Supplementary Information (SI) for Journal of Materials Chemistry B.
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Supporting Information for

High purity butoxydibutylborane catalysts enable the low-exothermic polymerization of PMMA bone cement with enhanced biocompatibility and osseointegration

Zhuo Wan, + [a] Yike Gao, + [b] Xianghao Zhang, + [c] Xiyin Gao, [d] Tuanfeng Zhou, [e] Zhishan Zhang, [f] Zijian Li, [f] Yunfei Lin, [g] Bing Wang, [g] Kun Chen, [a] Yang Wang, * [h] Chenggang Duan, * [c] and Zuoying Yuan * [a]

[a] Department of Mechanics and Engineering Science, College of Engineering, Peking University, Beijing 100871, P. R. China
E-mail: zy_yuan@pku.edu.cn
[b] Laboratory of Tissue Regeneration and Immunology and Department of Periodontics, Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, School of Stomatology, Capital Medical University, Beijing, P. R. China
[c] The Fifth Clinical Division, Peking University School of Stomatology, 100020, Beijing, P. R. China
E-mail: chenggang93@sina.com
[d] Key Laboratory of Science and Technology on High-tech Polymer Materials, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China
[e] The First Clinical Division, Peking University School of Stomatology, 100034, Beijing, P. R. China
[f] Department of Orthopedics, Peking University Third Hospital, Beijing 100191, P. R. China
[g] Department of Orthopaedic Surgery, Peking University First Hospital, Beijing 100034, P. R. China
[h] Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, Beijing 100081, P. R. China
E-mail: wyly78@sina.com

[+] These authors contributed equally to this work

Supplementary Methods

Cell culture

The cells were maintained in a-modified eagle medium (a-MEM) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 100 IU/mL penicillin (Sigma, USA) and 100 mg/mL streptomycin (Sigma, USA). The culture was performed at 37 °C under saturated humidity with 5% CO_2. For the osteogenic differentiation study, the inductive medium was made from culture medium by adding 0.05 mmol/L vitamin C (Sigma, USA), 10 mmol/L b-sodium glycerophosphate (Sigma, USA) and 1×10^{-8} mol/L dexamethasone (Sigma, USA). Media were refreshed every 2 days in all the cases. Prior to BMSCs seeding, bone cements were sterilized by being exposed to ultraviolet light for 12 h.

Cell viability

The cells were treated with trypsin-EDTA (Gibco) and then resuspended in the extracts. Cells at a density of 1×10^3 were seeded into each well of a 96 -well plate and allowed to grow for 24 or 72 hr. The cytocompatibility of the bone cements was analyzed by a cell counting kit-8 (CCK-8) assay (Bimake) and a live/dead assay. The CCK-8 assay was conducted as follows: after a specific period of incubation in a 96-well plate, CCK-8 solution was diluted 10-fold with the extracts. After the removal of the original medium, 100 µL of CCK-8 reagent was added to each well, which was cocultured with the cells in a 5% CO_2 incubator at 37 °C for 2 hr before measurement of the absorbance with a microplate reader at a wavelength of 450 nm. The live/dead assay was conducted as follows. First, 2 µM calcein AM (in DPBS) and 4 µM EthD-1 (Invitrogen) working solutions were added to the wells. The 96-well plate was then incubated in a 5% CO_2 incubator at 37 °C for 20 min. A confocal laser scanning microscope (CLSM, Nikon, Japan) was used to observe the morphologies of the cells. For cell morphology evaluation, cell or cell/bone cements complexes were stained with TRITC-labeled phalloidin for actin and counterstained with DAPI for nuclei, and florescent images were captured on CLSM.

In vitro osteogenic differentiation

In 24-well culture plates, ten thousand cells were seeded, with tissue culture plates (TCPs) serving as the control. After 24 hours, the medium was substituted with either osteoinductive medium or osteogenic extracts, maintaining the culture for 14 days, with medium replenished every 3 days. At designated intervals (3, 7, and 14 days), the medium in each well was discarded, and cell lysates were prepared for quantitative analysis. Alkaline phosphatase (ALP) and collagen I (Col-I) activities were assessed using the respective ALP assay kit (Sigma, USA) and Col-I ELISA kit (BlueGene, China), following manufacturers' protocols. All data were normalized to total protein content determined using the BCA protein assay kit (Thermofisher, USA), with bovine serum albumin (BSA) as the standard. For ALP staining, cells were stained with the BCIP/NBT Kit (C3206; Solarbio, China) for 15 minutes, followed by washing with distilled water. Alizarin red staining involved immersing cells in alizarin red working solution (1%, w/v) for 30 minutes and observation under an optical microscope.
For the quantitative reverse transcription polymerase chain reaction (qPCR) assay, at days 3, 7, and 14 of osteogenic induction, 1 mL of Trizol RNA extract kit (Invitrogen, Carlsbad, CA) was added to each well to extract total RNA. Two genes, osteopontin (OPN) and osteocalcin (OCN), were evaluated. Quantitative PCR was conducted using a Step-One-Plus real-time PCR system (Thermo Fisher, CA). Relative RNA expression was calculated using a ΔΔCt method by being normalized with a house-keeping gene 18s to exclude the influence of cell number. Briefly, rats were shaved and anesthetized with pentobarbital sodium.

