Enamel Biomineralization and Biomimetics

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Outlines

- Biomineralization
- Tooth Enamel; Structure, Properties, and Composition
- Molecular Events During Enamel Biomineralization
- In Vivo and In vitro Strategies to Study Protein Structure and Function
- Enamel Biomimetics
- Repairing Tooth Enamel
Biomineralization

http://www.src.wisc.edu/about/images/SRC_Biominerals.png
Biominerals have hierarchical structures

Silica

CaCO$_3$

Apatite

diatom

diatom

Coccolith

Enamel

Nacre

Power & Syred
Mechanical Properties of biomaterials

- collagen
- bone
- dentin
- enamel
- nacre
- aragonite
- hydroxyapatite

\[ k = \frac{F}{\delta} \]
Tooth Enamel

- Cusp
- Enamel
- Dentin
- Gum
- Pulp
- Cementum
- Periodontal ligament
- Alveolar bone
- Apex
How Enamel is Formed?
A multidisciplinary effort

- Biomineralization Mechanisms in General
- Developing “Biomimetic” Material
- Tooth Development and Genetic Disease, AI
Enamel Biomineralization

- Space delineation
- Secretion of an organic matrix
- Ion transport
- Setting up the saturated solution
- Protein supramolecular organization
- Nucleation of the Mineral
- Oriented and appositional Growth
- Protein degradation and removal
Stages of Enamel Formation

1. Basement Membrane
2. Ameloblasts
3. Enamel Matrix Formation
4. Enamel prism initiation
5. Enamel prism growth
6. Enamel prism mineralization
7. Enamel completion

Hu et al, Cells Tissues Organs, 2007
Nanci (2003) Ten Cate's Oral Histology, Chapter 7, pp. 149-150
Hierarchical Structures of Tooth Enamel


Dieckwisch et al 1995

Enamel mineralized matrix of 7 day postnatal mouse
Space delineation
Ameloblast Membrane Projections on Dentin Surface

Formation of the Enamel-Dentin Junction

Erik Rönnholm (1962) J Ultrastructure Res. 6:249-303
Calcium Hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6\,(\text{OH})_2$

- $\text{Pb}^{2+}$
- $\text{Sr}^{2+}$
- $\text{Na}^+$
- $\text{Mg}^{2+}$
- $\text{K}^+$, $\text{Li}^+$
- $\text{Fe}^{2+}$
- $\text{Mn}^{2+}$
- $\text{Zn}^{2+}$
- $\text{Cu}^{2+}$
- $\text{CO}_3^{2-}$, $\text{HPO}_4^{2-}$
- $\text{F}^-$, $\text{Cl}^-$, $\text{CO}_3^{2-}$
Ca_{10}(PO_4)_{6-x}(CO_3)x (F,OH)_2 Crystals at Different Stages of Enamel Formation

Nanci (2003) Ten Cate's Oral Histology, Chapter 7, pp. 149-150
Enamel Crystals Grow Thicker with Depth

The Extracellular Microenvironment
Proteins are secreted between DEJ and the secretory stage ameloblasts

Diekwisch et al 1995 (Cell & Tissue Research)
Extracellular Matrix Proteins of Enamel

- Amelogenin
- Enamelin
- Ameloblastin
- Amelotin
- Carbonic Anhydrase (pH control)
- Proteinases
  - MMP-20 or Enamelysin
  - KLK-4
Amelogenin Proteins (5-22kDa)

- The main protein component (>95%)
- Rich in Pro, His, Glx, Leu
- Hydrophobic-hydrophilic nature
- Highly conserved primary structure
- Self-assemble to form “nanospheres”
- Sex Chromosome (mostly X)
- Controls crystal growth and organization
- Has Signaling Activity
Enamelin (acidic protein)

- Binds to apatite crystals
- Maps to chromosome 4q21
- Glycosylated and phosphorylated
- It is presumed to play a role in control crystal nucleation and growth
Ameloblastin

- The second most abundant protein after amelogenin
- Human Chromosome 4q21
- Presumed to play a role in determining the prismatic structure
- Expressed by ameloblasts, HRS & pre-odontoblasts
- Processed after secretion
- It is a cell adhesion molecule and it plays a role in maintaining the differentiation stage of secretory stage ameloblasts.
In vivo Strategies to Access Protein Function

Application of Animal Models
Enamel without Amelogenin
Photographic examination of the wild type (Enam+/+; top row), heterozygous (Enam+/−; middle row), and null (Enam−/−; bottom row) mouse dentitions at 7 weeks.
Enamel Without Enamelin

Enamel Without Ameloblastin

Defects in enamel formation of ameloblastin-null mice.

