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Noninvasive Cellular Quantity Measurement in Bone Marrow Stromal Cells/ β-tricalcium phosphate

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***Abstract*— This paper describes noninvasive cellular quantity measurement in Bone Marrow Stromal Cells/ β-tricalcium phosphate. We attempt to identify cellular quantity with an ultrasonic system. The ultrasonic waves are reflected at boundaries where there is a difference in acoustic impedances of the materials on each side of the boundary. Therefore, we focus on the reflected signal. From the obtained ultrasonic data, we extract two features; amplitude and frequency. Amplitude is obtained from the raw ultrasonic wave, and frequency is calculated from frequency spectrum obtained by applying cross-spectrum method. Therefore, we suggest the superiority of frequency to analyze Bone Marrow Stromal Cells. This study shows the ability of intervention to produce the desired beneficial effect.**

I. Introduction

Regenerative medicine is working to restore structure and function of damaged tissues and organs. Typical examples of conventional filling material are autogeneous bone [1]-[3], allograft bone, and artificial bone. Autogeneous bone has the problem of quantitative limitation because the patients have to provide their own normal bone to heal the defects. Allograft bone has much trouble to find appropriate donors and to treat the administration of immunosuppressant. Artificial bone has the problem of low affinity to human bone. Recently, a composite of Bone Marrow Stromal Cells (BMSCs) is a potential solution for these problems [4]. The material has high affinity to human bone [5]-[6]. The BMSCs are mesenchymal stem cells. Generally, the cells are count by an electron microscope after crushed [7]. However, the crushed cells can never be used. This paper describes a novel nondestructive ultrasonic system for identifying the cellular quantity in the Bone Marrow Stromal Cells/ β-tricalcium phosphate with amplitude and frequency. In clinical setting, it is important to choose composites containing an appropriate number of cells before implanting them to patients [8]. The ultrasonic system has many advantages such as dynamic imaging, compactness and low cost. This system does not damage materials [9]. This study extended our works in [10]. This system measures the transmitted ultrasound wave through these composites. We employ 1.0 MHz ultrasonic wave, whose frequency can transmit the bone tissue [11]. For constructing the system and identifying the cellular quantity, two features; amplitude and frequency, are employed. The amplitude is measured from the raw wave, and the frequency is calculated from frequency spectrum of a transfer function using the cross-spectrum. We certify the superiority of the frequency data analysis. Moreover, we establish the validity for measuring the cellular quantity in BMSCs.

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II. Preliminary

The Bone Marrow Stromal Cells/ β-tricalcium phosphate used in our study is *Osferion* produced by Olympus Terumo Biomaterials Corp in Fig. 1. Osferion is macroporous ceramics with 75 % porosities based on beta-tricalcium phosphate. Figure 2 shows porosity of Osferion. This culture bone is commercially available. Its dimension is 10 mm × 10 mm × 10mm. Average pore size is 100-400 μm diameters. These are sterilized in a dry heater at 180°C for 4 hours.

Fig. 1. Bone Marrow Stromal Cells/ β-tricalcium phosphate.

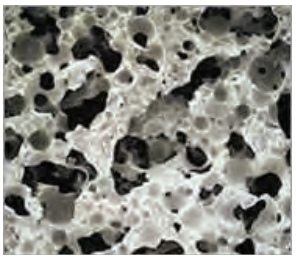

Fig. 2. Porosity of *Osferion.*

As shown in Fig. 3, we use the Bone Marrow Stromal Cells cleared under Japanese Government guidelines for the care and use of laboratory animals in this study. We obtain the cells by the bone marrow of 15-week-old Sprague-Dawley rats. Average diameter of BMSCs is about 40μm.

Fig. 3. Bone Marrow Stromal Cells.

The BMSCs cannot be alive in atmosphere. The raw BMSCs contains amount of air. First, we soak the BMSCs in culture medium in Fig. 4. Second, we decrease pressure to remove air from the bone in Fig. 5. The degassed culture bone

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is filled with medium.

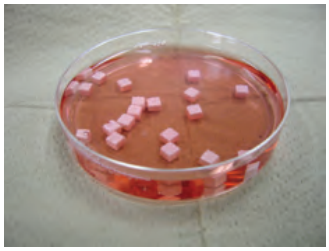


Fig. 4. Soak BMSCs.

