Why Enamel Cells?

Dental enamel cells offer many benefits as a research model for investigating biomedically important aspects of calcium cellular biology including:

- bulk calcium handling
- avoidance of calcium cytotoxicity
- transepithelial calcium transport
- biomineralization

What dental enamel cells do

- Enamel cells produce tooth enamel, the most highly calcified tissue (40% calcium by weight)
- The principal enamel cell type, termed 'ameloblast', forms a tight epithelial monolayer covering the developing tooth surface
- Accessory epithelial cells separate the ameloblast layer from the adjacent vascularised connective tissue which provides sustenance (and calcium) to the enamel epithelium [see below).

Enamel Epithelium

Histology

The principal enamel cell is termed 'ameloblast'. The ameloblast layer (A) faces developing enamel (E; not visible in this image) and is backed by accessory epithelial cells termed 'stratum intermedium' (SI) and 'stellate reticulum' (SR). Surrounding vascularised loose connective tissue (CT) was removed during microdissection. The dentine (D) core of the tooth underlies the enamel layer and likewise is not visible in this image.

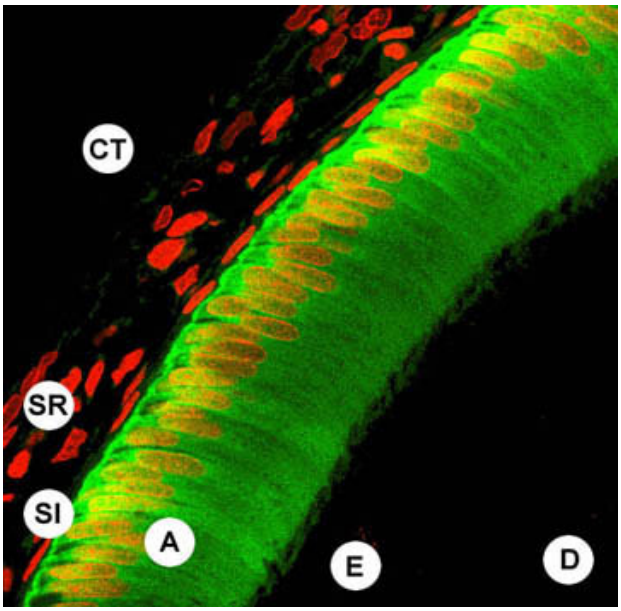

Figure 1: Confocal fluorescence microscopy of enamel cells from developing rat tooth

Dual colour confocal laser scanning microscopy

A cryosection of rat enamel epithelium (secretion phase) was labelled with antibodies to a calcium-binding protein (calbindin-28kDa, digitally coded as green) and with a DNA stain (propidium iodide, coded red). Ameloblasts contain high levels of calbindin in the cytosol (green) and lesser amounts in the nuclei (red/yellow). The accessory cells lack calbindin so only their red nuclei are seen. No calbindin or DNA was detected in the developing enamel and dentine (black).

Credit

Image by Chris Turnbull and Mike Hubbard (see: Sayer, R.J., et al 2000 [PMID: 11131128])

