Bull. Tokyo Med. Dent. Univ., 26: 57-61, 1979.

GROWTH BEHAVIOUR OF CONDYLAR CARTILAGE AND EPIPHYSEAL CARTILAGE ON THE DIFFERENT MEDIUM OF THE ORGAN CULTURE

BY

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ABSTRACT

In order to compare histological differences between the condylar and epiphyseal cartilages, an organ culture system was employed. Materials from 36 neonatal rabbits were cultured for 7 days on three different, chemically defined media (Ham F12, Medium 199, and Eagle's minimum essential medium) with the addition of various concentrations of ascorbic acid, fetal calf serum and $NaHCO_3$.

The epiphyseal cartilage was maintained *in situ* histological and biochemical features better than the condylar cartilage on any of the medium used. The maximum sensitivity to Toluidine Blue staining of the cultured condylar cartilage was observed on Ham F12.

For both the condylar and epiphyseal cartilages, Ham F12 with the addition of 50 μg/ml ascorbic acid and higher concentration of $NaHCO_3$ was more effective on the maintenance of cell organization. However, effect of the addition of fetal calf serum to the medium was quite different between the condylar and epiphyseal cartilages, that is, the former showed better histological features without the addition, but the latter showed features similar to that *in situ* with 20% addition of fetal calf serum.

INTRODUCTION

It has been assumed that the mandibular condylar cartilage has biological features different from those of the epiphyseal cartilage. It is generally accepted that the condylar cartilage cell has no columnar arrangement and capillary invasion is hardly find in its structure.[2,3] Furthermore, the amount of chondroitinsulfate is different from that in the epiphyseal cartilage.[10]

The purpose of this investigation was to compare histological and biochemical differences between the condylar and epiphyseal cartilages in organ culture. In addition, there was another object which was to examine the effect of medium on the tissue response.

MATERIALS AND METHODS

Experimental animals consisted of 36 Polish-Dutch neonatal rabbits, one to three days of age. After each rabbit was anesthetized and decapitated, the condylar cartilage and the proximal epiphyseal cartilage of tibia with some adjacent structures were removed. They were carefully prepared for organ culture in Earle's buffered solution. The epiphyseal cartilages were sliced sagitally in the central portion, about 2 mm in width, and used for organ culture.

A total of 144 explants were cultured up to 7 days on stainless steel grids (Falcon #3014) in plastic dishes (Falcon #3010), and maintained at 37°C in gassed (5% CO_2-air mixture) humidified incubator.

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Received for publication, March 7, 1979.

Table 1. Various combinations of the concentration of FCS, asorbic acid, and $NaHCO_3$ in three chemically defined media

Medium	FCS (%)	Abcorbic acid (μg/ml)
Ham F 12	10	10
	20	10
	0	50
	0	50[a]
	10	50
	20	50
	20	50[a]
Medium 199	0	50
	10	50
	20	50
MEM	10	10
	0	50
	10	50
	20	50

[a] With higher concentration of $NaHCO_3$ (1176 mg/l).
MEM: Eagle's Minimum essential medium

Three different media were used: (1) Ham F12 (Nissui), (2) Medium 199 (GIBCO), and (3) Eagle's minimum essential medium (GIBCO). Each of these media was modified by the addition of L-glucose (5 mg/ml), ascorbic acid (10 and 50 μg/ml), and fetal calf serum (GIBCO; 0, 10, and 20%). Each of them was supplemented with 100 IU/ml penicillin and 100 mcg/ml streptomycin, and adjusted to pH 7.4. Details of the media used in this study are shown in Table 1.

After 7 days, all of explants were fixed in 10% buffered Formalin and decalcified in EDTA neutralized with sodium hydroxide. After dehydration in graded alcohols and embedding in paraffin, explants were sectioned sagitally through the midline at 5 μm thickness. These sections were stained with Haematoxylin-Eosin and 0.1% Toluidin Blue (pH 4.0).

As the control, rabbits of 8–10 days of age were used.

Results

1) Effect of the concentration of ascorbic acid added

On all three media added with 50 μg/ml of ascorbic acid, both cultured condylar and epiphyseal cartilage cells appeared to be similar to *in situ* state regarding anatomical and histological features, such as cell density, cell division, cell hydration, and sensitivity for the staining, than those in the medium added with 10 μg/ml of ascorbic acid.

2) Effect of the concentration of fetal calf serum (FCS) added

Medium 199 and Eagle's minimum essential medium (MEM) added with higher concentration of FCS showed fairly good structural maintenance of the condylar cartilage cell *in situ*, but the metachromasy was not so clearly observed on either of these media. On Ham F12 without FCS addition, the structural maintenance of the cartilage cell showed the most similar status to *in situ* state and the same medium added with 50 μg/ml of ascorbic acid was the most suitable one for the condylar cartilage from the viewpoint of the structural maintenance and metachromasy.

