SDC Table 1: Experimental animals

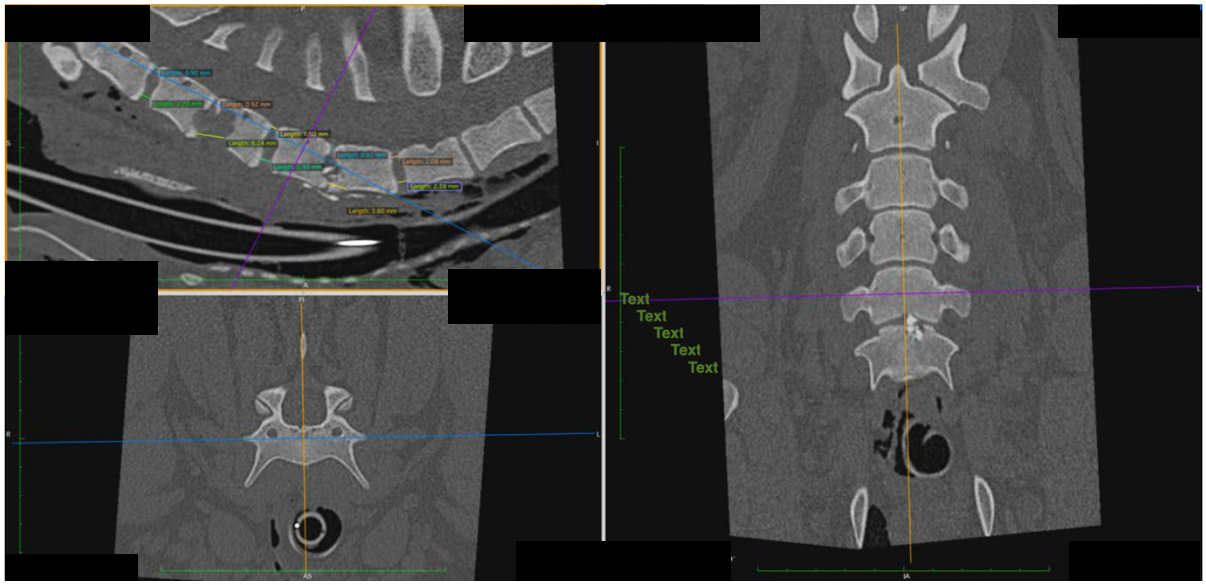
Demographics of the experimental animals in the present study. Values presented as mean (standard deviation).

Experimental animals	
Animal species (strain):	Pig (Ellegaard Göttingen Minipigs, Denmark)
Gender:	Female
Number of animals (N):	4
Age:	24 (3,5) months
Body weight at start of study:	55 (5) kg
Body weight increase over the study period:	16.2%
Diet:	Standard mini-pig diet containing 1,01% calcium and 0,59% phosphorous, Special Diets Services, UK and hay
Housing:	Single housed in pens around 3-5m^2, within sight, smell and sound of one another
Environment enrichments:	Straw bedding, daily 10-15 min exercise in walkway and socialization with the other study-pigs in walkway through the pen bars

SDC Table 2: Anesthesia, analgesia and euthanasia of experimental animals

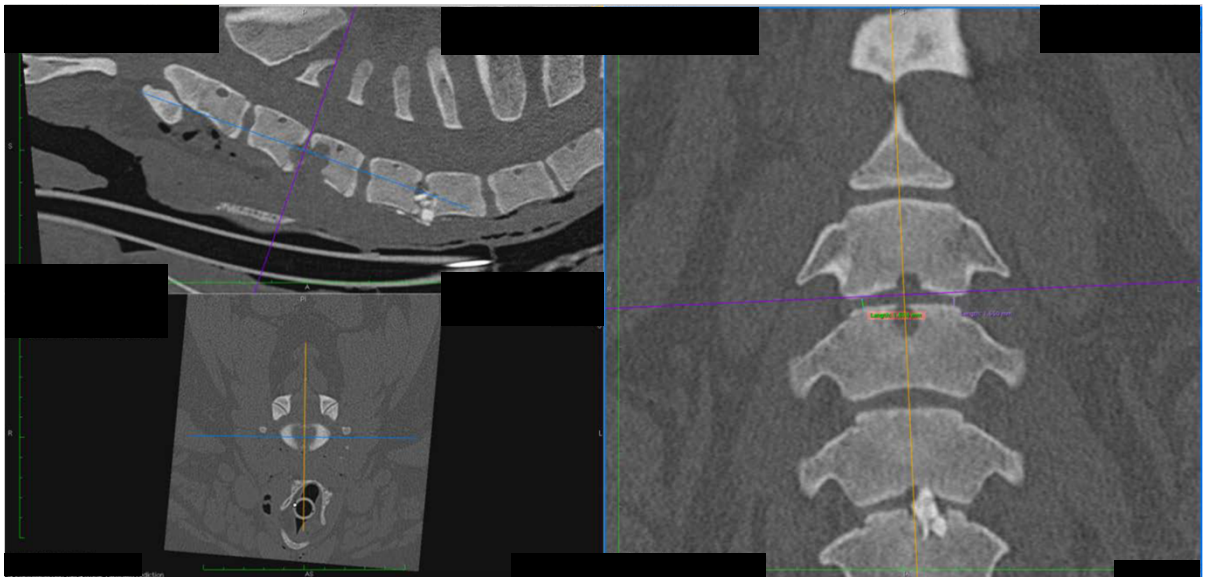
Drugs used for anesthesia, analgesia and euthanasia for the animals in the present study.

