Fluoride Modulations of Ferritin Expression and Impact in Enamel Structure

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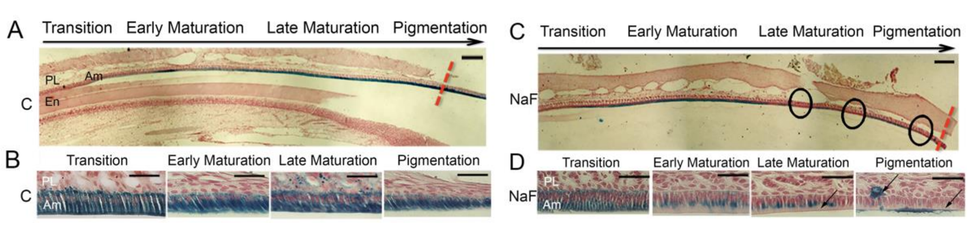
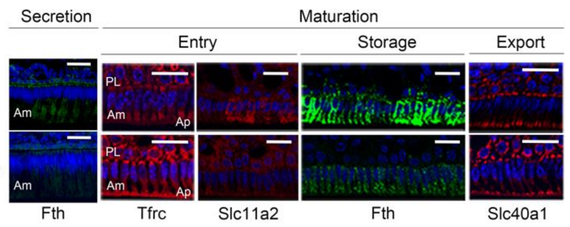
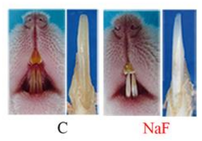
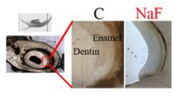
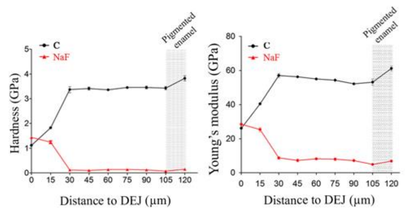
BACKGROUND OF THE STUDY : Enamel defects resulting from environmental conditions and lifestyle are public health concerns because of their high prevalence. We recently published that mice exposed to excessive fluoride present decreased iron storage in Ferritin Heavy Chain (Fth immunofluoresecence and Perl's blue staining) and disturbed iron transportation mRNA and proteins in maturation-stage ameloblasts with direct consequences on enamel structural and mechanical properties *(Houari et al. 2019).*









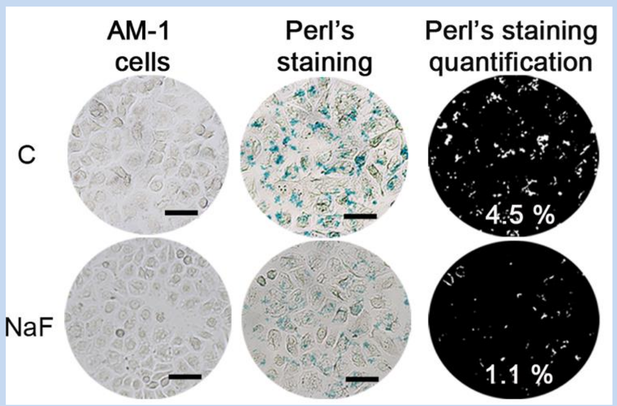


OBJECTIVES : In the continuity of this work, our goal was first to understand fluoride action on iron metabolism in human cells and second to compare structural and mechanical properties of fluorotic and sound human enamel.

MATERIALS & METHODS : Human AM1 ameloblastic cells were treated with increasing concentrations of NaF (0.25 to 1 mM) during 48 hours. Levels of cellular iron were evaluated by Perl's coloration, expression of genes involved in iron metabolism (*FTH, LFT, TFRC, DMT-1, FPN*, hepcidin and *IRE-BP*) were analyzed by RTqPCR and corresponding proteins by Quantified Western Blot and Immunohistochemistry. Oxidative stress was evaluated by measuring reactive oxygen species (ROS). In parallel, nano-hardness and stiffness of human fluorotic enamel were measured using the nano-indentation technique (n=6).

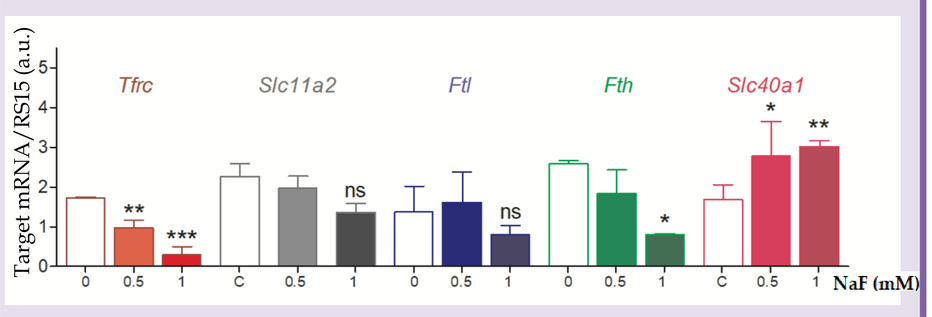
Results

Cellular iron (Fe^{3+}) level and distribution : Perl's blue staining in AM-1 cells



