Article Homework

Bioinspired Functionally Graded Composite Assembled Using Cellulose Nanocrystals and Genetically Engineered Proteins with Controlled Biomineralization

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Outline

Introduction

& Aim

Methods

Future
Aspects

Conclusion

Introduction & Aim

UUTISET

NEWS | HANNA'S CRISIS

"Järkeni ei riitä ymmärtämään"

Hanna during Wappu-week while doing this assignment: "I don't have the brains to understand".

According to Hanna Dahl this assignment should have been done in another week. WTF / HELP

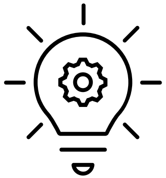
RESEARC

Bioin
Usin
Prot

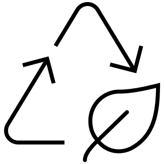
Pezh
Ali

Background

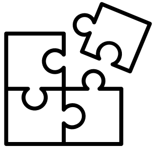
- **Bioinspiration**: Nature is the best engineer
- Multiphase bio-composites vs. traditional materials



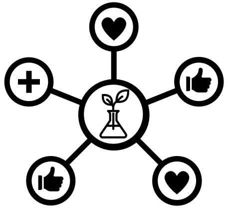
Improved mechanical properties



Sustainability



Customization



Versatility

Tougher and lighter dental implant crowns can be made of cellulose-based nanocomposites

News, Press release 02.09.2021 07:45 EEST

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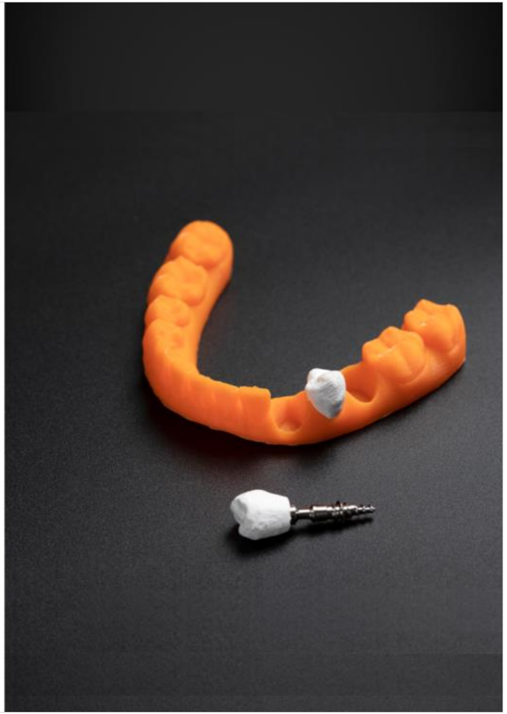

Tougher and lighter dental implant crowns can be made of cellulose-based nanocomposites

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Methods
& Approaches

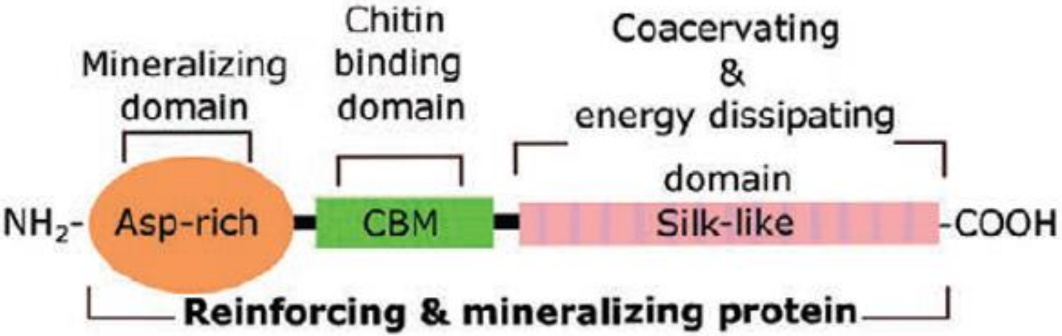
Inspiration from Nature

Peacock mantis shrimp club

- Exceptional structure
- Composition:
  1. Organized chitin fibrils
  2. Protein matrix
  3. $Ca_3(PO_4)_2$ & $CaCO_3$