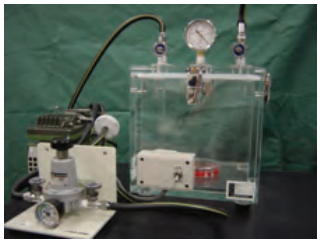


Fig. 5. Decrease Pressure.

As shown in Fig. 6, we performed the implantation of the BMSCs in the degassed bone. The seeding BMSCs has 4 cellular quantity, 1.0×10^6, 1.5×10^6, 2.0×10^6, 10×10^6 cells/ml.

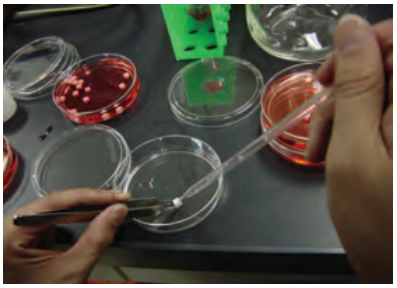


Fig. 6. Implantation of BMSCs.

References [12]-[13] described that mesenchymal stem cells cultured in vitro can differentiate immediate osteoblast cells. Thus we cultivate the seeded the BMSCs in 24 hours in Fig. 7.

Fig. 7. Cultivating the Seeded the BMSCs.

III. Measuring Cellular Quantity

When the Bone Marrow Stromal Cells/ β-tricalcium phosphate are implanted, it is important to choose composites that contain favorable number of cells before implantation. The number of seeded cells in the composites is a crucial factor for achieving successful bone tissue regeneration.

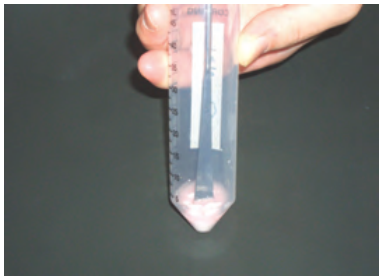


Fig. 8. Grinding Artifical Culture Bone.

Currently, the measurement of cellular quantity in the composite needs to collide the composite, and then we count the cells with an electron microscope [7]. After cultivated the seeded culture bone in 24 hours, we grind the Bone Marrow Stromal Cells/ β-tricalcium phosphate in test tube in Fig. 8. With an electron microscope, we obtained an image in Fig. 9. From the image, we count the number of the cells visually.

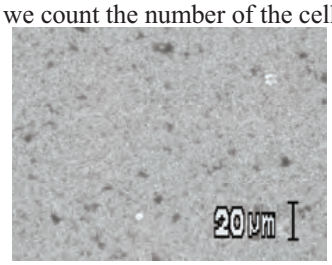


Fig. 9. Magnifying bone after cultivated.

However, the measured composite is not used for any future study or clinical practice. Therefore, we proposed the noninvasive measurement method using the ultrasonic system. On the image obtained by an electron microscope of the Bone Marrow Stromal Cells/ β-tricalcium phosphate, there are 800 blocks. In this experiment, we measured amount of cells in one block by the electron microscope. Therefore, the truth cellular quantity is obtained by multiplying 800. These ranged from 145 $^{(\times800)}$ to 836 $^{(\times800)}$ cells/ml. We assume that the cell number ranges from 0 to 1,000 $^{(\times800)}$ cells/ml.

IV. Ultrasonic Measuring System

For a noninvasive measurement of the Bone Marrow Stromal Cells/ β-tricalcium phosphate, we proposed the ultrasonic system. Our proposed system consists of an ultrasound caliper probe, a pulsar receiver, an oscilloscope, and a personal computer in Fig. 10. The center frequency of the probes is 1.0 MHz. The sampling interval is 5 ns. The ultrasound device constructs an ultrasound caliper probe produced by NSI Corporation. As shown in Fig. 11, the transmitting and receiving probes are attached to two points of outside jaws of the electronic caliper.

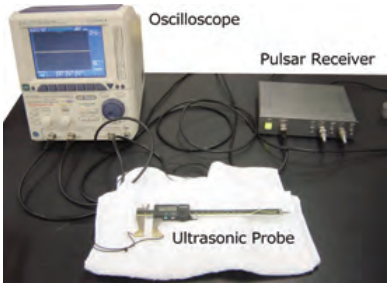


Fig. 10. Ultrasonic System.

