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# Bone Regeneration in Rat Critical-Size Calvarial Defects Using 3D-Printed Sphene Ceramic Scaffolds: A Micro-CT and Histological Study

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# Objectives

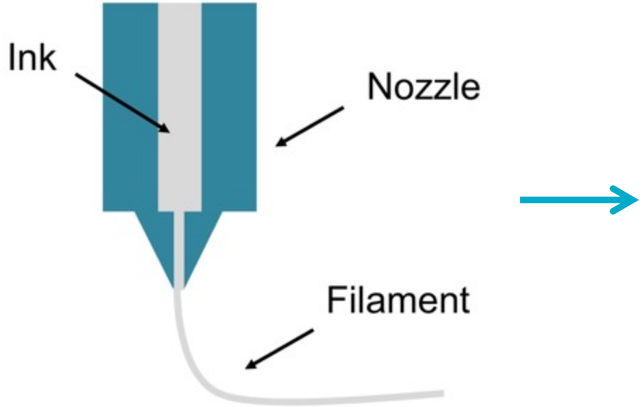
- Silica-based bioceramics are gaining increasing attention as bone grafting materials due to their bioactivity and adjustable mechanical properties → among these a calcium silica titanate, also known as sphene or titanite ($CaTiSiO_5$)

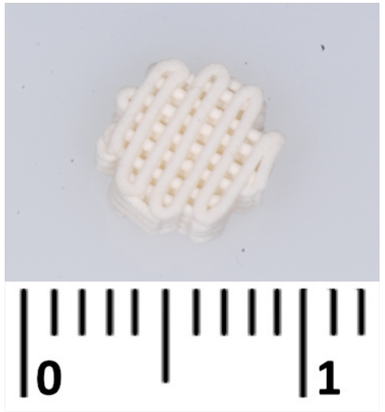
## PRODUCTION OF SPHENE-BASED SCAFFOLDS VIA DIRECT INK WRITING

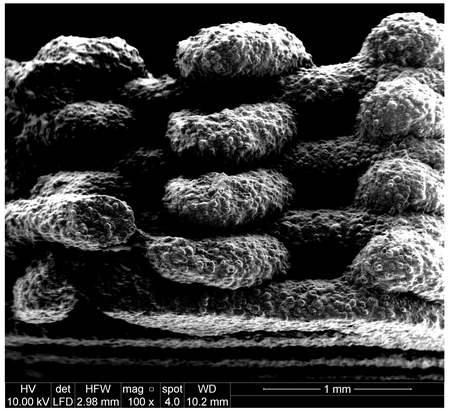
- Silicone (MK 25.7 wt%) paste incorporating fillers (fumed silica 2.4 wt%, $TiO_2$ 31.9 wt%, and $CaCO_3$ 40wt%) in isopropanol
- Heat treatment: 1300°C in air in a chamber furnace
- Porous structure with overlapping of cylindrical rods, periodically arranged along x- and y- axes

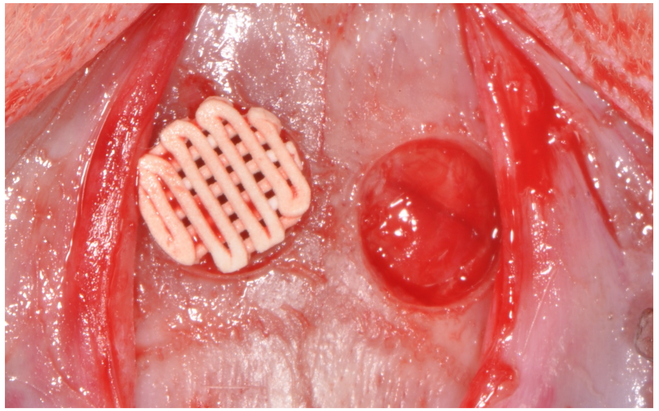
## AIM OF THIS *IN VIVO* STUDY

to evaluate bone healing in rat calvarial critical-size defects filled with 3D-printed sphene ceramic scaffolds fabricated via direct ink writing (DIW) or left empty



