Reasons for undertaking the research topic: One of the greatest achievements of mankind in recent decades is the increase in life expectancy, concomitantly associated with increased exposure to a variety of diseases. One of the most frequent causes of physical disability among middle-aged and older adults is osteoarthritis (OA). The affirmative diagnosis of OA has increased in recent years, and it is estimated that by 2040 the number of people suffering from arthritis will increase by 52%, to 34.6 million new patients in the US only. The direct cause of OA is the degeneration of joint cartilage, local inflammation, chronic pain and movement limitations, which causes major social and economic problem. Most of the current treatments available for OA provide short-term pain and inflammation relief only, but do not treat the cause of the disease, only temporary masking its symptoms. Hyaluronic acid (HA) injections improve joint mobility and in adequate form this biopolymer exhibits anti-inflammatory properties and lubricates the joint while reducing friction, but the effect is not permanent. The novel approach of the current project is to use synthetic polymers with greater than HA anti-inflammatory properties, that simultaneously inhibit further cartilage degeneration. In our previous studies, we discovered the analgesic effect of the novel synthetic polymers in an animal model of OA. This was an unexpected result, because the original purpose of synthesis of polymers aimed at creating cryopreserving substances - improving the survival of stem cells under freezing conditions. We observed not only a reduction of pain after intra-articular injection of polymers, but also diminution of cartilage degeneration at the end point of the experiment. This is a very promising result that requires further investigation. An innovative feature of tested polymers is that they exhibit protective properties, which means that they can counteract the degeneration of cartilage and thus slow down the progression of the disease. Currently, there is no therapy available that would allow the inhibition or reversal of cartilage degeneration. Therefore, the goal of the current project is to investigate the mechanism of action of polymers on patient-derived human chondrocyte (HC-OA) and synoviocyte (FLS-OA) cell lines. These two cell types build cartilage and synovial membrane, respectively - tissues located within the joint and involved in the development and progression of OA.

Aim of the project: The aim of the project is to determine the mechanism of analgesic and regenerative action of novel polymers on HC-OA and FLS-OA cell lines, and to determine which joint-building cell type plays a more important role in polymers protective action. All tissues within the joint are involved in the development of OA. Unfortunately, *in vitro* cell cultures are highly limiting in terms of the reflection of the joint conditions in patients. Mono-cultures also ignore biological and transport crosstalk which exists in the joint, e.g. FLS-OA might release important factors, which affect HC-OA and vice versa. What is more, a drug dose effective for one tissue might adversely affect another. Therefore, the results of potential therapeutic substances obtained in cell line studies may not reflect those obtained *in vivo*. The current project will address this issue as well and the interactions between HC-OA and FLS-OA cell lines using co-culture in special semi-permeable inserts, that allow the exchange of factors produced by the cells into the culture medium reflecting the closest image to the knee joint.

Research Description: In collaboration with the Department of Chemistry of the Jagiellonian University, new biotechnologically advanced polymers combining anti-inflammatory and regenerative properties will be synthesized. It is expected, that novel polymers will possess better anti-arthritic properties over those previously tested. Then, these polymers will be tested on cell lines - first separately on HC-OA and FLS-OA, to select the best promising polymer at a non-toxic and most effective dose. In a final research task, a co-culture of HC-OA and FLS-OA will be used to determine the role of cell interactions during polymer activity and biological and transport crosstalk between multiple joint tissue.

Expected Results: An important feature of the project is that the effects of polymers in an animal model of OA has recently been revealed by us. Since the effect observed in animals was unexpected, the current project aims to investigate the mechanism of action of compounds that already have proven high analgesic and anti-arthritic potential. An innovative feature of the project is the synthesis of unique polymers with even better anti-arthritic and anti-inflammatory properties. In the development of OA, degraded cartilage fragments released into the joint space cause pro-inflammatory activation of synoviocytes, which produce inflammatory factors (e.g. cytokines, chemokines, extracellular matrix metalloproteinases), that potentiate further cartilage degeneration. The whole process is therefore driven by a ”self-propelled wheel” that is likely broken by the anti-inflammatory effects of polymers. Reducing inflammation inhibits cartilage degradation, which in turn prevents the development of chronic pain. It is expected that both: new polymers and those previously tested in an animal model will exhibit anti-inflammatory effects. Moreover, it will be investigated which cell type (HC-OA or FLS-OA) plays a more important role in the action of polymers and what is the role of the interaction between these two types of cells.