FULL PAPER

Iodine doping of a photocuring, one-component embedding medium for \( \mu\)CT and histology

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Objective: Evaluation of \( \mu\)CT scans of bone implant complexes often shows a specific problem: if an implant material has a very similar radiopacity as the embedding medium (e.g. methacrylate resin), the implant is not visible in the \( \mu\)CT image. Segmentation is not possible, and especially osseointegration as one of the most important parameters for biocompatibility is not evaluable.

Methods: To ensure \( \mu\)CT visualisation and contrast enhancement of the evaluated materials, the embedding medium Technovit® VLC7200 was doped with an iodine monomer for higher radiopacity in different concentrations and tested regarding to handling, polymerisation, and histological preparation, and visualisation in \( \mu\)CT. Six different \( \mu\)CT devices were used and compared with regard to scan conditions, contrast, artefacts, image noise, and spatial resolution for the evaluation of the bone-implant blocks.

Results: Visualisation and evaluation of all target structures showed very good results in all \( \mu\)CT scans as well as in histology and histological staining, without negative effects caused by iodine doping. Subsequent evaluation of explants of in vivo experiments without losing important information was possible with iodine doped embedding medium.

Conclusion: Visualisation of implants with a similar radiopacity as the embedding medium could be considerably improved. \( \mu\)CT scan settings should be selected with the highest possible resolution, and different implant materials should be scanned individually for optimal segmentation. \( \mu\)CT devices with higher resolutions should be preferred.

Advances in knowledge: Iodine doped embedding medium is a useful option to increase radiopacity for better visualisation and evaluation of special target structures in \( \mu\)CT.
three-dimensional representation. However, there is no representa-
tion of the cellular level (e.g. foreign body responses, granulocytes, 
multinucleated giant cells), so a combination with cutting-grinding 
technique for histological sections of undecalcified bones (including 
ceramic and metallic implants) is recommended.

Using µCT, the quantitative evaluation of bone replacement mate-
rials depends on their X-ray absorption. Materials with higher X-ray 
absorption can be displayed well if their radiopacity and material 
thickness are not too high for the available X-ray energy spectrum 
to produce artefacts. On the other hand, radiopacity of the implant 
materials should differ from that of the surrounding tissues, in particular for segmentation. Some implant materials, especially made 
from specific polymers, show the same or a very similar radiopacity 
as the embedding medium. Their absorption of X-radiation is very 
low, so they cannot be visualised in µCT images if embedded in a 
medium used for hard tissue histology/cutting-grinding technique (fig. 1). This leads to the fact that especially the osseointegration as 
one of the most important parameter for biocompatibility is not 
evaluable.

To enable the assessment and analysability of methacrylate polymer 
implants, the embedding polymer used for the hard tissue histology 
was chemically modified, analogous to the addition of bromine or 
barium to bone cements. 

For the purpose of the present study, a polymerising iodine monomer 
was prepared and added in different concentrations to the embedding 

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Figure 1. 3D µCT image of an embedded bone-implant complex in Technovit® 7200 VLC (Heraeus Kulzer GmbH, Wehrheim, Germany). Upper left: partially degraded TCP (tricalcium phosphate) implant, upper right and lower left: composite implant, lower right: polymer implant (arrow, not visible or even segmentable because of similar radiopacity of the embedding medium).

Figure 2. Development of the different iodine monomer concentrations of the iodine-doped Technovit® 7200 VLC. DMSO, dimethyl sulfoxide; wt%, weight percent.

| Pre-tests | Concentration study |
|-----------|---------------------|
| 5 [wt%] TIP (5 g iodine compound, solved in 1 ml DMSO, filled up to 100 ml with Technovit) | 0.45 [wt%] (450 mg MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) |
| 1.8 [wt%] MIM monomer + 200 µl DMSO (1.8 g MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) | 0.3 [wt%] (300 mg MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) |
| 0.9 [wt%] MIM (900 mg MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) | 0.2 [wt%] (200 mg MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) |
| 0.45 [wt%] MIM (450 mg iodine MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) | 0.15 [wt%] (150 mg MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) |
| 1.8 [wt%] (1.8 g MIM, solved in 1.5 ml DMSO, filled up to 100 ml with Technovit) | |
| 0.9 [wt%] (900 mg MIM, solved in 1 ml DMSO, filled up to 100 ml with Technovit) | |

