GENETIC PORTRAIT OF OSTEOBLAST-LIKE CELLS CULTURED ON PERIOGLAS

Francesco Carinci; Annalisa Palmieri; Marcella Martinelli; Vittoria Perrotti; Adriano Piattelli; Giorgio Brunelli; Marzia Arlotti; Furio Pezzetti

PerioGlas (PG) is an alloplastic material used for grafting periodontal osseous defects since 1995. In animal models it has been histologically proven that PG achieves good repair of surgically created defects. In clinical trials, PG has been shown to be effective as an adjunct to conventional surgery in treating intrabony defects. Because the molecular events by which PG is able to alter osteoblast activity to promote bone formation are poorly understood, we investigated genes that are differently regulated in osteoblast-like cells exposed to PG. Bone formation can be attributable to ostegenesis (ie, direct stimulation of osteoblast to produce new bone), osteoconduction (which operates like a scaffold), or both processes. By using DNA microarrays containing 20 000 oligonucleotides, we identified several genes in which expression was significantly downregulated in a MG63 cell line cultured with PerioGlas (US Biomaterials Corp, Alachua, Fla). Specifically, PG is able to downregulate some functional activities of osteoblast-like cells: it acts on signal transduction, especially on the transforming growth factor beta (TGFB) paracrine network; it inhibits apoptosis; it decreases cell adhesion with consequent enhancement of cell mobility and migration; and it acts on bone marrow stem cells (ie, CD34). In conclusion, PG acts on bone formation by determining both osteoconduction (as demonstrated by the reduced cell adhesion) and ostegenesis (as shown by TGFB-related proteins and stem cell markers).

Key Words: alloplastic material; allograft; bone; DNA microarray; gene profiling

INTRODUCTION

PerioGlas (PG; US Biomaterials Corp, Alachua, Fla) is an alloplastic material that has been used for grafting periodontal osseous defects since 1995. In animal models it achieves histologically good repair of surgically created defects. In monkeys[1–3] PG demonstrates biocompatibility and osteoconductive activity. It is mostly resorbed and replaced by bone, and the remaining granules are in close contact with bone. In a rabbit model, PG is able to improve bone healing at the interface between titanium dental implants and bone,[4] whereas in ovariectomized rats it enhances newly bone formation into extraction sockets.[5]

In clinical trials, bioactive glass is effective as an adjunct to conventional surgery in treating intrabony defects[6] and dental extraction sites before dental implant placement, in order to regenerate bone and enhance early fixation of implants.[7] However, PG has no regenerative properties with regard to cementum and periodontal ligament.[8]

In previous in vitro studies on human cells, the osteoblast cell line MG63 was used as a prototype of human bone cells to test bioglass that found favorable results.[9] Human primary osteoblasts were used to

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investigate the osteogenic potential of a melt-derived bioactive glass (BG). It was shown that BG induces osteoblast proliferation and increases osteoblast activity; thus, it was hypotized that BG could be used as a template for forming bioengineered bone tissue.[10]

From a molecular point of view, it has been shown that ionic products of BG dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II messenger RNA expression and protein synthesis.[11] In addition, a gene-expression profiling of human osteoblasts after treatment with the ionic products of BG dissolution was performed by using complementary DNA (cDNA) microarray containing 1176 genes.[12]

To our best knowledge, however, no direct analysis on PG has been performed yet. Thus, because the mechanism by which PG stimulates osteoblast activity to promote bone formation is poorly understood, we attempted to address this question by using microarray techniques.

DNA microarray is a molecular technology that enables the analysis of gene expression in parallel on a very large number of genes, spanning a significant fraction of the human genome. Gene expression is performed by a process of (1) RNA extraction, (2) reverse transcription, and (3) labeling of cDNA. Reference (ie, untreated cells) and investigated (ie, cells cultured with PG) cDNA are labeled with different dyes and then hybridized on slides containing cDNA fragments. Then the slides are scanned with a laser system, and 2 false color images are generated for each hybridization with cDNA from the investigated and reference cells. The overall result is the generation of a so-called genetic portrait.[13–17] It corresponds to upregulated or downregulated genes in the investigated cell system. In the present study we define the genetic effect of PG on osteoblast-like cell line (ie, MG63) by using microarray slides containing 20 000 different oligonucleotides.

