MECHANISMS OF ACTION AND ROLE OF INTRA-ARTICULAR THERAPY IN THE MANAGEMENT OF CHRONIC SYNOVITIS

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# List of acronyms

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<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>RA</td>
<td>Rheumatoid Arthritis</td>
</tr>
<tr>
<td>ACPA</td>
<td>Anti-Citrullinated protein antibody</td>
</tr>
<tr>
<td>RF</td>
<td>Rheumatoid Factor</td>
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<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti-Inflammatory Drug</td>
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<tr>
<td>IA</td>
<td>Intra-articular</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>RSV</td>
<td>Radiosynovectomy</td>
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<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>Frβ</td>
<td>Folate Receptor Beta</td>
</tr>
<tr>
<td>mAb</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene Glycol</td>
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I. Introduction

Chronic synovitis is characterized by a chronic inflammation of the synovial membrane in the joint. The synovial tissue can be inflamed due to either infections, autoimmunity (of which rheumatoid arthritis (RA) is the most important) or crystal induced arthritis. Overall, these conditions are common, affecting around 3% of the western population. Although several treatments exist for each of these diseases, some patients remains unresponsive to therapies. For instance, biotherapies have changed the prognosis of patients with RA but some patients can have a persistent inflammation of one or 2 joints leading to local destructions. In other cases, the disease can involve only one joint and a systemic therapy is not indicated due to the risk of side effects. In this clinical context, it is better to use local treatment through intra-articular injections. These intra-articular injections however, are far from ideal, and have many shortcomings. Therefore, several recent studies have explored new approaches to improve their efficiency, trying to increase the retention time of the molecule in the joint to find new molecular drugs/targets. The goal of this dissertation is to describe these new prospective therapies. We will first, describe the normal synovial tissue and its histological modifications during chronic synovitis. We will then explain the technique of intra-articular injections and the pharmacokinetics and -dynamics that are involved. Finally we will discuss the new therapies that are under development.

II. Chronic synovitis

2.1 Normal synovial tissue

The synovial membrane (or synovium) is situated at the deep surface of the articular capsule. It is 60µm thick in the human knee and is characterized by many plica or folia. It is made of 2 strates : the intima and the subintima, which are separated by the subsynovial.

![Figure 1: Drawing of the synovial membrane with the intima and the subintima](http://biomedicaloptics.spiedigitallibrary.org)
The intima (20µm thick) is in contact with the articular cavity. It corresponds to 1 to 4 strates of 2 types of synoviocytes. There are two kinds of synoviocytes. First, the synoviocytes A (or macrophage-like), which originate from the yolk sac. They contain a developed Golgi apparatus, big vacuoles, and lysosomes (in relation with phagocytose function). The second type is the synoviocytes B (fibroblast-like), which are less numerous than the type A, and have a different origin, coming from a mesenchymal progenitor.

The second strate is the subintima. It is also rich in cells, but more vascularized (in the superficial part) than the intima. It contains fibroblasts (50%), histiocytes, mastocytes, and collagen fibres. The ground substance is made of mucopolysaccharides.

In between, the subsynovium is made of conjunctive cells, that make up the superficial strates. Whereas the deepest strates are invaded by adipose cells. The ground substance contains mucopolysaccharides and loose collagen fibres.

The synovial membrane plays many roles. It plays a role in the trophicity and lubrication of the joint, because the synoviocytes secrete the synovial fluid. This fluid is mainly composed of hyaluronic acid, which has viscoelastic, antalgic, anti-inflammatory, chondroprotectrices and healing properties. On the other hand macrophage-like synoviocytes are involved in the defense against pathogens and the elimination of intra-articular debris.

#### 2.2 Main causes of chronic synovitis

a) Autoimmune diseases

Rheumatoid Arthritis is the most frequent auto-immune cause of chronic synovitis. Genetic susceptibilities and environmental factors are both involved in the initiation of the inflammation. The disease is characterized by a synovial hypertrophy and a chronic joint inflammation. The auto-immune reaction is initiated by the presentation of an unknown antigen, endogenous or exogenous, to CD4 T cells in the joint. Activated lymphocytes produce cytokines and chemokines leading to the recruitment of other cells playing a key role in the inflammatory process, such as macrophages and neutrophils. Synovial fibroblasts get activated and are one of the main producers of pro inflammatory cytokine such as TNF-α and IL-1. Anti-citrullinated protein antibody (ACPA) is highly specific and is an early sign of rheumatoid arthritis. Rheumatoid factor (RF), an antibody directed against the Fc portion of IgG, constitutes deposits of immune complex RF-IgG leading to vessel damages. Ultimately, the inflammatory tissue turns into an aggressive tissue, the pannus. It covers the cartilage
and induces the activation of chondrocytes and osteoclasts responsible for the destruction of cartilage and bone.

