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Necessity of latency period in craniofacial distraction: Investigations with *in vitro* microdistractor and clinical outcomes☆

Ginger C. Slack, Kenneth L. Fan, Christina Tabit, Brian Andrews, David I. Hindin, Henry K. Kawamoto, James P. Bradley*

Division of Plastic and Reconstructive Surgery, David Geffen School of Medicine, Los Angeles, CA, USA

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Microdistraction;
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Summary *Background:* To determine the need for latency period in membranous bone distraction, we performed 1) *in vitro* comparison of preosteoblasts suspended in a 3D microdistraction model and 2) a clinical study comparing mandibular distraction cases with/without latency.
Methods: In the In Vitro study, Preosteoblasts polymerized in 3D-collagen gel were placed in a microdistractor and separated into three groups: 1) distraction with latency, 2) distraction without latency, and 3) static. After 2, 4, 6, and 8 days, cell proliferation, total protein levels, alkaline phosphatase activity, and osteogenic gene expression were assessed through RT-PCR. In the clinical study, patients underwent mandibular distraction in two groups: 1) latency and 2) no latency (n = 45). The rest of the distraction protocol was identical. Outcome was based on clinical examination, radiographs at six months, and 3D CT scans.
Results: In the In Vitro study, The distraction without latency group compared to the latency group had delays in: proliferation, total protein count, alkaline phosphatase activity, osteogenic gene expression in CBFA-1 (fourfold vs. eighteenfold), and in osteocalcin (twofold vs. sixfold). The distraction without latency group had higher apoptotic levels during the first four days compared to the latency group (68% vs. 14%). For the clinical study, similar perioperative complications (5% vs. 6%), X-ray mineralization (93% vs. 94%), bone volume, (8.6 vs. 9.1 cc) and bone density of central distraction zone (78% vs. 81%) were observed with or without latency.
Conclusions: In vitro studies showed poorer results in cell survival, proliferation and

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* Corresponding author. Division of Plastic and Reconstructive Surgery, David Geffen School of Medicine, University of California, Los Angeles, 200 UCLA Medical Plaza, Suite 465, Los Angeles, 90095 CA, USA.
E-mail address: jpbradley4@mac.com (J.P. Bradley).

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osteogenic activity compared to distraction with latency; yet, clinically, there were no differences in distraction with latency versus without.

Introduction

Recent investigations into craniofacial distraction osteogenesis have focused on 1) establishing appropriate indications, 2) improving instrumentation/devices, and 3) understanding the biology of membranous bone healing. This last area of investigation will allow clinicians and craniofacial surgeons to modify existing protocols to optimize results. Many distraction protocols used in the craniofacial skeleton are based on Ilizarov's investigations on endochondral bone healing from the 1950s.[1,2] Based on over 15,000 long bone distraction cases, he set up guidelines for latency, rate, rhythm, and consolidation times.[1,2]

While his concepts have formed the foundation of distraction osteogenesis, membranous bones of the craniofacial skeleton require different considerations than endochondral bone. Membranous bones are smaller than endochondral long bones and require finer instrumentation for distraction. In addition to having different germ layer derivations, they have different mechanisms of embryologic ossification. Specifically, endochondral ossification requires formation of a cartilaginous template that is replaced by woven bone, whereas intramembranous ossification entails mesenchymal precursors that differentiate directly into osteoblasts forming woven bone.[5] Membranous bone is known to have a more robust blood supply and thus, is associated with fewer perioperative infections. In-lay bone grafts undergo less resorption than endochondral bone.[6] Given membranous bone's unique biological characteristics, a refinement of Ilizarov's distraction protocols is necessary when applied to membranous bone in the craniofacial skeleton.

Latency is the time between placement of the distraction device and the beginning of lengthening. Ilizarov originally advocated a seven-day latency phase in order to allow healing after wide periosteal undermining.[1,2,7] In 1992, McCarthy applied a similar one-week period of fixation of the mandible before device activation.[3,8] More recently, latency has been shortened to 48–72 h to prevent premature union. However, some clinicians associated shorter latency with decreased callus volumes and inadequate osteogenesis.[9] The majority of craniofacial distraction cases are performed on the growing skeleton, which is know to heal fractures within two weeks. While recent studies have begun to elucidate the cellular mechanisms of distraction osteogensis,[10] no reported studies have examined the cellular responses to latency. Clinically, the optimal time of latency remains unknown.

To understand the cellular response to latency, we used a microdistractor system and compared varying latency durations to 'no latency' on murine preosteoblasts.[4,11] This system is capable of mimicking *in vivo* distraction forces using an *in vitro* system by suspending MC3T3 mice preosteoblasts within a collagen matrix so that distraction forces placed upon the scaffold are transmitted to the cells. A recent study demonstrated the ability of the microdistractor system to increase the proliferative and osteogenic expression of MC3T3 cells.[4,11] To determine the clinical effect of latency, we studied latency versus no latency in comparative mandibular distraction cases with regard to bone healing radiographic outcomes.

An improved understanding of the osteogenic effects of latency periods will provide necessary justification for clinical practices. Decreasing or even eliminating latency may shorten the treatment period required in craniofacial distraction, potentially opening the procedure for application in an even broader range of deformities.[12] Maintaining a necessary latency period may enhance bone formation resulting in improved outcomes. In this study, the cellular mechanisms of latency were explored and used to direct clinical management.

Methods

Section 1: in vitro – microdistraction of preosteoblasts

Cell isolation and culture

Mouse osteoprogenitor calvarial cells (MC3T3-E1, Clone 4, Lonza Inc, Allendale, N.J.) were cultured using control media comprised of alpha modified eagle's medium (αMEM, Sigma, St. Louis, MO), 10% fetal bovine serum (FBS, Omega Scientific, Tarzana, CA), and 5% streptomycin(100 ug/mL)/penicillin(100 U/mL) (Invitrogen, Grand Island, NY). At 90% confluence, cells were split using 0.25 trypsin-EDTA (Mediatech, Herndon, VA). Cells were used between passages 3 and 16.

Preparation of collagen gels

Delrin molds (McMaster Carr Supply, Los Angeles, CA) were devised to allow the liquid collagen to polymerize into a 3-D shape (3.5 cm × 3.5 cm × 1.5 cm). Within the molds, plasma etched 35 mm × 5 mm × 3 mm polyethyelene bars were fabricated and placed at opposite ends of the mold. Plasma etching sterilizes and induces hydrophilicity of the bars for improved cell attachment.[13] These sterile molds were housed in large petri dishes (150 mm × 15 mm) with edges sealed by silicone stopcock grease (Dow Corning, Midland, MI). Prior to gel polymerization, these petri dishes were blocked with 2 g/mL bovine serum albumin (BSA, EM science, Gibbstown, NK) for 24 h at 37 °C and rinsed three times with sterile 1× phosphate-buffered saline (PBS, Invitrogen, Grand Island, NY).