

## SUPPORTING INFORMATION

### Direct Immobilization of Glucose Oxidase in Magnetic Mesoporous Bioactive Glasses†

Dandan Min,<sup>a</sup> Xudong Zhang,<sup>\*a</sup> Wen He,<sup>\*a</sup> Yang Zhang,<sup>b</sup> Piwu Li,<sup>c</sup> Jianan Liu,<sup>a</sup>  
Shujiang Liu,<sup>a</sup> Fengxiu Xu,<sup>a</sup> Yi Du,<sup>a</sup> Meimei Zhang<sup>a</sup> and Caiyun Sun<sup>d</sup>

<sup>a</sup>Shandong provincial key laboratory of processing and testing technology of glass and functional ceramics, Shandong Polytechnic University, Jinan 250353, China. Fax: +86 531 89631080; Tel: +86 531 89631518; \*E-mail: [zxd1080@126.com](mailto:zxd1080@126.com); [hewen@126.com](mailto:hewen@126.com)

<sup>b</sup> College of Engineering, Ocean University of China, Qingdao 266100, China.

<sup>c</sup> Shandong key laboratory of Fermentation Engineering, Shandong Polytechnic University, Jinan 250353, China.

<sup>d</sup> State Key Laboratory of microbial technology, Shandong University, Jinan, 250100, China.

\*e-mail: [zxd1080@126.com](mailto:zxd1080@126.com); [hewen1960@126.com](mailto:hewen1960@126.com).

**S1: Biocompatibility tests in vitro (Fig. S1)**

**S2: Magnetic test (Video)**

**S3: Enzymatic activity in comparison with those reported in literatures (Table S1)**

**S4: Details of measurements by atomic force microscopy (AFM) in lift mode**

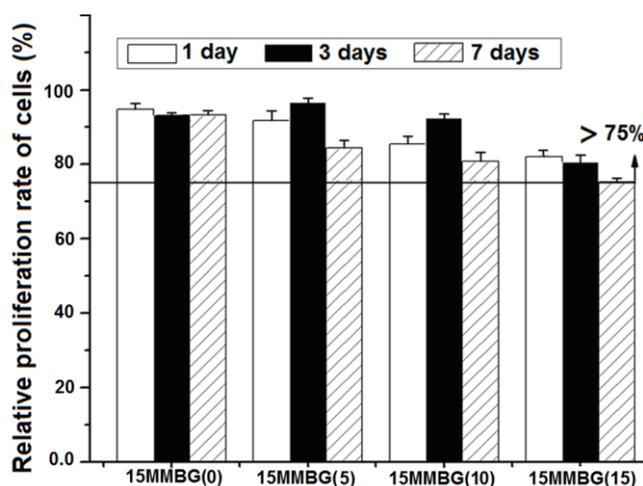
**References**

## **S1: Biocompatibility tests in vitro**

**Cell culture:** L-929 fibroblasts were obtained from Nanjing Kaiji Biological Technology Development Co., Ltd.. L-929 fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone SH30022.02, USA ) supplemented with 10% fetal bovine serum (FBS, Hangzhou sijiqing biological engineering materials co., LTD), 100 U/ml penicillin and 100 g/ml streptomycin. The cells were maintained at 37 °C in a 5% CO<sub>2</sub> humidified atmosphere and pH 7.4. Every 2 - 3 days, L-929 fibroblasts were passaged by removing 90% of the supernatant and replacing it with fresh medium. In all experiments, viable cells were checked at the beginning of the experiment by Trypan Blue exclusion. When the endocrine fuction of cultured cells is normal, they can be used for experiment study.

**Viability assay:** The MMBG powder samples were repeatedly washed to neutral with a ultrasonic cleaner, distilled water and ethanol, and then dried in the drum wind drying oven. The MMBG powders (2 g) were soaked in 20 mL dual evaporate water, leaching for 1 h under 121 °C for according to the weight/leaching medium=0.1 g/mL. After cooling the centrifugal take its supernatant fluid into sterile glass container seal. The cytotoxicity of MMBG samples was qualitatively evaluated by 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl -tetrazolium bromide (MTT) assay.<sup>1</sup> The MTT assay relies on the ability of viable cells to metabolically reduce a yellow tetrazolium salt (MTT) to purple formazan product. This reaction takes place when mitochondrial reductase enzymes are active. L-929 fibroblasts ( $2 \times 10^4$ /ml) were exposed to the leaching solution of different MMBG samples in 96-well plates for