Use	Drug (trade name), dose, administration	Manufacturer
Induction:	Dexmedetomidine, (Dexdomitor), 0,025 mg/kg IM	Orion, Finland
	Zolazepam/Tiletamine (Zoletil), Zolazepam 2,5 mg/kg, Tiletamine 2,5 mg/kg IM	Virbac, Switzerland
	Butorphanol (Dolorex), 10mg/ml, 0,1 mg/kg IM	Intervet, Netherlands
Maintenance:	Sevoflurane (SevoFlo), 100%, inhelative	Zoetis, Finland
Peri-operative analgesia:	Dexmedetomidine (Dexdomitor), 4 mg/kg/h CRI	Orion, Finland
	or fentanyl (Fentadon), 7mcg/kg/h CRI	Dechra, Netherlands
Post-operative analgesia 3-5 days:	Buprenorphine (Temgesic) 0,03mg/kg IM	Indivior, Switzerland
	or transdermal fentanyl (Durogesic), 100 -150µg/h TD, ~2,4-3,6 mg/day	TD Janssen, Belgium
Post-operative analgesia 5 days:	Meloxicam (Metacam), 0,6-0,7 mg/kg PO	Boehringer Ingelheim, Germany
Sedation:	Zolazepam/Tiletamine (Zoletil), Zolazepam 2,5 mg/kg, Tiletamine 2,5 mg/kg IM	Virbac, Switzerland
	Butorphanol (Dolorex), 10mg/ml, 0,1 mg/kg IM	Intervet, Netherlands
Euthanasia:	Pentobarbital (Euthanimal Vet), 200mg/ml, 100mg/kg IC	VM Pharma, Netherlands



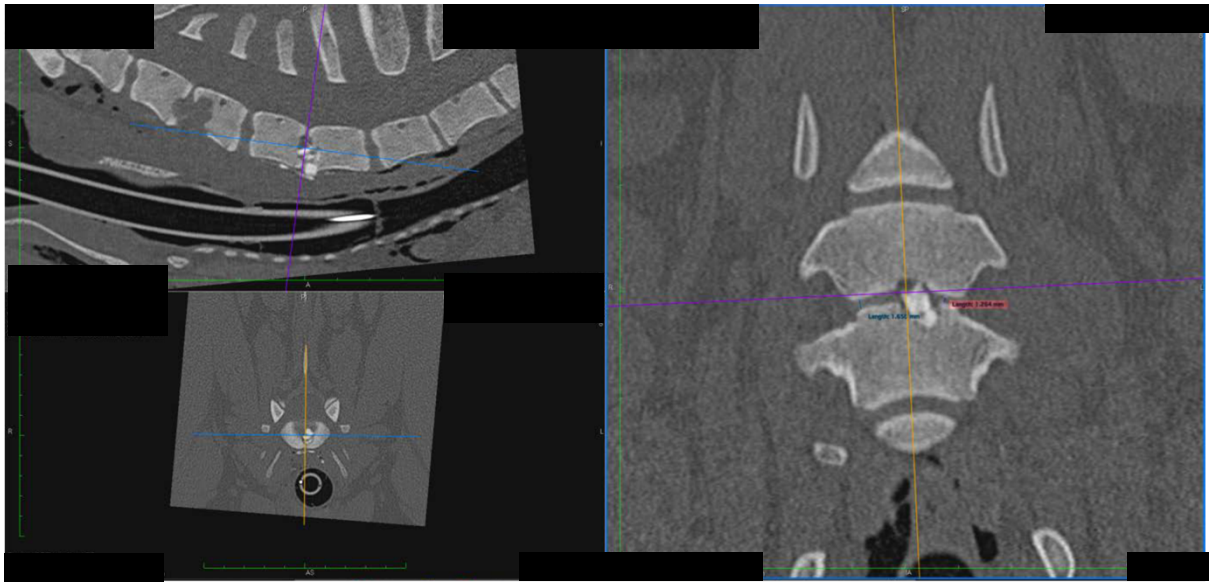
SDC Figure 1: Positioning of animals for cervical spine computer tomography measurements

Use 3D MPR of the Spine Helical 0.6 B70s series. If the computer asks you a 'High Dynamic Values' question when you do the 3D MPR then select 'Clip' (don't select resample). Measurement of dorsal and ventral intervertebral disc space dimension. Align sagittal image so that it is mid sagittal in the dorsal plane image. Measure the lowest width of the dorsal half and the ventral half of each disc space. Minimum distance (use 0.1 mm unit) between the vertebrae (width of intervertebral disc space) in 4 locations, dorsal, ventral, right and left margins of the intervertebral disc space.



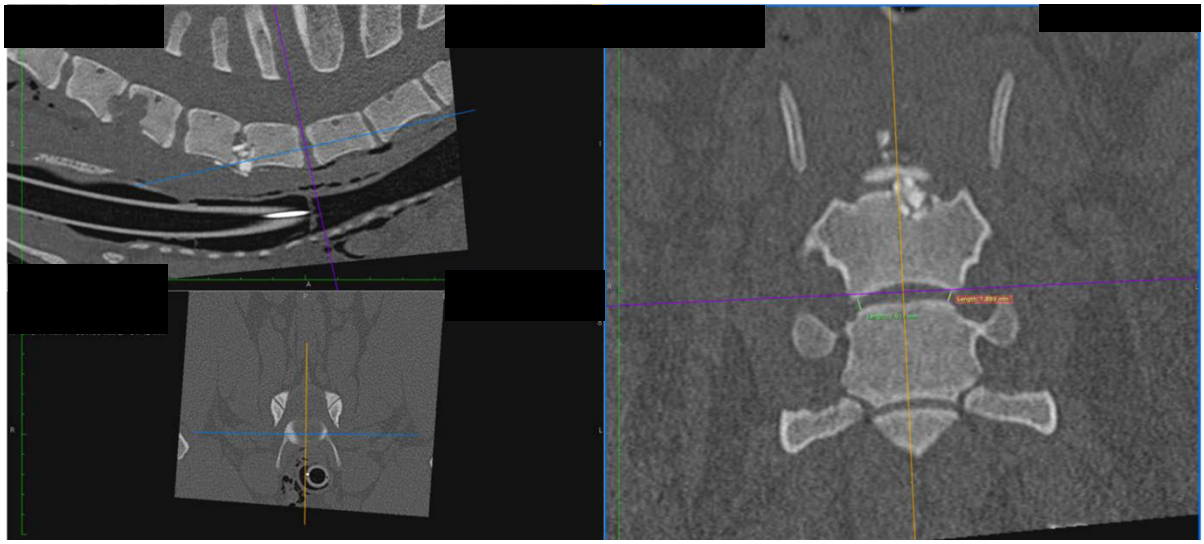
SDC Figure 2: Alignment of image when measuring control operated level.

Alignment of the 3D MPR image to measure the lowest left and right width of cervical (C) C3-4 intervertebral disc space in a control operated disc space. Mid sagittal plane, in the middle of the disc space with transverse plane parallel to the disc space.