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\[\text{Figure 2. Development of the different iodine monomer concentrations of the iodine-doped Technovit® 7200 VLC. DMSO, dimethyl sulfoxide; wt%, weight percent.} \]
medium Technovit® 7200 VLC (Heraeus Kulzer GmbH, Wehrheim, Germany), a photocuring one-component methacrylate-based resin. The study includes evaluation of the iodine monomer doping of the radiopaque embedding medium, evaluation of handling and properties of the modified embedding medium (infiltration, polymerisation/curing, histological methods), μCT scanning using different μCT devices, visualisation of methacrylate polymer implants as well as possible effects on the visualisation of other implant materials or tissues embedded in the modified medium. Further, image quality of the different devices, based on image contrast, spatial resolution, image noise, and artefacts, was evaluated.7,8

METHODS AND MATERIALS

2.1 Doping with iodine

The polymerising iodine monomer was synthesised using a previously reported procedure.9 Briefly, the iodine compound, 2,4,6-triiodophenol (TIP, Sigma-Aldrich Chemie, Taufkirchen, Germany) and dibutyltin dilaurate (DBTL, Sigma-Aldrich Chemie, Taufkirchen, Germany) were dissolved in dichloromethane (Sigma-Aldrich Chemie, Taufkirchen, Germany) and dibutyltin dilaurate (DBTL, Sigma-Aldrich Chemie, Taufkirchen, Germany) were dissolved in dichloromethane (Sigma-Aldrich Chemie, Taufkirchen, Germany) at room temperature under a nitrogen atmosphere. 2-isocyanatoethyl methacrylate (IEM, Sigma-Aldrich Chemie, Taufkirchen, Germany), dissolved in dichloromethane, was slowly added, and the reaction mixture was stirred for 4 h at 50°C. After cooling to room temperature, the solvent was evaporated with a rotary evaporator. The residue was washed several times with petroleum ether and dried under vacuum at ambient temperature to isolate the methacrylated iodine monomer (MIM). The monomer was characterised in the usual way by FTIR and NMR spectroscopic techniques.

During various pre-tests, the iodine monomer was added to pure embedding medium Technovit VLC 7200 (Heraeus Kulzer GmbH, Wehrheim, Germany) in different concentrations, partially with additives (Figure 2).

In the pre-tests, the addition of the iodine compound in a concentration of 5 [wt%] TIP formed brownish viscous supernatants during polymerisation. Here, the iodine concentration was too high. The embedding medium appeared too bright in a test scan. The subsequent polymerisation of 5 [wt%] TIP formed brownish viscous supernatants, which led to exclusion of the pre-tests and further evaluation. The iodine concentrations of 1.8 [wt%] and 0.9 [wt%] MIM without additives were still too high in test scans and were also excluded.

The four lowest iodine monomer concentrations without initiator (0.45, 0.30, 0.20, and 0.15 [wt%]) were used for further extensive tests of the respective properties, including embedding, infiltration and polymerisation, and eventually the radiopacity of specifically made test specimens with different implant materials by evaluation with μCT.

The quantitative evaluation of the optimal iodine monomer concentration for the intended application was based on a developed score (see Table 1), from 1 (not suitable) to 3 (well suited). The overall evaluation could reach a value up to a maximum of 6.