Rat calvarial defect model

A 5 mm defect was created on each side of the calvarium using a trephine bur. Subsequently, each bone cement component was filled into the defects, and the overlying tissue was closed with surgical staples. Half of the rats were euthanized at 4 and 8 weeks post-surgery, with 3 samples obtained for analysis. Retrieved skulls were fixed in 10% neutral buffered formalin for 48 hours before further analysis.

Characterizations on bone regeneration

The calvaria samples were analyzed using micro-CT (Skyscan 1176, Bruker, Belgium) with 3D reconstruction and image viewing facilitated by NR econ software version 1.6.

A circular region of 5 mm diameter at the center of the calvarial defect area was selected to locate the cylindrical volume of interest perpendicularly for analysis. Bone volume fraction (BV/TV) and bone mineral density (BMD) were quantified using CT An software version 1.14 (Bruker micro-CT, Kontich, Belgium).

Following micro-CT analysis, the calvaria underwent decalcification in a rapid decalcifier (RapidCal. Immuno, ZS-Bio, China) for 48 hours at room temperature, followed by dehydration and embedding in paraffin. Sections of 5 μm thickness were prepared for histological and immunohistochemical analysis. Hematoxylin-eosin (H&E) and Masson's trichrome staining (Senbeijia, China) were conducted on consecutive tissue sections, with digital images captured using Nanozoomer digital slice scanning equipment (Hamamatsu, Japan). Immunohistochemical staining for OPN was performed, wherein endogenous peroxidase was blocked with 3% hydrogen peroxide, followed by treatment with 10% horse serum for 10 minutes to prevent nonspecific binding. OPN was detected using anti-OPN primary antibody (Abcam, UK, cat. no. ab8448) at a 1:500 dilution in antibody diluent (ZSGB-BIO, China). Subsequently, sections were labeled using the Polink-1 HRP DAB detection system (GBI, USA), and antibody complexes were visualized with 3,3-diaminobenzidine (DAB kit, ZSGB-BIO, China). Stained sections were dehydrated in ethanol and xylene, mounted with a permanent medium, and photographed using an optical microscope.

Supplementary Figures

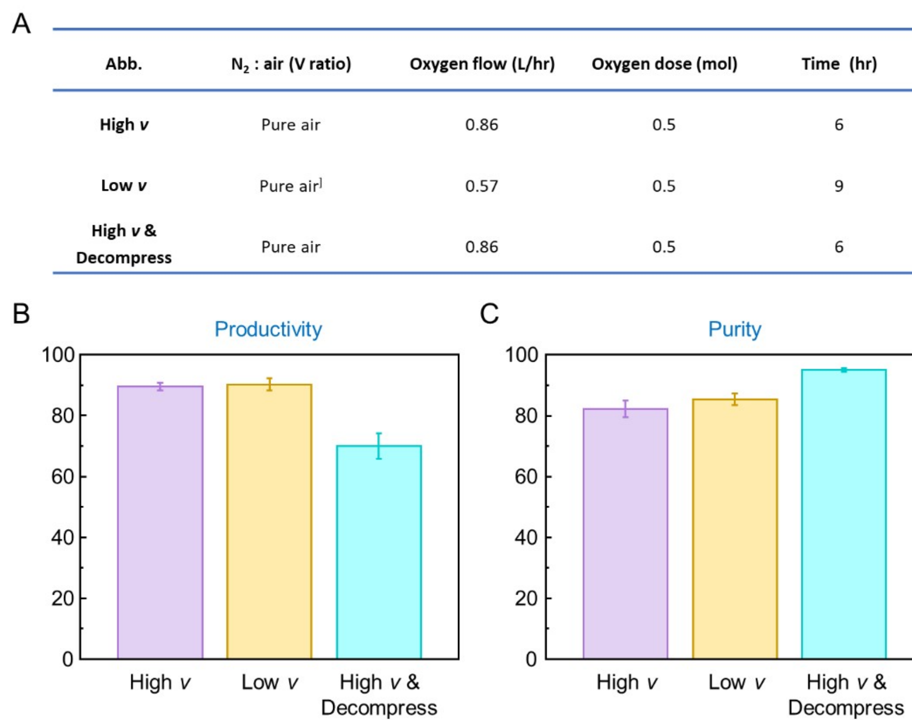
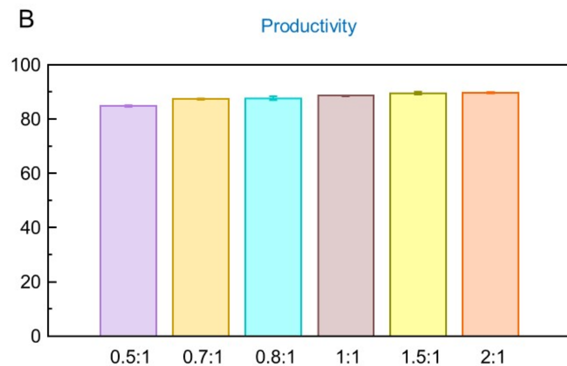


