# Micro-CT as an evaluation tool for first line screening of the bone forming capacity of human periosteum-derived cells in nude mice

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## Aims

Large bone defects due to tumor resection and trauma require extensive surgical therapy. The "gold standard" to treat these large defects is the use of autologous bone grafts (1). Autologous bone chips are harvested from the iliac crest and transplanted into the defect. The drawbacks in using this include the limitation of autologous bone available, donor site morbidity as well as the risk for incomplete graft integration and graft resorption when the defect exceeds more than 5 cm. This has led to the development of novel methods based on Tissue Engineering (TE).

One of the promising bone TE approaches is the use of osteo/chondro-progenitor cells in combination with 3D open porous biomaterials, i.e. 3D scaffolds (2). In a study of Roberts *et al.* (2) and Perka *et al.* (3), the bone forming capacity of periosteum-derived cells (PDCs) are identified as potential cell source for bone TE. Human PDCs (hPDCs) were seeded onto a 3D scaffold composed of calcium phosphate (CaP) particles in an open collagen network and evaluated for their capacity to form bone in an *in vivo* ectopic mouse model as a first line screening model for their quality assessment. To enable a fast, reliable and quantitative assessment, the explanted cell-scaffold combinations are evaluated using microfocus X-ray computed tomography, i.e. microCT (4) to visualize and quantify the newly formed bone volume, and provide real 3D information. In the study of Roberts *et al* (2), it was shown that the volume of newly formed bone analyzed by microCT corresponds well to the histomorphometric results, still used as 'gold standard' for evaluating the bone forming capacity of cell/scaffold combinations. As an additional benefit of micro-CT, the information gathered from the 3D image analysis can be produced within one day instead of at least 3 weeks when using histology and histomorphometric 2D analysis.

The aim of this study was twofold: to use micro-CT as a quality assay to (i) evaluate whether NuOss™ can be used to direct bone formation of any progenitor population by testing the best performing CaP scaffold from the study of Roberts *et al.* (2), namely NuOss™, with other alternative progenitor populations than the hPDCs (i.e. **cell type-specific variability**) and (ii) screen different individual hPDCs by seeding them onto NuOss™ to evaluate the **donor-specific variability**.

## Materials and methods

To assess the cell type-specific variability, two different alternative progenitor populations were used in comparison with hPDCs (coming from 3 individual donors), namely (i) human synovium derived cells (hSDCs) from 3 individual donors and (ii) human mesoangioblasts (hMES) from 2 individual donors. All cells were screened in the in-house standard ectopic nude mouse assay and evaluated by microCT to evaluate their direct bone formation on

NuOss™. To evaluate the patient-specific variability, the bone forming capacity of 15 individual hPDC donors seeded onto NuOss™ was quantified and compared.
For all experiments, one million of cells were seeded onto a 21 $mm^3$ cylindrical 3D scaffold. Allowing cell attachment, the cell-scaffold combinations were incubated overnight at 37°C. The next day, the constructs were implanted in duplicate subcutaneously in the back of NMRI-nu/nu mice. After 8 weeks, the constructs were explanted and immediately fixed in 4% paraformaldehyde, and scanned using high-resolution micro-CT on a Skyscan 1172 system [Skyscan NV, Kontich, Belgium] at an isotropic voxel size of $(4.5\ \mu m)^3$. Since each construct had a similar composition, for all explants a source voltage and current of 60kV and 167 µA respectively and a filter of 0.5mm Al were applied. Using a rotation step of 0.3° over a total of 180° resulted in 640 radiographic images. After reconstruction using dedicated software [NRecon, Skyscan NV, Kontich, Belgium], a total of about 900 greyscale axial micro-CT images per sample were generated. Manual, but consistent global segmentation of the newly formed mineralized tissues from the CaP particles present in the explants was carried out based on the greyscale histogram to allow quantification of parameters such as the volume fraction of newly formed bone within the available scaffold volume using CTAn [Skyscan NV, Kontich, Belgium].

## Results

- Cell type-specific variability

MicroCT image analysis of the explants revealed between the different cell types (fig. 1). For both the hMESs and the hSDCs, a limited amount of bone (on average 6.10 % and 7.45 % respectively) and only small bone fragments lying adjacent to the CaP particles were observed. The hPDCs resulted in a larger amount of newly formed bone (on average 12.68 %) compared to the hMESs and the hSDCs. This showed that (i) hPDCs perform well in NuOss™, as was also shown in Roberts *et al.* (2) and (ii) the bone forming capacity of a specific 3D scaffold is highly cell type-dependent. Thus, in order to optimize the scaffold design and characteristics, one always needs to evaluate the cell/scaffold combination rather than only focusing on one of both.
A large standard deviation on the results of the hPDCs can be noticed in fig. 1. This implies that, even within the same cell type, a large variability in bone forming capacity can be present.