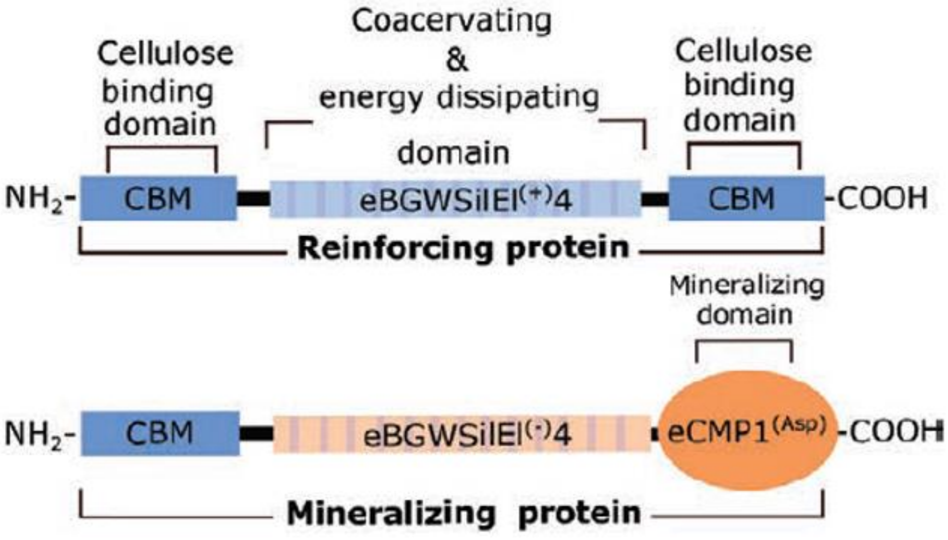
Club Mineralization Protein-1 (CMP-1)

- The main protein in the protein matrix
- Three block structure:
    - Asp-rich
    - CBM
    - Silk-like



Selection of Building-Block Components

- **Cellulose nanocrystals (CNC)**
- Matrix was mimicked by two types of proteins:
    1. Reinforcing protein
    2. Mineralizing protein
- Synthesized in *E. coli*



Reinforced protein samples

RP 1-4 proteins with different AA arrangement and composition in the middle block

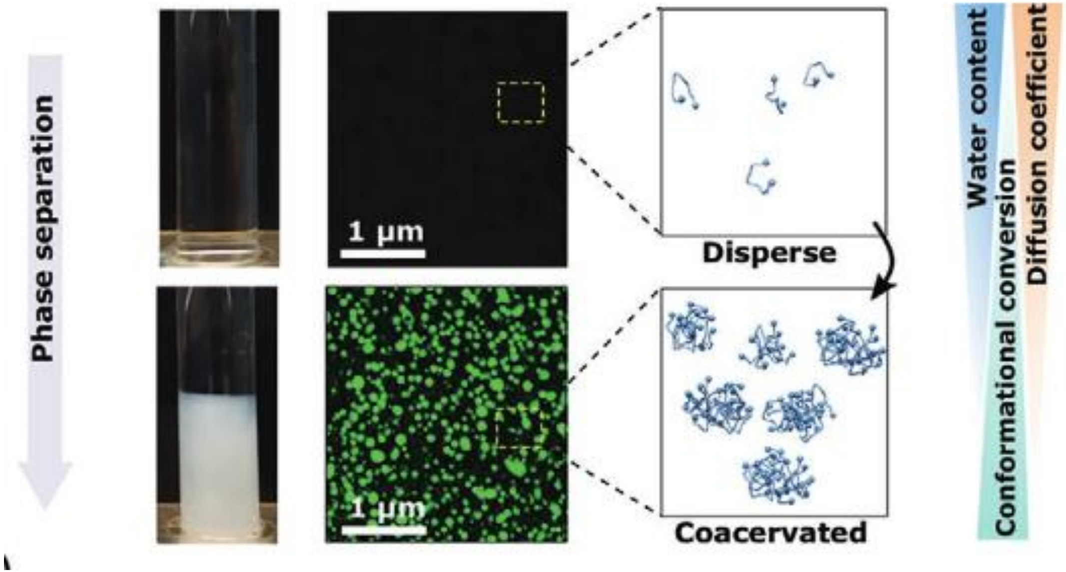
- **RP1**- six repeats of motif 1 of the *Eumeta variegate* silk fibroin (BGW6)
- **RP2** - 80 repeats of the elastin-like polypeptide (ELP)_$(VPGVG)_{80}$
- **RP3-** four repeats of the tandem repeat $((A)_{25}K(VPGVG)_{15}GD)_4$,
- **RP4** - four-motif repeating sequence $((YKYKYKY(VPGVG)_5)_3GD(A)_9\ K(A)_{12}\ KSVVYV)_{4,}$ combination of RP1+RP3 parts with lysine- (Lys) and Tyrosine-rich (Tyr) stretch (YKYKYKY) to enhance binding interactions with negatively charged CNCs through electrostatic interactions.

