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Restoring the electrical microenvironment using ferroelectric nanocomposite membranes to enhance alveolar ridge regeneration in a mini-pig preclinical model

Yiping Li[a,b,c,‡], Yanze Meng[a,‡], Yunyang Bai[c,‡], Yijun Wang[a,‡] , Jiaqi Wang[b,‡], Boon Chin Heng[d], Jinqi Wei[e], Xi Jiang[f], Min Gao[c,g,*], Xiaona Zheng[c,g,*], Xuehui Zhang[a,g,*], Xuliang Deng[d,g]

[a]Department of Dental Materials & Dental Medical Devices Testing Center, Peking University School and Hospital of Stomatology, Beijing, 100081, PR China.

[b]Department of Prosthodontics, Xiangya Stomatological Hospital & School of Stomatology, Central South University, Changsha, 410078, PR China.

[c]Department of Geriatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, 100081, PR China.

[d]Central Laboratory, Peking University School and Hospital of Stomatology, Beijing, 100081, PR China.

[e]First Clinical Division, Peking University School and Hospital of Stomatology, Beijing, 100081, PR China.

[f]Department of Oral Implantology, Peking University School and Hospital of Stomatology, Beijing, 100081, PR China.

[g]National Engineering Research Center of Oral Biomaterials and Digital Medical Devices, NMPA Key Laboratory for Dental Materials, Beijing Laboratory of Biomedical Materials & Beijing Key Laboratory of Digital Stomatology, Peking University School and Hospital of Stomatology, Beijing, 100081, PR China.

*Corresponding authors:

Prof. Xuehui Zhang, E-mail: zhangxuehui@bjmu.edu.cn

Dr. Xiaona Zheng, E-mail: zhengxiaona@pku.edu.cn

Dr. Min Gao, E-mail: dzgaomin@163.com

[#]These authors contributed equally to this work.

Supplemental Materials and Methods

Sub-chronic systemic toxicity assay

The experiment was approved by the Animal Care and Use Committee of Peking University. This assay was performed according to the standard ISO 10993-11: 2006 protocols. Healthy adult rats (n = 20) (male: n = 10; female: n = 10) were randomly assigned to 2 groups as follows: BTO/P(VDF-TrFE) group (male: n = 10; female: n = 10) and blank control group (male: n = 10; female: n = 10). After general anesthesia, the skin was cut along the midline of the rat's back to expose the subcutaneous tissue, and the tissue on both sides was bluntly separated by hemostatic forceps to prepare a subcutaneous skin sac, and the BTO/P(VDF-TrFE) membranes was implanted to the surface of the subcutaneous tissue. The amount of animal implantation was determined by equivalent conversion of 100 times the maximum human clinical dose. Each female animal in BTO/P(VDF-TrFE) group was implanted with a 7 cm^2, and each male animal in BTO/P(VDF-TrFE) group was implanted with a 9.3 cm^2. In blank control group, the rats were subjected to the same surgical treatment as the BTO/P(VDF-TrFE) group, but no stimulation was given. All rats were observed daily and fed rations for three months. All rats were weighed once every week.

Examination of blood biochemistry and cytology

After implantation for three months, the rats were sacrificed and the blood was collected from the abdominal aorta for biochemical and cytological examination. Following hematological parameters were determined: total protein (TP), Albumin (ALB), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine (CRE), blood glucose (GLU), alanine aminotransferase (ALT), total bilirubin (TBIL), Triglyceride (TG), calcium (Ca), Inorganic Phosphorus (IP), Urea (UREA), Cholesterol (CHO), White blood cell (WBC), Albumin (ALB), Hemoglobin (HGB), Hematocrit (HCT), Platelet (PLT), Activated partial thromboplastin time (APTT),

Prothrombin time (PT), International normalized ratio (INR) and Prothrombin activity (PTA) between the BTO/P(VDF-TrFE) group versus blank control group.

