Dept. of Cranio-Maxillofacial and Oral Surgery

Oral Biotechnology & Bioengineering

3D-printed bone substitutes with triply periodic minimal surface microarchitectures

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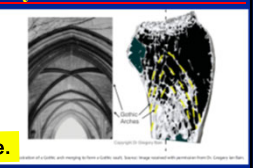
Natural bone microarchitecture is gothic style

Microstructure of the Distal Radius and Its Relevance to Distal Radius Fractures



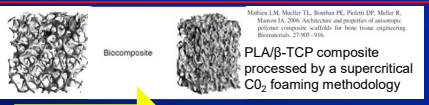
Load orchestrates microarchitecture.

Trabecular microarchitecture takes up the load directly and is constantly adjusted to actual loads during bone turnover (Wolff's law)

There is no evolutionary pressure on the microarchitecture of bone for optimization of osteoconductivity.

No need for biomimetics, since osteoconduction is an artificial although clinically highly relevant phenomenon.

Biomimetic or technomimetic microarchitecture



PLA/β-TCP composite processed by a supercritical $C0_2$ foaming methodology

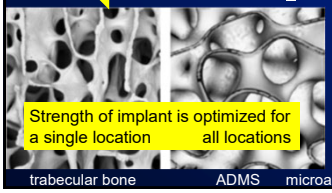
bone is a lightweight, load-driven, gothic style 3D- structure aiming towards high strength

Biomimetic look alike

"technomimetics"

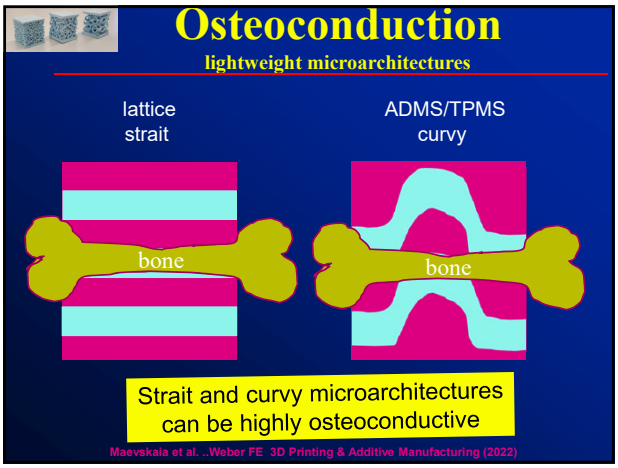
Adaptive Density Minimal Surface (ADMS) algorithm

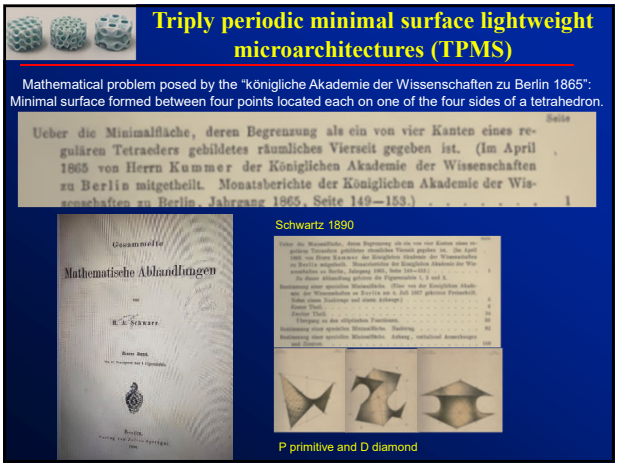
lightweight 3D- structure high strength/low material in aerospace, aviation and EV

