Open Science Repository Dentistry

doi: 10.7392/openaccess.70081966

In Vitro levels of Calcium, Phosphate and Alkaline Phosphate Activity in Media of Rat Osteoblasts Grown in the Presence of Various Implant Materials

Fiayaz A Shaama, M.Phil., D.D.S., M.Sc.[1], Shivananda B Nayak, M.Sc., Ph.D.[2], Valerie A Stoute, Ph.D., M.Sc.[3]

[1]Lecturer, Restorative Dentistry, Prosthetics unit, Faculty of Medical Sciences, the University of the West Indies, EWMSC, Mt Hope, Trinidad and Tobago

[2]Professor, Department of Preclinical Sciences, Biochemistry unit, Faculty of Medical Sciences, EWMSC, Mt Hope, Trinidad and Tobago

[3]Professor, Departments of Environmental and Graduate Studies, the University of Trinidad and Tobago, O'Meara Campus, O'Meara Industrial Estate, Lots 74-98

Citation: Shaama, F. A., Nayak, S. B., & Stoute, V. A. (2013). In Vitro Levels of Calcium, Phosphate and Alkaline Phosphate Activity in Media of Rat Osteoblasts Grown in the Presence of Various Implant Materials. Open Science Repository Dentistry, Online(open-access), e70081966. doi:10.7392/openaccess.70081966

Received: May 21, 2013

Published: May 27, 2013

Author for correspondence:
Fiayaz Aleem Shaama
University of the West Indies
Faculty of Medical Sciences
Restorative Dentistry, Unit of Prosthetics.
EWMSC, Trinidad and Tobago, West Indies.
Phone: 1-868-384-0707,
Fax: 1-868-645-3823. E-mail: fiayaz.shaama@sta.uwi.edu

ABSTRACT

We studied the *in vitro* levels of calcium, phosphate, and alkaline phosphate (ALP) activity in media of rat osteoblasts grown in the presence of various implant materials. Osteoblast cells from newborn Sprague-Dawley rats were harvested from calvaria. Cells were cultivated in the presence of Cobalt-Chrome (Co-Cr), cpTitanium (Ti), polymethylmethacrylate (PMMA), and wood in an artificial medium in a humidified atmosphere (5% CO_2) at 37^0 C. Cellular function was monitored over two weeks. Statistical tests (2-way ANOVA) of the results indicated that changing the disc material did not have a significant effect ($p>0.05$) on the amounts of Ca^{2+} and PO_4^{2-} produced but it did on the level of ALP activity in the medium ($p<0.05$). The culture time impacted significantly ($p<0.05$) on the levels of all 3 markers. Further, the interaction effect between the disc material and culture time was significant ($p<0.05$) for all markers. Wood produced the highest activity for ALP. Electron Microscopic studies captured cells growing over all materials, but the presence of $Ca^{2+}$was detected in wood samples only. With respect to markers for bone function, wood is comparable to known implant materials.

Key words: *Rat Osteoblasts; Dental Implant materials; Calcium/ Phosphate/ALP levels; ANOVA tests*

INTRODUCTION

Artificial bone and prostheses are used for the rehabilitation and restoration of function in the human body. The materials used for implants and artificial bone are evolving. However, a 'one-type-fits-all" material for implants is still eluding scientists worldwide. Materials used have been made from stainless steel [1], cobalt-chrome alloys (Co-Cr) [2], and titanium alloy (Ti), cpTitanium [3],*inter alia*.

Most of the implants used today are generally successful. With some patients, however, revision surgery or removal of implants after several years may be needed. This is especially probable where fixtures are subjected to considerable torque and movement. Hence, reports of implant success, with any material, must be interpreted with caution. Most implants exhibit immediate integration. Note, for example, that short term success was recently found when Co-Cr and Ti implants were fixed in pigs [4]. However, the host response, over a period of time and under different environmental conditions, affects overall outcome. This may be so because of the nature of chronic rejection, a poorly understood chronic inflammatory and immune response against implants in general. The adaptive immune system confronts a combination of slowly released antigens that are dispersed at very low concentrations. Antibodies and cell mediated immunity responses are designated by many researchers as the number one cause of implant failure [5,6,7].

Several materials are being tried in the search for a better product. For example, tantalum [8], ceramics [9] ,and composite materials [10] are some of those studied for osseointegration and applicability. Artificial bone made from wood has also been investigated. Several studies have addressed the ability of wood- natural or treated- to withstand the rigors of orthopedic or dental implantation. Investigations with pinus wood [11], beach wood [12], birch [13], and Juniper [14],*inter alia*, have led to information about wood's good (comparable to bone) mechanical performance (insertion torque and pullout force) [11], fracture behaviour (Young's modulus and failure load under compression) [12], and its stability/decomposition behaviour – the latter being indirectly represented by wood's favorable osteoconductive [13] and osseointegration [14-16] properties, necessary for bone fusion.