2.2 Test specimens

Based on the Jena skull model, corresponding test specimens were produced, made from a combination of bone and various implant materials. Freeze-dried pieces of porcine Os nasale (without soft tissue) in two different sizes were the basis of the test specimens (approx. 2 x 2 cm for four implants, and approx. 2 x 1 cm for two implants), sawn with a bone cutting disc. Defects were made with a trephine drill (diameter 5 mm) to guarantee an exact fitting of the implants which consisted of four different

Table 1. Score to assess the suitability of iodine monomer-doped Technovit® 7200 VLC (iodine monomer concentrations 0.45, 0.30, 0.2, 0.15 [wt%]) for the visualisation of embedded materials by μCT scanning

| Score value for visualisation | Specification |
|-------------------------------|---------------|
| 1                             | Not suitable - poor visualisation of one or more implant materials |
| 2                             | Suitable - limited visualisation of one or more implant materials |
| 3                             | Well suited – good visualisation of all implant materials |

Table 2. Manufacturer specifications of the six different high resolution μCT systems, used for scanning the test specimens

| Trade name | Producer | Type | X-ray source | Detector |
|------------|----------|------|--------------|----------|
| SkyScan® 1178 | Bruker-microCT, Belgium | In vivo scanner | 40 W, 20–65 kV | Digital 12 bit X-ray camera, 1280 x 1024 Pixel |
| Tomoscope® Synergy Twin | CT Imaging, Germany | In vivo scanner | Two microfocus tubes, 20–65 kV | Hamamatsu flat panel detector |
| Fraunhofer CT portable | Fraunhofer EZRT Fürth, Germany | Portable system | 50 W, 50 kV | DALSA, RadEye 2 X-ray camera |
| SkyScan® 1173 | Bruker-microCT, Belgium | High resolution laboratory μCT system | 40–130 kV, 8 W X-ray source | 12 Bit 2240 x 2240 pixel flat-panel detector |
| Fraunhofer Sub-μCT | Fraunhofer EZRT, Fürth, Germany | High resolution laboratory μCT system | Yxlone FXE trans-mission tube with 64 W, max. 160 kV | Paxscan® 4343CB (Varian Medical Systems, Palo Alto, USA) |
| phoenix nanotom | GE Sensing & Inspection Technologies GmbH, Germany | High resolution laboratory μCT system | 180 kV, 15 W high-power nanofocus tube | GE DXR detector |
Table 3. Scan parameters of the six µCT devices for different configurations of the test specimens (implant materials)

|                      | Tomoscopy® Synergy Twin | Dynamic scan | SkyScan® 1178 | Framhofer Cpartable | SkyScan® 1173 | Framhofer sub-µCT | phoenix nanotom® m |
|----------------------|-------------------------|--------------|--------------|---------------------|--------------|-------------------|--------------------|
| X-ray source (1/2)   |                         |              |              |                     |              |                   |                    |
| Tube voltage         | 65 kV / 40 kV           | 65 kV / 40 kV| 65 kV / 40 kV| 65 kV / 40 kV       | 65 kV / 40 kV| 65 kV / 40 kV     |                    |
| Tube current         | 450 µA / 1000 µA        | 450 µA / 1000 µA | 450 µA / 3000 µA | 450 µA / 1000 µA | 450 µA / 1000 µA | 450 µA / 1000 µA |                    |
| Filter               | 0.5 mm Al               | 0.5 mm Al    | 0.5 mm Al    | 0.5 mm Al           | 0.5 mm Al    | 0.5 mm Al         | 0.5 mm Al          |
| detector             |                         |              |              |                     |              |                   |                    |
| Exposure time        | 40 ms                   | 40 ms        | 40 ms        | 40 ms               | 40 ms        | 40 ms             | 40 ms              |
| Image resolution     | 73 µm                   | 73 µm        | 73 µm        | 82 µm               | 82 µm        | 82 µm             | 82 µm              |
| Image matrix         | 484 x 477               | 488 x 469    | 366 x 329    | 367 x 330           | 367 x 330    | 367 x 330         | 367 x 330          |
| Number of images     | 424                     | 392          | 360          | 360                 | 360          | 360               | 360                |
| Scan                 |                         |              |              |                     |              |                   |                    |
| Rotation step        | 0.514°                  | 0.514°       | 0.514°       | 0.503°              | 1.080°       | 1.080°            | 1.080°             |
| Averaging            | no                      | no           | no           | no                  | no           | no                | no                 |
| Scan time            | 28.8 s                  | 28.8 s       | 28.8 s       | 2 min 24 s          | 1 h 2 min    | 1 h 19 min        | 53 min             |
materials: methacrylate polymer (pure, hardened in a silicone mould), a composite material (base material: 3D printed and sintered tricalcium phosphate, infiltrated and hardened with the methacrylate polymer), as well as medical-grade titanium (Ti 6Al-4V ELI), and the bioglass-ceramic Bioverit®, both CNC milled. The implants were inserted in various combinations into the bone pieces (Bioverit®-Titanium, Composite-Polymer etc.).

