Charakterisierung von organisch-anorganischen Kompositen auf der sub-µm Skala

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Research topics

Influence of molecular structure, thermal and mechanical previous history in the phase formation in the melt and in solid state

Physical-chemical characterization on the sub-µm scale

→ Optimization of mechanical properties due to variation in constructional morphology and chemical composition

Hierarchical structure formation in biological materials
Introduction

• Development of new polymer based composites requires improved knowledge in the structure-properties relationship

• Biological materials
  • Reveal much better mechanical properties than man made composites
  • Optimized over long period of time assigned to specific use
  • Unique combination of organic and inorganic components
  • Special conditions for material synthesis
  • Hierarchical structures on different levels
Introduction

• Exoskeleton of crustaceans
  • The cuticle covers the whole body to provide stability, protection and motility
  • Internal variations necessary to achieve the specific functions

• Isopods
  • well adapted to their habitat and their life style
  • broad variety of sizes

⇒ All crustacean cuticles are a mineralized chitin composites with similar assembly of the organic matrix!
Hierarchical structure of the chitin matrix of crustacean cuticle

Chitin crystal

Crystalline nanofibrils coated with protein

Chitin-protein plane

Chitin-protein layer

Chitin-protein fiber

Minerals reinforce the organic matrix!!

⇒ Minerals reinforce the organic matrix!!
Calcium carbonate

• Appearing in different modifications

  ⇒ Calcite
  • Rhombohedral crystal lattice
  • Biogenic calcite
    ⇒ Micro crystalline morphology

  ⇒ Amorphous CaCO₃ (ACC)
  • High solubility (10 - 30 times >> calcite)
  • Crystalline precursor
    • precipitation from oversaturated alkaline solutions in spherical aggregates with Ø ≈ 25 nm
    ⇒ recrystallizes to calcite
  • Transient calcium reservoir
    • stabilized by magnesium, phosphate and specific proteins or macromolecules
The terrestrial isopod *Porcellio scaber*

**Antrittsvorlesung, Linz, 12. 04. 2010**

- **Living space:** Humid environment
  - Below stones, coppice, garden, compost pile

- **The terrestrial isopod Porcellio scaber**
  - *F. Neues, M. Epple, University Duisburg-Essen*

- **Synchrotron-radiation μCT**
  - *F. Neues, M. Epple, University Duisburg-Essen*

- **Mineral Composition**
  - **Water:** 67%
  - **Organic:** 25%
  - **Calcite:** 15%
  - **Calcium Carbonate (CaCO₃):** 11%
  - **HAP:** 6%
  - **Unknown:** 8%

- **Tergite cuticle**

- **compost pile**
Cuticle of *Porcellio scaber*: SEM analysis *

* S. Seidel, A. Ziegler, University Ulm
SFM investigation of the microtom polished cuticle

Outside

Sample

Exocuticle

Endocuticle

Membranous layer

$Z_{\text{max}} = 200 \text{ nm}$

$Z_{\text{max}} = 20 \text{ nm}$

$Z_{\text{max}} = 30 \text{ nm}$

$Z_{\text{max}} = 165 \text{ nm}$

Antrittsvorlesung, Linz, 12. 04. 2010
Testing the local mechanical properties using the SFM

Are the structural differences in the distal exocuticle and the endocuticle sufficient to explain the variation in local stiffness?
Chemical characterization by Raman spectroscopy

Organic compounds

Chitin
- CH stretching: 2800 – 3020 cm\(^{-1}\)
- Amide: 1550, 1625 cm\(^{-1}\)
- Amide vibrations: 3275 cm\(^{-1}\)

Protein (BSA)
- CH stretching: 2800 – 3020 cm\(^{-1}\)
- Amide: 1550, 1625 cm\(^{-1}\)

Inorganic compounds

Calcite
- CaCO\(_3\) stretching: 1087 cm\(^{-1}\)
- Lattice vibrations: 154 cm\(^{-1}\)
- H\(_2\)O stretching: 3450 cm\(^{-1}\)

ACC*
- CaCO\(_3\) stretching: 1080 cm\(^{-1}\)
- Lattice vibrations: 711 cm\(^{-1}\)
- H\(_2\)O stretching: 2850 – 3850 cm\(^{-1}\)

Raman spectral imaging in confocal mode

Synthetic coccolith

⇒ Scanning spectra
⇒ Single spectra
• 0.25 s integration time
• 10 spectra per time
⇒ about 20000 single spectra

CaCO$_3$ stretching $1087 \text{ cm}^{-1}$

Counts / a.u.

CaCO$_3$ lattice vibration $280 \text{ cm}^{-1}$

CaCO$_3$ bending $712 \text{ cm}^{-1}$

⇒ Film thickness $\sim 400 \text{ nm}$
Calcite is found only close to the outer side.