On the other hand, cultured epiphyseal cartilages on all of the media with higher concentration of FCS appeared to be almost similar to *in situ* features, and best of all, Ham F12 with 20% FCS and 50 μg/ml of ascorbic acid seemed to be the most suitable medium for the epiphyseal cartilage.

3) Effect of the concentration of $NaHCO_3$ added.

Comparison of the concentration of $NaHCO_3$ in Nissui's Ham F12 (540 mg/liter) with the one of the prescription by Ham[5] (1176 mg/liter) shows that the former contains about one-half of that in the latter. In the present study, the effect

Table 2. Histological and biochemical results of cultured condylar and epiphyseal cartilages

Medium	FCS	AA	Condylar cartilage						Epiphyseal cartilage				
			A	B	C	D	E	F	A	B	C	D	E
Ham F 12	Control		±±±	±±±	±±±	±±±	±±±	±±±	±±±	±±±	±±±	±±±	±±±
	10	10	±	±	±	+	±	+	±±	+	±	±±	±±±
	20	10	±	−	±	±	−	−	±±±	±±±	+	±±	±±±
	0	50	±±	±±	+	±±	±±	±±	+	−	±	+	±±
	0	50[a)]	±±±	±±±	±±	±±±	±±	±±±					
	10	50	±±	±±	+	±±	+	±±	+	±±±	+	±±	±±±
	20	50	+	±±	+	+	+	±±	±±±	±±±	±±	±±±	±±±
	20	50[a)]							±±±	±±±	±±±	±±±	±±±
Medium 199	0	50	±	±	±	±	±	±	±	±±	±±	+	±±
	10	50	+	±	±	±	−	±	+	±±	+	±±	±±±
	20	50	+	±	±	±	+	±	+	+	±±	±±	±±±
MEM	10	10	±	±	−	±	−	−	±	+	+	±±	±±±
	0	50	−	+	±	±	±	±	±	−	+	+	±±
	10	50	+	±	−	−	±	−	+	±±	±±	+	±±±
	20	50	+	±	+	±	−	+	±±	±±±	±±	±±±	±±±

AA=ascordic acid [a)] With higher concentration of $NaHCO_3$ (1176 mg/l).
MEM=Eagle's minimum essential medium
A: cell density
B: cell vacuolation
C: nuclear condensation
D: cell division
E: metachromasy
F: zone distinction

of these two different concentrations of $NaHCO_3$ on the cartilaginous growth was compared on Ham F12 added with 50 μg/ml of ascorbic acid without FCS for the condylar cartilage and with 20% FCS for the epiphyseal one.

The cultured condylar cartilage showed a character such as the metachromasy and cell organization similar with *in situ* state on Ham F12 with higher concentration of $NaHCO_3$ than the one with lower concent tration. On the other hand, no particular differences regarding histological and biochemical observations were found in the cultured epiphyseal cartilage on Ham F12 with higher and lower concentration of $NaHCO_3$. (Figures 1~6, Table 2)

Discussion

The effect of various concentrations of ascorbic acid, FCS, and $NaHCO_3$ in three different chemically defined media on the cartilaginous growth was compared, judging from the maintenance of the cell organization and metachromasy.

It has been assumed that ascorbic acid is effective for collagen synthesis and for prevention of the hydration of cartilage explants[7,9,10] and that FCS is related to the maintenance, growth, and differentiation of explants.[6] Regarding the concentration of $NaHCO_3$, there was an observation that the increase in length of the cartilaginous tissue did occur when the concentration of