Histological examination

After implantation for three months, the rats were sacrificed and main organs of rats were weighed and collected (liver, brain, thyroid, heart, lung, thymus, intestine, kidney, pancreas, adrenal gland, spleen and salivary gland). These organs were fixed in 10% formalin, embedded in paraffin blocks and colored by haematoxilin-eosine staining. Sections were evaluated with help a light microscope. Also, calculate the ratio of organ weight to body weight.

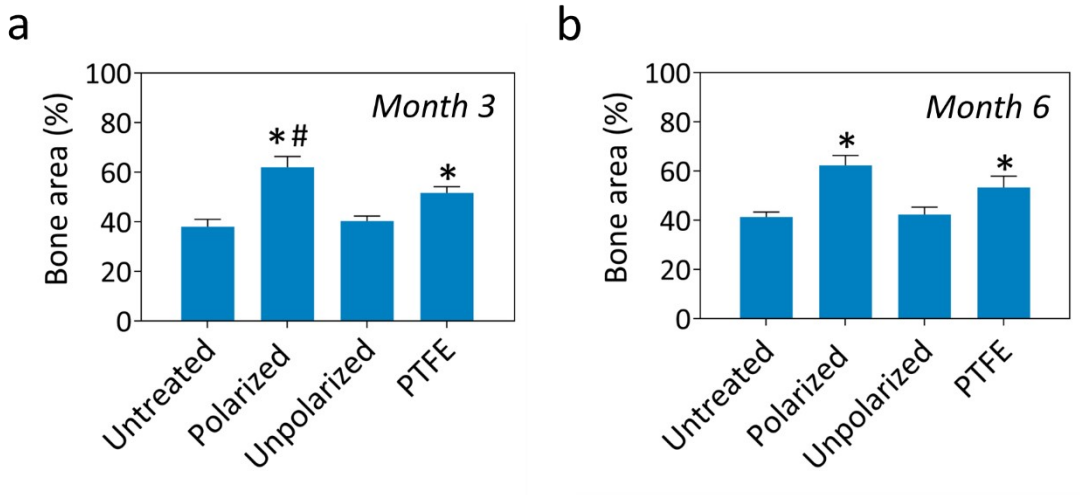

Figure S1. Quantitative histological analyses of the percentages of newly-regenerated bone areas upon alveolar ridge preservation. (a) After 3-months of healing. (b) After 6-months of healing. *$p<0.05$ compared to Untreated control. $^{\#}p<0.05$ compared to PTFE.

