial depends on the physicochemical properties of this biomaterial. This adhesion is a very important factor for the cellular activity (Anselme et al., 2000; Ignatius et al., 2005; Jayaraman et al., 2004).

In order to better understand the mechanical strain and vibrational content involved, a signal acquisition and signal processing study have been performed on the BSD. More precisely, the purpose of this study was to analyse the vibrational content resulting from a

shock on a biomaterial. Biomaterial biocompatibility can be tested with the BSD, and the size of the biomaterial enables imaging and force investigations by using Atomic Force Microscopy (AFM) (with 10 mm diameter and 2 mm thickness disc). The BSD will be presented in this paper in section 2. Then, the experimental setup allowing the signals to be recorded is detailed in section 3. Next, in section 4, results of the signal acquisition will be analyzed. Furthermore, a synthesis on this study and a conclusion will be given.

2 EXPERIMENTAL DEVICE (BSD)

A schematic diagram of the BSD is shown in Fig. 1. Four different biomaterial discs (Titanium (Ti6Al4V), Hydroxy-apatite (HAP), cortical bone and trabecular bone) have been used. The discs are normally sealed into a culture chamber filled with culture medium, and cells are seeded on the top of the disc. However, all signal acquisitions have been performed without medium and cells in order to only characterise the mechanical behaviour of the disc. This is a fundamental step that is necessary to better understand what kind of vibratory stimuli the cells can perceive at the top of the biomaterial disc. During the mechanical stimulations, a vertical actuator is activated with a small and short stroke, high force solenoid, and a titanium hammer head is adapted to the solenoid stalk.

The mechanical impacts are directly driven through a current amplifier. Square type stimulation signals are used according to the current supply mode of the solenoid (DC current). Since the input signal waveform is not sinusoïdal, the signal frequency is defined in terms of hits per second (Hps) rather than in Hz. This notation is used to avoid misunderstanding between shocks application and resulting frequency content. Some authors have shown that in vitro cultured osteoblasts respond to mechanical strain at frequency values between 1 Hz and 10 Hz (Lanyon, 1984; Neidlinger-Wilke et al., 1994; Kaspar et al., 2000). The 1 Hz stimulation frequency corresponds to the human locomotor behaviour. In this study, mechanical shocks are applied at these two stimulation rates : 1 Hps and 10 Hps.

3 SIGNAL ACQUISITION

Signal acquisition is performed using two sensors coupled on the BSD, as shown in Fig. 2.

An Integrated Circuit Piezoelectric (*ICP*®) accelerometer (model 352C23, PCB Piezotronics, Inc., NY, USA) is fixed on the discs top surfaces by direct adhesive mounting and an Impulse Force Test Hammer (model 086D80, PCB Piezotronics, Inc., NY, USA) is adapted to the solenoid stalk and used as mechanical impactor. These sensors are connected to a signal conditioner (model 442B104, PCB Piezotronics) and acquired signals are recorded in a PC by means of an acquisition card (NI DAQ Pad-6015, National Instruments) with a sampling rate of 100 kHz using a program written with *LabVIEW*® software. A functional diagram of sensors monitoring, and main sensors characteristics are given in Fig. 3, 4 respectively. Accelerometer and Impulse force Test Hammer signals are recorded simultaneously during the impact. Acceleration and Force signals samples are interlaced to ensure synchronized acquisition for future joint analysis.

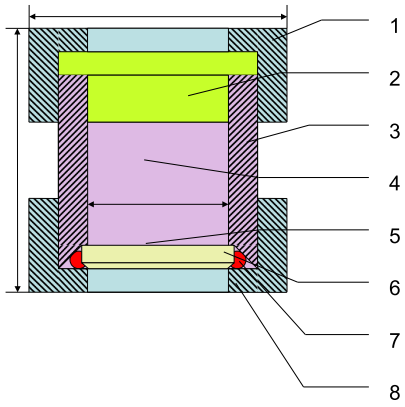

Figure 1: Schematic diagram of the BSD culture chamber used for in vitro experiments. Impact hammer is activated by a solenoid, the head of the impact hammer strikes the external surface of the biomaterial disc. Bone cells are cultured on the surface of biomaterial and the culture chamber is filled with medium. The impact hammer strikes the biomaterial with predefined frequency and duration. The different components of culture chamber and hammer head represented in the figure : (1) *Macrolon*® top lid, (2) teflon cork,(3) *Macrolon*® main part of culture chamber, (4) culture medium, (5) cell culture, (6) biomaterial disc, (7) *Macrolon*® bottom lid, (8) seal.

