Calcium Phosphate Ceramics

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Outline

1. Synthetic bone grafts: concept and applications
2. Why Calcium phosphates?
3. Calcium phosphate sources: natural vs synthetic
4. High-T vs low-T calcium phosphates
5. Porosity in Calcium Phosphates
6. Biological performance of Calcium Phosphates: resorption, bioactivity and osteoinduction
Bone Grafting procedures

2 million bone grafting procedures worldwide every year

≈ 1 million in Europe

Allografts  Autografts  Xenografts

Applications of synthetic bone grafts
Applications of synthetic bone grafts
Applications of synthetic bone grafts

Treatment of bone tumors is one of the clinical fields where bone grafts are required

Maxillofacial reconstruction (cleft lip and palate)
Why Calcium Phosphates?

Composition

Matrix 30%
- Organic 95%
  - Collagen
  - Mucopolysaccharides
  - Non-Collagenous Proteins
- Non-Orgainc 5%

Cells 2%
- Inorganic 70%
  - Calcium
  - Phosphorus

Hydroxyapatite (95%) (Biological apatite)
Why Calcium Phosphates?

- Tropocollagen
  - Collagen fibrils
  - 300 nm
- Hydroxyapatite crystals
- Microfibril
  - 1 nm
  - 70 nm
- Osteon
  - 100 - 500 μm
- Bone
“Form follows function” Louis Sullivan (1896)
<table>
<thead>
<tr>
<th>Bone</th>
<th>Dental Enamel</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Mechanical: support and protection – strength and toughness</td>
<td>✓ Mechanical: to tear and chew food - hardness, resistance to abrasion</td>
</tr>
<tr>
<td>✓ Metabolic: Ion reservoir / Protein and growth factor regulation</td>
<td>✓ Resistance to acidic dissolution</td>
</tr>
<tr>
<td>✓ Autorepair</td>
<td>✓ Partial re-mineralization</td>
</tr>
</tbody>
</table>
Conventional Calcium Phosphates

Calcium Phosphates:
- Hydroxyapatite
- β-Tricalcium Phosphate
- Biphasic Calcium Phosphates (HA/β-TCP)
Shapes and Forms

- Granules (0.1-5 mm)
- Block
- Cement
- Putty
- Coating

Origin of commercial CaP

**Natural**
- Bovine bone
- Coral

**Synthetic**
- High temperature
- Low temperature
CaP of natural origin

**Coralline Hydroxyapatite**

- Hydrothermal conversion (260 °C, 15,000 psi) of coral (mostly CaCO₃, calcite form) in the presence of ammonium phosphate to hydroxyapatite (*Interpore™ and Pro-Osteon™ manufactured by Interpore International, Inc, Irvine, CA)*

\[
4\text{CaCO}_3 + 6(\text{NH}_4)_2\text{HPO}_4 \rightarrow \text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 + \text{H}_2\text{O} + \text{CO}_2
\]

- F, Sr, and CO₃ present in the coral become incorporated in the resulting hydroxyapatite. Other ions (Mg) become incorporated in the minor β-TCP component that forms after hydrothermal conversion.

CaP of natural origin

Bovine-bone Derived Apatites

2 types, depending on the method of preparation:

(1) unsintered (Bio-Oss™, by Geistlich Biomaterials, Geistlich, Switzerland)
(3) sintered (Osteograf-N™ by CeraMed Co Denver, CO and Endobon™ by Merck Co, Darmstadt, Germany).

• The unsintered bone mineral consists of small crystals of bone apatite (carbonatehydroxyapatite) and other trace elements originally present in bone, whereas the sintered bone mineral consists of much larger apatite crystals without CO$_3$ when sintered above 1000 °C.

• Safety concerns after mad cow disease (Creutzfeldt–Jakob disease): bovine-derived graft biomaterials may carry a risk of prion transmission to patients.

Synthetic CaP

High Temperature
Sintered ceramics
Coatings (plasma spray)

Low Temperature: Biomimetic CaPs
Calcium phosphate cements
Biomimetic coatings
CaP Nanoparticles
High Temperature CaPs: Sintered ceramics

- Precursor powders
- Mixing
- Compactation
- Furnace

- High-T Sintered ceramic
- Hydroxyapatite 1050-1300 °C
- β-TCP 1000-1100 °C
High Temperature CaPs: Sintered ceramics

[Diagram showing the process of sintering with precursor powders, mixing, compactation, and a furnace leading to hydroxyapatite 1100-1300 °C and β-TCP 1000-1100 °C.

