

IN-VIVO ULTRASOUND ASSISTED TISSUE ENGINEERED ARTICULAR CONDYLE

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1. INTRODUCTION

The increased prevalence of joint degeneration has led to incredible progress in the field of bone and cartilage tissue engineering [1,2]. Regardless of the current applications of cell and tissue-based therapies, such as chondrocyte transplantation [3] for the treatment of articular cartilage defects, prosthetic replacement of the entire articular surface continues to be the foremost practice for total joint replacement [4,5]. In-vitro and in-vivo experiments for tissue engineering articular condyles were proposed to replace synovial joints such as the temporomandibular joint (TMJ) [6,8]. However these trials did not provide convincing evidence of functional integration of the tissue engineered articular condyles [8]. Low Intensity Pulsed Ultrasound (LIPUS) has been shown to enhance osteogenic and chondrogenic differentiation [9-10]. Also, it has been shown that LIPUS enhances bone formation and growth by bone and cartilage matrix production [11-12]. In addition, it has been shown that LIPUS enhances tissue engineering of bone construct in-vitro [12]. The aim of this study was to evaluate the effect of LIPUS on in-vivo functional integration of autologous tissue engineered mandibular condyle (TEMC) for replacement of excised temporomandibular articular condyles.

2. METHOD

2.1 Animals.

This research was approved by the University of Alberta Animal Care Committee. Eight skeletally mature rabbits were selected and divided into three groups. Group 1 [3 rabbits] (Ultrasound with tissue engineered mandibular condyle), group 2 [3 rabbits] (Ultrasound with empty scaffold), and group 3 [2 rabbits] (empty scaffold, no ultrasound).

2.2 Stem cell isolation and conditioning.

All rabbits had bone marrow stem cells (BMSCs) isolated from their femur bones according to the previously established protocol [13]. BMSCs were differentiated into chondrogenic or osteogenic cells. Chondrogenic medium contained (DMEM+10% FBS + 1% pen-strep^{*}fungizone [as a base medium] \ in addition to 0.1mM None essential amino acid + 50ug/ml ascorbic acid-2-phosphate + 10nm dexamethazone + 5ug/ml insulin + 5ng/ml TGF-B1) while osteogenic medium contained (base medium in addition to 50ug/ml ascorbic acid + 2-phosphate + 10nm dexamethazone + 7mM + 1ug/ml BMP-2). LIPUS was applied for 20 minutes /day for four weeks to the cells to enhance their differentiation.

2.3 Experiment procedure

The chondrogenic and osteogenic differentiated cells were seeded into collagen sponges (Halistate, Dental Implant

Technology, Scottsdale, AZ, USA) that were housed into biodegradable extra-cellular matrix (ECM) scaffold to form TEMCs. The biodegradable scaffold was prepared as follows. The urinary bladder of market weight (6 months of age) pigs were harvested following euthanasia and cleaned of excess connective tissues and immersed in 1.0N saline to remove the urothelial cells on the luminal surface. The resulting material was referred to as urinary bladder matrix (UBM). The UBM ECM was then lyophilized. Six sheets of the UBM ECM material were laminated by vacuum pressing with placement of a central accumulation of comminuted (powdered) ECM. This local accumulation of powdered ECM was placed between the third and fourth layers, effectively creating a "pillow". The construct was then sterilized with ethylene oxide (ETO). The central pillow region of the construct served as the meniscal substrate site while the adjacent multi-laminate sheets served as suture anchoring points.

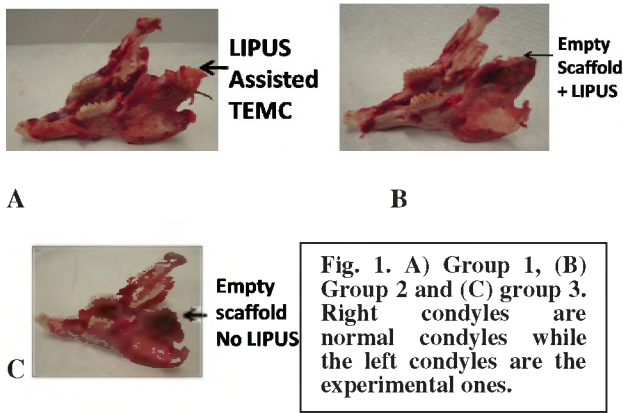
2.3 In-vivo TMJ condylectomy and implantation of the TEMC.

