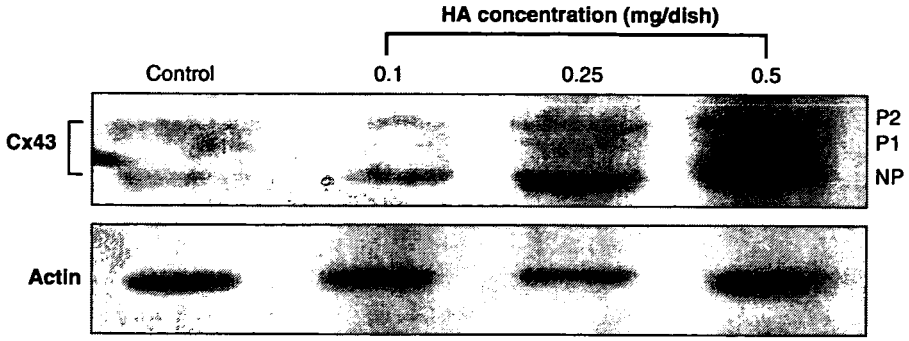

Fig. 5. Identification of Cx43 in HIT-T15 cells grown on the HA-coating dish by Western blot analysis. After HIT-T15 cells were incubated into HA-coated dish for 24 h, cells were lysed and proteins (20 μg) were separated by SDS–PAGE followed by Western blotting using rabbit anti-Cx43 antibody. Actin immunostaining was used to assess equivalent protein loading. This is a representative autoradiogram of three experiments.

FBS and the nutrients in media such as hormone, growth factor (FGF, etc.), cell adhesion molecule (N-CAM and cadherins), and transportation protein [6,17]. As a result, the HIT-T15 cells can use these nutrients and the nutrient-enriched substrata (e.g. natural ECMs) by ionic interaction and the binding of HMW HA to various kinds of cytokines, to change the cell aggregations, resulting in the increase of GJIC. With the evidence above, the enhancement of GJIC activity induced by HA-coating participated in the regulation of insulin release and insulin biosynthesis. On the other hand, the glucose stimulus-secretion coupling in β-cells generated several signals, including a signal to secrete preformed insulin stored in secretory vesicles, a signal, which may be the same or different, to secrete newly made insulin, and a signal to synthesize more insulin. The mechanism of glucose-induced insulin secretion is distinct from that of glucose-induced proinsulin biosynthesis and insulin gene transcription [18]. Moreover, the qualities of ECM affect the insulin release [19]. Therefore, it is possible that HA-coated dishes promoted a large increase in insulin synthesis but only a modest increase in insulin release. The detailed action mechanism should be investigated in the next study.

In native and tumoral insulin-producing pancreatic β-cells, gap-junction protein Cx43 has been identified. Furthermore, the stable transfection of the gene coding for Cx43 induces the expression of functional gap-junction channels and improves both the biosynthetic and secretory defects of the cells. Cx43-transfection and incidence of junctional coupling also secrete more insulin than wild-type and noncommunicating cells, the absence of Cx43 implicated in the loss of β-cell-specific functions in vitro and in vivo [9,14]. In this study, HA-coating expressing high levels of the Cx43, gap junctions, and coupling, showed the striking enhancement of the amounts of stored hormone in HIT-T15 cells and promoted the glucose-induced insulin release, indicating that adequate levels of Cx43 and coupling are required for proper insulin production. These results provide further evidence that HA-coating increases the pancreatic β-cells function by enhancing the function of Cx43-mediated GJIC.

5. Conclusion

In conclusion, the function of GJIC is considered to be a useful marker for evaluating tissue-engineered products. The data obtained in this study show that gap junctions contribute to regulating some still-unknown mechanism to couple the stimulus-secretion of HIT-T15 cells under the condition of low concentration HA-coating. The growth regulation with a bioartificial pancreatic construct using HA is achievable. These results give useful information on design biocompatibility of HA when the HA is used as a biomaterial for bioartificial pancreas. HA-coating may be a new technique for constructing three-dimensional bioartificial pancreas in tissue engineering.

