FREEZE-FOAMING: A PROMISING NEW APPROACH TO MANUFACTURE STRENGTH ENHANCED BIOACTIVE MATERIALS

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AGENDA

1. Motivation

- Biomaterials & implants for: necessary surgeries
- demographic change

Bioreactors (Cell Cultivation)

- Implants
- Asservation-and Cultivation media

Medical technology

- (artificial) bone scaffolds
- Tissue-Engineering
- Etc.

Manufacturing of porous, cellular structures

1. Motivation

- Initial situation:
  - Hydroxyapatite (HAp) is biocompatible
    - bioactive
    - relatively low compressive strength → no application as long-term implant
  - ZrO_2 is biocompatible
    - bioinert
    - high toughness → use as long-term implant (dental-,endoprosthetics...)

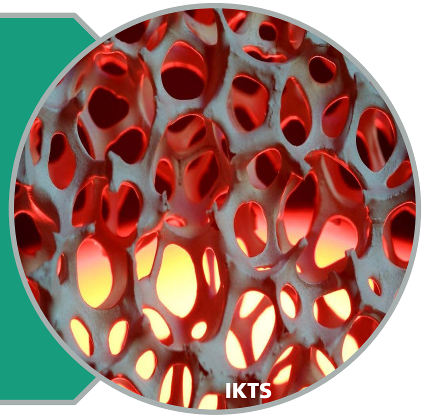
- Approach
  - **Combination of both materials** to strength-enhanced porous structures while maintaining the biocompatibility/-activity

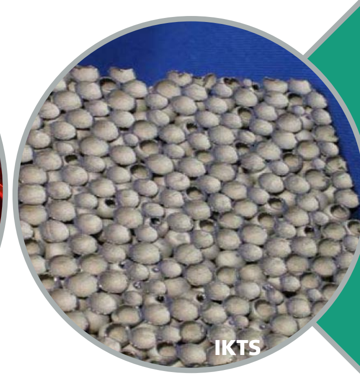
    → Possible use as all-ceramic implant

2. State of the Art

Replica Method
(PU-foam)

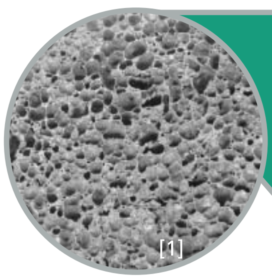
- Dipping in the suspension
- Drying
- Burnout of the PU-foam





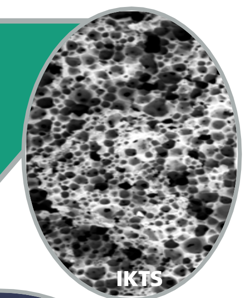
Placeholder Method

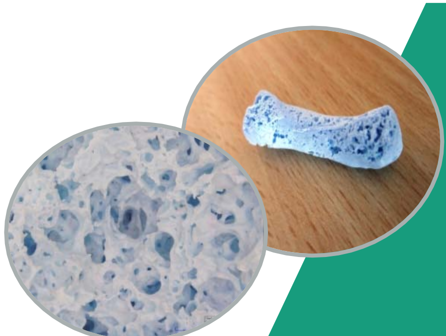
- Hollow beads
- Volatile polymers, sawdust, carbon black, wax
- Burnout of the placeholder



Direct Foaming

- Gasinjection
- Gas development through chemical reaction
- Mechanically mixed in air





Freeze Foaming

- Pore generation:
  - Mechanically mixed in air
  - Rising water vapour
  - Sublimation
- Patent disclosed[2]

[1] Colombo, P.; Conventional and novel processing methods for cellular ceramics, Phil.Trans.R.Soc.A, Vol. 364, **2006**, S.109–124
[2] Moritz, T.; Offenlegungsschrift DE 10 200800100A1

3. Freeze Foaming

…Experimental Setup

Starting material

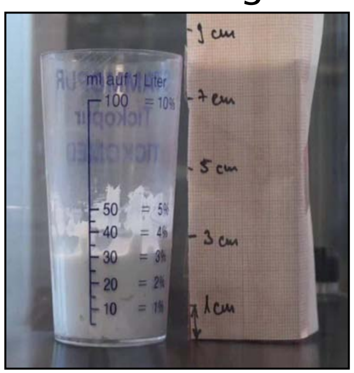
- Hydroxyapatite[1]
- ZrO_2 [2]

+ Water

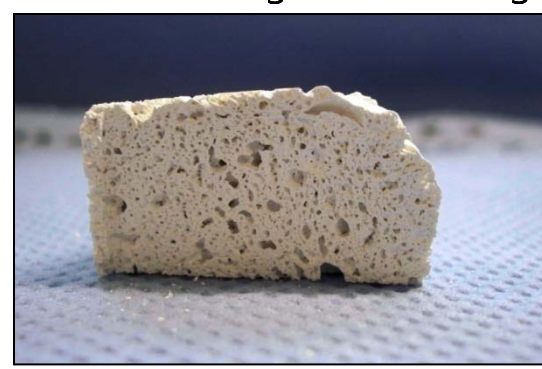
+ organic additives (Suspension/Foam stabilizers)