4 RESULTS

4.1 Signal Analysis

The experimental BSD produces two different types of signal : force signals from the instrumented impact hammer and acceleration signal from the accelerometer. Ten discs of each biomaterial have been pro-

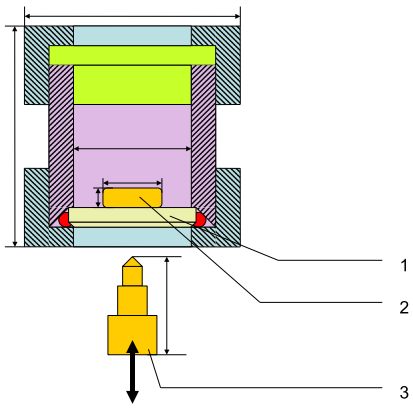

Figure 2: Schematic diagram of the culture chamber used for signal acquisition in air : (1) Biomaterial disc, (2) *ICP*®. accelerometer, (3) *ICP*® Impulse Force Test Hammer.

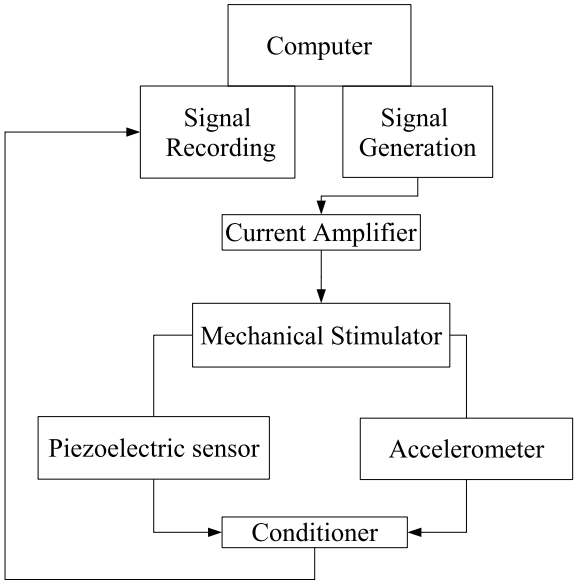

Figure 3: BSD is driven by amplified voltage current signals from the computer. During mechanical shocks, signals of *ICP*®. accelerometer and *ICP*® impulse hammer connected to BSD are simulteanously transmitted to the computer.

Accelerometer : 5 mV/g (15%) sensitivity
50 kHz frequency range

Impact hammer : 22.5 mV/N sensitivity
12 kHz Frequency Range (5%)

Figure 4: Sensitivity and frequency range given by the manufacturer for the two sensors.

cessed. Standard mean deviation have been calculated for each acceleration signals: Ti6Al4V, 783±31 g; HAP, 1197±87 g; Cortical bone, 1215±21 g; Trabecular bone, 225±24 g; and for each force signals: Ti6Al4V, 34±0.6 N; HAP, 28±0.2 N; Cortical bone, 23±0.2 N; Trabecular bone, 8±0.6 N. These values exhibit that Trabecular bone is softer than the other biomaterials.

The first step of the signal analysis consists in a zoom on the force and acceleration signals (Fig. 5) of the four materials : Ti6Al4V, Hydroxy-apatite (HAP), cortical bone and trabecular bone. This analysis enables to show important characteristics that can not be seen easily. For example, several rebounds between the impactor and the disc can be seen for only one hit. The delay between two rebounds is shortening as their amplitude decreases. A flat part can also be seen at the end of all the signals : it corresponds to the sustain time of the solenoid due to the square driving signal.