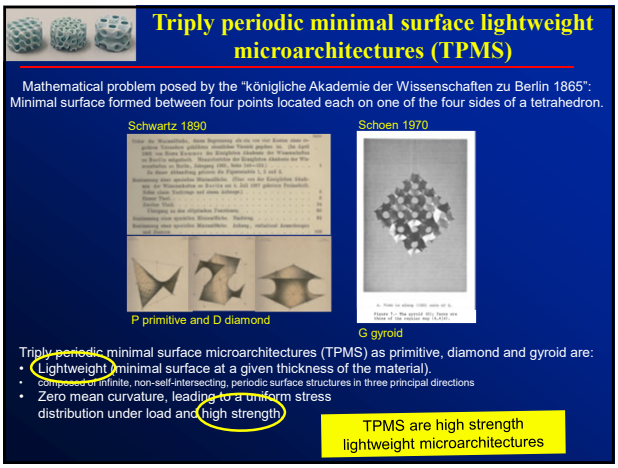


Strength of implant is optimized for a single location all locations

trabecular bone ADMS microarchitecture


Osteoconduction
lightweight microarchitectures
lattice
strait
ADMS/TPMS
curvy
bone
bone
Strait and curvy microarchitectures
can be highly osteoconductive
Maevskaia et al. ..Weber FE 3D Printing & Additive Manufacturing (2022)


Triply periodic minimal surface lightweight
microarchitectures (TPMS)
Mathematical problem posed by the "königliche Akademie der Wissenschaften zu Berlin 1865":
Minimal surface formed between four points located each on one of the four sides of a tetrahedron.
Ueber die Minimalfläche, deren Begrenzung als ein von vier Kanten eines re-
gulären Tetraeders gebildetes räumliches Vierseit gegeben ist. (Im April
1865 von Herrn Kummer der Königlichen Akademie der Wissenschaften
zu Berlin mitgetheilt. Monatsberichte der Königlichen Akademie der Wis-
Mathematische Abhandlungen
Schwartz 1890
P primitive and D diamond


Triply periodic minimal surface lightweight
microarchitectures (TPMS)
Mathematical problem posed by the "königliche Akademie der Wissenschaften zu Berlin 1865":
Minimal surface formed between four points located each on one of the four sides of a tetrahedron.
Schwartz 1890
P primitive and D diamond
Schoen 1970
G gyroid
Triply periodic minimal surface microarchitectures (TPMS) as primitive, diamond and gyroid are:
Lightweight (minimal surface at a given thickness of the material).
composed of infinite, non-self-intersecting, periodic surface structures in three principal directions
Zero mean curvature, leading to a uniform stress
distribution under load and high strength
TPMS are high strength
lightweight microarchitectures

Natural TPMS-lightweight microarchitectures

Gyroid

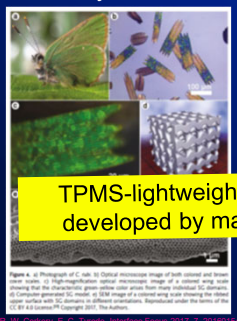
36 gyroids were designed by
Nintendo to be found in Animal Crossing ©

Natural TPMS-lightweight microarchitectures

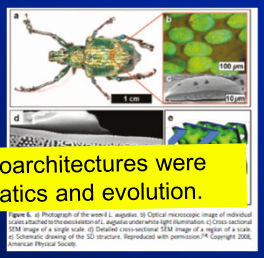
Gyroid in butterfly wings

Diamond in exoskeleton of a beetle

microarchitecture in nature



R. W. Corkery, E. C. Tyrode, Interface Focus 2017, 7, 20160154.
Callophrys rubi (Linnaeus, 1758) Grüner Zipfelfalter



J. W. Galusha, L. R. Richey, J. S. Gardner, J. N. Cha, M. H. Bartl, Phys. Rev. E. 2008, 77, 050904
Lamprocyphus augustus, Rüsselkäfer

TPMS-lightweight microarchitectures were developed by mathematics and evolution.

