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TITLE: Shock Wave-Stimulated Periosteum for Cartilage Repair

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14. ABSTRACT The primary objective of this project is to determine if extracorporeal shock wave (ESW)-stimulated periosteum improves cartilage repair when it is used as an autograft to fill a defect in the articular surface of goats. A miniature fiber optic pressure sensor will be inserted into the tibial periosteum of 6 animals to measure the actual shock waveform in the tissue for two ESW doses (energy densities). In 12 goats, tibial periosteum stimulated by one of the 2 doses of ESWs (n=6) will be harvested, 4 days post-treatment, as an autograft for implantation into one 1 cm2 defect surgically produced in the trochlear groove of the knee joint of the same goat. Non-ESW-treated periosteum will serve as the control group (n=6). All animals will be sacrificed after 16 weeks, and the reparative tissue will be quantified histomorphometrically by determining the areal percentage of selected tissues in the original cartilage defect area.					
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I. INTRODUCTION

The primary objective of this project is to determine if extracorporeal shock wave (ESW)-stimulated periosteum improves cartilage repair when it is used as an autograft to fill a defect in the articular surface of goats. Periosteum, which contains cells with chondrogenic potential, has been investigated as an autograft for cartilage repair procedures. However, this approach is limited because the cambium layer of the periosteum is normally only 2-5 cells thick, and some of these cells are lost during the harvest procedure. We have recently demonstrated that extracorporeal shock waves, at doses approved by FDA for treatment of certain disorders, can stimulate up to a 10-fold increase in the thickness of rat periosteum after only 4 days.

This protocol deals with the therapeutic use of shock waves. Shock waves are pressure waves of very short duration (a few microseconds). The initial peak compressive wave is followed by a lower amplitude tensile wave. The ESWs can be produced by apparatus that focus the waves at a certain location in the body or produce waves which radiate from the shock wave head. Normally treatments apply up to 3000 shocks in a session. The “dose” of shock waves is measured in energy density. For our studies the energy densities to be used will be: 0.15 mJ/mm² (“low”) and 0.45 mJ/mm² (“high”). This range is approved by FDA for other indications than the one we will be investigating, but it demonstrates that shock waves in this dose range have an acceptable safety profile.

During this period of the project we investigated: 1) the type of shock waves (focused versus radial); 2) the specific locations at which we administer the shock waves, because the periosteum varies with respect to its thickness at various anatomic locations; and 3) the dose of shock waves (*i.e.*, energy density and number of shocks).

II. BODY

During the period since the last Annual Report, research focused on:

1. the dose response of goat periosteum to unfocused (radial) ESWs;
2. the effects of periosteum at 3 sites to ESWs.

Unfocused ESWs (Fig. 1) were investigated because of their potentially more desirable safety profile when compared to focused ESWs. Focused ESWs expose tissues around the target site to what may be unwanted exposure to ESWs. Table 1 shows the doses of unfocused shock waves employed. The ESWs were applied to the periosteum in the proximal medial aspect of the tibia and to the mandible and maxilla.

Table 1. Doses of unfocused ESWs employed in the pilot experiment, n=1.

Goat	Dose mJ/mm ² x no. of shocks
1	0.4 X 3000
2	0.4 X 2000
3	0.4 X 1000
4	0.3 X 1000
5	0.18 X 1000
6	0.1 X 1000



Fig. 1. Unfocused shock waves were applied to the skin overlying the periosteum of the proximal tibia.