Further test specimens were pieces of rat tibiae with surrounding soft tissue (fixed in 5% formalin, without implants), which were kindly provided by Experimental Trauma Surgery, University Hospital Jena. In contrast to the test specimens with implants, these specimens consisted of intact bone and soft tissue, including bone marrow, muscles, tendons, and connective tissue, and were used for the suitability of the cutting/grinding process as well as the histological stainings.

2.3 Embedding of the test specimens
The test specimens (bone with implants) were initially fixed in 5% formalin for 2 weeks, then dehydrated by ethanol solutions of increasing concentration, followed by infiltration and embedding with the iodine monomer-doped Technovit® 7200 VLC, a light-curing one-component poly(methyl methacrylate) (PMMA) resin (Heraeus Kulzer GmbH, Wehrheim, Germany). All four iodine monomer concentrations (0.45, 0.30, 0.2, 0.15 [wt%]) were used.

The formalin-fixed rat tibia pieces with surrounding soft tissue were also dehydrated and embedded in the four concentrations of iodine doped Technovit® 7200 VLC. The subsequent handling tests of the resin blocks of the modified embedding medium included sawing with a diamond bond saw (EXAKT GmbH, Norderstedt, Germany), and grinding and polishing with a micro grinding system (EXAKT GmbH, Norderstedt, Germany) to a thickness of 5–15μm. The polished thin sections were stained with hematoxylin-eosin staining or modified Masson-Goldner staining.

2.4 µCT scanning
The bone-implant test specimens were scanned in six different µCT devices. We used the two in vivo scanner SkyScan® 1178 (Bruker-microCT, Kontich, Belgium) and Tomoscope® Synergy Twin (CT Imaging GmbH, Erlangen, Fürth, Germany) and the portable µCT scanner Fraunhofer CTportable (Fraunhofer EZRT, Fürth, Germany). As high resolution lab µCT systems, we used SkyScan® 1173 (Bruker-microCT, Kontich, Belgium), Fraunhofer Sub-µCT (Fraunhofer EZRT, Fürth, Germany), and Phoenix nanotom (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). The µCT systems were compared regarding X-ray tube/performance, voltages, detector, and voxel size/spatial resolution (manufacturer specifications in Table 2, used scan parameters in Table 3).

The image quality of the different devices was evaluated by scoring image contrast, spatial resolution, image noise, and artefacts (Table 4). The score values range from 0 (poor/no contrast, evaluation not possible) up to 3 (excellent contrast). Intermediate values (e.g. 1–2) were achieved by different score results depending on the implant materials. The maximum reachable score was 12.

2.5 Visualisation and processing of the µCT data
2.5.1 Used hardware and software
Visualisation and evaluation of the µCT data were performed under MS Windows 7 (Microsoft Corp., Redmont, WA, USA) with a Xenon (INTEL Corp., Santa Clara, CA, USA) based workstation, equipped with 48GB ECC RAM and a Quadro 4000 (Nvidia Corp., Santa Clara, CA, USA) graphics card.

