Cartilage Tissue Engineering for Ear as in Rabbit Model with Perforated Polyurethane Prosthesis: In Vivo Assay

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ABSTRACT

Attempts have been done in this study to assay in vivo implantation of the perforated polyurethane prosthesis as ear cartilage in a rabbit model. The suitable prosthesis, compatible with ear cartilage in rabbit (300–400 μm thickness) was synthesized and implanted in the left ear of 18 rabbits as cartilage substitution. Cartilage from the left ear with the same area of the implant was trans-implanted on the right ear as control. After 1 to 5.5 months en bloc resection of prosthesis and surrounding tissues for histopathological and biocompatibility studies were performed. Of the 18 inserted implants, complete healing was observed in 8 (45%) cases. Four (22%) of the remaining 10 implants showed partial healing and remaining six (33%) showed non-healing. It was, therefore, concluded that perforation might improve the cartilage call ingrowth, when perforated polyurethane used as artificial cartilage for the repair of ear cartilage defects.

Key Words: perforated polyurethane (PPU), biocompatibility, ear cartilage, tissue engineering

INTRODUCTION

Biomedical implants are used to resolve defects that cannot be corrected either by the natural healing process or traditional surgical interventions.

Successful use of implants requires materials exhibiting specific characteristics particular to applications. Prosthetic implants must fulfill two criteria:

1. Biocompatibility: the material from which we construct the implant, must not elicit an adverse response once inside the body.
2. Functional characteristics: the implant must perform as the tissue for which it is substituted.

Polyurethane prepolymer was first applied to living biological tissue in 1958. The results of
subsequent studies were generally very disappointing [1]. Later, another preparation was used satisfactorily in vascular surgery [2].

Certain polyurethane materials have shown to have good biocompatibility and favorable mechanical properties [3].

Biocompatibility has been postulated to depend on many contradictory factors [5-7]. On the other hand, the biocompatibility of a material is determined by its surface properties.

Porous membranes are often used to support enhancement of the cell ingrowth. The cell culture intermembrane due to the porosity will penetrate and form a monolayer to enhance the cell growth [8].

Superstructure engineering provides optimal spatial and nutritional conditions for cell maintenance by the arrangement of structural elements (e.g., pores or fibres) so as to vary the order of cell-to-cell contact [9]. Certain cells, such as the chondrocytes, which comprise cartilage tissue, will be survived on the surface of such superstructures. It has been reported that special knits with open pores, have the potential for use in reconstructive surgery [9].

It has been reported that, perforation in biomaterials will improve the cell tissue ingrowth and cell communications [4].

Physical and chemical properties of implant have an important role on the results of in vitro assays. Porosity and/or perfusion of the implant facilitate infiltration of cells would lead to biocompatibility between tissues and implant. It has been documented that porosity and perforation have critical role in biostability of implant and the cell growth on it [10].

**EXPERIMENTAL**

Polyether polyol, polyethylene glycol (PEG 1000, Sigma), caster oil (Aldrich), were dried at 80 °C under vacuum. 2,4-Toluene diisocyanate (TDI, Riedel-de Haen), polysulfone hollow fibre (Fresenius-Germany) were used as received.

**Synthesis**

As shown below, the synthesis was carried out by

\[
(O=\text{C}=N-R-N=\text{C}=O)_n + \text{HO}--(-\text{polyol})-\text{OH} \rightarrow \\
O=\text{C}=N-R-N-\text{NH}-\text{COO}--(-\text{polyol})- \\
\text{OCO}=\text{NH}-R-N=\text{C}=O + (O=\text{C}=N-R-N=\text{C}=O)_{n-2}
\]

A weighed quantity of dried polyol was charged into flask equipped with stirrer, thermometer, dropping funnel, gas inlet, and air condenser with a drying tube. During flushing with a slow stream of nitrogen the mixture was heated and stirred. Then an excess of TDI was added to the flask with stirring so that the NCO/OH ratio became 2. The reaction was controlled and to be carried out at a temperature below 70 °C under a nitrogen blanket.

Analytical measurements and mechanical and surface properties of the cured polyurethane have been reported [11]. The urethane prepolymer has a potential to be cured with air moisture. PU patches were prepared and perforated using a special method as mentioned below:

Before complete curing the prepolymer which is a clear yellow viscous liquid was poured around a glass cylinder packed with polysulfone hollow fibre. After curing time of the prepolymer the PU patches

![SEM photomicrograph of the PPU sample from above.](http://www.sid.ir)
with a desired thickness (300 µm) have been prepared using a precise and sharp cutting. The PU patches have 36 perforations per 1 mm² using packed polysulfone hollow fibres with 150 µm internal diameter.

Figures 1 and 2 show scanning electron microscopy of the perforated PU prosthesis.

In Vivo Assays
18 Adult Dutch rabbits (average 2 kg) were selected for the study.

General conditions of animals were assessed pre-operatively. During physical examination, careful evaluation of ears was performed. Animals were anesthetized by I.M. injection of ketamine HLC (50 mg/kg) and xylazine HLC (5 mg/kg).

Under sterile conditions, a curvilinear skin flap, measuring 2×2 cm was performed on the centre part of the inner surface of the left ear and cartilage was exposed onto the ear surface. A 1.5×1.5 cm semicirculate segment of the cartilage, after separation from the contra-lateral skin surface, was resected. After sterilization by ethylene oxide, the prosthesis was implanted in the left ear of 18 rabbits as cartilage substitution. Right ear was used as control, by re-implantation of de novo cartilage, in each instance, as shown in Figures 3-5.