The second step of the signal analysis is the calculation of the Fourier Transform (FT) and the representation of the magnitude (Flandrin, 1993). The Fourier Transform (FT) $X(f)$ of a signal $x(t)$ is expressed as :

$$FT_x(f) = \int_{-\infty}^{+\infty} x(t)e^{-i2\pi ft}dt$$

where t denotes the time and f the frequency.

The FT has the property of expressing a signal in a frequency space.

The magnitude of the Fourier transform gives the global frequency content of a signal (Fig. 5). Usually, only the positive frequencies of the Fourier Transform magnitude are shown in Fig. 6, since they possess a physical meaning. In addition, the Shannon principle is taken into account to avoid aliasing effect. This principle is respected by the BSD signal acquisition process.

The frequency spectrums of the signals are multimodal and different for the four discs. The Ti6Al4V force signal frequency spectrum (Fig. 6a) shows that a slightly higher amplitude occurs for the Ti6Al4V than for the other materials. It is interesting to notice that HAP and cortical bone force frequency spectrums are regular (Fig. 6b,c), it might be due to the fact that they have almost the same chemical composition. Nevertheless, the cortical bone force frequency spectrum's pattern is smooth, whereas the HAP force frequency spectrum pattern is sharper. The frequency spectrum of the trabecular bone is very low, up to 1500 Hz, because the trabecular bone is softer than the cortical bone. The acceleration frequency spectrums are not regular and present several peaks, but relevant peaks can not be observed on these spectrums. However, it