b) Crystal induced arthritis

In crystal induced arthritis, the inflammation is characterized by the presence of crystals in the synovial fluid.

Gout is a type of inflammatory arthritis that is initiated by the crystallization of uric acid within the joints. An increase of uric acid in the blood, called hyperuricemia, is directly associated with a risk of gout. It results from exogenous sources (food rich in purines) or endogenous metabolism. Moreover, the solubility of urate in joint fluids may be influenced by other factors such as temperature, pH or the concentration of cation. The acute gouty attack occurs in three steps: the initiation, the amplification and the termination. Two processes allow interactions between urate crystals and phagocytes. The first pathway involves common mechanisms: the opsonization and phagocytosis, inducing lysosomal fusion, respiratory burst and release of inflammatory mediators. The other pathway consists of the direct interaction of the urate crystals with the membranes of the phagocytes, leading to the activation of several transduction signals. Consequently, a key mediator is expressed in monocyctic cells for the neutrophilic recruitment: IL-8. During this early phase of inflammation, monocytes and mast cells play a central role by releasing inflammatory mediators. Then, cytokines and chemokines induce the neutrophil migration. The neutrophils then amplify the inflammation and are mainly responsible of the synovitis in an acute gouty attack.

Chronic gouty arthritis results from years of acute gouty attacks. Low-grade synovitis persists in the joint during the remission periods of acute flares.

c) Infectious arthritis

Infectious arthritis is the less common cause of chronic synovitis. Two ways of entry explain the infection of the joint: by a direct inoculation or by a hematogenous spread following an infection.

Most types of infectious arthritis are caused by bacteria. The most frequent cause for adults are Gram positive bacteria, in particular *Staphylococcus Aureus*. Viruses can also induce infectious arthritis, some examples are: parvovirus B19, hepatitis B and C virus and rubella virus. In rare cases, the infection of the joint is caused by fungi. As this infectious arthritis is most of the time acute, it will not be part of our dissertation.
2.3 State of the art treatment strategy for inflammatory arthritis and role of intra-articular injections

a) Definition

A joint injection (intra-articular injection) is a local procedure used in the treatment of inflammatory joint conditions, such as rheumatoid arthritis, psoriatic arthritis, gout, or osteoarthritis. Its role is to increase the efficacy of administered substances by reducing systemic exposure to drugs, off-target effects and by enhancing bioavailability and the delivery of molecules that would be incompatible with systemic delivery.

b) Injection Technique

Physicians should perform all joint and soft tissue injections wearing gloves and using aseptic technique. Surface anatomy and joint landmarks should be identified and the point of entry can be marked with a small indentation from a needle cap, before skin cleaning.

The main concern associated with IA injections is the increased risk of iatrogenic joint infections, which can be prevented with rigorous skin preparation. Over the last few years the way of doing intra-articular injections has improved with the use of guiding imaging techniques such as ultrasound and x-ray. With the use of these imaging techniques the administration site of intra-articular injections and the needle can be made visible while performing the injections. Studies have shown that this greatly increases the accuracy of the injections and thus more drugs reach the intended target site.