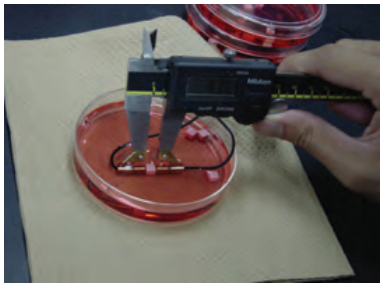


Fig. 11. Ultrasound Caliper Probe.

As shown in Fig. 12, we acquire the ultrasound wave by using the ultrasonic system. We transmit the wave to each of three directions, three times for each bone. In total, these are the obtained nine waves.

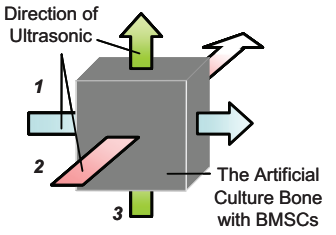


Fig. 12. Measuring Direction of Ultrasound Wave.

V. Ultrasonic Data Analysis

The Sound travels through materials under the influence of sound pressure. The acoustic impedance is important in the determination of acoustic transmission and reflection at the boundary of two materials having different acoustic impedances. The ultrasonic waves are reflected at boundaries where there is a difference in acoustic impedances of the materials on each side of the boundary. We show the reflected signal intensity as to the product of its density in Fig. 13. The higher the product of its density is, the higher the reflected signal intensity appears. Therefore, we focus on the reflected signal. From the obtained ultrasonic data, we extract two features. The first one is the amplitude [8], and the second one is the frequency.

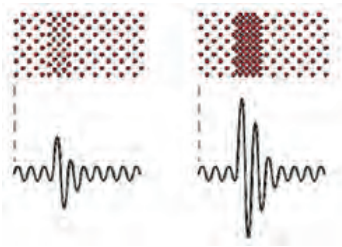


Fig. 13. Reflected Signal Intensity.

A. Amplitude

The amplitude is the value between maximum and minimum in the wave in Fig. 14. The amplitude is calculated as the mean of the obtained nine waves.

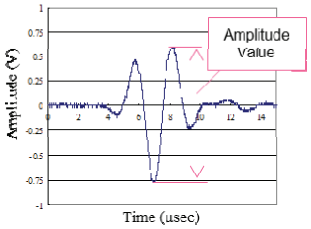
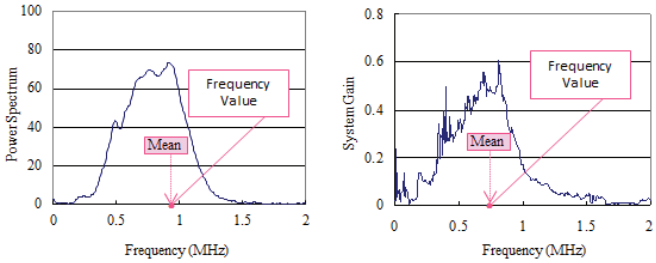


Fig. 14. Amplitude.

A. Frequency

In order to calculate the frequency value, we suggest two approaches, which is Fast Fourier Transform in Fig. 15 (a) and Cross-Spectrum in Fig. 15 (b). The frequency values using both approaches are defined with center of gravity. The center frequency of the ultrasound is 1.0 MHz, thus the frequency response in more than 2.0 MHz is almost never intensity. Therefore, the center of gravity is calculated from 0 to 2.0 MHz.



(a) Fast Fourier Transform (b) Cross-Spectrum

Fig. 15. Frequency Analysis

We focus on the attenuation due to viscosity, which is proportional to the squares of the frequency [14]. We have a discussion about the attenuation of frequency. According to this law attenuation of sound α is proportional to the dynamic viscosity η, square of the sound frequency ω, and reciprocally proportional to the fluid density ρ and cubic power of sound speed V:

$$\alpha = \frac{2\,\eta\,\omega^2}{3\,\rho\,V^3} \qquad (1)$$

We employ the ultrasonic waves with the center frequency of 1.0 MHz. As given (1), the attenuation is proportional to the squares of the frequency. The frequency range of approximate 1.0 MHz value attenuates.

