**The Effects of TNF-alpha Inhibition on Cartilage: a Systematic Review of preclinical studies.**

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**Running headline: TNF-α inhibition effects on articular cartilage**

**Abstract:**

**Objective:** To report the most up-to-date evidence on the effects of TNF-alpha inhibition on cartilage with a focus on its clinical relevance.

**Design:** A systematic review was performed by searching PubMed, Embase and Cochrane Library databases. Inclusion criteria were studies of any level of evidence published in peer-reviewed journals reporting clinical or preclinical results written in English. Relative data were extracted and critically analysed. PRISMA guidelines were applied, and risk of bias was assessed as well as the methodological quality of the included studies.

**Results:** 13 studies were included after applying the inclusion and exclusion criteria. Three were *in vitro* human studies from OA patients. Ten were animal modal studies including two *in vitro* studies, and eight *in vivo* studies. TNF-alpha inhibition in *in vitro* studies was generally reported beneficial due to the improved osteochondral viability, proliferation and chondrogenesis. In addition, TNF-alpha inhibition was noted to be beneficial in promoting the natural repair of osteochondral lesions and has a chondroprotective effect in *in vivo* studies.

**Conclusion**: Based on current evidence, TNF might have the potential to interfere with the healing process of chondral and osteochondral defects occurring naturally or in low inflammatory environment after a cartilage repair procedure. Therefore, the use of biological agents to inhibit its action in cartilage repair surgery could be beneficial, and this could translate into a promising therapy that improves the outcome of currently available cartilage procedures.

**Keywords:** TNF-alpha; tumour necrosis factor; TNF-alpha inhibition; articular cartilage; early osteoarthritis; chondrocytes

Introduction

Human cartilage is a unique avascular, aneural and alymphatic tissue with limited cellular mitotic activity 1,2. It has highly specialized cells, chondrocytes, contained in an extracellular matrix (ECM) mainly composed of type II collagen and proteoglycans 3. An overview of chondrogenesis is important to help understanding cartilage disease. Chondrogenesis begins *in utero* as a result of aggregation and condensation of mesenchymal cells 1,2. The condensation of mesenchyme is a crucial step as it allows the production of several factors such as ECM, cell adhesion molecules, and transcription factors [SRY-box 9 (SOX9)]. SOX9 plays an essential role in chondrogenesis and mesenchymal cell differentiation into chondrocytes 3. Cartilage damage is the leading cause of restricted daily life activities in patients with chronic musculoskeletal conditions, and has long lasting physical and psychological effects 4–6. In many chronic autoimmune and inflammatory diseases involving the cartilage, several cytokines are released as part of the pathophysiologic response including Tumour Necrosis Factor (TNF). TNF is a proinflammatory cytokine and, along with other cytokines, is a catabolic factor for cartilage 4,7,8. TNF promotes the release of matrix metalloproteinases (MMPs) from synovial fibroblasts resulting in cartilage destruction 4,7, and inhibits chondrogenesis through the nuclear factor-kB (NF-kB) pathway 8 by down regulating the production of SOX9 8,9. A specific function carried out by TNF that is of great interest in the current literature is the ability to signal chondrocyte apoptosis 10. TNF-alpha also inhibits the ability of mesenchymal stem cells (MSCs) to differentiate into chondroblasts affecting chondrogenesis 11.

TNF exists in either secreted or membrane-bound form 12, and with two extensively investigated subsets- TNF-alpha and TNF-beta 13. It is isolated primarily from monocytes/macrophages, but other cell types such as T and B lymphocytes, mast cells, natural killer cells, neutrophils, fibroblasts, and osteoclasts can also produce TNF 12. TNF is first expressed as a transmembrane protein (mTNF) on the cell surface, and is then transformed into soluble TNF (sTNF) form by TNF-converting enzyme 12. This soluble form is subsequently released and becomes detectable in the blood plasma. Both mTNF and sTNF function through two receptors, each carrying out a particular function: TNFR1 (TNFRSF1A or CD120a) is expressed across all human tissues, and TNFR2 (TNFRSF1B or CD120b) is expressed primarily in immune cells, neurons, and endothelial cells 12. The majority of TNF proinflammatory functions are believed to be mediated through TNFR1. The function of TNFR2 on the other hand remains only partially understood, especially in the disease setting 12,13. Upon receiving a signal from TNF, these receptors can carry out the various functions of TNF that include inflammatory immune response, lymphoid tissue development and bone remodelling 12,13. TNFR1’s cytoplasmic tail contains a death domain (DD) thereby allowing it to recruit the TNFR1-associated DD (TRADD); TNFR2, on the other hand, does not have an intracellular DD and recruits the TNFR-associated factor (TRAF) 1 and 2 proteins instead. Both TNF receptors signalling pathways may lead to the activation of NF-kB and the induction of a cell survival response. In addition TNFR1 is also capable of inducing cell death 12,13.