Nanocomposite Assembly of Impact Interior Region of the Crown

2D (films) → 3D (bulk material)

Mix of CNC+RP (reinfrorcing protein)

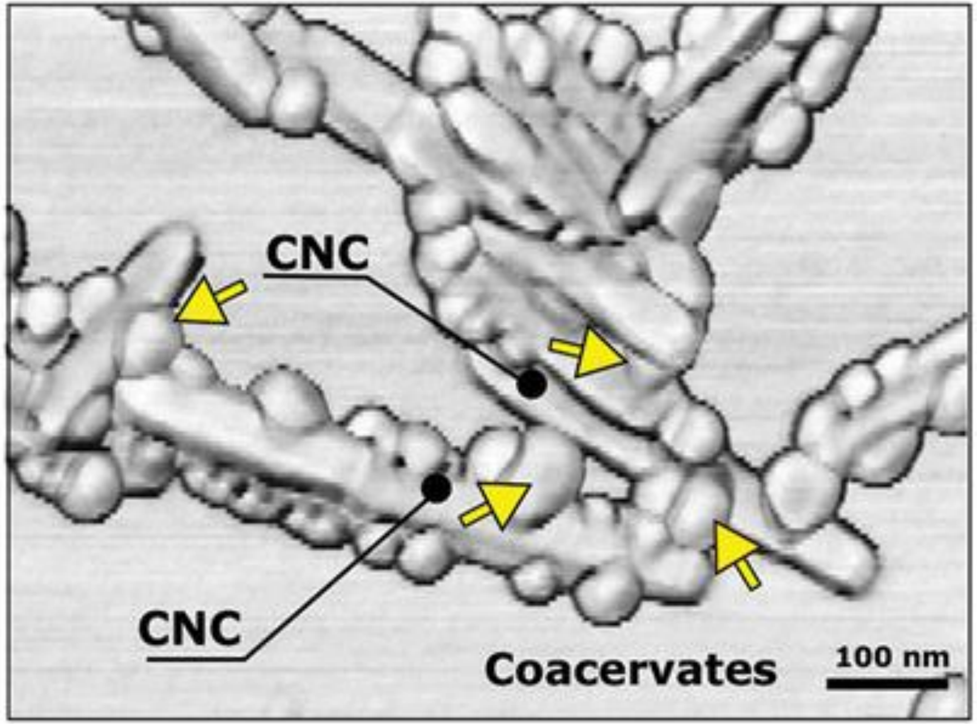
- RP mixed with buffer – LLPS - dense coaservate phase
- CNC 3% (w/v) + RP 10% mix
- Concentrated to CNC 6%
- Dried to assemble nanocomposite films



Picture. Phase separation of RP in buffer

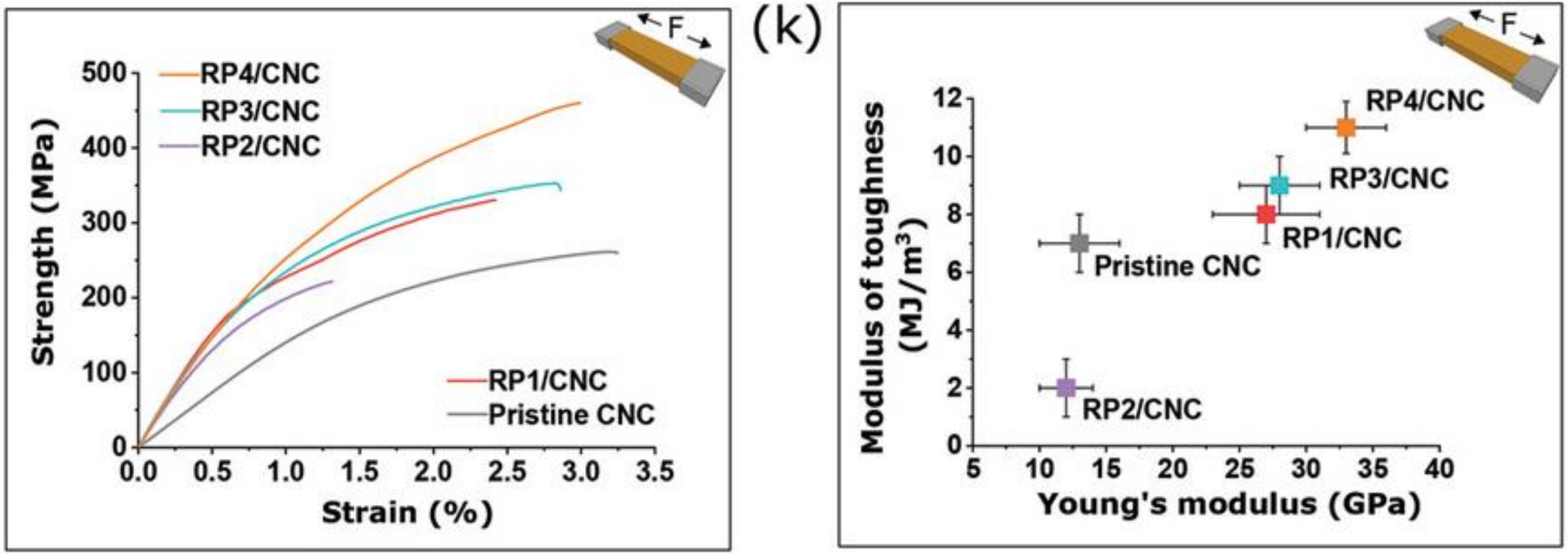
Tests

Binding of RP to CNC structure



Picture. AFM image, binding of protein coacervates to CNC. Arrows indicate bound RP

