Did you know that more than 100 million people in developed countries are affected by skin fibrosis each year? It is a condition where the skin forms excessive scars, and it can cause all sorts of problems like changes in appearance, difficulty with normal functions, and even emotional distress. In some serious cases, it can even be life-threatening. Current therapeutic approaches with their empirical effects are unreliable and unpredictable. That is why we really need better therapies that target the problem directly and have a clear understanding of how they work to heal wounds. We are on the lookout for effective and precise treatments that can prevent and treat skin fibrosis. Scientists are exploring new approaches using regenerative therapies. They are trying to transform a type of cell called myofibroblasts, which play a role in hypertrophic scarring. It is like reprograming those cells to do their job better. There are some substances called chalcones, like isoliquiritigenin, flavokawain A, and butein, that have shown some promising abilities. They are known to have strong anti-inflammatory properties, fight off microbes, and even reduce fibrosis.

The goal of the presented project is to evaluate for the first time the impact of selected chalcones on the anti-fibrotic activity in the skin - an *in vitro* study in the modified skin model on model substrates as well as on hydrogels with incorporated chalcones which would be used as drug sustained-release matrix.

Description of research:

Firstly, we want to improve the skin fibrosis model. To optimize the model, we are going to use different substances like dextran sulfate with certain molecules called TGFβ or a combination of pro-inflammatory cytokines like TGFβ, IL-6, and IL-8. We will test these substances in different amounts and for different lengths of time. The aim is to find the best conditions that make the fibroblasts transition into myofibroblasts (FMT) more efficient. To check if the transformation works, we will analyze the gene expression profiles and protein levels. We will look at specific markers related to myofibroblasts, as well as the signaling pathways involved. We plan to use techniques like **qRT-PCR** (a way to measure gene activity), **western blotting** and **ELISA** (methods to measure protein levels). Secondly, we want to find out the right concentrations of chalcones that will not harm the skin cells. To do that, we will check how the chalcones affect the cells' shape, how many of them survive, and how they grow while being exposed to different chalcone concentrations. We expect that using lower concentrations will not be harmful to the skin cells. Next, we are going to test the anti-fibrotic effects of the selected chalcones in the modified skin model. We want to see how well the chalcones can stop the skin fibroblasts transition into myofibroblasts, which are the cells involved in fibrosis. We will also check the levels of certain markers that indicate fibrosis. We hope that some of the chalcones will lower the levels of these markers, indicating a positive anti-fibrotic effect. After that, we will prepare hydrogel substrates that contain the chalcones. We will examine the properties of these hydrogels before and after the skin cells culturing on them using special techniques like X-ray photoelectron spectroscopy **(XPS)**, Raman spectroscopy **(RS)**, and atomic force microscopy **(AFM)**, scanning electron microscopy **(SEM)**. Finally, we want to see how the chalcones, when released slowly from the hydrogels, affect the skin cells' shape, movement, and structure of their cytoskeleton. We expect that hydrogel will function as a drug (chalcone) sustained-release matrix. We hope that this will reduce the skin fibroblasts' transition into myofibroblasts and the levels of expression of fibrotic markers. In summary, we are evaluating how the chalcones affect skin cells and fibrosis in different settings, including in the skin model and with hydrogel delivery.

We expect that the obtained results will help in developing new methods for treating fibrosis. The special wound dressings created, combining hydrogel and chalcone, would have two important features. First, the hydrogel material used in the dressings would be very safe and compatible with the body. Second, the dressings would slowly release the chalcone, which has properties that can prevent scarring. If these dressings prove successful, it could be a big difference in the lives of patients. It could mean improved healing, reduced scarring, and better overall outcomes for those who need treatment for their skin conditions. So, the hope is that this research will bring about new and effective ways to help people with skin fibrosis and enhance their quality of life.