

CARTILAGE REPAIR USING PORCINE SMALL INTESTINAL SUBMUCOSA IN A RABBIT MODEL: A PRELIMINARY STUDY

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REZUMAT

În acest studiu a fost cercetată capacitatea submucoasei intestinului subtire (SIS porcin) de a ajuta la reconstrucția cartilajului auricular. Au fost folosiți 13 iepuri adulți din Noua Zeelandă, la fiecare animal s-a creat un defect de 1,1 cm² la nivelul cartilajului auricular, ambele urechi. Animalele au fost împărțite în 4 grupuri. La grupul 1, defectul cartilajului a fost reparat utilizând SIS de aceeași dimensiune și un transfer liber de pericondru pediculat. La grupul 2 s-a folosit pentru repararea defectului doar SIS, iar la grupul 3 doar transfer liber de pericondru pediculat. La grupul 4 nu s-a practicat repararea defectelor. Animalele au fost sacrificate la intervale de 3 și 6 săptămâni. Țesutul cartilajinos adiacent defectului reparat a fost biopsiat și examinat macroscopic și microscopic. S-a observat că la grupul 1 și 2 defectele au fost umplute cu țesut cartilajinos format din țesut conjunctiv colagenos cu organizare moderată împreună cu condrocite încorporate în cartilajul adiacent. La grupurile 3 și 4 la nivelul defectului s-a constatat țesut cicatricial. Acest studiu demonstrează calitativ că SIS poate constitui un suport natural pentru încorporarea condrocitelor gazdă. SIS este un biomaterial de încredere ce poate susține vindecarea leziunilor de cartilaj și merită continuarea cercetărilor în acest sens.

ABSTRACT

The capacity of porcine small intestinal submucosa (SIS) to support repair of auricular cartilage was examined in this study. Thirteen adult New Zealand white rabbits were used. 1.1 cm² auricular cartilage defects were created on both ears. The animals were divided into 4 groups. In group 1, the cartilage defect was repaired with the same size of SIS and a pedicled perichondrium flap. In group 2, the defect was repaired with SIS only. In group 3, the defect was repaired with a pedicled perichondrium flap only. In group 4, no repair was performed for the defect. At the intervals of 3 and 6 weeks, the animals from each group were sacrificed. The cartilage tissues adjacent to the defect repair site were biopsied for gross and histologic examinations. The results showed that the defects were filled with cartilagenous tissue, consisting of moderately organized collagenous connective tissue along with chondrocytes incorporated into the adjacent cartilage, in groups 1 and 2 during postoperative follow-up. Scar tissue was found in the defects in groups 3 and 4. This study qualitatively demonstrated that small intestinal submucosa can provide a natural scaffold for host chondrocyte incorporation. SIS is a reliable biomaterial that may support cartilage wound healing and is worthy of further investigation.

INTRODUCTION

Reconstruction of the framework of lost or damaged auricular and nasal cartilage represents an ongoing challenge. Currently the most commonly used material is autogenous rib cartilage.¹⁻⁵ Due to the

inherent shortcomings of cartilage, the high technical demands to accurately carve a realistic framework, and potential risk of donor site complications, ideal synthetic materials that could be used as the substitute for cartilage framework have been investigated in the last two decades. These biomaterials included cartilage allograft, silicone-Dacron mesh, porous high-density polyethylene, polytetrafluoroethylene patch, and trabecular demineralized bone matrix.⁶⁻¹¹ Inconsistent results from the use of these materials and complications from material incompatibility with the host have limited the wide application of these materials in cartilage repair. Tissue engineered cartilage grafts with biodegradable polymers and chondrocytes may provide a new tool for cartilage reconstruction, but these techniques still remain in experimental trials.

12-14

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Porcine small intestinal submucosa (SIS) is a collagen glycosaminoglycan material that has been experimentally investigated for several uses, including vascular grafts, tendon repairs, urinary bladder defect repairs, as well as body wall reconstruction.¹⁵⁻¹⁹ This acellular extracellular matrix material is believed to consist of structural and biochemical elements capable of functioning as an interactive scaffold for host cell incorporation and tissue remodeling.²⁰ The current study was designed to evaluate SIS in repair of auricular cartilage defects.

MATERIALS AND METHODS

Thirteen adult New Zealand white rabbits weighing between 2.0 kg and 2.5 kg were used. The National Research Council's guidelines for the care and use of laboratory animals were followed. The rabbits were anesthetized using sodium pentobarbital administered by intraperitoneal injection (30 mg/kg body weight), followed by intramuscular injection of cephalothin sodium (20 mg/kg body weight). All surgical procedures were performed under sterile conditions. Porcine small intestinal submucosa (SIS) material was provided by Cook Surgical Inc. (Bloomington, IN).

Surgical procedures: The convex side of the ear was shaved and disinfected with povidone iodine and 70% alcohol. An incision was made 1.5 cm from the base of the ear, and carried down to the depth of the auricular cartilage. A perichondrium flap was then elevated off the auricular cartilage. Hemostasis was maintained throughout the procedure with a handheld electrocautery. A 1.1 cm² auricular cartilage along with the opposing side perichondrium was sharply excised (Fig. 1). Depending upon the type of defect repair, 26 ears from thirteen animals were divided into 4 groups.