Biological drugs that modify disease progression are increasingly used to manage chronic autoimmune and inflammatory diseases. These drugs include anti-TNF-alpha therapy that include etanercept (ETA- a recombinant human TNF-alpha soluble receptor fusion protein), infliximab (INF- chimeric mouse/human monoclonal antibody), adalimumab (ADA- recombinant human mono-clonal antibody), golimumab and certolizumab pegol 12,14. Many studies have investigated the effect of anti-TNF-alpha drugs on cartilage. Some studies have reported a potential benefit in cartilage repair models with anti-TNF-alpha drugs by lowering its inflammatory actions 5,6,15,16, but this finding has also been challenged 17. Early intervention to prevent and limit progression of cartilage destruction is crucial to improve patient outcome and quality of life.

The inflammatory processes involving the joint as a whole and the cartilage in particular have been linked to several surrogate biomarkers of cartilage degradation 18–20. Recent literature however suggests a nonlinear relationship between these biomarkers and some structural variables of cartilage 19,20. A urinary or blood biomarker could reflect a metabolic change that is not necessarily related to structural, radiographic or clinical changes 19,20. A soluble biomarker used to investigate changes in tissue or organ metabolism may be an easier and less invasive measure but is of doubtful or limited value in assessing structural changes in the cartilage. Our aim is to report the most up-to-date evidence on the effects of TNF-alpha inhibition on cartilage in preclinical studies with a focus on its clinical relevance.

**Methods**

*Literature search Strategy*

            This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 21 and MOOSE guidelines 22. A comprehensive search was performed on three medical electronic databases (PubMed, Embase and Cochrane Library) by two independent authors (E.C. and K.M.Y.) from the 1st of January 1998 to the 12th of June 2019. Our main aims were to: (1) evaluate available quality of evidence on TNF-alpha as a target for cartilage restoration through the review of animal and *in vitro* studies, and (2) discuss its possible implementation in further experimental studies. To achieve the maximum sensitivity of the search strategy, we combined the terms: ‘‘anti-TNF” OR “TNF inhibition”, as well some common names of the anti-TNF drugs such as “Adalimumab OR (certolizumab pegol) OR etanercept OR golimumab OR infliximab” with “cartilage OR chondrocytes OR MSCs” as either key words or MeSH terms. The reference lists of all included articles, previous literature reviews on the topic and top hits from Google Scholar were reviewed for further identification of potentially relevant studies and were assessed using the inclusion and exclusion criteria. In order to avoid overlapping of other ongoing reviews, we first searched PROSPERO site for any similar review and then prospectively registered our study (registration number: CRD42019118710 and CRD42019119796).

*Selection Criteria*

Eligible studies for our systematic review included those investigating the effects of TNF-alpha inhibition on cartilage tissue. Primary screening of the titles and abstracts was performed by including studies of any level of evidence published in peer-reviewed journals reporting preclinical results in English. Moreover, articles discussing the effect of the inhibition in OA patients were reviewed. Exclusion criteria included studies investigating biomarkers for treatment response and/or not strictly related to cartilage changes, due to the weak scientific evidence on the topic 19,20, and articles investigating use of TNF-alpha inhibition on rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis as well as other rheumatologic condition which would make the included studies too heterogenous to draw conclusions. Additionally, we excluded studies in which data were not accessible, missing, without an available full text, or not well reported. We also excluded all the remaining duplicates, and the studies with poor scientific methodology. Abstracts, case reports, conference presentations, reviews, editorials and expert opinions were excluded.  The study selection was performed independently by two authors (E.C. and K.M.Y.), and any discrepancies in the selection process were resolved by discussion amongst the authors. A senior investigator (W.K.) was consulted to revise the selection process.

*Data Extraction and Criteria Appraisal*

All data were extracted from article text, tables and figures. Data were extracted using the Population, Intervention, Comparison, Outcome (PICO) framework and included title, year of publication, study design, sample size, study population, patient characteristics, intervention and comparator (where applicable), outcomes, funding and conclusions. Two investigators independently reviewed each article (E.C. and K.M.Y.). Discrepancies between the two reviewers were resolved by discussion and consensus. The final results were reviewed by the senior investigator (W.K.).

*Risk of Bias Assessment*

            Risk of bias assessment was performed according to the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)’s risk of bias tool 23 for preclinical studies (Supplementary material Table 1)﻿. SYRCLEs tool has been designed for the sole purpose of assessing risk of bias in systematic reviews of animal studies. Although it is designed using cochrane risk of bias tool as a platform, it has been modified for components of bias that have a considerable impact on animal interventional studies. This assessment used “Low,” “Moderate” and “High” as judgement keys where “Low” indicated a low risk of bias, “Moderate” indicated that the risk of bias was moderate, and “High” indicated a high risk of bias. The assessment was performed by two authors (E.C. and K.M.Y.) independently. Inter-rater agreement was 92%. Any discrepancy was discussed with the senior investigator (W. K.) for the final decision.

*Study Quality Assessment*

            The methodological quality of evidence was assessed according to Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist with supporting guidance from the CAMARADES website 24, giving one point for each of (1) publication in a peer-reviewed journal; (2) statement of temperature control; (3) random allocation to groups; (4) allocation concealment; (5) blinded assessment of outcome; (6) use of anesthetic without significant internal protection of blood vessel; (7) appropriate animal model (aged, healthy, diabetic, or hypertensive); (8) sample size calculation; (9) compliance with animal welfare regulations; (10) statement of potential conflict of interests. Each study was assessed and scored on a scale from 0 (lowest) to 10 (highest) points. This quality assessment instrument has been introduced to assess quality of animal studies since previous quality assessment tools lacked validity. The assessment was performed by two authors (E.C. and K.M.Y.) independently. Inter-rater agreement was 94%. Any discrepancy was discussed with the senior investigator (W. K.) for the final decision.