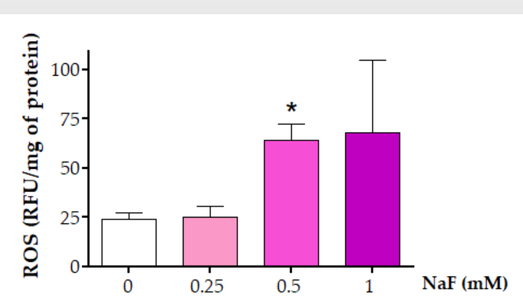
- **Fluoride reduced cellular iron level**

RT Q-PCR QUANTIFICATION OF RNAs EXTRACTED FROM AM-1 CELLS



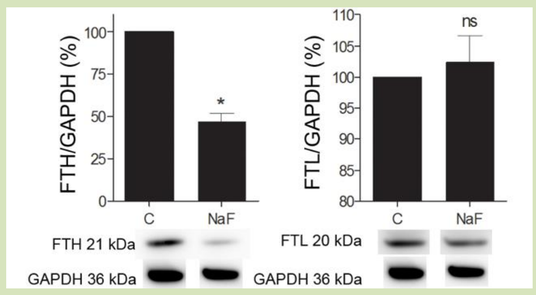
- **All genes involved in iron metabolismthat were investigated here for their expression, were detected in AM1 cells.**
- **Ferritin (Fth) and transferrin receptor 1 (Tfrc) were down-regulated by fluoride in a dose-dependent manner.**

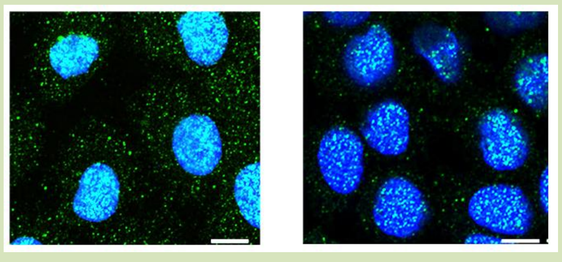
REACTIVE OXYGEN SPECIES ISOLATED FROM AM-1 CELLS MITOCHONDRIAL FRACTIONS



An induction of ROS production suggested the fluoride-induced oxidative stress, thus raising the question of ferritin in this process (current experiments)

WESTERN BLOT & IMMUNOFLUORESCENCE OF FTH PROTEIN (GREEN)

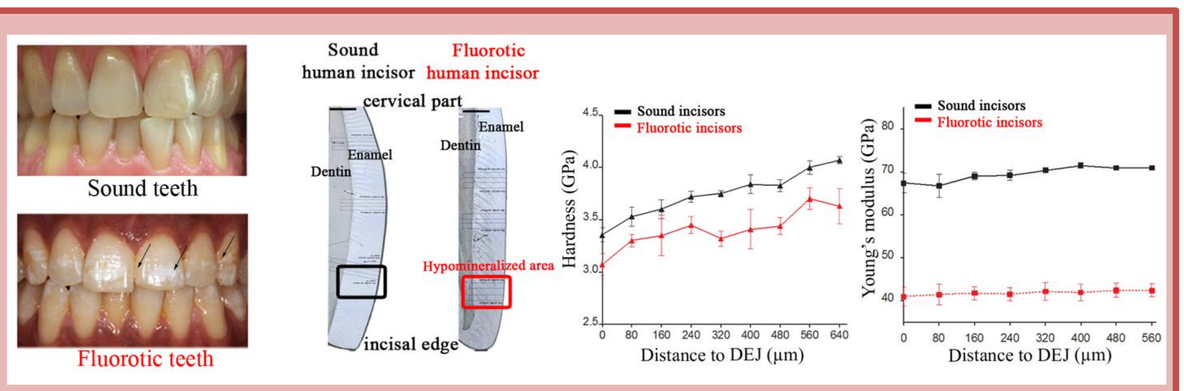




C 1 mM NaF

- **Cell treatment with 1 mM NaF decreased FTH protein expression in AM-1 cells while the level of expression of the FTL protein is invariant**
- **Additional result available concerning a decrease of Slc11a2 following NaF treatment (Fadia's work).**

HUMAN DENTAL FLUOROSIS: MINERAL STRUCTURAL ASPECTS



- **Human enamel structural properties are illustrated by nano-hardness and stiffness.**
- **Young's modulus values are at least 2 times lower in fluorotic enamel than in sound enamel.**

Conclusion

✓ The metabolism of iron may be important to take into account in the mechanism of action of fluoride which contributes to the dental fluorosis pathophysiology.

✓ Biochemical studies involving cellular mechanics and signalization are in course.

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