Lu Han* and Shunai Che, Adv. Mater. 2018, 30, 1705708

TPMS-lightweight microarchitectures

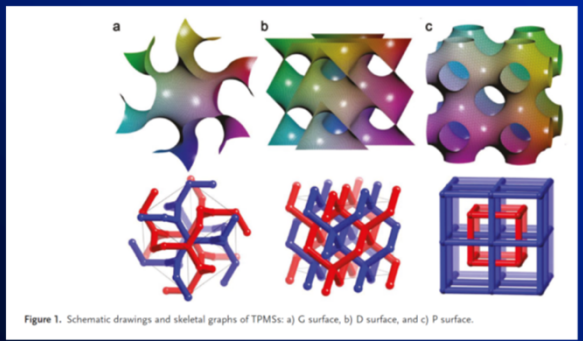
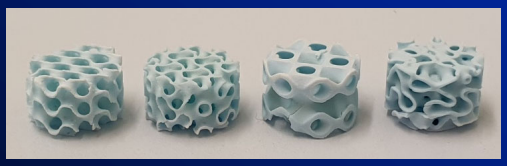


Figure 1. Schematic drawings and skeletal graphs of TPMSs: a) G surface, b) D surface, and c) P surface.

Lu Han* and Shunai Che, Adv. Mater. 2018, 30, 1705708

TPMS-lightweight microarchitectures

Additive manufacturing enables the production of
D-, G-, P- triply periodic minimal surface microarchitectures and
ADMS adaptive density minimal surface (only periodic)



D- diamond G-gyroid P-primitive ADMS

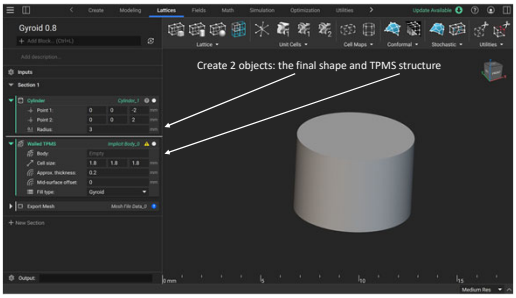
How to create TPMS designs

Cell unit	Final shape	TPMS equation
		**Gyroid**: sin(x) cos(y) + sin(y) cos(z) + sin(z) cos(x)
		**Diamond**: sin(x)*sin(y)*sin(z)+sin(x)*cos(y)*cos(z)+cos(x)*sin(y)*cos(z)+cos(x)*cos(y)*sin(z)
		**Primitive**: cos(x)+cos(y)+cos(z)

The order of actions

- Create a structure with nTopology
- Check parameters with Fusion 360
- Prepare the file for printing with the slicer
- Print the scaffolds
- Sinter at the desired temperature

nTop



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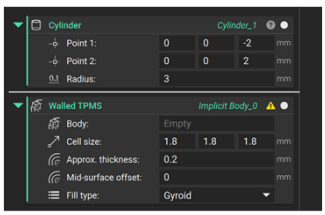
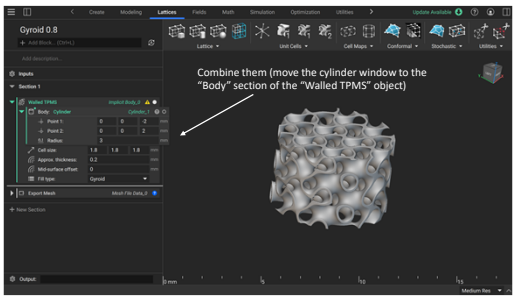


The shape (a cylinder in our case) can be determined by the points in space (x, y, and z axis) and its radius

There are preinstalled TPMS types (Gyroid, Diamond, Primitive, and some others) that can be chosen; the size of the cell unit as well as the thickness of the walls can be changed

nTop



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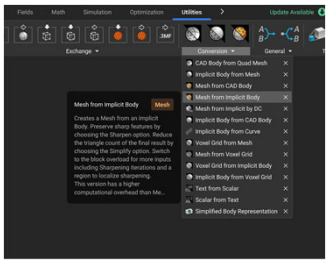
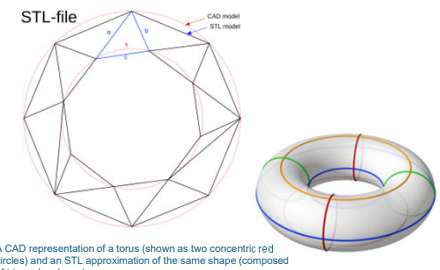


To create a .stl file for the printer's slicer, we need to convert the resulting object to the mesh. The conversions between the mesh and bodies are available under the "Utilities" section