**Results**

A total of 647 studies were collected from the databases according to the aforementioned inclusion and exclusion criteria. Overall 363 papers were screened through abstract and title reading after the removal of the duplicates. Due to abstract and title screening we were able to exclude 325 articles. Ultimately, after full text reading and reference list check, we selected 13 articles to the purpose of the present manuscript**.** A PRISMA 21 flow chart of the selection process and screening is provided (Figure A).

**Figure A.**

Thirteen articles were included after applying the inclusion and exclusion criteria 2,6,14,16,17,25–32. Three studies were human *in vitro* studies 2,14,28. Ten were animal model studies 6,16,17,25–27,29–32 including two *in vitro* studies 6,29 and eight *in vivo* studies 16,17,25–27,30–32. Of these animal studies, five were in an OA model 25,26,31–33, three were on cartilage graft models 6,16,29, one was from a polyarthritis model 17, and one was on a temporomandibular primary chondrocyte model 27. Overall, the quality of the studies was assessed as moderate. The quality assessment was high for only one study 27, moderate for five of the included studies 6,16,17,29,30 and moderate to low in four studies25,26,31,32 (see Supplementary Material Table 2). Based on the risk of bias and quality assessment it was not necessary to exclude any article due to high risk of low quality. The main characteristics of the included studies are detailed in Table 1.

**Table 1**

*TNF inhibition in in vitro studies of chondrocytes*

Human *in vitro* studies have shown variable results of TNF-alpha inhibition on cartilage. Isyar et al. 28 investigated the effects of 10μg/mL of ADA (500mg/ml), ETA (50mg/ml), and INF (100mg/ml) on human gonarthrotic chondrocytes in monolayer that had been harvested from the cartilage of six distal femora. All three drugs demonstrated significant toxic effects on the viability and proliferation of chondrocytes compared to the control group. Complete chondrocyte inhibition was reported with all three drugs at 48 hours. This chondrotoxic effect of anti-TNF drugs on chondrocytes was further confirmed by Guzelant et al. 2 in a study on human gonarthrotic chondrocytes in monolayer that had been harvested from the cartilage of three distal femora and proximal tibias. They demonstrated impaired human OA chondrocyte viability and proliferation at 24, 36 and 48 hours with ADA (40mg at 10mg/ml). A more recent human study however showed that the cytotoxic effect of TNF-alpha on microspheroids containing human OA primary chondrocytes or chondrogenically differentiated MSCs, and the inflammation gene expression triggered by TNF-alpha, was prevented by ADA, ETA and INF 14.

Animal *in vitro* studies showed a beneficial effect of TNF-alpha inhibition on cartilage. Ossendorff et al. 29 simulated early osteoarthritic inflammation with TNF-alpha in a standardized joint-like Autologous Chondrocyte Implantation (ACI) bioreactor model using chondrocytes from five bovine fetlock joints. They found that although TNF-alpha down-regulated anabolic factors and up-regulated catabolic factors, these effects were counteracted by ADA. Linn et al. 6 investigated chondrocyte viability when applying ETA (10μg/ml) into an osteochondral sample from the femoral condyles of eight Boer goats. They noted that ETA substantially maintained chondrocyte viability after 28 days of storage in the superficial zone of cartilage, but found no difference in chondrocyte viability or glycosoaminoglycan (GAG) content across all three zones.

*TNF inhibition effects on cartilage metabolism in in vivo studies*

All studies 25,26,31,32 investigating *in vivo* effects of anti-TNF drugs on animal OA models concluded that inhibiting TNF has substantial chondroprotective effect. Ma et al. 30 assessed knee cartilage in 24 Female Sprague-Dawley OA rats macroscopically and microscopically after subcutaneous injection of 20 mg/kg ADA. They found that ADA improved the structure of subchondral bone, and reduced the degradation of the cartilage matrix using Mankin score and MMP-13 expression on immunohistochemical staining. Yang et al. 27 showed that intra-articular injection of the TNF inhibitor partially reduced the loss of proteoglycans and prevented the caspase-8 increase when assessing the Caspase 8 death pathway, but did not prevent cartilage loss, in their rat temporomandibular primary chondrocyte model.

Shealy et al. 31 studied the effect of administering anti-TNF-alpha monoclonal antibody by intraperitoneal injection in 25 Tg197 transgenic mice with OA. It was reported that TNF inhibition reduced degenerative changes clinically and histologically in the mice at 6 and 16 weeks. Zhang et al. 32 investigated the effect of 0.5ml of low dose (10mg/ml) and high dose (20mg/ml) INF on 20 New Zealand white OA rabbits injected intrarticularly, compared to 0.5ml of normal saline in 10 rabbits in the control group. Macroscopically it was demonstrated that INF treated rabbits had reduced signs of OA compared to the control group. This chondroprotective effect was further supported by the histological examination that revealed a significantly lower Mankin score for the INF treated groups compared to the control group. No difference between low and high dose of INF was found.