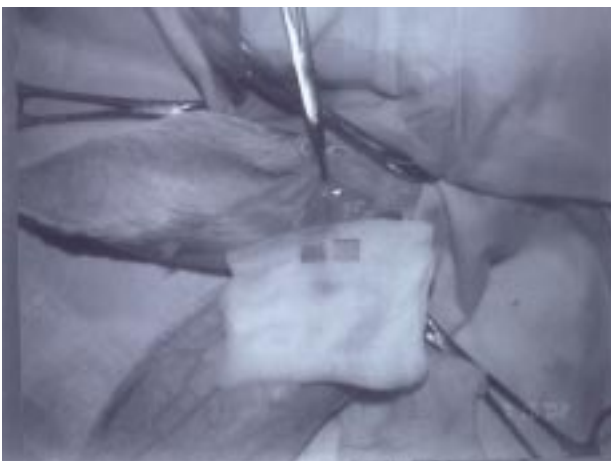


Figure 1. A 1.1 cm² auricular cartilage was created.

In group 1 (n=8), the cartilage defect was repaired

with the same size of SIS patch. The biomaterial was attached with 5-0 nylon sutures. The pedicled perichondrium flap was then sutured back into its original position (Fig. 2). The skin incision was closed with 4-0 nylon sutures. In group 2 (n=6), the pedicled perichondrium flap was excised. The defect was repaired with SIS patch only. In group 3 (n=6), the defect was repaired with a pedicled perichondrium flap only. No SIS was used. In group 4 (n=6), the pedicled perichondrium flap was excised. No repair was performed for the defect. The skin incision was closed with 4-0 nylon sutures in an interrupted pattern.

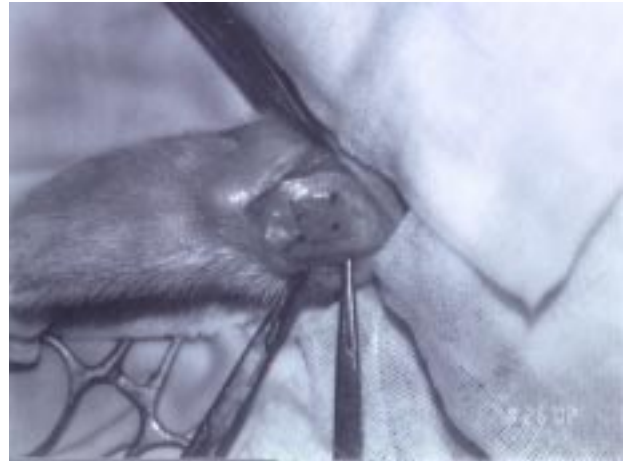


Figure 2. The cartilage defect was repaired with the same size of SIS patch and pedicled perichondrium flap.

The animals were allowed to recover from surgery. At intervals of 3 weeks (2 rabbits from group 1, 1 rabbit from group 2 to 4) and 6 weeks (2 rabbits from each group) after cartilage repair, the animals were sacrificed with an overdose of pentobarbital. The cartilage tissues adjacent to the defect repair site were biopsied for gross and histologic examination. Tissue samples were fixed in 10% formalin solution, after which the specimens were mounted, sectioned and stained with hematoxylin and eosin for microscopic examination of the cartilage healing processes.

RESULTS

All animals survived after surgery. At the end of postoperative week two, mild swelling at the cartilage repair sites was observed in animals of all groups, however all wounds were well healed. No extrusion of repair material or infection were observed over six postoperative weeks.

On gross examination at the end of postoperatively week three, the repair sites were filled with cartilagenous tissue when the skin wounds were opened in groups 1 and 2 (repaired with SIS and perichondrium and SIS alone, respectively). However,

in groups 3 and 4 (without SIS or perichondrium only), only fibrous tissue was present in the defect. At 6 weeks postoperatively, the cartilaginous tissue at the repair sites was integrated with surrounding cartilage in groups 1 and 2 (Fig. 3) while only scar tissue was seen in groups 3 and 4.



Figure 3. The SIS repair cartilaginous tissue from group 1.

Histology showed that sheets of moderately organized collagenous connective tissue along with a few chondrocytes were incorporated into the adjacent cartilage in groups 1 and 2 by the end of the postoperative week three. At 6 weeks postoperatively, the regenerated cartilage was significantly increased, and was in line with the surrounding cartilage in these two groups (Fig. 4). The thickness of generated cartilage in the repair with combined SIS and perichondrium was slightly greater than the repair with SIS only. However, this difference was not significant. In groups 3 and 4, at the cut edges of the defect, vital cartilage was observed, but only fibrous tissue was found in the defect. No cartilaginous bonding was seen in these two groups even in the sixth postoperative week (Fig. 5).