1 day, 3 days and 7 days, respectively. The cells were treated with pancreatin (0.25%). After incubation, the cells were washed with fresh culture medium and 20  $\mu$  L of MTT was added, followed by incubation for 2 h at 37 °C. The precipitated formazan was dissolved in 150  $\mu$  L of DMSO and the absorbance (optical density, OD) was measured at 490 nm using a micro-well system reader. L-929 fibroblast proliferation was calculated by the formula: mean OD=blank control mean OD  $\times$  100. The data are presented as means  $\pm$  standard errors of the means (n = 3). A one-way ANOVA was used to compare the means of different data sets and a statistical significance was accepted at a 0.05 confidence level (P).



**Fig. S1.** The relative proliferation rates of L-929 fibroblasts assessed using MTT-based methods at different time points of incubation and in the leaching solution of different substrates (P>0.05). The data are presented as means  $\pm$  standard errors of the means (n = 3).

**Cell toxicity:** The MTT assay is used to evaluate the cytotoxicity of biomaterials, and the OD value can provide an indication of cell growth and proliferation on various materials. Measurements of cell proliferation is often used to determine cell response to a particular stimulus or toxin.<sup>2</sup> To determine the biocompatibility of different MMBG samples, L-929 fibroblasts were exposed to the leaching solution of

different MMBG samples. The relative proliferation rate values of L-929 fibroblasts assessed using MTT-based methods at different time points of incubation are shown in Fig. S1. It can be seen that the relative proliferation rate values for all samples are greater than 75% , which indicates that all MMBG samples have no negative effect on the viability proliferation of L-929 fibroblasts, and that they have good biocompatibility.<sup>1</sup>

### S3: Enzymatic activity in comparison with those reported in literatures

**Table S1.** Enzymatic activity in comparison with those reported in literatures (40 °C, p H =6.5)

GOD immobilization supports	Average enzyme activity recovery (%)	Ref
Calcium alginate gels	61.69	[3]
Hydroxyapatite	61.92	[4]
Calcium alginate	59.64	[4]
Chitosan membrane	57.71	[4]
FDU-1 silica	45	[5]
Porous silica	69.1	[6]
15MMBG(15)	71.09	In this study

### S4: Details of measurements by atomic force microscopy (AFM) in lift mode

A droplet of ethanol suspension with the dispersed 15MMBG(15) particles was deposited onto a freshly cleaved mica surface and subsequently dried in air. During the drying process, the sample was placed on a strong magnet. The drying process lasted for 15 min, allowing the 15MMBG(15) particles to be well aligned. The samples were analyzed by AFM in lift mode with a DI multimode nanoscope IV, applying the two-pass technique. The sample's topography is obtained during the first pass very close to the sample surface, and magnetic contrast is subsequently obtained during the second pass at a constant lift height of the sample surface. The employed magnetic tips were made from commercial AFM tips (NSC18/ALBS, Mikromasch,

Estonia).

## References

- [1] T. Mosmann, *J. Immunol Methods*, 1983, **65**, 55.
- [2] P. Sangsanoh, O. Suwanton, A. Neamnark, P. Cheepsunthorn , P. Pavasant, P. Supaphol ,  
*European Polymer Journal*, 2010, **46**, 428.
- [3] Z. F. Xiao, L. Wu, D. L. Yi, X. R. Qin, C.Q. Yang, *J. chemical and biological Engineering*. 2008, **25**, 42.
- [4] Y.X. Zhang, T. F. Wang, R. M. Wang, *Shandong food fermentation*, 2010, **4**, 14.
- [5] L. C. Cides da Silva, C. M. C. Infante, A. W. O. Lima, I. C. Cosentino, M. C. A. Fantini, F. R.  
P. Rocha, J. C. Masini, J. R. Matos, *J. Molecular Catalysis B: Enzymatic*, 2011, **70**, 149.
- [6] F. van de Velde, N. D. Lourenco, M. Bakker, F. van Rantwijk, R. A. Sheldon, *Biotechnol. Bioeng.*, 2000, **69**, 286.