Abb.	N_2 : air (V ratio)	Oxygen flow (L/hr)	Oxygen dose (mol)	Time (hr)
High *v*	Pure air	0.86	0.5	6
Low *v*	Pure air	0.57	0.5	9
High *v* & Decompress	Pure air	0.86	0.5	6

Figure S1. The (A) parameters, (B) productive rate, and (C) the purity of BODBB synthesized through Route 1.

A

Abb.	N_2 : air (V ratio)	Oxygen flow (L/hr)	Oxygen dose (mol)	Time (hr)
0.5:1	0.5:1	0.57	0.5	9
0.7:1	0.7:1	0.57	0.5	9
0.8:1	0.8:1	0.57	0.5	9
1:1	1:1	0.57	0.5	9
1.5:1	1.5:1	0.57	0.5	9
2:1	2:1	0.57	0.5	9



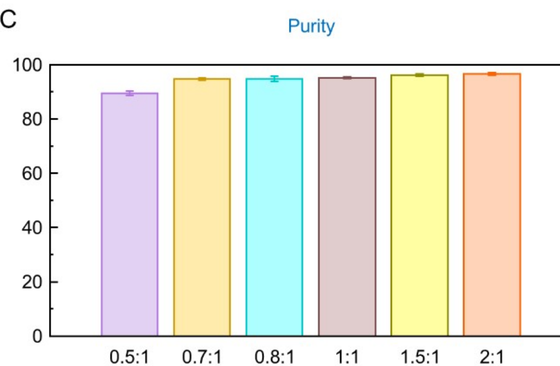


Figure S2. The (A) parameters, (B) productive rate, and (C) the purity of BODBB synthesized through Route 2 with various volume ratio of nitrogen to air.