Kawaguchi et al. 16 investigated osteochondral defects in a cartilage model of 133 Japanese white rabbits exposed to subcautaneous ETA at low (0.05μg/kg), intermediate (0.4μg/kg) and high dose (1.6μg/kg). Inhibiting TNF promoted enhanced repair of the osteochondral defects in the rabbits demonstrating early remodelling and improved cell structure histologically. Urech et al. 25 investigated the effect of an intrarticular injection of ESBA105 (a single-chain Fv antibody) against TNF-alpha in three OA model Lewis rats when compared to INF. Analysing tissue penetration, biodistribution and pharmacokinetics, they showed that ESBA105 has the ability to infiltrate the osteoarthritic cartilage and prevent further arthritic damage induced by TNF resulting in 90% inhibition of knee joint swelling, inflammatory infiltrates and proteoglycan loss from cartilage.

Zwerina et al. 17 looked at inhibition of TNF-alpha mediated cartilage damage with INF in 64 Human TNF–transgenic mice with polyarthritis by quantitatively assessing proteoglycan loss by toluidine blue staining and expression of MMPs 3, 9, and 13 in articular chondrocytes. Inhibition of proteoglycan loss was associated with gradually decreased expression of MMPs 3, 9, and 13 in articular chondrocytes. Although MMP expression was significantly inhibited by anti-TNF, articular changes caused by chronic overexpression of TNF were not completely blocked by monotherapies targeting TNF, IL-1, or RANKL. A combined approach with the blockade of TNF and IL-1, and to a lesser extent TNF and RANKL, lead to almost complete remission of disease. Elsaid et al. 26 administered ETA (0.5 mg/kg) by subcutaneous injection in 18 Lewis rats with transected anterior cruciate ligaments. Measures included lubricin, sulfated GAG, and histological evaluation of articular cartilage with lubricin-specific antibody staining and Safranin O. They demonstrated substantial chondroprotective effect with ETA as evident by the upregulation of lubricin deposition on cartilage and a decrease in sulphated GAG release from the cartilage.

*Anti-TNF agents in cartilage repair surgery models*

Ossendorff et al. 29 simulated early osteoarthritic inflammation in chondrocytes with TNF-alpha in a bovine ACI bioreactor model. The mechanical conditions after implantation were recreated with a custom-built multiaxial load bioreactor. The use of ADA significantly counteracted the TNF-alpha effects on chondrocytes gene expression, DNA, GAG, and collagen production and on histological analysis at two weeks 29. Similar findings were reported by Linn et al. 6 who investigated the chondrocytes viability in osteochondral graft at 28 days with or without ETA in an *in vitro* study. Although no significant histological differences were noted on safranin-O staining, the percentage viability of the superficial zone in ETA-treated allografts was maintained at significantly higher levels than those measured in the untreated group (69.3 ± 9.4 compared to 47.8 ± 19.1, *p* = 0.01). In an *in vivo* study, Kawaguchi et al. 16 demonstrated that subcautaneous injections of varying doses of ETA promoted enhanced repair of the osteochondral defects in a cartilage model of Japanese white rabbits. ETA at all doses promoted early subchondral bone remodeling of the osteochondral defects and, at higher doses, cell morphology and matrix staining indicated good repair. Adjacent cartilage did not show any degeneration in any of the samples examined, indicating that ETA does not affect metabolism of normal cartilage.

**Discussion**

The available literature on the effects of TNF alpha inhibition in cartilage is highly heterogenous and made up by a majority of moderate to low quality of studies. Although TNF interferes with the healing process of chondral and osteochondral defects occurring naturally or after a cartilage repair procedures, the role of TNF-alpha inhibition in these settings is still controversial 15,34–40. In this review we have systematically analysed the evidence in the literature on the effects of TNF-alpha inhibition on chondrocytes, cartilage remodelling, and their differentiation and proliferation that is integral to cartilage repair procedures.

Our study shows that TNF-alpha inhibition in *in vitro* cartilage repair models generally improved osteochondral viability 6, improved proliferation and chondrogenesis enhancing the natural repair of chondral lesions 29, and demonstrated chondroprotection 14,29,30. Although anti-TNF-alpha drugs are currently used for several rheumatic and autoimmune diseases, two human *in vitro* studies included in this review have raised some concerns on their chondrotoxic effect 2,28. In a study comparing the effects of ETA, ADA and INF on chondrocyte viability, INF was reported to be the most toxic drug when compared to ETA and ADA (p < 0.01) 28. The same research group later investigated the effects of ADA, the least toxic drug in their earlier study, on OA chondrocytes cell viability and reported impairment of proliferation at 24, 36, and 48 hours 2. Although the concentrations used in these two studies is similar to those in other studies included in our review, these two human *in vitro* studies exposed chondrocytes in monolayer culture to these high concentrations directly, where as *in vivo* they would be protected by the ECM. A human study included in our review looked at the effects of these biological agents on microspheroids containing 10,000 cells and did not identify this chondrotoxic effect 14. A 3D cell culture system can mimic physiological tissue microenvironments, and is a more useful predictive model for drug testing than monolayer culture41. The two animal *in vitro* studies included in our review were more representative of a cartilage construct rather than monolayer chondrocytes, i.e. a bovine ACI bioreactor model 29 and an osteochondral graft model 6, and did not show a chondrotoxic effect. The ease of performing future studies on chondrocyte toxicity in monolayer culture needs to be balanced with its relevance and limitations in clinical applicability. Chondrotoxic effect with TNF-alpha inhibition was only seen at high dosage; this highlights the need to carefully select the drug, dose, and route of administration when managing patients on anti-TNF medication to limit any adverse events.