- Donor-specific variability

MicroCT images of the explants of the different hPDCs donors revealed, for each individual donor, bone spicules in between the CaP particles or in close relation to these particles (fig.3). However, quantification of the volume fraction of the newly formed bone in the available scaffold volume revealed a large spread between the individual donors, which shows the importance of individual donor screening as it influences donor selection in function of the release criteria (fig.2). Within the screened hPDCs a group of donors expressed a bone forming capacity above the 10 % threshold that showed a significantly higher percentage of newly formed bone (up to 13.99 % on average) (fig.3A), compared to another group of donors that only expressed a limited amount of newly formed bone (average of 8.61 %) which is below the 10 % threshold (fig.3B).

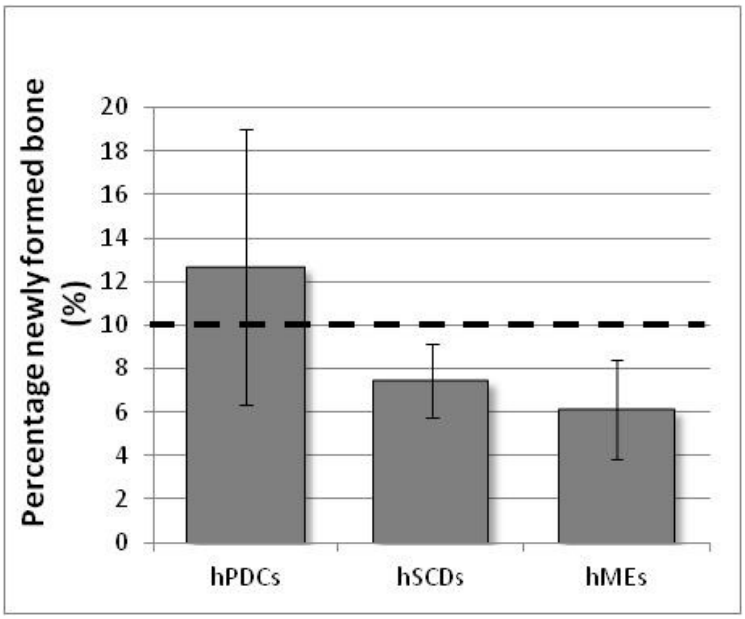


Fig. 1: The percentage of newly formed bone for the different cell types (n = 2 or 3 per cell type). 10% of newly formed bone is defined as the threshold above which the donors are assigned as having a good bone forming capacity.