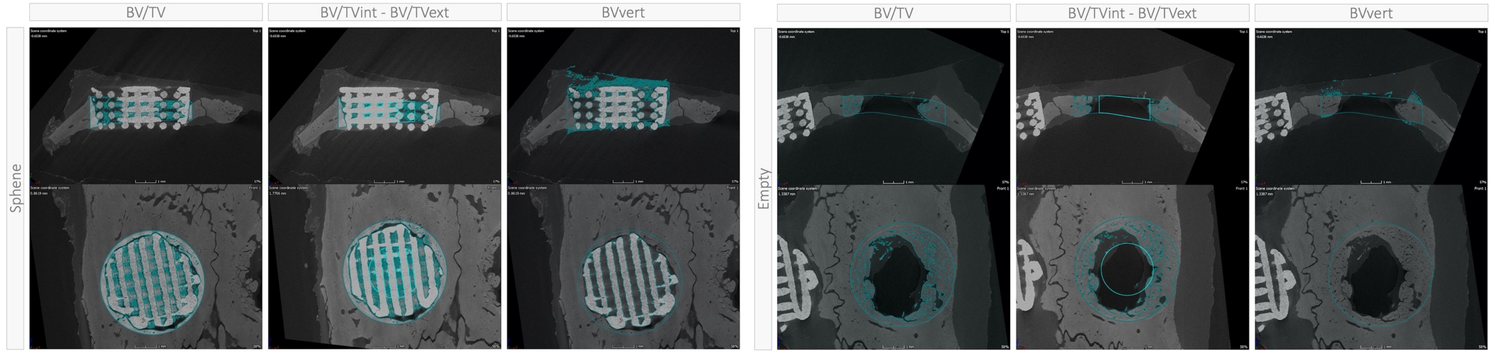



# Material and Methods

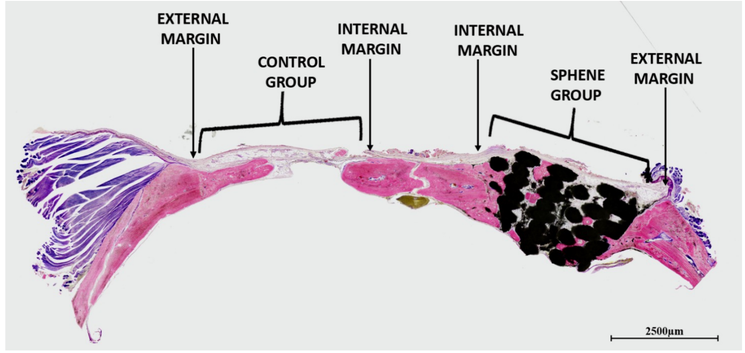
- Bilateral 5-mm calvarial defects in 20 Sprague–Dawley rats (sphene scaffold vs empty)
- Killing 4 or 8 weeks (n=10 pet time point)

## MICRO-COMPUTED TOMOGRAPHY (MICRO-CT) ANALYSIS




- Metrological X-ray CT system Nikon MCT 225
- Scanning parameters: 120 kV, 57 µA, 1415 ms, voxel size 9 µm
- Parameters:
  - Bone volume fraction (BV/TV) within the defect area
  - BV/VT in central (BV/TVint) and peripheral (BV/TVext) regions
  - New bone volume beyond original cortical boundary (BVvert)

## UNDECALCIFIED HISTOLOGY




- Cut and grinding
- Acid fuchsin and toluidine blue staining
- Histological analysis
- Histomorphometric analysis
  - internal bone margin thickness
  - external bone margin thickness

## STATISTICAL ANALYSIS

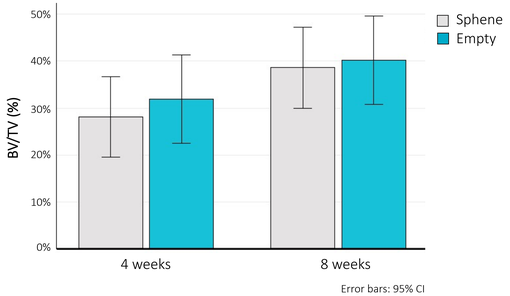
- Micro-CT outcomes: ANOVA test with Bonferroni correction, except for BVvert (non-parametric tests)
- Histomorphometric outcomes: paired t-test for intergroup comparison, unpaired t-test for overtime comparison, with Bonferroni correction
- Results considered significant with $p<0.05$

