論文内容の要旨

論文題目 **Administration of growth factors into the atelocollagen for human tissue-engineered cartilage**

和訳　**アテロコラーゲンを用いたヒト再生軟骨に対する成長因子の投与法に関する研究**

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1. Introduction

When the chondrocytes are isolated from the native cartilage and proliferate in vitro, they soon lose their original ability to express GAG and type II collagen, which is termed dedifferentiation, or decrease cell viability. As the dedifferentiated chondrocytes seemed sensitive to malnutrition or hypoxia, the larger tissue-engineered constructs containing the abundant dedifferentiated cells may worsen the central necrosis, and eventually lead to the deterioration of cartilage regeneration.

Therefore, we should induce the re-differentiation for the chondrocytes suffering from the dedifferentiation that inevitably occurs during the proliferation culture. The purposes of the present study were to increase the cell viability and the cartilage maturation of the tissue-engineered constructs by the optimization for the place or the timing in the administration of growth factors.

2. Methods

We firstly examined the in vitro cartilage regeneration of tissue-engineered pellets that consisted of human auricular chondrocytes and atelocollagen, and that were incubated in vitro under the stimulation with BMP-2, insulin and T_3. We then examined the administration of those growth factors into the scaffold, instead of the medium, and explored the possibility that the atelocollagen, the hydrogel scaffold of chondrocytes, may work as drug delivery of the factors. Lastly, we investigated the timing and the duration of administration of growth factors on the 3D culture of the tissue-engineered cartilage.

3. Results and discussion

We measured the GAG contents in the pellets, in which BMP-2, insulin or T_3 were placed into the medium or the atelocollagen hydrogel. We prepared 27 groups in which three factors (BMP-2, insulin and T_3) were treated with 3 options (in the medium, in the atelocollagen gel, and no administration), and incubated those pellets for 3 weeks. In the 27 groups, we noticed that BMP-2 and insulin took the major role in the production of GAG. The six combination: BMP-2 and insulin in the atelocollagen; BMP-2 in the atelocollagen and insulin in the medium; insulin in the medium; BMP-2 and insulin in the medium; insulin and T_3 hormone in the medium; BMP-2 in the atelocollagen, insulin and T_3 hormone in the medium showed high GAG production. Especially, BMP-2 in the atelocollagen with the supplement of insulin in the medium could not only produce the higher GAG matrix in a shorter period but also sustain the cell viability with lower mortality. The insulin in the medium could better be administered only for 2 weeks, rather than 3 weeks, which would save the time and the economical cost and hence shortening the in vitro culture of chondrocytes. Our protocol of BMP-2

embedded within pellets with insulin supplement just shed a light on the strategy of making tissue-engineered cartilage with a larger size. In the clinical practice, the present size of the tissue-engineered cartilage in the conventional method has often been restricted to less than 1 mL, which indication was confined to focal cartilage defects. The tissue-engineered constructs with a larger size would broaden the indication range of the cartilage regenerative medicine.