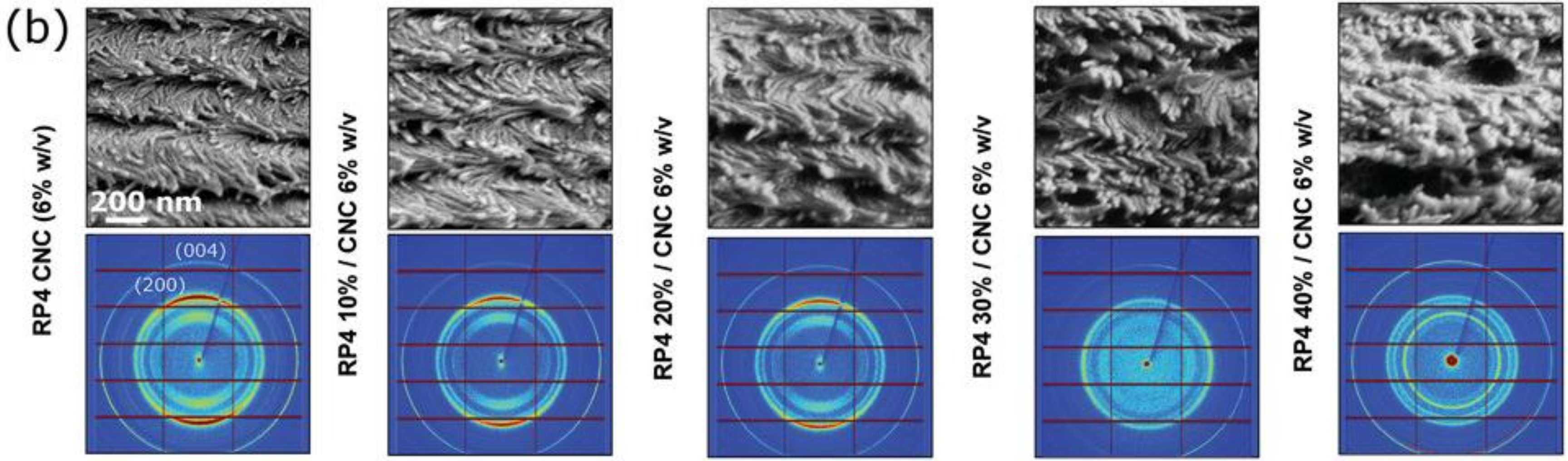
Tensile measurements



Graphs. The tensile measurements of samples RP 1-4, Pristine CNC as a control

Test of different ratios of RP to CNC

4%RP is the optimum



Picture. High resolution SEM imaging (top pictures) of films with different mixing ratios

2D film measurements' results

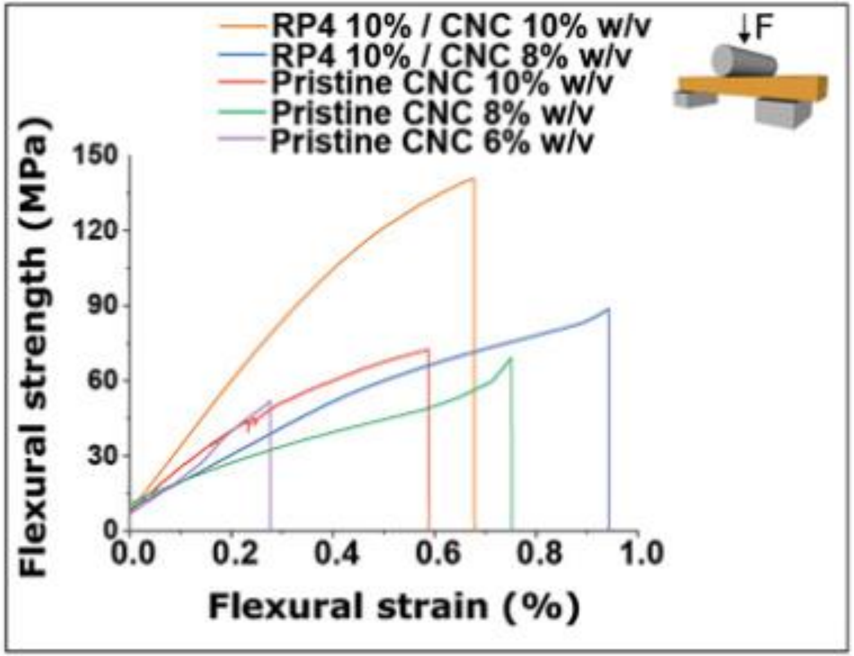
All except RP2 showed increased strengths, stiffness and toughness.

