



Osteoinductive Potential of In Vitro Elaborated Bone Matrix

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Introduction

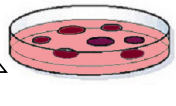
Bone marrow cells have frequently been cultured with the goal of establishing an osteogenic cell pool for tissue engineering applications. While such cell populations can be highly expanded in culture, their functional osteogenic phenotype may be compromised as demonstrated by the elaboration, in culture, of a non-specific, or dystrophic, form of mineralization [1]. One of the hallmarks of bone matrix is that it can, following demineralization, induce new bone formation in an ectopic, or non-bony, implantation site, as first described as osteoinduction by Urist [2]. The purpose of the present work was to grow bone in vitro to address the question: **Is bone matrix grown by osteogenic cells in culture osteoinductive?**

Materials and Methods

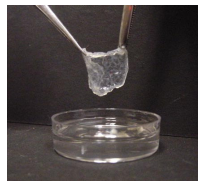
Osteoinduction Assay



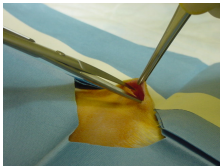
1. Rat bone marrow cells (RBMC) were harvested from the femurs of young Wistar rats and cultured under osteogenic conditions (15% (v/v) fetal calf serum, 50 µg/ml L-ascorbic acid, 5mM β-glycerophosphate and 10⁻⁸ M dexamethazone) for 6-8 weeks. Cultures were characterized using scanning and transmission electron microscopy (SEM/TEM).



2. Bone matrix was isolated from mature cultures according to standard protocols used in the preparation of demineralized bone matrix (DBM):
i. Devitalization in H₂O
ii. Demineralization in 0.6N HCl
iii. Freeze drying.



3. In order to assess its osteoinductive potential, the in vitro elaborated matrix was implanted subcutaneously in young rats. Histological analysis was used to evaluate the osteoinductive response in comparison to the positive control, demineralized bone matrix (DBM) prepared from rat long bones.



Results

1. Characterization of rat bone marrow cell (RBMC) cultures established the presence of abundant bone nodules.

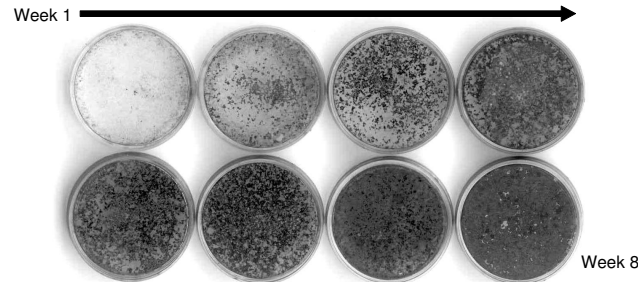


Figure 1. Von Kossa labeled rat bone marrow cultures at 1 to 8 weeks after subculture. The darkly stained areas represent areas of mineralization.

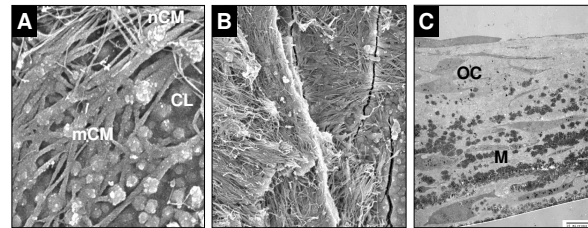


Figure 2. Using SEM, several hallmarks of new bone formation were identified in culture (A,B), including the deposition of the cement line (CL) and mineralized and non-mineralized collagen matrix (mCM/nCM). Areas of mineralization (M) and embedded osteocyte cells (OC) were further visualized in the cross-section of a bone nodule using TEM (C). These observations are consistent with previous reports characterizing in vitro bone nodule formation [3].

2. Bone matrix harvested from mature RBMC cultures consisted of a network of collagen fibers devoid of viable cells and mineral.

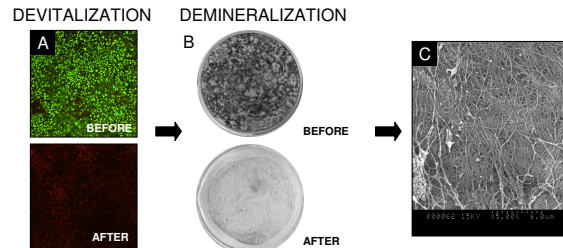


Figure 3. Using the Live/Dead assay (A), and von Kossa staining (B), it was confirmed that viable cells and mineral were removed from in vitro elaborated matrix. Following processing, a matrix of collagen fibers (C) was obtained.

3. Upon implantation of in vitro elaborated bone matrix at an ectopic site, new bone and cartilage formation was observed.

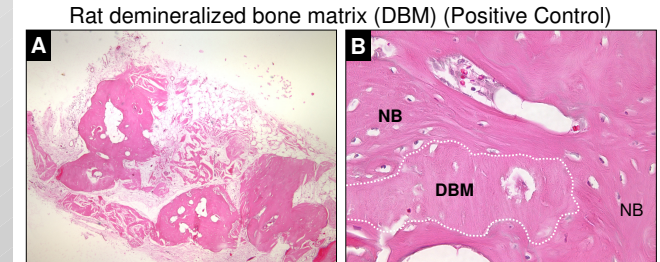


Figure 4. DBM was used as a positive control in the osteoinduction assay. At 28 days post implantation, new bone formation (NB), characterized by the presence of osteocytes within lacunae, was observed on the surface of the implanted particles.

Demineralized bone matrix from RBMC cultures

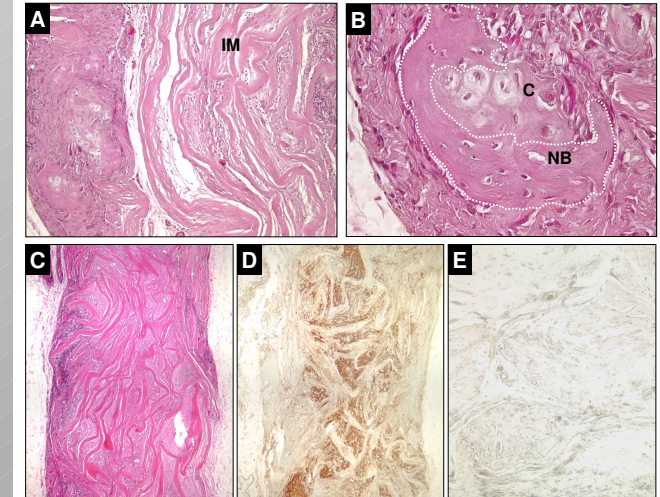


Figure 5. At 28 days following ectopic implantation of devitalized and demineralized bone matrix harvested from RBMC cultures, new bone and cartilage formation were observed. The implanted matrix (IM) appeared as thin sheets, while the morphologically identifiable new bone (NB) was often found in conjunction with cartilage (C). Immunohistochemical labeling for Osteocalcin, a bone specific protein, further confirmed the presence of induced bone tissue (C and D). Non-specific labeling was not detected in control samples (E).

Conclusions

These findings indicate that bone matrix isolated from osteogenic cultures is osteoinductive, and may provide an important component of a cell/scaffold-based bone tissue engineering strategy.