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Research Paper

# The effect of polymer size and charge of molecules on permeation through synovial membrane and accumulation in hyaline articular cartilage

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ABSTRACT

The treatment of joint related diseases often involves direct intra-articular injections. For rational development of novel delivery systems with extended residence time in the joint, detailed understanding of transport and retention phenomena within the joint is mandatory. This work presents a systematic study on the *in vitro* permeation, penetration and accumulation of model polymers with differing charges and molecular weights in bovine joint tissue. Permeation experiments with bovine synovial membrane were performed with PEG polymers (6–200 kDa) and methylene blue in customized diffusion chambers. For polyethylene glycol, 2-fold (PEG 6 kDa), 3-fold (PEG 10 kDa) and 13-fold (PEG 35 kDa) retention by the synovial membrane in reference to the small molecule methylene blue was demonstrated. No PEG 200 kDa was found in the acceptor in detectable amounts after 48 h. This showed the potential for a distinct extension of joint residence times by increasing molecular weights.

In addition, experiments with bovine cartilage tissue were conducted. The ability for positively charged, high molecular weight chitosans and HEMA-Co-TMAP (HCT) polymers (up to 233 kDa) to distribute throughout the entire cartilage matrix was demonstrated. In contrast, a distribution into cartilage was not observed for neutral PEG polymers (6–200 kDa). Furthermore, the positive charge density of different compounds (chitosan, HEMA-Co-TMAP, methylene blue, MSC C1 (neutral NCE) and MSC D1 (positively charged NCE) was found to correlate with their accumulation in bovine cartilage tissue.

In summary, the results offer pre-clinical *in vitro* data, indicating that the modification of molecular size and charge of a substance has the potential to decelerate its clearance through the synovial membrane and to promote accumulation inside the cartilage matrix.



## 1. Introduction

The treatment of joint related diseases such as osteoarthritis and rheumatoid arthritis involves highly potent actives, entailing severe side effects in systemic therapy. As osteoarthritis is a locally limited disease, affecting only few joints, direct intra-articular drug administration via injection into the joint cavity as local treatment is an advantageous alternative [1–3]. However, local injections into the joint also bear some challenges. The clearance of small molecules from the joint is relatively fast, resulting in insufficient concentration levels inside the joint cavity [4,5]. Further, the number of injections that can be administered to a patient is limited due to pain, risk of infection, iatrogenic inflammation and poor patient compliance. Hence, new alternatives to extend the residence time of an injected drug inside the joint have to be explored in order to increase injection intervals. As a consequence, patient compliance and therapeutic success can be improved [6,7].

In this context, the unique physiology of synovial joints has to be considered. The joint capsule surrounds the joint cavity, with

*Abbreviations:* SM, synovial membrane; MW, molecular weight; PBS, phosphate buffered saline; ECM, extracellular matrix; HCT, hydroxyethylmethacrylate-Co-tri methylammoniumpropylacrylamid (HEMA-Co-TMAP); GAG, glycosaminoglycan; BSA, bovine serum albumin; PEG, polyethylene glycol; MB, methylene blue; DD, degree of deacetylation; CD, charge density; $P_{coeff}$, partition coefficient.

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its inner layer formed by the synovial membrane. Besides its secreting properties for lubricants (lubricin, hyaluronic acid), the synovial membrane acts as a physical barrier between the synovial fluid and the blood- and lymph vessels in the underlying subsynovial tissue [8–10]. In addition, the synovial membrane is known to act as a size-selective barrier, which is illustrated by the fact that the quantitative occurrence of plasma proteins in synovial fluid correlates with their molecular size [11–14]. This size-selectivity is also reflected in the clearance of intra-articular administered macromolecules: While half-lives of small molecules such as NSAIDs are reported to be in the range of 1–4 h, macromolecules such as albumin and hyaluronic acid show prolonged half-lives of 13–26 h [3]. These characteristics bare the potential to provide extended pharmacological action of intra-articular administered solutions (e.g. macromolecular drugs such as proteins and antibodies, or prodrug approaches with small molecules being linked to biocompatible polymeric carriers [15–17]). However, synovial retention may not suffice to gain controlled drug delivery over several days or weeks. Thus, an additional potential retention mechanism was identified and investigated.