SDC Figure 3: Alignment of image when measuring synthetic bone graft operated level.

Alignment of the 3D MPR image to measure the lowest left and right width of C5-6 intervertebral disc space in an synthetic bone graft operated disc space. Mid sagittal plane, in the middle of the disc space with transverse plane parallel to the disc space.



SDC Figure 4: Alignment of image when measuring non-operated level.

Alignment of the 3D MPR image to measure the lowest left and right width of C6-7 intervertebral disc space in a non-operated disc space. Mid sagittal plane, in the middle of the disc space with transverse plane parallel to the disc space.

SDC Figure 5: CT grading protocol

Pig name and journal number:

Radiologist name:

Examination date:

Date images graded:

Location of the control operated disc space:

Location of the synthetic bone graft operated disc space:

Intervertebral disc space (3D MPR of the Spine Helical 0.6 B70s series)

1. Minimum intervertebral disc space (use 0.01 mm unit) between the vertebrae (width of intervertebral disc space) in 4 locations, dorsal (posterior), ventral (anterior), right and left halves of the intervertebral disc space. Total intervertebral disc space was normalized to baseline for each level each timepoint (post-op disc space – pre-op disc space).

	Minimum width of disc space (0.01 mm)				
	Dorsal (posterior)	Ventral (anterior)	Right	Left	Tot. disc space, normalized to baseline
C2-3					
C3-4					
C4-5					
C5-6					
C6-7					

2. Defect size estimation (mm^3)

3D MPR alignment at a sagittal plane centered over the defect, in the middle of the defect with transverse plane parallel to the disc space. Measure the longest distance of the defect (use 0.01 mm unit) length, width and height.

	Maximum defect size (0.01 mm)			
	Length	Width	Height	Volume of defect (mm^3)
SBG defect				
Control defect				

3. Fusion Evaluation

- Interbody bony fusion present (Yes/No), evaluated through the entire intervertebral disc space, scroll back and forth through the disc space. Interbody fusion defined as: continuous trabecular bony bridge within the disc space.

- Fusion by spondylosis present (Yes/No), defined as continuous trabecular bony bridge between two vertebras outside the disc space, scroll back and forth through the vertebral bodies. Use 3D MPR of the Spine Helical 0.6 B70s series.

	Interbody fusion Yes/No (Y/N)		Fusion by spondylosis Yes/No (Y/N)	
	Yes	No	Yes	No
C2-3				
C3-4				
C4-5				
C5-6				
C6-7				

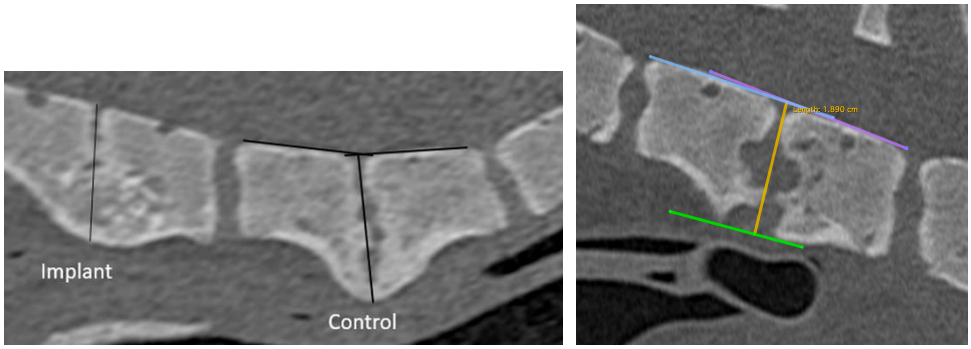
4. Spondylosis anterioposterior dimension

Always do this measurement for the operated disc spaces, whether or not there is ventral (anterior) spondylosis (but also always answer the question ‘Ventral spondylosis Y/N). The

measurement must be done in the region of the intervertebral disc space (or fused intervertebral disc space) and is done on the same images used to measure the intervertebral disc space (so done in the mid-sagittal plane). Ratio is calculated according to formula below and reported reported as median (range). R_{ab}: spondylosis anterioposterior dimension ratio. (a): spondylosis anterioposterior dimension 12 months after surgery. (b): disc space anterioposterior dimension before surgery

$$R_{ab} = (a) / (b)$$

For non-operated disc spaces only do the measurement if spondylosis is present



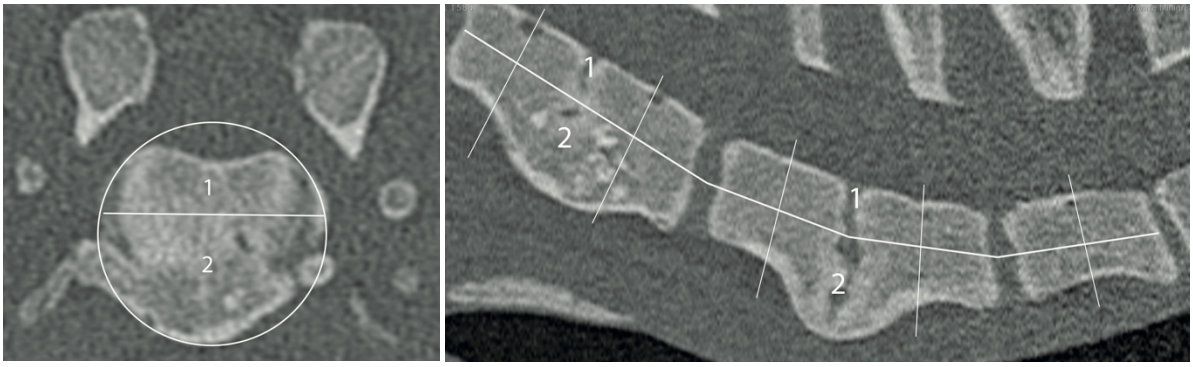
Measure the longest distance (use 0.01 mm unit) between bone formation on the dorsal (posterior) and ventral (anterior) aspects of the epiphyses of the vertebral bodies. If the bone formation is not fused/bridging then draw a line between the most ventral margins of the bone formation on each vertebrae and measure to the line.

