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The effect of hyaluronan injections into human knees on the number of bone and cartilage wear particles captured by bio-ferrography

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ABSTRACT

Osteoarthritis is characterized by degradation of cartilage and subchondral bone, releasing wear particles into the synovial fluid. Intra-articular injections of exogenous hyaluronan are often given to patients suffering from osteoarthritis in order to compensate for the reduction in the level of endogenous hyaluronan and to restore the rheological properties of the synovial fluid. The exact effect of these injections is still ambiguous. In this work bio-ferrography was used to capture magnetically labeled cartilage and bone debris from the synovial fluid in human knees before each of four injections (Euflexxa™). The wear particles were counted and characterized microscopically and chemically. WOMAC, VAS, SF-36 and KS questionnaires indicated significant pain relief during the treatment, but suffered from inconsistency. Bio-ferrography showed a reduction in the concentration of both cartilage and bone particles, with a minimum after the third hyaluronan injection. The advantages of bio-ferrography as a primary assessment tool are discussed. The results indicate that while hyaluronan treatment may temporarily slow the wear rate to an extent beyond a placebo effect, it does not prevent joint degradation altogether.

1. Introduction

Osteoarthritis (OA) is a heterogeneous group of conditions that lead to degradation of cartilage and, in severe cases, to related changes in the subchondral bone [1]. OA is the main cause of disability of elder persons and the major reason for total joint replacement. It is most common in weight-bearing joints, the knee joint being most affected. Its prevalence is expected to continue increasing in the coming years [1,2]. Wear particles, such as bone and cartilage fragments, are released into the synovial fluid (SF) of the affected joint [3,4]. This debris may trigger the release of enzymes, such as collagenase [3], which results in inflammation of the synovial membrane.

SF is an ultra-filtrate of plasma, which has non-Newtonian properties and is thixotropic [5]. It enables efficient movement of the joint, acting as a lubricant, supplying nutrients and removing catabolic products. The synovial membrane secretes and removes SF from the joint space, adjusting both the volume of SF and its macromolecular composition [6]. Endogenous hyaluronan (HA) is the main constituent of SF [7]. This polysaccharide acts as a lubricant during low impact joint movement and as a shock absorber during high impact movement [8]. It is secreted in high concentrations in the extracellular matrix of connective tissues, although its highest concentration is in the SF (2.5–4.0 mg ml^{-1} in normal joints) [6,9]. The molecular structure of HA is shown in Fig. 1a. The polysaccharide chain is made of repeating disaccharide units of N-acetylglucosamine and glucuronic acid. The average molecular weight of healthy human HA is 5000 kDa [9,10]. The viscosity of the SF is affected by the length and conformation of the polymer chains, as well as by interaction between adjacent chains [6].

In arthritic joints the concentration and average molecular weight of HA are lower, at 0.8–2.0 mg ml^{-1} and 3000 kDa, respectively. Consequently, the viscosity of the SF is abnormally low, its functionality is lost and rubbing of the cartilage occurs. Intra-articular knee injection of exogenous HA (a treatment also known as viscosupplementation [9]) has been suggested in order to compensate for the reduction in the concentration of endogenous HA. It was assumed that the exogenous HA could restore the rheological, analgesic and anti-inflammatory effects of SF, which are lost in OA [2,10,11]. The US Food and Drug Administration (FDA) approved exogenous HA injections in 1997, but classified it as a device, not as a medicine. Nevertheless, numerous studies have reported significant pain relief and a better knee function that can persist for up to 6 months due to treatment with three to five weekly intra-articular HA injections [2,10–19]. A few studies have even reported that HA treatment prevents cartilage degradation and release of proteoglycans [7,20–24]. As the half-life of HA is a few days,