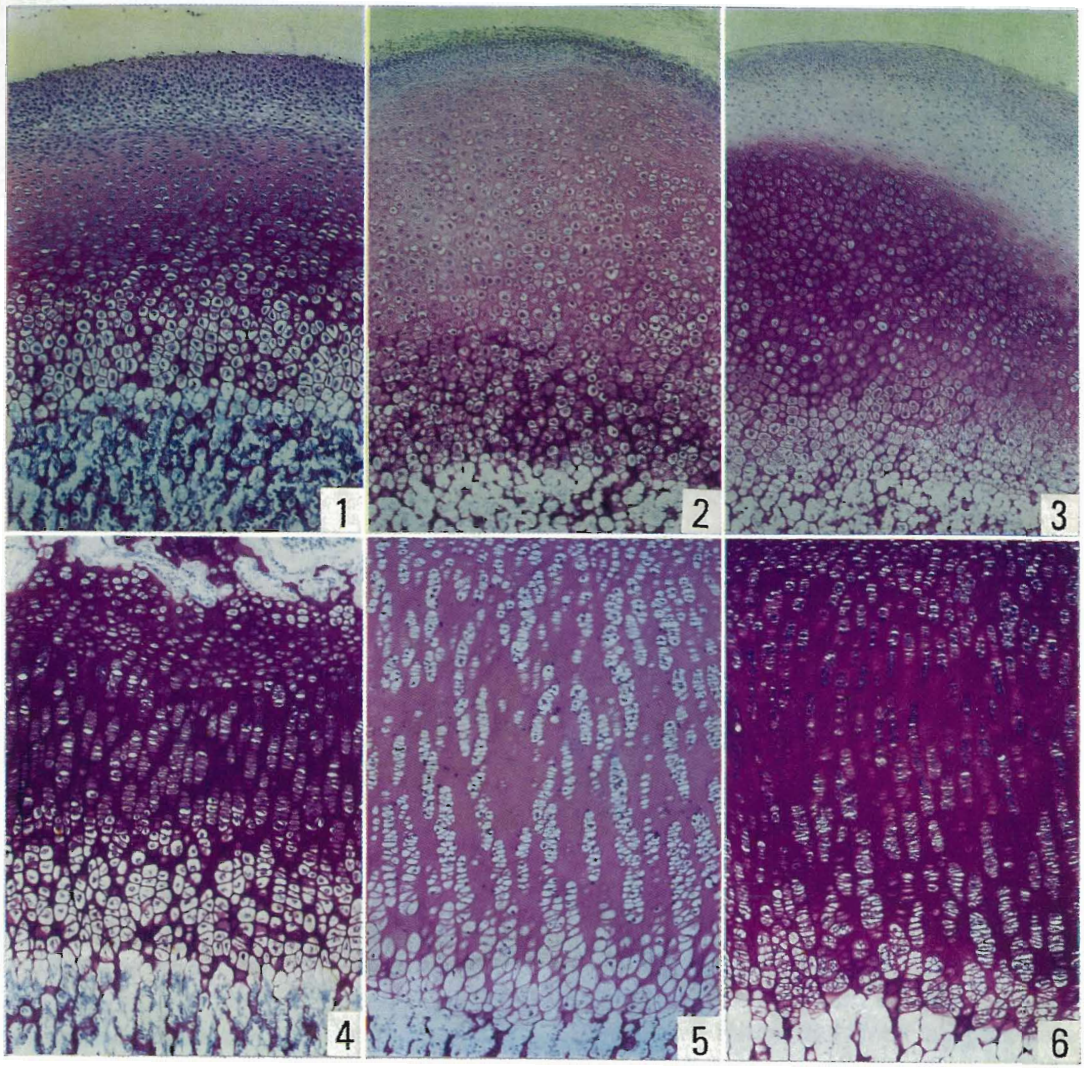

Fig. 1. Condylar cartilage (control).
Fig. 2. Condylar cartilage cultured on Ham F 12 with 50 μg/ml of ascorbic acid and 1176 mg/l of $NaHCO_3$ without FCS
Fig. 3. Condylar cartilage cultured on Ham F 12 with 50 μg/ml of ascorbic acid, 540 mg/l of $NaHCO_3$, and 20% FCS
Fig. 4. Epiphyseal cartilage (control)
Fig. 5. Epiphyseal cartilage cultured on Ham F 12 with 50 μg/ml of ascorbic acid and 450 mg/l of $NaHCO_3$ without FCS
Fig. 6. Epiphyseal cartilage cultured on Ham F 12 with 50 μg/ml of ascorbic acid, 1176 mg/l of $NaHCO_3$, and 20% FCS

Toluidin Blue staining ($\times$50)

$NaHCO_3$ was increased.[4]

In the present study, both the condylar and epiphyseal cartilages showed the same response to the concentration of ascorbic acid and $NaHCO_3$, that is, showed similar histological features as *in situ* in higher concentration. The results of this experiment corresponded with generally accepted effect of ascorbic acid on cultured explants. As a rule, the addition of $NaHCO_3$ in the medium is involved in pH adjustment, and the fact that the higher concentration of $NaHCO_3$ maintained cartilaginous cell organization well would be related to the increase of the buffering activity.[6]

Regarding the response to FCS concentration, the cultured epiphyseal cartilage seemed to maintain its *in situ* feature well with higher concentration. On the other hand, the cultured condylar cartilage cells were hypertrophied, cell density decreased, and also cell vacuolation and nuclear condensation were found. However, metachromasy of the cultured condylar cartilage on Ham F12 without FCS was positively shown as *in situ* and the structural cell organization was considerably well maintained.

It is not clearly defined yet from this study whether this different response depended on the essential biological character of these two cartilages or the biological adaptability of FCS to them. Bremers[1] reported in his study of the organ culture on BGJ, that the serum might disturbed the growth and histological organization of the condylar cartilage.

Findings from this experiment inferred that the condylar cartilage might have a physiological specificity to the chemically defined medium. Though metachromasy was taken as an indicator of the cellular activity in this study, it does not always indicate the evidence for proliferative activity and matrix synthesis in the particular explants, because the observed positive response to Toluidin Blue staining might be considered as the rudiments of the mucopolysaccharide products at the time of decapitation. Accordingly, in order to make clear these problems, autoradiographic studies by using ^{3}H-thymidine or ^{35}S-sulfate should be necessary.

Acknowledgements

The authors wish to thanks Prof. Fujio Miura of Department of Orthodontics, Tokyo Medical and Dental University for his helpful suggestion and encouragement.

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