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Original article

The effect of trace elements on BMP-2, BMP-7 and STRO-1$^+$ cells in hip replacement

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ABSTRACT

To explore the correlation between the trace elements in the proximal femur and BMP-2, BMP-7 and STRO-1+ cells in hip replacement, and analyze the therapeutic effect of prosthesis loosening in clinic. Fifty-one patients undergone the first hip replacement in xxx hospital from August 2016 to August 2019 were selected as the study subjects, including 26 females and 25 males, aged 52–89 years. The bone marrow mesenchymal stem cells (BMSCs) were cultured in vitro for flow cytometry, and the string-1+ in BMSCs was detected and analyzed. After that, the expression of bone morphogenetic protein 2 (BMP-2) and bone morphogenetic protein 7 (BMP-7) in the cells were detected by enzyme-linked immunosorbent assay, the content of trace elements in the supernatant was detected by radioimmunoassay, and the collected data were analyzed statistically. In the analysis of the content of trace elements, it was found that the correlation between trace elements was dependent on the separation area, and all trace elements had no correlation with BMP2. Ca2$^+$, Mg2$^+$ were correlated with the level of BMP7 and Ca2$^+$, VD3 was correlated with the percentage of STOR-1$^+$ cells. Further analysis showed that the correlation between trace elements was dependent on bone mineral density (BMD) area, and there was a positive correlation between vitamin D3 (VD3), parathyroid hormone (PTH), zinc, and BMD in zone 7. To sum up, it is found that trace elements may be related to prosthesis loosening, which provides experimental basis for the treatment of prosthesis loosening later.

1. Introduction

Artificial joint replacement especially hip arthroplasty has become increasingly popular (Berry et al., 2002). Improving the fusion of prosthesis and prolonging the survival rate of prosthesis has become a research hotspot in joint surgery. The current research mainly focuses on prosthesis design, lacking of consideration of the patient's factors. This study discussed the prophylaxis for aseptic loosening of hip prosthesis on the basis of the clinical research of patients.

As a kind of non-hematopoietic stem cells, BMSCs can support hematopoiesis in vitro and in vivo by secreting a variety of growth factors. Because BMSCs are easy to be isolated and cultured and have strong proliferation ability in vitro, they have a broad application prospect in the treatment of bone tissue cells. Stromal cell antigen (STRO-1) is the surface antigen of human bone marrow cells. STRO-1$^+$ stem cells have strong osteogenic differentiation. STRO-1$^+$ bone marrow stem cell cells have strong mesenchymal differentiation, such as fat cells, osteoblasts and chondrocytes (Bidwell et al., 2013). Many factors lead to Stro-1+ cell differences, such as age, gender and other factors (Ganguly et al., 2017; Zheng et al., 2018). Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-β super family and were originally identified to induce ectopic bone growth and cartilage formation as osteoinductive cytokines (Urist, 1965; Wozney et al., 1988). In particular, BMP-2 and BMP-7 have been approved for clinical use in the United States, Europe and Australia (Reddi, 2005). BMP-2 is most extensively studied for its ability to induce bone regeneration in tissue engineering (Carreira et al., 2014). It has been reported that BMP-7 causes the inhibition of the intracellular signaling initiated by TGF-β, through the blockage of Smad 3/4, and diminishes the effects of TGF-β (Pegorier et al., 2010; Lim et al., 2016).

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Microelement are essential to maintain normal physiological function and stable internal environment. Microelement also play an important role in bone metabolism. For example, Zinc is an important auxiliary factor of a variety of bone metabolic enzymes like alkaline phosphatase and collagenase, which is closely related with osteogenesis (Qiao et al., 2014; Luo et al., 2014).

From the point of view of the content of trace elements around the prosthesis, the trace elements and bone mineral density (BMD) of 51 patients undergoing hip replacement were investigated, and the relationship between trace elements and BMP-2, BMP-7, STRO-1+ cells was analyzed, thereby providing a reference for the clinical patients after hip replacement to improve the life of the prosthesis.

2. Materials and methods

2.1. Patients

51 cases of patients with the first hip replacement were enrolled from xxx hospital between August 2016 and August 2019. The study was approved by the ethics committee of the hospital. The patients and their families signed the informed consent. The enrolled criteria was list as following: Patients with osteoarthritis and femoral neck fractures; Ages between 50 and 90 years old; With standard pelvic flat and full-length femoral head.

Exclusion criteria: Patients with rheumatoid arthritis and other autoimmune diseases; Patients with non-steroid analgesics, steroid hormones, estrogen replacement therapy, bisphosphonates, and calcium treatment two weeks prior to hip replacement; Complications after hip replacement include infection, leave Implant failure, wound healing and vascular, nerve injury.

2.2. Cell culture

BMSCs were extract from proximal femoral head grooved and discarded bone mass during the hip replacement. Cells were cultured in α-MEM with 20% FBS. And cells were identified by flow cytometric analysis.