ACC in combination with higher amount of organic matrix is located in the middle.

At the inside of the cuticle a chitin/protein layer appears.
Raman imaging of the tergite cuticle of *P. scaber*

$$\Rightarrow$$ At the inside of the cuticle a chitin layer appears

$$\Rightarrow$$ At the outer side a calcite layer is formed

$$\Rightarrow$$ ACC in combination with higher amount of organic matrix is located in the middle
Correlation between chemical and mechanical properties

**Graph:**
- Force vs. Indentation
- Raman spectra for Calcite, Calcite + ACC, and ACC

**Images:**
- AFM images of Calcite and Calcite + ACC at 1 μm scale
Raman spectra at different crystalline orientations

† Intensity of the lattice vibrations (280 cm\(^{-1}\)) depends on the crystal orientation

\[
\frac{h_{1087}}{h_{712}} = 15.3 \\
\frac{h_{1087}}{h_{280}} = 3.9 \\
\frac{h_{1087}}{h_{712}} = 15.2 \\
\frac{h_{1087}}{h_{280}} = 1.6
\]
Determine crystalline orientation within the exocuticle

Tangential cut cuticle

⇒ Raman images taken from depth scan of the tangential cut
Determine crystalline orientation within the exocuticle

- Average spectra of calcite region recorded from sagital cut

- Average spectra of calcite region recorded from depth scan of a tangential cut

⇒ Microcrystalline calcite has a preferred orientation
Biphasic molt of *Porcellio scaber*

- When the crustaceans grow, the cuticle is periodically replaced
  ⇒ Molting: Cuticle is periodically decalcified and shed
  ⇒ Terrestrial isopods developed sophisticated strategies for mineral recycling

- Demineralization of the old cuticle
  - Premolt (~ 7 days)

- Remineralization of the new cuticle
  - Intramolt (24 hours!)
To quantify the change in mineral content and the ACC/calcite ration within the molting cycle Xray in combination with elementary analysis has been used.

⇒ No information about absolute content of different components!
Changes in CaCO$_3$ distribution during natural decalcification

- Total thickness of the cuticle decreases
- The thickness of the calcite layer is nearly unchanged
  ⇒ The amount of ACC is reduced due to resorption
- Preferably calcite is given away in natural decalcification
Changes in CaCO$_3$ distribution during re-mineralization

- The calcite layer is immediately reformed but with reduced thickness
  - Protective outer layer which has to be quickly re-established
  - Recrystallization of ACC
- Total thickness of the cuticle quickly increases
- With further increase of the cuticle thickness only the ACC layer extends
- After one week the cuticle is nearly rebuild
Quantitative analysis of chemical composition

The amount of calcite in the exocuticle is nearly unchanged within the molting cycle
⇒ Protective outer layer which is quickly re-established

The amount of ACC in the exocuticle is varies within the molting cycle
⇒ Indicate resorptioin and recrystallization
CaCO$_3$ distribution within the cuticle of *Tylos europeus*

- Living space: Beach
  - Digged into the sand
  - Different crystalline domains
CaCO$_3$ orientation within the cuticle of *Tylos europeus*

$\Rightarrow$ Crystalline orientation within the cuticle changes
• SEM and SFM imaging reveals three different layers within the cuticle the terrestrial isopod *P. scaber*

• Raman microscopy allows the discrimination and localization of different compounds on the sub µm-scale

• The different layers reveal different material properties
  ⇒ Variation in mineralization cause differences mechanical behavior
  ⇒ Microcrystalline calcite shows preferred orientation although homogeneously distributed

• Monitoring the changes in mineral distribution during the molting cycle
  ⇒ Calcite forms the protective outer layer, which is immediately re-established after molting
  ⇒ ACC is located inside to improve the mechanical stability and for quick recycling during the molt

• Mineral distribution and microcrystalline structure are optimized for the living conditions of isopods
  ⇒ Variation in the crystalline orientation in the exocuticle of *Tylos europeus*
Acknowledgments

• Deutsche Forschungsgesellschaft DFG
  • SSP 1420 “Functionality by Hierarchical Structuring of Materials”

• A. Ziegler, B. Seidel, P. Walther
  • Central Facility of Electron Microscopy, University of Ulm

• MPI für Eisenforschung
  • D. Raabe, H. Fabritius, T. Fischer

• F. Neues, M. Epple
  • Inorganic Chemistry, University of Essen

• O. Marti
  • Experimental Physics, University of Ulm

• U. Schmidt, W. Ibach, J. Müller
  • WiTEC GmbH, Ulm

• Institute of Polymer Science

• Colleagues at JKU

• Collaborators from Industry