MATERIALS AND METHODS

Cell culture

Osteoblast-like cells (MG63) were cultured in sterile Falcon wells (Becton Dickinson, Franklin Lakes, NJ) containing Eagle's minimum essential medium supplemented with 10% fetal calf serum (Sigma Chemical Co, St Louis, Mo) and antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL; Sigma Chemical Co). Cultures were maintained in a 5% CO_2 humidified atmosphere at 37°C.

MG63 cells were collected and seeded at a density of 1×10^5 cells/mL into 9 cm^2 (3 mL) wells by using 0.1% trypsin, 0.02% ethylene diamine tetraacetic acid in Eagle's buffer free of Ca++ and magnesium for cell release. One set of wells received PG at the concentration of 0.04 g/mL. After 24 hours, when cultures were subconfluent, cells were processed for RNA extraction.

DNA microarray screening and analysis

The protocol was the same used in previous experiments.[13–17] RNA was extracted from cells by using RNAzol (RNAzol Molecular System, San Diego, Calif); 10 μg total RNA was used for each sample. The cDNA was synthesized using Superscript II (Life Technologies, Invitrogen, Milano, Italy) and aminoallyl dUTP (Sigma Chemical Co). Monoreactive Cy3 and Cy5 esters (Amersham Pharmacia, Little Chalfont, UK) were used for indirect cDNA labeling. RNA extracted from untreated cells was labeled with Cy3 and used as a control against the Cy5 labeled treated (PG) cDNA in the first experiment and then switched. For 20 000 human DNA microarray slides (MWG Biotech AG, Ebersberg, Germany) 100 μL of the sample and control cDNAs in DIG Easy hybridization solution (Roche, Basel, Switzerland) was used in a sandwich hybridization of the 2 slides constituting the 20 000 set at 37°C overnight. Washing was performed 3 times for 10 minutes with 1 × saline sodium citrate (SSC), 0.1% sodium dodecyl sulfate at 42°C, and 3 times for 5 minutes with 0.1 × SSC at room temperature. Slides were dried by centrifugation for 2 minutes at 2000 rpm. The experiment was repeated twice and the dyes were switched. A GenePix 4000a DNA microarrays scanner (Axon, Union City, Calif) was used to scan the slides, and data were extracted with GenePix Pro (Axon). Genes with expression levels <1000 after removing local background were not included in the analysis, because ratios are not reliable at that detection level.

RESULTS

DNA microarrays

After scanning the 2 slides containing the 20 000 human genes in duplicate, local background was calculated for each target location. A normalization factor was estimated from ratios of median. Normalization was performed by adding the $\log_2$ of the normalization factor to the $\log_2$ of the ratio of medians. The $\log_2$ ratios for all the targets on the array were then calibrated using the normalization

TABLE 1

Upregulated genes

Accession Gene Bank	Name	Symbol	Cytoband	Score(d)
AF107495	Growth and transformation-dependent protein	E2IG5	3q21.1	3,218625599
NM_018452	Chromosome 6 open reading frame 35	C6orf35	6q25.3	2,150043216
AK022322	Retinoblastoma-associated factor 600	RBAF600	1p36.13	1,97987665

factor, and $\log_2$ ratios outside the 99.7% confidence interval (the median ± 3 times the SD $= 0.52$) were determined to be significantly changed in the treated cells. Thus, genes are considered significantly modulated in expression when the absolute value of their $\log_2$ expression level is higher than 1.56 or there is a 3-fold difference in expression between treated cells and reference. GenePix Pro software was used to report genes above the threshold and with $<10\%$ difference in 3 different statistical evaluations of the intensity ratio, thus effectively enabling an automated quality control check of the hybridized spots. Furthermore, all the positively passed spots were finally visually inspected. Statistical analysis of microarray (SAM) program was then performed, and the SAM score was obtained (*t* statistic value).[13–17] The genes differentially expressed in cells treated with PG are shown in Tables 1 and 2, and the SAM plot is shown in Figure 1.

