High-resolution x-ray diffraction microscopy
applied to engineered bone study

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OUTLINE

- **Scientific problem**
  - Engineered bone sample
  - Opened scientific problems

- **Set-up**

- **Qualitative analysis**
  - SAXS and WAXS combination
  - Mineralization distribution

- **Quantitative analysis**
  - Bone-Scaffold interaction
Scientific Problem: Sample

1. Bone marrow harvest
2. Amplification of osteoprogenitor cells
3. Loading on bioceramic sub.

Removed sample

In vivo subcutaneously implant

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AIM of X-ray $\mu$-diffraction measurements is the determination of:

- grain shape and orientation of new bone
- bone growth mechanism
- bone-scaffold interaction
X-ray diffraction microscopy

standard diffraction + sub-micron beam

Micrometer-resolution mapping (or imaging)

of atomic structural information
Scanning micro-diffraction set-up

Simultaneous acquisition of WAXS and SAXS

ID13 at ESRF

Beam size: 

WG: 3 μm, 0.3 μm
KB: 1 μm

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100 µm

ST

Scaf

NB

air
HA (scaffold)

Spotty rings

Large grains (50 nm)

new bone (HA)

HA reflections:
Continuous rings

Small grains (3-5 nm)

Soft tissue

Diffuse scattering

Amorphous organic tissue

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Qualitative analysis
New bone – Orientation at pore surface

Azimuthal intensity

WAXS

[002]
New bone – Orientation at pore surface

Unit cell

WAXS from [002]

Pore surface

HA

NB

ST

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Digital zoom at small angles of diffraction pattern to obtain SAXS information in the same sample point.
New bone – Orientation at pore surface

Grain

Anisotropy of SAXS shows that the elongated shape of bone mineral particles are non-random distributed around the pore surface (// to the c axis).
New bone – Growth mechanism

From WAXS and SAXS combination:

The collagen fibers (bone seed) are oriented parallel to the pore surface.

The bone mineral crystals are elongated along the collagen fibers with the c-axis parallel to the elongation direction.

AS FOR NATURAL BONE

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Quantitative analysis
Study of a new scaffold composed of two different phases: HA and TCP

The final goal of the Tissue Engineering is the replacement of the scaffold with the engineered tissue.

We look for the best re-absorbable scaffold.

The study of the Interaction scaffold-bone is necessary.
SAXS intensity distribution:
High resolution spatial distribution of bone mineral crystals
After implantation the scaffold is strongly modified and a large amount of new bone is formed. What happens?

Depletion of TCP scaffold phase when bone increases
Conclusions

- grain shape and orientation of new bone

- bone growth mechanism

- Interaction bone-scaffold

Good degree of organisation

Orientation of the collagen fibers and c-axis as for natural bone

In (HA+TCP) scaffold strong depletion of the TCP promotes bone formation