Sintering process depicted with powder particles and pores.]
High Temperature CaPs: Sintered ceramics

High T CaP ceramics vs low T biomimetic CaP

**High Temperature**
- Sintered ceramics
- Coatings (plasma spray)

**Low Temperature: Biomimetic CaPs**
- Calcium phosphate cements
- Biomimetic coatings
- CaP Nanoparticles
Low T setting reaction

- Powder
- Liquid
- Plastic paste
- Setting
- Rigid paste
- Hardening
- Solid body
- Dissolution
  + Precipitation at physiological temperature
Micro & nanoporosity

a) Particle Size

- Fine Particles
- Coarse Particles

Needle-like crystals
Plate-like crystals

b) Liquid to Powder ratio

- Low L/P
- High L/P

Low inter-aggregate distance
High inter-aggregate distance

Fixed L/P=0.65mL/g

Entrance pore size (µm)

Fixed particle size: Fine

Entrance pore size (µm)

High T CaP ceramics vs low T biomimetic CaP

Sintered CaP ceramic

CaPs obtained by a low-temperature self-setting reaction
The role of porosity

**Micro/nanoporosity:**
- Increased bioactivity
- Effect on protein adsorption, and cell adhesion/proliferation
- Permeability: nutrient and cell metabolic waste substances diffusion.

**Macroporosity:** cell/tissue colonisation.
- migration, proliferation and differentiation of osteoblast progenitor cells.
- angiogenesis.

Size > **decens/hundreds μm**

*Need of interconnected porosity*
Macroporous CaPs: Injectable Calcium phosphate foams

\[ 3 \alpha\text{-Ca}_3(\text{PO}_4)_2 + \text{H}_2\text{O} \rightarrow \text{Ca}_9(\text{HPO}_4)(\text{PO}_4)_5(\text{OH}) \]

Macroporous CaPs: Injectable Calcium phosphate foams

Macroporous CaPs: 3D printing strategies

Ceramic ink
- Non-reactive suspension: Printing + Sintering
- Suspension of reactive ceramic particles → Self-setting scaffolds

Tomsia et al (2007)
Self-setting Ceramic Ink: Gelatin-αTCP

- Gelatine gellation: initial consolidation of the scaffold
- Subsequently: αTCP hydrolysis to Hydroxyapatite

Biological performance of CaP

- Resorption
- Bioactivity / Osteoconduction
- Osteoinduction
# Biological performance of CaP: Resorption