DISCUSSION

PG is a silicate-based synthetic bone augmentation material that has been used to fill periodontal defects with binding and integration to soft tissue and bone. Previous histologic studies in animal models have shown that PG achieves good repair of surgically created defects.[1–5] In clinical trials, PG has been shown to be effective as an adjunct to conventional surgery in treating intrabony defects[6] and dental extraction sites.[7]

With regard to molecular studies, a gene-expression profiling of human osteoblasts after treatment with the ionic products of BG dissolution was performed by using cDNA microarray containing 1176 genes.[12] To our best knowledge, however, no direct analysis has been performed on PG. Thus, because the mechanism by which PG stimulates osteoblast activity to promote bone formation is poorly understood, we attempted to address this question by using microarray techniques to identify genes that are differently regulated in osteoblasts exposed to PG. Bone formation can be attributable to ostegenesis (ie, direct stimulation of osteoblast to produce new bone), osteoconduction (which operates like a scaffold), or both processes.

Hybridization of cDNA (derived form MG63 cultured with 0.04 g/mL of PG) to cDNA microarrays allowed us to perform systemic analysis of expression profiles for thousands of genes simultaneously and to provide primary information on transcriptional changes related to PG. We identified several genes whose expression was definitely upregulated and downregulated. Very few genes are upregulated and none has a major regulatory role (Table 1).

Among the downregulated genes (Table 2), some are involved in signal transduction like TGFB3 and GHRHR, a receptor for growth hormone-releasing hormone. Binding of this hormone to the receptor leads to synthesis and release of growth hormone. Mutations in this gene have been associated with isolated growth hormone deficiency, also known as dwarfism of Sindh, a disorder characterized by short stature.[18] Another signal transductor is PLCB1, which catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. This reaction uses calcium as a cofactor and plays an important role in the intracellular transduction of many extracellular signals.[19]

Cell-cycle regulation, proliferation, and apoptosis are modulated by PG. PCSK6 belongs to the subtilisin-like proprotein convertase family. Members of this family are proprotein convertases that process latent precursor proteins into their biologically active products. PCSK6 is a calcium-dependent serine endoprotease that can cleave precursor protein at their paired basic amino acid processing sites. Some of its substrates are transforming growth factor beta (TGFB)-related proteins.[20]

DAPK3 (death-associated protein kinase 3) induces morphologic changes in apoptosis when overexpressed in mammalian cells; thus, it has been suggested that DAPK3 may play a role in the induction of apoptosis.[21] PG acts also on genes related to the immune system. NFAT5 is a member of the nuclear factors of activated T cells family of transcription factors. Proteins belonging to this family play a central role in inducible gene transcription during the immune response.[22] CCL26 is 1 of 2 Cys-Cys (CC)