Most of the clinical evidence available on the effect of TNF-alpha inhibition is based on the evaluation of biomarkers of cartilage metabolism that poorly correlate to the status of cartilage due to possible confounding factors influencing the serum or urinary results 19,20. We recommend that further preclinical and clinical studies evaluating cartilage should primarily be based on histological and cytological changes in cartilage. We only included studies in our review where direct measures of cartilage were taken rather than surrogate markers. In our eight *in vivo* studies, the most common method of assessment was either histological alone 16,17,25–27,30–32 or coupled with a GAG measure 16,17,25–27,30–32 . The Mankin score was used in two studies 30,32 and another histological score in one study 16. The joints were also assessed clinically 31 or macroscopically 16,17,25–27,30–32. Two studies looked at MMP expression 17,30. One study looked at tissue penetration, biodistribution and pharmacokinetics 25. Our review shows that TNF-alpha inhibition in the *in vivo* studies had a beneficial effect on cartilage metabolism, demonstrated a chondroprotective effect, and promoted the natural repair of osteochondral lesions.

Some studies included in our review attempted to identify pathways by which the anti-TNF-alpha agents were acting on cartilage. The role of MMPs was highlighted by two studies due to their role in articular cartilage destruction 42. MMP 13 cleaves type II collagen more so than types I and III, and may be involved in articular cartilage turnover and cartilage pathophysiology associated with osteoarthritis. Ma et al. 30 showed that ADA decreases TNF-alpha induced MMP-13 expression and improve the subchondral bone microstructure to inhibit cartilage degeneration and alter the subchondral bone quality in a rat model of OA. MMP-3 is a broadly-active enzyme with capacity to degrade a number of non-collagenous components of the extracellular matrix. Karsdal et al 40 demonstrated that MMP-3 induction of TNF-alpha is irreversible leading to aggrecan and collagen 2 degradation. Vasara et al 39 investigated biomarkers following ACI procedure and found that MMP-3 expression was highly elevated even one year after the procedure, and correlated with the degenerative process and higher risk of graftfailure 39.MMP 9 is a key MMP in osteoclasts. In their Human TNF–transgenic mice with polyarthritis, Zwerina et al. 17 looked at MMPs 3, 9, and 13 expression as surrogates for the effector molecules of cytokine-driven synovial inflammation. They found that the MMP expression in articular chondrocytes was significantly inhibited by anti-TNF although the articular changes caused by chronic overexpression of TNF were not completely blocked by targeting TNF alone. This suggests that, in addition to reducing synovial inflammation, TNF inhibition alters the molecular composition and invasive properties of the tissue that facilitates catabolic changes in the osteochondral tissue. Their work also suggests that there may be a role for combined blockade of more than one proinflammatory pathway rather than addressing TNF alone 17. Yang et al. 27 showed that an anti-TNF monoclonal antibody reduced the mRNA expression level of caspase-8 and the levels of active caspase-8 units, but had no effect on the mRNA expression levels of caspase-9, the levels of active capsase-9, or the cytosolic levels of cytochrome c. This suggests that caspase-8 is involved in the TNF-stimulated apoptosis of chondrocytes without involvement of either caspase-9 or cytochrome c.

Cell-based cartilage repair procedures, such as ACI and osteochondral allograft implantation, are successful and cost-effective solutions for focal cartilage defects 43,44. There is however a lack of evidence supporting these procedures in patients with OA 45. The failure of cell-based repair surgery in OA patients could be due to dysregulation of the healing process due to high levels of proinflammatory cytokines related to the chronic pathological process 46,47. The chronic inflammatory environment seen during various stages of OA 36 creates a degenerative environment that adversely affects the graft healing in the short to midterm 47. Of all cytokines involved, TNF-alpha steps in as a major actor being able to induce chondrocyte death 10,34,35,37 and inhibit chondrocyte differentiation and proliferation 1,8,11,13,17,35,48,49 both in OA and in other chronic musculoskeletal conditions 36,50,51. TNF-alpha also has a role in stimulating other inflammatory molecules involved in both the pathogenesis of OA as well as traumatic and non-traumatic chondral defect. Fukui et al. 52 reported that it can interfere with the mobilization, proliferation, and/ or differentiation of mesenchymal osteochondral-progenitor cells.

**Limitations**

The major limitation of this systematic review is the heterogeneity and quality of the included studies. Most of the studies were preclinical studies, with no clinical randomized controlled trials. Despite applying strict methodological evaluation through quality and risk of bias tool, treatment variables including dose, drug delivery and population used differed across the included studies. The findings of our review will however hopefully help direct future research as well as clinical randomized controlled trials exploiting the use of TNF-alpha inhibitors in cartilage disease and injuries.