Besides the synovial membrane, cartilage tissue is an important structure in this context, consisting of an extracellular matrix (ECM) essentially produced by the embedded chondrocytes. However, the cellular component is only about 3–5% (w/w), while the major content of total cartilage mass is water (~80% w/w), bound to the ECM [1,18]. The ECM essentially consists of glycosaminoglycans (e.g. hyaluronic acid) and proteoglycans (e.g. aggrecan). This composition forms a strong hydrophilic and negatively charged matrix. The high water binding capacity of the ECM enables significant volume expansion of the tissue and thus tolerating high mechanical stress. Thereby, cartilage tissue literally acts like a sponge, releasing water under mechanical load and aspirating water during relaxation. Besides their water binding capacity, the negatively charged components of the ECM bare the potential to attract and bind positively charged compounds through electrostatic interaction. For instance, van Lent et al. demonstrated, that, in contrast to anionic bovine serum albumin, cationic BSA showed significant absorption in cartilage tissue [19]. In addition, the size dependency of cartilage permeability was shown before, demonstrating a significant decline of solute transport through cartilage tissue with increasing size of the solute [20]. Concerning the potential for positively charged, macromolecular compounds to penetrate cartilage tissue, it seems to be a reasonable approach to consider cartilage tissue as a deep compartment, where an injected compound can accumulate and be retained over a longer period of time. In combination with the size selective retention by the synovial membrane, positive charge may enable prolonged residence times of injected drugs inside the synovial cavity. In addition, the cartilage itself may be a reasonable drug target, for example for growth factors such as FGF-18 (stimulating the chondrocytes to produce matrix components) or for enzyme inhibitors (preventing degradation of cartilage matrix). However, only limited information about the complex physiological interactions inside the joint affecting drug therapy is available, yet.

The described characteristics of synovial joints can be expressed by a kinetic model. Elimination of injected drugs from the joint capsule occurs in two steps: elimination through the synovial membrane and subsequent elimination from systemic circulation. Re-distribution into the joint from systemic circulation can be neglected due to the high concentration gradient after intra-articular administration. Thus, elimination through the synovial membrane can be defined as the rate limiting step and the entire elimination process can be expressed by a combined elimination constant "$k_e$". Kinetically, this can be described by an open 2-compartment model (Fig. 1).

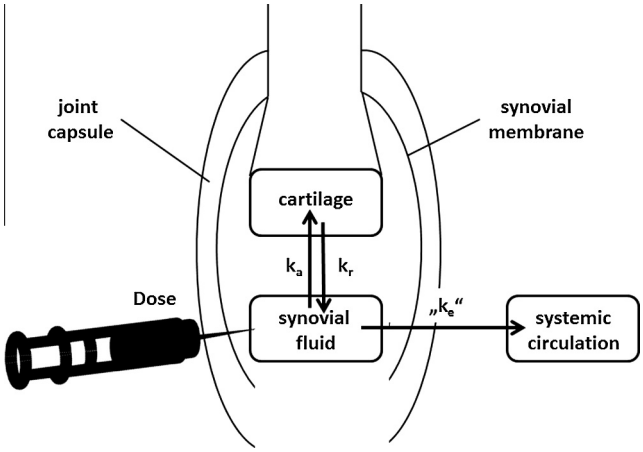


**Fig. 1.** Schematic illustration of the kinetic situation of an active compound upon intra-articular injection. $k_a$ describes the absorption in, $k_r$ the release from cartilage tissue.

Based on this model, the concentration of the injected solute in synovial fluid, $c_{SF}$, can be calculated by (modified according to [21])

$$c_{SF}(t) = \frac{D \cdot [(k_r - \beta) \cdot e^{-\beta \cdot t} + (\alpha - k_r) \cdot e^{-\alpha \cdot t}]}{V_i \cdot (\alpha - \beta)} \quad (1)$$

where "$D$" is the administered dose, "$V_i$" is the initial distribution volume, "$k_r$" is the release constant, "$t$" is the timepoint and

$$\alpha, \beta = \frac{1}{2} \cdot [(k_a + k_r + k_e) \pm \sqrt{(k_a + k_r + k_e)^2 - 4 \cdot k_r \cdot k_e}] \quad (2)$$

As demonstrated by Eqs. (1) and (2), the residence time of injected compounds in the synovial fluid is mainly controlled by "$k_e$" (which is defined by the retention through the synovial membrane), and "$k_a$" and "$k_r$" the ratio of which is described as the partition coefficient ($P_{coeff}$) as follows:

$$P_{coeff} = \frac{k_a}{k_r} = \frac{c_{analyte}(cartilage)}{c_{analyte}(donor)} \quad (3)$$

Thus, the evaluation of both the retention by the synovial membrane, and the distribution into cartilage tissue (described by the partition coefficient, Eq. (3)), is a crucial step in the development of new drugs to be retained inside the joint cavity over a longer period of time.

The following work presents 2 systematic approaches, independently investigating the described retention mechanisms:

(i) Size-selective retention by the synovial membrane ("$k_e$").
(ii) Retention through charge and size-dependent accumulation inside the cartilage matrix ($P_{coeff}$).

The results provide pre-clinical data, demonstrating the relationship between the molecular size of a substance and its retention by the synovial membrane as well as between the positive charge of a compound and its tendency to accumulate inside the cartilage matrix.

## 2. Materials and methods

### 2.1. Materials

Polyethylene glycols with 6, 10, 35 and 200 kDa (PEG 6–PEG 200) were purchased from Sigma–Aldrich (St. Louis, USA). Chitosan 95/5 (95% degree of deacetylation/viscosity, measured in 1% acetic acid), chitosan 70/5 (70% degree of deacetylation/viscosity, measured in 1% acetic acid) as well as chitosan oligomers were purchased from Heppe Medical (Halle, Germany).