	Control = C, Synthetic bone graft = S	Ventral (anterior) spondylosis Y/N	Size (0.01 mm)	R_{ab}
C2-3				
C3-4				
C4-5				
C5-6				

C6-7				

Vertebral body (use 3D MPR of the Spine Helical 0.6 B70s series)

Note that lysis, sclerosis and bone formation are determined by comparing to immediate postop CT images. The endplate defects present immediately after the surgery are not considered lysis, rather it is areas of hypoattenuation in the vertebrae that develop later. The gradings are done centered on the disc space and take into consideration the entire volume of the vertebrae (so scroll back and forth). The regions are 1) the caudal half and cranial half of the vertebral body with the disc space in the middle if there are changes (see vertical lines in picture below) then localize them to dorsal (posterior) (1) or ventral (anterior) (2) in the transverse and sagittal plane.



1. Sclerosis present Yes/No (Y/N), scroll back and forth through the disc space, Grade 0 = 0%, Grade 1 = 1-25%, Grade 2 = 26-50%, Grade 3 = 51-75%, Grade 4 = 75-99%, Grade 5 = 100%. For borderline grades always choose the lower grade. Localize any changes to dorsal (posterior) or ventral (anterior) half.
2. Lysis present Y/N, scroll back and forth through the disc space, Grade 0 = 0%, Grade 1 = 1-25%, Grade 2 = 26-50%, Grade 3 = 51-75%, Grade 4 = 75-99%, Grade 5 = 100%. For borderline grades always choose the lower grade. Localize any changes to dorsal (posterior) or ventral (anterior) half.

3. Bone formation within the intervertebral disc space Y/N. Localize any changes to dorsal (posterior) or ventral (anterior) half.

	Sclerosis			Lysis			Bone formation	
	Y/N	Grade	P/A	Y/N	Grade	P/A	Y/N	D/V
C2-3 Cr								
C2-3 Cd								
C3-4 Cr								
C3-4 Cd								
C4-5 Cr								
C4-5 Cd								
C5-6 Cr								
C5-6 Cd								
C6-7 Cr								
C6-7 Cd								

Other findings

Briefly describe any other lesions (not included in the grading) observed in the C2-C7 region (for example fractures, luxation/subluxation, angulation). When describing include location, attenuation, size, shape, number, margination information.

SDC Table 3: Semiquantitative scoring scheme according to ISO 10992-6:2016

Histopathological scoring scheme used to evaluate local tissue response to the synthetic bone graft 15 months post-surgery.

Score					
Cell type/response	**0**	**1**	**2**	**3**	**4**
Polymorphonucl. cells	0	Rare, 1 to 5/phf[a]	5-10/phf	Heavy infiltrate	Packed
Lymphocytes	0				
Plasma cells	0				
Macrophages	0				
Giant cells	0	Rare, 1 to 2/phf	3-5/phf		Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe
[a] phf = per high-powered (400x) field.					
Score					
Response	0	1	2	3	4
Neovascularization	0	Minimal capillary proliferation, focal, 1 to 3 buds	Groups of 4 to 7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting fibroblastic structures	Extensive and of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells around the synthetic bone graft site	Extensive fat completely surrounding the synthetic bone graft
Traumatic necrosis	None	Mild	Moderate	Obvious	Extensive area of necrosis
Foreign debris (synthetic bone graft)	None	Mild	Moderate	Obvious	Pronounced area of debris
Degradation of synthetic bone graft	None	Mild	Moderate	Strong	Heavy
Conclusion tissue reaction	Minimal or no reaction (0,0 to 2,9)	Slight reaction (3,0 to 8,9)	Moderate reaction (9,0 to 15,0)		Severe reaction (15,1)

SDC Figure 6: Summary of necropsy report

At necropsy, none of the four animals had observable lesions in the tissues surrounding the surgery site. One of the animals had unilateral moderate osteoarthritis with focal erosion of the central part of the joint cartilage of the head of humerus, consistent with repaired lesions of osteochondrosis. Another had similar lesions bilaterally on the trochlea of humerus, while a third had mild discoloration and fibrillation in the same locations of both the condyle and the trochlea of humerus. In the fundus part of the ventricle of the first animal, several circular hyperemic foci, up to 10 mm diameter, were observed, but histological examination did not reveal pathological changes. The kidneys of all four animals were pale and had moderately increased texture. Histological examination showed mild to moderate diffuse interstitial fibrosis of both the renal medulla and cortex that might be consistent with previous episodes of contrast-induced acute kidney injury.

SDC Table 4: Vertebral body sclerosis

Vertebral body sclerosis evaluated by computed tomography graded from Grade 1 (1-25% sclerosis) to Grade 5 (100% sclerosis) at cranial half and caudal half of the vertebral body with the disc space in the middle, at control (C) and synthetic bone graft (SBG) levels, over 12 months. M=months post-surgery.

	3M		6M		9M		12M	
Animal	**SBG**	**C**	**SBG**	**C**	**SBG**	**C**	**SBG**	**C**
1	1	0	1	0	1	1	1	2
2	1	1	1	1	1	1	1	1
3	1	1	1	1	2	1	1	1
4	1	1	1	1	2	1	2	1

