

**“VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND PHARMACY
FROM TIMISOARA**

FACULTY OF MEDICINE

Department IV – BIOCHEMISTRY AND PHARMACOLOGY

ȘTEFAN IULIAN STĂNCIUGELU



PHD THESIS

**Modern diagnostic and treatment methods in
pathologies of the osteo-articular system**

A B S T R A C T

Scientific Coordinator:

PROF. UNIV. DR. MARIAN CĂTĂLIN

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ABSTRACT

According to the World Health Organization (WHO), over 343 million individuals globally are affected by osteoarthritis (OA), with a higher prevalence in women compared to men. Furthermore, the incidence of OA is notably higher in Europe and the United States than in other regions, which suggests a greater disease burden in these areas. This geographic variation may be influenced by a mix of genetic, environmental, and lifestyle factors that are more prevalent in these populations. The rising healthcare demands linked to OA are proving unsustainable, particularly in developed nations. For example, in Spain and Italy, where life expectancy is among the highest in Europe, the annual medication cost for OA per patient is estimated between €1,000 and €1,500. The Robert Koch Institute's 2014/2015 "German Health Update" (GEDA) study reported a 12-month OA prevalence of 17.9% among German adults, with a significant increase with age: 48.1% of women and 31.2% of men aged 65 and older are affected.

Research has highlighted a moderate correlation between OA and an increased risk of cardiovascular diseases and conditions associated with atherosclerosis. Individuals with OA in the lower limbs are also more susceptible to depressive symptoms, largely due to chronic pain, which is the most common and severe symptom of OA. Chronic pain significantly influences mental health, often leading to depressive episodes by limiting physical activity. This inactivity further exacerbates knee pain and weight gain, creating a harmful cycle. The mental health impacts of OA, including the increased risk of suicidal thoughts, demonstrate that OA is not only an economic burden but also a profound social issue with wide-reaching effects on individual well-being and public health.

Despite the predominant role of pain in OA, treatment options remain challenging. Standard strategies like pain management and lifestyle changes often fall short, particularly in advanced cases where surgical intervention, such as joint replacement, becomes the only viable option. While knee arthroplasty is a major advancement in OA treatment, it also underscores the limitations of medical interventions in fully addressing the underlying disease.

Genetic factors play a crucial role in OA, accounting for 40% to 80% of hip and hand OA cases, though the influence on knee OA is relatively lower. Genome-wide association studies (GWAS) have identified 90 genetic risk loci linked to OA, though most of these have small effect sizes. Beyond genetic predisposition, epigenetic mechanisms also play a key role in the onset and progression of OA, adding complexity to the disease. Geographic and ethnic disparities are evident, with African Americans having a higher risk of developing symptomatic knee OA, while hip OA is less common in Asian populations.

Against this backdrop, I chose to focus my doctoral research on OA, with the goal of contributing to the growing body of international research on the disease. My work aims to enhance the understanding of OA and improve patient outcomes by refining diagnostic methods, preventing complications, and reducing the global burden of OA-related morbidity and mortality.

The research started with a thorough literature review aimed at examining differences in miRNA levels among OA patients. The objective was to synthesize existing data to better understand the role of miRNAs in OA pathogenesis and their potential as diagnostic tools. We sought to identify new biomarkers by analyzing plasma samples from OA patients and comparing them with healthy controls, focusing on the disease's molecular profile to improve diagnostic and therapeutic approaches.

From the 35 case-control studies including OA patients compared to healthy controls, a total of 54 human miRs were identified to be dysregulated in OA. In total, 41 miRs were involved in the pathophysiological processes of OA, including apoptosis, inflammation, and proliferation, having either a protective or a progressive role in OA. The discovery of altered miR levels in OA patients compared to healthy controls determines a better understanding of the molecular mechanisms involved in the pathophysiology of OA and could open novel horizons in the field of orthopedics.

MiRs appear to regulate the expression of target genes in OA cartilage, suggesting that they might be involved in the pathogenesis of OA through various mechanisms. It appears that apoptosis, inflammation, and proliferation are important processes involved in the pathophysiology of OA.

The included studies provided hypothesized data on the pathophysiological effects of miRs in OA. Thus, 19 miRs are involved in apoptosis, 23 miRs are involved in inflammation, and 16 miRs play a role in cartilage proliferation.

MiRs involved in OA pathophysiology (apoptosis, inflammation, and/or proliferation) seem to have either a protective or destructive role, the latter leading to the progression of the disease.

In conclusion, understanding the exact roles and mechanisms of non-coding RNA species (such as miRs) in the pathogenesis of OA could bring new insights into disease management and future therapeutical approaches and improve the overall quality of life for patients suffering from this degenerative disorder.

