



## Supporting Information

for *Adv. Sci.*, DOI: 10.1002/adv.201900209

**A Magnesium-Enriched 3D Culture System that Mimics the Bone Development Microenvironment for Vascularized Bone Regeneration**

*Sihan Lin, Guangzheng Yang, Fei Jiang, Mingliang Zhou, Shi Yin, Yanmei Tang, Tingting Tang, Zhiyuan Zhang, Wenjie Zhang,\* and Xinquan Jiang\**

Copyright WILEY-VCH Verlag GmbH & Co. KGaA, 69469 Weinheim, Germany, 2019.

## Supporting Information

### **A magnesium-enriched 3D culture system that mimics the bone development microenvironment for vascularized bone regeneration**

*Sihan Lin<sup>#</sup>, Guangzheng Yang<sup>#</sup>, Fei Jiang, Mingliang Zhou, Shi Yin, Yanmei Tang, Tingting Tang, Zhiyuan Zhang, Wenjie Zhang<sup>\*</sup>, Xinquan Jiang<sup>\*</sup>*

S. Lin, G. Yang, F. Jiang, M. Zhou, S. Yin, Y. Tang, Dr. W. Zhang, Prof. X. Jiang  
Department of Prosthodontics  
Shanghai Engineering Research Center of Advanced Dental Technology and Materials  
Shanghai Research Institute of Stomatology  
National Clinical Research Center for Oral Diseases  
Shanghai Key Laboratory of Stomatology  
Ninth People's Hospital, College of Stomatology, Shanghai JiaoTong University School of  
Medicine  
639 Zhizaoju Road, Shanghai 200011, China  
E-mail: xinquanj@aliyun.com; zhangwenjie586@126.com

Prof. T. Tang  
Department of Orthopaedic Surgery  
Ninth People's Hospital affiliated to Shanghai JiaoTong University School of Medicine  
639 Zhizaoju Road, Shanghai 200011, P. R. China

Prof. Z. Zhang.  
Department of Oral and Maxillofacial-Head and Neck Oncology  
Shanghai Research Institute of Stomatology  
National Clinical Research Center for Oral Diseases  
Shanghai Key Laboratory of Stomatology  
Ninth People's Hospital, College of Stomatology, Shanghai JiaoTong University School of  
Medicine  
639 Zhizaoju Road, Shanghai 200011, P. R. China

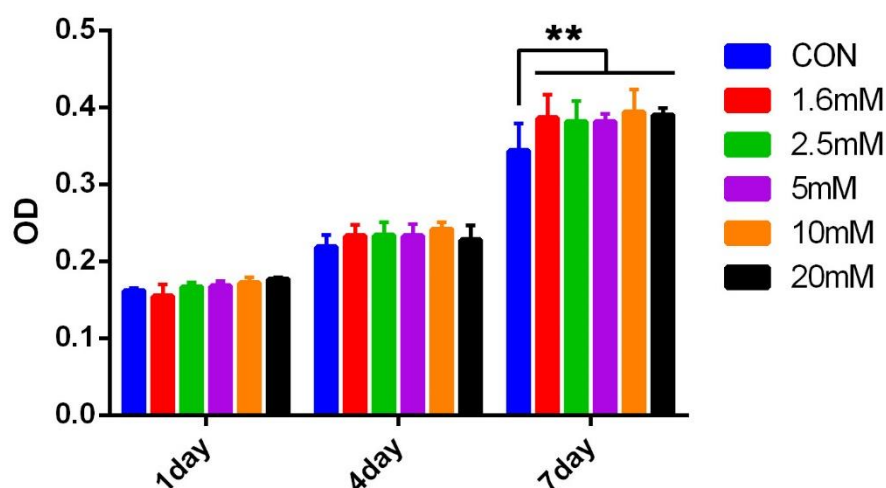
<sup>#</sup> These authors contributed equally.

## 1. Experimental section

### Analysis of BMSC proliferation in medium with a gradient concentration of $Mg^{2+}$

Gradient concentrations of  $Mg^{2+}$  (1.6 mM, 2.5 mM, 5 mM, 10 mM, and 20 mM) were created by adding  $MgCl_2$  (Sigma, USA) to high-glucose DMEM (HyClone, USA). Normal DMEM was used as a control. Briefly, BMSCs were seeded in 96-well plates at a density of 1000 cells per well. Medium with different concentrations of  $Mg^{2+}$  was added 12 hours later. Six replicates in each group were analyzed in this experiment. The MTT assay was conducted after 1, 4, and 7 days of incubation.

## 2. Supporting figures



**Figure S1.** MTT assay of cell proliferation in different magnesium environment.

## 3. Supporting table

**Table S1.** Primer sequences for qPCR.

| Gene  | Forward primer           | Reverse primer          |
|-------|--------------------------|-------------------------|
| GAPDH | GTCTCCTCTGACTTCAACAGCG   | ACCACCCTGTTGCTGTAGCC    |
| MagT1 | TGACATGTTTTCAAGCTTAGGC   | GACCAGCTTTCAAGGAACAATT  |
| ALP   | CCCACAAGAGCCCACAAT       | AGAGCCAGGAATCCGACCC     |
| Osx   | TCCTGCGACTGCCCTAATTGC    | TCCGAACGAGTGAACCTCTTGC  |
| Runx2 | ACCAGCAGCACTCCATATCTCTAC | CTTCCATCAGCGTCAACACCATC |
| OCN   | ATTGTGACGAGCTAGCGGAC     | GCAACACATGCCCTAAACGG    |
| PDGF  | TCTCTGCTGCTACCTGCGTCTG   | AAGGAGCGGATGGAGTGGTCAC  |
| SDF-1 | ACCTCGGTGTCCTCTTGCTG     | GATGTTTGACGTTGGCTCTGG   |
| VEGF  | GGCTCTGAAACCATGAACTTCT   | GCAGTAGCTGCGCTGGTAGAC   |

#### 4. Supplementary files

**File S1.** Result of Gene sequencing of MagT1 in BMSCs infected by lentivirus carrying negative control.

**File S2.** Result of Gene sequencing of MagT1 in BMSCs infected by lentivirus carrying sgRNA.

#### 5. Supplementary movies

**Movie S1.** A remarkable increase of intracellular fluorescence intensity resulting from addition of  $Mg^{2+}$ .

**Movie S2.** The slightly change in intracellular fluorescence intensity indicates the inhibitory effects of knockout of MagT1 on uptake of  $Mg^{2+}$  in BMSCs.