SDC Table 5: Vertebral body lysis

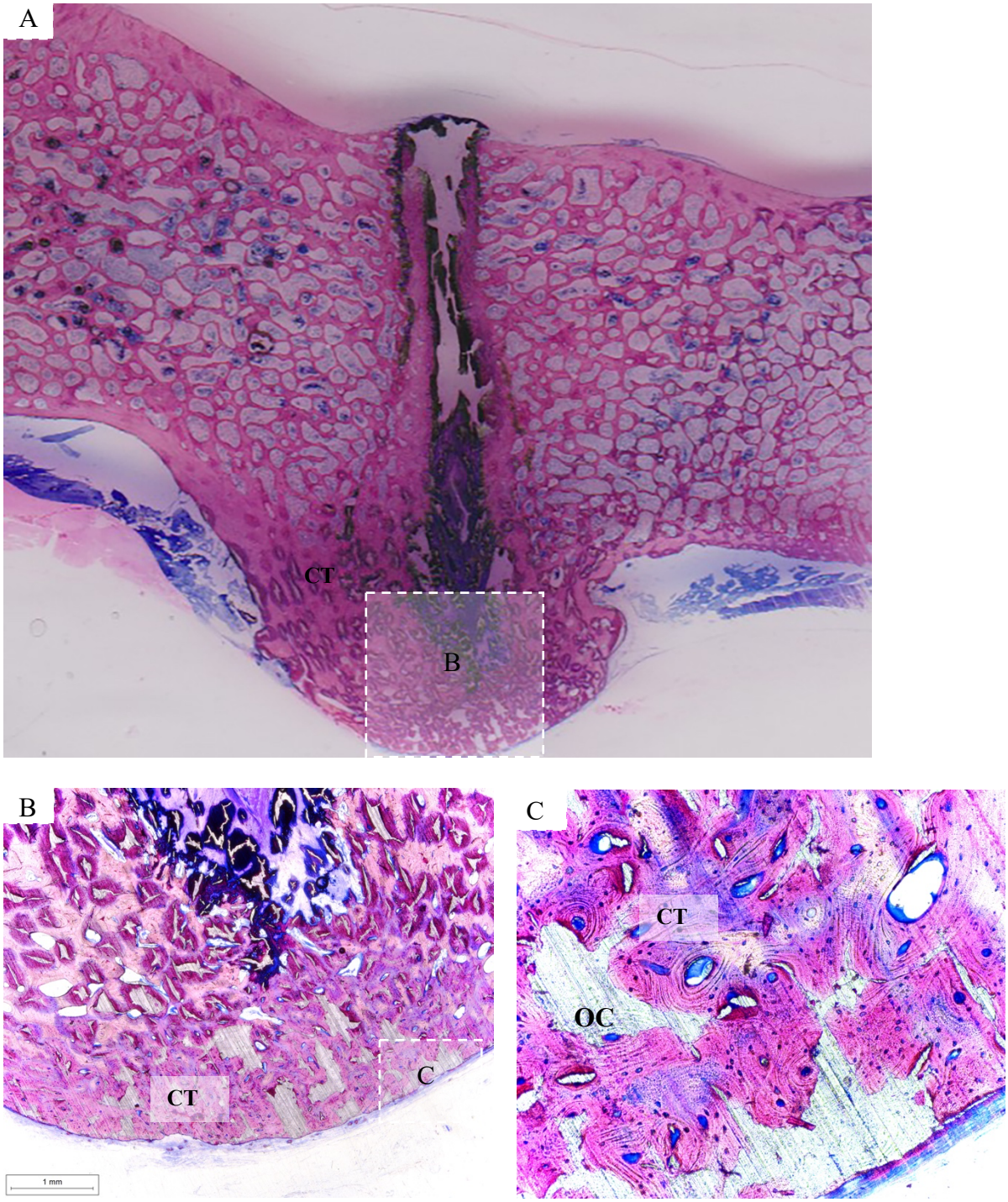
Vertebral body lysis evaluated by computed tomography graded from Grade 1 (1-25% lysis) to Grade 5 (100% lysis) at cranial half and caudal half of the vertebral body with the disc space in the middle, at control (C) and synthetic bone graft (SBG) levels, over 12 months. M=months post-surgery.

	3M		6M		9M		12M	
Animal	**SBG**	**C**	**SBG**	**C**	**SBG**	**C**	**SBG**	**C**
1	0	0	0	0	0	0	0	0
2	0	0	0	1	0	1	0	1
3	0	0	0	0	0	0	0	0
4	1	0	1	0	0	0	0	0

SDC Table 6. Bone formation within intervertebral disc space

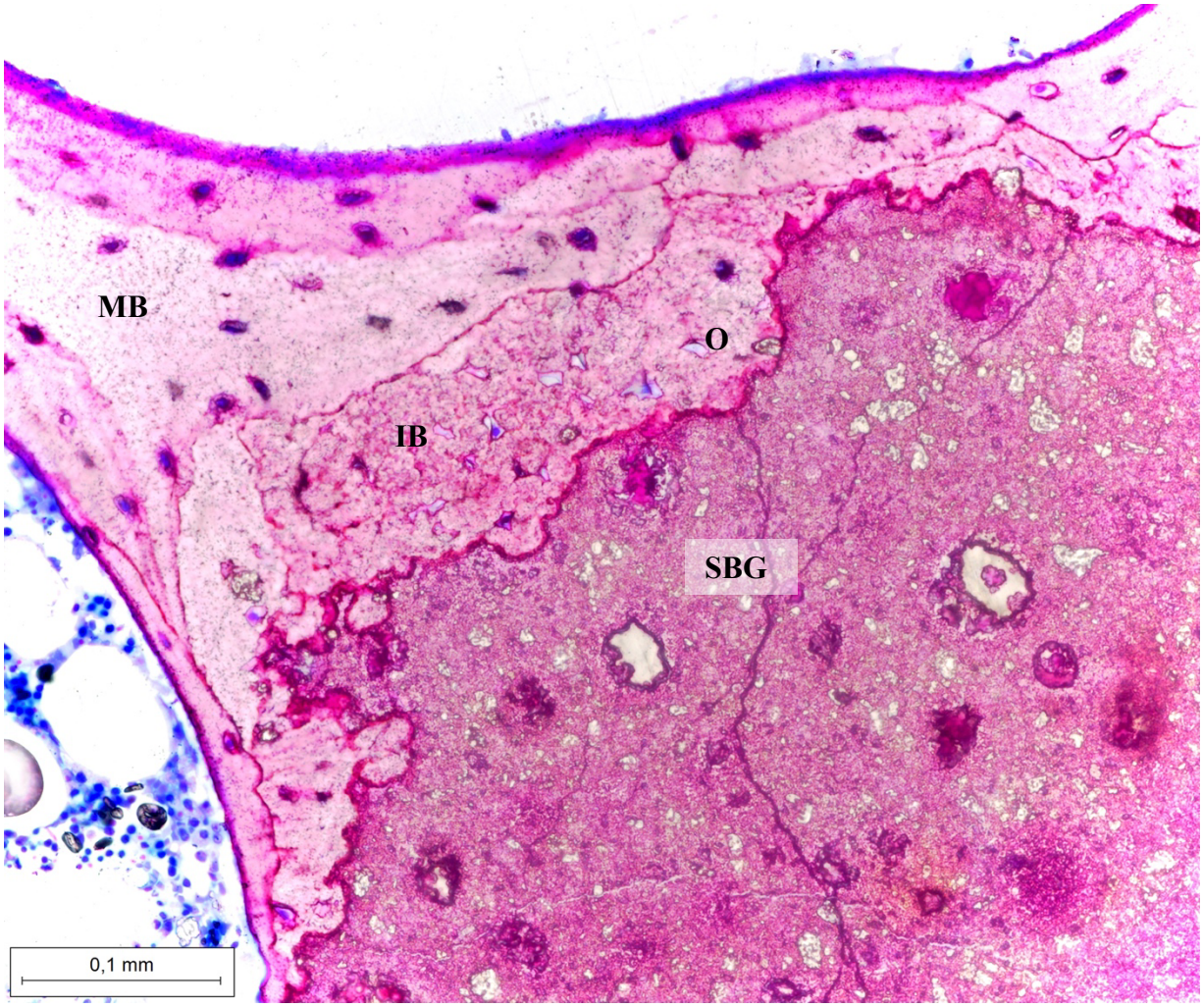
Presence of bone formation within the intervertebral disc space at anterior or posterior half evaluated by computed tomography, for control (C) and synthetic bone graft (SBG) levels, over 12 months. M = months post-surgery. A = anterior half of disc space, P = posterior half of disc space