Wikipedia

STL is a file format native to the stereolithography CAD software created by 3D Systems.[3][4][5] Chuck Hull, the inventor of stereolithography and 3D Systems' founder, reports that the file extension is an abbreviation for *stereolithography*.[6]
An STL file describes a raw, unstructured triangulated surface by the unit normal and vertices (ordered by the right-hand rule[7]) of the triangles using a three-dimensional Cartesian coordinate system.[7]

3 StereoLithography Interface Specification, 3D Systems, Inc., July 1988
4 StereoLithography Interface Specification, 3D Systems, Inc., October 1989
5 SLC File Specification, 3D Systems, Inc., 1994
6 Grimm, Todd (2004). "3. The Rapid Prototyping Process". User's Guide to Rapid Prototyping. Society of Manufacturing Engineers. p. 55. ISBN 0-87263-697-6.
7 Burkardt, John (2014-07-10). "STLA Files - ASCII stereolithography files". Retrieved 2022-05-30.



A CAD representation of a torus (shown as two concentric red circles) and an STL approximation of the same shape (composed of triangular planes)

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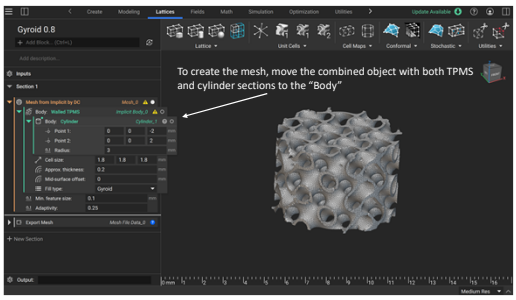

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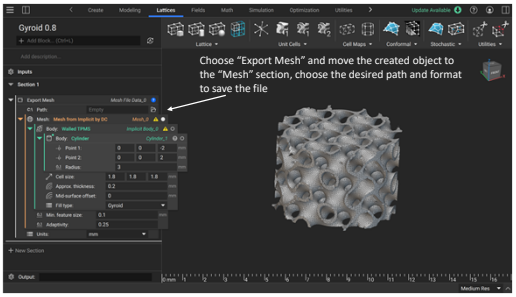
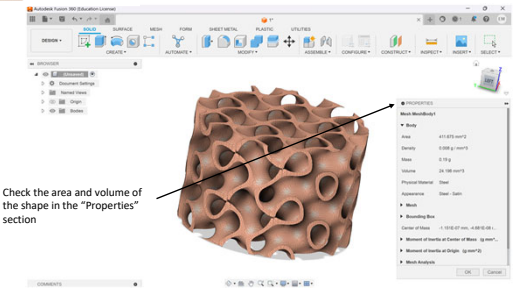
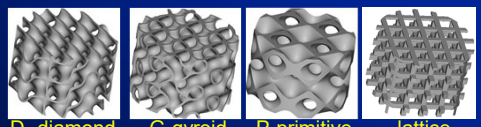


AUTODESK
Fusion 360

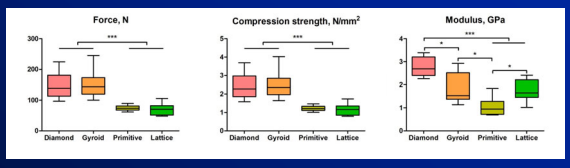


TPMS-lightweight microarchitectures

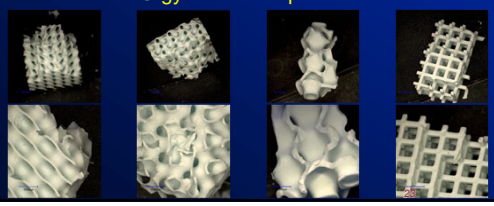


	D- diamond	G-gyroid	P-primitive	lattice
Porosity:	82 %	79%	69%	83%
Surface area/vol.	21 $\frac{1}{mm}$	20 $\frac{1}{mm}$	9 $\frac{1}{mm}$	13 $\frac{1}{mm}$
Bottle neck percolation	0.8 mm	0.8 mm	0.8 mm	0.8 mm

