Engineered Cartilage Covered Ear Implants for Auricular Reconstruction

Sang Jin Lee, Dong Joon Lee, Christopher Broda, Anthony Atala, James J. Yoo
Wake Forest Institute for Regenerative Medicine, Wake Forest University Health Sciences
Winston-Salem, North Carolina

Introduction

Cartilage tissues are often required for auricular tissue reconstruction. Currently, alloplastic ear shaped implants such as silicone and MedPor® are being used clinically. However, the use of these implants is often associated with complications, including inflammation, infection, erosion and dislodgement. To overcome these limitations, we have developed a system in which tissue engineered cartilage serves as a shell that entirely covers the alloplastic implants. To optimize the *in vivo* stability of cell-fibrin constructs, variations of fibrinogen and thrombin concentration as well as cell density were tested. 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.

Methods

Rabbit auricular chondrocytes were isolated and expanded *in vitro* and 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 and characterized by measuring the clotting time and initial mechanical properties with various concentrations of fibrinogen and thrombin.

To optimize the stability of chondrocyte-fibrin construct, various concentrations of fibrinogen and thrombin, cell density, Ca^{2+}, and pH were tested to achieve mature engineered cartilage.

The ear implants (MedPor®) were modified by surface oxidation and analyzed by contract angle and fluid uptake ability. The optimized cell-fibrin suspension was covered onto the surface modified ear implants. After then, the constructs 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 analyses.

Results

Variation of the fibrin hydrogel parameters resulted in different clotting time and initial mechanical properties (Figure 1). The clotting time was significantly accelerated by increasing the concentration of thrombin (Figure 1b). Compression testing indicated that the stiffness of fibrin hydrogels containing 10 U/mL thrombin and 25 mg/mL fibrinogen was significantly different than the stiffness of hydrogels containing 80 mg/mL fibrinogen (Figure 1c). In addition, when the concentration of fibrinogen in the gels was held constant at 80 mg/mL and the thrombin concentration was varied, the stiffness of a hydrogel containing 10 U/mL thrombin was significantly different than that of a gel containing 100 U/mL of thrombin (Figure 1d).

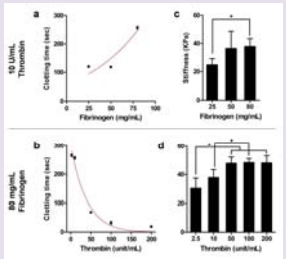

Figure 1. Fibrin hydrogels; (a,b) clotting time and (c,d) stiffness of fibrin hydrogels with various concentrations of fibrinogen and thrombin (without cells). *P<0.05.

Six experimental groups were implanted with constructs containing varying concentrations of fibrinogen and thrombin and cell density (n=3) (Table 1).

Table 1. Summary of variation of fibrinogen and thrombin concentration and initial cell density of the constructs.

Experimental groups	Fibrin hydrogels		Cell density (cells/mL)
	Fibrinogen (mg/mL)	Thrombin (U/mL)	
EC-1	25	10	1×10^7
EC-2	50	10	1×10^7
EC-3	80	10	1×10^7
EC-4	80	10	5×10^6
EC-5	80	10	4×10^7
EC-6	80	100	1×10^7

To evaluate the dimensional changes of cell-fibrin constructs after implantation, dimensions of the retrieved samples were measured prior to implantation and at retrieval (Figure 2). Initial cell density significantly influenced the dimensional maintenance of the constructs over time. At 24 weeks, the dimensions of the constructs in group EC-5, which contained the highest cell density, were approximately 83% of the initial size (Figure 2f).

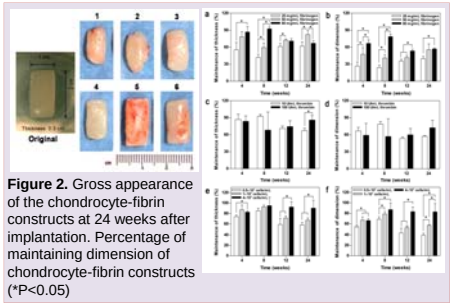

Figure 2. Gross appearance of the chondrocyte-fibrin constructs at 24 weeks after implantation. Percentage of maintaining dimension of chondrocyte-fibrin constructs (*P<0.05)

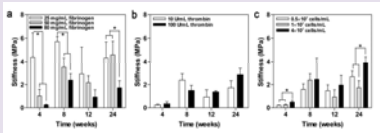

Figure 3. Stiffness of the retrieved chondrocyte-fibrin constructs at 4, 8, 12 and 24 weeks after implantation (*P<0.05).