Freeze Foaming

Mold Filling



Debinding and Sintering



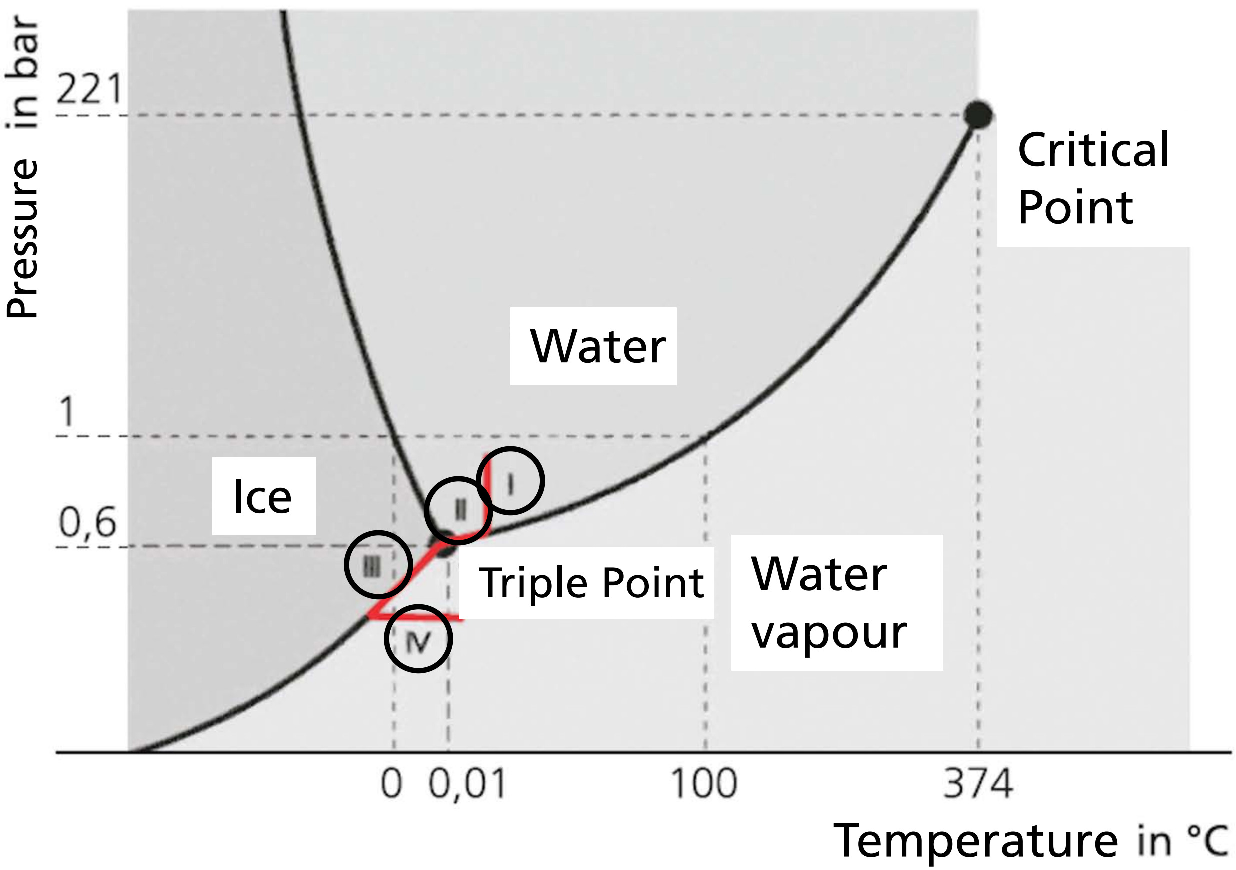
[1] HAp (Fa.SIGMA-ALDRICH), d_{50} = 2,6 µm

BET = 72,76 m²/g

[2] ZrO2 (TZ3-Y-E, TOSOH), d_{50} = 0,7 µm, BET = 15,4 m²/g

3. Freeze Foaming

...The Technique



I: Pressure reduction in a freeze dryer

- aqueous suspension starts to foam

II: System moving along the equilibrium line to the triple point (TP)

- increasing foaming
- decreasing foam/suspension temperature
- transition aqueous/gaseous state to solid state at TP

III: Further pressure reduction

- instantaneous freezing of the porous foam structure→ stabilization

IV: Sublimation

- Foam drying through sublimational heat (panel heating)

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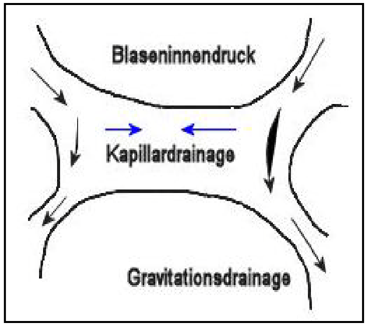
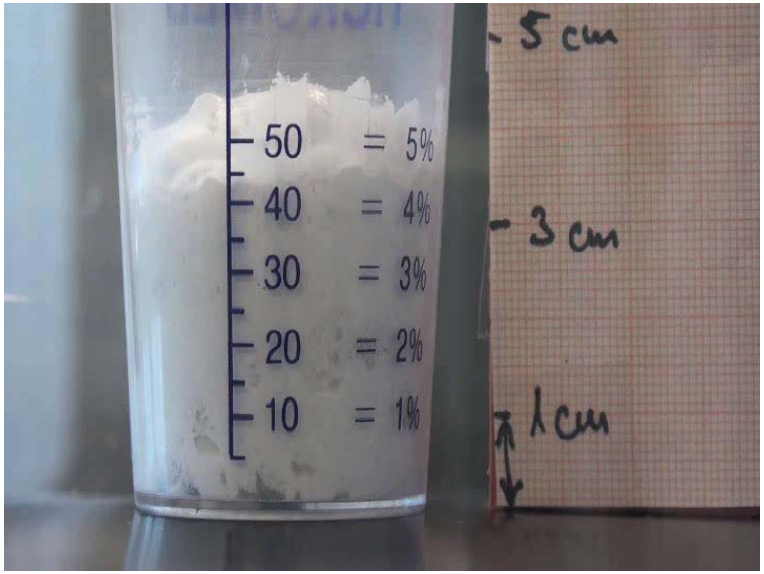
Foaming process