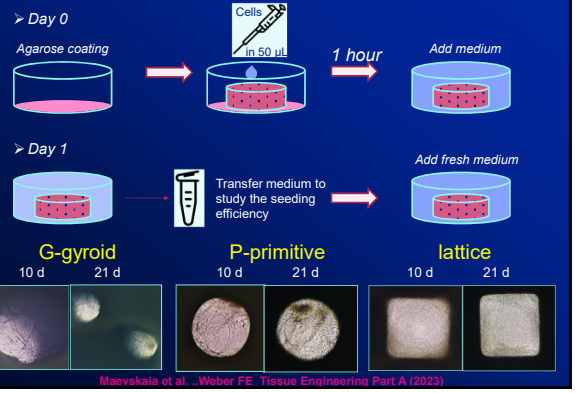
TPMS-lightweight microarchitectures mechanics



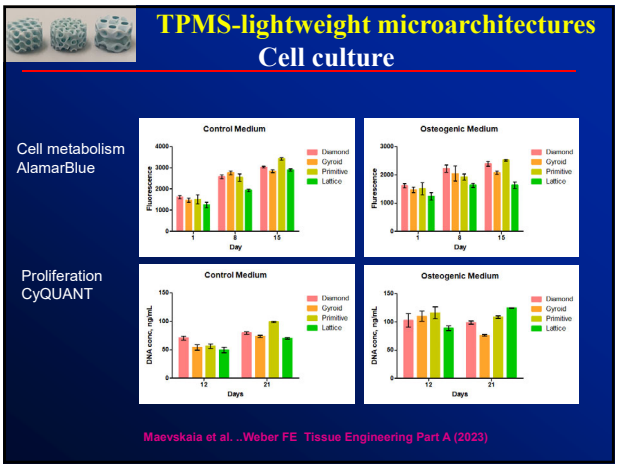
D- diamond G-gyroid P-primitive lattice

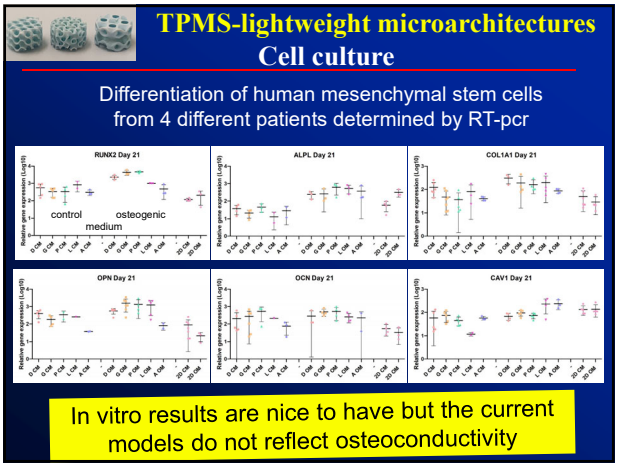


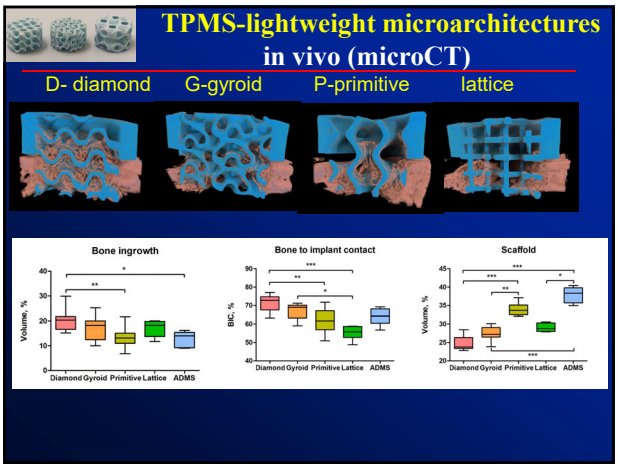
TPMS-lightweight microarchitectures Cell culture