TABLE 2

Downregulated genes

Accession Gene Bank	Name	Symbol	Cytoband	Score(d)
AB047801	Carbohydrate (*N*-acetylgalactosamine 4–0) sulfotransferase 8	CHST8	19q13.1	–2,261658038
AF468030	Pyridoxine 5′-phosphate oxidase	PNPO	17q21.32	–1,949000132
AK025439	Ring finger protein 34	RNF34	12q24.31	–1,841587535
L07594	Transforming growth factor, beta receptor III (betaglycan, 300kDa)	TGFBR3	1p33-p32	–1,827350394
NM_057170	G protein–coupled receptor kinase interactor 2	GIT2	12q24.1	–1,726393384
AK025813	Armadillo repeat containing 7	ARMC7	17q25.1	–1,724121674
NM_032442	G protein pathway suppressor 2	KIAA1787	17p13	–1,714251438
AB000463	SH3-domain binding protein 2	SH3BP2	4p16.3	–1,70775054
NM_005578	LIM domain containing preferred translocation partner in lipoma	LPP	3q28	–1,689453141
NM_000811	Gamma-aminobutyric acid (GABA) A receptor, alpha 6	GABRA6	5q34	–1,688344372
S50537	Upstream transcription factor 2, c-fos interacting	USF2	19q13	–1,685617301
AF148464	Phosphate cytidylyltransferase 1, choline, beta isoform	PCYT1B	Xp22.11	–1,664173755
AK027694	Fibronectin leucine rich transmembrane protein 3	FLRT3	20p11	–1,661955598
BC011620	Chromosome 9 open reading frame 74	C9orf74	9q34.11	–1,660978099
NM_021188	Zinc finger protein 410	ZNF410	14q24.3	–1,640684056
AK097391	Family with sequence similarity 11, member A	FAM11A	Xq28	–1,640083267
AF317840	Glucosidase, beta, acid 3 (cytosolic)	GBA3	4p15.31	–1,637275905
BC001875	Antigen p97 (melanoma associated) identified by monoclonal antibodies 133.2 and 96.5	MFI2	3q28-q29	–1,633157687
AB080265	Cytochrome P450, family 2, subfamily J, polypeptide 2	CYP2J2	1p31.3-p31.2	–1,631395594
M29551	Protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform (calcineurin A beta)	PPP3CB	10q21-q22	–1,619516222
NM_138324	Proprotein convertase subtilisin/kexin type 6	PCSK6	15q26	–1,602048597
NM_147158	Opioid receptor, sigma 1	OPRS1	9p13.3	–1,591437887
U62432	Cholinergic receptor, nicotinic, alpha polypeptide 3	CHRNA3	15q24	–1,581405968
AB024313	Polymerase (DNA directed), eta	POLH	6p21.1	–1,575326792
AB024742	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6	B4GALT6	18q11	–1,571530546
AF314959	Solute carrier family 26, member 8	SLC26A8	6p21	–1,568899225
NM_001829	Chloride channel 3	CLCN3	4q33	–1,551651404
AY137773	Chromosome 1 open reading frame 35	C1orf35	1q42.13	–1,549781561
BC018370	Fibronectin leucine rich transmembrane protein 1	FLRT1	11q12-q13	–1,541067052
S76942	Dopamine receptor D4	DRD4	11p15.5	–1,536007655
AB020522	Deleted in lung and esophageal cancer 1	DLEC1	3p22-p21.3	–1,521316905
BC004154	Nuclear receptor subfamily 2, group F, member 1	NR2F1	5q14	–1,519248943
NM_021233	Deoxyribonuclease II beta	DNASE2B	1p22.3	–1,507429065
Z97016	Nuclear factor of activated T-cells 5, tonicity-responsive	NFAT5	16q22.1	–1,50608166
AF152307	Protocadherin alpha 6	PCDHA6	5q31	–1,503829565
AF077953	Protein inhibitor of activated STAT, 2	PIAS2	18q21.1	–1,503124198
BC026313	E2a-Pbx1-associated protein	EB-1	12q23.1	–1,50024403
NM_004035	Acyl-Coenzyme A oxidase 1, palmitoyl	ACOX1	17q24-q25	–1,494275077
BC005926	Ecotropic viral integration site 2B	EVI2B	17q11.2	–1,493591132
AB010447	Chemokine (C-C motif) ligand 26	CCL26	7q11.23	–1,489787816
BC028393	MYCBP associated protein	MYCBPAP	17q21.33	–1,482931383
AF282262	Growth hormone releasing hormone receptor	GHRHR	7p14	–1,482681773
NM_002023	Fibromodulin	FMOD	1q32	–1,476803591
NM_000073	CD3G antigen, gamma polypeptide (TiT3 complex)	CD3G	11q23	–1,46730377
AF112227	Tumor differentially expressed 1	TDE1	20q13.1–13.3	–1,466837673
U26554	Calcitonin receptor	CALCR	7q21.3	–1,465212182
AB035305	Cadherin 8, type 2	CDH8	16q22.1	–1,46239539
AF026548	Branched chain ketoacid dehydrogenase kinase	BCKDK	16p11.2	–1,461531413
U23946	RNA binding motif protein 5	RBM5	3p21.3	–1,457397202
AF084555	Cyclic AMP phosphoprotein, 19 kD	ARPP-19	15q21.2	–1,456933396
BC001173	Eukaryotic translation initiation factor 3, subunit 9 eta, 116kDa	EIF3S9	7p22.3	–1,455193406
NM_002838	Protein tyrosine phosphatase, receptor type, C	PTPRC	1q31-q32	–1,454518379
AF305779	Sorting nexin 16	SNX16	8q21.13	–1,453279952
AF275813	McKusick-Kaufman syndrome	MKKS	20p12	–1,446178881
AB040913	Neuroligin 3	NLGN3	Xq13.1	–1,438524833
AY004175	Phospholipase C, beta 1 (phosphoinositide-specific)	PLCB1	20p12	–1,436380353
NM_002290	Laminin, alpha 4	LAMA4	6q21	–1,434222763
AB033888	SRY (sex determining region Y)-box 18	SOX18	20q13.33	–1,433885836
AB000815	Aryl hydrocarbon receptor nuclear translocator-like	ARNTL	11p15	–1,430033382
BC021288	Lysosomal-associated membrane protein 1	LAMP1	13q34	–1,4229506
M64073	Aspartylglucosaminidase	AGA	4q32-q33	–1,422393567
NM_000799	Erythropoietin	EPO	7q22	–1,421414252