…Occuring effects

- Drainage
  → Struts are getting thinner
- Ostwald ripening
- Coalescence
  → small pore structures go amiss





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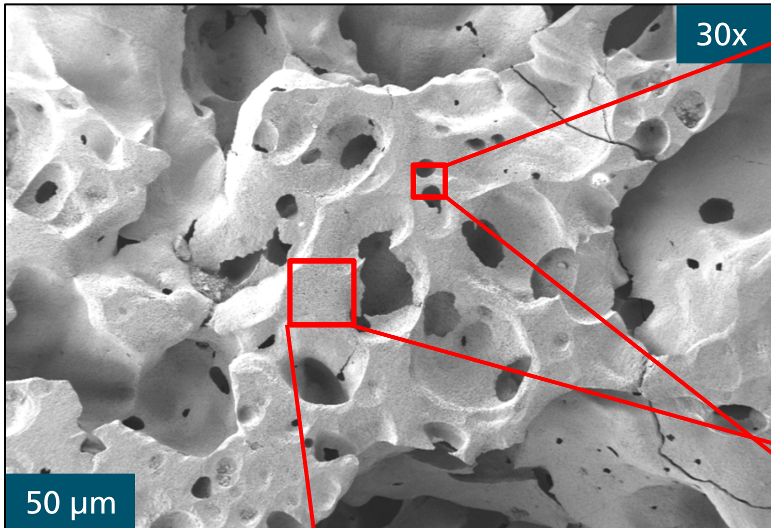
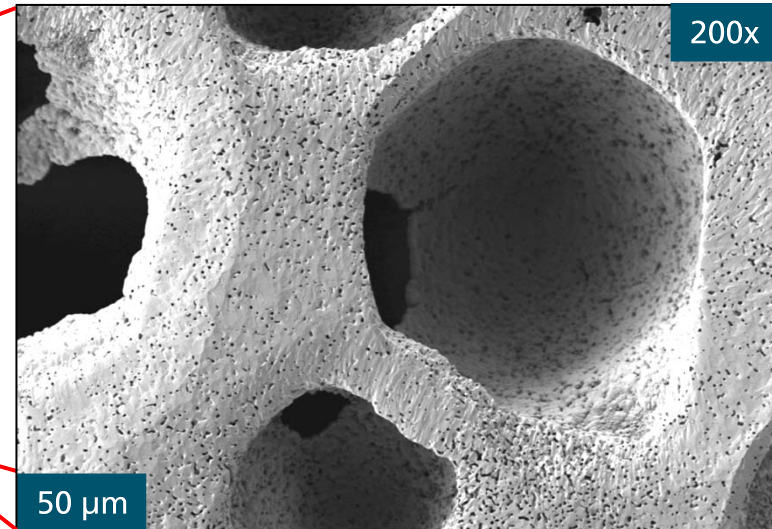
4. Results