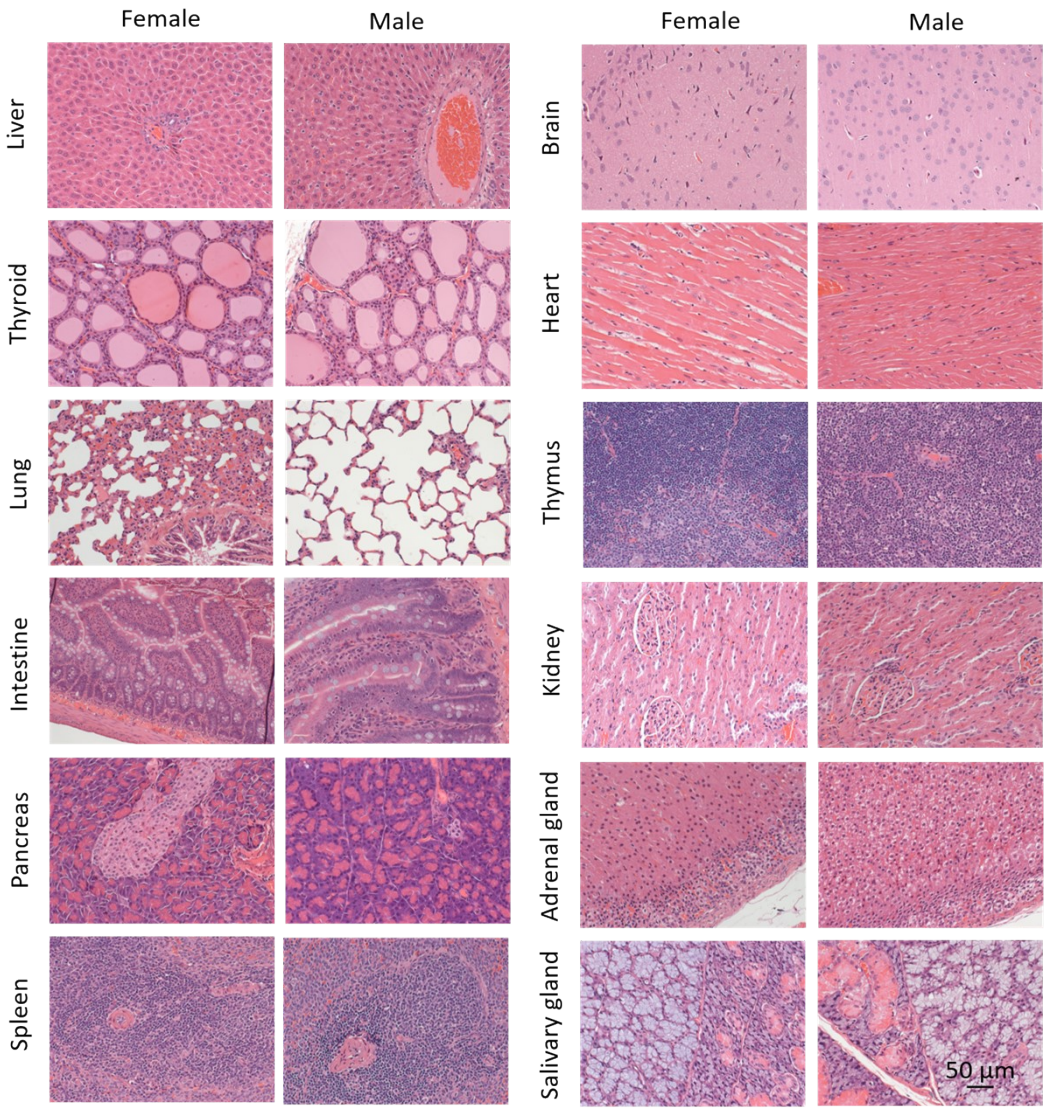

Figure S2. Histological analyses results of preclinical subchronic systemic toxicity evaluation of the electroactive BTO/P(VDF-TrFE) nanocomposite membranes.

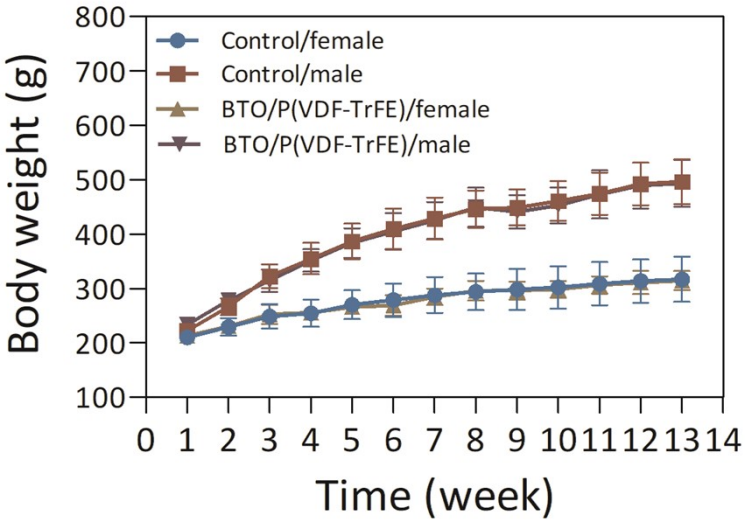

Figure S3. The body weight of after the electroactive BTO/P(VDF-TrFE) nanocomposite membranes were implanted in rats at different time points among 13 weeks. (X±S, n=10)

Table S1. Ratio of organ weight to body weight after the electroactive BTO/P(VDF-TrFE) nanocomposite membranes were implanted in rats for three months. (X±S, n=10)