The 2D morphology software “HistoGap” was implemented using the C++ compiler of GNU Compiler Collection 9.2 (The GNU Project) with Qt 5.13 (Qt Group Oyj, Espoo, Finland) as framework for graphical user interface. The MATLAB script for morphometric assessment of segmented 3D images was created and run using MATLAB v.19 (The MathWorks, Inc., Natick, MA, USA).

Table 4. Scoring system for image quality, ranging from 0 to 4

| Score | Image contrast | Spatial resolution/small structures | Image noise | Artefacts |
|-------|---------------|------------------------------------|-------------|-----------|
| 0     | Poor/no contrast, evaluation not possible | Poor/no details, evaluation not possible | High level of image noise, evaluation not possible | High level of artefacts, evaluation not possible |
| 1     | Moderate contrast, evaluation is limited | Low level of detail, evaluation is limited | Moderate level of noise, evaluation is limited | Strong artefacts, evaluation is limited |
| 2     | Good contrast | Moderate level of detail | Low level of noise | Moderate artefacts |
| 3     | Excellent contrast | High level of detail | Minimal noise | Minimal or no artefacts |
2.5.2 Avizo® fire 8.0
Avizo® Fire (FEI Visualization Sciences Group, Bordeaux, France) is a commercial 3D data processing and visualisation software that can display and edit data in various data formats. The software has a graphical user interface for automated image processing. The data records can be viewed, edited, and relevant structures or materials can be segmented. Meanwhile, the software has been updated to Avizo 2019.2.

2.5.3 ImageJ
Similar to Avizo® Fire, the software ImageJ (National Institutes of Health, Bethesda, MD, USA) can display, analyse and process a wide variety of image data. The program supports numerous image formats and has the ability to display complete image stacks. The software is freely accessible and freely expandable. It has various user-selectable options (angles, distances, histograms and line profiles). Furthermore, standardised image processing functions such as contrast manipulation, sharpening and smoothing, edge detection and various filtering options are supported. We used the software to visualise the data sets created with all six different μCT scanners. Parameters required for a comparison were collected, e.g. histograms with associated standard deviation and average of the displayed grey values.

2.5.4 Segmentation
Segmentation is used to divide an image into regions with similar properties such as texture, grey values, color, brightness, and contrast. For the present study, Avizo® Fire and ImageJ were used for segmentation of the requested structures. Segmentation in both programs was done manually using threshold-based segmentation techniques. The basis for setting the threshold values were the mean grey values taken from the images of the different implant materials and bones.

RESULTS

3.1 Evaluation of monomer dilutions
After the pre-tests, the iodine monomer concentrations of 0.45, 0.3, 0.2, and 0.15 [wt%] were used for further tests. All of these showed good properties in terms of handling, polymerisation, processing, and grindability. The cutting-grinding-technique was tested successfully on polymerised blank samples, as well as the infiltration, embedding, and grinding of the rat femora specimens and subsequent standard histological stainings. Thin sections could be made with a minimal thickness of 15μm.

Due to the good results, the four concentrations were used as embedding medium for the test specimens with bone and different implant materials. In the μCT scans, the relevant polymer implant in all four concentrations was distinguishable in all μCT images. Other materials, such as bone and other implant materials were not negatively affected in the CT image in general. Only small differences occurred between the four different concentration levels in the μCT scans. At a concentration of 0.15 wt% iodine monomer, the methacrylate-polymer implant could not be differentiated optimally from the modified Technovit® embedding medium. The peaks are too close to each other in the histogram (Figure 3). At a concentration of 0.2 wt% iodine monomer, the polymer implant can be demarcated well, and the histogram shows a better separation of the peaks (which nevertheless are relatively close to each other, Figure 4). At a concentration of 0.3 wt% iodine monomer, clear differences in the grey values and the peaks of the histogram are visible. Bone can also be easily demarcated from other materials (Figure 5). At a concentration of 0.45 wt% iodine monomer, the polymer implant also can be demarcated easily from the modified Technovit®, but the embedding material has high absorption. Thus, bones and composite materials cannot be differentiated optimally from the light background (Figure 6).