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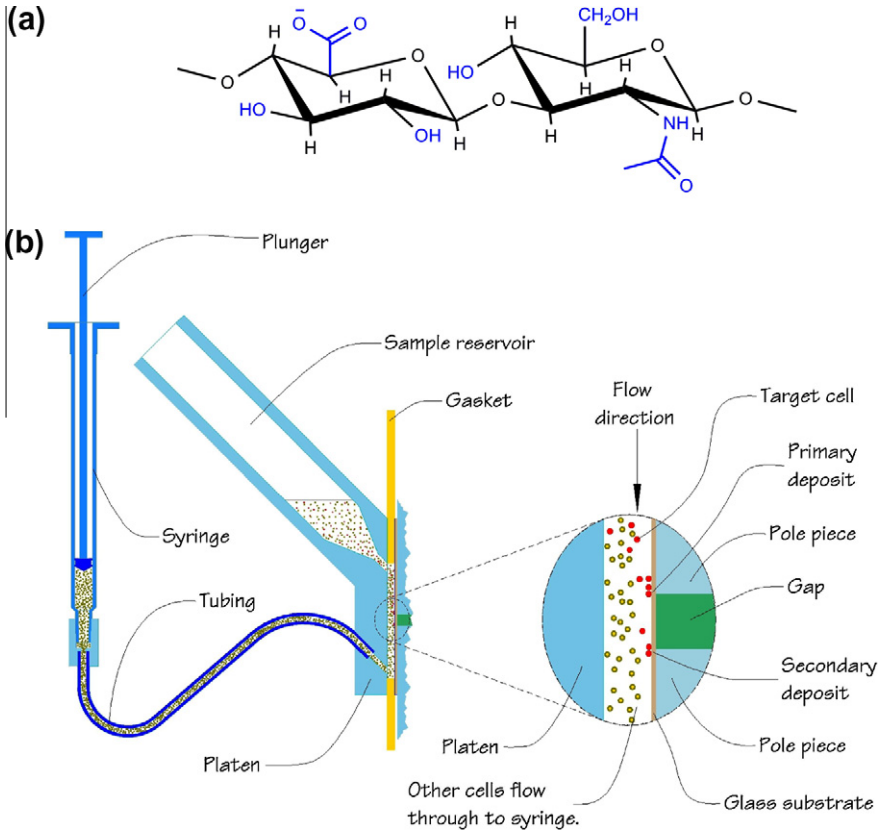

Fig. 1. (a) The repeat disaccharide of hyaluronan: –D-glucuronic acid-β-1,3-N-acetylglucosamine-β-1,4–. (b) The principles of bio-ferrography: the deposition scheme of captured particles on a glass slide [48].

several injections are given to provide sufficient efficacy, although some producers seek to minimize the treatment to one injection only.

There are various HA products on the market, which differ with respect to origin, method of production, treatment schedule, molecular weight, half-life within the synovium, rheological properties, pharmacodynamics and cost. Their efficacy has been evaluated using biomarkers of bone and cartilage present in the SF, serum and urine [4,13,25,26], questionnaires [8,13,15–19,27–29], in combination with measuring joint space narrowing [30], and biopsy of cartilage samples [31]. This study used Euflexxa™ (1% sodium hyaluronate, Ferring Pharmaceuticals Inc., Parsippany, NJ) [8,10]. This is the first HA to be produced from a non-avian source (it is bioengineered by biological fermentation of bacteria) and it has one of the highest molecular weights (3000 kDa) of all available HA products [10].

Surprisingly, none of the studies cited above has studied the effect of serial HA injections on the number of cartilage and bone wear particles over time. Hence, the exact effect of HA therapy is still unknown. Most of the existing studies cannot be claimed to be placebo controlled – as soon as a needle is inserted into the joint (either to remove excess SF or to inject saline solution or for active treatment) the patient experiences some relief.

The objective of the current study was to evaluate the effect of serial Euflexxa™ injections on the concentration of cartilage and bone particles in the SF of osteoarthritic human knee joints. Isolation of these wear particles was achieved by means of bio-ferrography. To the best of our knowledge this is the first ever study that has demonstrated the use of this technique in evaluating the efficacy of a drug.

Ferrography is a non-destructive method of particle separation from a suspension onto a glass slide based upon interaction between an external magnetic field and the magnetic moments of the particles [32–34]. This method was developed by Westcott and co-workers in the early 1970s in order to investigate the occurrence of ferrous wear particles in lubricated dynamic components. By determining the number, shape, size, texture and composition of particles on the ferrogram (i.e. a glass slide with wear particles), the origin, mechanism and level of wear can be determined. Bio-ferrography [35–41] is the latest modification of conventional analytical ferrography that was specifically developed to allow magnetic isolation of target cells or tissues. Its strengths of interest for the current study include: (1) the ability to quantify biological matter and, at the same time, analyze its microscopic and chemical features; (2) extremely high selectivity and sensitivity; (3) applicability to any liquid sample; (4) the ability to analyze samples as small as 1 μl and target particles as small as several nanometers; (5) the possibility of simultaneously processing up to five samples within bracketed areas (channels) on a single slide, without cross-contamination.

2. Experimental

2.1. Selection of patients, sample collection and storage

The study protocol and informed consent form were approved by the Helsinki committee of Assaf Harofeh Medical Center. The criterion for patient inclusion was a diagnosis of OA of the knee based on the Kellgren–Lawrence (K-L) grading of radiographs, from 0 (normal) to 4 (most severe) [42]. The exclusion criteria were: (1) patients that suffered from acute septic arthritis; (2) patients that were treated with Coumadin and/or other anti-coagulant drugs; (3) patients that showed mental or physical conditions which