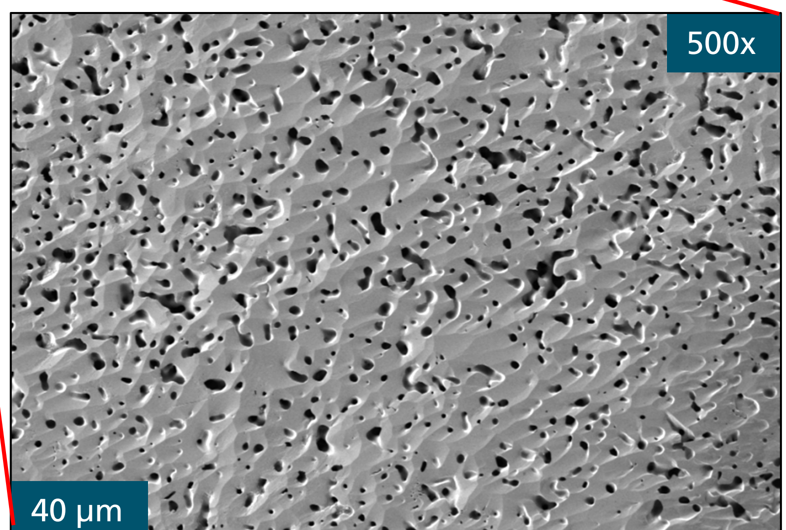
…Microstructure





FESEM

Ion polished



- Open porosity
- Interconnectivity
- Microporosity

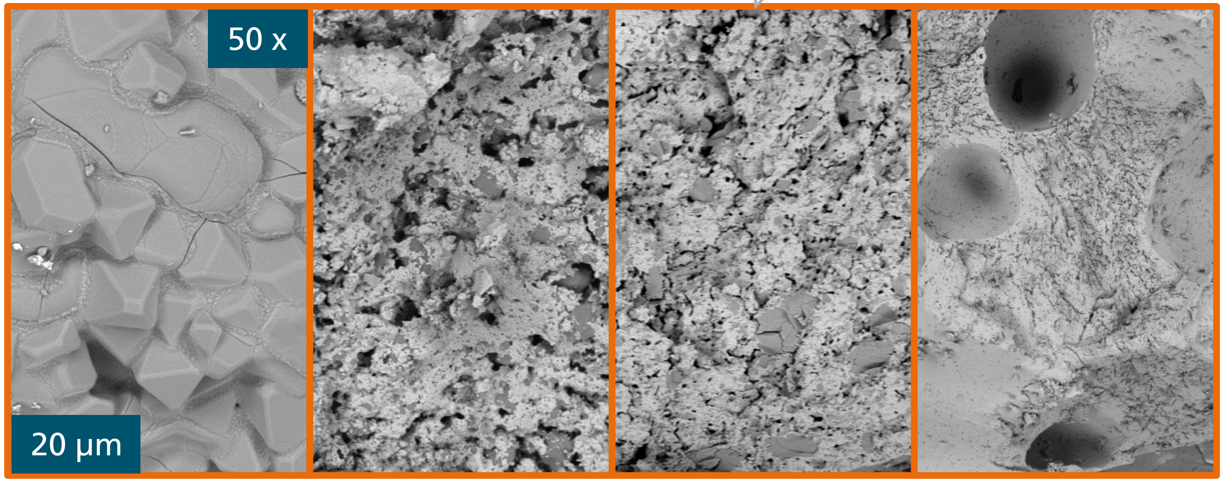
Structural requirements for cell cultivation