VI. Experimental Results

In this study, 24 Bone Marrow Stromal Cells/ β-tricalcium phosphate were employed. By using the ultrasonic waves, we acquired two features: amplitude and frequency. Lastly, the truth cellular quantities were obtained by using an electron microscope. The results show in Table. 1. In order to obtain the frequency value, we apply two techniques: FFT and Cross-Spectrum. As to the correlation coefficient(R) between the frequency and the cellular quantity, the FFT result has R=0.659 (R^2=0.434) and the Cross-Spectrum result has R=0.766 (R^2=0.587). When the coefficient of determination (R^2) is more than 0.50, these data are considered highly correlated. Therefore, Cross-Spectrum is superior to analyze the frequency of the Bone Marrow Stromal Cells/ β-tricalcium phosphate. Moreover, Table 1 shows all the frequency results of Cross-Spectrum are low ones of FFT in each composite. The experimental results show the features of the frequency attenuations for the reason that the higher the product of its density is, the higher the reflected signal intensity appears.

Figure 16 and 17 show the relationship of the cellular quantity to the amplitude and frequency. The correlation coefficient between the amplitude and the cellular quantity is 0.750, and the correlation coefficient between the frequency and the cellular quantity is 0.766.

TABLE I. DATA ANALYSIS OF CELL QUANTITY

NO.	Cellular Quantity	Amplitude	Frequency	
			FFT	Cross-Spectrum
#01	215	1.128	0.769	0.678
#02	145	0.856	0.781	0.661
#03	240	1.248	0.781	0.687
#04	199	0.994	0.766	0.671
#05	185	0.968	0.758	0.672
#06	171	0.758	0.770	0.704
#07	481	1.819	0.814	0.746
#08	439	1.532	0.805	0.723
#09	271	1.105	0.786	0.698
#10	440	1.672	0.820	0.748
#11	195	0.958	0.769	0.683
#12	281	1.237	0.797	0.704
#13	680	1.674	0.807	0.735
#14	430	1.229	0.772	0.678
#15	418	0.716	0.747	0.656
#16	728	0.864	0.764	0.670
#17	792	1.210	0.784	0.695
#18	470	1.084	0.766	0.677
#19	426	0.996	0.755	0.654
#20	676	1.350	0.788	0.699
#21	488	0.656	0.735	0.650
#22	836	1.481	0.782	0.700
#23	544	1.096	0.772	0.685
#24	514	1.173	0.775	0.689

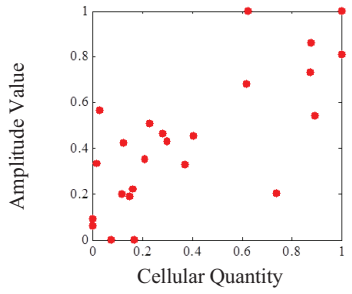


Fig. 16. Amplitude and Cellular Quantity (correlation coefficients: 0.750).

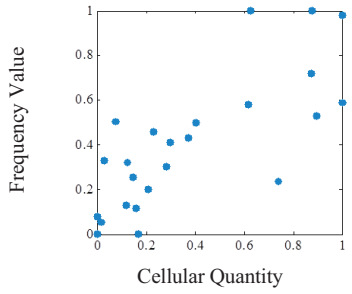


Fig. 17. Frequency and Cellular Quantity (correlation coefficients: 0.766).

VII. CONCLUSION

In this paper, we proposed a noninvasive ultrasound system for identifying cellular quantity with two features. This ultrasonic device has two probes attached an electric caliper with the center frequency of 1.0 MHz. This fact demonstrates that the ultrasonic approaches are valuable for identifying cellular quantity in the Bone Marrow Stromal Cells/ β-tricalcium phosphate by using two features: amplitude and frequency. We suggested that the frequency features were valuable to analyze the BMSCs with the features of the frequency attenuations. This study showed the ability of intervention to produce the desired beneficial effect. We certified the superiority of the frequency data analysis. Moreover, we established the validity for measuring the cellular quantity in BMSCs by using the feature of frequency with Cross-Spectrum focusing on the attenuation. It remains as the future studies to apply this system to more samples.

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