Summary

Hyaluronic acid (HA) and dental pulp stem cells (DPSCs) are attractive research topics and their combined use in tissue engineering is a promising direction in regenerative medicine. DPSCs are a mesenchymal population of stem cells that are obtained from the soft tissues within the dental pulp cavity. Their advantages are easy availability, high proliferative activity, and wide differentiation potential. HA is a natural extracellular biopolymer occurring across human body tissues, including dental pulp. Due to its biocompatibility and biodegradability, HA is a suitable scaffold materal. Degradation of high molecular weight (HMW) HA chains, which are enzymatically cleaved in tissues, results in low molecular weight (LMW) HA fragments. Compared to the HMW HA, LMW HA exhibits markedly different bioactive properties. Thus, it is necessary to assess the effect of HA on tissues and cells separately for its distinct fractions.

The results of two *in vitro* experiments are presented in this work. The first of them was focused on the influence of a wide spectrum of HA (116 kDa, 540 kDa, 1500 kDa) on two DPSC lines. This pilot experiment verified the basic assumption that cells in the environment of selected molecules adhere, survive, proliferate, maintain their typical phenotype and osteogenic and chondrogenic differentiation potential. The second experiment was focused on LMW HA and its effect was tested on 5 DPSC lines. Molecules of the particular sizes corresponding with *in vivo* degradative HA fragments (800 Da, 1600 Da, 15 kDa) were used. After application of HA to the culture media, an acute reduction in proliferative activity was observed in the experimental groups compared to the control group ($p = 0.0033$, $p = 0.0033$, $p = 0.1416$). This reduction was limited to the passage in which the cells were initially seeded in LMW HA enriched media and was not observed in subsequent passages. At the end of the experiment, statistically significant difference in the cumulative number of population doublings was observed between the control group and experimental groups ($p = 0.015$, $p = 0.0143$, $p = 0.0864$). Additionally, DPSCs cultured in media with LMW HA were statistically significantly smaller compared to the control group ($p = 0.0078$, $p = 0.0019$, $p = 0.0098$). The relative telomere length was shorter in experimental groups of 4 of 5 lines compared to the control group. A statistically significant difference was detected in cells cultured in medium with the addition of 800 Da HA ($p = 0.0275$, $p = 0.1416$, $p = 0.0864$). All cells maintained high expression of surface markers typical of DPSCs (CD29, CD44, CD73, CD90). Compared to the control, a statistically significant reduction in the expression of phenotypic markers was observed in the experimental groups ($p < 0.05$; CD29, CD34, CD90, CD106, CD117, CD146, CD166). The cells retained the features of mesenchymal stem cells and the naive undifferentiated phenotype even after long-term cultivation in the presence of LMW HA.

This work provides data on the effect of HA on DPSCs *in vitro* with emphasis on LMW HA. The results show that LMW HA affects their proliferative activity, size, telomerase activity and expression of phenotypic traits. The work presents important findings about the influence of HA degradation products, which must be taken into account in the further development of HA scaffolds for DPSCs.