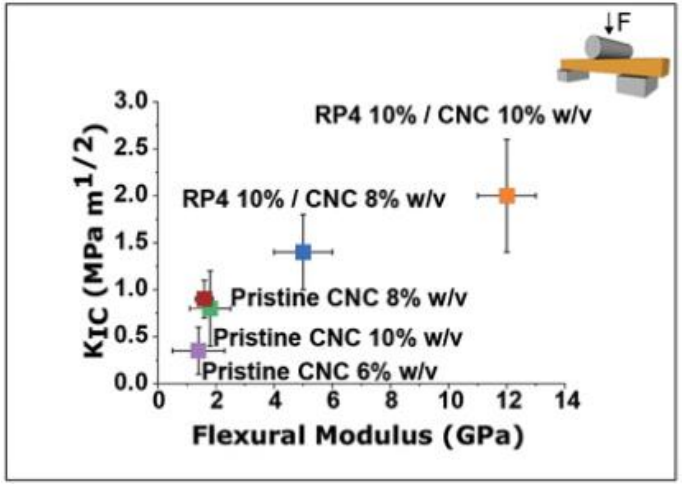
- Alanine rich sequence → conformational conversion from random coil/α-helical to β- sheets → better protein-protein interaction → more efficient load distribution
- RP4 showed best results with highest stiffness, strength and modulus of toughness thanks to Lysine rich stretch that forms ionic interaction with CNC
- RP4 effectively absorbed to the CNC

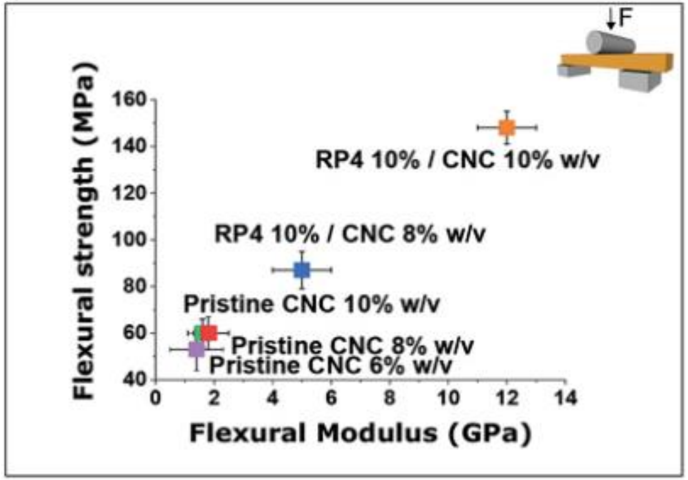
RP4 was chosen for 3D material tests

3D bulk material test

High flexural strength and high modulus in
RP4 10%/CNC 10%







Graphs. Flexural strength measurements

Nanocomposite Assembly of a Highly Mineralized Stiff and Hard Exterior region of the Crown

- mineral formation by highly ordered 3D fibrillar frameworks and intrinsically disordered proteins
- CMP-1 for nucleation and growth of apatite crystals
- CMP-1 undergoes LLPS which is a a key step in bio fabrication
- MP1 explored if it could induce biomimetic apatite mineralization on the surface of the organized RP4/CNC 3D framework

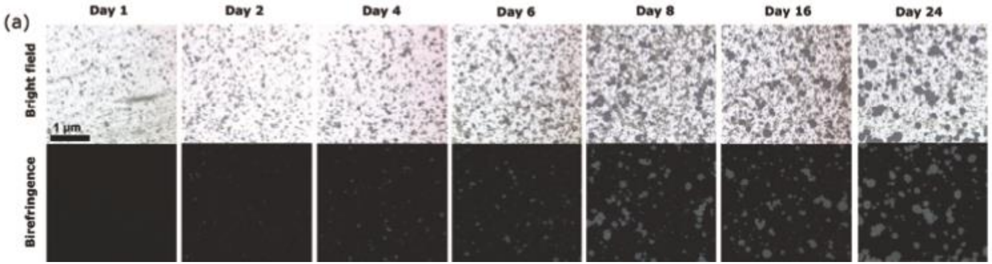


Figure. LLPS of MP1 and apatite biomineralization for 30 days

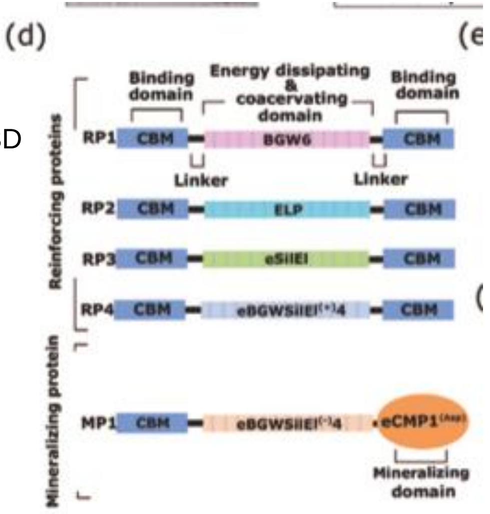


Figure. Representation of RP1-RP4 and MP1.