**Conclusion**

TNF-alpha is a proinflammatory cytokine involved in the inflammatory reaction in cartilage due to trauma and systemic diseases. Biologic therapy inhibits and counteracts the effects of TNF-alpha in cartilage with possible benefit for OA patients. Although there are some concerns regarding chondrotoxicity, there is good quality evidence of a beneficial effect of TNF-alpha in both *in vitro* and *in vivo* studies of OA, cartilage repair and autoimmune diseases models. The use of biologics to inhibit TNF-alpha action in cartilage repair surgeries should be evaluated as this promising therapy has the potential to improve outcomes. Its potential to improve outcome in cartilage procedure such as ACI and osteochondral transplantation is promising and should be evaluated in double blind randomized clinical trial in human.

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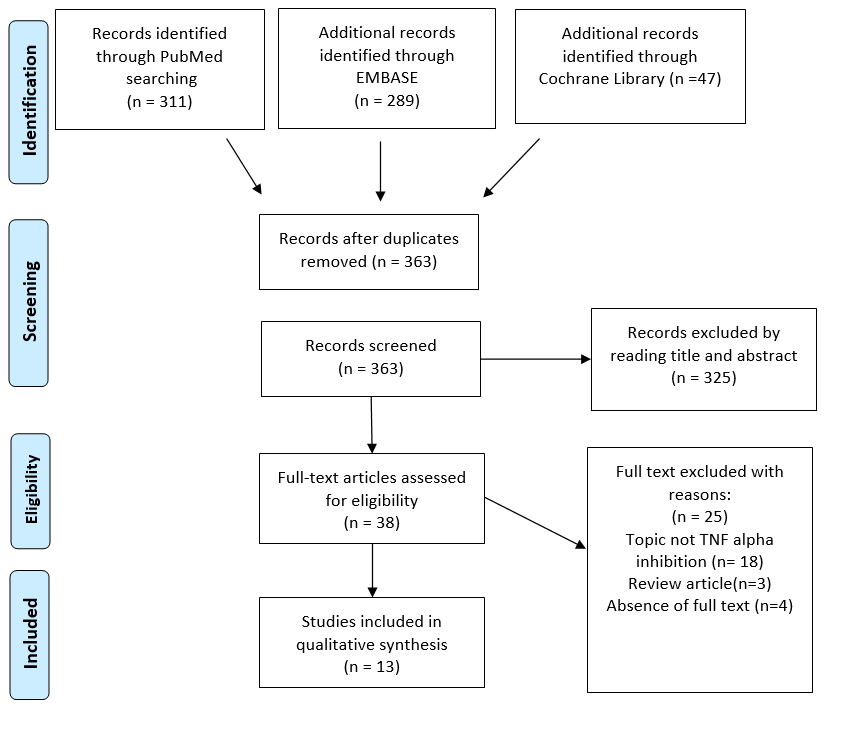
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**Figure A.** *PRISMA flowchart of the systematic literature review.*

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**Table 1.** Main findings of studies