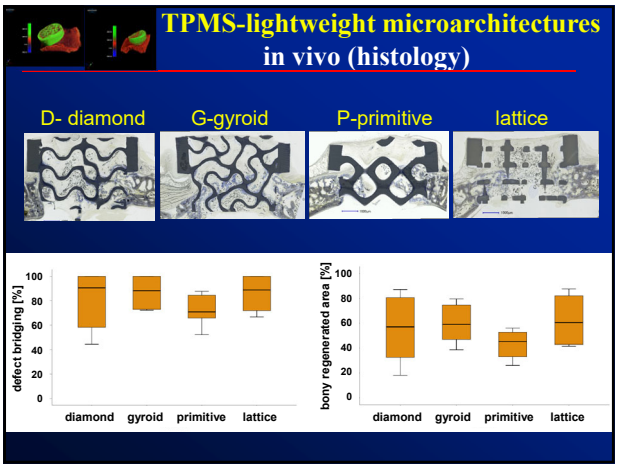
Maevskaia et al. ..Weber FE Tissue Engineering Part A (2023)


TPMS-lightweight microarchitectures
Cell culture
Cell metabolism
AlamarBlue
Proliferation
CyQUANT
Control Medium
Osteogenic Medium
Diamond
Gyroid
Primitive
Lattice
Fluorescence
Day
DNA conc, ng/mL
Days
Maevskaia et al. ..Weber FE Tissue Engineering Part A (2023)


TPMS-lightweight microarchitectures
Cell culture
Differentiation of human mesenchymal stem cells
from 4 different patients determined by RT-pcr
RUNX2 Day 21
ALPL Day 21
COL1A1 Day 21
OPN Day 21
OCN Day 21
CAV1 Day 21
control
osteogenic
medium
In vitro results are nice to have but the current
models do not reflect osteoconductivity

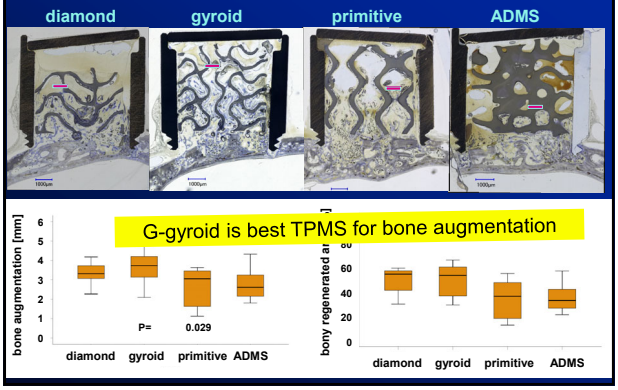

TPMS-lightweight microarchitectures
in vivo (microCT)
D- diamond
G-gyroid
P-primitive
lattice
Bone ingrowth
Bone to implant contact
Scaffold
Volume, %
BIC, %
Diamond Gyroid Primitive Lattice ADMS

TPMS-lightweight microarchitectures in vivo (histology)



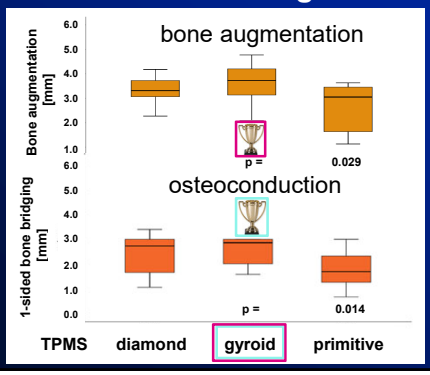
Bone augmentation

periodic minimal surface microarchitectures

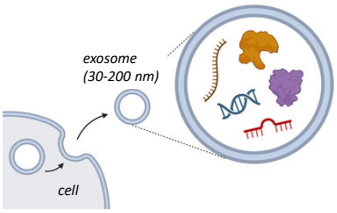


Augmentation/osteoconduction

TPMS-designs



Exosomes



- Low immunogenicity and toxicity
- Biocompatibility
- Stability
- Less strict storage condition