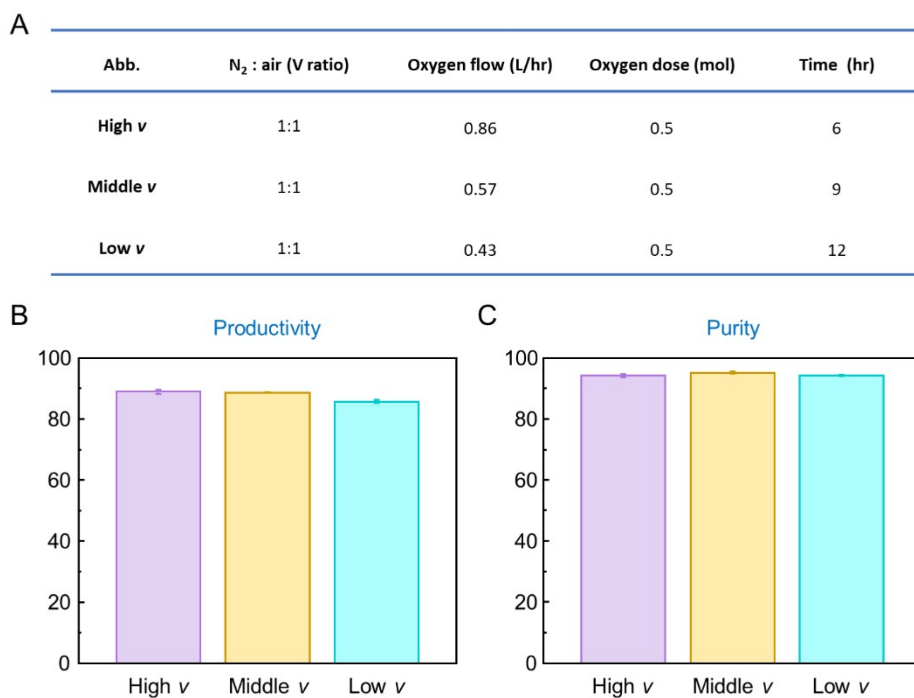


Abb.	N_2 : air (V ratio)	Oxygen flow (L/hr)	Oxygen dose (mol)	Time (hr)
High *v*	1:1	0.86	0.5	6
Middle *v*	1:1	0.57	0.5	9
Low *v*	1:1	0.43	0.5	12

Figure S3. The (A) parameters, (B) productive rate, and (C) the purity of BODBB synthesized through Route 2 with various oxygen flow.

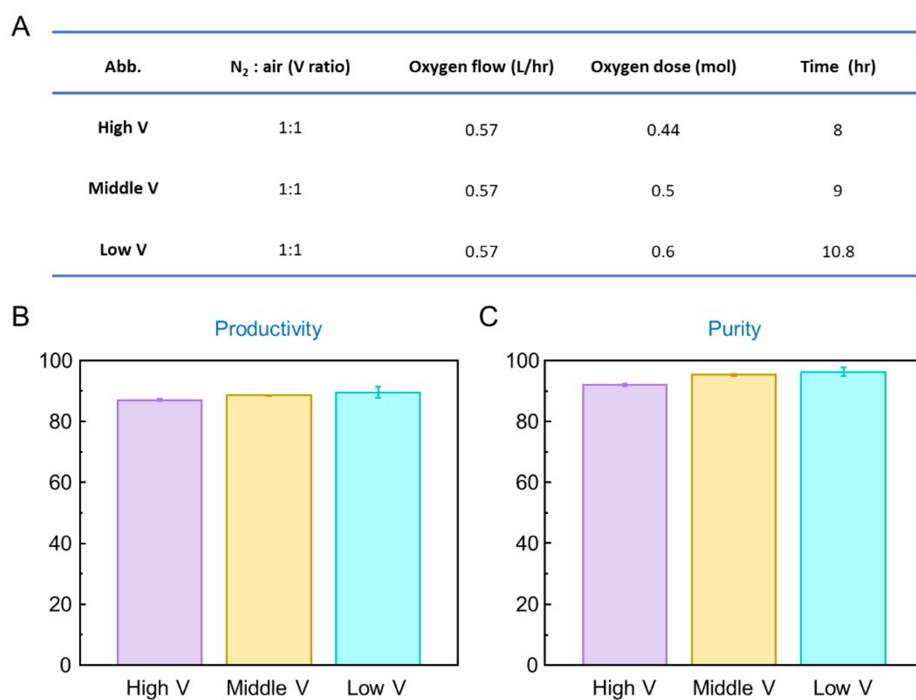


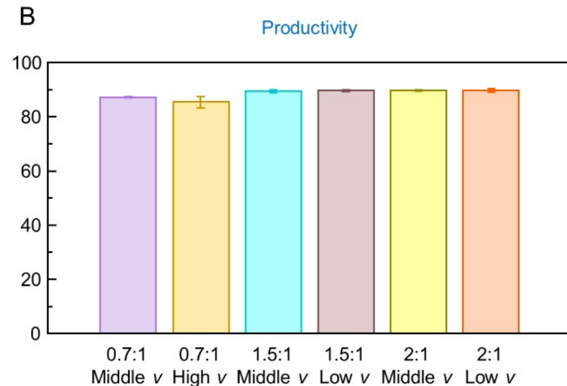
Abb.	N_2 : air (V ratio)	Oxygen flow (L/hr)	Oxygen dose (mol)	Time (hr)
High V	1:1	0.57	0.44	8
Middle V	1:1	0.57	0.5	9
Low V	1:1	0.57	0.6	10.8

Figure S4. The (A) parameters, (B) productive rate, and (C) the purity of BODBB synthesized through Route 2 with various total oxygen dose.

