The Genetic Basis for Osteogenesis Stimulation by Controlled Release of Ionic Dissolution Products

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Introduction

Autografts are the gold standard for bone grafts due to release of growth stimulating proteins. This paper presents a genetic theory for stimulation of bone growth by controlled release of critical concentrations of inorganic ions (Ca and Si) that control the cell cycle of osteogenic precursor cells. Gene array analyses of five different in-vitro models using five different sources of inorganic ions provide the experimental evidence for a genetic theory of osteogenic stimulation. The cell and organ culture models are listed in Tables 1 and 2.

Table 1. Cell and Organ Culture Models Used to Establish Genetic Basis for Osteostimulation by Bioactive Glass Dissolution Products

Model	Description	Code
1	Primary Human Osteoblasts	pHOBs
2	Foetal Human Osteoblasts	fHOBs
3	Murine Embryonic Stem Cells	mES
4	Human Embryonic Stem Cells	hES
5	Murine Foetal Long Bone Cells	mFLBs

Table 2. Source of Ionic Dissolution Products Used in Studies of Osteostimulation by Gene Activation

Source	Description
A	45S5 bioactive glass culture disks
B	45S5 bioactive glass particulate (NovaBone)
C	58S sol-gel bioactive glass
D	70/30 sol-gel bioactive glass porous scaffold
E	Ionic dissolution products of B, C, D above

Materials and Methods

The composition of the melt-derived 45S5 bioactive glass[1] culture discs (A) and particulate (B) was 45% (by weight) SiO_2, 24.5% CaO, 24.5% Na_20, and 6% P_2O_5. Samples of (A) were obtained from US Biomaterials Corp., Alachua, FL from a certified batch. Commercial powders of (B) with a particle size of 90-710 pm were obtained from NovaBone Products, LLC, Alachua, FL. The 58S sol-gel derived particulate (C) composition (58% SiO_2, 36% CaO, 6% P_20_5)[2] and the 70/30 sol-gel sample (D) composition (70% SiO_2, 30% CaO)[3] were made by the Dept. of Materials, Imperial College London. Sample (E), the ionic dissolution products of (B), (C), and (D) were obtained by immersing particulates of (B), (C), and (D) in simulated body fluid solution at 37°C for various times to achieve concentrations of 15-30 ppm of soluble Si ions and 60-90 ppm of soluble Ca ion.[4,5] A prior study of dose dependence of ionic dissolution products showed this range of concentrations led to enhanced proliferation of osteoblasts.

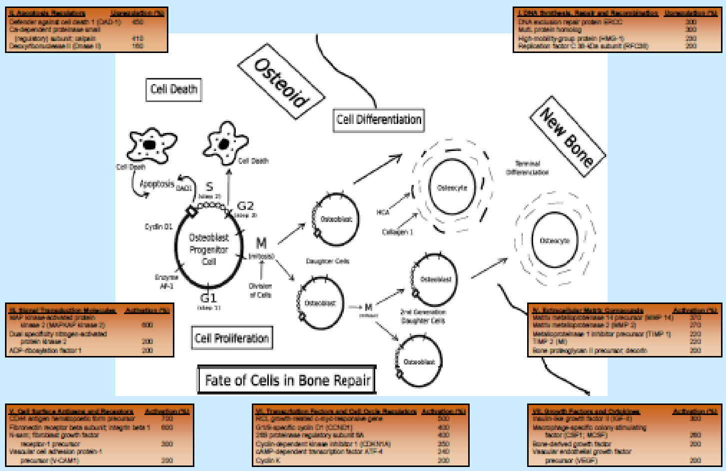
Cell Sources: Human primary osteoblasts were obtained from excised femoral heads of total hip arthroplasty patients aged 50-70 years.[4] The first cell cycle and gene array experiments compared samples (A) with Thermanox plastic controls; the 2nd experiment compared ionic dissolution products of (B) with Thermanox controls;[5] experiment 3 used PCR methods to confirm effects of the ionic dissolution products of (B) on expression of specific genes from osteoblasts obtained from excised femoral heads of five individual patients. Student's t-tests were used to determine statistical significance of the results.

