Low Intensity Pulsed Ultrasound May Accelerate Physeal Closure in Rabbit Growth Plate Damage Repaired by Bioengineered Cartilage Pellet

[1]Chow S K H; [1]Cheung W H; [2]Lee K M; [1]Qin L; +[1]Leung K S

[1]Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong
[2]Lee Hysan Clinical Research Laboratories, The Chinese University of Hong Kong
Senior author: ksleung@ort.cuhk.edu.hk

INTRODUCTION

Growth plate injury is a common paediatric orthopaedic condition. In our previous report, the Bioengineered Cartilage Pellet (BCP) showed high restorative potential in terms of femoral lengthening when applied to a physeal defect rabbit model mimicking a Salter-Harris type II fracture [Ref: ORS 2008]. Moreover, various in vitro studies have demonstrated that Low Intensity Pulsed Ultrasound (LIPUS) is capable of increasing the synthesis of extracellular matrix as well as proteoglycan in chondrocytes. In this study, we hypothesized that the biophysical stimulation of LIPUS would further improve the growth potential of the BCP transplant in a physeal defect, by stimulating increased chondrocyte proliferation and matrix production.

METHODS

A partial physeal defect at the distal femur was created on 10 weeks old rabbits using a sagittal saw. Growth plate cartilage exposed on the metaphyseal and epiphyseal surfurces of the anterior defect was removed by a curette, whereas the posterior half of the physis was left intact. Temgesic was given for three consecutive days post surgery. (Animal Ethics Approval by the Chinese University of Hong Kong, reference number 04/004/ERG.)

Chondrocytes disaggregated from 8 weeks old rabbit ribs were pellet cultured into the bioengineered cartilage pellet (BCP) with diameter no smaller than 12 mm. The transplantation was performed on the 14th day in culture. 54 Rabbits were randomly divided into three groups: NC, GC and GT with the specified treatment scheme in the Table 1. Sample numbers with n = 6 in each group, at each time-point. Standardized Low Intensity Pulsed Ultrasound (Exogen, Memphis, USA) was given to each rabbit in the GT group for 20 minutes per day, 5 days per week, and for time-points of 4, 8 and 16 weeks post surgery.

Group	BCP	LIPUS
NC		
GC	•	
GT	•	•

Table 1 Animal grouping.

After euthanasia of rabbits by overdose pentobarbital at endpoints, femurs were dissected. The effect of the combined treatment of LIPUS and BCP was accessed by femoral lengths and qualitative histology. Lengths between contralateral limbs were compared using paired t-test, whereas between-group lengths were compared by independent t-tests by SPSS 13.0.

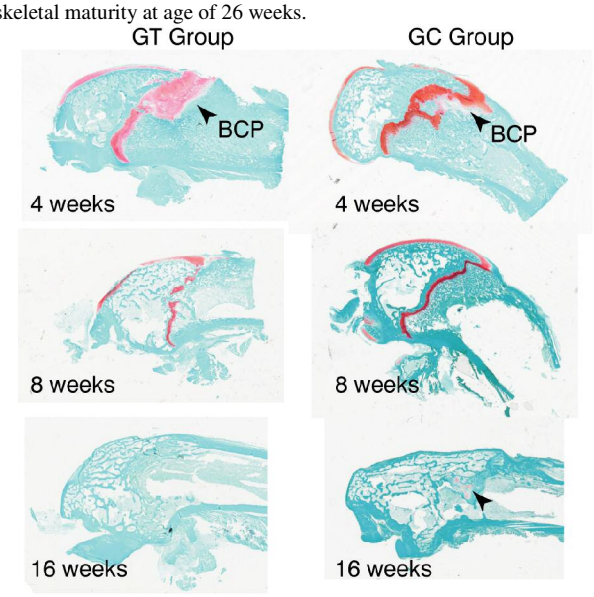
RESULTS

Femoral lengths on the defective side were found to be 5.6%, 6.7% and 11.7% shorter then the contralateral control at 4, 8 and 16 weeks respectively across all groups. Compared to NC group, the GC and GT groups with the BCP transplantation generally demonstrated better femoral lengthening. However, significant difference was only found between GC group and NC group at 16 weeks post surgery with lengthening advantage of 4.1%.

Time-point	GC	GT
4 weeks	1.8% *p = 0.361*	2.7% *p = 0.123*
8 weeks	1.9% *p = 0.402*	2.3% *p = 0.316*
16 weeks	4.1%* *p = 0.040*	2.3% *p = 0.313*

Table 2 Percentage length advantage over NC group compared to contralateral control.

Histological sections showed that the BCP transplant remained highly compatible with the host tissue at up to eight weeks post surgery as demonstrated by the strong proteoglycan signal in Safranin O staining, in both the GC and GT group. Minimal to no proteoglycan was found at the transplantation site at 16 weeks post surgery as the rabbits reached skeletal maturity at age of 26 weeks.



DISCUSSION

In this study, the BCP used to repair a growth plate defect was shown to be promising in reducing the length difference from 11.7% to 7.6% in the GC group. The BCP remained in active growth at up to eight weeks in both the GC and GT group.

At earlier stage of the transplantation, the GT group showed better lengthening than the GC group which can be explained by other in vitro studies reporting the increased extracellular matrix production under the stimulation of LIPUS. However, at longer time-points, the GT group showed reduced amounts in lengthening. This may due to the accelerated maturation of the BCP under the influence of the biophysical stimulation similar to that found in accelerated endochondral ossification in fracture healing. (Figure 2)

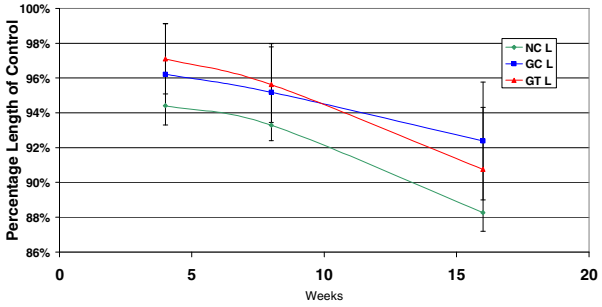


Figure 2 Percentage lengths versus control at time-point from 4 to 16 weeks. GT group showed better lengthening before 8 to 10 weeks, and earlier physeal closure after 10 weeks post surgery.

These results strongly suggest that Low Intensity Pulsed Ultrasound may accelerate physeal closure of physeal defects that is augmented with bioengineered cartilage pellet. Further experiments required to confirm the effect of LIPUS on normal and injured physis without any transplantation.

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