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| --- | --- | --- | --- | --- | --- |
| **Author** | **Drug investigated** | | **Population** | **Outcome** | **Main findings** |
| ***In Vitro* Human Studies** | | | | | |
| *Guzelant et al.* | ADA (40 mg). Final concentration: 10 mg/ml | Human knee OA chondrocyte in monolayer culture  n=3 | | **Viability:** Absent at 24, 48h  **Proliferation:** impaired cell proliferation at 24,36,48h  **Toxicity:** Chondrotoxicity present at 24h, 48h | Biologic agent dosage should be determined taking into consideration their cellular-level toxic effects on chondrocytes. |
| *Isyar et al.* | ADA (500 mg/ml), ETA (50 mg/ml),INF (100mg/ml). Final concentration: 10 μg/mL | Human knee OA chondrocyte in monolayer culture  n=6 | | **Viability:** Decreased at 24h (ETA, INF > ADA), absent at 48h.  **Proliferation:** Complete inhibition at 48h  **Toxicity:** INF>ETA, ADA | The concentration of the preferred biological agents after inhibition of inflammation needs to be checked more strictly. |
| *Žigon-Branc et al.* | ADA, ETA, INF; 1μg/mL | Human OA chondrocytes and chondrogenically differentiated  Mesenchymal Stem Cells (MSC) in 10,000 cell microspheroids  n=3 | | Cytotoxic effect of TNF-alpha on chondrocytes and chondrogenically differentiated MSCs could be prevented by ADA, ETA, or INF.  Inflammation gene expression triggered by TNF-alpha, could be prevented by the addition of ADA, ETA and INF. | The tested drugs significantly decreased the inflammation induced by TNF-alpha |
| ***In Vitro* Animal Studies** | | | | | |
| *Ossendorff et al.* | ADA 10 mg/mL | Study with novel bioreactor  Bovine autologous chondrocyte implantation (ACI) model  n=5 | | Metabolic markers: downregulation of anabolic factors and upregulation of catabolic markers induced by TNF-alpha. This effect was antagonized by ADA.  Chondrogenic differentiation: Supplementation of ADA without addition of TNF-alpha did not show any influence on chondrogenic maturation.  Apoptosis: TNF-alpha increased apoptosis, ADA could inhibit this increase. | TNF-alpha negatively influences the chondrogenesis in simulated ACI and early rehabilitation. These effects can be counteracted by ADA |
| *Linn et al.* | ETA 10 μg/ml | Boer goats’ osteochondral allograft  n=8 | | ETA was found to significantly maintain the cell viability in the superficial zone but no difference in other zones or all zones in total.  The glycosaminoglycan (GAG) component of the cartilage matrix, did not change significantly after 28 days of storage  Histomorphometry did not demonstrate any changes in height of cartilage or surface roughness of the articular surface with and without addition of ETA at 4°C. | ETA maintained cell viability of osteochondral allografts significantly better than the current storage paradigm after 28 days storage. |
| ***In vivo* Animal Studies** | | | | | |
| *Ma et al.* | ADA (20 μg/kg) alone or ADA + Anterior Cruciate Ligament transection (ACLT) or ACLT alone | Female Sprague-Dawley OA rats articular cartilage ACLT + ADA  n=24 | | **Macroscopic and microscopic assessment of the knee** ADA (alone): Healthy cartilage without osteophyte formation in the intervention group ACLT + ADA: Mild sign of degeneration without osteophytes ACLT: Clear cartilage degeneration  **Subcondral bone** The Tb.N of the ACLT+ADA group were significantly increased while Tb.Sp was markedly decreased (P<0.05) compared to ACLT +NS.  **The Mankin score** in the ACLT group was dramatically higher than that in the SP group, and significantly higher than that in the ACLT+ADA group.  **Immunohistochemical staining for MMP-13 expression:** Staining for MMP-13 was less detectable in the ADA group.  In the ACLT+ADA group, MMP-13 was mainly detected in chondrocytes at and close to the articular surfaces;  In ACLT group as a control, MMP-13 expression was found throughout the articular cartilage. | ADA treatment probably protects articular cartilage by improving the structure of the subchondral bone and reducing the degradation of the cartilage matrix. |
| *Yang et al.* | TNF inhibitor 10 ug/ml | Rat temporo-mandibular primary chondrocyte model  n=32 | | **Proteoglicans.** Intra-articular injection of the TNF inhibitor partially reduced the loss of proteoglycans.The mRNA expression levels of IL-1β and IL-6 were not affected (p>0.05)  **Caspase 8 death pathway. C**aspase-8 is involved in the TNF-stimulated apoptosis of chondrocytes without involvement of either caspase-9 or cytochrome c at all three dosages. At the highest dose of TNF (100 ng/ml) used to treat chondrocytes, the TNF inhibitor (10ug/ml) prevented the caspase-8 increase without affecting either caspase-9 or cytochrome c. | TNF accelerated the unilateral anterior crossbite induced chondrocytes apoptosis via death-receptor pathway. However, anti-TNF therapy does not prevent cartilage loss in this model of temporomandibular joint. |
| *Kawaguchi et al.* | ETA low-dose group (0.05 μg/kg) or Intermediate-dose group (0.4 μg/kg)  or  High- dose group (1.6 μg /kg) | Japanese white rabbits osteochondral graft defect cartilage model  n=133 | | **Histological observation:** The findings suggest that ETA (in all groups) promoted early subchondral bone remodeling of osteochondral defects.  The cell morphology and matrix staining indicated good repair with 4 injections in the intermediate and high-dose subgroups.  Adjacent cartilage did not show any degeneration in any of the samples examined, indicating that etanercept does not affect metabolism of normal cartilage.  **Western blot analysis** confirmed that etanercept has a substantial affinity for rabbit intra-articular TNF-alpha | Blocking of TNF by etanercept enabled repair of osteochondral defects in rabbit knee. ETA therapy could be a strategy for the use of tissue engineering for bone and cartilage repair. |