TABLE 2

Continued.

Accession Gene Bank	Name	Symbol	Cytoband	Score(d)
AF151022	Brain protein 16	LOC51236	8q24.3	–1,417739446
AF100745	PTD016 protein	LOC51136	17q23.2	–1,410125596
AF123074	Dynein, cytoplasmic, intermediate polypeptide 1	DNCI1	7q21.3-q22.1	–1,400708494
BC032346	Arrestin domain containing 1	ARRDC1	9q34.3	–1,400166823
AF523361	CD34 antigen	CD34	1q32	–1,398625428
AF115323	Syntaxin 8	STX8	17p12	–1,395847064
AB022341	Death-associated protein kinase 3	DAPK3	19p13.3	–1,394098637
NM_003947	Kalirin, RhoGEF kinase	HAPIP	3q21.1-q21.2	–1,392429156
AY028424	Cell division cycle 2-like 6 (CDK8-like)	CDC2L6	6q21	–1,391459912
AK022611	Pericentrin 1	PCNT1	17q25.1	–1,391093533
M15353	Eukaryotic translation initiation factor 4E	EIF4E	4q21-q25	–1,388239379
AF100781	WNT1 inducible signaling pathway protein 3	WISP3	6q22-q23	–1,385884118
M96652	Interleukin 5 receptor, alpha	IL5RA	3p26-p24	–1,385457043
M15533	Amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease)	APP	21q21.2	–1,384635032

cytokine genes clustered on the q arm of chromosome 7. Cytokines represent a family of secreted proteins involved in immunoregulatory and inflammatory processes. The CC cytokines are proteins characterized by 2 adjacent cysteines. The cytokine encoded by CCL26 displays chemotactic activity for normal peripheral blood eosinophils and basophils.[23] CD3 is a complex of protein associated to T cell antigen receptor on the T cell surface. CD3G (gamma chain) is 1 of the 4 peptides (gamma, delta, epsilon, and zeta) that form CD3. Defects in CD3G are associated with T cell immunodeficiency.[24]

