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The multiple biological and clinical facets of osteoarthritis

Osteoarthritis (OA) is characterized by cartilage damage with a narrowing of joint space, synovitis, osteitis, osteosclerosis and osteophyte formation. OA is not a single entity but has several subtypes with considerable differences which are not only related to genetic defects, mechanical factors such as trauma, joint architecture, repeated minor trauma, inflammation and aging, but also to "enhancer" factors such as obesity, metabolism, smoking, hypermobility. Does the disease originate in the cartilage, in the synovium or in the subchondral bone? Probably all three answers are partially correct but could represent different forms of the "osteoarthritis syndrome". In this syndrome, a gradation between at least two types of disease: one strongly linked to the innate immunity or autoimmunity, the other where the autoimmunity aspect will be preeminent. Furthermore, some OA are linked to specific genetic defects in the extracellular components of the matrix.

Genetic predisposition of siblings is 2-8x and 2x in monozygotic twins versus dizygotic twins. Crystal-induced OA is a prototypical example involving auto-inflammatory pathways where the disease starts mainly in the synovial space and appears more predominant in man. In contrast, severe erosive arthritis may rather be linked to autoimmune; it may start in the subchondral bone and affects more frequently the hands and more women. Pro- and anti-inflammatory mediators and cytokines dictate the intensity, fluctuation and remission of pain, inflammation, tissue destruction and fibrosis. An example is the balance between IL-1/IL-1Ra, MMP/TIMP.

IL-1 is an important cytokine involved in cartilage destruction (induction of aggrecanases, MMPs, cathepsins), whereas IGF, TGFβ and FGF18 are important for the repair process. IL-1 and TNF block biosynthesis of proteoglycans and collagens. In OA the metabolic and endocrine components play important roles. Adipose tissue releases multiple pro- and anti-inflammatory mediators which affect not only directly the joint tissue but also distal endocrine organs, as well as the hypothalamic centers involved in fever, mood and appetite. It is therefore crucial to link the local and systemic clinical manifestations to pathophysiological mechanisms in order to match therapeutic intervention to the stage of the disease and the predominant symptoms.

References:
The composition and cellular organization of human adult articular cartilage is complex with qualitative and quantitative differences in and matrix constituents ranging from the superficial through deep zones and between the interterritorial and territorial, or pericellular, regions. Chondrocytes, the unique cellular component of articular cartilage, maintain the matrix components under normal, low turnover conditions in which the proteoglycans and other non-collagen molecules can be replaced. One of the first steps at the initiation of osteoarthritis (OA) is the degradation of the pericellular matrix with distinctive constituents such as collagen VI, fibromodulin, and matrilin 3, but little or no type II collagen. During the development of OA, the normal quiescent chondrocytes become activated and undergo a phenotypic shift, resulting in fibrillation and degradation of cartilage matrix, the appearance of chondrocyte clusters, increased cartilage calcification associated with tidemark advancement or duplication, and vascular penetration from the subchondral bone. With the coincident upregulation of cartilage-degrading proteinases, matrix degradation products can further promote catabolic activation, aberrant hypertrophy-like differentiation, and apoptosis.

Once the collagen network, containing type II, IX, and XI collagens, is degraded, it cannot be repaired to its original state. Thus, the therapeutic challenge is to prevent the damage or promote repair to recapitulate the physiological and functional properties of the original cartilage.