- Polarized light microscopy, technique in which changes in birefringence and crystallinity are directly correlated
- Biomineralization in the form of granule-shaped precipitates
- A gradual increase in the size which had a direct correlation with the incubation time

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ATR-FTIR, CP/MAS Spectrum of mineralized specimens

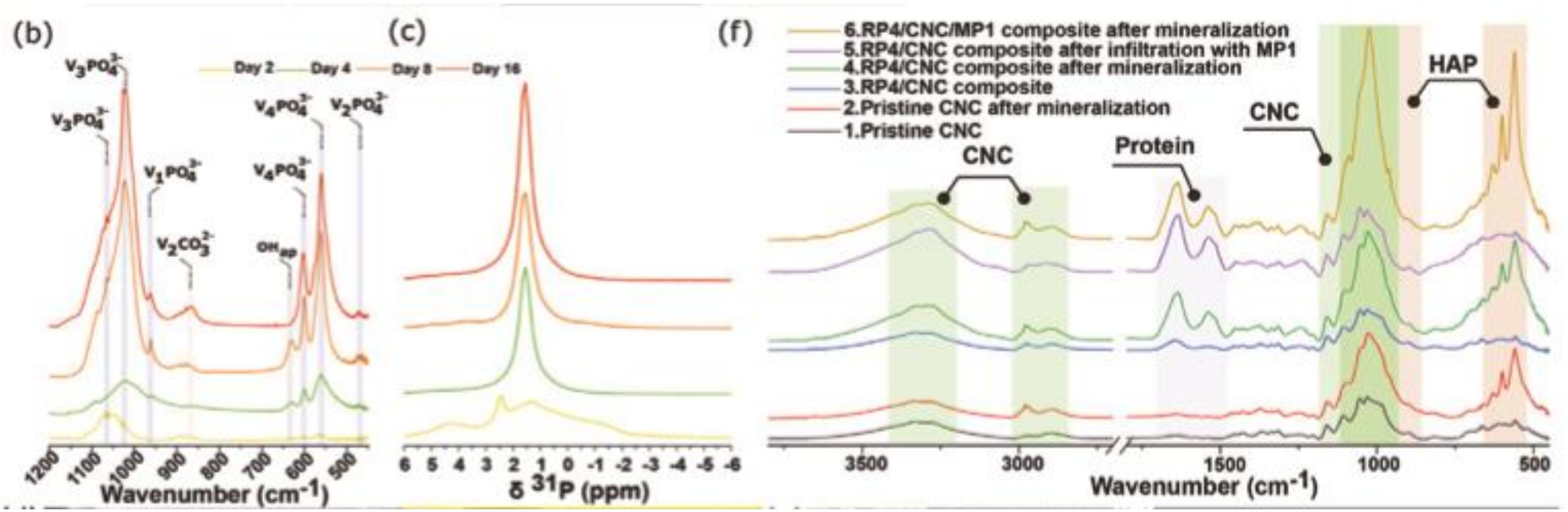


Figure. b) ATR-FTIR spectrum, c) Solid-state CP/MAS spectrum f) After three-point flexural bending testing

