

Intercellular Communication via Gap Junctions in the Annulus Fibrosus

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INTRODUCTION: Annulus fibrosus cells have an elaborate morphology[1,2] and complex mechanical[3] interaction with the extracellular matrix. Evidence of the gap junction protein connexin-43 has been found in bovine and human annulus fibrosus cells[2,4]. To fully determine their mechanobiological behaviour it is essential to investigate possible intercellular communication. Fluorescence recovery after photobleaching (FRAP) has demonstrated functional gap junctions in the nucleus pulposus and cartilage[5,6]. The aim of this study was to determine the activity of gap junctions in the *intact* annulus fibrosus.

MATERIALS AND METHODS: Discs were obtained from 8 bovine tails within 4 hours of slaughter. Circumferential outer (OA) and inner (IA) annular specimens (~1 mm thick) were bluntly dissected from the posterior disc. All specimens were incubated in calcein-AM (5 μ M, Molecular Probes) in Ca^{2+} -free PBS (1.5 h, 37°C) and then washed in 100 times volume PBS for 10 minutes (3x). Some specimens were treated with the gap junction blocker 1-octanol (1 mmol/L, Sigma-Aldrich). Cells were imaged on a Zeiss LSM 510 confocal microscope (63x 1.2 NA water immersion objective; 488 nm argon laser; 1.3 μ m optical slice). An initial image was recorded (t_{ini}). An oval region of interest (ROI) was then fit around the cell body and visible processes of a cell. This ROI was then photobleached at 100% intensity for 10-15 seconds (until ~40% original fluorescence). Images were obtained immediately following photobleaching (t_0), after 10 min (t_{10}) and 20 min (t_{20}) of recovery. The mean pixel intensity within a cell of interest was determined and normalized to an uninvolved cell in the image field (Image J, NIH). Percent recovery was calculated from: $[(t_{20} \text{ or } t_{10} - t_0) / t_{ini}] \times 100$.

RESULTS: A clear return in fluorescence was observed in cells of the OA, which did not occur when treated with octanol (Fig. 1). In the OA, a significant difference was observed between the percent recovery of untreated (n=17) and octanol treated (n=15) cells at both t_{10} and t_{20} (Fig. 2, $p < 0.05$). In the IA, only 50% of the untreated cells showed a greater than

10% recovery in fluorescence. No significant difference was observed between the percent recovery of untreated (n=14) and octanol treated IA cells (n=10) at t_{10} or t_{20} ($p > 0.05$).

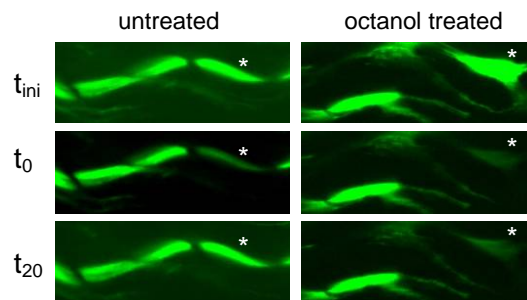


Fig. 1 Return of fluorescence in cells() of the OA and no return when treated with octanol.*

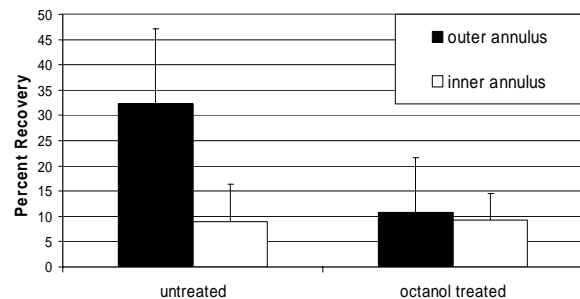


Fig. 2 Percent recovery (t_{20}) of OA and IA cells untreated and treated with octanol.

DISCUSSION: This study demonstrates functional gap junctions in the *intact* annulus fibrosus. The percent recovery of fluorescence in untreated outer annulus cells is similar to the 25-35% recorded in chondrocytes[6]. Functional gap junctions have been demonstrated in cultured human annulus fibrosus cells with decreased communication observed with aging[4]. Future research will explore the potential role of functional gap junctions in the mechanobiology of healthy and degenerated annulus fibrosus cells.

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