Also, cytoskeleton, cell adhesion, and extracellular matrix components are modulated by PG. FLRT3 is a member of the fibronectin leucine rich transmembrane protein family. It may function in cell adhesion and/or receptor signaling. Its protein structure resembles small leucine-rich proteoglycans found in the extracellular matrix.[24]

B4GALT6 is 1 of the 7 beta-1,4-galactosyltransferase (beta4GalT) genes. They encode for type II membrane-bound glycoproteins that seem to have exclusive specificity for the donor substrate UDP-galactose; all transfer galactose in a beta 1,4 linkage to similar acceptor sugars: GlcNAc, Glc, and Xyl. Each beta4GalT has a distinct function in the biosynthesis of different glycoconjugates and saccharide structures. As type II membrane proteins, they have an *N*-terminal hydrophobic signal sequence that directs the protein to the Golgi apparatus and then remains uncleaved to serve as a transmembrane anchor. By sequence similarity, the beta4GalTs form 4 groups: beta4GalT1 and beta4GalT2, beta4GalT3 and beta4GalT4, beta4-GalT5 and beta4GalT6, and beta4GalT7. B4GALT6 is a lactosylceramide synthase important for glycolipid biosynthesis.[25]

FMOD is a member of a family of small interstitial proteoglycans. It participates in the assembly of the extracellular matrix as it interacts with type I and type II collagen fibrils and inhibits fibrillogenesis in vitro. It also regulates TGF-beta activities by sequestering TGF-beta into the extracellular matrix.[26] Also LAMA4 is downregulated. It belongs to laminins, a family of extracellular matrix glycoproteins that are the major noncollagenous constituents of basement membranes and have a role in adhesion and migration of human bone marrow progenitor cells.[27]

Among the receptors are CDH8 (that encodes for a

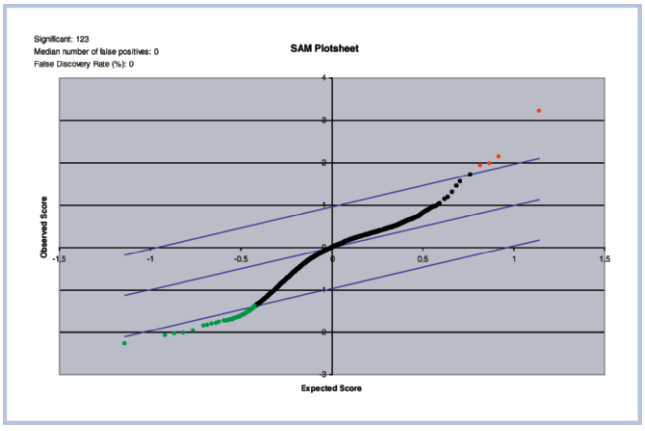

FIGURE 1. Statistical analysis of microarray plot (SAM) of MG63 treated for 24 hours with PerioGlas at the concentration of 0.04 g/mL. Expected differentially expressed genes are reported in the x axis whereas observed differentially expressed genes are in the y axis. Downregulated genes (green dots) are located in the lower left-hand side of the diagram; upregulated genes (red dots) are in the upper right-hand side; genes with different expression that are not statistically significant differences are black dots. Blue parallel diagonal lines represent the cutoff limits; the middle diagonal line indicates the equal value of observed and expected differentially expressed genes.

type II classical cadherin from the cadherin superfamily, integral membrane proteins that mediate calcium-dependent cell-cell adhesion[28]) and CD34 (that is a sialomucin-like adhesion molecule only expressed on a few percent of primitive bone marrow cells; in human bone marrow, virtually all colony-forming unit activity resides in the population expressing human CD34[29]).

The genes discussed are only a limited number among those differentially expressed and reported in Table 1 and 2. We briefly analyzed some of those with a better-known function.