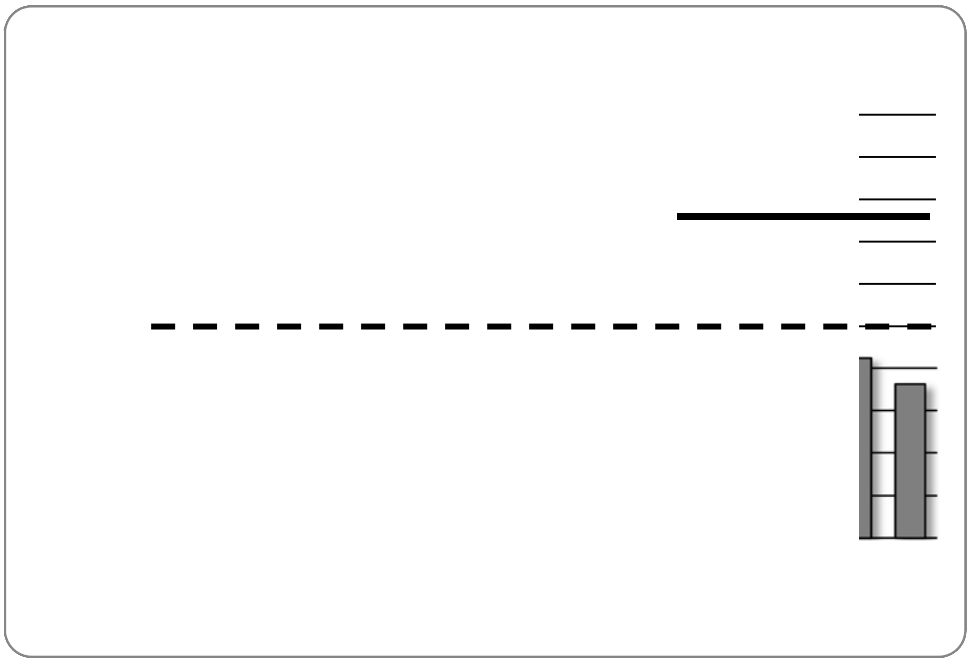

Fig. 2: The percentage of newly formed bone for the different donors. 10% of newly formed bone is defined as the threshold above which the donors are assigned as having a good bone forming capacity. * $p < 0.05$ = significantly different.

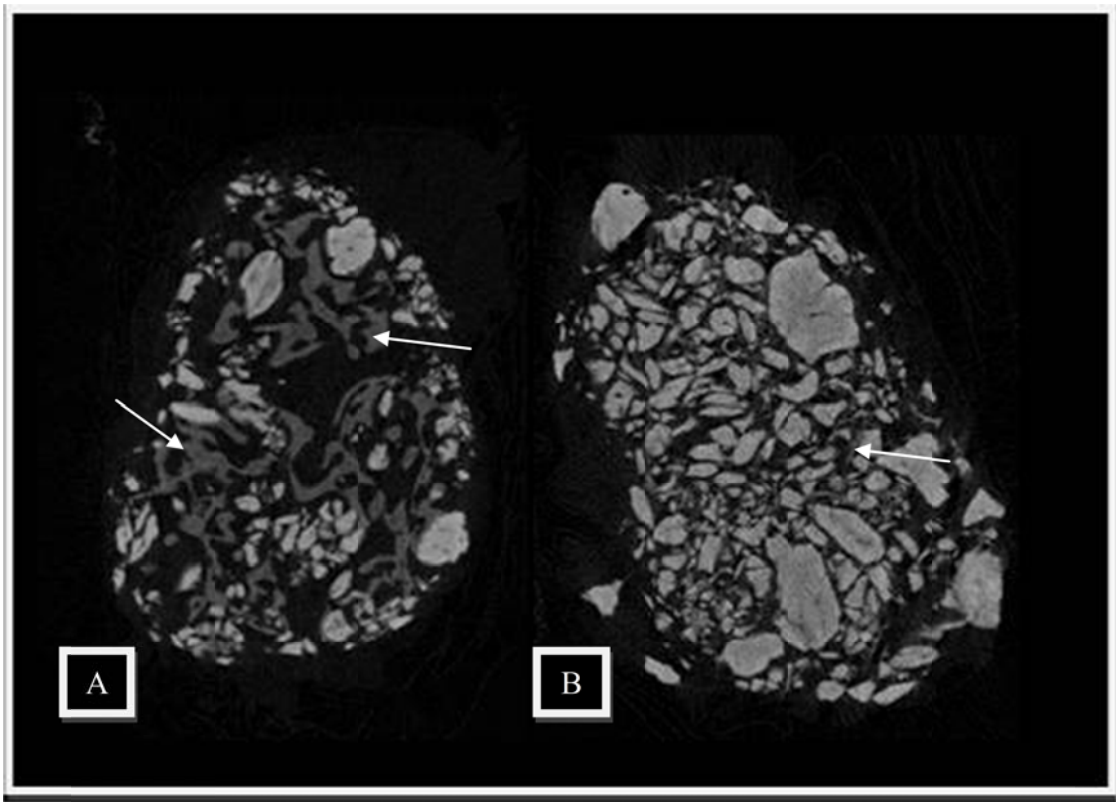


Fig 3: Two different hPDC donors screened by microCT. One donor has a large amount of newly formed bone in between the CaP particles (A). Another donor has a very limited amount of bone spicules in between CaP particles (B).

## Conclusion

MicroCT image analysis of explanted cell-scaffold combinations is a very powerful quantitative first-line screening tool to investigate the cell type-specific and donor-specific variability of their bone forming capacity. Using 3D micro-CT image analysis, it was shown that hPDCs can form a significant amount of bone between and in close contact with the CaP particles of NuOss™, while hMESs and hSDCs did not perform well within NuOss™. This indicates the importance of a proper interplay between cells and scaffolds to obtain a relevant biological outcome. On the other hand, micro-CT image analysis also revealed a large spread in bone forming capacity within one specific cell type (i.e. hPDCs) depending on the donor specificities.

## References:

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