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A Novel Non-Destructive Method for Measuring Elastic Moduli of Cultivated Cartilage Tissues

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Abstract. Accurate measurement of the mechanical properties of artificial or cultivated cartilage is a major factor for determining successive regeneration of defective soft tissues. In this study, we developed a novel method that enabled the bulk modulus (k-modulus) to be measured nondestructively using the relationship between volume and pressure of living soft tissues. In order to validate this method we estimated the bulk modulus of soft silicone rubbers using our new method and a conventional method. The results showed a 5 ~ 10% difference between the results obtained with the two methods. Our method was used subsequently to measure the mechanical properties of cultivated cartilage samples (collagen gel type), that had been incubated for four weeks in the presence or absence of human articular chondrocytes (HACs). Our experiments showed that cultivated cartilage tissues grown in the presence of HACs had a higher bulk modulus (120 ± 20 kPa) than samples grown without HACs (90 ± 15 kPa). The results indicated that our novel method offered an effective method for measurement of volume changes in minute living soft tissues, with the measurements having a high degree of accuracy and precision. Furthermore, this method has significant advantages over conventional approaches as it can be used to rapidly and accurately evaluate the strength of soft tissues during cultivation without causing damage to the specimen.

Introduction

Recently, artificial cartilage, obtained by cultivation with human articular chondrocyte (HACs) and mesenchymal stem cell (MSCs) on collagen scaffolds, has provided a method with the potential to regenerate damaged articular cartilage [1, 2]. In order to achieve successfully regeneration of damaged soft tissues, it is well established that biomechanical function and the biological construct of the artificial tissues play an important role in the tissue engineering [3-5]. For this reason, many experimental approaches have been used to assess the mechanical properties of factitious soft tissues. Some researches recently proposed a method for measuring mechanical properties with micro-needles or other specially designed devices [6-8]. However, using these approaches it has proved very difficult to accurately assess the mechanical properties of biological materials, such as skeletal muscles, cartilage, or other soft tissues including artificial tissues. This is due to the technical difficulties associated with non-constructive measurements, in addition to the irregular geometries of living tissues. In spite of these practical limitations, mechanical assessments are crucial when determining the maturity of cultivated soft tissues for transplantation and to ensure the success of regenerative medicine. We therefore developed a novel method that enabled the bulk modulus of elasticity (modulus of volume elasticity) to be measured rapidly, using the relationship

between volume and pressure of living soft tissues. This method was then applied to measure the elastic modulus of cartilage cultivated for four weeks on collagen scaffolds with and without human articular chondrocyte (HACs).

Materials & Methods

Theory and Experiment. Figure 1 shows a diagrammatic illustration of our device, which was used in a pressure pot under different air pressures. The system was composed of Chambers 1 and 2, a pressure sensor, an A/D converter and an air compressor. The sample was placed in Chamber 1, with Chamber 2 acting as a reference for the device. The pressure sensor detected the difference in pressure (ΔP) produced when a pressure change occurred in Chamber 1. The volume change (ΔV) was expressed as an electronic signal using a FFT (Fast Fourier Transform) analyzer as shown in Fig. 1-(A). In order to calculate the relationship between volume (V) and pressure (P), we used the classical equation of the relationship between volume and pressure [6]. If the volume of the sample changed (V_x) in chamber 1, the equation (1) can be written as:

$$\Delta P_1 - \Delta P_2 = \frac{nP_1\Delta V_1}{V_1 - V_X} - \frac{nP_2\Delta V_2}{V_2} \quad . \qquad (1)$$

where P_1, V_1 are the pressure and volume in chamber 1, respectively, and P_2, V_2 are the pressure and volume in chamber 2, respectively. n represents the number of the polytropic index. When the pressure increases, ΔP is inserted into the equation (1). The realtionship between pressure and volume in this system can be rewritten as equestion (2).