Therefore, optimal properties for segmentation were found in the dilutions of 0.2 and 0.3 wt% iodine monomer. Specimens with 0.15 wt% iodine monomer showed a too low contrast, 0.45 wt% iodine monomer specimens showed too high contrast.

The following table (Table 5) shows the score values of the four concentrations.

Table 5. Score values of the four dilutions of iodine monomer

| Monomer dilution/ iodine monomer concentration | Score of visualisation               |
|-----------------------------------------------|-------------------------------------|
| 0.15 wt% iodine monomer                       | 2        | Suitable - limited visualisation of one or more implant materials |
| 0.2 wt% iodine monomer                        | 3        | Well suited – good visualisation of all implant materials         |
| 0.3 wt% iodine monomer                        | 3        | Well suited – good visualisation of all implant materials         |
| 0.45 wt% iodine monomer                       | 2        | Suitable - limited visualisation of one or more implant materials |

No impairments or worsening or deterioration of the visualisation of other materials were found in the μCT scans, and no
significant changes in the mechanical properties of the embedding medium or in the different histological stainings of the embedded test specimens were determined.

3.2 Evaluation of the µCT devices
The following six different µCT devices were used in the study, and basically valued regarding image contrast, spatial resolution/details, image noise, and artefacts.

**SkyScan® 1178 (in vivo scanner)**
In almost every scan made with SkyScan® 1178, ring artefacts appeared. Moderate beam hardening artefacts occurred in scans of composite and Bioverit® implants, and strong beam hardening and contrast reduction were visible in the titanium implant scans (Figure 7). Image contrast was rated ‘good’, but image quality (spatial resolution/details and image noise) is in the lower score range.

**Tomoscope® synergy twin (in vivo scanner)**
The use of the standard scan protocol of the Tomoscope® Synergy Twin led to high noise in all scans. Beam hardening occurred in scans of composite and Bioverit® implants, but was almost covered by strong image noise. Many artefacts appeared in scans of titanium implants. By averaging several CT images (dynamic scan), the noise of the images could be considerably reduced, but led to more visible artefacts such as beam hardening and weak ring artefacts in some scans (Figure 8). Image contrast was good, but image quality (spatial resolution/details and image noise) is in the lower score range.

**Fraunhofer CTportable**
Scans made with Fraunhofer CTportable showed good image contrast. Weak beam hardening are visible in composite, Bioverit® and polymer implant scans, and high beam hardening artefacts in titanium implants (Figure 9). Image quality (spatial resolution/details and image noise) is in the lower score range.

**SkyScan® 1173**
Scans made with the SkyScan® 1173 showed very detailed and high-contrast images with only weak ring artefacts in some scans. Scans of titanium implants showed weak beam hardening. Structures with low contrast differences could be recognised well, as well as textures of composite implants and interfaces between the implants and the surrounding bone tissue (Figure 10). Image quality (spatial resolution/details and image noise) was rated in a high level.

**Fraunhofer sub-µCT**
These scans showed very detailed images of the test specimens, with only a few artefacts (beam hardening in titanium implant, composite implant and polymer implant). Titanium implants appeared slightly less contrasted, but allowed the recognition of the surrounding bone (Figure 11). Image quality (spatial resolution/details and image noise) was rated in a high level.
Similar to Fraunhofer CTportable, scans made with phoenix nanotom® m showed weak beam hardening in composite implants and Bioverit® implants. Moderate beam hardening artefacts were visible in the titanium implant scans (Figure 12). Image quality (spatial resolution/details and image noise) was rated in a high level.