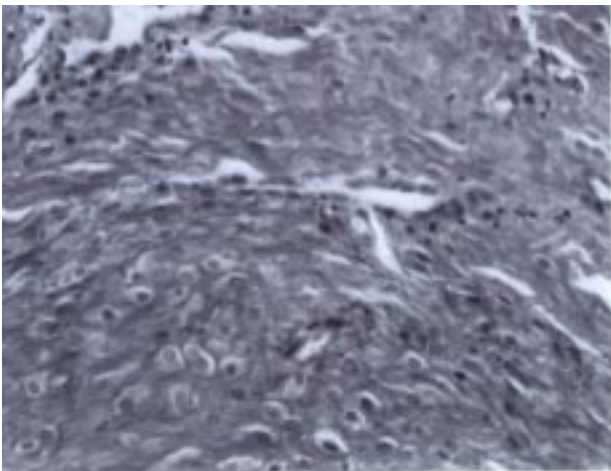


Figure 4. The regenerated chondrocytes were incorporated into the adjacent cartilage in the repair with SIS and perichondrium, and SIS only.

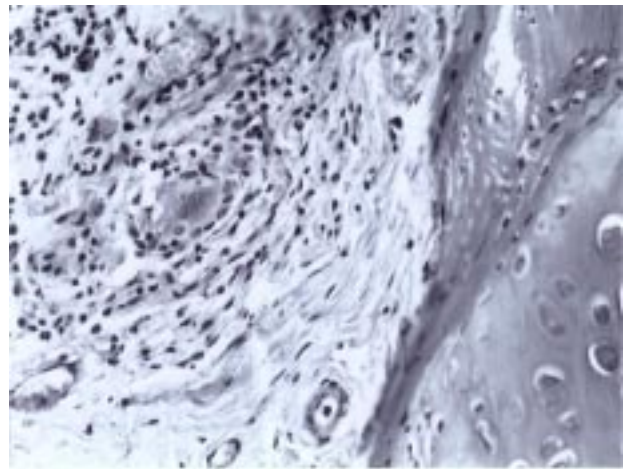


Figure 5. No cartilaginous bonding was seen in the repair with perichondrium only and no repair.

DISCUSSIONS

The ideal synthetic material that is used to substitute for cartilage repair should show excellent host tissue tolerance, should be easily manipulated to produce the required shape, show minimal recipient capsule formation, and demonstrate host tissue ingrowth, making it similar to native tissue.⁸ Despite recent advances in biological and tissue engineering techniques, an ideal material that matches all of the above requirements has still not been established.

Silicone rubber was previously a widely used biologically inert substance for the re-establishment of a cartilage framework. Some authors reported excellent results with silicone materials for external ear reconstruction.^{7,21} However, experience has revealed that infection and extrusion rates were high in this setting.² The porous material can allow collagen tissue ingrowth and provide a large interface between the material and the recipient soft tissue. Porous high-density polyethylene has recently been a frequently used material for cartilage framework reconstruction.^{2,22} This material can be anchored into the tissue and tolerate wound exposure.^{8,9} Polytetrafluoroethylene has been demonstrated to increase tissue stability over time, with a minimal inflammatory response.¹⁰ However, all of these porous materials can not integrate with the recipient tissue, foreign body reaction may result in capsule formation, and furthermore, contracture of a fibrous capsule would force the repair material toward the surface of the skin.

SIS is a collagen-based biomaterial extracted from the porcine small intestine. This glycosaminoglycan material has been demonstrated to provide a collagen scaffold and cytokine elements capable of sustaining host cell migration and in corporation with recipient tissue.^{20,23,24} When used as a biologic vascular graft

material in a canine model, complete endothelialization was found at 28 days postimplantation.¹⁵ In an Achilles tendon repair model, the tendon repaired with SIS showed organized collagen-rich connective tissue similar to the contralateral normal tendons.¹⁶ SIS serves as a structural framework and has also been observed to function in the repair of a urinary bladder defect model.¹⁷ In this study we evaluated the capacity of SIS in the repair of cartilage defects. The results showed that a SIS patch could incorporate into the adjacent cartilage and induce chondrocyte generation.

The repair of cartilage defect by perichondrium has been documented. When the perichondrium was left intact while the cartilage was removed, some cartilage neoformation was observed. However the quantity of new cartilage was highly variable.^{25,26} In our study, the use of a perichondrium flap failed to repair the cartilage defect. When a perichondrium flap was supported by a SIS scaffold, its function in chondrocyte generation was enhanced. Our results showed that the generated cartilage in the repair with combined SIS and perichondrium was greater than in the repair with SIS alone. This finding suggests an interaction between the perichondrium and SIS.

Recent research has been focused on techniques of tissue engineered cartilage grafts with biodegradable polymers and cultured chondrocytes. These biodegradable polymers include polyglycolic acid (PGA), poly-L-lactic acid (PLLA), benzyl ester of hyaluronic acid (HYAFF 11), and a collagen sponge.^{12-14,27} These investigations still remain in experimental trials. SIS might be an ideal biomaterial for tissue engineering in cartilage reconstruction.

In conclusion, this study demonstrated that SIS can provide a natural, three-dimensional structure that functions as an interactive scaffold for host chondrocyte incorporation. SIS is a reliable biomaterial that may support cartilage wound healing and is worthy of further investigation.

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