Osteogenic Embryoid Body-Derived Material Induces Bone Formation In Vivo

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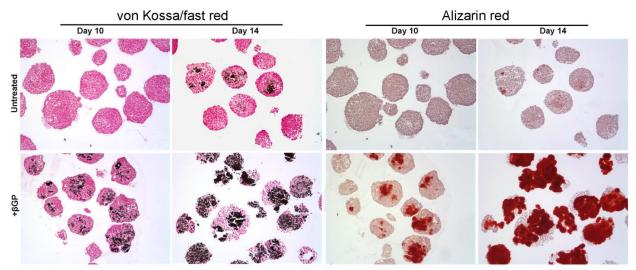
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Supplementary Table

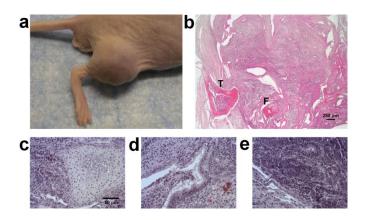
| Osteoinduction Score | Observation Within Entire Limb Section |
|----------------------|---|
| 0 | No residual DBM or EBM |
| 1 | Residual DBM or EBM but no new bone formation |
| 2 | One single ossicle |
| 3 | Ossicle formation at multiple sites |

Supplementary Table 1. Osteoinduction Scoring

Supplementary figures



Supplementary Fig. S1 Mineralization in EBs cultured under different conditions. EBs differentiated in the absence or presence of β GP (10 μ M) beginning at day 5 of EB differentiation. The mineralization of EBs was evaluated using both von Kossa and Alizarin Red staining (scale bar = 400 μ m).



Supplementary Fig. S2 Teratoma formation 28 days post-implantation of viable day 10 EBs. Viable day 10 EBs, with and without β GP treatment, were implanted into mouse hindlimbs. Large masses in the hindlimbs were apparent by 28 days post-implantation (a), and when evaluated histologically, the masses were confirmed to be teratomas (b) (T: Tibia, F: Fibula,

scale bar = 250 μ m), which comprised of cells from all three germ lineages: mesoderm (c), endoderm (d), and ectoderm (e), scale bar = 50 μ m.

Supplementary methods

Histology analysis of mineralization in ESC aggregates

Paraffin-embedded ESC aggregates were sectioned at a thickness of 5 µm and subjected to routine von Kossa/fast red and alizarin red staining to visualize mineralization within aggregates. Stained sections were imaged using a Nikon Eclipse 80i equipped with a SpotFlex digital camera (Diagnostic Instruments, Sterling Heights, MI).

Teratoma formation assay

All studies were performed with a Georgia Institute of Technology Institutional Animal Care and Use Committee approved protocol. Male SCID mice (8-week-old) were used for all experiments. D10 ESC aggregates were harvested after *in vitro* culture and resuspended in saline and injected into the hindlimbs of the mice. After 28 days of implantation, the tissues were harvested for histological analysis. Paraffin-embedded teratoma tissues were sectioned at a thickness of 5 μ m and subjected to routine hematoxylin and eosin staining to examine the tissue formation.