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EXPERIMENTAL ARTHRITIS

# Neutrophil microvesicles protect cartilage in arthritis

Microvesicles (MVs) released from neutrophils have chondroprotective properties that could be harnessed for the treatment of diseases associated with cartilage degeneration, suggests new research published in *Science Translational Medicine*. The study shows that MVs deliver bioactive molecules, including the anti-inflammatory protein annexin A1 (AnxA1), to chondrocytes — through the 'impenetrable' cartilage matrix — in an inflammatory setting.

"Vesicles are emerging as a novel, massively understudied means of communication between cells," contends Mauro Perretti, corresponding author of the paper reporting the findings. "These microstructures contain proteins, lipids, microRNA, mRNA and more, [and] hence seem to enable a reasonably efficient way to transfer 'cellular information.'"

The researchers quantified MVs present in plasma and blood samples from patients with rheumatoid arthritis (RA), and found that MVs derived from neutrophils ($CD66b^+$), monocytes ($CD14^+$) and T cells ($CD3^+$) were more abundant in synovial fluid than in plasma for each patient ($n = 7$). Furthermore, as Perretti reports, "a subset of neutrophil-derived $AnxA1^+$ vesicles was enriched in the synovial fluid, when measured against paired blood samples, of RA patients." Higher numbers of $AnxA1^+CD66b^+$ MVs, in comparison with $CD14^+$ or $CD3^+$ MVs, were also found in synovial fluid samples from a separate cohort of patients with RA ($n = 22$).

> "...neutrophil MVs migrated into the cartilage matrix and delivered AnxA1 to chondrocytes..."

"We queried whether this enrichment of $AnxA1^+$ vesicles in the synovial fluid might be of biological relevance," Perretti explains. To investigate their effects *in vivo*, purified MVs from human neutrophils (treated with TNF to induce MV shedding) were injected into the knee joints of mice with inflammatory arthritis and nonarthritic control mice. "Against our initial prediction, $AnxA1^+CD66b^+$ vesicles exerted protective reparative effects on cartilage," recounts Perretti. In the K/B×N serum transfer model of inflammatory arthritis, the loss of sulfated glycosaminoglycans (sGAGs, a marker of cartilage integrity) observed in the joints of arthritic mice was prevented by treatment with MVs. Similarly, in the glucose-6-phosphate isomerase (GPI)-induced model of RA, intra-articular injection of MVs reduced sGAG loss.

By contrast, cartilage erosion was exacerbated in mice with inadequate production of neutrophil MVs owing to deficiency of the phospholipid scramblase anoctamin-6 (encoded by *ANO6*, also known as *TMEM16F*). In response to K/B×N serum transfer, synovitis and inflammation scores were similar in *Tmem16f*$^{+/-}$ mice and wild-type littermates, but sGAG loss was approximately twofold greater in the joints of *Tmem16f*$^{-/-}$ arthritic mice.

The effects of MVs on cartilage were further explored *in vitro* using 3D micromass cultures of the human chondrocyte cell line C28/I2 and of primary adult articular chondrocytes. Stimulation of these chondrocytes with IL-1β induced release of sGAGs, reduced expression of the cartilage matrix genes *ACAN* and *COL2A1* as well as the transcription factor *SOX9*, increased release of IL-8 and prostaglandin $E_2$, and increased chondrocyte apoptosis. However, co-treatment with MVs protected chondrocytes against these effects of IL-1β treatment, by inducing the synthesis of transforming growth factor β (TGF-β).

In rat cartilage explants, neutrophil MVs migrated into the cartilage matrix and delivered AnxA1 to chondrocytes, whereas macrophage MVs did not penetrate the cartilage. *In vivo*, MVs derived from systemically injected murine neutrophils were detected in articular cartilage in mice with active arthritis but not in nonarthritic mice. Data from further mechanistic studies suggest that the protective effects of MVs are dependent on AnxA1 and its receptor, *N*-formyl peptide receptor 2 (FPR2, also known as ALX), upstream of TGF-β production.

Regarding the clinical development of MVs, "[Further studies are needed] to define the effects of MVs modified by the addition of molecules to augment their biological effects on cartilage and to determine the longevity of the pharmacological effects we describe here for $AnxA1^+CD66b^+$ vesicles," suggests Perretti.

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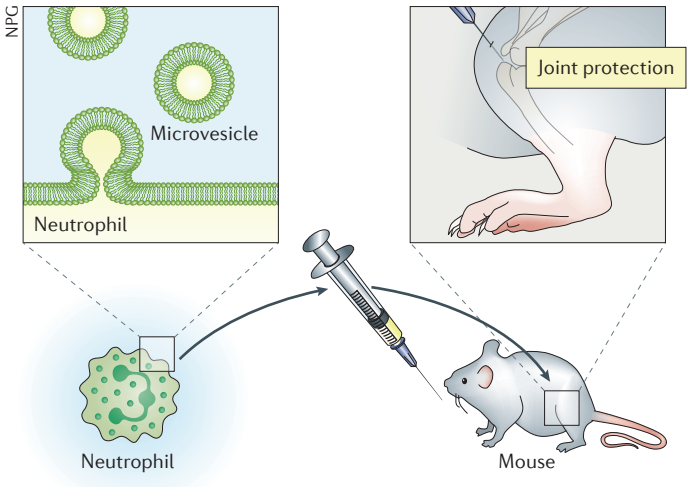


**ORIGINAL ARTICLE** Headland, S. E. *et al.* Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis. *Sci. Transl. Med.* **7**, 315ra190 (2015)