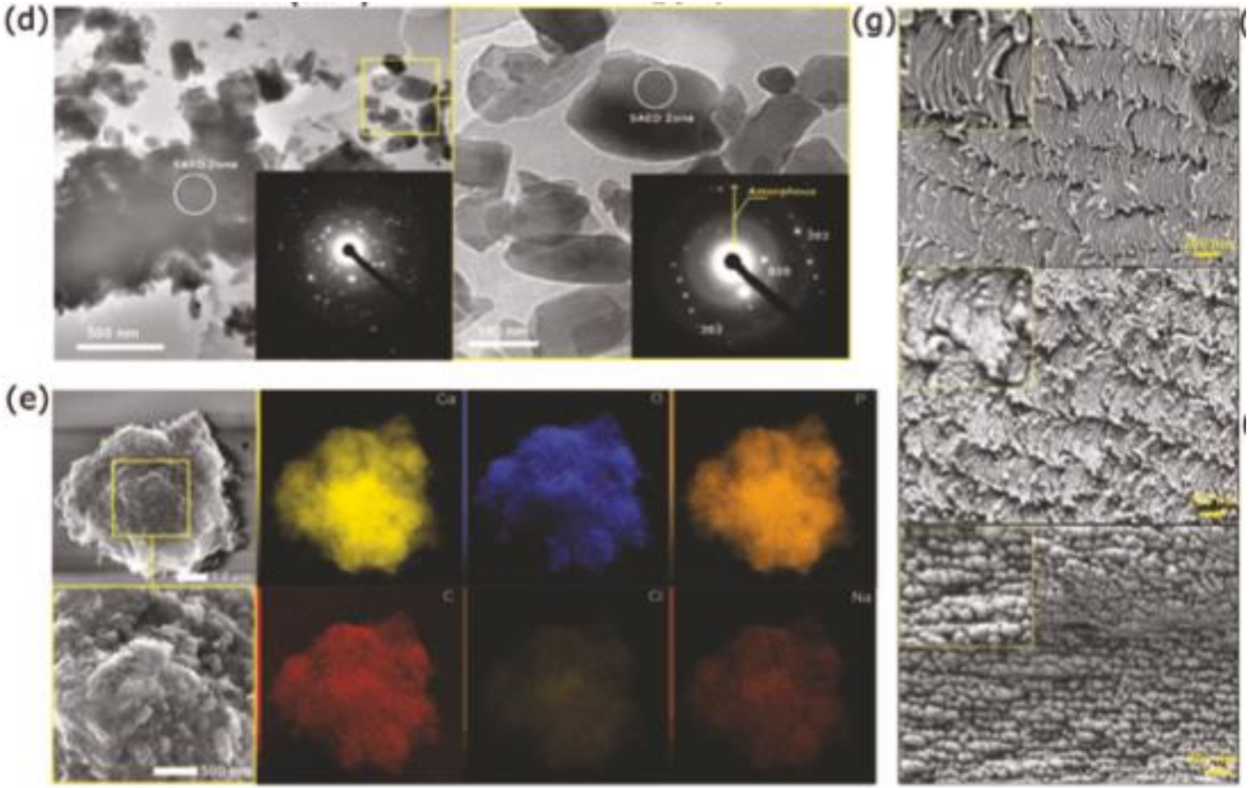
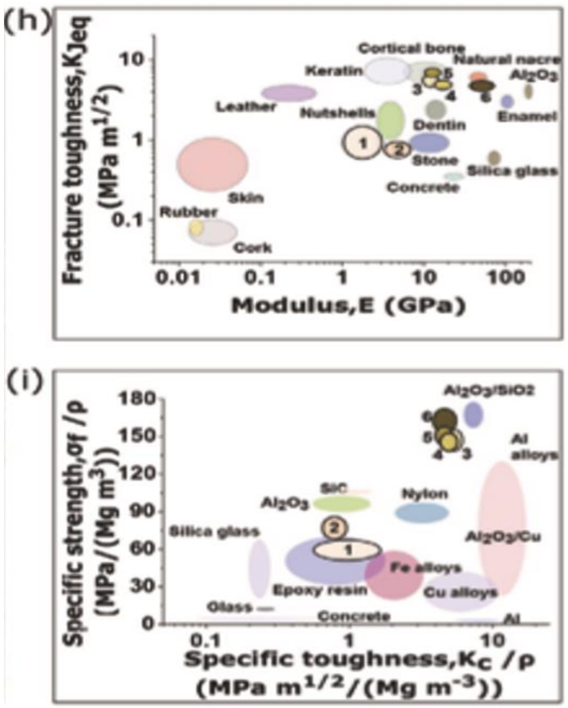


Figure. d) TEM images, e) EDS elemental maps g) SEM images

Ashby Plots



These findings imply that ;

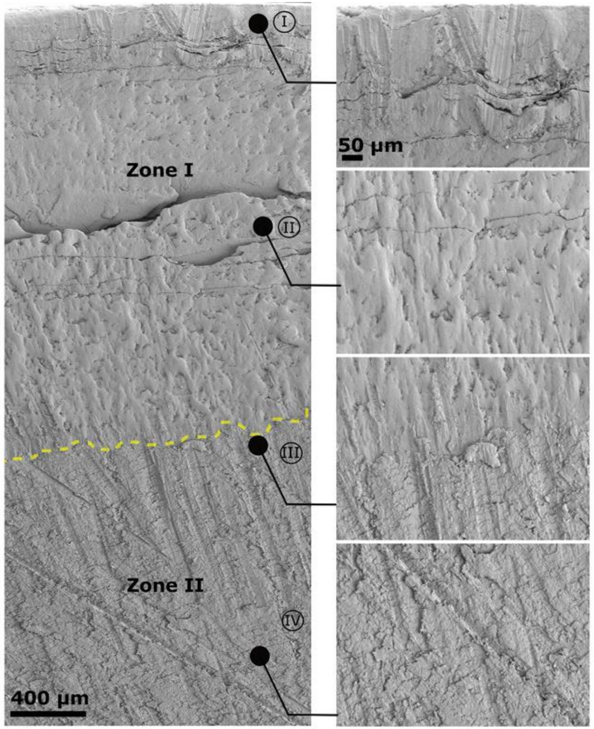
- **Sequestration of MP1 could induce biomimetic apatite mineralization**
- **Emulates the highly mineralized stiff and hard exterior impact region**
- **CNCs bearing negatively charged carboxyl groups synergistically work in concert with MP1 to enhance sequestration of inorganic ions and apatite crystallization**
  - ✓ Improving surface hardening
  - ✓ HAP can form granule shapes with isotopic distribution on the surface of the anisotropic chitin-protein scaffold