…Microstructure

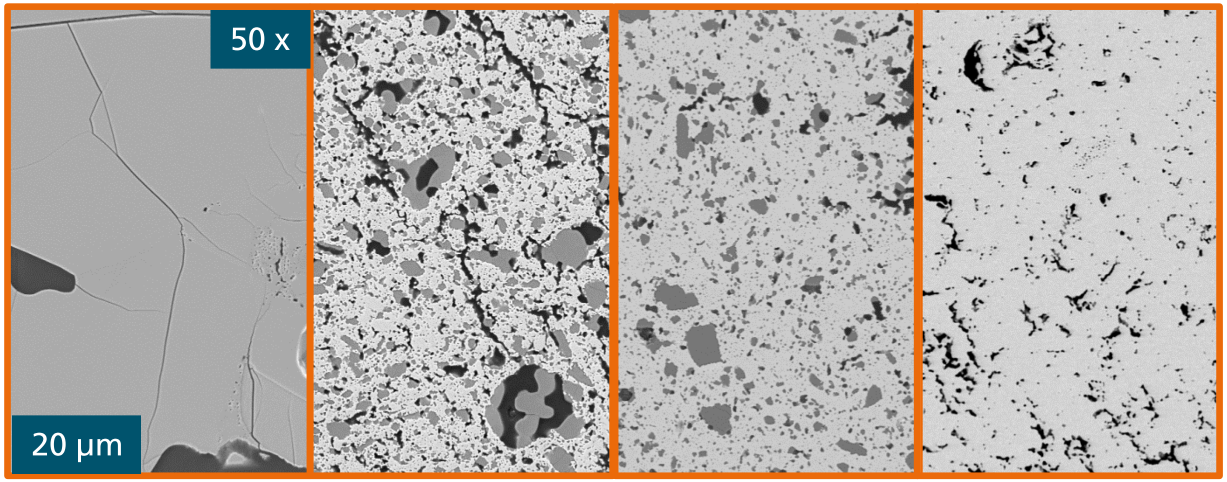
Amount ZrO_2

Hydroxyapatit
e

Fractured
view



Polished view



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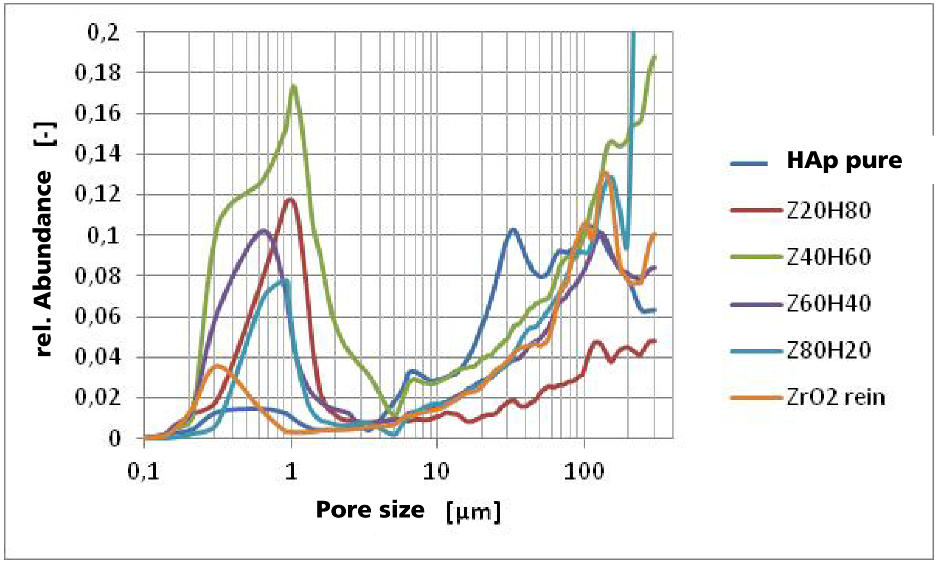
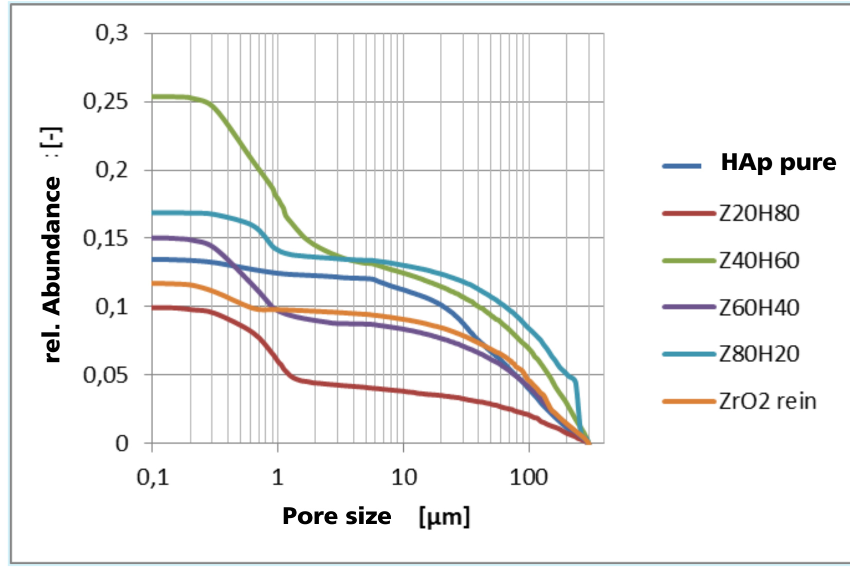
…Pore size distribution

Mercury intrusion porosimetry

Distribution



Intrusion



Amount ZrO_2 [Vol.-%]	0	20	40	60	80	100	
Pore $size_{max}$ [µm]	0,6	1	0,2	0,6	0,9	0,3	HAp SA
Porosity [%]	27	33	50	41	33	40	

+ Makropores between 300 and 700 µm (per X-ray computed tomography)

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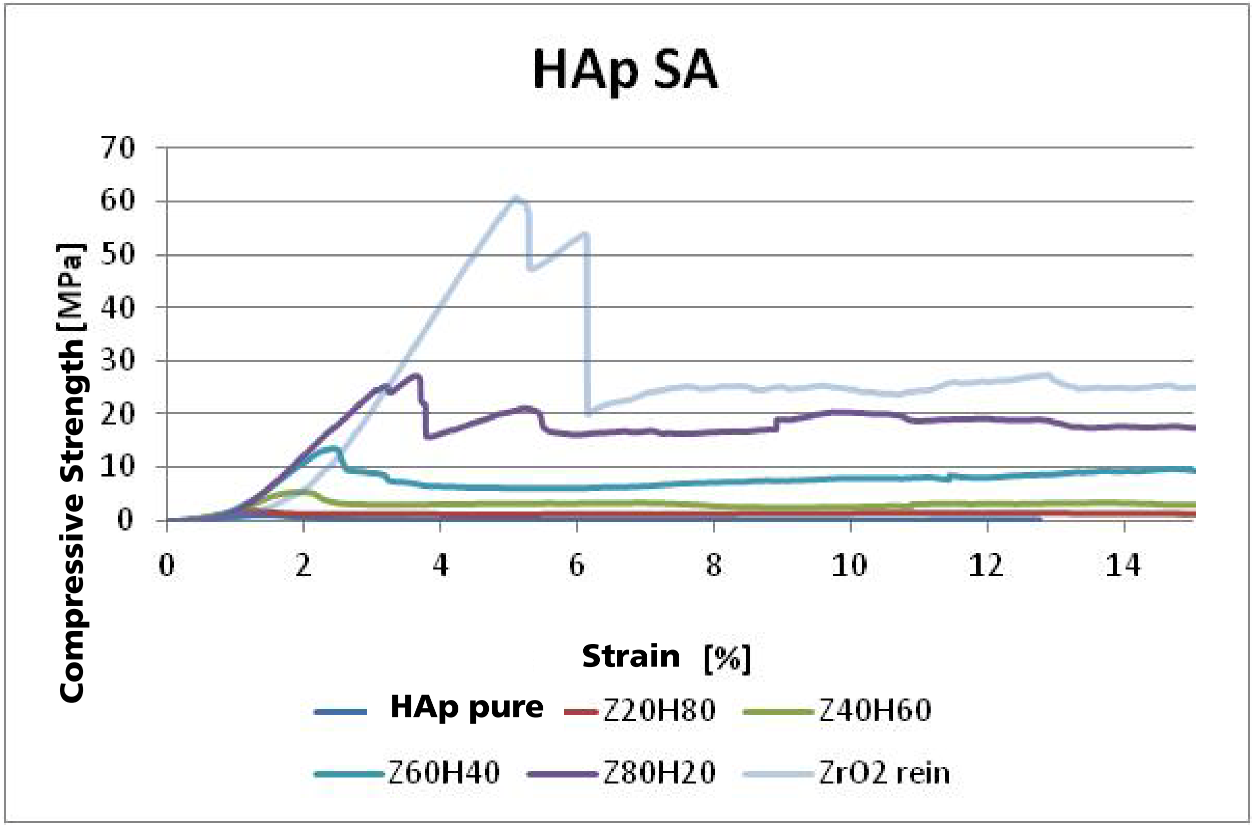
…Compressive Strength