This research utilized a multidisciplinary approach, employing advanced technologies to explore a range of molecular species, particularly non-coding RNAs like microRNAs. Techniques such as real-time polymerase chain reaction and microarray analysis were employed, along with lipidomic studies to assess the diagnostic potential of lipid metabolites in 53 subjects (33 plasma and synovial fluid samples from OA patients and 20 control subjects). Ultra-high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-ESI+MS) was used for these analyses, with the findings compared to existing literature.

In plasma samples, 25 metabolites had area-under-the-curve (AUC) values higher than 0.9, suggesting a very good diagnostic potential for phosphatidic acid PA (16:0/16:0), PA (34:0), phosphatidylethanolamine PE (34:2), glucosylceramide, phosphatidylcholine PC (32:1), and other metabolites while in SF 20, metabolites had AUC values higher than 0.8, the vast majority belonging to lipid metabolism as well.

The methods employed in this study are considered modern diagnostic techniques, offering a personalized evaluation of each patient's unique epigenetic and metabolic profile. This approach holds promise for generating new insights into the diagnosis and management of osteoarthritis.

In addition, this research was also focused on sticky bone as a new type of autologous bone grafting in schatzker type II tibial plateau fracture. Schatzker type II fractures usually need to be grafted. Autograft bone from the iliac crest represents the gold standard, but it comes with high rates of morbidity on the donor side. Sticky bone is one of the regenerative therapies that aims to find new solutions to treat bone defects and to overcome the limitation of conventional options regarding bone grafts, due to their content in growth factors, which offer osteo-induction and osteo-conduction properties. Notably, regenerative dentistry has been at the forefront of applying these products in bone regeneration, demonstrating that PRF produces a highly promising “sticky bone” when combined with bone chips. To the best of our knowledge, this grafting technique has not been used in the orthopedic field to date. The subject was a 53-year-old woman with a Schatzker type II tibial plateau fracture, for which a new autologous bone grafting technique, i.e., sticky bone, was used for the treatment of the fracture. This case reports the effectiveness of sticky bone as autologous bone graft used in Schatzker type II tibial plateau fracture. As an indispensable component of regenerative medicine, it seems to be an ideal biologic graft with a fibrin-rich structure that provides effective treatment in impressed tibial plateau fractures. Sticky bone showed promising results and should be considered in the future as an appropriate bone implant.

This doctoral thesis offers an in-depth exploration that showcases how a multidisciplinary research approach, utilizing various methodologies and focusing on molecules from different biochemical classes, marks a significant step forward in personalized, evidence-based medicine. Specifically, it advances the identification of biomarkers for osteoarthritis (OA). By examining genetic material in circulating biological fluids and analyzing a variety of metabolites, this research deepens the understanding of OA's underlying mechanisms and uncovers reliable biomarkers for early and specific diagnosis, which could lead to more targeted and effective treatment strategies.

Additionally, the thesis presents an innovative clinical application of "sticky bone," a grafting method that combines autologous platelet-rich fibrin (PRF), platelet-rich plasma (PRP), and bone chips. This method proved to be an effective biological scaffold for treating tibial plateau fractures, especially Schatzker type II fractures.

Case study findings reveal that the sticky bone technique not only promotes bone healing and provides stable fracture fixation but also minimizes bone loss during recovery, offering a promising alternative to traditional grafting techniques.

From the research conducted, the following conclusions were reached:

Study 1: A literature review confirmed the significant role of microRNAs (miRs) in maintaining articular cartilage homeostasis and regulating key processes like inflammation, apoptosis, and proliferation. MiRs were also shown to provide high diagnostic accuracy in distinguishing OA patients from healthy individuals.

Study 2: This study demonstrated that miRs, such as miR-34a-5p, miR-140-5p, and miR-181a-5p, show distinct expression patterns across different biological fluids, with significant functional implications. These miRs emerged as promising biomarkers for both diagnosis and prognosis of OA, as well as potential therapeutic targets.

Study 3: Research on metabolomics revealed that OA patients exhibit significant alterations in lipid profiles, identifying 25 plasma metabolites and 20 synovial fluid metabolites with high diagnostic accuracy. These findings suggest new avenues for diagnostic biomarkers in OA.

Study 4: The introduction of sticky bone grafting demonstrated its effectiveness in treating periarticular fractures, providing both fracture stabilization and enhanced functional recovery.

In conclusion, the thesis highlights the importance of integrating molecular biomarkers with clinical and imaging tools for early and precise OA diagnosis, which may lead to more personalized treatments. Additionally, sticky bone offers a new, promising option in orthopedic care, specifically for periarticular fractures.