PG is able to downregulate some functional activities of osteoblast-like cells: it acts on signal transduction especially on TGFBs paracrine network; it inhibits apoptosis and thus increases cell proliferation; it decreases cell adhesion with a consequent enhancement of cell mobility and migration; finally, it acts on bone marrow stem cells (ie, CD34). In conclusion, PG acts on bone formation by stimulating both osteoconduction (as demonstrated by the reduction in cell adhesion) and ostegenesis (as shown by TGFB-related proteins and stem cell markers).

It is worth noting that MG63 is a cell line and not normal osteoblasts. One of the advantages of using a cell line is related to the fact that the reproducibility of the data is higher because there is not the variability of the patient studied. Primary cell cultures provide a source of normal cells but they also contain contaminating cells of different types and cells in variable differentiation states. Moreover, we have chosen to perform the experiment after 24 hours to get information on the early stages of stimulation. It is our knowledge, therefore, that more investigations, with other osteoblast-like cell lines, primary cultures, and different time points, are needed to get a global comprehension of the molecular events related to PG action. Finally, we believe the reported data can be a model to compare different substances with similar effects.

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REFERENCES

1. Fetner AE, Hartigan MS, Low SB. Periodontal repair using PerioGlas in nonhuman primates: clinical and histologic observations. *Compendium.* 1994;15:932, 935–938.

2. Karatzas S, Zavras A, Greenspan D, Amar S. Histologic observations of periodontal wound healing after treatment with PerioGlas in nonhuman primates. *Int J Periodont Restorative Dent.* 1999;19:489–499.

3. Cancian DC, Hochuli-Vieira E, Marcantonio RA, Garcia IR Jr. Utilization of autogenous bone, bioactive glasses, and calcium phosphate cement in surgical mandibular bone defects in Cebus apella monkeys. *Int J Oral Maxillofac Implants.* 2004;19:73–79.

4. Johnson MW, Sullivan SM, Rohrer M, Collier M. Regeneration of peri-implant infrabony defects using PerioGlas: a pilot study in rabbits. *Int J Oral Maxillofac Implants.* 1997;12:835–839.

5. Teofilo JM, Brentegani LG, Lamano-Carvalho TL. Bone healing in osteoporotic female rats following intra-alveolar grafting of bioactive glass. *Arch Oral Biol.* 2004;49:755–762.

6. Zamet JS, Darbar UR, Griffiths GS, et al. Particulate bioglass as a grafting material in the treatment of periodontal intrabony defects. *J Clin Periodontol.* 1997;24:410–418.

7. Gatti AM, Simonetti LA, Monari E, Guidi S, Greenspan D. Bone augmentation with bioactive glass in three cases of dental implant placement. *J Biomater Appl.* 2006;20:325–339.

8. Nevins ML, Camelo M, Nevins M, et al. Human histologic evaluation of bioactive ceramic in the treatment of periodontal osseous defects. *Int J Periodont Restorative Dent.* 2000;20:458–467.

9. Price N, Bendall SP, Frondoza C, Jinnah RH, Hungerford DS. Human osteoblast-like cells (MG63) proliferate on a bioactive glass surface. *J Biomed Mater Res.* 1997;37:394–400.

10. Xynos ID, Hukkanen MV, Batten JJ, Buttery LD, Hench LL, Polak JM. Bioglass 45S5 stimulates osteoblast turnover and enhances bone formation in vitro: implications and applications for bone tissue engineering. *Calcif Tissue Int.* 2000;67:321–329.

11. Xynos ID, Edgar AJ, Buttery LD, Hench LL, Polak JM. Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis. *Biochem Biophys Res Commun.* 2000;276:461–465.

12. Xynos ID, Edgar AJ, Buttery LD, Hench LL, Polak JM. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution. *J Biomed Mater Res.* 2001;55:151–157.

13. Carinci F, Volinia S, Pezzetti F, Francioso F, Tosi L, Piattelli A. Titanium-cell interaction: analysis of gene expression profiling. *J Biomed Mater Res B Appl Biomater.* 2003;66:341–346.