Amount ZrO_2 [Vol.-%]	0	20	40	60	80	100	
Compressive strength$_{max}$ average [MPa]	1,2	2,0	5,7	15,1	36,2	68,7	HAp SA
Porosity [%]*	73	75	71	72	60	72	

* geometrical

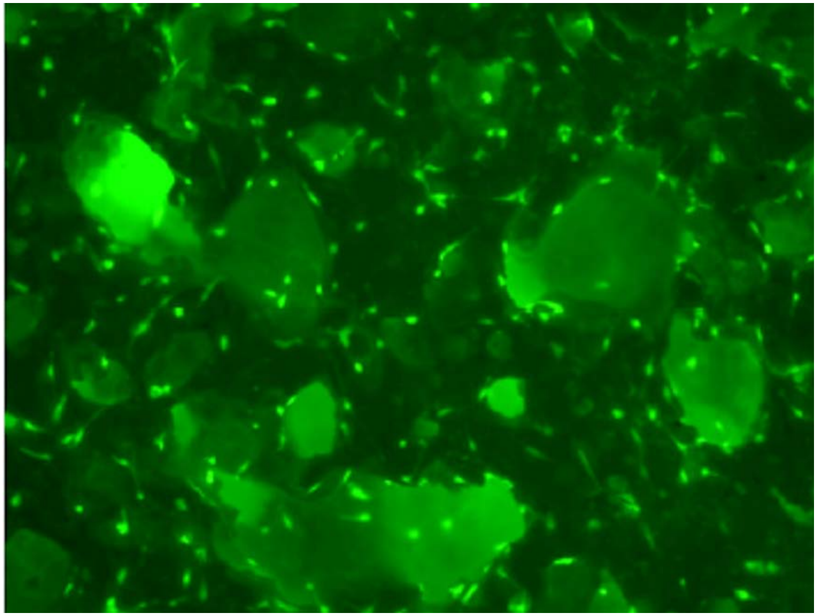
4. Results

…Biological Tests

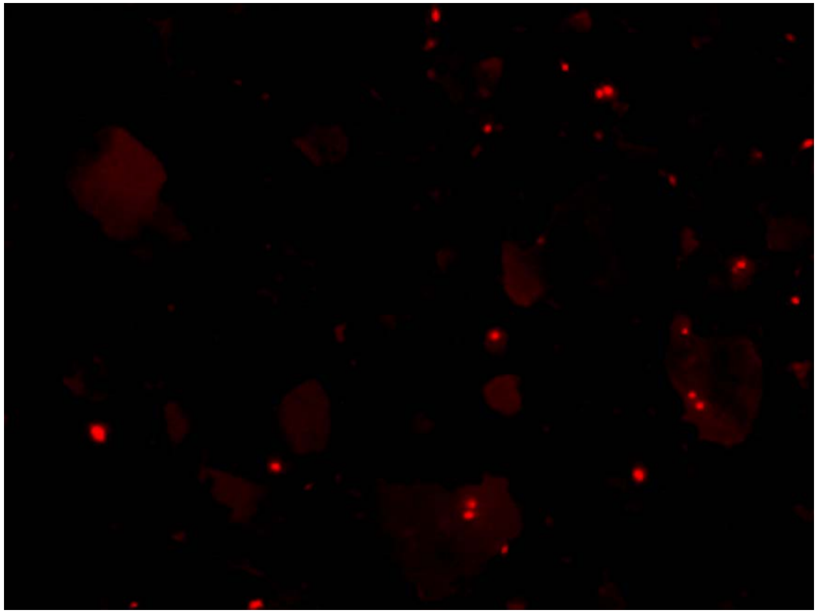
In cooperation with the Fraunhofer Institute for Biomedical Engineering IBMT
Sankt Ingbert, Saarbrücken

- Starting with tests of cytotoxicity
  - Cell type: adulte stem cells from human bone marrow, mesenchymal origin
  - Morphology: fibroblast-like growing, adherent
  - Incubation: 37 °C, 5 % CO_2, ca. 90 % relative moisture

Flurorescein diacetate – Live staining



Propidium iodide – Dead staining



4x lens, incident fluorescent WIB (1x70)