Assembly of the dental implant crown

Assembly of the dental implant crown

Verification

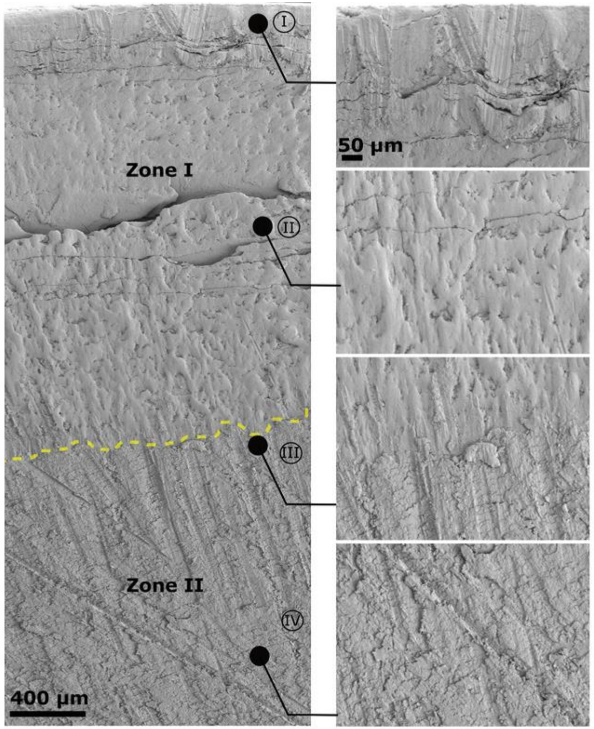
- SEM (Scanning electron microscopy)
- Phase mapping
- WAXS and XRF (Simultaneous synchrotron wide/small-angle X-ray scattering and X-ray fuorescence)
- Nanoindentation mapping
- In vitro cytocompability assesment



Assembly of the dental implant crown

Nanoidentation mapping

		Biocomposite	Human tooth
Exterior	Elastic modulus	25–36 GPa	70–80 GPa
	Hardness	1.9–3.2 GPa	4–4.5 GPa
Interior	Elastic modulus	18 – 22 GPa	15–20 GPa
	Hardness	1.5–1.7 GPa	0.5–0.8 GPa

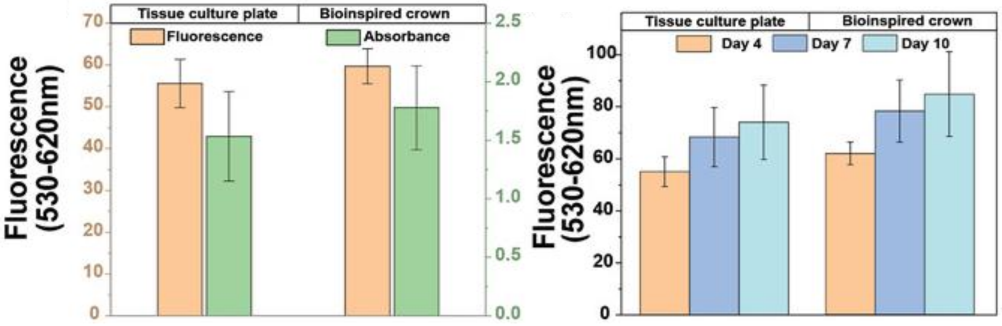


Assembly of the dental implant crown

In vitro cytocompatibility assesment

- **AHDFs cultured on 2D film** (exterior crown composition)
- **Could it support adhesion, growth and proliferation** without apparent cytotoxicity?

Yes it could!



Future Aspects

Future Aspects

Why and how is this study important?

- Novelty aspect

  "… a graded microstructural design as well as surface hardening <u>has not been previously achieved</u> in engineered composites."

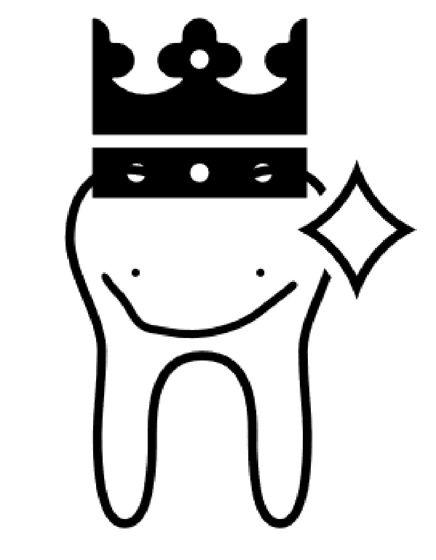
- Path forward

  "Future developments will include scaling-up of material fabrication and prototyping for orthopedic applications, bone repair, and bioengineering."

Conclusion

Conclusion

- Exploring the structural compability of CNCs with CMP-containing proteins
- How to produce tough energy-dissipating matrix → **biocomposites**
- **Successful**
- Important path forward, many possible applications!



Thank you!
Questions?