To identify and characterize mediators of cartilage remodeling common to these processes, we are using culture models of primary human and mouse chondrocytes and cell lines and mouse models to manipulate and compare gene expression with complementary approaches. MMP-13, the major type II collagen-degrading collagenase, is regulated by both stress and inflammatory signals that not only contribute to irreversible joint damage (progression) in OA, but importantly, also to the initiation/onset phase, wherein chondrocytes in articular cartilage leave their natural growth- and differentiation-arrested state. We and other investigators have found that there are common mediators of these processes in human OA cartilage and from early through late stages of OA in mouse models, including the surgical models and the genetic models during aging. We are validating our in vitro analyses of the signaling and transcriptional mechanisms that determine the expression and activities of these mediators by in vivo analyses of the consequences of knockout or transgenic overexpression of these genes in mouse models. In current studies, we are examining the epigenetic mechanisms and using proteomics and genomics approaches to map the signaling networks and microRNA targets that impact on gene expression programs during the onset and progression of OA in both human and murine cartilage. Since the chondrocytes in adult human cartilage are normally quiescent and maintain the matrix in a low turnover state, understanding how they undergo phenotypic modulation and promote matrix destruction and abnormal repair in OA may lead to identification of critical targets for therapy to block cartilage damage and promote effective cartilage repair.
Osteoarthritis (OA) is the most common rheumatic disease in the world, with a considerable economical importance. This disease leads to the degeneration of the articular cartilage due to the release of extracellular matrix degrading enzymes with the release of inflammatory molecules. In advanced stages, the patients suffer from severe pain associated with the destruction of the joint surfaces resulting in restriction of mobility. The consequence, in many cases, is an inability to work and often the inevitable substitution of the diseased joint with an artificial implant. In the next future, the prevalence of OA will increase dramatically with the increase of aged population.

Early or moderate OA can be treated by pharmacological, surgical or physiological interventions, but no treatment is still available to reverse the process of cartilage degeneration.

In the last decade, many groups have used autologous cartilage implantation (ACI) to treat traumatic cartilage injuries and osteochondritis dissecans. The data obtained at long-term follow-up showed good clinical results, together with the formation of new tissues with hyaline and/or fibrous features.

This treatment requires the harvest of healthy cartilage tissue from which chondrocytes are isolated, expanded in vitro and implanted usually after loading on a suitable scaffold. Some clinical data suggest that ACI can also be useful in focal lesion in early OA and preclinical data on OA animal model show an improvement in cartilage lesion after ACI treatment.

Recently adult mesenchimal stem cells (MSC) derived from the bone marrow (BM-MSC) or the adipose tissue (ASC) have been proposed as an alternative source for tissue repair. These cells have the ability, under appropriate conditions, to differentiate in cartilage, bone, adipocytes and tenocytes, and display also some immunosuppressive and anti-inflammatory properties.

Using BM-MSC loaded on a hyaluronan scaffold (Hyaff®-11) we could slower OA progression in a preclinical model of OA in rabbits. At present, our group is involved in a research project funded by the European Community (Adipose-derived stromal cells for osteoarthritis treatment – ADIPOA, Grant n. 241719) that foresees an open multi dose phase I clinical trial for therapeutic applications of ASCs in human OA and a phase II controlled study in OA. Preliminary results obtained in different animal models of OA have shown that ASCs injected intraarticularly can induce an improvement in cartilage and in subchondral bone morphology, and a decrease in the activation of extracellular matrix degrading enzymes. After intraarticular injection, labelled ASC moves to the synovial tissue and the meniscus and probably exert their therapeutical effect by releasing anti-inflammatory molecules and/or growth factors.

In cell transplantation another important issue to consider is the safety of the expanded cells used for autologous implantation. Different groups have tested the stability of the kariotype, alteration of the mechanisms of DNA repair and the activation/mutation of protooncogenes. Adult human MSC and ASC seem to present no substantial alteration during short-term cell culture before implantation.
Diagnostic of osteoarthritis (OA) is based on history, physical examination and radiographic features. Cartilage damage in OA is detected radiographically by a decrease in joint space width. However, radiographic evidence is seen only after significant cartilage degradation has already taken place. At this late stage, cartilage lesions are irreversible. The early stages of the disease may be asymptomatic for many years and are characterized by molecular/metabolic changes in joint tissues. To detect this molecular/metabolic phase before the occurrence of imaging signs is a key challenge for the next decade. Therefore, there is an acute need for reliable biological markers that can facilitate earlier diagnosis of OA, and inform the prognosis, monitoring and therapeutic strategies for chronic and disabling forms of the disease. Biomarkers of tissue turnover in joints have the capacity to reflect disease relevant biological activity and provide information that may be useful diagnostically and therapeutically, potentially enabling a more rational and personalised approach to healthcare management.