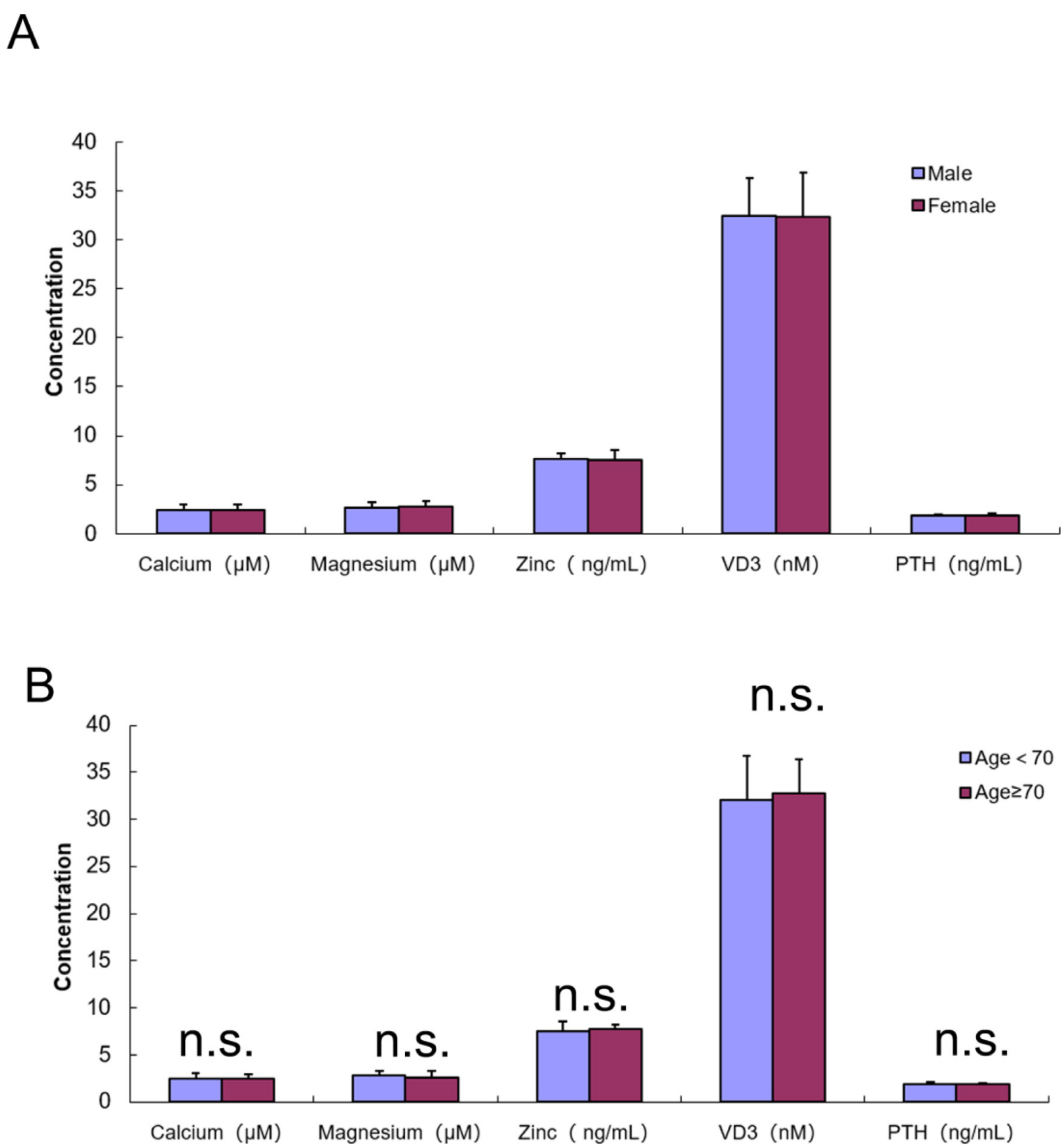

Fig. 1. Comparison of microelements in patients of different genders and ages.

2.3. Flow cytometric analysis

Flow cytometric analysis was performed to analyzed the STRO-1$^+$ cells. The specific steps were as follows: (1) recognize and combine different antigens (staining) on cells by antibodies with different fluorescent groups; (2) cell with fluorescence passes through laser one by one; (3) different fluorescent groups have different emission spectra, and the principle of fluorescent dye selection: it must be able to be excited by the laser equipped on the flow cytometer, and the excitation spectrum must be within the proper range that the filter can accept, and the overlap of the fluorescein spectrum should be minimized; (4) the complex fluorescence signal is decomposed by different spectroscopes and filters; (5) the photomultiplier tube (PMT) turns the optical signal into the electrical signal; (6) the analog signal is further transformed into the digital signal that can be processed by the computer through the analog-to-digital converter (ADC).

2.4. Enzyme-linked immunosorbent assay (ELISA)

Human IL-11 ELISA kits (Neobioscience, China) were used in accordance with the manufacturer's instructions manual to quantify concentrations of the BMP-2 and BMP-7.

2.5. Radio immunofluorescence (RIA)

The level of microelement was measured by radio immunofluorescence assay. According to the principle of antigen antibody reaction, the known antigens or antibodies were labeled with fluorescent groups, and then the fluorescent antibodies (or antigens) were used as probes to check the corresponding antigens (or antibodies) in cells or tissues. The fluorescence microscope can be used to see the cells or tissues where the fluorescence is located, thereby determining the nature and location of antigens or antibodies, and

Table 1
The baseline information of the patients enrolled in the present study.

Number	Gender	Age	BMP2 (pg/ml)	BMP7 (pg/ml)	Stro-1 %
1	Female	62	85.577	167.033	6.99
2	Female	83	75.072	167.033	9.54
3	Female	75	85.577	190.020	26.62
4	Male	50	109.213	181.400	8.42
5	Male	71	80.324	198.640	30.27
6	Male	89	101.334	195.766	27.11
7	Male	65	80.324	187.146	8.50
8	Female	75	72.445	227.373	33.00
9	Male	82	88.203	158.413	12.36
10	Male	52	148.607	184.273	22.16
11	Female	65	101.334	169.906	8.85
12	Female	87	80.324	169.906	34.16
13	Female	62	80.324	192.893	12.53
14	Male	55	80.324	175.653	15.52
15	Female	78	75.072	172.780	37.82
16	Female	66	90.829	204.387	19.63
17	Male	54	75.072	181.400	17.29
18	Female	45	75.072	187.146	38.67
19	Female	80	85.577	235.994	23.29
20	Male	89	93.456	190.020	3.59
21	Female	65	101.334	195.766	46.36
22	Female	75	96.082	169.906	21.16
23	Male	82	103.961	184.273	18.89
24	Female	52	80.324	161.286	26.12
25	Male	56	109.084	175.097	23.42
26	Male	67	154.679	140.206	15.71
27	Male	52	66.132	133.228	13.33
28	Male	65	109.084	161.140	6.29
29	Male	63	63.489	129.739	8.65
30	Male	70	81.727	157.651	14.46
31	Female	59	55.251	161.140	7.61
32	Male	56	118.203	175.097	11.09
33	Male	70	99.965	154.162	15.66
34	Female	77	145.560	140.206	29.72
35	Female	56	109.084	133.228	17.75
36	Female	52	81.727	115.782	13.11
37	Female	77	99.965	126.249	11.07
38	Female	87	90.846	178.586	21.44
39	Male	77	99.965	154.162	8.01
40	Male	52	109.084	143.695	11.42
41	Male	52	145.560	199.520	34.83
42	Female	72	136.441	143.695	15.82
43	Female	58	90.846	126.249	8.73
44	Male	63	63.489	112.293	3.07
45	Female	58	191.155	133.228	23.14
46	Female	58	136.441	143.695	21.38
47	Male	86	72.608	185.564	14.88
48	Female	59	127.322	182.075	19.41
49	Male	78	163.798	178.586	20.08
50	Female	79	172.917	230.922	36.99
51	Male	77	118.203	157.651	15.48

deciding the content by quantitative technology (such as flow cytometry).