A piece of perforated polyurethane (36
Figure 4. Prosthesis implantation in the rabbit ear as the cartilage substitute.

Figure 5. Skin closure after prosthesis implantation.
Table 1. Prosthesis implant and control tests behaviour towards healing process.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Complete healing</th>
<th>Partial healing</th>
<th>Non-healing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prosthesis implant test</td>
<td>18</td>
<td>8(45%)</td>
<td>4(22%)</td>
<td>6(33%)</td>
<td>18</td>
</tr>
<tr>
<td>Control</td>
<td>18</td>
<td>14(77.7%)</td>
<td>4(22%)</td>
<td>0</td>
<td>18</td>
</tr>
</tbody>
</table>

perforation/mm²) with the same shape and size, was replaced and sutured to the surrounding cartilage, using 6-0 prolin suture material. Right ear was operated similarly as control, and the resected cartilage was replaced again and sutured to the adjacent cartilage by the same method. Skin incision was sutured, using 6-0 prolin.

Each rabbit was allowed to recover from anesthesia and to have regular nutrition using routine protocols.

After a medium period of 15 weeks (30 days to 165 days), under the same condition re-operation was scheduled, during which en bloc resection of central area of both ears was performed. Pathological study of resected specimen by light microscopy and H&E staining method was performed in all instances.

RESULTS AND DISCUSSION

After 1 to 5.5 months, complete resection of both the prosthesis and control area was performed.

All microscopic findings are tabulated by classic criteria of acute, chronic, foreign body type granulomatous reaction and healing process by degree of fibrosis and peri-implant changes, as shown in Table 1.

The non-healing (complete rejection) cases are those which have exposed prosthesis and chronic inflammatory reaction accompanied by acute inflammatory cell infiltration. The presence of many polymorphonuclears (PMN) infiltrated in the foreign granulomatous reaction background (Figure 6).

The incomplete rejected cases are those with partially healing of the wound and subacute inflammatory reaction associated with mild to moderate foreign body type granulomatous reaction (Figure 7) or collagenization foreign body type granulomatous reaction is the only pathological findings.

In the complete healed cases fibrosis of peri-implant region was found with minimal histological reaction, (Figures 8 and 9) and normal chondrocyte adjacent to the polymer implant.

In the control group we have observed no non-healing case in the control group. However, 4 (22%) cases showed partial healing in this group. Figure 10 shows the normal chondroid tissue of the rabbit ear.

Cartilage is a tissue rich in collagen, which has two main physiological functions. First is to maintain the shape and consistency of the structures such as ear, nose, tracheal rings, etc., and second would be to bear the weight on the joint surfaces.

Cartilage contains high amounts of mucopolysaccharide molecules (MPS), making a gel, which interacts with collagen molecules. Such combination satisfies the mechanical properties of the cartilage and

![Figure 6. Polymer implant adjacent to the chondroid tissue peri-implant fibrosis with 3-5 μm of thickness and macrophagic reaction is noted (non-healing). P: indicates the prosthesis; Arrows indicate macrophages presence.](www.SID.ir)
Figure 7. Polymer implant showing fibrosis and foreign body type reaction. Macrophagic reaction is minimal (partial healing). P: indicates the prosthesis; Arrows indicate macrophages presence; T: the surrounding tissues.

Figure 8. Normal appearance of chondroid tissue adjacent to the polymer implant. Mild inflammatory reaction is present (complete healing). P: indicates the prosthesis; T: the surrounding tissues.

Figure 9. Polymer implant showing fibroin of 3-5 μm of thickness and foreign body type reaction macrophagic reaction is minimal. P: indicates the prosthesis; Arrows indicate macrophages presence.

Figure 10. Normal chondroid tissue of the rabbit ear.
its movements at collagen network interfaces.

It has been reported that formation of fibrocartilage tissue takes about 20 weeks [9]. Elasticity (10.3–20.7 MRA) and tensile strength (3.4 MPA) are very low [12].

Friction coefficient is very low at the level of joint cartilage (<0.01). This is due to a small layer of synovial fluid between the joint and cartilage. MPS provide softness and mobility of the cartilage.

Damage of ear cartilage occurs in accidents and its defect happens during surgical resection of deeply invaded tumors. The resultant deformity is badly tolerated and has several socio-psychological effects on the patients.

In the past, porous materials made of aromatic polyurethane were successfully used for meniscal reconstruction in dogs [13]. Porous materials of this polymer were also successfully implanted for meniscal reconstruction.

Efforts to satisfy the later criterion in terms of mechanical properties led to the investigation of structural biomaterial composites. Since portions of the human body are composite structures, a progression toward the use of composite materials for application in the human body is natural.

**CONCLUSION**

Histopathological studies of reaction to implanted polyurethane in ear lobe, show tolerable prosthesis with 67% complete and partial healing process in test compared to 99% complete and partial healing in control cases.

Our results show that the perforated polyurethane caused chondrocyte cell ingrowth. Furthermore, perforated polyurethane (PPU) provided a favorable environment for chondrocyte cell attachment. This may be due to the combined effect of polyurethane properties as well as perforated surface, which enable cell communication, adhesion, proliferation and ingrowth. Pathological investigation confirmed the above-mentioned conclusion. These findings are promising for the use of these scaffolds for tissue engineering of cartilage replacement.

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