	3M		6M		9M		12M	
Animal	**SBG**	**C**	**SBG**	**C**	**SBG**	**C**	**SBG**	**C**
1	A	A	A/P	A/P	A/P	A/P	A/P	A/P
2	A/P	A	A	A	A/P	A	A/P	A
3	A/P	A	A/P	A/P	A/P	A/P	A	A/P
4	A/P	A	A	A	A/P	A	A/P	A/P



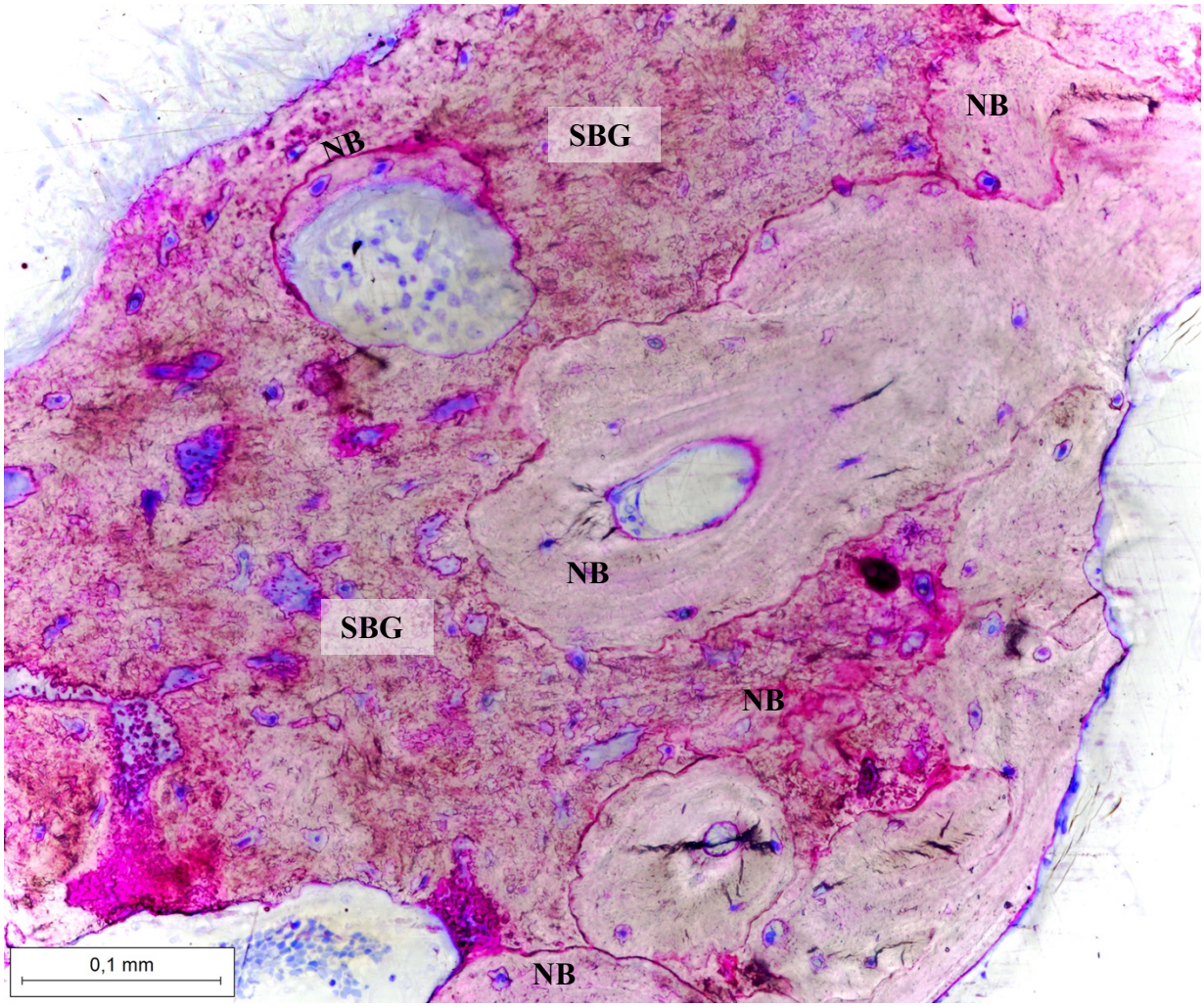
SDC Figure 7: Fusion by spondylosis in control sample.

Histopathological sagittal section of bony bridge at the one control level (A, B and C), where fusion had occurred, consisted of irregular and to some degree contorted trabeculae (CT) with active osteoclasts (OC), small marrow spaces and less mature bone.