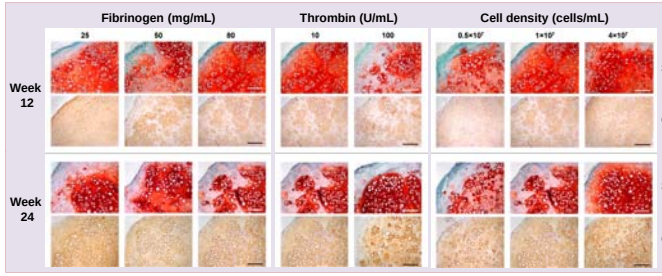

Figure 4. Histological evaluations of the retrieved chondrocyte-fibrin constructs with various concentrations of fibrinogen/thrombin and cell density after 12 and 24 weeks *in vivo* .The sections of the constructs were stained with safranin O staining for GAG and immunohistochemistry for collagen type II. Scale bar indicates 200 μm.

Histological staining of the retrieved samples revealed that a new cartilaginous matrix had been formed in the chondrocyte-fibrin constructs in all experimental groups (Figure 4). The chondrocytes in the newly formed tissue demonstrated the same morphological characteristics as those in native cartilage, with cells located within typical chondrocyte lacunae, surrounded by cartilaginous matrix. The newly formed matrix generated in all experimental groups stained intensely with safranin-O, demonstrating the presence of sulfated proteoglycans.

The water content of the constructs was determined by comparing wet and dry weights (Figure 5A). All experimental groups exhibited a slight decrease in water content with time, most likely due to gel contraction or matrix production.

Elastin content per wet weight was increased over time with increasing initial cell density. In general, the constructs exhibited an increase in cartilaginous matrix production with increasing initial cell density. These results correlated with the results of histomorphologic observations (Figure 4).

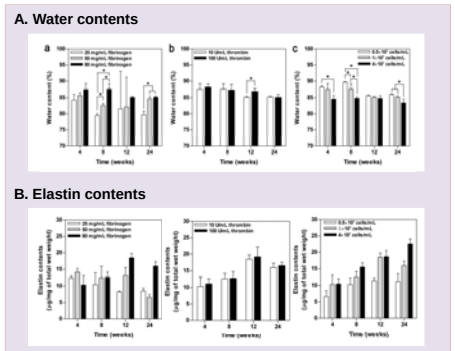

Figure 5. [A] Water contents and [B] elastin contents of the retrieved chondrocyte-fibrin constructs at 4, 8, 12 and 24 weeks after implantation; (a) 10 U/mL thrombin and 1×10^7 cells/mL, (b) 80 mg/mL fibrinogen and 1×10^7 cells/mL, and (c) 80 mg/mL fibrinogen and 10 U/mL thrombin. *P<0.05.

The procedure of developing cartilage to cover this ear implant consists of two main steps: 1) surface modification by oxidation to achieve hydrophilicity and 2) attachment of the chondrocyte-fibrin hydrogel to the ear implant.

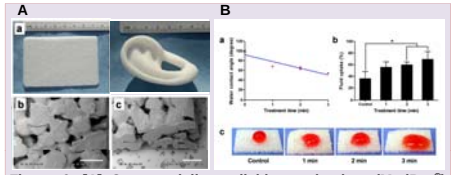

Figure 6. [A] Commercially available ear implant (MedPor®) sheet type; (a) gross appearance and SEM images of (b) surface and (c) cross-section of the implants. Scale bar indicates 500 μm. **[B] Surface hydrophilicity of the ear implants: (a) Water contact angle, (b) fluid** uptake, and (c) gross wetting appearance by the drop of red dye solution of the ear implants after surface oxidation.

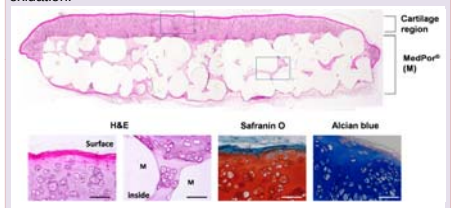

Figure 7. Engineered cartilage covered ear implants at 12 weeks after implantation. With histological staining, the implant revealed the presence of evenly dispersed triangular and ovoid-shaped chondrocytes inhabiting typical appearing lacunae, and surrounded by perichondrium.

Conclusions

This study demonstrates that cartilage tissues can be engineered to serve as a biological cover for an ear implant. The cell-fibrin constructs successfully formed neocartilage tissue with adequate mechanical strength and the long-term stability required for successful clinical application. This system may improve the structural and functional interactions between the implant and recipient tissue, and this in turn may enhance the outcome of auricular cartilage reconstruction.

AFIRM

Wake Forest Institute *for* REGENERATIVE MEDICINE