III. Intra-articular therapy

3.1 Pharmacokinetics/pharmacodynamics/technical consideration

To be able to design drugs with optimal efficacy, knowledge of its pharmacokinetics and -dynamics is essential. Intra-articular therapy is applied, because it has several advantages (see 2.3) over systemic therapies when the inflammation is localized to one joint. Soluble substances that are taken systemically enter the synovial fluid through the capillaries of the subsynovium. This is also the route through which intra-articularly delivered soluble substances leave the synovial fluid, when the molecules are small. For small molecules the main barrier is the extra-cellular matrix of the synovial interstitium. The bigger molecules leave the synovial fluid through lymphatic vessels. There are many factors that influence the
uptake of drug molecules from the synovial fluid to either the blood or the lymph, including
the dissociation constant, molecular size, protein binding and solubility of the drug. Interestingly, the inflammation itself plays an important role in drug retention time as well. Because of inflammation the capillary permeability is increased and this results in faster uptake of small molecular solutes, including the drug molecules, into the capillaries. In contrast however, it has also been suggested that in rheumatoid arthritis the proliferated cells decrease the uptake of small molecules into the capillaries. For larger molecule removal the inflammation itself also plays an important role, because during inflammation there in an increase in lymphatic drainage, which increases the removal of larger drug molecules. Large molecules are normally very efficiently drained from the joint through the lymphatic vessels, also in a non-inflamed state. The retention time for drugs composed of macromolecules is therefore only a few hours or less. Larsen et al. investigated the half life of different drugs within the synovial fluid. The results vary from 0.23 hours for acridine orange to 1.23-13.1 hours for albumin and 26.3 hours for hyaluronic acids, while the half lives of NSAIDs and soluble steroids range from 1-4 hours. What this shows is that there is still a lot to improve for intra-articular therapies, especially when they are used to treat chronic synovitis.

3.2 Current therapy used in inflammatory arthritis

a) Corticosteroid injections

There are 5 injectable corticosteroids that have a current Food and Drug Administration (FDA) label for IA injections. These are: methylprednisolone acetate, triamcinolone acetate, betamethasone acetate and betamethasone sodium phosphate, triamcinolone hexacetonide, and dexamethasone.

Corticosteroids have both anti-inflammatory and immunosuppressive effects, but their mechanism of action is complex. Corticosteroids act directly on nuclear steroid receptors. Because of this, they reduce vascular permeability and inhibit accumulation of inflammatory cells, phagocytosis, production of neutrophil superoxide and prevent the synthesis and secretion of several inflammatory mediators, such as prostaglandins and leukotrienes. The clinical anti-inflammatory reflections of these actions are decreases in erythema, swelling and heat.

The clearance from the joint space is prevented by complexing the drugs with salts or polymers and suspending them in aqueous solutions that sequester the agents from the synovial fluid. Because of their low molecular weight, the intra-articular half-lives achieved for corticosteroids have rarely been found to exceed 12h.
b) Isotopes

Radiosynovectomy (RSV) is a local form of radiotherapy used for treatment of resistant synovitis of individual joints after failure of intra-articular steroid injections. It is an intra-articular injection of a radionuclide in colloidal form. Three radionuclides are in current use: 90Y-silicate/citrate, 186Re-sulfide, and 169Er-citrate. Radionuclide-loaded colloidal particles are rapidly phagocytized by macrophages in the inflamed synovial membrane (Fig. 2A).

The ideal radiopharmaceutical for RSV should meet the following 3 requirements: it should be attached to a particle that is sufficiently small to be phagocytized, but not so small that it might leak from the joint before being phagocytized (2-10 μm), binding between radionuclide and particle should be stable throughout the course of the RSV and radiolabeled particles should be distributed homogeneously in the intraarticular space without initiating an inflammatory response.

These particles cause ultimate damage to the absorbing medium—the cells of the synovial membrane—beginning with excitation and ionization of the atoms and molecules within this medium, creating a large number of secondary particles. Thus, free radicals known to initiate biochemical effects are created, with subsequent evolving apoptosis and ablation of inflamed synovial membrane (Fig. 2B). Radiation synovectomy can be complicated by rare cutaneous radiation necrosis and concerns about genotoxic effects.

3.3 Therapies under development/new opportunities

a) New target molecules

• TNF-α

Aside from improving the current intra-articular injection therapies by increasing the drug retention time in joints, there are also new therapies being developed to better treat
inflammatory arthritis. One molecular target that has been under scrutiny for quite some time now is the cytokine TNF-α (Tumor Necrosis Factor). TNF-α is an inflammatory cytokine that plays an important role in many inflammatory reactions and immune responses, some of which are involved in inflammatory arthritis. TNF-α is important for recruiting neutrophils by increasing the production of cytokines that enhance the expression of adhesion molecules on endothelial cells. Besides this, it is also a co-stimulator for T-cell activation and antibody production. Because of the essential role of TNF-α in inflammation, systemic TNF-α antagonists can be very effective in treating inflammatory arthritis. Several studies have tried to use this drug in the context of chronic resistant synovitis. One recent review article suggests that anti-TNF-α may be a good alternative to corticosteroids in this clinical context. However, most of the clinical studies conducted so far are small size low quality studies. Moreover, the cost and secondary effects of these new drugs are some of the barriers that exist against their use in every-day clinical practice.