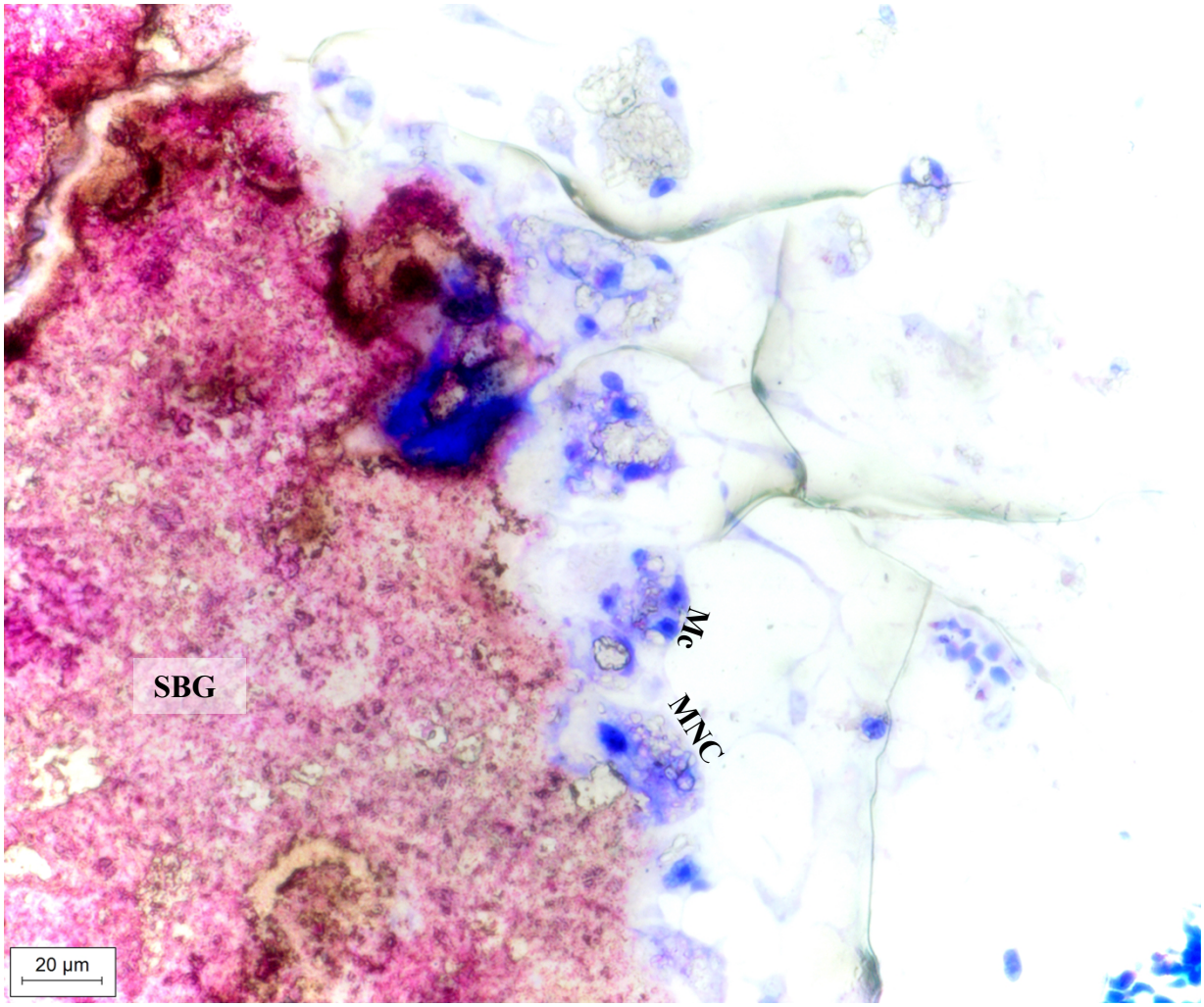
SDC Figure 8: Synthetic bone graft aggregate.

Histopathological section of synthetic bone graft level. Synthetic bone graft aggregate (SBG) were surrounded by a rim of carmoisine red material also presumed to represent osteoid (O). Similar material seemed to be present also within the aggregates without any obvious connection with bone, but confirmation of this observation would have required serial sectioning which hitherto not have been performed. The aggregates were variably surrounded by mature (MB) or immature bone (IB) or bone marrow.



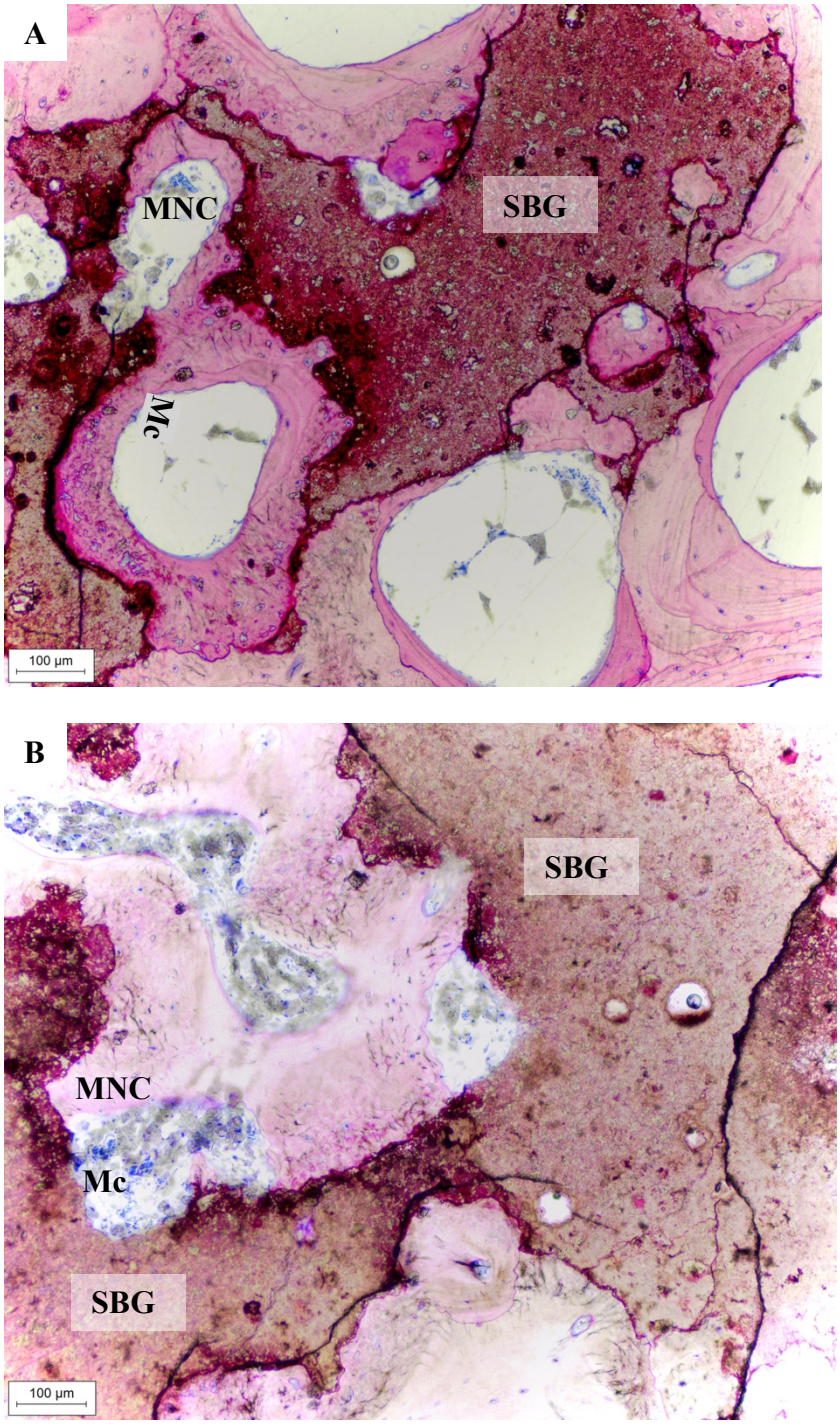
SDC Figure 9: Synthetic bone graft aggregate.