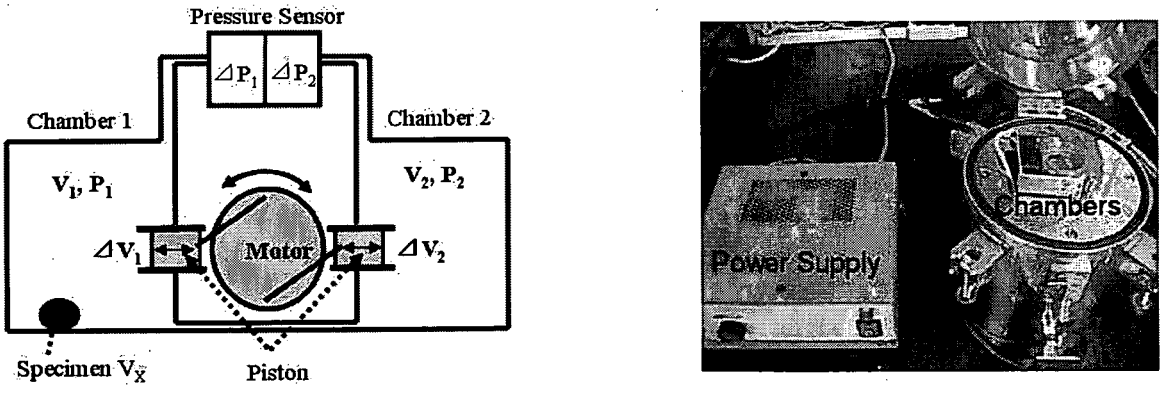
$$\Delta P_1 - \Delta P_2 = \frac{n(P+\Delta P)\Delta V}{V_1 - V_X} - \frac{n(P+\Delta P)\Delta V}{V_2} = n(P+\Delta P)\Delta V\left(\frac{1}{V_1 - V_X} - \frac{1}{V_2}\right) \qquad (2)$$

The bulk modulus is calculated and converted by the following equation (3), (4).

$$k = P \ / \varepsilon_v \qquad (3)$$

$$k = E / 3\,(1 - 2\nu) \qquad (4)$$

where k, P, ε_v, E, and ν are the bulk modus, pressure, volume strain, elastic modulus, and possion ratio, respectively.



(A) (B)

Fig. 1 Schematic diagram (A) and photograph (B) of the novel testing device using the principle of the relationship between pressure and volume changes

Validation of New Testing Method. Before calculating the bulk modulus of the samples, the relationship curves between the known volumes and pressure changes between 0 ~ 120 kPa in this system were first defined using stainless steel balls. Soft silicone rubber, which had similar mechanical properties to human soft tissues, was then used to validate our specially-designed device. The elastic modulus of the silicone rubbers was measured by two methods: 1) the conventional dynamic elastic modulus (E') test and 2) our method. In the dynamic test, the silicone samples were loaded with a cyclic strain of amplitude 0.5% at a frequency of 0.5 ~ 100 Hz by Rheogel-E4000 (UBM Co., Japan). The dynamic elastic moduli at 1 Hz were converted to bulk moduli using equation (4). The two bulk moduli calculated using the conventional dynamic test and our method were then compared using the statistical t-test ($p < 0.05$).

HACs and Culture Methods. Two types of the cultivated cartilage grown with HACs (+) and without HACs (−) on collagen scaffolds were used to measure the bulk modulus. The artificial cartilages were prepared by the following process [2]. HACs of the knee joint were commercially obtained from BioWhittaker (Walkersville, USA) and cultured in chondrocyte growth medium (Walkersville, USA). After the collagen scaffold (BD Science, USA) was placed in a 24-well tissue culture micro-plate (Coring, USA), high-density micromass cultures were started by seeding 4×10^4 HACs in 20 μL of medium onto the collagen scaffold. After a 2 h attachment period in a 5% CO_2 incubator at 37°C, 1 mL of culture medium was added to each well. The cultures were then incubated for a further 4 weeks with the medium being changed twice weekly. Figure 3-(A) shows samples of the cultivated cartilages on the collagen scaffolds grown with and without HACs.

Results & Discussions

In order to validate this method, we mesured the bulk modulus of soft silicone rubber using our proposed method and the conventional method, The results were then compared. The results obtained using the novel method (409 ± 14 kPa) compared favorably with those obtained using the conventional biomechanical measurements (417 ± 22 kPa). As shown in Table 1, this result represented a mere 5 ~ 10% difference between the two methods, thereby confirming the accuracy of our new method. We found no significant difference between the two measuring methods in two types of silicone ($p > 0.05$).

We then applied our method to measure the bulk moduli of cultivated cartilage samples incubated for four weeks in the presence or absence of HACs. Figure 2 shows the relationship between volume and pressure changes of the cultivated cartilages. The volume of the cultivated cartilages was decreased with increasing pressure. As shown in Fig. 3-(B), cultivated cartilage tissues grown in HACs had a higher bulk modulus (120 ± 20 kPa) than samples grown without HCAs (90 ± 15 kPa). This differenence between the two samples of cultivated cartilage was statistically significant ($p < 0.05$). These findings indicated that growing cells such as HACs could increase the mechanical property of cultivated cartilages. Although the results showed a lower elastic modulus than that reported for normal human articular cartilage of 0.3 ~ 1.5 MPa, the lower mechanical property of an initial artifical cartilage would be suitable for assimilation around normal living cartilage [8,9].