2.6. Statistical analyses

SPSS 18.0 software was applied for statistical analysis. Pearson correlation coefficient was used for correlation analysis. r was used to represent the correlation coefficient between variables in the sample, and the size of the correlation. P was used to test whether the two variables have the same correlation in the population from which the sample comes. $P < 0.05$ indicated that there was correlation between variables.

3. Results

3.1. The concentration of microelement in the culture medium of cell extracted from proximal femur bone

Fig. 1 shows the comparison of the content of trace elements in the body of patients of different genders and ages. To determine the essential role of microelement, we first collected and organized the basic information of enrolled patients. As revealed in Table 1, there are 26 females and 25 males, aged 52–89 years old, with an average age of 68.3. The BMD analysis results were presented in Table 2. Then, we measured the level of five different microelement Ca^{2+}, Mg^{2+}, Zn^{2+}, VD3 and PTH in the culture medium of cell extracted from proximal femur bone. As demonstrated in Table 3–5 and Fig. 1, the level of these five microelements were consistent in male and female patients, and the trend not correlate with age.

3.2. The level of BMP2, BMP7 and the percent of STRO-1⁺ cells

Next, we evaluated the expression level of BMP2 and BMP7 in the culture medium of extracted cell by using ELISA. And we also sorting the STRO-1+ cells by applying flow cytometric analysis. All the data are presented in Table 1.

3.3. The correlation between microelements and BMD

We analysis the correlation between those microelements and BMD. As demonstrated in Table 6, in Zone 1 when followed up for six months, the level of VD3 (r = 0.326, P = 0.019), PTH (r = 0.325, P = 0.020) and Zinc (r = 0.346, P = 0.013) was positively correlated with BMD; in Zone 2, no significantly relationship between those microelements and BMD; in Zone 3, the level of Mg^{2+} and Zinc waspositively correlated with BMD when followed up for three months (r = 0.294, P = 0.036; r = 0.285, P = 0.043, respectively) and six months (r = 0.292, P = 0.038; r = 0.284, P = 0.050, respectively); in Zone 4, only the level of Zinc at the follow-up time of three month was positively correlated with BMD (r = 0.317, P = 0.023); in Zone 5, only the level of Zine at the follow-up time of three and twelve months was positively correlated with BMD (r = 0.305, P = 0.029; r = 0.288, P = 0.040, respectively); in Zone 6, the level of Ca^{2+} at the follow-up time of six and twelve months was positively correlated with BMD (r = 0.290, P = 0.039; r = 0.279, P = 0.048, respectively) and the level of PTH and Zinc at the follow-up time of twelve months was positively correlated with the level of BMD (r = 0.280, P = 0.022; r = 0.321, P = 0.022, respectively); and in Zone 7, the level of VD3, PTH, Zinc was positively correlated with BMD at the follow-up time of one (r = 0.571, P = 0.025; r = 0.280, P = 0.047; r = 0.456, P = 0.001, respectively), three (r = 0.557, P = 0.001; r = 0.370, P = 0.008; r = 0.447, P = 0.001, respectively), six (r = 0.551, P = 0.001; r = 0.352, P = 0.011; r = 0.437, P = 0.001, respectively) and twelve months (r = 0.541, P = 0.001; r = 0.343, P = 0.014; r = 0.420, P = 0.002, respectively).

3.4. The correlation between microelements and BMP2, BMP7 and STRO-1⁺ cells

We analyzed the correlation between microelements and BMP2. As demonstrated in Table 7, none of the microelements was correlated with BMP2. Then we analyzed the correlation between microelements and BMP7. We found that the level of Ca^{2+} was positively correlated with the level of BMP7 (r = 0.32448, p = 0.0202); the level of Mg^{2+} was negatively correlated with the level of BMP7 (r = -0.30196, p = 0.0313); while the level of Zinc, VD3 and PTH showed no significant correlation with BMP7. Additionally, we also analyzed the correlation between those microelements with STRO-1+ cells. We revealed that the level of Ca^{2+} was positively correlated with the percent of STRO-1 + cells (r = 0.28654, p = 0.0415); and the level of VD3 was positively correlated with the percent of STRO-1+ cells (r = 0.2683, p = 0.050); while the level of Mg^{2+}, Zinc and PTH exerted no significantly correlation with STRO-1+ cells. All the data were present in Table 7.

Table 2
The BMD of the patients enrolled in the present study.

Number	Follow-up time	Zone1	Zone2	Zone3	Zone4	Zone5	Zone6	Zone7
1	1 week	0.88	1.485	1.425	1.491	1.428	1.334	0.877
	3 month	0.872	1.288	1.375	1.267	1.149	1.07	0.679
	6 month	0.717	1.349	1.355	1.358	1.401	1.18	0.688
	12 month	0.783	1.385	1.378	1.34	1.351	0.98	0.696
2	1 week	0.899	1.19	1.499	1.39	1.531	1.308	0.56
	3 month	0.705	1.299	1.597	1.431	1.48	1.249	0.39
	6 month	0.613	1.22	1.59	1.487	1.399	1.216	0.439
	12 month	0.443	1.266	1.45	1.349	1.348	1.231	0.452
3	1 week	0.666	1.404	1.498	1.497	1.618	1.256	1.028
	3 month	0.722	1.326	1.509	1.451	1.672	1.378	1.065
	6 month	0.688	1.385	1.575	1.519	1.75	1.406	1.124
	12 month	0.849	1.411	1.588	1.535	1.731	1.255	1.17
4	1 week	0.908	1.533	1.471	1.539	1.474	1.377	0.905
	3 month	0.900	1.329	1.419	1.308	1.186	1.104	0.701
	6 month	0.740	1.392	1.398	1.401	1.446	1.218	0.710
	12 month	0.808	1.429	1.422	1.383	1.394	1.011	0.718
5	1 week	0.928	1.228	1.547	1.434	1.580	1.350	0.578
	3 month	0.728	1.341	1.648	1.477	1.527	1.289	0.402
	6 month	0.633	1.259	1.641	1.535	1.444	1.255	0.453
	12 month	0.457	1.307	1.496	1.392	1.391	1.270	0.466

