CAMBIUM CELL STIMULATION RESPONSE TO SURGICAL RELEASE OF OVERLAPPING PERIOSTEAL TISSUE

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Introduction

Resurfacing of full thickness articular cartilage defects by implantation of periosteal autografts with cambium cell layer oriented upward (toward intraarticular space) has been reported (1). Periosteal flaps are also used with cambium cell layer oriented downward (toward base of defect) for covering articular cartilage defects implanted with cultured chondrocytes. The presence of the cambium cell layer with its progenitor cells and matrix factors appear to be important in the success of articular cartilage repair using periosteal flaps. Maintaining an intact cambium cell layer during harvesting of the periosteum is technique dependent. Other non-technical factors that can contribute to variable cambium cell yield include age, harvest site, and harvest time interval.

It would be desirable to optimize or enhance the cambium cell yield as a source of progenitor cells for transplantation. The cambium cells reside in the layer adjacent to the bone and mechanical alterations in the periosteal layer may induce them to respond, i.e., bone fracture with callus tissue formation and osteogenesis. In situ stimulation of cambium cells to increase their number by mechanical release of the overlying periosteal tissue was studied. It was our intent that the stimulated cambium cells that would develop in this “in situ incubator” would be then transplanted to enhance articular cartilage repair. It was the purpose of this study to evaluate the effects of mechanical release of periosteum on the stimulation of cambium cells and gene expression of mRNA for BMP2 during the early stimulation interval.

Methods

In this IACUC/IRB approved study, aseptic bilateral surgery was performed to mechanically release the periosteum in the proximal tibia of 9 adult female Spanish goats. Two techniques for periosteal release were used and included: 1) scoring all 4 sides of the tissue, and 2) scoring only the proximal and distal side of the tissue flap. A 25mm incision was made beginning 20mm distal to the tibial tubercle and centered over the medial tibial shaft. Fascia was dissected to reveal the periosteal layer and the corners of a rectangle (20x15mm) were marked on the tissue with a surgical pen. On one leg all four sides of the periosteal rectangle were scored to the bone using a scalpel blade and the marks as a guide (4 sided release). In the opposite leg, the periosteum was scored and released from only the proximal and distal sides of the rectangle (2 sided release). Immediately after scoring, the periosteum was observed to contract 1-2 mm in the proximal/distal plane. The wound was closed in layers and the animals recovered and resumed activity without incident. Three animals were euthanized at each evaluation interval of 4, 8, and 16 days. The periosteal release sites were exposed, grossly evaluated and harvested by cutting the tibial shaft above the below treatment sites. Major changes to the periosteal tissues to mechanical release. Major changes to the periosteal tissue included proliferation of cambium cells that underwent a rapid transition to bone formation in 4 days, development of a vascular overlaying tissue, trabecular bone formation over the cortical bone layer, and an increase in cambium cells. We expected to see a slower transition in the cambium cells production of osteogenesis that would have allowed us to harvest them in a chondrocytic phase. Instead we observed that periosteal tissue release resulted in nearly immediate osteogenesis. The 2 and 4 sided release scoring of the periosteum induced similar stimulatory responses in the cambium cells. Increased gene expression of mRNA for BMP2 suggests that the cells in the periosteal tissues respond quickly to stimulatory events such as tissue release. Such information may be helpful in the manipulation and use of stimulated periosteal tissue for use in articular cartilage repair strategies.

Results

At the evaluation times there was no evidence of lameness in any of the animals. Physical examination of the treatment site demonstrated that the soft tissues overlying the release sites were highly vascular and markedly swollen and warm at 1-4 days but appeared less swollen and warm by 16 days after surgery. The release sites typically formed mounds over the scored lines at 4 days postop and the entire release site was more uniformly raised 2-3mm at 16 days. No signs of wound infection were observed grossly or histologically.

The gross evaluation of the periosteal release sites demonstrated the development of a robust vascular tissue over the treatment site. Cell proliferation and new bone formation in response to the stimulation in this in situ incubator were consistently separate from the underlying cortical bone. The histology of the normal periosteal tissue in this region shows a relatively thick fibrous layer that is oriented parallel to the long axis of the bone and which covers the 2-3 cambium cell thick layer which reside adjacent to the outer cortical bone surface. At 4 days after surgery, the cambium cell layer was 7-8 cells thick in the 2 sided release and 10-12 cells thick in the 4 sided release. At 8 days after surgery, the release sites were covered with a fibrovascular tissue overlying this layer, trabecular bone formation was observed parallel to the bone shaft axis but separate from the cortical bone surface. The cambium cells were still 7-8 cells thick in the 2 sided release and 10-14 cells thick in the 4 sided release. At 16 days there were mounds of woven trabecular bone developing over the cortical bone layer at the release site. The fibers of the overlying tissue were being incorporated into the newly forming trabeculae.

An important observation was the increased level of gene expression for BMP-2 mRNA from the time-zero baseline values. The increase in mRNA expression was detected as early as 24 hours after the stimulation event (release of periosteal tissue). The level of expression may peak as early as 72 hours but remained high at 96 hours after the stimulation event.

Discussion

In the native state, cambium cells form a sparse cell layer under the fibrous periosteum and are adjacent to and in close contact with the cortical bone surface. Rupture or release of the superficial layers has a strong stimulatory effect on the normally quiescent appearing cambium cells stimulating them to synthesize and meet production. This study demonstrated a robust and rapid response of the periosteal tissues to mechanical release. Major changes to the periosteal tissue included proliferation of cambium cells that underwent a rapid transition to bone formation in 4 days, development of a vascular overlaying tissue, trabecular bone formation over the cortical bone surface, and an increase in cambium cells. We expected to see a slower transition in the cambium cells production of osteogenesis that would have allowed us to harvest them in a chondrocytic phase. Instead we observed that periosteal tissue release resulted in nearly immediate osteogenesis. The 2 and 4 sided release scoring of the periosteum induced similar stimulatory responses in the cambium cells. Increased gene expression of mRNA for BMP2 suggests that the cells in the periosteal tissues respond quickly to stimulatory events such as tissue release. Such information may be helpful in the manipulation and use of stimulated periosteal tissue for use in articular cartilage repair strategies.

The advantage of stimulated cambium cells for use in articular cartilage repair has yet to be determined.


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