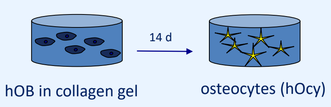
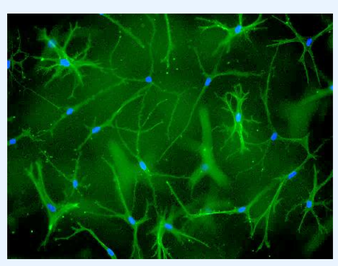
Advancing alginate-based bioinks through collagen incorporation and biomimetic mineralization to study osteoblast-to-osteocyte differentiation

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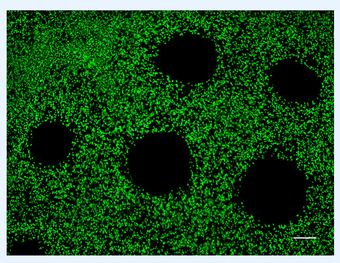
Introduction and preliminary work

Osteocytes
Most prevalent cells in bone tissue, responsible for mechanosensation and orchestration of bone remodeling.
In vitro they can be differentiated from osteoblasts (hOB) in 3D environment under low serum conditions



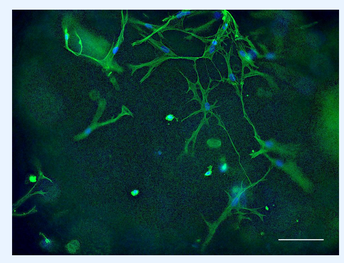

hOcy in collagen gels, nuclei blue, cytoskeleton green, scale bar: 100 µm

Bioprinting of osteoblasts
Human osteoblasts can be bioprinted in an ink composed of 3 % alginate (Alg) and 9 % methyl cellulose (MC), dissolved in human plasma [1]. The addition of plasma improves cell spreading compared to pure Alg/MC inks.


Bioprinted hOB in Alg/MC/plasma ink, Live/Dead staining day 3, scale bar 100 µm

Osteoblast-to-osteocyte transition in bioprinted constructs

hOB bioprinted in Alg/MC/plasma ink were successfully differentiated into osteocytes (manuscript in revision)


Bioprinted hOB in Alg/MC/plasma ink, differentiated into osteocytes, cytoskeleton green, nuclei blue, day 14
Scale bar 100 µm

Aim: Create a bone-like environment for osteocyte differentiation by introducing collagen I and calcium phosphate into alginate-based ink

Results & discussion

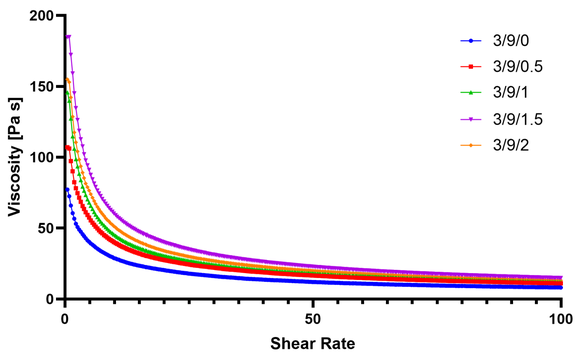
Preparation of collagen-containing inks

Collagen I from calf skin (Collaplex 1.0, GfN) was introduced as a viscous solution (18 mg/ml)

name	Alg (%)	MC (%)	collagen (%)	plasma (%)
3/9/0	3	9	0	100
3/9/0.5	3	9	0.45	80
3/9/1	3	9	0.9	66.6
3/9/1.5	3	9	1.35	57
3/9/2	3	9	1.8	50

Inks were prepared from Alg and MC powders (sterilized by autoclaving), mixed with human plasma and collagen solution. Collagen solution was sterilized by supercritical CO_2 treatment [4].

Rheology



All ink formulations showed shear thinning behaviour. Initial viscosity was highest for 3/9/1.5 and lowest for the collagen-free 3/9/0 formulation. All formulations except 3/9/1.5 showed good printability.

Biomimetic mineralisation of 3D printed structures

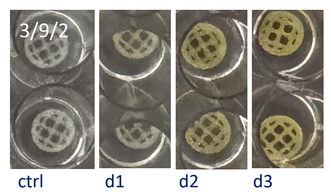
A previously published method for biomimetic interfibrillar mineralisation of collagen [2] was slightly adapted to mineralise 3D printed structures of Alg/MC/plasma and its collagen-containing blends



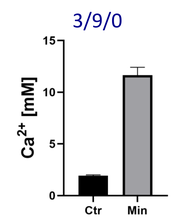
non-mineralised

mineralised

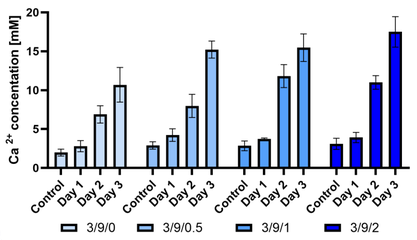
Photographic images of collagen-free 3D constructs, non-mineralised and after 3 d of mineralisation



von Kossa staining (yellow staining as published according to Puchtler and Meloan [3]) after 1,2 or 3 days mineralisation of 3D printed constructs



All ink formulations were biomimetically mineralised, with increasing Ca content with mineralisation time.

