OSTEOGENIC PROTEIN-1 PROMOTES THE FORMATION OF TISSUE-ENGINEERED CARTILAGE USING THE ALGINATE-RECOVERED-CHONDROCYTE METHOD

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INTRODUCTION

Most attempts to form cartilage in vitro use cells cultured within or on a biological or synthetic scaffold. We have developed a novel two-step culture method (alginate-recovered-chondrocyte method, ARC method) for the production of cartilaginous tissue in vitro that does not require exogenous matrices [1]. The first step consists of culturing phenotypically stable chondrocytes under conditions optimal for the formation of a cell-associated matrix (CM). The second step allows these cells with their CM to rapidly form and become integrated into a solid mass of cartilage on a porous insert. Cartilage tissue engineered in vitro using this approach is softer than normal cartilage: it is rich in proteoglycan (PG), mostly aggrecan and has an immature collagen network. We also have shown that recombinant human osteogenic protein-1 (OP-1) can stimulate PG and collagen synthesis by human and bovine articular chondrocytes. OP-1 promotes both the rate of formation and the size of the CM by these cells. The enhancement of matrix formation in the CM might enable the application of the tissue-engineered tissue to a larger area. For application in humans, the enhancement of the CM formation is preferable, because matrix formation by cells from aged cartilage is limited.

We present here the results of a study aimed at testing the hypothesis that exposure of young adult articular chondrocytes to OP-1 promotes the formation of cartilaginous tissue engineered for transplantation using the ARC method.

MATERIALS AND METHODS

The ARC Method was used as follows to form cartilage in vitro.

Step 1: Assessment of Formation of the CM in Alginate Beads

Bovine articular chondrocytes from the metacarpophalangeal joints of 14-18 month old steer were isolated by sequential enzyme digestion. Chondrocytes were cultured within or on a biological or synthetic scaffold. We have developed a novel two-step culture method (alginate-recovered-chondrocyte method, ARC method). Most attempts to form cartilage use cells cultured within or on a biological or synthetic scaffold. We have developed a novel two-step culture method (alginate-recovered-chondrocyte method, ARC method) for the production of cartilaginous tissue in vitro that does not require exogenous matrices [1]. The first step consists of culturing phenotypically stable chondrocytes under conditions optimal for the formation of a cell-associated matrix (CM). The second step allows these cells with their CM to rapidly form and become integrated into a solid mass of cartilage on a porous insert. Cartilage tissue engineered in vitro using this approach is softer than normal cartilage: it is rich in proteoglycan (PG), mostly aggrecan and has an immature collagen network. We also have shown that recombinant human osteogenic protein-1 (OP-1) can stimulate PG and collagen synthesis by human and bovine articular chondrocytes. OP-1 promotes both the rate of formation and the size of the CM by these cells. The enhancement of matrix formation in the CM might enable the application of the tissue-engineered tissue to a larger area. For application in humans, the enhancement of the CM formation is preferable, because matrix formation by cells from aged cartilage is limited.

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Step 2: Characterization of Cartilage Tissue Formed In Vitro

The cells with their CM, recovered after 7 days of culture in alginate, were resuspended in complete medium containing 10% FBS or 10% FBS + OP-1 (100 ng/ml). The cells were cultured with OP-1 was approximately 4 times that of the tissue cultured with 10% FBS alone (Fig 2). The stimulatory effects of OP-1 on matrix formation were observed in both steps of the ARC method. As the ability of human adult chondrocytes to form a cohesive matrix is limited, the results suggest that human chondrocytes should be stimulated by growth factors, such as OP-1, when the goal is to produce cartilage tissue for transplantation using the ARC method.

RESULTS

Step 1: Formation of the CM in Alginate Beads

On day 7 of culture, the CM formed by chondrocytes cultured in the presence of OP-1 was more voluminous when observed under the microscope. The thickness of the tissue cultured with OP-1 was approximately 4 times that of the tissue cultured with 10% FBS alone (Fig 2). The stimulatory effects of OP-1 on matrix formation were observed in both steps of the ARC method. As the ability of human adult chondrocytes to form a cohesive matrix is limited, the results suggest that human chondrocytes should be stimulated by growth factors, such as OP-1, when the goal is to produce cartilage tissue for transplantation using the ARC method.

Table Biochemical Analyses of the Tissue-engineered Tissues

<table>
<thead>
<tr>
<th></th>
<th>10% FBS</th>
<th>10% FBS + OP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Weight</td>
<td>1.99 ± 0.08</td>
<td>3.91 ± 0.26 *</td>
</tr>
<tr>
<td>PG (µg/tissue)</td>
<td>171.4 ± 9.5</td>
<td>653.6 ± 17.4 *</td>
</tr>
<tr>
<td>Collagen (µg/tissue)</td>
<td>120.3 ± 3.8</td>
<td>304.7 ± 9.1 *</td>
</tr>
<tr>
<td>PG/Collagen (µg/µg)</td>
<td>1.43 ± 0.11</td>
<td>2.15 ± 0.04 *</td>
</tr>
<tr>
<td>HA (ng/tissue)</td>
<td>969.3 ± 34.9</td>
<td>1565.3 ± 87.3 *</td>
</tr>
</tbody>
</table>

(p < 0.01: vs 10%FBS, mean ± SD)

REFERENCES


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Poster Session - Tissue Engineering - Hall E