The µCT devices achieved the following total score values: SkyScan® 1178: 6–7; Tomoscope® Synergy Twin – standard: 6–8, dynamic (averaging): 8–10; Fraunhofer CTportable: 6–10; SkyScan® 1173: 11–12; Fraunhofer sub-µCT: 12; and phoenix nanotom® m: 12 (Table 6).

**DISCUSSION**

### 4.1 Iodine monomer

A specifically developed iodine monomer was added to the commercially available embedding medium Technovit® 7200 VLC. The subsequently evaluated X-ray absorption was significantly higher than the previously used pure embedding medium.

In the literature, doping of the embedding medium (methacrylate or epoxy resin) with iodine or other substances to increase radiopacity in µCT is not common, although it has a lot of benefits regarding to the visualisation of a wide range of materials. Iodine is non-toxic, easy to use and has no profound effects to further histological processings and further analysis. The specimens, fixated in a solid block, can be handled easily, are dry, storable, and do not decay.

Various groups evaluated bone cements qualitatively, which have a similar radiopacity as bone, with added barium\textsuperscript{6,10} or bromine\textsuperscript{15} to increase radiopacity. Different acrylic bone cements were used, e.g. partially replacing the methyl methacrylate (MMA) monomer phase with 2-(2-bromopropionyloxy) propyl methacrylate (BPPM)-co-monomer, and included the evaluation of different concentrations. As a result, strength of bone cement was reduced, depending on the barium concentration (up to 20%). This should be comparable with higher concentrations of the iodine doped Technovit\textsuperscript{8}, which leads to too high radiopacity, and possible negative effects on hardening process and stability.

Iodine staining (iodine potassium iodine staining (IKI), triiodine, or Lugol’s staining) is widely used as a contrast agent for the visualisation of soft, undecalcified tissues, e.g. in µCT or scanning electron microscope (SEM). It is cost-efficient, non-toxic, and simple. Boyd\textsuperscript{11} evaluated the staining of the surface of specimens embedded in plastic blocks with triiodine to visualise cells and soft tissues in SEM.\textsuperscript{11} A similar method was applied for mouse and horse bone samples by use of MMA and 2-[4'-iodobenzoyl]-oxo-ethylmethacrylate (4-IEMA) as embedding medium.\textsuperscript{12} The block surfaces were prepared with diamond ultramilling and carbon coated and evaluated with digital backscattered electron (BSE) SEM. In 2014, Boyd et al\textsuperscript{13} had the similar intention and tested the use of iodine vapor. Busse et al\textsuperscript{14} used, e.g. a cytoplasm-specific X-ray stain to visualise mouse kidneys in µCT and nanoCT. Though, the triiodine (IKI) staining has an evident disadvantage: it causes a concentration-dependent shrinkage of the tissue volume.\textsuperscript{15}

Further elements and chemical compounds to increase the X-ray density/radiopacity of soft tissues are barium, osmium tetroxide (OsO4), galloyanine chromealum (GC), and phosphotungstic acid (PTA),\textsuperscript{16} but all are associated with high costs and/or toxicity.

The evaluation of our modified embedding medium showed that a concentration of 0.3 wt% iodine monomer was ideal to ensure the visualisation of weakly absorbing materials such as methacrylate polymer implants in contrast to the embedding medium with almost the same radiopacity.

The modified Technovit\textsuperscript{8} with the concentration of 0.45, 0.3, 0.2, and 0.15 wt% iodine monomer was easy to process, did not show any deviations during polymerisation, and showed normal properties during the cutting/grinding process. The higher concentrations, partly with added initiator, showed unwanted properties in polymerisation and handling in general.

The only restrictions are in storage of the modified embedding medium. The freshly prepared iodine-Technovit\textsuperscript{8} should be used as soon as possible for embedding, since the monomer mixture of the embedding medium has a short durability and is unstable after a long storage. The longer the storage, the stronger appears a reddish colour caused by oxidation reaction of the
iodine monomers with oxygen in the ambient air which causes the typical colouration.

