

## **Hyaluronan-dependent matrix is involved in cell-cell coupling and cell migration following micromanipulation: implications for mechanotransduction and tissue changes following fascial manipulation.**

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**BACKGROUND** Hyaluronan (HA) is a multifunctional glycosaminoglycan providing lubrication in synovial tissues, and together with bound proteoglycans in the pericellular space, contributes to tissue swelling pressure and viscoelasticity. An important regulator of cellular adhesion, HA is extruded through the plasma membrane by one of three hyaluronan synthases, and provides a hydrated interface conducive to cell migration and proliferation. Clinical fascial manipulative techniques involve the application of a wide range of pressure, tension and shear and produce palpable tissue softening, suggesting that HA may play a role in the tissue response to manipulation. Recent data also show that cells can make massive HA-based cables, particularly under inflammatory conditions, implying that structured HA may be important for mediating mechanotransduction, early contractile events, or cell-cell communication within inflamed connective tissues or nerve sheaths.

**METHODS** In this study, we have used time-lapse microscopy, in combination with a particle exclusion assay, to examine the immediate and early effects of micromanipulation of individual human fibroblasts or smooth muscle cells and confluent cell sheets on the formation of HA-dependent pericellular matrix. Matrix components were also located by immunohistochemistry.

**RESULTS.** In cells with no manipulation, formation of HA and versican-rich cell coats preceded spontaneous release of cellular tension. In one sequence, the same matrix provided guidance for filopodial reextension within minutes of initial recoil. In migrating cells, thick concentrations of HA and versican were present at sites of high focal adhesion turnover, such as ruffling membrane at the lamellipodium and along the cell flanks. Stretching of single cells or long filopodia with a micropipette caused the formation of multiple cytoplasmic dilatations that tended to move along the cell process toward the cell body, usually followed by catastrophic retraction and/or fragmentation of the filopodium. Following recoil, the cells assumed a more rounded shape. HA-dependent matrix formed around the cell body or stretched process within as little as 20 minutes. HA coats were also seen around cell fragments. During manipulation of confluent cell sheets, the forces experienced by any individual cell were complex and varied. Retraction of the torn edges depended on the level of pre-stress in the local vicinity, cellular adhesiveness, the degree of intercellular connection and orientation relative to the direction of force. Prominent HA-dependent pericellular matrices were seen by 6 hours around the cells migrating from the retracted edges of the cell sheet. At 24 h, fluorescent staining revealed long HA and versican-rich cables that connected cells, and occasionally completely bridged the wound gap (>1 mm long). In the presence of inflammatory mediators poly I:C or IL-1 plus TNF- $\alpha$ , increased HA cables were seen, but cell migration was impaired.

**CONCLUSION** These data suggest that structured hyaluronan may form an early mode of cellular mechanical recoupling following manipulation and are consistent with the hypothesis that alterations in hyaluronan amount and organization may underlie tissue changes following fascial manipulation.