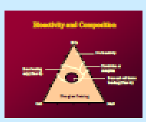
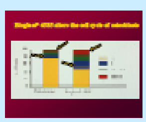
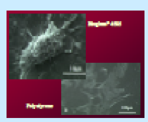
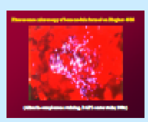
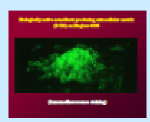
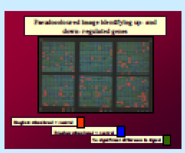
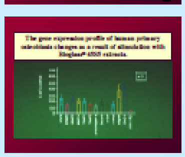
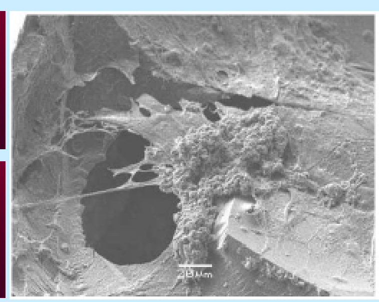
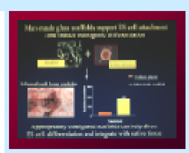
The 4th and 5th experiments tested the effects of sample (E) on fHOBs[6] and hES cells. The 6th and 7th experiments confirmed the findings of experiments 1-5 by comparing dosage effects of samples A and E on murine foetal metatarsals grown for 4 days in organ culture post day 14 gestation,[7] and growth of pHOBs within 3-D scaffolds (sample D).[8]

Results

All seven experiments showed enhanced proliferation and differentiation of osteoblasts towards a mature, mineralizing phenotype without the presence of any added bone growth proteins, such as dexamethasone. Shifts in osteoblast cell cycles were observed as early as six hours, with elimination (by apoptosis) of cells incapable of differentiation. The remaining cells exhibited enhanced synthesis and mitosis. The cells quickly committed to generation of extracellular matrix (ECM) proteins and mineralization of the matrix.

Figure 1. Fate of Cells During Bone Repair and the Activation of Multiple Gene Families by Bioactive Glasses




Results (cont.)

Shown in Figure 1, gene array analyses at 48 hours showed early up-regulation or activation of seven families of genes that favored both proliferation and differentiation of the mature osteoblast phenotypes:

- I. DNA synthesis, repair and recombination (four with increases of 200-300%);
- II. Apoptosis regulators (three at 160-450%);
- III. Signal transduction molecules (three at 200-600%);
- IV. ECM compounds (five at 200-370%);
- V. Cell surface antigens and receptors (four at 200-700%, especially CD44);
- VI. Transcription factors and cell cycle regulators (six with increases of 200-500%);
- VII. Growth factors (four at 200-300%) including IGF-II and VEG F.

All seven experiments showed enhanced rates of collagen I production and mineralization of bone modules. The murine foetal long bone cultures (Exp. #6) showed that sequential dosages of the inorganic osteostimuli were most effective. This effect is achieved in-vivo by use of a range of particle sizes of bioactive particles (Sample B) where the rate of release is controlled by the radius of curvature (r) of the particles, i.e.:

$$[Ca, Si] = (l/r)[-k_1 t^{0.5} - k_2 t^{1.0}].$$

Discussion

These findings demonstrate that the full range of cell sources of the osteoblast lineage (ES cells, foetal cells and adult primary cells) are stimulated at a genetic level by critical dosages of Ca and Si ionic dissolution products. The up-regulated or activated genes control the osteoblast cell cycle to favor proliferation and subsequent differentiation of only the cells that can proceed towards creation of a mineralized ECM, osteocytes, and new bone.
The critical dosages and kinetics of release of the ionic osteostimuli can be achieved by controlling the particle size range, composition, processing method or nano-structure of Ca, Si-containing materials.

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