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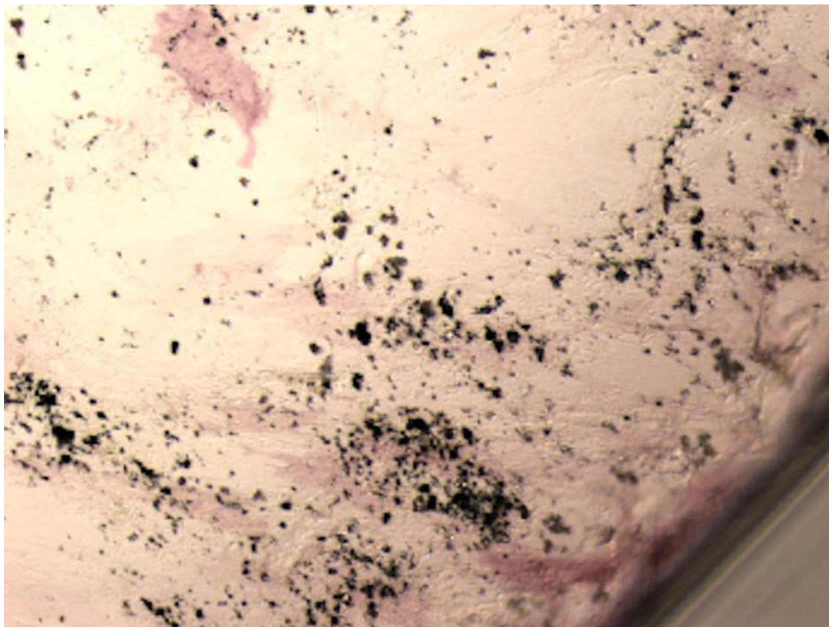
4. Results

...Biological Tests

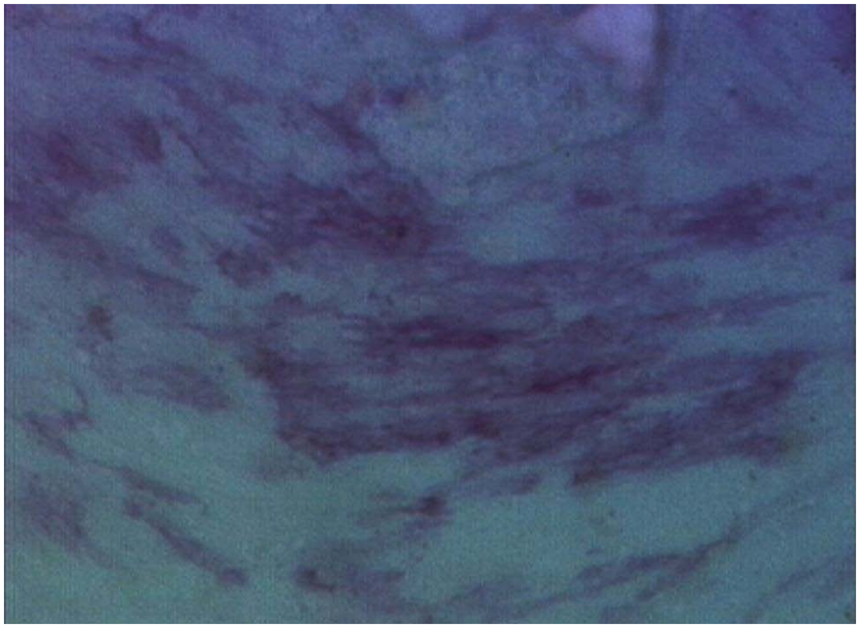
- Differentiation approach with alkaline phosphatase (ALP)

After 9 days



10x lens, transmitted light (IX70)

Well sorrounding



5x lens, incident light (BX51)

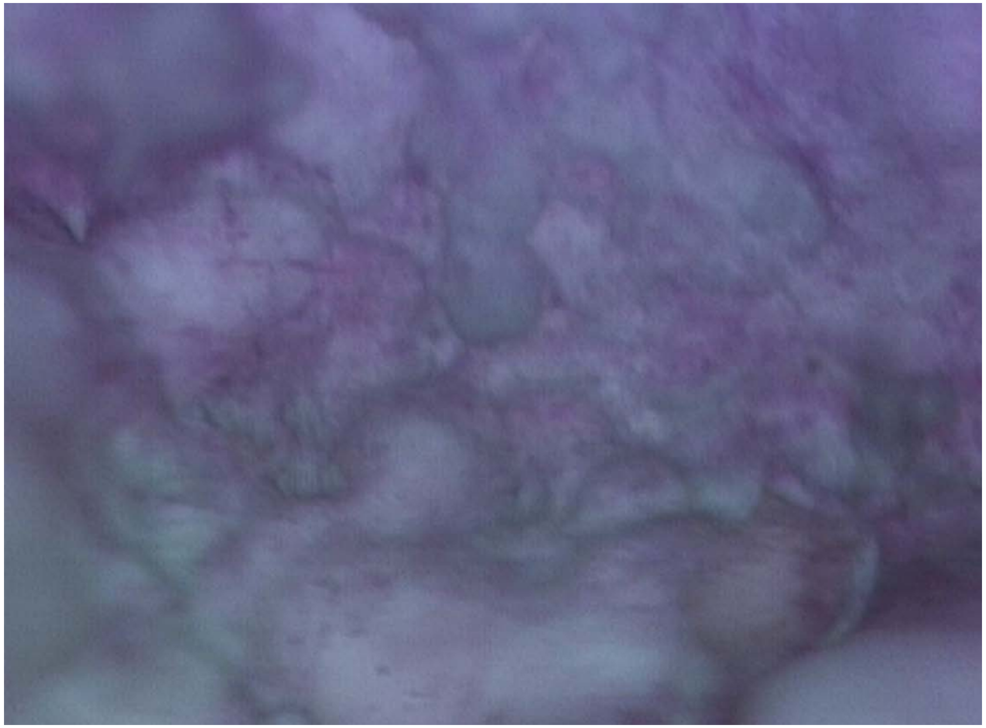
Sample surface

4. Results

…Biological Tests

- Differentiation approach with alkaline phosphatase (ALP)