Table 1 Comparison of the elastic moduli calculated using elastic modulus and our novel method

Samples	Bulk Modulus by Conventional Method	Bulk Modulus by Our Novel Method
Silicone gel (n=3)	176 ± 34 [MPa]	120 ± 20 [MPa]*
Silicone rubber (n=3)	417 ± 22 [kPa]	409 ± 14 [kPa]**

*, **: were converted with $\nu = 0.48$ and $\nu = 0.42$

Conclusions

On the basis of these results, it can be concluded that our novel method offers an effective method for measurement of the biomechanical properties of artificial or cultivated soft tissues as well as living soft tissues. The method has a high degree of accuracy and precision. In addition, the method can be used for rapid and accurate evaluation of changes in strength of soft tissues during cultivation without causing damage to the specimen.

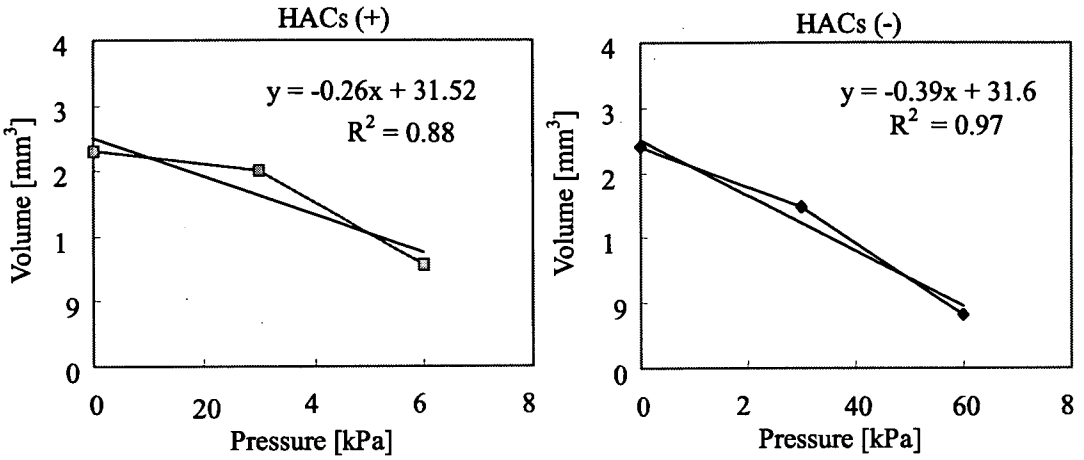


Fig. 2 Comparison of volume changes in relationship to changes in pressure in two types of cultivated cartilages grown with HACs (+) and without HACs (-)

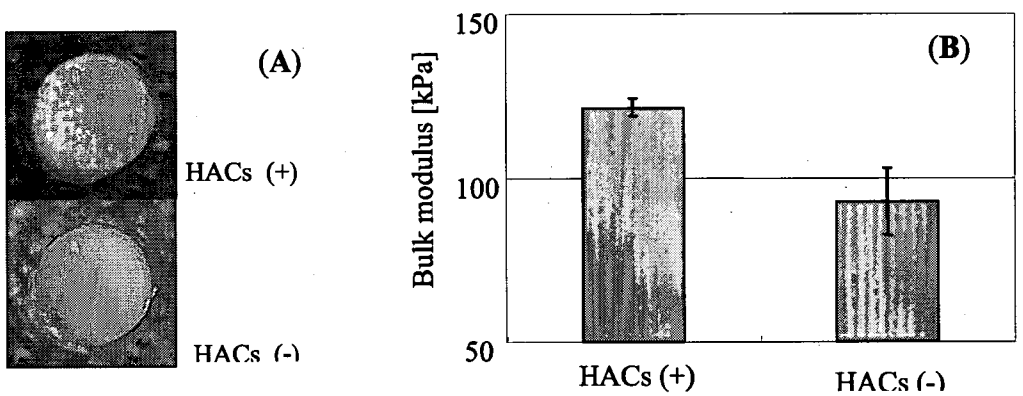


Fig. 3 Samples (A) and bulk moduli (B) of the cultivated cartilages grown with HACs and without HACs on collagen scaffolds for four weeks

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