- **VEGF**

TNF-α also leads to the expression of another cytokine that is important in inflammation and that is being targeted in cancer. This cytokine is called Vascular Endothelial Growth Factor (VEGF). VEGF stimulates endothelial permeability, swelling and angiogenesis in joints, which all contribute to the inflammation in chronic synovitis. Furthermore, a recent study has shown that VEGF expression was associated with the risk of failure of intra-articular therapy in RA. Animals studies have shown the interest of blocking VEGF to reduce articular inflammation. The efficacy of intra-articular bevacizumab injection (anti-VEGF antibody) is currently being investigated in a pilot study for secondary prevention of recurrent hemarthroses for hemophilia patients. Therefore, we think that targeting VEGF could be a future option that need to be investigated in the context of chronic synovitis.

b) New drug delivery

- **Nanoparticles**

A new field of research that seems to hold good prospects of increasing the drug retention time in joints is the use of synthetic polymeric nanoparticles. These particles can be used as capsules for drugs in order to increase the retention time of the drugs in the joints and ensure sustained release of the drugs in the joint. Besides the fact that these particles must have certain properties to make sure that they work properly, it is also very important to make sure that these particles are safe drug-carriers and do not elicited inflammation themselves. Many studies have been done to find the right nanoparticles that have good
drug-delivery properties, that increase the drug-retention time significantly and that do not cause any inflammation themselves.

There are different ways in which drugs can reach their target cells when injected with nanoparticles. The encapsulated drugs may be phagocytosed by synovial macrophages, in which they are released over time. They can also remain within the synovial fluid, where they can attach to cartilage or synovium or become entrapped within the synovial folds. The drug is then released into the synovial fluid and diffuses into target cells, but also out of the joint and into the blood or lymph. Once the particles are injected and have reached the target sites they will over time release the drug and degrade. The rate of release and biodegradation is under the influence of a number of factors, including composition, molecular weight and crystallinity of the particle as well as the load, nature and size of the drug.

The nanoparticles are a very interesting new possibility with the potential of providing a 20 times longer drug retention time. However, much research is still to be done to fully understand the pharmacodynamics and kinetics of these particles and then to develop the ideal particle.

• Antibodies directed against specific cells

Synovial tissue consists of macrophages, fibroblasts, and T cells, which produce a variety of cytokines and growth factors that may elicit the inflammatory reaction. The severity of the synovitis is correlated with the number of macrophages and their product, TNF-α, in the tissues. Therefore, anti-TNF biological agents were designed to target synovial sublining macrophages, but they have been of variable efficacy.

It has been shown that the synovial sublining inflammatory macrophages express folate receptor beta (FRβ) as a receptor for oxidized folate. Because FRβ-expression is limited in normal tissues, researchers have hypothesized about the removal of only the inflammatory macrophages and thereby minimizing adverse side effects. One of these studies presents the effect of monoclonal antibody (mAb) produced against Frβ coupled with an immunotoxin.

After the intra-articular treatment of the joint with different doses of this molecule, a reduction in a dose-dependent manner of the joint swelling has been noticed. The medium and high doses lead to a positive result even at 14 days post-injection. From a histological point of view, these doses lead to lower grades of inflammation and synovial membrane thickness.
Moreover, due to the local efficiency of the treatment and the reduced sera half-life (less than 30 min), this treatment has the advantage to not have significant systemic effect.

• **PEG**

One method to modify the residence of the drug into the joint is the PEGylation. Indeed, conjugation of polyethylene glycol (PEG) for hydrophobic and small molecules increases their molecular weight and also leads to delay of systemic elimination. An example of PEGylation is the corticosteroids. Corticosteroids are highly hydrophobic and small (<700Da), so they can be transported into the joint space by trans-capillary diffusion after the systemic injection. To increase the time of retention we can reduce the clearance through the use of excipients like polyethylene glycol (PEG), dextran or polysorbate-based suspension. This promotes retention time of the drug in an aqueous solution. Salts also promote the retention of the steroid in a crystalline form. In this manner, the drugs are complexed with salts or polymers and suspended in aqueous solutions that act to sequester the drug from the synovial fluid and delay clearance from the joint space. Nevertheless, the intra-articular half-lives achieved for corticosteroids have rarely been found to exceed 12 h, owing to the very low molecular weights of these compounds.