Dry and dark storage at room temperature is recommended to avoid premature polymerisation of the iodine-doped embedding medium. When embedding hard tissue specimens in particularly large or wide vessels during previous tests, we observed that the polymerisation process takes more time and does not proceed completely from bottom to top. Viscous supernatant can occur on the surface, caused by the large interface between embedding medium and air. Oxygen in the ambient air inhibits polymerisation reaction, binds active radicals very quickly at the end of the polymer chain, and blocks further polymerisation processes, so that the monomer mixture may partly remain liquid. For this reason, the specimens should be embedded and cured individually in narrow tubes with a small diameter in order to keep the reaction surface as small as possible so that a complete polymerisation can be guaranteed.

4.2 Test specimens and implant materials
The configuration of the test specimens, specifically the combination of methacrylate polymer and titanium, led to a reduction in contrast around the methacrylate polymer implant, and thus to difficulties in differentiating the methacrylate polymer from the background. The combination of composite and Bioverit® was also not ideal, since all existing materials have a similar radiopacity. If the spatial resolution is too low, problems occur in differentiating the individual implants from another as well as in segmentation using threshold-based segmentation methods.

The best quality in the visualisation of the test specimens was achieved with the combination of methacrylate polymer and composite as well as Bioverit® and titanium. All implants could be well represented and differentiated in these combinations. For optimal visualisation of all implant materials, e.g. in animal experiments, however, the different specimens should be scanned individually.

The iodine doping of the embedding medium was primarily developed to visualise a non-resorbable implant material. Partially or fully degradable implant materials which can be replaced by adjacent bone tissue and/or connective tissue and with a similar radiopacity of the used embedding medium Technovit® VLC 7200 require further investigations, preferably in different stages of degradation and osseointegration, and additionally with different iodine concentrations of the embedding medium.

4.3 Comparison of the six µCT devices
For a comparative study, we used six different µCT devices: SkyScan® 1178 and Tomoscope® Synergy Twin (both in vivo µCT scanning devices), the portable system Fraunhofer CT portable, as well as three high resolution µCT systems (phoenix nanotom® m, SkyScan® 1173 and Fraunhofer sub- µCT). The latter three high resolution devices showed the best results as expected, because of their sufficiently powerful X-ray tubes which ensured an adequate visualisation of the test specimens.

Nevertheless, none of the scans was completely artefact-free. Beam hardening and ring artefacts occurred relating to the detector. Typical localisations of these artefacts were the implant tops of Bioverit® implants, composite implants and titanium implants, more or less in all data sets.

Beam hardening occurs when a polychromatic X-ray beam passes through an object, whereby low energy X-ray photons are attenuated more easily. The edges of this object are brighter than the centre, and dark streaks can appear. Appropriate pre-filtering and sufficiently high tube voltages, as used in all tested µCT systems to pre-harden the beam, can reduce these artefacts effectively. Strongly absorbing materials such as titanium should be scanned using a dual-energy imaging approach.

For the individual scans of the polymer implants, tube voltages in the range up to 65 kV have proven to be optimal to achieve a good contrast between the embedding medium and the polymer implant. Stronger absorbing and dense materials can be scanned with significantly higher voltages, at least at 100 kV in the present study.

Beneficial for subsequent segmentation of the implants and other structures like bone, the use of an averaging method can reduce noise (as realised with Tomoscope® Synergy Twin), which improves image quality.
CONCLUSION
Iodine doping of the embedding medium has a lot of benefits. The visualisation of implants with a similar radiopacity as the embedding medium could be considerably improved. μCT scan settings should be selected with the highest possible resolution, and different implant materials should be scanned individually for optimal segmentation (methacrylate polymer implants: 65kV, composite implants and bones: 80–100 kV, titanium implants at least 100kV). The use of an averaging method can reduce noise and thus improve image quality. During scanning, a high resolution should always be preferred. With regard to our aim, materials and sample size, μCT devices with lower resolutions were (as expected) only usable to a limited extent.

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