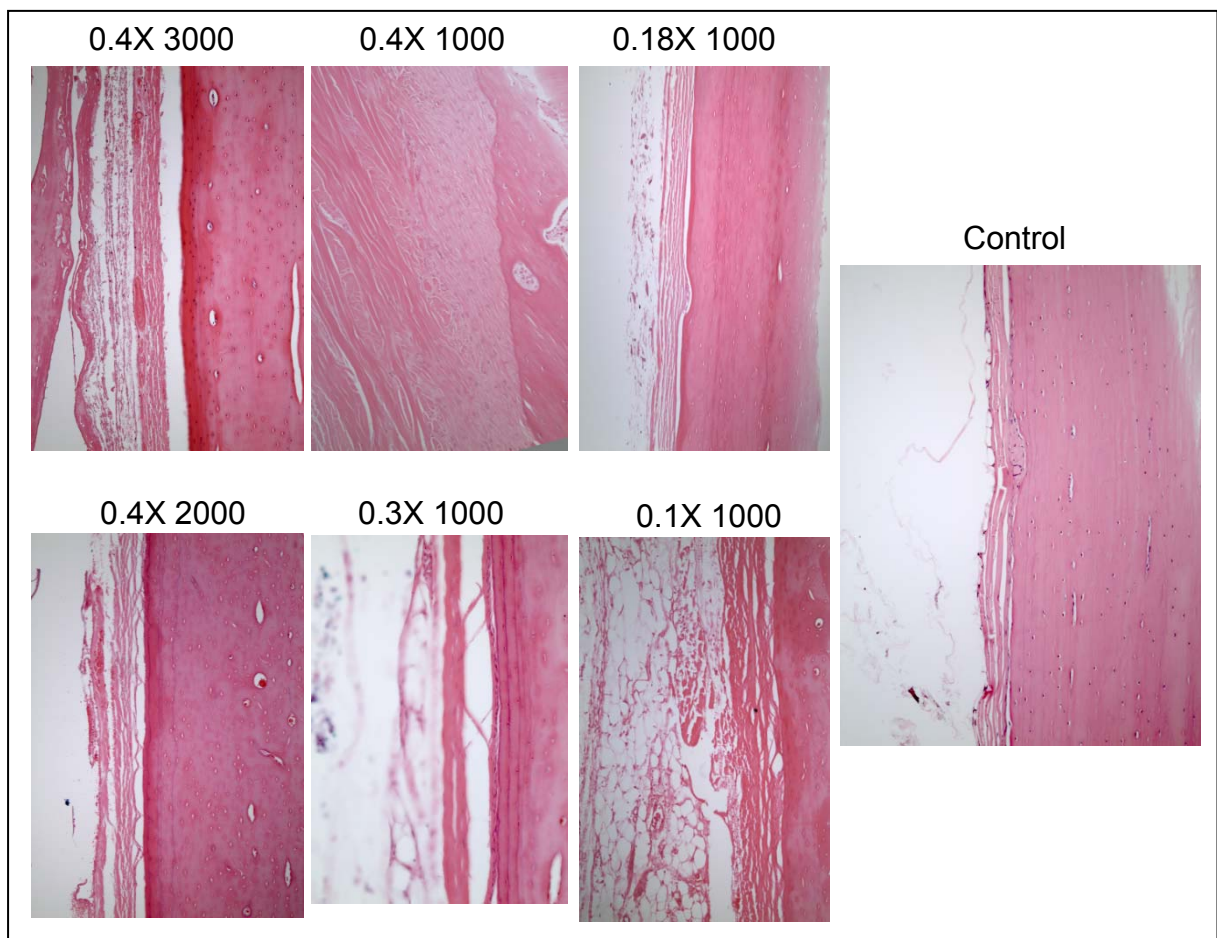


Fig. 2. Histology of the tibial periosteum in the animals treated with different doses of unfocused ESWs.

All 6 goats were sacrificed 4 days after application of the ESWs. The tissues were fixed in formalin and decalcified and embedded in paraffin. The histological processing is currently still in progress. Histological evaluation of the tibial periosteum has begun (Fig. 2). The inflammation was graded based on the following scale:

- 0: No inflammation
- 1: 1 or more small (barely visible through 10x objective) inflammatory cell infiltrates
- 2: 1 or more medium (visible through 10x objective) inflammatory cell infiltrates
- 3: 1 or more large (immediately visible in 10x objective) inflammatory cell infiltrates
- 4: Tissue necrosis and/or granulation tissue formation
- 5: Abscess

Hemorrhage was graded with the following scores:

- 0: No hemorrhage
- 1: 1 or more small (barely visible through 10x objective) hematomas
- 2: 1-3 or more medium (visible through 10x objective) hematomas
- 3: >3 or more medium (immediately visible in 10x objective) hematomas
- 4: 1 or more large (immediately visible in 10x objective) hematomas

The results obtained to date are shown in Table 2

Table 2. Scores for the degree of inflammation and hemorrhage.

Dose	Inflam. Score	Hemor. Score
0.4X 3000	4	4
0.4 X2000	2	4
0.4X 1000	3	4
0.3X 1000	3	3
0.18X1000	2	2
0.1 X 1000	2	4

III. KEY RESEARCH ACCOMPLISHMENTS

- The effects of unfocused ESWs of various doses on periosteum at several locations in the goat has begun to be evaluated.

IV. REPORTABLE OUTCOMES

None

V. CONCLUSIONS

These results demonstrate the range of histological changes that can be induced by unfocused ESWs.

VI. REFERENCES

None

VII. APPENDICES

None