**Amelogenesis Imperfecta**

- **Phenotypes**
  - Hypoplastic (Thickness, thin enamel)
  - Hypocalcified or Hypomaturation (Hardness, poorly mineralized enamel)
  - Combined Types

www.dental.mu.edu/oralpath/spresent/amelogenesis/sld021.htm
Let’s take a break
In Vitro Models to Access Protein Function
In Vivo Source, Pig Mandibular Molars

[Image of pig mandibular molars]

[Image of gel electrophoresis with markers p173, p161, p148]
Recombinant technology

DNA

Extraction

Purification (HPLC)

Cell growth
Organic Matrix Assembly

- Amelogenin Supra-Molecular Self-Assembly
- Protein-Protein interactions
  - Dynamic Light Scattering
  - Atomic Force Microscopy
  - Transmission & Scanning Electron Microscopy
  - High Performance Liquid Chromatography
  - Circular Dichroism Spectroscopy
  - Fluorescence Spectroscopy
Amelogenin is a proline-rich protein

MPLPPHPGHPGYINFSYEVLTPLKWFYQNMRHPYTSYGYEPMPGG
WLHHQIIIPVVSQQTPQSHALQPMVPAQQPGIPQQPMMPLP
GQHSMTPTQHHQPNLPLPAQQPFQPQPQVQPQPHQPPLQPQSPMH
PIQPMLQPPLPPMFSMQSLLPDLPLEAWP<PASSWORD>
Amelogenin self assembles into “nanospheres’, nanochains, nanoribbons

Du et al 2005, Science
Moradian-Oldak J. & Goldberg M. (2005) CTO
Wen HB, Finchem AG, Moradian-Oldak J, Matrix Biology, 2002
Organic Matrix Assembly

Organic Matrix Processing

Mineral Nucleation & Growth
WHY??

Enamel Biomimetic

- the hardest mineralized human tissue
- injury
  - Erosion
  - Caries
- can not self-regenerate
- common drilling
- enamel regeneration in gel-like matrix containing amelogenin
Enamel Defects

- Environmental Defects
  - Caries (tooth decay)
  - Acid Erosion
Developmental Defects

Fluorosis,

*$Amelogenesis Imperfecta*$

1 in 700 people in northern Sweden to 1 in 14,000 people in the United States
Enamel-like HAP synthesis

- 2. Assemble with the aid of AOT isoctane surfactant (Journal of Colloid and Interface Science 2005, 288:97),
- 3. Directly from solution by bis(2-ethylhexyl)sulfosuccinate sodium salt (AOT) (J. Mater. Chem., 2005, 15, 3317)
“Synthetic Enamel”

Organic Matrix
- Composition
- Structure
- Genetic determination

Mineral Phase
- Crystal Nucleation
- Crystal Growth (orientation and morphology)
Controlled remineralization of enamel in the presence of amelogenin and fluoride

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Self-assembly of rP172 and rP148 in ELD

172+NaP
R=20-30nm
UA Stained
172+CaP
R=80-100nm

rP172 in NaP

rP172 in CaP

rP148 + NaP
An amelogenin–chitosan matrix promotes assembly of an enamel-like layer with a dense interface

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Biomimetic Enamel Repair in Amel-Chitosan

(a) [Image of a tooth]

(b) [SEM image of a tooth surface]

CS-AMEL hydrogel
**Why Chitosan?**

*Chitosan* is an amino-polysaccharide obtained by deacetylation of natural chitin.

**Properties:**
- Biocompatible
- Biodegradable
- Mucoadhesive
- Non-toxic
- Bacteriostatic
- pH-sensitive

![Chemical structure of chitosan](image)
1. Tooth slicing and acid-etching

Preparation of CS-AMEL Hydrogel

Application of CS-AMEL Hydrogel

Enamel Regrowth

Artificial Saliva

37°C, 3-7 days

Tooth slice

Chitosan

Ca^{2+}, PO_4

Amelogenin
Enamel remineralization in CS-AMEL hydrogel
Composition of the newly-grown layer

![Graph showing the composition of the newly-grown layer with and without rP172.](image)

**Graph a:**
- Intensity vs. 2θ (degree)
- Peaks at 002, 211, and 004
- Comparison between 'With rP172' (red) and 'No rP172' (blue)

**Graph b:**
- Counts vs. Energy (KeV)
- Peaks at O, P, C, F, Mg, and Ca
Fluorescence labeling
Interface between enamel and regrown crystals

FIB-SEM

HRTEM image taken from the interface between the enamel and regrown crystal, showing seamless growth of repaired crystal on the enamel.
Function of Amelogenin

TEM images of the original hydrogel with (left) and without (right) amelogenin. Amelogenin assemblies stabilized Ca-P clusters in CS-AMEL hydrogel and guided their arrangement into linear chains.
The hardness and elastic modulus of etched enamel were increased by 9 and 4 times after treatment with amelogenin-chitosan hydrogel.
Solution

Biomimetic Enamel Reconstruction

CS-AMEL
Figure 1- Classification of *in vitro* pH-cycling models according to the flux of minerals.
Remineralization Solution:

- 1.5 mM Ca$^{2+}$
- 0.9 mM H$_2$PO$_4^-$
- 130 mM KCl
- 20 mM HEPES
- pH 7.0

Demineralization Solution:

- 1.5 mM Ca$^{2+}$
- 0.9 mM H$_2$PO$_4^-$
- 50 mM Actate Buffer
- pH 4.6

Rinse the samples,
Apply the Hydrogel,
Dry for 15 min

Repeated for 7 days
Repair of caries (Caries_3days)

With hydrogel

7 pH cycles (7days)

18h Re- ----- 5 h De
Can you imagine growing your enamel while sleeping?