Histopathological section of synthetic bone graft level. Remains of synthetic bone graft (SBG) material were visible in one animal that appeared to have been integrated into the bone tissue, new bone (NB).



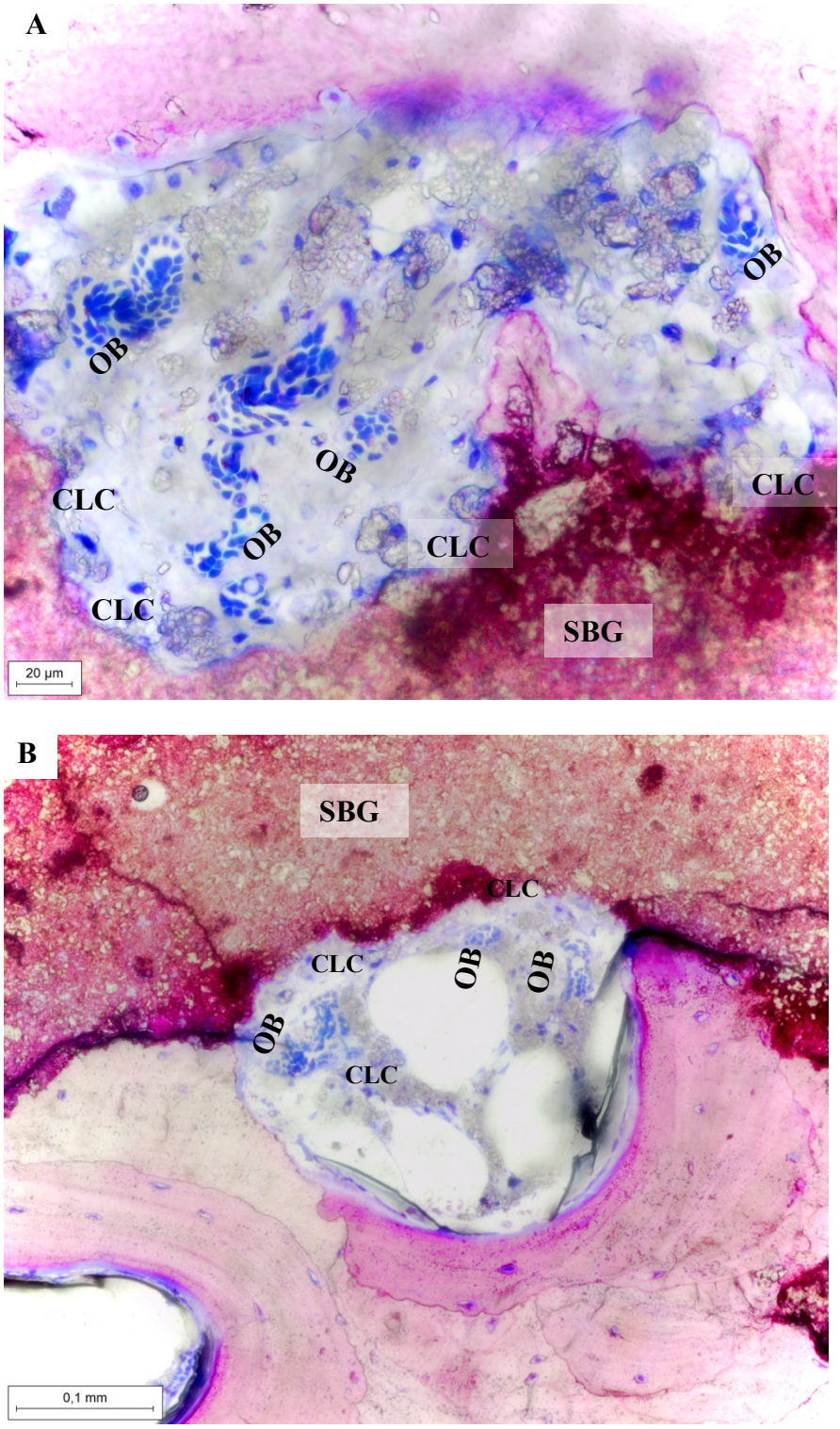
SDC Figure 10: Macrophages and multinucleated giant cells.

Histopathological section of synthetic bone graft level. Foci where the margins of the synthetic bone graft (SBG) aggregates were populated with macrophages (Mc) and multinucleated giant cells (MNC) filled with granular material resembling the synthetic bone graft was detected in three out of four animals.



SDC Figure 11: Multinucleated giant cells.

Histopathological section of synthetic bone graft level (A and B). In a few foci, adjacent bone marrow to synthetic bone graft (SBG) aggregates was filled with multinucleated giant cells (MNC) and macrophages (Mc) filled with granular material resembling the synthetic bone graft.



SDC Figure 12: Clast-like cells and osteoblasts.

Histopathological section of synthetic bone graft level (A and B). Osteogenic buds with clast-like multinucleated cells (CLC) and osteoblasts (OB) seemed to grow towards the aggregates of synthetic bone graft (SBG).