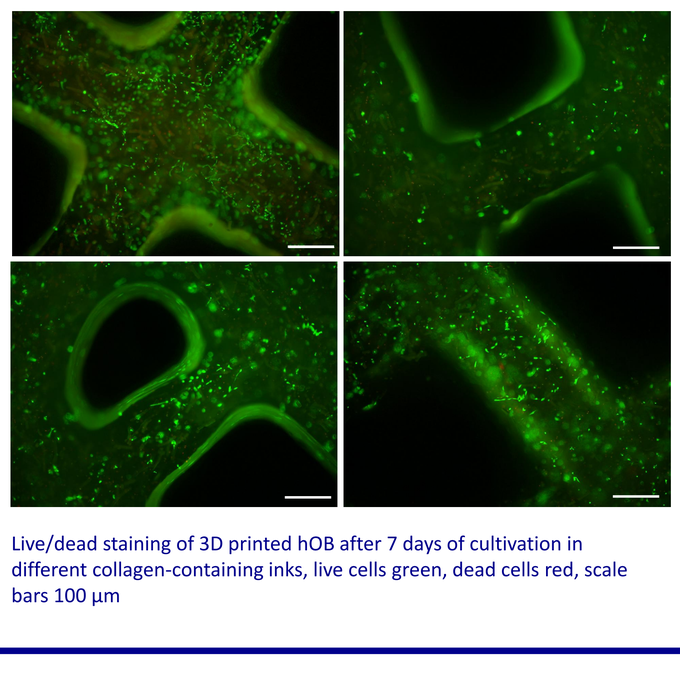


Calcium content of mineralised samples (after acidic dissolution, colorimetric assay)

Bioprinting and osteocytic differentiation

Cytotoxicity test

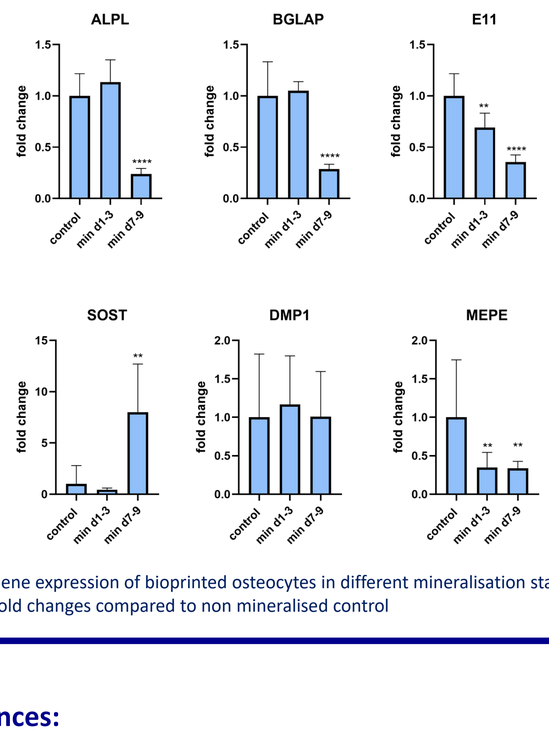
Collagen containing inks did not show any cytotoxic effects (additives from $scCO_2$ sterilisation were successfully removed)



Live/dead staining of 3D printed hOB after 7 days of cultivation in different collagen-containing inks, live cells green, dead cells red, scale bars 100 µm

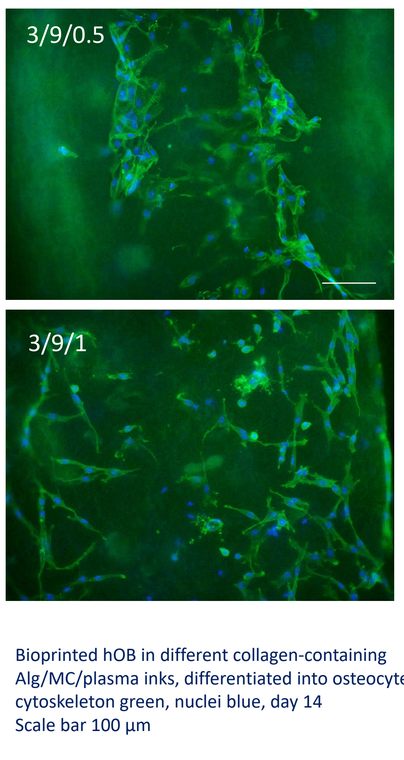
Effect of mineralisation on osteocyte differentiation

Bioprinted samples (3/9/0 ink) cultivated for 14 d for osteocytic differentiation. Biomimetic mineralisation performed either at day 1-3 or at day 7-9.



Gene expression of bioprinted osteocytes in different mineralisation states. Fold changes compared to non mineralised control

Osteocytic differentiation



Bioprinted hOB in different collagen-containing Alg/MC/plasma inks, differentiated into osteocytes, cytoskeleton green, nuclei blue, day 14
Scale bar 100 µm

Conclusion and Summary

- Shear thinning behaviour and printability of Alg/MC/plasma inks blended with collagen
- Supercritical CO_2 treatment is suitable for collagen sterilisation for ink preparation
- Osteocytes can be obtained in 3D printed Alg/MC/plasma from osteoblasts
- Printed constructs from Alg/MC with and without collagen can be biomimetically mineralised, Ca content increased with mineralisation duration
- Osteoblast-to-osteocyte transition successful in all examined inks
- Mineralisation did not change osteocyte differentiation, when applied in the beginning of the osteoblast-to-osteocyte transition

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References:

[1] Ahlfeld, T. et al. ACS Appl. Mater. Interfaces 12, 12557-12572 (2020)
[2] Thrivikraman, G. et al. Nat. Commun. 10, 350 (2019)
[3] Puchtler, H. & Meloan; S. Histochemistry 56, 177-185 (1978)
[4] Bernhardt, A. et al. PloS One 10, e0129205 (2025)

Die TUD ist Gründungspartnerin der Forschungsallianz DRESDEN-concept e.V.
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