A

Abb.	N_2 : air (V ratio)	Oxygen flow (L/hr)	Oxygen dose (mol)	Time (hr)
0.7:1 Middle v	0.7:1	0.57	0.5	9
0.7:1 High *v*	0.7:1	0.86	0.5	6
1.5:1 Middle v	1.5:1	0.57	0.5	9
1.5:1 Low *v*	1.5:1	0.43	0.5	12
2:1 Middle v	2:1	0.57	0.5	9
2:1 Low *v*	2:1	0.43	0.5	12

B



C

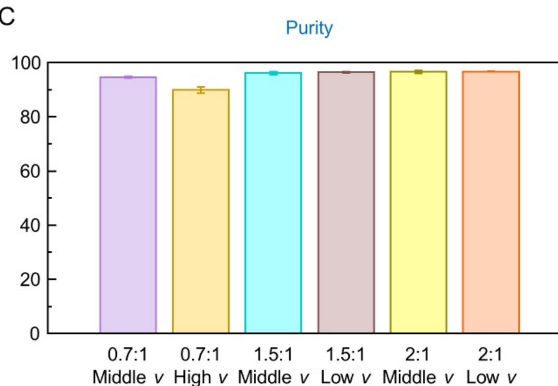


Figure S5. The (A) parameters, (B) productive rate, and (C) the purity of BODBB synthesized through Route 2 with various oxygen flow and volume ratio of nitrogen to air.

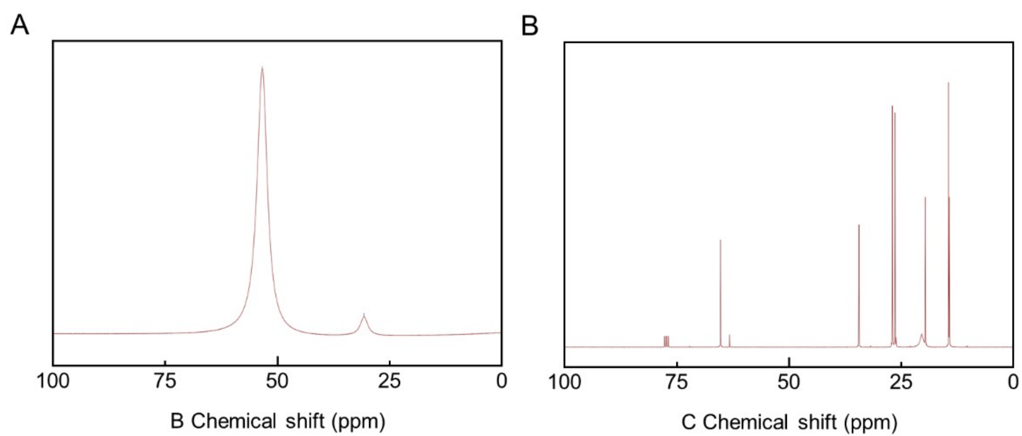


Figure S6. The representative (A) B11 and (B) C13 nuclear magnetic resonance (NMR) spectrum of BODBB.

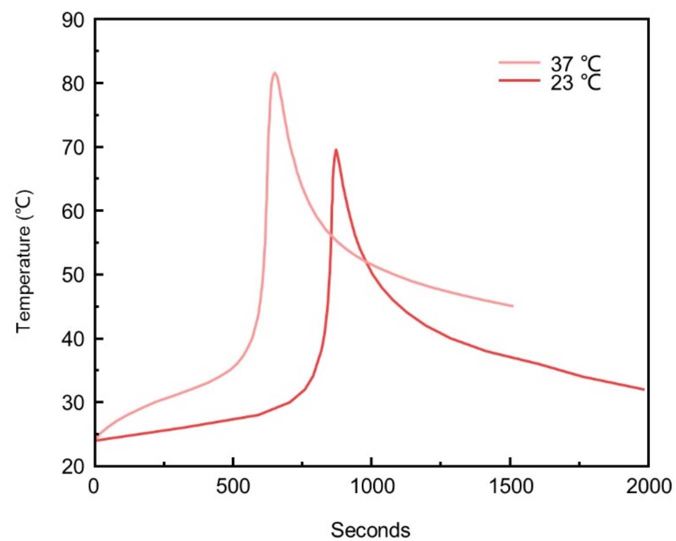


Figure S7. Temperature changes in the process of polymerization using BPO as a catalyst.