<table>
<thead>
<tr>
<th>Ca/P ionic ratio</th>
<th>Compound</th>
<th>Chemical formula</th>
<th>Solubility at 25 °C, (-\log(K_s))</th>
<th>Solubility at 25 °C, g/L</th>
<th>pH stability range in aqueous solutions at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Monocalcium phosphate monohydrate (MCPM)</td>
<td>Ca(H_2PO_4)_2H_2O</td>
<td>1.14</td>
<td>~ 18</td>
<td>0.0 – 2.0</td>
</tr>
<tr>
<td>0.5</td>
<td>Monocalcium phosphate anhydrous (MCPA)</td>
<td>Ca(H_2PO_4)_2</td>
<td>1.14</td>
<td>~ 17</td>
<td>[1]</td>
</tr>
<tr>
<td>1.0</td>
<td>Dicalcium phosphate dihydrate (DCPD), mineral brushite</td>
<td>CaHPO_4·2H_2O</td>
<td>6.59</td>
<td>~ 0.088</td>
<td>2.0 – 6.0</td>
</tr>
<tr>
<td>1.0</td>
<td>Dicalcium phosphate anhydrous (DCPA), mineral monetite</td>
<td>CaHPO_4</td>
<td>6.90</td>
<td>~ 0.048</td>
<td>[1]</td>
</tr>
<tr>
<td>1.33</td>
<td>Octocalcium phosphate (OCP)</td>
<td>Ca(HPO_4)_2(PO_4)_2·5H_2O</td>
<td>96.6</td>
<td>~ 0.0081</td>
<td>5.5 – 7.0</td>
</tr>
<tr>
<td>1.5</td>
<td>α-Tricalcium phosphate (α-TCP)</td>
<td>α-Ca_3(PO_4)_2</td>
<td>25.5</td>
<td>~ 0.0025</td>
<td>[1]</td>
</tr>
<tr>
<td>1.5</td>
<td>β-Tricalcium phosphate (β-TCP)</td>
<td>β-Ca_3(PO_4)_2</td>
<td>28.9</td>
<td>~ 0.0005</td>
<td>[1]</td>
</tr>
<tr>
<td>1.2 – 2.2</td>
<td>Amorphous calcium phosphate (ACP)</td>
<td>CaH_2(PO_4)_3·nH_2O, n = 3 – 4.5, 15 – 20% H_2O</td>
<td>[8]</td>
<td>[8]</td>
<td>~ 5 – 12 [13]</td>
</tr>
<tr>
<td>1.5 – 1.67</td>
<td>Calcium-deficient hydroxyapatite (CDHA)</td>
<td>Ca_{10-x}(HPO_4)<em>2(PO_4)</em>{25-x}(OH)_{25-x} (0&lt;x&lt;1)</td>
<td>~ 85.1</td>
<td>~ 0.0094</td>
<td>6.5 – 9.5</td>
</tr>
<tr>
<td>1.67</td>
<td>Hydroxyapatite (HA)</td>
<td>Ca_{10}(PO_4)_2(OH)_2</td>
<td>116.8</td>
<td>~ 0.0003</td>
<td>9.5 – 12</td>
</tr>
<tr>
<td>1.67</td>
<td>Fluorapatite (FA)</td>
<td>Ca_{10}(PO_4)_2F_2</td>
<td>120.0</td>
<td>~ 0.0002</td>
<td>7 – 12</td>
</tr>
<tr>
<td>2.0</td>
<td>Tetracalcium phosphate (TTCP), mineral higgenstockite</td>
<td>Ca_4(PO_4)_3O</td>
<td>38 – 44</td>
<td>~ 0.0007</td>
<td>[14]</td>
</tr>
</tbody>
</table>
**Chemical dissolution**
- Plaster of Paris (CSH)
- Gypsum (CSD)
- Dicalcium phosphate dihydrate (DCPD)

**Cell-mediated**
- osteoclasts, macrophages...
- Precipitated Hydroxyapatite *
- β-Tricalcium phosphate
- Biphasic calcium phosphates
Biological performance of CaP: Resorption

**Passive resorption**
- Chemical dissolution
  - Plaster of Paris (CSH), Gypsum (CSD), Dicalcium phosphate dihydrate (DCPD)

**Active resorption**
- Cell-mediated
  - Osteoclasts, macrophages...
- *Precipitated Hydroxyapatite*
  - β-Tricalcium phosphate
  - Biphasic calcium phosphates

**Reactivity:**
- ✓ Stoichiometry
- ✓ Cristallinity
- ✓ Specific surface area
- ✓ Porosity

- Sintered HA
  - Non resorbable
- HA ➔ Biological HA
  - Resorbable
Biological performance of CaP: Bioactivity

1. Surgeon implants biomaterial
2. The biomaterial adsorbs a layer of proteins
3. Cells (neutrophils and macrophages) interrogate the biomaterial
4. Cells fuse to form giant cells and secrete protein signaling agents (cytokines)
5. In response to the cytokines, fibroblasts arrive and begin synthesizing collagen
6. The biomaterial is encapsulated in an acellular, collagenous bag

Biological performance of CaP: Bioactivity

- **Bioceramics**
  - **Bioinert**
    - Alumina
    - Zirconia
  - **Bioactive**
    - Bioactive glasses and glass ceramics
    - Calcium phosphates

+/− biodegradable
Biological performance of CaP: Bioactivity

Biological performance of CaP: Osteoinduction

La osteoinduction: differentiation of mesenchymal stem cells (pluripotent) to the osteoblastic phenotype.
Two strategies to trigger osteoinduction:

1. Incorporation of bone morphogenetic proteins (BMPs) in the biomaterials.

2. Designing intrinsically osteoinductive biomaterials
Biological performance of CaP: Osteoinduction

Summary

• CaP have unique properties as synthetic bone grafts

• CaP can be obtained either from natural sources or by synthetic processes either at high-T or low-T

• The biological performance of CaP (resorption, bioactivity and osteoinduction) depends not only on their composition but also on their porosity and textural properties. Their control can allow actively directing the material-cell/tissue interaction.

• CaP processing is compatible with promising strategies for the fabrication of macroporous CaP scaffolds for tissue engineering and bone regeneration.
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