- Long waiting times due to cell expansion
- Large doses

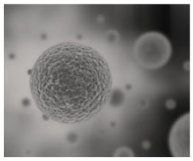
- Cargo
- Optimal source
- Dosage
- Duration of treatment

The goal is the development of ceramic-based bone grafts functionalized with exosomes

Strategies:

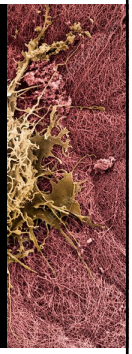
1. Scaffold design

2. Functionalization of scaffolds

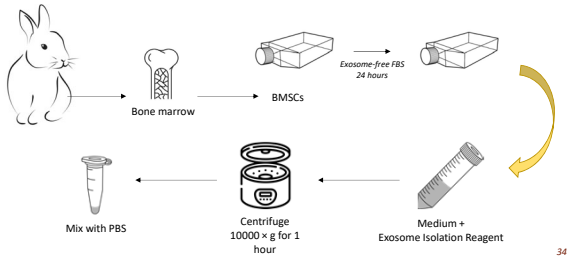


Functionalization of scaffolds with BMSCs exosomes

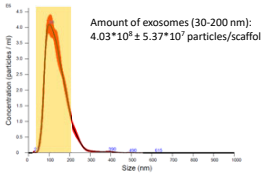
- Characterization of cells and exosomes
- Interaction of cells with exosomes
- Interaction of cells with exosomes within scaffolds
- Implantation of scaffolds with exosomes



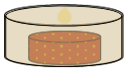
Extraction of BMSCs and exosomes

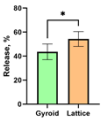


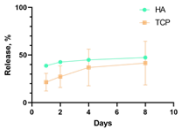
Nanoparticle tracking analysis



Amount of exosomes (30-200 nm):
$4.03*10^8 \pm 5.37*10^7$ particles/scaffold







➢ Slower release with *Gyroid* and TCP scaffolds

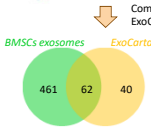
Maevskaia E *et al.* Int. J. Mol. Sci. **2024**

Proteomics analysis

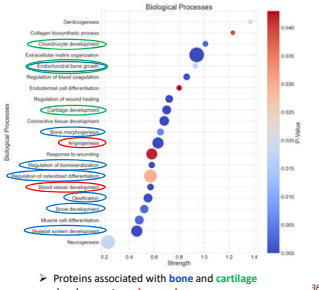
Functional Genomics Center Zurich
by LC-MS/MS DIA method

Bioinformatics assay

Comparison with
ExoCarta website



➢ Proteins were extracted from exosomes



➢ Proteins associated with **bone** and **cartilage** development, **angiogenesis**

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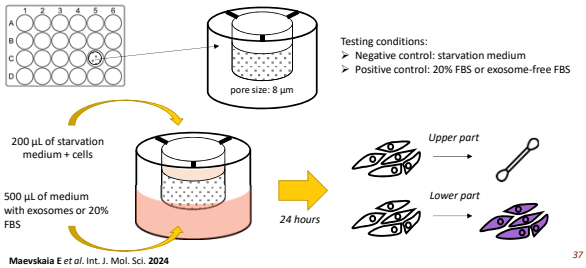
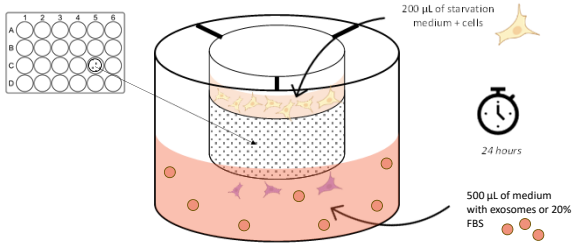
Migration of BMSCs



Testing conditions:
- Negative control: starvation medium
- Positive control: 20% FBS or exosome-free FBS