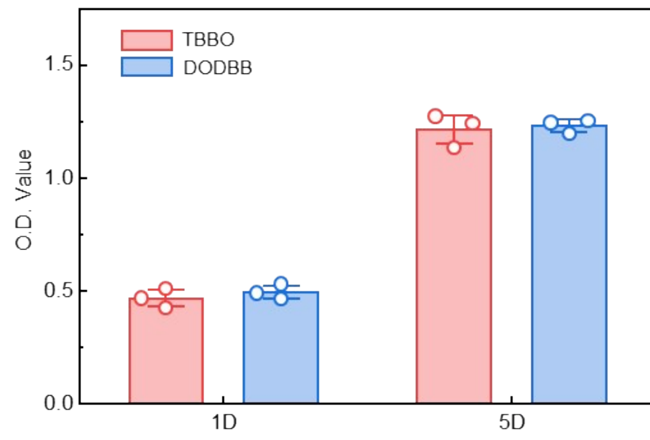


Figure S8. CCK8 test on cells cultured on the surface of the materials for 5 days.

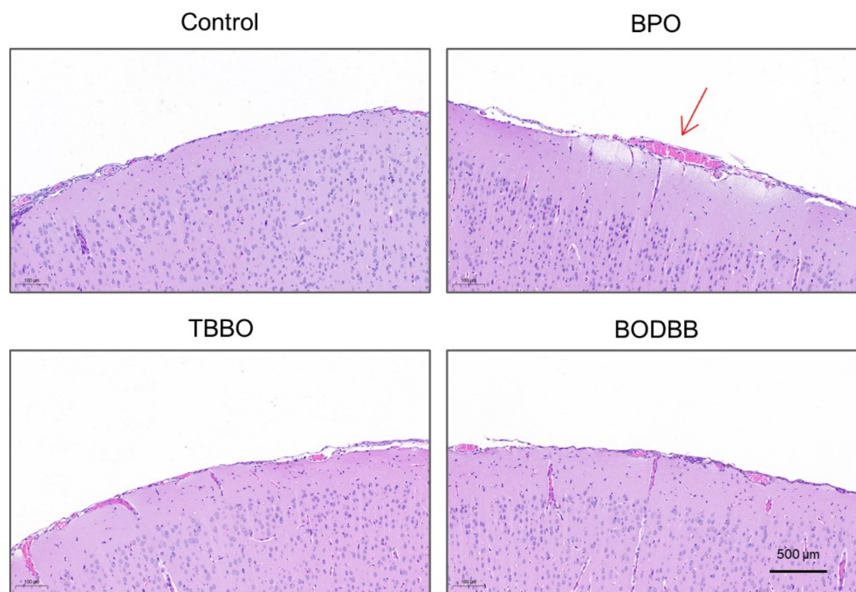


Figure S9. The H&E staining image of obtained brain tissue section 4 weeks post-operation.

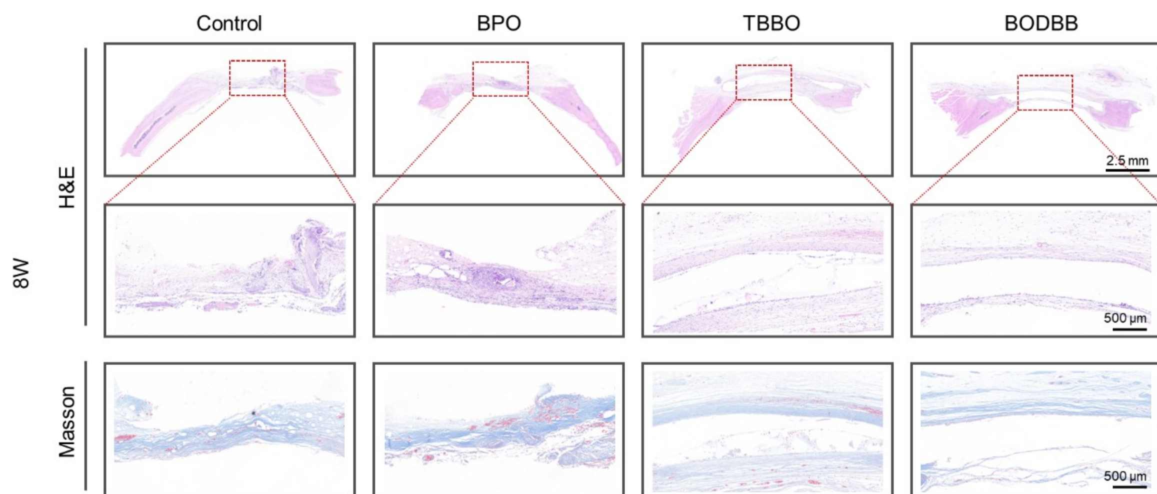


Figure S10. The H&E staining and Masson's trichrome staining image of obtained tissue section 8 weeks post-operation.