(continued on next page)

Table 2 (*continued*)

Number	Follow-up time	Zone1	Zone2	Zone3	Zone4	Zone5	Zone6	Zone7
6	1 week	0.687	1.449	1.546	1.545	1.670	1.296	1.061
	3 month	0.745	1.368	1.557	1.497	1.726	1.422	1.099
	6 month	0.710	1.429	1.625	1.568	1.806	1.451	1.160
	12 month	0.876	1.456	1.639	1.584	1.786	1.295	1.207
7	1 week	0.719	1.201	1.629	1.597	1.625	1.144	0.486
	3 month	0.795	1.374	1.639	1.621	1.657	1.245	0.446
	6 month	0.504	1.179	1.735	1.718	1.679	1.368	0.454
	12 month	0.535	1.439	1.838	1.818	1.703	1.57	0.383
8	1 week	0.674	1.420	1.515	1.514	1.636	1.270	1.040
	3 month	0.730	1.341	1.526	1.467	1.691	1.394	1.077
	6 month	0.696	1.401	1.593	1.536	1.770	1.422	1.137
	12 month	0.859	1.427	1.606	1.552	1.751	1.269	1.183
9	1 week	0.705	1.177	1.596	1.565	1.593	1.121	0.476
	3 month	0.779	1.347	1.606	1.589	1.624	1.220	0.437
	6 month	0.494	1.155	1.700	1.684	1.645	1.341	0.445
	12 month	0.524	1.410	1.801	1.782	1.669	1.539	0.375
10	1 week	0.861	1.062	1.072	1.285	1.185	1.072	0.833
	3 month	0.669	1.026	1.216	1.319	1.243	1.255	0.584
	6 month	0.439	1.055	1.137	1.291	1.222	1.200	0.593
	12 month	0.593	1.075	1.179	1.329	1.202	1.178	0.600
11	1 week	0.870	1.141	1.455	1.348	1.479	1.283	0.581
	3 month	0.683	1.262	1.551	1.390	1.437	1.213	0.370
	6 month	0.595	1.187	1.575	1.450	1.371	1.185	0.488
	12 month	0.320	1.228	1.403	1.313	1.305	1.191	0.441
12	1 week	0.831	1.454	1.666	1.619	1.543	1.540	1.686
	3 month	0.743	1.440	1.591	1.630	1.579	1.565	1.623
	6 month	0.809	1.457	1.581	1.610	1.527	1.641	1.643
	12 month	0.767	1.371	1.505	1.680	1.545	1.595	1.700
13	1 week	0.550	1.304	1.389	1.273	1.409	1.366	0.938
	3 month	0.633	1.341	1.371	1.267	1.355	1.347	0.856
	6 month	0.480	1.310	1.392	1.380	1.377	1.360	0.917
	12 month	0.392	1.333	1.391	1.358	1.405	1.417	0.947
14	1 week	0.711	1.569	1.524	1.537	1.961	1.78	0.818
	3 month	0.68	1.364	1.56	1.604	2.051	1.841	0.781
	6 month	0.654	1.629	1.662	1.608	1.951	1.746	0.784
	12 month	0.675	1.555	1.678	1.816	1.975	1.642	0.775
15	1 week	0.720	1.588	1.542	1.555	1.985	1.801	0.828
	3 month	0.688	1.380	1.579	1.623	2.076	1.863	0.790
	6 month	0.662	1.649	1.682	1.627	1.974	1.767	0.793
	12 month	0.683	1.574	1.698	1.838	1.999	1.662	0.784
16	1 week	0.739	1.632	1.585	1.598	2.039	1.851	0.851
	3 month	0.707	1.419	1.622	1.668	2.133	1.915	0.812
	6 month	0.680	1.694	1.728	1.672	2.029	1.816	0.815
	12 month	0.702	1.617	1.745	1.889	2.054	1.708	0.806
17	1 week	0.748	1.651	1.604	1.618	2.064	1.873	0.861
	3 month	0.716	1.436	1.642	1.688	2.159	1.938	0.822
	6 month	0.688	1.714	1.749	1.692	2.053	1.838	0.825
	12 month	0.710	1.637	1.766	1.911	2.079	1.728	0.816
18	1 week	0.875	1.201	1.241	1.33	1.66	1.348	1.018
	3 month	0.531	1.291	1.429	1.311	1.598	1.239	1.089
	6 month	0.87	1.165	1.437	1.371	1.712	1.388	1.031
	12 month	0.722	1.235	1.399	1.465	1.752	1.425	1.029
19	1 week	0.831	1.454	1.666	1.619	1.543	1.540	1.686
	3 month	0.743	1.440	1.591	1.630	1.579	1.565	1.623
	6 month	0.809	1.457	1.581	1.610	1.527	1.641	1.643
	12 month	0.767	1.371	1.505	1.680	1.545	1.595	1.700
20	1 week	0.829	1.262	1.646	1.618	1.644	1.212	0.621
	3 month	0.897	1.419	1.654	1.640	1.673	1.303	0.583
	6 month	0.635	1.245	1.745	1.729	1.695	1.413	0.590
	12 month	0.663	1.478	1.837	1.815	1.711	1.589	0.527
21	1 week	0.661	1.192	1.258	1.244	1.340	1.310	1.067
	3 month	0.615	1.185	1.278	1.240	1.402	1.301	1.067
	6 month	0.587	1.191	1.365	1.228	1.375	1.262	0.907
	12 month	0.552	1.195	1.261	1.237	1.462	1.284	1.003
22	1 week	0.530	1.265	1.418	1.293	1.456	1.448	1.226
	3 month	0.594	1.410	1.485	1.360	1.505	1.562	1.244
	6 month	0.767	1.318	1.510	1.394	1.526	1.450	1.261
	12 month	0.717	1.270	1.584	1.500	1.549	1.461	1.318
23	1 week	0.527	1.109	1.481	1.365	1.670	1.592	1.119
	3 month	0.545	1.199	1.511	1.312	1.647	1.601	1.113
	6 month	0.542	1.237	1.527	1.420	1.763	1.661	1.137
	12 month	0.594	1.301	1.508	1.348	1.721	1.674	1.164

Table 2 (*continued*)