The left TMJ condyle in each rabbit was excised under general anesthesia (Ketamine (70 mg/mL) and Xylazine (10 mg/mL) at a rate of 1 mL/kg IM) according to the previously published technique [14]. Post-surgical care included antibiotic (Baytril 5mg/kg) twice a day SC for about 3 - 4 days and analgesic (Metacam 0.2 mg/kg) once a day for two days SC then 0.1 mg/kg once a day as needed. Rabbits were fed a soft diet for few days then they resumed normal food. The tissue engineered condyles were implanted into the amputated temporomandibular joint (TMJ) articular condyle and were fixed in place using a hardening calcium sulfate/phosphate material (ProDense, Wright Medical Technology Canada Ltd., Mississauga, ON, Canada). In groups 2 and 3, the amputated TMJ articular condyles were replaced by empty scaffolds. Groups 1 and 2 were treated daily for 20 minutes by an ultrasound device that delivers a power of 30 mW/cm² with pulse frequency of 1.5 M Hz, pulse repetition frequency of 1 K Hz. Four weeks after implantation of the TEMC or empty scaffolds, rabbits were euthanized and evaluated by microCT scanning as well as by histological examination.

3. RESULTS

3.1 Gross anatomy of the dissected mandibles.

Figure 1 shows the gross pictures of the dissected mandibles in each group. It can be seen that group 1 mandible shows comparable morphological and dimensional features of the TEMC and normal condyle. However in all other groups there was no noticeable growth of the excised mandibular condyles.



3.2 MicroCT evaluation.

Figure 2 reveals that the MicroCT evaluation of the TEMC in groups 1 has similar features of the normal condyle, while the empty ECM scaffold with LIPUS shows more hard tissue formation than the implanted ECM without LIPUS.

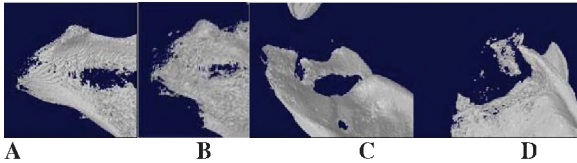


Figure 2. MicroCT scanning of the normal (A); LIPUS assisted TEMC (B); Empty ECM scaffold + LIPUS (C) and site of implanted empty ECM scaffolds without LIPUS (D).

3.3. Histological examination

Figure 3 shows the photomicrographs of the histological sections of normal (right) and left TEMC in group 1 as well as the site of implanted ECM scaffold with or without LIPUS.

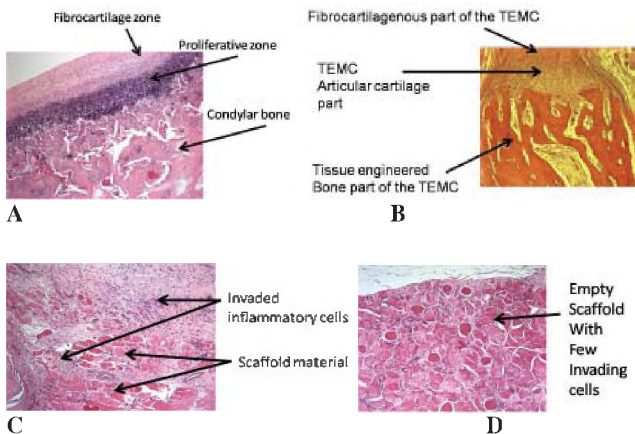


Figure 3. Photomicrograph of the histological examination of normal condyle (A); LIPUS assisted TEMC in group 1 (B); Empty scaffold with LIPUS (C) and empty scaffold without LIPUS (D).

It can be seen that the LIPUS assisted TEMC had similar morphological features of the normal control mandibular condyle. The implanted ECM with LIPUS showed inflammatory cell invasion of the empty scaffold with

LIPUS and empty scaffold TEMCs without LIPUS showed minimal cell invasion of the ECM scaffold.

4. DISCUSSION

To our knowledge, this is the first report that TEMC can successfully replace excised articular joint condyle. Our results support previous findings that LIPUS enhances bone and cartilage matrix formation and integration. However, this preliminary result requires further investigation regarding gene expression in each group as well as mechanical testing of the TEMC. One limitation of this study is that we did not test a positive control of the in-vivo TEMC without LIPUS. This was based on our in-vitro study that showed LIPUS enhanced matrix formation by the differentiated chondrogenic and osteogenic BMSCs.

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