Index	Unit	Normal control (Male)	Normal control (Female)	BTO/P(VDF-TrFE) (Male)	BTO/P(VDF-TrFE) (Female)
Heart/body	(%)	0.34±0.03	0.36±0.05	0.32±0.03	0.30±0.11
Liver/body	(%)	2.09±0.16	2.34±0.27	2.15±0.16	2.25±0.83
Spleen/body	(%)	0.15±0.01	0.18±0.03	0.16±0.02	0.16±0.06
Kidney/body	(%)	0.54±0.05	0.58±0.06	0.55±0.07	0.51±0.18
Adrenal gland/body	(%)	0.01±0.00	0.02±0.00	0.01±0.01	0.02±0.01
Thymus/body	(%)	0.08±0.01	0.12±0.02	0.09±0.01	0.10±0.05
Brain/body	(%)	0.41±0.03	0.60±0.09	0.40±0.10	1.08±1.70

Table S2. Blood biochemical analysis after the electroactive BTO/P(VDF-TrFE) nanocomposite membranes were implanted in rats for three months. (X±S, n=10)

Index	Unit	Normal control (Male)	Normal control (Female)	BTO/P(VDF-TrFE) (Male)	BTO/P(VDF-TrFE) (Female)
TP	(g/L)	61.96±1.90	70.26±4.86	63.40±2.09	70.48±2.36
ALB	(g/L)	31.00±1.09	37.59±2.52	30.76±1.89	38.34±1.97
ALP	(U/L)	64.08±7.61	31.04±6.22	69.06±19.55	36.93±10.94
AST	(U/L)	89.90±12.01	91.50±24.76	99.20±19.98	126.30±57.16
CRE	(μmol/L)	30.88±2.88	35.69±4.55	31.52±3.02	36.14±5.28
GLU	(mmol/L)	8.41±0.68	8.16±1.25	7.94±0.70	8.02±0.60
ALT	(U/L)	38.60±5.87	36.60±9.16	37.30±5.40	36.00±11.11
TBIL	(μmol/L)	0.81±0.22	1.23±0.34	0.77±0.21	1.51±0.45
TG	(mmol/L)	0.66±0.22	1.16±0.79	0.60±0.09	0.76±0.34
Ca	(mmol/L)	2.71±0.07	2.82±0.04	2.74±0.08	2.83±0.07
IP	(mmol/L)	1.97±0.11	1.61±0.11	2.10±0.13	1.75±0.17
UREA	(mmol/L)	5.85±0.67	5.97±1.15	5.67±0.74	5.73±0.89
CHO	(mmol/L)	1.33±0.24	2.00±0.50	1.57±0.26	1.86±0.40

Table S3. Blood cytology analysis after the electroactive BTO/P(VDF-TrFE) nanocomposite membranes were implanted in rats for 13 weeks. (X±S, n=10)

Index	Unit	Normal control (Male)	Normal control (Female)	BTO/P(VDF-TrFE) (Male)	BTO/P(VDF-TrFE) (Female)
WBC	($\times10^9$/L)	3.57±0.56	1.93±0.77	4.07±1.06	1.87±0.88
ALB	($\times10^{12}$/L)	7.23±0.17	6.27±0.63	7.40±0.25	6.39±0.29
HGB	(g/L)	136.50±2.68	123.60±10.84	140.10±3.60	126.70±3.77
HCT	(L/L)	38.75±1.09	34.68±3.16	39.59±1.42	35.75±1.61
PLT	($\times10^9$/L)	846.50±88.81	818.70±239.08	882.90±77.91	888.80±71.33
APTT	(s)	21.09±0.92	18.85±2.22	21.02±2.30	17.03±1.37
PT	(s)	16.53±1.25	13.12±1.99	17.20±0.90	12.76±1.03
INR	/	1.36±0.10	1.09±0.16	1.41±0.07	1.06±0.08
PTA	(%)	72.06±5.70	93.62±11.41	68.66±4.41	96.16±7.16