Number	Follow-up time	Zone1	Zone2	Zone3	Zone4	Zone5	Zone6	Zone7
24	1 week	0.984	1.247	1.265	1.266	1.464	1.188	1.262
	3 month	0.861	1.265	1.322	1.436	1.522	1.373	1.272
	6 month	0.753	1.239	1.482	1.400	1.487	1.319	1.321
	12 month	0.753	1.192	1.459	1.487	1.496	1.340	1.360
25	1 week	0.721	1.202	1.629	1.598	1.627	1.147	0.49
	3 month	0.797	1.377	1.638	1.622	1.659	1.248	0.448
	6 month	0.506	1.183	1.739	1.721	1.683	1.37	0.455
	12 month	0.537	1.442	1.841	1.817	1.701	1.566	0.385
26	1 week	0.534	1.124	1.198	1.182	1.289	1.256	0.985
	3 month	0.483	1.117	1.22	1.178	1.358	1.245	0.985
	6 month	0.452	1.123	1.317	1.164	1.328	1.202	0.808
	12 month	0.413	1.128	1.201	1.174	1.424	1.227	0.914
27	1 week	0.389	1.206	1.376	1.237	1.418	1.409	1.162
	3 month	0.46	1.367	1.45	1.311	1.472	1.536	1.182
	6 month	0.652	1.264	1.478	1.349	1.495	1.411	1.201
	12 month	0.597	1.211	1.56	1.467	1.521	1.423	1.264
28	1 week	0.386	1.032	1.445	1.317	1.655	1.569	1.043
	3 month	0.405	1.132	1.479	1.258	1.63	1.579	1.037
	6 month	0.402	1.174	1.497	1.378	1.759	1.645	1.063
	12 month	0.46	1.245	1.476	1.298	1.712	1.66	1.093
29	1 week	0.709	1.567	1.522	1.533	1.955	1.779	0.819
	3 month	0.678	1.365	1.556	1.606	2.048	1.838	0.782
	6 month	0.656	1.627	1.659	1.6	1.946	1.743	0.785
	12 month	0.673	1.553	1.68	1.814	1.977	1.64	0.775
30	1 week	0.768	1.429	1.576	1.771	1.662	1.349	1.049
	3 month	0.771	1.436	1.456	1.867	1.728	1.389	1.15
	6 month	0.781	1.41	1.567	1.856	1.786	1.396	1.132
	12 month	0.765	1.529	1.609	1.887	1.802	1.409	1.12
31	1 week	0.664	1.402	1.494	1.495	1.617	1.252	1.022
	3 month	0.723	1.325	1.508	1.449	1.674	1.389	1.063
	6 month	0.688	1.381	1.57	1.518	1.748	1.404	1.123
	12 month	0.852	1.408	1.587	1.536	1.727	1.257	1.169
32	1 week	0.977	1.65	1.852	2.038	1.628	1.133	1.17
	3 month	1.045	1.631	1.866	2.044	1.681	1.084	1.001
	6 month	1.08	1.66	2.026	1.997	1.851	1.066	1.115
	12 month	0.817	1.621	1.985	1.955	1.802	1.082	1.081
33	1 week	0.893	1.185	1.205	1.207	1.427	1.12	1.202
	3 month	0.757	1.205	1.269	1.395	1.491	1.326	1.213
	6 month	0.637	1.177	1.447	1.356	1.452	1.265	1.268
	12 month	0.637	1.124	1.421	1.452	1.462	1.289	1.311
34	1 week	0.719	1.044	1.338	1.121	1.289	1.31	0.601
	3 month	0.795	1.017	1.37	1.139	1.336	1.297	0.692
	6 month	0.784	1.185	1.423	1.191	1.35	1.278	0.754
	12 month	0.808	1.066	1.369	1.163	1.365	1.301	0.784
35	1 week	0.385	0.938	1.285	1.026	1.106	0.945	1.206
	3 month	0.525	0.904	1.353	1.16	1.216	1.073	1.172
	6 month	0.531	0.895	1.4	1.252	1.265	1.038	1.165
	12 month	0.496	0.869	1.533	1.361	1.276	1.325	1.237
36	1 week	0.839	1.469	1.683	1.635	1.559	1.556	1.703
	3 month	0.75	1.455	1.607	1.646	1.595	1.581	1.639
	6 month	0.817	1.472	1.597	1.626	1.542	1.658	1.66
	12 month	0.775	1.385	1.52	1.697	1.561	1.611	1.717
37	1 week	0.816	1.727	1.702	1.804	1.658	1.321	0.831
	3 month	0.784	1.625	1.726	1.716	1.693	1.281	0.896
	6 month	0.766	1.628	1.559	1.616	1.54	1.299	0.863
	12 month	0.719	1.622	1.577	1.771	1.587	1.279	0.896
38	1 week	0.686	0.966	1.268	1.313	1.542	1.421	0.454
	3 month	0.588	0.987	1.374	1.402	1.649	1.542	0.463
	6 month	0.496	0.927	1.414	1.262	1.61	1.214	0.413
	12 month	0.396	0.893	1.504	1.344	1.636	1.384	0.452
39	1 week	0.542	1.201	1.447	1.351	1.469	1.335	1.044
	3 month	0.535	1.169	1.264	1.338	1.257	1.335	1.047
	6 month	0.544	1.103	1.404	1.403	1.457	1.323	1.013
	12 month	0.563	1.236	1.491	1.463	1.412	1.349	1.002
40	1 week	0.49	1.194	1.323	1.402	1.456	1.319	0.775
	3 month	0.439	1.268	1.289	1.367	1.482	1.323	0.563
	6 month	0.396	1.271	1.463	1.438	1.509	1.412	0.693
	12 month	0.402	1.3	1.546	1.45	1.515	1.466	0.716
41	1 week	0.963	1.971	1.634	1.733	1.515	1.688	0.999
	3 month	0.771	1.621	1.611	1.63	1.483	1.42	0.829
	6 month	0.852	1.653	1.626	1.734	1.583	1.582	0.868
	12 month	0.588	1.578	1.601	1.702	1.517	1.562	0.868

(continued on next page)

Table 2 (*continued*)