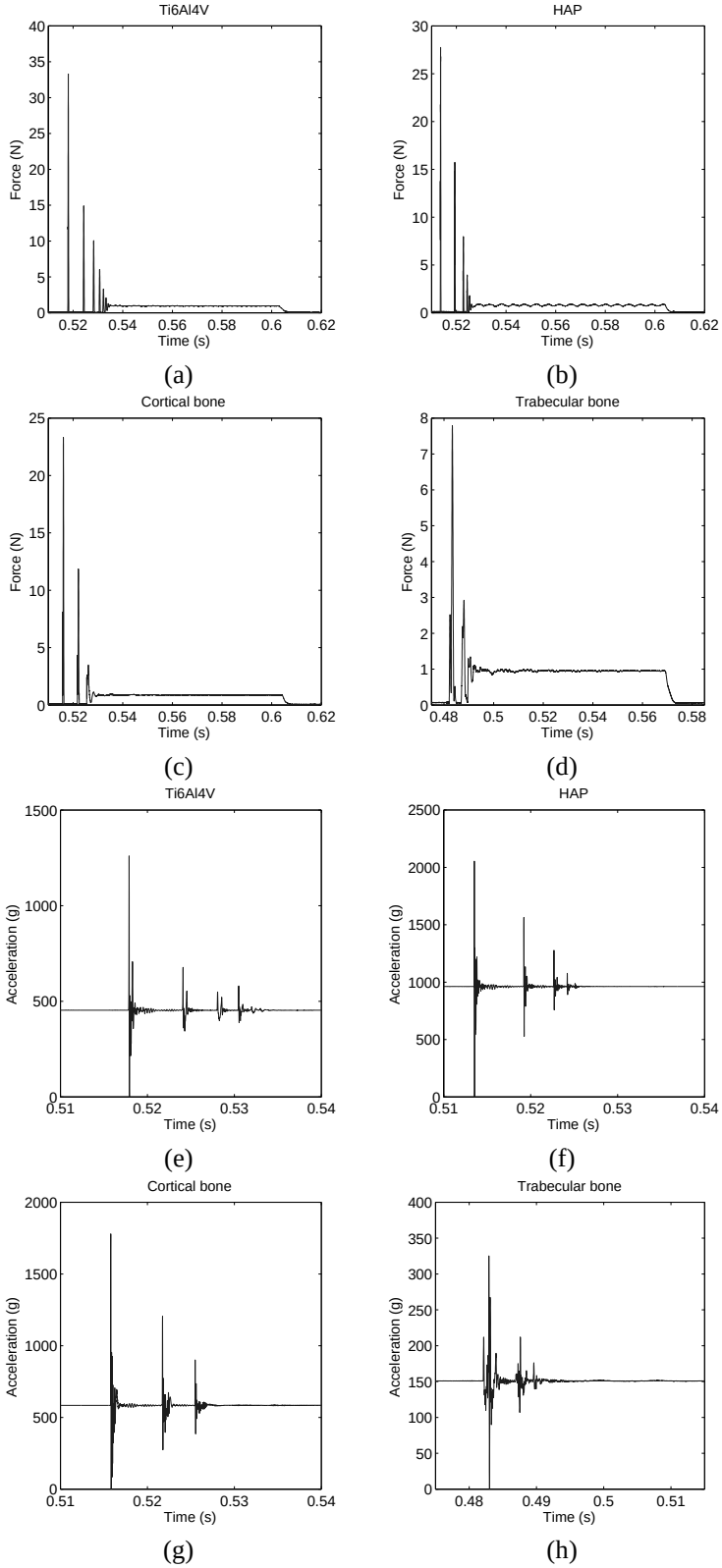

Figure 5: Force and Acceleration signals, recorded at 100 kHz sampling frequency with 12 bit amplitude resolution. (a) : Ti6Al4V acceleration signal. (b) : HAP acceleration signal. (c) : Cortical Bone acceleration signal. (d) : Trabecular bone acceleration signal. (e) : Ti6Al4V force signal. (f) : HAP force signal. (g) : Cortical Bone force signal. (h) : Trabecular bone force signal.