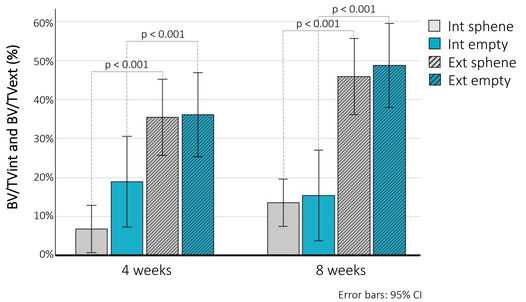
# Results

- No adverse event during surgery and healing phase

## MICRO-CT ANALYSIS

### BV/TV, BV/TVint and BV/TVext (%)



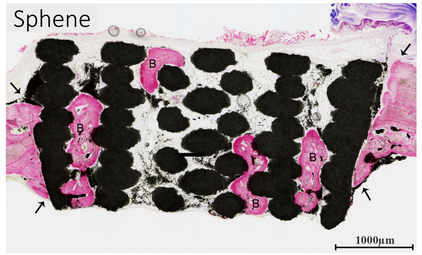

- BV/TV tended to increase over time in both groups
- No significant differences in BV/TV among groups and timepoints
- Within the two timepoints, BV/TVext resulted significantly higher than BV/TVint in both groups

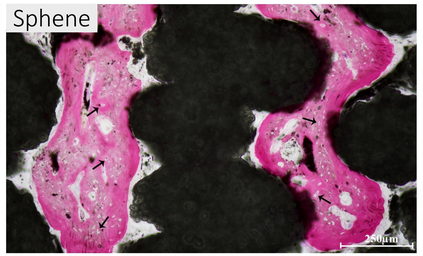
### BVvert ($mm^3$)

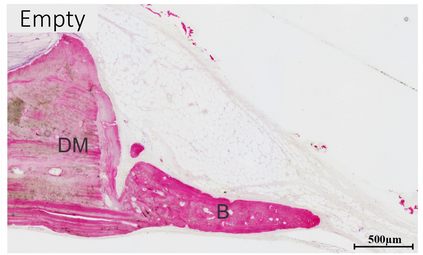
| Timepoint | Group | Mean (SD) |
|---|---|---|
| 4 weeks | Sphene | 3.07 (2.70) |
| | Empty | 1.28 (1.87) |
| 8 weeks | Sphene | 1.93 (1.63) |
| | Empty | 0.63 (0.72) |

Higher at 4 (p=0.013) and 8 weeks (p=0.013) in sphene group

## HISTOLOGICAL AND HISTOMORPHOMETRICAL ANALYSES



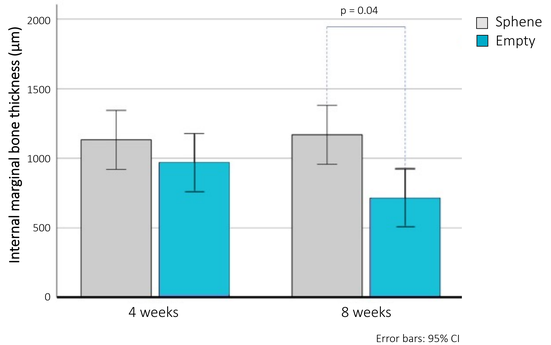



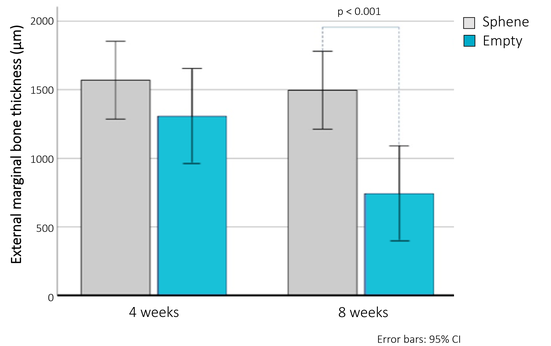

In sphene group, increased cortical bone thickness at the defect margins and within the pores of the scaffold, near the marginal portions of the defects

## HISTOMORPHOMETRICAL ANALYSIS

### Internal marginal bone thickness (µm)




### External marginal bone thickness (µm)



# Conclusions

## 3D-PRINTED SPHENE CERAMIC SCAFFOLDS

- Good biocompatibility
- Supported bone formation at the periphery of critical-size defects
- Combined with mechanical stability and compatibility with DIW manufacturing → promising material for bone tissue engineering

## FUTURE STUDIES

- Refining scaffold architecture to improve internal bone ingrowth
- Longitudinal imaging methods to monitor healing progression
- Experiments in larger animal models or load-bearing sites