Number	Follow-up time	Zone1	Zone2	Zone3	Zone4	Zone5	Zone6	Zone7
42	1 week	0.873	1.194	1.237	1.319	1.592	1.302	1.007
	3 month	0.515	1.281	1.417	1.308	1.588	1.22	1.071
	6 month	0.867	1.165	1.42	1.331	1.612	1.328	1.02
	12 month	0.702	1.231	1.389	1.412	1.649	1.425	1.013
43	1 week	0.329	1.239	1.499	1.627	1.437	1.217	0.92
	3 month	0.537	1.282	1.576	1.558	1.421	1.224	0.914
	6 month	0.819	1.298	1.58	1.675	1.473	1.312	0.992
	12 month	0.797	1.218	1.467	1.662	1.509	1.348	1.147
44	1 week	0.37	0.987	1.142	1.234	1.233	1.241	0.932
	3 month	0.451	1.111	1.174	1.292	1.263	1.07	0.617
	6 month	0.656	1.209	1.194	1.238	1.278	1.182	0.784
	12 month	0.515	1.227	1.187	1.313	1.412	1.341	0.688
45	1 week	0.879	1.484	1.423	1.488	1.426	1.333	0.874
	3 month	0.873	1.287	1.378	1.267	1.156	1.069	0.684
	6 month	0.716	1.35	1.356	1.359	1.411	1.2	0.699
	12 month	0.784	1.381	1.372	1.342	1.353	0.99	0.699
46	1 week	0.885	1.092	1.102	1.321	1.218	1.102	0.857
	3 month	0.688	1.055	1.25	1.356	1.278	1.291	0.601
	6 month	0.451	1.085	1.169	1.328	1.257	1.234	0.61
	12 month	0.61	1.105	1.212	1.367	1.236	1.211	0.617
47	1 week	0.895	1.173	1.496	1.386	1.521	1.319	0.597
	3 month	0.702	1.298	1.595	1.429	1.478	1.247	0.38
	6 month	0.612	1.221	1.62	1.491	1.41	1.219	0.502
	12 month	0.329	1.263	1.443	1.35	1.342	1.225	0.453
48	1 week	0.519	1.23	1.31	1.201	1.329	1.289	0.885
	3 month	0.597	1.265	1.293	1.195	1.278	1.271	0.808
	6 month	0.453	1.236	1.313	1.302	1.299	1.283	0.865
	12 month	0.37	1.258	1.312	1.281	1.325	1.337	0.893
49	1 week	0.895	1.173	1.496	1.386	1.521	1.319	0.597
	3 month	0.702	1.298	1.595	1.429	1.478	1.247	0.38
	6 month	0.612	1.221	1.62	1.491	1.41	1.219	0.502
	12 month	0.329	1.263	1.443	1.35	1.342	1.225	0.453
50	1 week	0.519	1.23	1.31	1.201	1.329	1.289	0.885
	3 month	0.597	1.265	1.293	1.195	1.278	1.271	0.808
	6 month	0.453	1.236	1.313	1.302	1.299	1.283	0.865
	12 month	0.37	1.258	1.312	1.281	1.325	1.337	0.893
51	1 week	0.57	1.187	1.342	1.434	1.433	1.441	1.132
	3 month	0.651	1.311	1.374	1.492	1.463	1.27	0.817
	6 month	0.856	1.409	1.394	1.438	1.478	1.382	0.984
	12 month	0.715	1.427	1.387	1.513	1.612	1.541	0.888

Table 3
The level of microelements in enrolled patients.

Number	Gender	Age	Ca^{2+} (μM)	Mg^{2+} (μM)	Zn (ng/ml)	VD3 (nM)	PTH (ng/ml)
1	Female	62	2.21	3.23	7.37	28.75	1.90
2	Female	83	2.56	2.87	7.76	34.36	1.95
3	Female	75	2.50	2.87	8.25	36.46	1.99
4	Male	50	2.81	1.54	8.04	35.11	1.96
5	Male	71	2.75	1.18	8.19	36.27	1.99
6	Male	89	2.58	3.34	6.58	25.92	1.65
7	Male	65	2.67	3.16	7.57	29.75	1.91
8	Female	75	2.75	1.73	7.57	30.26	1.95
9	Male	82	2.23	3.42	6.86	27.23	1.82
10	Male	52	2.64	2.92	8.14	35.99	1.99
11	Female	65	2.75	3.22	7.33	31.98	1.85
12	Female	87	2.45	3.37	8.16	36.11	1.97
13	Female	62	2.57	3.03	7.13	30.42	1.84
14	Male	55	2.41	3.21	8.09	35.78	1.98
15	Female	78	2.36	1.89	8.00	35.75	1.93
16	Female	66	2.04	2.41	7.92	34.67	1.96
17	Male	54	2.77	2.75	6.98	25.25	1.68
18	Female	45	3.96	2.85	7.81	34.18	1.95
19	Female	80	3.37	2.50	8.24	36.57	1.98
20	Male	89	3.53	2.65	7.20	29.33	1.84
21	Female	65	2.90	1.98	8.03	35.83	1.96
22	Female	75	2.16	3.18	7.18	29.88	1.84
23	Male	82	3.38	2.71	7.95	34.69	1.94
24	Female	52	2.08	3.24	8.26	36.58	1.99
25	Male	56	2.42	2.15	6.95	27.43	1.77

Table 3 (*continued*)

Number	Gender	Age	Ca^{2+} (μM)	Mg^{2+} (μM)	Zn (ng/ml)	VD3 (nM)	PTH (ng/ml)
26	Male	67	2.03	3.02	7.86	34.24	1.90
27	Male	52	2.03	3.35	8.12	36.05	1.98
28	Male	65	2.80	2.47	8.26	36.84	2.00
29	Male	63	2.42	2.93	7.67	31.70	1.93
30	Male	70	1.36	2.59	7.83	30.37	1.88
31	Female	59	2.38	3.29	4.13	21.11	1.23
32	Male	56	2.75	2.44	7.88	34.88	1.94
33	Male	70	1.42	1.51	8.01	35.60	1.95
34	Female	77	2.20	2.31	7.58	34.42	1.89
35	Female	56	1.71	2.97	8.14	35.93	1.98
36	Female	52	2.92	3.25	7.12	26.40	1.86
37	Female	77	1.56	2.48	8.03	34.76	1.96
38	Female	87	2.42	3.34	8.13	36.08	1.94
39	Male	77	2.01	2.38	7.89	34.64	1.93
40	Male	52	3.50	2.62	7.17	29.30	1.81
41	Male	52	2.05	3.21	8.23	36.55	1.96
42	Female	72	2.39	2.12	6.92	27.40	1.74
43	Female	58	2.77	2.44	8.23	36.81	1.97
44	Male	63	1.33	2.56	7.80	30.34	1.85
45	Female	58	2.35	3.26	4.10	21.08	1.20
46	Female	58	1.39	1.48	7.98	35.57	1.92
47	Male	86	2.17	2.28	7.55	34.39	1.86
48	Female	59	2.18	3.20	7.34	28.72	1.87
49	Male	78	2.47	2.84	8.22	36.43	1.96
50	Female	79	2.64	3.13	7.54	29.72	1.88
51	Male	77	2.20	3.39	6.83	27.20	1.79

Table 4
The level of microelements in enrolled Female patients.