Maevskaia E *et al.* Int. J. Mol. Sci. **2024**

Migration of BMSCs



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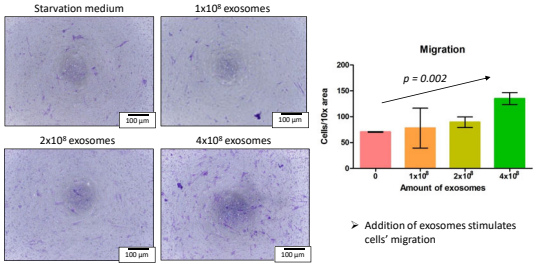
Migration of BMSCs



- Addition of exosomes stimulates cells' migration

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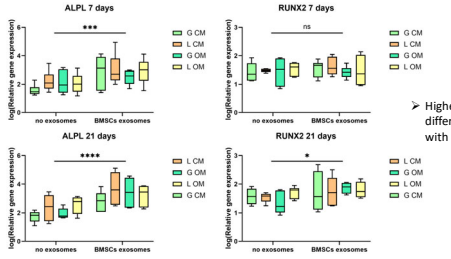
Differentiation of BMSCs with exosomes



- Higher and faster differentiation of BMSCs with exosomes

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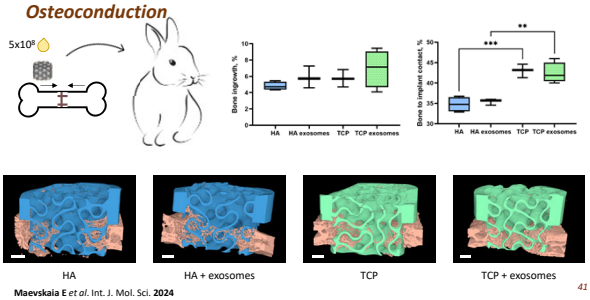
Osteoconduction



HA | HA + exosomes | TCP | TCP + exosomes

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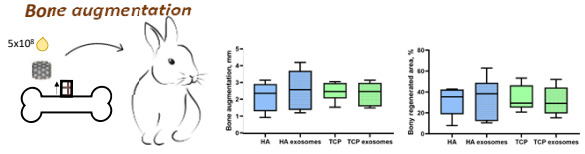
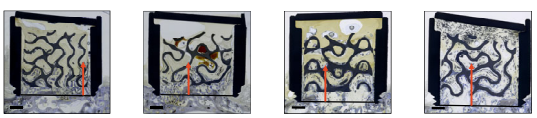
Bone augmentation





HA | HA + exosomes | TCP | TCP + exosomes

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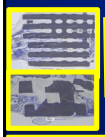
Conclusion of the second part

- Slower release of exosomes from **TCP** and ***Gyroid*** scaffolds
- Proteins associated with bone development
- Earlier differentiation of cells cultured with exosomes
- Higher bone to implant contact with **TCP** scaffolds

Maevskaia E, Guerrero J, Ghayor C, et al. Functionalization of Ceramic Scaffolds with Exosomes from Bone Marrow Mesenchymal Stromal Cells for Bone Tissue Engineering. International Journal of Molecular Sciences 2024. 25(7):3826. https://doi.org/10.3390/ijms25073826

Microarchitecture orchestrates osteoconduction and bone augmentation



Reduction of filament dimension and distance from 1.25 to 0.50 mm induces early stage differentiation and angiogenesis and translates into osteoconductivity

The choice of the microarchitecture matters for osteoconduction and bone augmentation. Gyroid and diamond appear superior.

Strait and curvy microarchitectures can be highly osteoconductive. No need for biomimetics

Gyroid and diamond combine good osteoconductivity and high mechanics of scaffold at minimal surface
=minimal material and minimal problems with degradation

Principles of bone tissue engineering

Biomaterial Scaffold

Microarchitecture is a key factor for optimized osteoconduction and bone augmentation

can be realized and studied by additive manufacturing

Additive manufacturing will be center stage for personalized bone substitutes
in
Cranio-maxillofacial Surgery
Orthopedics
Dentistry


University Hospital Zurich
Center of Dental Medicine
Thank you
Center of Dental Medicine
Cranio-Maxillofacial and Oral Surgery
Oral Biotechnology & Bioengineering
Zurich, Switzerland
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Christian Waldvogel
Daniel Salamon
Niklaus Arn
for your attention