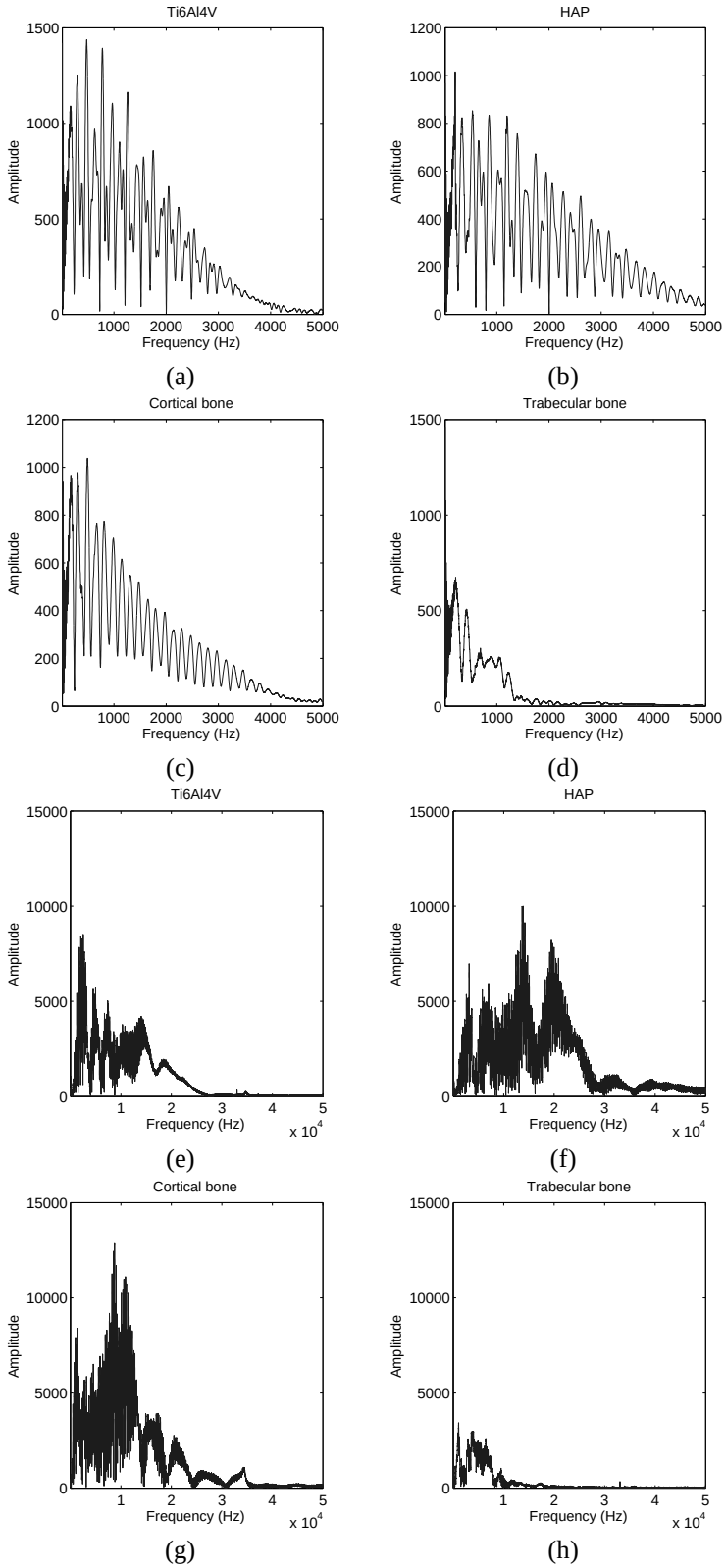

Figure 6: Fourier transform (FT) spectrums of the acquired force and acceleration signals. (a) : magnitude of Ti6Al4V force FT. (b) : magnitude of HAP force FT. (c) : magnitude of cortical bone force FT. (d) : magnitude of trabecular bone force FT. (e) : magnitude of Ti6Al4V acceleration FT. (f) : magnitude of HAP acceleration FT. (g) : magnitude of cortical bone acceleration FT. (h) : magnitude of trabecular bone acceleration FT.

is noticeable that the cortical acceleration frequency spectrum is higher than the others.

4.2 Energy

From the acceleration signal recorded, the kinetic energy of the Ti6Al4V disc/accelerometer unit during the mechanical shock is computed with the classical formula

$$E_k = \frac{1}{2} m \int v(t)^2 dt.$$

(with E_k: kinetic energy, m: mass , v: speed, given for each disc-accelerometer unit.)

The speeds of the different disc-accelerometer units are computed by integration of the acceleration signals, which occurs in the data processing sequence after application of 30 Hz Butterworth high-pass filter to remove continuous component. The frequency response curve of the Butterworth filter is practically flat in the passband. However, the use of this kind of filter introduces a non linear frequency dephasing. In this study, only the frequency content is expected and not the signal phase, thus the application of Butterworth filter is well adapted. The kinetic energy is calculated per surface unit: Ti6Al4V, 4.8$\pm$0.1 pJ/μm^2 for 1 Hps and 50.2$\pm$1.1 pJ/μm^2 for 10 Hps; HAP, 2.0$\pm$0.04 pJ/μm^2 for 1 Hps and 20.6$\pm$0.6 pJ/μm^2 for 10 Hps; Cortical bone, 8.5$\pm$0.2 pJ/μm^2 for 1 Hps and 65.1$\pm$3.6 pJ/μm^2 for 10 Hps; Trabecular bone, 0.9$\pm$0.2 pJ/μm^2 for 1 Hps and 8.8$\pm$3.2 pJ/μm^2 for 10 Hps. Notice that values of kinetic energy at 10 Hps are 10 times higher than those at 1 Hps.

5 SYNTHESIS

This new BSD was initially developed to simulate the effects of mechanical impacts on bone cells cultured on biomaterials to compare them with the effects of impacts during walking or running activities. A previous study on Ground Reaction Forces (GRF) (Giakas G, 2001) has shown that frequency content during impact phase of running is limited to 100 Hz, acceleration magnitude is $\sim$10 g. During shocks applied with the BSD, a frequency range comprised between 100 Hz and 4 kHz, an acceleration magnitude of 783,1$\pm$ 32,5 g at 1 Hps and an acceleration time of $\sim$1 ms have been found on Ti6Al4V for example. So it is quite difficult to directly put in relation the results obtained during this kind of mechanical impact with those obtained by GRF studies.

