Intracellular Tension in Periosteum/Perichondrium Cells Regulates Long Bone Growth

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Introduction
Circumferential periosteal incision is used to correct unilateral long bone growth retardation.1 Extended growth upon removal of the perichondrium (PC) and periosteum (PO), can be inhibited when tibiotarsi are cultured in conditioned medium from these PO/PC cells, cultured on glass substrates. Incision might reduce the stress of mechano-sensitive PO/PC cells, decreasing the expression of growth inhibiting biochemical signals.

We therefore hypothesized that the ability of the periosteum/perichondrium cells to carry intracellular tension through their actin microfilament network is at the base of the signaling cascade, eventually resulting in the expression of soluble factors that modulate cartilage growth.

Method
Cells from the PO/PC were seeded on glass or polyacrylamide gels with various stiffnesses (3, 14, 21, 48 and 80kPa), to alter intracellular tension. For cultures treated with cytochalasinD (CD), which disrupts the actin network, 6µM CD in DMEM was added. After 30min of incubation, CD was removed and 4mL serum-free DMEM was added. Cultures, not treated with CD, were handled exactly the same, except for CD incubation. After 24h, conditioned medium was collected.

Dissected tibiotarsi, treated as experimental pairs, were left intact or disposed of PO/PC (stripped) and provided with conditioned or unconditioned medium. After 3 days of culture distal cartilage length was determined (fig 1).

Results
A significant relation was detected between substrate stiffness and length ratio (fig 2b, linear regression analysis, R²=0.30). Only at high substrate stiffness (80kPa & glass), a significant difference was no longer detected (fig 2a; paired t-test).

In stripped experimental pairs, distal cartilage length was significantly longer when cultured in conditioned medium from 3kPa compared to 80kPa substrates (fig 3). This effect was counteracted upon CD treatment (fig 3), i.e. upon release of intracellular tension. The effect of CD treatment was confirmed by the significant difference between stripped experimental pairs in conditioned medium from 80kPa substrates (fig 3). Similar trends were observed in intact bones, however not significant (fig 3).

Discussion
Results support our hypothesis that loss of intracellular tension upon periosteum incision alters gene expression patterns of the PO/PC cells, through which long bone growth is modulated. Differences in cartilage length due to PO/PC removal is counteracted by the conditioned medium originating from high substrate stiffness, but not by low stiffness substrates or when CD is added; conditions that both result in low intracellular tension.

References