M U N I

COMMENTARY TO HABILITATION THESIS

Teeth exhibit a complex internal organization in terms of their cellular composition and microstructure of hard matrices, which gives them unique mechanical properties but also allows them to respond actively to various changes in the external environment. This ensures their sensitivity, long-term viability and also adaptability to some extent.

During the evolution of vertebrates, due to the pressure to adapt to changing environmental conditions, dentition in different organisms have undergone several major innovations accompanying the changes in their lifestyles. One of the interesting adaptations was the development of hypsodont teeth with high crowns and subsequently hypselodont teeth whose growth never stops. These types of teeth are often, but not always, associated with animals specialized feeding habits and have brought a great evolutionary advantage to their holders. From a scientific point of view, hypselodont teeth serve as great models for studying tooth development, epithelial-mesenchymal interactions, stem cell dynamics, or investigating the microstructure and patterning of enamel and dentin.

Although all the research work presented here is focused on dental biology, the aims and results can be divided into two main subgroups. The first part aimed to comprehensively map the cellular composition of the tooth and to understand it at both the cellular and molecular levels. Here, using advanced single-cell RNA-sequencing techniques and subsequent experiments which included bioinformatic analyses, lineage tracing and various *in vivo* validations, new cell types in the teeth were discovered and their expression profiles described. Furthermore, differentiation trajectories leading to the formation of odontoblasts and ameloblasts from undifferentiated cells were mapped, and new types of quiescent mesenchymal and epithelial stem cells were discovered.

The second part of the research work was focused on tooth development and patterning of hard dental tissues. At the beginning of this part, a new genetically engineered mouse strain that allows studying various microstructural aspects of odontoblasts was introduced. In these mice, the expression of two major odontoblasts-specific genes (*Dspp* and *Dmp1*) is coupled with the production of cyan and red fluorescent proteins. Using this new strain, a different dentin structure in the crown and root of the tooth has been described. Further experiments have uncovered the molecular basis standing behind the formation of these differences and different

elemental composition in these two studied dentin types was revealed. These findings have uncovered an essential inductive role of different types of dental epithelium on the microstructure of the adjacent root or crown dentin. The second part of this research also included the study revealing the important role of enamel knots during the morphogenesis of reptile teeth.

Taken together, this work brings new insights into the field of dental biology from the perspective of studying different organisms and teeth types and thus provides a broader context, allowing for better understanding of several previously separately observed phenomena.

[1] Krivanek, J., Soldatov, R.A., Kastriti, M.E., Chontorotzea, T., Herdina, A.N., Petersen, J., Szarowska, B., Landova, M., Matejova, V.K., Holla, L.I., Kuchler, U., Zdrilic, I.V., Vijaykumar, A., Balic, A., Marangoni, P., Klein, O.D., Neves, V.C.M., Yianni, V., Sharpe, P.T., Harkany, T., Metscher, B.D., Bajénoff, M., Mina, M., Fried, K., Kharchenko, P.V., Adameyko, I., 2020. Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth. Nat. Commun. 11, 4816.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
80	20	50	50

[2] Landova Sulcova, M., Zahradnicek, O., Dumkova, J., Dosedelova, H., **Krivanek, J.**, Hampl, M., Kavkova, M., Zikmund, T., Gregorovicova, M., Sedmera, D., Kaiser, J., Tucker, A.S., Buchtova, M., 2020. Developmental mechanisms driving complex tooth shape in reptiles. Dev. Dyn. Off. Publ. Am. Assoc. Anat. 249, 441–464.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	0	10	10

[3] Book chapter "Dental stem cells: Developmental aspects" in: Gruber, R., Stadlinger, B., Terheyden, H., 2022. Cell-to-Cell Communication: Cell-Atlas - Visual Biology in Oral Medicine.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	-	70	-

[4] Krivanek, J., Adameyko, I., Fried, K., 2017. Heterogeneity and Developmental Connections between Cell Types Inhabiting Teeth. Front. Physiol. 8.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
-	-	50	60

[5] Vijaykumar, A., Ghassem-Zadeh, S., Vidovic-Zdrilic, I., Komitas, K., Adameyko, I., Krivanek, J., Fu, Y., Maye, P., Mina, M., 2019. Generation and characterization of DSPP-Cerulean/DMP1-Cherry reporter mice. Genes. N. Y. N 2000 57, e23324.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	0	20	10

[6] Lavicky, J., Kolouskova, M., Prochazka, D., Rakultsev, V., Gonzalez-Lopez, M., Steklikova, K., Bartos, M., Vijaykumar, A., Kaiser, J., Pořízka, P., Hovorakova, M., Mina, M., Krivanek, J., 2022. The Development of Dentin Microstructure Is Controlled by the Type of Adjacent Epithelium. J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 37, 323–339. https://doi.org/10.1002/jbmr.4471

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	100	70	90

[7] Krivanek, J., Lavicky, J., Bouderlique, T., Adameyko, I., 2021. Rapid Isolation of Single Cells from Mouse and Human Teeth. J. Vis. Exp. JoVE.

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	100	90	70