Number	Gender	Age	Ca^{2+} (μM)	Mg^{2+} (μM)	Zn (ng/ml)	VD3 (nM)	PTH (ng/ml)
1	Female	62	2.21	3.23	7.37	28.75	1.90
2	Female	83	2.56	2.87	7.76	34.36	1.95
3	Female	75	2.50	2.87	8.25	36.46	1.99
8	Female	75	2.75	1.73	7.57	30.26	1.95
11	Female	65	2.75	3.22	7.33	31.98	1.85
12	Female	87	2.45	3.37	8.16	36.11	1.97
13	Female	62	2.57	3.03	7.13	30.42	1.84
15	Female	78	2.36	1.89	8.00	35.75	1.93
16	Female	66	2.04	2.41	7.92	34.67	1.96
18	Female	45	3.96	2.85	7.81	34.18	1.95
19	Female	80	3.37	2.50	8.24	36.57	1.98
21	Female	65	2.90	1.98	8.03	35.83	1.96
22	Female	75	2.16	3.18	7.18	29.88	1.84
24	Female	52	2.08	3.24	8.26	36.58	1.99
31	Female	59	2.38	3.29	4.13	21.11	1.23
34	Female	77	2.20	2.31	7.58	34.42	1.89
35	Female	56	1.71	2.97	8.14	35.93	1.98
36	Female	52	2.92	3.25	7.12	26.40	1.86
37	Female	77	1.56	2.48	8.03	34.76	1.96
38	Female	87	2.42	3.34	8.13	36.08	1.94
42	Female	72	2.39	2.12	6.92	27.40	1.74
43	Female	58	2.77	2.44	8.23	36.81	1.97
45	Female	58	2.35	3.26	4.10	21.08	1.20
46	Female	58	1.39	1.48	7.98	35.57	1.92
48	Female	59	2.18	3.20	7.34	28.72	1.87
50	Female	79	2.64	3.13	7.54	29.72	1.88

Table 5
The level of microelements in enrolled male patients.

Number	Gender	Age	Ca^{2+} (μM)	Mg^{2+} (μM)	Zn (ng/ml)	VD3 (nM)	PTH (ng/ml)
4	Male	50	2.81	1.54	8.04	35.11	1.96
5	Male	71	2.75	1.18	8.19	36.27	1.99
6	Male	89	2.58	3.34	6.58	25.92	1.65
7	Male	65	2.67	3.16	7.57	29.75	1.91
9	Male	82	2.23	3.42	6.86	27.23	1.82
10	Male	52	2.64	2.92	8.14	35.99	1.99
14	Male	55	2.41	3.21	8.09	35.78	1.98
17	Male	54	2.77	2.75	6.98	25.25	1.68
20	Male	89	3.53	2.65	7.20	29.33	1.84
23	Male	82	3.38	2.71	7.95	34.69	1.94
25	Male	56	2.42	2.15	6.95	27.43	1.77
26	Male	67	2.03	3.02	7.86	34.24	1.90
27	Male	52	2.03	3.35	8.12	36.05	1.98
28	Male	65	2.80	2.47	8.26	36.84	2.00
29	Male	63	2.42	2.93	7.67	31.70	1.93
30	Male	70	1.36	2.59	7.83	30.37	1.88
32	Male	56	2.75	2.44	7.88	34.88	1.94
33	Male	70	1.42	1.51	8.01	35.60	1.95
39	Male	77	2.01	2.38	7.89	34.64	1.93
40	Male	52	3.50	2.62	7.17	29.30	1.81
41	Male	52	2.05	3.21	8.23	36.55	1.96
44	Male	63	1.33	2.56	7.80	30.34	1.85
47	Male	86	2.17	2.28	7.55	34.39	1.86
49	Male	78	2.47	2.84	8.22	36.43	1.96
51	Male	77	2.20	3.39	6.83	27.20	1.79

Table 6
The correlation between microelements and BMD.

