

**Inhibition of chondrocyte death at the wound edge enhances integrative cartilage repair.****S.J. Gilbert, S.K. Singhrao, I Khan, V.C. Duance, C.W. Archer**

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**Introduction.** Experimental wounding of articular cartilage leads to necrotic and apoptotic cell death at the lesion edge (1). It is hypothesised that the presence of healthy chondrocytes at the wound edge is critical for producing a repair tissue that is capable of integrating with the surrounding cartilage (2,3). Since repair of cartilage lesions often requires surgical debridement, associated chondrocyte death at the wound edges will be an inevitable problem. An earlier study has shown that short-term intra-articular administration of a caspase inhibitor decreases chondrocyte apoptosis at the wound edge and subsequently reduces cartilage degeneration following experimental osteochondral injury in rabbits (4). This study investigates whether inhibiting the cell death that occurs as a response of articular cartilage to wounding will result in enhanced cartilage integrative repair.

**Materials and Methods.** Full depth articular cartilage cores (6mm) were incubated for 3 hours in media containing inhibitors of necrosis (Necrostatin-1; 30 $\mu$ M; Biomol) or apoptosis (Z-VAD-FMK; 20 $\mu$ M; Calbiochem). At the end of this period, a 3mm inner core was cut and left *in situ* thereby creating a 'doughnut' model to study cartilage-cartilage integration. Cartilage was cultured for 2-weeks in media containing inhibitors with cultures being fed with fresh media and inhibitors every three days. Cartilage was analysed for necrotic (LDH release) and apoptotic (TUNEL) cell death, sGAG release (DMMB) and tissue integration (histology, immunohistochemistry, SEM).

**Results.** Within 48 hours of creating the wound, a significant ( $p=0.003$ ) increase in LDH release was observed compared to unwounded cartilage. Treatment with Z-VAD-FMK significantly reduced the extent of LDH release ( $p<0.02$ ) and apoptosis occurring at the wound edge. Necrostatin-1 reduced LDH release but the response was more variable ( $p<0.058$ ). Treatment with either of the inhibitors reduced the level of sGAG lost into the media that resulted from wounding the cartilage. Toluidine blue staining of cartilage sections revealed significant integration of the wound edges in 'doughnuts' treated with Z-VAD-FMK. Necrostatin-1 improved integration but to a lesser extent. Immunohistochemistry revealed that in untreated doughnuts, there was an increase in pericellular staining for type III

collagen extending out from the wound edge within the mid zone. Necrostatin-1 treatment resulted in an apparent increase in type I and III collagen whereas this was not observed in cartilage treated with Z-VAD-FMK. SEM analysis showed that Z-VAD-FMK treatment enhanced tissue integration.

**Discussion.** This study shows that treatment of articular cartilage with cell death inhibitors prior to wounding increases the number of viable cells at the wound edge, prevents matrix loss and results in a significant improvement in cartilage-cartilage integration.

**References.**

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