Engineered Cartilage Covered Ear Implants for Auricular Reconstruction

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Statement of Purpose: Cartilage tissues are often required for auricular tissue reconstruction. Currently, ear shaped medical implants such as MedPor® implant, is being used clinically. This medical device is composed of porous high density polyethylene (HDPE) and it is considered nontoxic and causes little foreign body reaction [1]. However, the use of this implant is often associated with complications, including inflammation, infection, erosion and dislodgement. To minimize these problems, we have developed a system in which tissue engineered cartilage serves as a shell that entirely covers the implant. In this study we investigated the feasibility of using this system to minimize the morbidity involving implant dislodgement.

Methods: Rabbit auricular chondrocytes were isolated and expanded *in vitro*. The cells were characterized by cell growth, maintenance of phenotypic and functional expression, and the quality of neocartilage formation. A fibrin hydrogel was used as a cell delivery vehicle. The fibrin hydrogels were characterized by measuring the clotting time and initial mechanical properties with various fibrinogen/thrombin ratios. To optimize the *in vivo* stability of chondrocyte-fibrin construct, various concentrations of fibrinogen and thrombin, cell density, Ca^{2+}, and pH were tested to achieve mature engineered cartilage.

Although the commercially available ear implant (MedPor®) is approved by the FDA, the hydrophobic nature may not be optimal for cell ingrowth, as well as combination with engineered cartilage. To this end, the ear implants were modified by surface oxidation techniques analyzed by contract angle and fluid uptake ability before combining the cell-fibrin and the ear implant. Engineered cartilage covered ear implants were implanted into the dorsal subcutaneous space of athymic mice. The animals were sacrificed at 4, 8, 12 and 24 weeks after implantation for histomorphological, structural and biomechanical analyses.

Results: The concentration of fibrinogen and thrombin, cell density, Ca^{2+}, and pH were readily modified to achieve optimal fabrication conditions. The retrieved constructs confirmed the presence of cartilage tissue as demonstrated by a series of histological evaluations (Figure 1). Dimensional analysis of the retrieved implants showed the maintenance of tissue thickness and total dimension. The stiffness of cell-fibrin constructs was significantly increased with time. The water content of the chondrocyte-fibrin constructs was decreased with time.

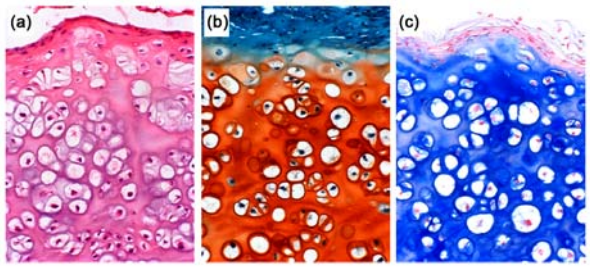

Figure 1. Cartilage tissue formation at 12 weeks after implantation: (a) H&E, (b) Safranin O, and (c) Alcian Blue staining.

After surface modification by oxidation, the water contact angle of ear implant (MedPor®) was decreased. The fluid uptake ability of the ear implant increased with prolonged treatment time of surface oxidation. This indicates that ear implant can be changed more hydrophilic by surface oxidation.

The cell-fibrin constructs were covered on the sheet-shaped implant. The histomorphological evaluations consistently showed neocartilage formation in the implants retrieved 12 weeks after implantation. Histologically, the implants demonstrated the presence of evenly dispersed triangular and ovoid-shaped chondrocytes inhabiting typical appearing lacunae, and surrounded by perichondrium. Safranin O staining confirmed the presence of sulfated glycosaminoglycans (GAGs), indicating a mature neocartilage framework had formed (Figure 2).

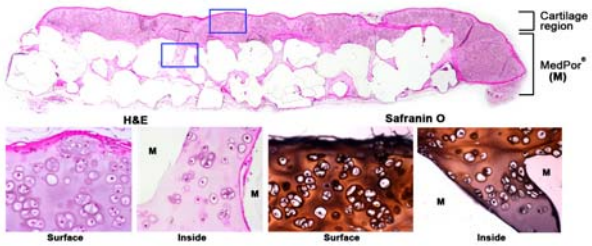

Figure 2. Engineered cartilage covered ear implant after 12 weeks implantation. Cartilage was formed in surface and inside areas of ear implant as stained by H&E and safranin O for sulfated GAG production. M: MedPor®

Conclusions: This study shows that cartilage tissues can be engineered to serve as a shell that entirely covers ear implant. This system is designed to improve the structural and functional stability between the implant and recipient tissue which may minimize the morbidity involving implant dislodgement.

References:

[1] Williams JD, et al. (1997) Porous high-density polyethylene implants in auricular reconstruction. *Arch Otolaryngol Head Neck Surg* 123:578.