After 21 days



5x lens, incident light (BX51)

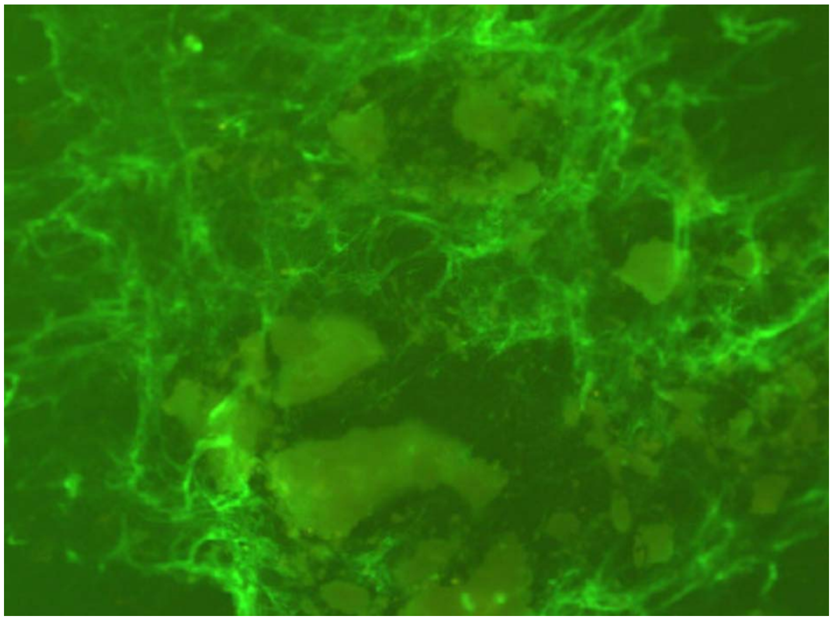
Sample surface

4. Results

...Biological Tests

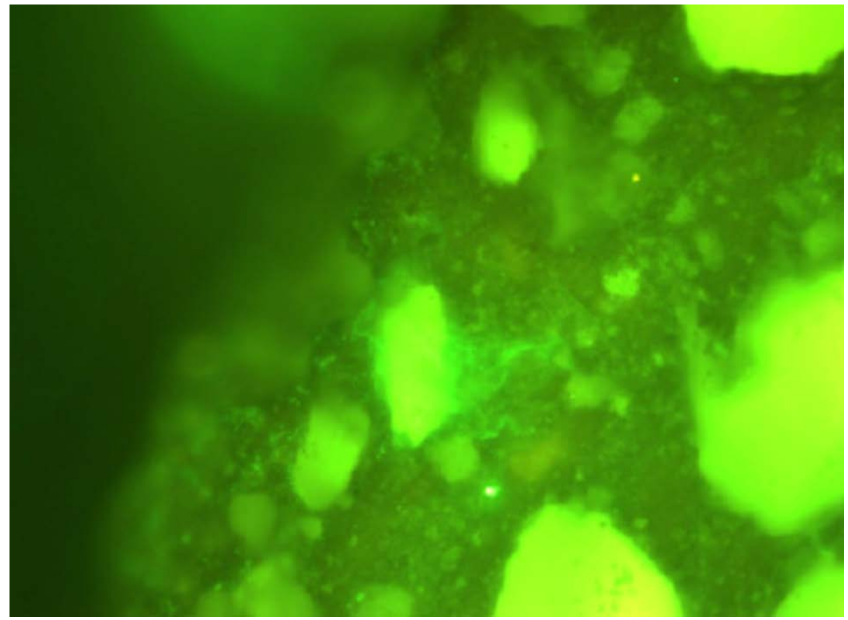
- Differentiation approach with Collagene (Col1)

After 21 days



10x lens, incident fluorescent WIB (1x70)

Cells in the well in the sample's sorrounding



10x lens, incident fluorescent WIB (1x70)

Surface sample

5. Conclusion and Future Prospects

- Freeze foaming as environmentally method to achieve porous structures enabling cell growth
- Succeeded hybrid combination of two biocompatible materials
  - Successful enhancement of the compressive strength with rising amount of ZrO_2
  - Successful cell cultivation
  - Successful differentiation approaches
- Future Prospects
  - Cytotoxicity tests and
  - Differentiation approaches for all HAp-ZrO_2 pairings
  - Metabolic activity
  - Increasing the compressive strength
  - in-vitro und in-vivo tests
  - Customer adapted shaping and design of implants

5. Conclusion and Future Prospects

- Possibilities of the Additive Manufacturing (SLS, 3D printing, LOM, STL, etc.)
  - 3D printing

HAp (Co. MERCK) – pig bone (un-sintered)

HAp (Co. MERCK) – pig bone (sintered)

Thumb bone replica

Freeze-foamed, 3D-printed

- **Aim: potential use as all-ceramic implant a huge step closer**
- additionally: use as bio reactors, cultivation media etc.

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Many Thanks
to you,
to the Fraunhofer IBMT and the Fraunhofer Society!