Type II collagen is the ideal source of biomarker for studying cartilage remodelling. First, this collagen is relatively specific to articular cartilage, although it is also present in the vitreous humour of the eye, the nucleus pulposus of vertebral discs and the meniscus. Second, it is the most abundant protein in cartilage, representing 25% of the wet weight, 50% of the dry weight, and 90-95% of the total collagen content. Recently, we have developed a method for the assessment of oxidative damage of type II collagen in cartilage and biological fluids (serum, urine and synovial fluid). This original approach is based on the detection in biological fluids or in tissue of a nitrated peptide release from type II collagen during proteolytic and/or oxidative cartilage degradation.

Our strategy was based on the following points: 1) type II collagen is specific for cartilage and is the most abundant collagen in the extracellular matrix; 2) peptide nitrination results from the reaction of aromatic acid with -ONOO-; 3) in pathological circumstances, chondrocytes, but also synoviocytes (mainly macrophages) produce high levels of \( \cdot \mathrm{NO} \) and \( \mathrm{O}_2^- \); 4) type II collagen contains two tyrosine residues, but no other aromatic amino acids, one located in the triple helix (Coll2-1) and the other in the telopeptide of the C-terminal end (Coll2-2).

We have then developed specific immunoassays, one for the peptide 108HRGYPGLDG116 (Coll2-1) and the other for the nitrated form of this peptide 108HRGY(NO2)PGLDG116 (Coll2-1NO2). This strategy allows the calculation of the ratio Coll2-1NO2 / Coll2-1 which reflects the oxidative-related damage of the triple helical area of the molecule. These immunoassays have been validated in animal models and in human clinical surveys. For the first time, we show data from two independent NHI studies named “Progression” and “Doxycycline”. Coll2-1NO2 appears to be a relevant prognosis biomarker. Coll2-1NO2 variation over one month in urine is highly predictive of joint space width changes over three years. These data support the use of Coll2-1NO2 in the daily practice to predict knee OA progression at an individual level.
The main objectives of management of osteoarthritis (OA) are: education of patients, alleviate pain, improve function and prevent or retard OA progression. There are overarching principles of EULAR guidelines and also 10 recommendations (1). They are similar for knee and hip OA. In hip OA recommendations, topical NSAID are not included, but recommended is osteotomy for young patients with dysplasia. Optimal management of patients with knee or hip OA requires a combination of non-pharmacological and pharmacological modalities of therapy.

OARSI guidelines included 12 non-pharmacological modalities, 8 pharmacological modalities and 5 surgical modalities in 2008 publication (2). Evidence from 2006-2009 was analysed in update 2011 (3). It included 64 metaanalyses or systemic reviews, 266 randomised, controlled trials and 22 economic evaluations. Among non-pharmacological therapies effect size (ES) for pain relief was unchanged for self-management, education, exercise and acupuncture. There is new evidence the ES for pain relief for weight reduction reached statistical significance.

Among pharmacological therapies, cumulative evidence was same for NSAIDs, i.a. steroids and diacerein. Effect size decreased for glucosamine sulphate, while increased for chondroitin sulphate and hyaluronic acid. ES for paracetamol decreased. New evidence increased risk of PUB after dosing > 3 g daily of paracetamol. Among surgical therapies further negative trials have been published on debridement / lavage and ES of their modality is not higher than placebo.

**Conclusion:** Risk / benefit ratio of some treatment may be different after publication of knew studies.

**Key words:** osteoarthritis, therapy

There is increasing evidence that synovial inflammation plays a significant role in the symptoms and progression of osteoarthritis (OA).

The cellular elements of the synovial membrane (SM) are a source of the major synovial fluid (SF) components, which contribute to the unique low friction properties of articular surfaces, and modulate the activity of the chondrocytes. Lubricin and hyaluronic acid (HA) are two important molecules produced by synovial lining cells that help to protect and maintain the integrity of articular cartilage surface in diarthrodial joints.

These two molecules together provide boundary lubrication at the articular surface, which is essential for normal joint function. In the setting of OA or after joint injury, the concentration and molecular weights of HA and lubricin are reduced and the alterations in the SF composition of these two key joint lubricants adversely affects chondrocyte function and homeostasis of the articular cartilage.