In this study, recording conditions for acceleration and force signals were slightly different from conditions used in case of in vitro experiments, particularly concerning the sealing of the culture chamber.

The disc should be excited by a frequency higher than his resonnant frequency to enter in resonnance. To verify this assumption, a numerical simulation was perfomed and the first resonant frequency mode of the Ti6Al4V disc was found to be at 144 kHz, which is much higher than the frequencies observed on the force signal Fourier transform. Consequently, the disc can not enter in resonance and induces some self-frequencies. The results found during signal recording can be extrapolated to in vitro conditions.

When mechanical shocks were applied to the discs, whatever the signal stimulation frequency was (1 Hps and 10 Hps), one could observe that the shape and the mean maximal value of the force and acceleration signals were identical. Analysis of acceleration signals leads to an estimation of the kinetic energy of the tested disc during one impact. It has been found that the amount of kinetic energy at 10 Hps is 10 times greater than that calculated at 1 Hps. Considering these results, in the case of mechanical shock, it appears that it is more interesting to analyse more accurately the frequency content of force signals, to compare kinetic energy derived from accelerations signals, and to focus on the force perceived by cells when they are subjected to acceleration phase. By applying the fundamental law of dynamic, the acceleration force during impact has been calculated by estimating cellular mass (1 picogramm): a force of $\sim$1 nN per cell has been found. This acceleration force is comparable to forces for which biological events have been previously observed. Consequently, it can be expected that this new kind of mechanical stimulation would have biological effects on bone cells.

A critical analysis can be done concerning the reproducibility of the acceleration and force peaks' measurements. The values and their errors are for each material: Ti6Al4V, 783$\pm$31 g; HAP, 1197$\pm$87 g; Cortical bone, 1215$\pm$21 g; Trabecular bone, 225$\pm$24 g; and for each force signals: Ti6Al4V, 34$\pm$0.6 N; HAP, 28$\pm$0.2 N; Cortical bone, 23$\pm$0.2 N; Trabecular bone, 8$\pm$0.6 N. The error is less important in the case of hard materials (cortical bone acceleration error is $< 2\%$ for example). However, a 10% error occurs on the measurements of acceleration and force peaks on the trabecular bone. This can be due to the non-heterogeneity of the material and probably to the non-flatness of the surface. In fact, the guidance of the hammer is not perfect so the head of the hammer does not hit in the same place every time. This is an improvement to ensure to the BSD for future work. An other improvement has been already done, it consists in a screw that allows to ajust the stroke of the

hammer. This leads to the possibility of controlling the peak value and it reduces drastically the errors between different discs (The error for one disc is about 10 times smaller than that between discs).

6 CONCLUSIONS

A new device designed to apply mechanical shocks to bone cells cultured on biomaterials has been developed. It allows to measure and compute shock parameters during impact : value and frequency content of force impact, acceleration and kinetic energy for each disc. When signals' characteristics during impact at different stimulation frequencies (1 Hps and 10 Hps) are compared, similar characteristics are found for force signals but acceleration signals and kinetic energy are different. Moreover, the computed value of the acceleration force should lead to the observation of cellular responses. In conclusion, this new mechanical stimulator could be used for in vitro studies to better understand bone cells mechanotransduction during impacts.

Further studies, particularly concerning the biological effects of mechanical shocks on bone cells, will be presented in future papers. Studies concerning other signal analysis tools like time-frequency or time-scale representations will also be held, since it seems interesting to know if the BSD is a new way to characterize biomaterials. Other biomaterial will be tested, and Atomic Force Microscopy (AFM) will be used in order to observe the bone cells behaviour before and after mechanical shocks loading.

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