| *Zwerina et al.* | INF 10 mg/kg | Human TNF–transgenic (hTNFtg) mice suffering from polyarthritis  n=64 | | Inhibition of TNF-alpha mediated cartilage damage by blockade of TNF was performed by quantitatively assessing proteoglycan loss by toluidine blue staining of articular cartilage. Inhibition of proteoglycan loss was associated with gradually decreased expression of MMPs 3, 9, and 13 in articular chondrocytes. MMP expression was significantly inhibited by anti-TNF. | Articular changes caused by chronic overexpression of TNF are not completely blockable by monotherapies that target TNF, IL-1, or RANKL. However, combined approaches, especially the combined blockade of TNF and IL-1 and, to a lesser extent, TNF and RANKL, lead to almost complete remission of disease.  This further strengthens the rationale for using combined blockade of more than one proinflammatory pathway. |
| *Shealy et al.* | Anti-TNF-alpha mAb 10 mg/kg  or  saline | Tg197 transgenic mice with OA  n=25 | | Anti-TNF-alpha treatment for 6weeks significantly reduced cartilage degradation young mice (7- or 8-week- old). After 16 weeks, a significant reduction in cartilage degradation in comparison with baseline was maintained in young mice. Cartilage degradation in aged (27- or 28-week-old) mice treated with anti- TNF-alpha was similar to that at the baseline measurement and significantly lower than that in the saline treatment group. | Progression of established polyarthritis in the Tg197 hTNF-alpha transgenic mouse can be reversed by treatment with anti-TNF-alpha mAb, as shown by significant improvement in clinical and histological scores. |
| *Zhang et al.* | INF (0.5 ml) of 10 mg/ml OR 20 mg/ml or Saline | New Zealand white OA rabbits  n=30 | | **Macroscopic observation:** In interventional group, in both the 10 and 20 mg/ml doses, cartilage lesions and osteophytes were reduced when compared with the control group  **Histological evaluation:** In both the treatment groups, histological changes associated with OA were clearly seen, but the severity was milder than in the control group, as was the proteoglycan loss  Mankin scores in the rabbits administered infliximab 10 mg/ml (2.7 0.9) or infliximab 20 mg/ml (2.4 0.7) were significantly lower than in the control group (6.4 1.2) (p < 0.05). No significant differences between the two treatment groups were observed.  **Immunohistochemistry.** The levels of both TNF-α and nitric oxide (NO) were significantly decreased in the treatment groups compared with the control group (p < 0.05), No significant differences were detected between the two treatment groups (p > 0.05). | This study demonstrates that intra-articular injections of infliximab can protect against the development of experimentally induced OA. |
| *Urech et al.* | ESBA105 [a humanised single-chain Fv antibody (scFv)] (1 mg/ml) and INF (1 mg/ml or 2.2 mg/ml) | Bovine cartilage specimens  *In vivo* efficacy was measured in knee joint OA in Lewis rats  n=21 | | Cartilage penetration, biodistribution and  pharmacokinetic studies performed.  ESBA105 blocks the apoptotic effect of rhTNFa in a concentration-dependent manner similar to infliximab.  Time course studies revealed that ESBA105-FITC but not infliximab-FITC penetrated into bovine cartilage within a few hours, Penetration of ESBA105-FITC was time- dependent and in cartilage the concentration of ESBA105-FITC increased linearly with time. In contrast to ESBA105- FITC, infliximab-FITC did not penetrate into cartilage.90% inhibition of knee joint swelling, inflammatory infiltrates and proteoglycan loss from cartilage was seen. | ESBA105 potently inhibits inflammation and prevents cartilage damage triggered by TNF-alpha.  ESBA105 also penetrates into cartilage and can be expected to reverse the TNF-alpha- induced catabolic state of articular cartilage in arthritides. |
| *Elsaid et al.* | ETA 0.5 mg/kg   Treatment A animals received early and high doses of etanercept administered 1,3,5,7,9,11, and 13 days following ACLT.   Treatment B dosing was performed only on days 7 and 14 following ACLT.  Treatment C dosing was performed only on days 14 and 21. | Male Lewis rats OA model  n=12 | | Measures included synovial fluid lavage levels of  lubricin, synovial tissue lubricin gene expression, sulfated glycoaminoglycans (sGAG), and histological evaluation of articular cartilage with lubricin-specific antibody staining and Safranin O.  Cartilage degeneration is associated with a decrease in either synovial fluid (SF) or cartilage bound lubricin:  **Treatment A and B.** SF lubricin concentrations were significantly higher (p=0.021, 0.025, respectively) than untreated ACLT lubricin SF concentrations.  **Treatment C.** There were no differences in adjusted SF lubricin concentrations when compared with untreated ACLT lubricin SF concentrations (p=1.0).  **Lubricin staining.** Were significantly higher (p<0.001) than that of **Treatment A**, with no significant difference between **Treatments B and C.**  **sGAG concentrations** were lower in **Treatments A** (p<.0001), **B** (p=0.0001), and **C** (p=0.0055), relative to untreated ACLT limbs. **Treatment A** was significantly lower than **Treatment C** (adj. p=0.0117), and **Treatment B** did not differ significantly from either **treatment A** (adj. p=0.1358), or **treatment C** | Blocking TNF-α resulted in a chondroprotective effect, exemplified by increased lubricin deposition on articular cartilage and a decrease in sGAG release from articular cartilage in a post-traumatic arthritis animal model. |

ADA: Adalimumab, OA: Osteoarthritis, ETA: Etanercept, INF: infliximab, TNF: Tumour necrosis factor, MSCs: Mesenchymal stem cells, ACI: Autologous chondrocyte implantation, GAG: Glycosaminoglycan, ACLT: Anterior cruciate ligament transection, Tb.N: Trabecular bone number, Tb.Sp: Trabecular bone separation, NS: Normal saline, SP: Sham-operated, MMP-13: Matrix metalloproteinase 13, hTNFtg: Human TNF–transgenic, IL-1: Interlukin-1, RANKL: Receptor activator of nuclear factor kappa-Β ligand, mAb: monoclonal antibody, Tg197: Transgenic, hTNF-alpha: human TNF-α, rhTNFα: recombinant human TNFα, FITC: Fluorescein isothiocyanate, sGAG: Sulphated glycosaminoglycan.