The histological SM changes observed in OA exhibit cellular features indicative of an inflammatory “synovitis” and encompass a range of abnormalities, including synovial lining hyperplasia, infiltration of macrophages and lymphocytes, neoangiogenesis and fibrosis. The pattern of synovial reaction varies with the duration of the disease process and the associated advancement of structural deterioration in the joint tissue components.

Imaging modalities including Magnetic Resonance (MRI) and ultrasound (US) have proved useful in detecting and quantifying the synovial reaction and have provided convincing evidence that the presence of synovitis in OA is associated with more severe symptoms, including pain and joint dysfunction. In addition, the presence of synovitis is predictive of more rapid rates of cartilage loss in certain patient populations. Recent studies have implicated activation of Toll-like receptors by degradation products of the extracellular cartilage matrix and products of chondrocyte cellular stress in the pathogenesis of the SM inflammation.

The ensuing synovial inflammation can lead to the synthesis and release of a broad spectrum of proinflammatory products, including metalloproteinases and aggrecanases, which contribute to articular matrix degradation, as well as a wide variety of cytokines and chemokines, which enhance synovial leukocyte infiltration and activation.

Many chemokines and cytokines beyond IL-1 and TNF-α are detected in OA joint tissues and SF, and also have catabolic effects on chondrocytes, and these mediators represent potential targets for therapeutic intervention to impact both symptoms and structural progression in OA.
Abstract

Obesity is the most modifiable risk factor for knee osteoarthritis (OA). The mechanisms by which obesity contributes to the onset of knee OA are not fully understood, but the increase in biomechanical loading to cartilage seems to play a major role.

Recent data have also suggested that metabolic factors and low-grade inflammation in obese patients might contribute to the genesis of the OA process. Weight loss is recommended by international bodies (EULAR and OARSI) as a treatment modality for obese patients with knee OA.

RCTs have demonstrated that moderate dietary weight loss of about 5% improves function but pain only slightly. Few open studies have investigated the effect of massive weight loss induced by bariatric surgery in knee OA.

Although the results from these studies should be cautiously interpreted, it seems that drastic weight loss could be more effective to reduce pain and disability in obese patients.

Moderate weight loss significantly reduces several markers of systemic inflammation (TNF\(\alpha\), IL-6 and CRP) but the search for a correlation between theses changes and an improvement in clinical outcomes has remained inclusive in different studies.
Hyaluronic matrix based autologous chondrocytes transplantation: long-term experience

Introduction
The incidence of articular cartilage pathology has grown due to the marked increase in sports participation and greater emphasis on physical activity in all age groups. Unfortunately, articular cartilage lesions, with their inherent limited healing potential, are hard to treat and remain a challenging problem for orthopedic surgeons. In the last years matrix autologous chondrocyte transplantation has become a possible solution in the treatment of chondral lesions. We used a biodegradable, hyaluronan-based biocompatible scaffold for cell proliferation (Hyalograft® C). The easy handling of Hyalograft® C has permitted to develop an arthroscopic procedure for chondrocyte implant.

Material and methods
Arthroscopic technique has been used from December 2000 in more than 150 cases. All the patients prospectively clinically evaluated using the International Repair Cartilage Society score. Actually 83 patients achieved at least 7 years follow-up. Tegner score was applied to evaluate the sport activity level. MRI evaluation was also performed.

Results
At the 7 years follow-up evaluation we had 8 failed patients, who were re-operated for the chondral lesion. However, all the scores analysed still presented good results with a significant improvement compared to the basal level. The mean IKDC subjective score obtained was 77.5 at 7 years. Self-assessment of quality of life, assessed by EQ-5D, showed a statistically significant improvement, too: 85/100. MRI evaluation showed good healing of the defect, as assessed by the MOCART score.

Conclusion
This matrix autologous chondrocyte transplantation procedure avoids the use of periosteal flap, simplify the surgical procedure and permit to perform the arthroscopic implant reducing the morbidity of the procedure.