• **Liposomes**

A liposome is a spherical vesicle made up of a lipid bilayer. Into contact with the aqueous environment, hydrophobic phospholipids spontaneously form a sphere. This potential drug transporter is characterized by two main advantages. First, liposomes improve the therapeutic index by achieving a high level concentration of the drug at the active site. The loading of hydrophobic drugs into the liposome reaches 90% while the loading ratio is lower with polar drugs such as methotrexate. Secondly, they reduce the toxicity of drugs by reducing exposure to unaffected tissues.

![Figure 3: Effects of anti-Frβ immunotoxin on the histology of knee joints in rat. S=synovium; B=bone; C=cartilage; E=erosion.](http://arthritis-research.com)

![Figure 4: Drawing of a liposome.](http://wikipedia.commons.com)
In comparison, NSAIDs and glucocorticoids present limited benefits due to side effects. However, conventional liposomes are rapidly eliminated after an intravenous injection by the reticuloendothelial system of phagocytes due to plasma protein coating. To avoid this rapid clearance, a new type of liposomes has been developed: long circulation “stealth” liposomes. They are coated with polyethylene glycol (PEG), a linear synthetic polymer, which increases hydrophilicity. Interactions with plasma proteins are reduced, which results in an increase of the bioavailability of drugs. Another important characteristic of “stealth” liposomes is their capacity to go through vascular endothelium with high permeability, like in inflammatory sites.

Several studies have tested beneficial effects of liposomes loading lipophilic analog of methotrexate, superoxide dismutase, lactoferrin or clodronate. All demonstrate beneficial effects. Therefore, liposomes are an encouraging therapeutic approach of chronic synovitis.

IV. Conclusion

Chronic synovitis is a common problem in people affected with autoimmune or crystal induced diseases. Current therapies are effective but a significant number of patients are still resistant to these treatment and are at risk of long term joint destruction. Therefore research is being done to find new target molecules and new drug delivery systems to improve the efficiency of intra-articular treatments. These target molecules include TNF-α and VEGF, and the most promising new drug delivery systems are nanoparticles, antibodies, and liposomes. However, most of the clinical trials currently available are case series or small size randomised controlled studies. Furthermore, as some of these new molecular target drugs are very expensive, more studies need to be done in order to establish whether the cost effectiveness is better than the current available drugs. On the other hand, other prospective treatment area’s, such as gene or cell therapies are being developed, but these were outside the scope of our dissertation. For the future we suggest that more clinical trials need to be done to compare current treatment with the use of new molecular target drugs and new ways of delivery.
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Abstract

Synovitis is characterized by an inflammation of the synovial membrane of the joints. This tissue has many physiological functions, such as lubrication of the joint and nutrition of the cartilage. In pathological conditions, immune cells are the leading cause of inflammation, either because of auto-immunity in RA (induced by genetic susceptibility and environmental factors) or crystallization of uric acid in crystal induced arthritis.

Local intra-articular therapy has many benefits over the current systemic treatment in cas of mono-articular involvement. Current treatment available are mainly corticosteroid that are efficient but not in all cases. Indeed, some patients remains unresponsive to these therapies, leading to a need to more efficiency drugs. Therefore, several strategies are under development to reach this goal. First, new molecular targets are under investigation including TNF-α or VEGF. Second, it seems important to improve the retention time of the drugs, in the joint as they leave rapidly their site of action either through blood for small molecules or lymphatic vessels for macromolecules. Therefore, new drug delivery systems are also being developed in order to improve the drug retention time. So far the most promising new drug carriers seem to be nanoparticles, antibodies, and liposomes. Once the proof of concept of the interest of these new drugs will be demonstrated in vitro or in animal models, there will be a need for high quality clinical trials to confirm their efficiency compared to current available drugs and to prove their cost-efficiency. Our dissertation shows that finding new intra-articular therapies is a field of exiting future research and the opportunity for innovation.

Key words

Synovitis, Intra-articular treatment, new therapies