Immunohistochemistry

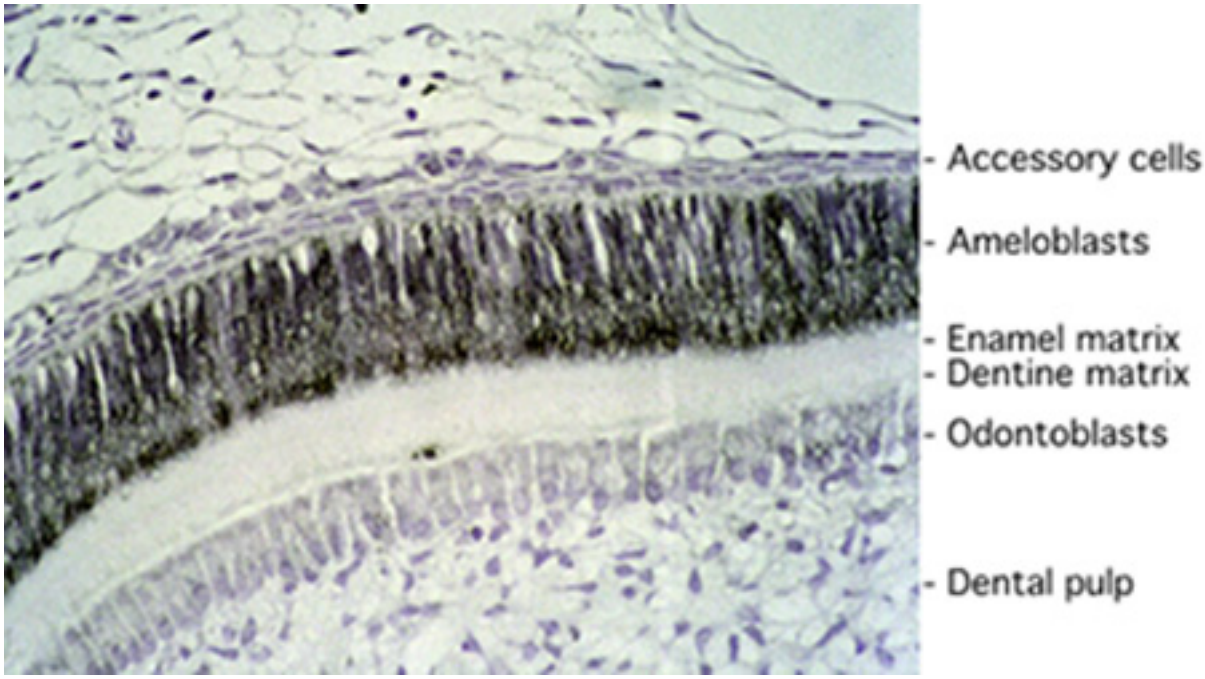

Figure 2: Immunohistochemistry of developing rat tooth

Light microscopy of a paraffin section of developing rat molar labelled with antibodies to ERp29 (an endoplasmic reticulum resident) and counterstained with haematoxylin. The principal enamel cells (ameloblasts) contain high levels of ERp29 (dark brown) compared with the other cell types present. ERp29 is not detected in the developing hard tissues (enamel and dentine matrix).

Credit

Image by Steve Shnyder and Mike Hubbard. (see Shnyder SD and Hubbard MJ 2002 [PMID: 11897809])

Functionally distinct developmental stages

- Enamel is produced in two major stages termed secretion and maturation. First a protein-rich extracellular matrix is secreted on top of dentine to form a template layer of immature enamel. Second the soft enamel matrix is highly calcified and dehydrated to produce steel-hard mature enamel
- Enamel cells undergo distinct developmental phases of cytodifferentiation, matrix secretion, and maturation and then finally disappear by physiological cell death ('apoptosis') before tooth eruption
- Protein production and export is the principal cell function during secretion phase. Calcium transport is the dominant function during maturation but the cells are also involved in deproteinating, dehydrating and neutralising the extracellular enamel matrix
- Calcium has important roles in cell differentiation, protein secretion and cell death besides its central role in enamel mineralization. Accordingly we anticipated that distinct calcium-oriented molecular phenotypes would accompany the major stages of enamel cell development

Elongate cell morphology

- Ameloblasts are elongate and polarised cells with distinct morphologies at each of the major developmental stages
- These features provide an excellent opportunity to elucidate the topography and function of calcium-handling machinery in enamel cells

Calcium-handling proteins are developmentally regulated and hyperabundant

- We have found that enamel cells contain an unusually high abundance of intracellular calcium homeostasis proteins, consistent with their calcium-centric biology. It appears that enamel epithelium could surpass brain as the richest tissue expressing multiple calcium-binding proteins
- Our findings show that distinct 'calcium-binding protein fingerprints' exist for the major developmental stages of enamel formation. Already this has provided several novel insights to structure-function associations
- All calcium-handling proteins characterised in enamel cells are structurally identical to those expressed in other cell types (see ToothPrint database - http://tooth-print.mdhs.unimelb.edu.au/fmi/xsl/toothprint/home.xsl)

Accordingly findings from enamel cells will likely be of broader biological relevance [see review - http://www.ncbi.nlm.nih.gov/pubmed/12016000?dopt=Abstract)

A scarce tissue source

- Human enamel epithelium is not readily available and no fully authentic enamel cell lines have been reported for any species

Rat and mouse enamel formation has been characterised most comprehensively and appears to be a good model for human. However murine enamel epithelium is a very small tissue. We have found that biochemical investigations of rat and mouse enamel cells are feasible subject to use of appropriately high sensitivity and microscale approaches (see review - http://www.ncbi.nlm.nih.gov/pubmed/12016000?dopt=Abstract)