Microelements		Zone1			
		1 W	3 M	6 M	12 M
Calcium (μM)	r	0.06861	−0.013	0.040	0.031
	P	0.6324	0.930	0.780	0.829
Magnesium (μM)	r	−0.19171	−0.070	−0.001	−0.053
	P	0.1778	0.627	0.994	0.712
VD3 (nM)	r	0.241	0.166	0.229	0.326
	P	0.089	0.244	0.107	0.019
PTH (ng/mL)	r	0.107	0.170	0.211	0.325
	P	0.898	0.287	0.697	0.020
Zinc (ng/mL)	r	0.121	0.150	0.217	0.346
	P	0.399	0.294	0.127	0.013
Microelements		Zone 2			
		1 W	3 M	6 M	12 M
Calcium (μM)	r	0.013	0.149	0.042	0.115
	P	0.928	0.298	0.768	0.422
Magnesium (μM)	r	0.040	0.088	0.028	0.017
	P	0.778	0.541	0.847	0.905
VD3 (nM)	r	−0.111	−0.180	−0.115	−0.291
	P	0.437	0.205	0.422	0.038
PTH (ng/mL)	r	−0.109	−0.069	−0.079	−0.167
	P	0.447	0.630	0.582	0.241
Zinc (ng/mL)	r	−0.098	−0.096	−0.068	−0.184
	P	0.493	0.501	0.636	0.195
Microelements		Zone 3			
		1 W	3 M	6 M	12 M
Calcium (μM)	r	0.237	0.294	0.292	0.248
	P	0.094	0.036	0.038	0.079
Magnesium (μM)	r	0.067	0.007	−0.002	0.066
	P	0.638	0.963	0.989	0.644
VD3 (nM)	r	−0.104	−0.009	−0.036	−0.151
	P	0.466	0.952	0.804	0.289
PTH (ng/mL)	r	−0.030	0.269	0.251	−0.042
	P	0.833	0.050	0.076	0.767
Zinc (ng/mL)	r	−0.059	0.285	0.274	−0.074
	P	0.680	0.043	0.050	0.607
Microelements		Zone 4			
		1 W	3 M	6 M	12 M
Calcium (μM)	r	0.149	0.030	0.144	0.041
	P	0.297	0.837	0.314	0.777
Magnesium (μM)	r	−0.016	−0.020	−0.003	0.039
	P	0.914	0.890	0.981	0.784
VD3 (nM)	r	−0.132	−0.026	−0.102	−0.129
	P	0.356	0.854	0.478	0.367
PTH (ng/mL)	r	−0.074	0.057	−0.001	0.005
	P	0.608	0.692	0.994	0.972
Zinc (ng/mL)	r	−0.071	0.317	0.255	0.236
	P	0.621	0.023	0.070	0.096
Microelements		Zone 5			
		1 W	3 M	6 M	12 M
Calcium (μM)	r	0.246	0.155	0.236	0.196
	P	0.082	0.276	0.096	0.167
Magnesium (μM)	r	−0.036	−0.012	0.005	0.032
	P	0.804	0.936	0.971	0.821
VD3 (nM)	r	−0.102	−0.029	−0.134	−0.157
	P	0.477	0.840	0.350	0.271
PTH (ng/mL)	r	−0.045	0.255	−0.079	−0.066
	P	0.752	0.070	0.580	0.645
Zinc (ng/mL)	r	0.219	0.305	0.258	0.288
	P	0.122	0.029	0.067	0.040
Microelements		Zone 6			
		1 W	3 M	6 M	12 M
Calcium (μM)	r	0.168	0.105	0.290	0.279
	P	0.240	0.463	0.039	0.048
Magnesium (μM)	r	0.088	0.071	0.091	0.147
	P	0.539	0.621	0.524	0.302

Table 6 (*continued*)

Microelements		Zone 6			
		1 W	3 M	6 M	12 M
VD3 (nM)	r	0.050	0.246	−0.039	0.241
	P	0.726	0.081	0.786	0.088
PTH (ng/mL)	r	0.056	0.125	0.038	0.280
	P	0.694	0.381	0.789	0.046
Zinc (ng/mL)	r	0.192	0.238	0.045	0.321
	P	0.176	0.093	0.754	0.022
Microelements		Zone 7			
		1 W	3 M	6 M	12 M
Calcium (μM)	r	0.019	0.029	0.026	0.034
	P	0.895	0.839	0.857	0.815
Magnesium (μM)	r	0.116	0.112	0.129	0.116
	P	0.416	0.435	0.367	0.418
VD3 (nM)	r	0.571	0.557	0.551	0.541
	P	0.025	0.001	0.001	0.001
PTH (ng/mL)	r	0.280	0.370	0.352	0.343
	P	0.047	0.008	0.011	0.014
Zinc (ng/mL)	r	0.456	0.447	0.437	0.420
	P	0.001	0.001	0.001	0.002

Table 7
The correlation between microelements and BMP2, BMP7 and STRO-1+ cells.

		Ca2+ (μM)	Mg2+ (μM)	Zn2+ (ng/mL)	VD3 (nM)	PTH (ng/ml)
BMP2	r	−0.1816	−0.03489	−0.04287	0.09361	−0.09286
	P	0.2022	0.808	0.7652	0.5135	0.5169
BMP7	r	0.32448	−0.30196	0.16096	0.24256	0.11778
	P	0.0202	0.0313	0.2592	0.0864	0.4104
Stro-1(%)	r	0.28654	−0.13449	0.19462	0.2683	0.1763
	P	0.0415	0.3467	0.1711	0.05	0.2158

4. Discussion

Hip replacement is one of the most commonly used adult joint reconstruction surgery, which has been widely used for the treatment of severe arthritis, rheumatoid arthritis, aseptic necrosis of femoral head. With the improvement of surgical technique and the normalization of perioperative treatment, the incidence of early complications like infection, hip dislocation and sciatic nerve injury has been decreased significantly. However, due to many factors such as the fabrication process, biomechanics and the ability of individual bone reconstruction, long-term complications such as prosthesis loosening still have a high incidence (Chen et al., 2017; Hoskins et al., 2017; Bovonratwet et al., 2018; Lovelock and Broughton, 2018; Makela et al., 2014). At present, the research mechanism for aseptic loosening after the hip replacement surgery is mainly summarized into mechanistic and biological factors. In terms of the mechanistic factors, fretting wear, prosthetic wear, stress shielding and the size, materials, properties and surface features of the prosthesis may cause osteolysis. Among the biological factors, the generation and diffusion of wear debris, the histogenesis of interfacial film, osteoclast activation and osteolysis as well as certain cytokines are key inducers. In brief, all of the researches above are mainly focused on prosthesis design, fixation techniques and wear debris. Nevertheless, patients themselves are not included. This study discussed the prophylaxis for aseptic loosening of hip prosthesis on the basis of the basic and clinical research of patients.

In our study, we determined the BMD and measured the level of microelements in all 51 enrolled patients and also the level of BMP2, BMP7 and the percent of STOR-1+ cells. Based on our data, we found that not all the microelements were correlated with BMD. The correlation between microelements were dependent on the Zone. In Zone 7, the level of VD3, PTH, Zinc was positively correlated with BMD. And in our study, we found that the level of all the detected microelements was not correlated with BMP2 but some microelements like Ca^{2+} and $Mg^{2+}$was correlated with the level of BMP7 and Ca2+ and VD3 was correlated with the percent of STOR-1+ cells. It was found that active VD3 maintained the stability of serum calcium and phosphorus concentration. When serum calcium concentration was low, PTH secretion was induced and released to kidney and bone cells. Calcium, as the raw material of osteogenesis, is closely related to osteogenesis. Vitamin D promotes the absorption of calcium and phosphorus, mobilizes calcium and phosphorus from bone, makes the plasma calcium and phosphorus reach the normal value, and promotes bone mineralization and constantly renews.

In general, our study revealed that microelements might be associated with the prosthesis loosening and we hope our study will be useful for exploring novel therapy method for clinical treatment of prosthesis loosening.

Declaration of Competing Interest

There is no conflicts of interest.

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