The medium-long term clinical and MRI results at 7 minimum years follow-up are positive, confirming the positive results previously obtained and the perseverance of the beneficial outcome offered by this bioengineered approach.
Evolution of surgical treatment for the repair of osteochondral lesions

Introduction
Osteochondral lesions of the talus (OLT) frequently require surgery. Different methods have been proposed to achieve the regeneration of hyaline cartilage, with good results but also with well-known drawbacks. The purposes of this work is to describe evolution in cartilage repair from open field autologous chondrocyte implantation to regeneration by arthroscopic bone-marrow-derived cells (BMDCs) “one step” technique and to present the results of a series of patients consecutively treated.

Methods
154 patients (mean age 29±7 years) were treated between 1996 and 2009. Patient evaluation included clinical AOFAS score, X-Rays and MRI preoperatively and at different established follow-ups. All the lesions were > 1.5 cm² and received open autologous chondrocyte implantation (10 cases), arthroscopic autologous chondrocyte implantation (46 cases), and “one step” arthroscopic repair by BMDC transplantation (98 cases). For arthroscopic repair techniques a hyaluronic acid membrane was used to support cells and specifically designed instrumentation was developed.

Results
Mean AOFAS score before surgery was 59.4±15.8 and 90.4±14.2 (p<0.0005) at mean 6±2.4 years. A similar pattern of AOFAS improvement in results was found in the 3 different techniques. Histological evaluations highlighted type II collagen and proteoglycan expression. MRI and bioptic control showed a progression of the reparative process in the lesions. T2-mapping MRI showed a mean T2 value of 46 msec, typical of healthy cartilaginous tissue for all the three techniques described.

Conclusions
The cartilage repair techniques described were able to provide a repair tissue which closely approximates the characteristics of the original hyaline cartilage. Evolution in surgical technique, new biomaterials and more recently the use of BMDCs permitted a marked reduction in procedure morbidity, duration and costs up to a “one step” technique able to overcome all the drawbacks of previous repair techniques.
Articular cartilage regeneration with autologous peripheral blood progenitor cells and hyaluronic acid

Introduction
One of the most common and challenging injuries for physicians to treat is cartilage damage in joints. The structure and function of articular cartilage leads to non-healing lesions or the formation of fibrocartilage after injury occurs.

Well-established arthroscopic methods utilize controlled healing with marrow stimulation or transferring of non-injured cartilage to areas of injury. Historically, these arthroscopic methods as well as open and two-stage methods have shared common marginal outcomes. Recent study has investigated synthetic and biologic adjuncts to current methodology, including the use of: hyaluronic acid (HA), platelet rich plasma, mesenchymal stem cells (MSC) and peripheral blood progenitor cells (PBPC).

Cell therapy has produced exciting results in animal models and has been shown to regenerate hyaline cartilage clinically in the knee joint.

Our current method utilizes arthroscopic subchondral drilling of cartilage lesions in combination with a postoperative adjunct treatment involving: stimulation of the release of PBPC with filgrastim, harvest of PBPC with apheresis, and postoperative intraarticular injection of PBPC in combination with HA.

Our early results lead us to the conclusion that cell therapy will have an integral part in the future treatment of cartilage damage as well as other potential orthopedic, surgical, and medical applications.

Abstract
Patients with chondral lesions underwent arthroscopic subchondral drilling followed by postoperative intraarticular injections of peripheral blood progenitor cells (PBPCs) in combination with hyaluronic acid (HA). Continuous passive motion was used on the operated knee two hours per day for 4 weeks. Partial weight bearing was observed for the first 6 to 8 weeks.

Autologous PBPCs were harvested one week after surgery. One week after surgery, 8mls of the harvested PBPCs in combination with 2mls of HA were injected intraarticularly into the operated knee.

The remaining PBPCs were divided into vials and cryo-preserved. A total of 5 weekly intraarticular injections were given.

Second-look arthroscopy confirmed articular cartilage regeneration and histologic sections showed features of hyaline cartilage.

Apart from the minimal discomfort of PBPC harvesting and localized pain associated with the intraarticular injections, there were no other notable adverse reactions.

Articular hyaline cartilage regeneration is possible with arthroscopic subchondral drilling followed by postoperative intraarticular injections of autologous PBPCs in combination with HA.