14. Carinci F, Pezzetti F, Volinia S, et al. Zirconium oxide: analysis of MG63 osteoblast-like cell response by means of a microarray technology. *Biomaterials.* 2004;25:215–228.

15. Carinci F, Pezzetti F, Volinia S, et al. Analysis of MG63 osteoblastic-cell response to a new nanoporous implant surface by means of a microarray technology. *Clin Oral Implants Res.* 2004;15:180–186.

16. Carinci F, Pezzetti F, Volinia S, et al. P-15 cell-binding domain derived from collagen: analysis of MG63 osteoblastic-cell response by means of a microarray technology. *J Periodontol.* 2004;75:66–83.

17. Carinci F, Piattelli A, Stabellini G, et al. Calcium sulfate: analysis of MG63 osteoblast-like cell response by means of a microarray technology. *J Biomed Mater Res.* 2004;71B:260–267.

18. Salvatori R, Hayashida CY, Aguiar-Oliveira MH, et al. Familial dwarfism due to a novel mutation of the growth hormone-releasing hormone receptor gene. *J Clin Endocrinol Metab.* 1999;84:917–923.

19. Martelli AM, Fiume R, Faenza I, et al. Nuclear phosphoinositide specific phospholipase C (PI-PLC)-beta 1: a central intermediary in nuclear lipid-dependent signal transduction. *Histol Histopathol.* 2005;20:1251–1260.

20. Tsuji A, Sakurai K, Kiyokage E, et al. Secretory proprotein convertases PACE4 and PC6A are heparin-binding proteins which

are localized in the extracellular matrix. Potential role of PACE4 in the activation of proproteins in the extracellular matrix. *Biochim Biophys Acta.* 2003;1645:95–104.

21. Kawai T, Akira S, Reed JC. ZIP kinase triggers apoptosis from nuclear PML oncogenic domains. *Mol Cell Biol.* 2003;23:6174–6186.

22. Jauliac S, Lopez-Rodriguez C, Shaw LM, Brown LF, Rao A, Toker A. The role of NFAT transcription factors in integrin-mediated carcinoma invasion. *Nat Cell Biol.* 2002;4:540–544.

23. Abonyo BO, Alexander MS, Heiman AS. Autoregulation of CCL26 synthesis and secretion in A549 cells: a possible mechanism by which alveolar epithelial cells modulate airway inflammation. *Am J Physiol Lung Cell Mol Physiol.* 2005;289:L478–488.

24. Torres PS, Alcover A, Zapata DA, et al. TCR dynamics in human mature T lymphocytes lacking CD3 gamma. *J Immunol.* 2003;170:5947–5955.

25. Lacy SE, Bonnemann CG, Buzney EA, Kunkel LM. Identification of FLRT1, FLRT2, and FLRT3: a novel family of transmembrane leucine-rich repeat proteins. *Genomics.* 1999;62:417–426.

26. Fan Y, Yu L, Tu Q, et al. Molecular cloning, genomic organization, and mapping of beta 4GalT-VIb, a brain abundant member of beta 4-galactosyltransferase gene family, to human chromosome 18q12.1. *DNA Seq.* 2002;13:1–8.

27. Sjoberg A, Onnerfjord P, Morgelin M, Heinegard D, Blom AM. The extracellular matrix and inflammation: fibromodulin activates the classical pathway of complement by directly binding C1q. *J Biol Chem.* 2005;280:32301–32308.

28. Gu YC, Kortesmaa J, Tryggvason K, et al. Laminin isoform-specific promotion of adhesion and migration of human bone marrow progenitor cells. *Blood.* 2003;101:877–885.

29. Shimoyama Y, Tsujimoto G, Kitajima M, Natori M. Identification of three human type-II classic cadherins and frequent heterophilic interactions between different subclasses of type-II classic cadherins. *Biochem J.* 2000;349:159–167.