The idea of wood being used to replace bone is appealing since wood is an inexpensive natural product. Additionally, the physical structure of wood is porous and somewhat similar to bone. The hierarchical micro-fibre structure of cellulose, hemi-cellulose, and lignin has variable porosity and high strength even at low densities [15]. Researchers in Italy have devised a novel system for converting wood to a scaffold of hydroxyapatite [16].Their method basically involves slow

pyrolysis at 1000^0 C to reduce the material to a carbon frame, followed by conversion of this frame to hydroxyapatite. In another study, wood was pretreated differently [13].The organic components were removed by heating in a much lower temperature range, specifically between200^0 C and 140^0 C. Under these conditions, there was more integration and less fibrosis found at the interface of the bone and implant when applied in rabbits. At this temperature, the reactive interface of wood would be composed mainly of hydroxyl groups, unlike the carbon frame expected at 1000^0 C.

The use of polymethylmethacrylate (PMMA) as a bone cement has persisted since 1959 [17]. PMMA in preformed beads containing benzoyl peroxide is mixed with methyl methacrylate (MMA) containing *N, N*-dimethyl-*p*-toluidine (DMPT).

Alkaline phosphate activity (ALP) measurements have been used as a marker for bone formation. It is produced by osteoblasts to provide a high PO_4^{2-} concentration at the cell surface during mineralization. Osteoblast maturation is associated with the expression of ALP [18].The presence of Ca^{2+} and an increase in inorganic phosphate have been found in the extracellular vesicles at the stage of precipitation of hydroxyapatite or mineralization [19].

The aim of this study was to determine if there is a difference in the production of ALP, **Ca^{2+},** and **PO_4^{2-}**for several different materials, over various time periods. The data was analyzed in two-factor ANOVA tests using SPSS V.17. The significance of the main and interaction effects is assessed at the ά =0.05 critical level.

MATERIALS AND METHODS

All the materials used in this research were procured from the standard companies. This research proposal was approved by the ethics committee, The University of the West Indies, Faculty of Medical Sciences, Trinidad (Ref number: EC65:21/12-06/07).

Cell **c**ultures of rat osteoblasts were grown in the presence of discs (0.8 x 0.25 mm) made of wood and of the various implant materials currently used. The control experiment had no disc. In this, the cells were cultured in the presence of the growth medium alone. Scanning Electron Microscope (SEM) images and surface elemental analyses were performed on all disc materials, before they were placed in and after they were removed from the cell cultures.

Cell Harvest

Calvaria from eight newly born Sprague-Dawley rats were cut out from the skull under a dissecting microscope. The suture areas were removed in such a way as to obtain only the central component of the bone. This method has been described previously [20]. The periosteum and endosteum of the dissected bone were gently scrapped off and washed with sterile Ca^{2+}-free and Mg^{2+}-free Tyrode's solution (Sigma-Aldrich). The bone fragments were cut into fragments of 0.5 mm before being subjected to enzyme digestion. This latter involved the sequential treatment with 2.5 mL of 1.0 mg/mL trypsin (Sigma-Aldrich) in Phosphate Buffer solution (PBS) for 10.0 minutes. The action of the trypsin was stopped by the addition of 5 mL of Medium 199, with glutamine and Earle's salts (MEM), containing 10% fetal calf serum (FCS) (all from Sigma-Aldrich). The liquid was decanted and 5.0 mL of PBS added. The bone fragments were then digested using 5.0 mL of 0.25% collagenase (Fisher Bioreagents) in PBS for one hour with slight agitation in a water bath at 37^0 C. The bone fragments were allowed to settle after 3 minutes. The suspension was decanted, then centrifuged at 400*g* for five minutes to allow for pellet formation. This pellet was washed using 5.0 mL of PBS before being re-suspended in 10.0 ml of growth medium. This consisted of MEM containing 10% FCS and 0.2% antibiotics (100 units penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B) (Sigma-Aldrich).

Experimental Template

One milliliter of the osteoblast cell suspension in the growth medium was evenly distributed in each well of a 24-well sterile cell culture plate, containing discs of Ti (American Elements, Merelex Corp.), Co-Cr alloy (Nobilium Inc.), PMMA (SuperDental Co.) and wood (*Pinus)*. Five (5) replicates of each disc (and of the blank medium) were used. All the discs used for the experiments were made to identical dimensions: 0.8 x 0.25 mm. The wood discs were pre-treated by placing them in an oven for 12 hours at 200^0 C. All the wells were topped up by the addition of 2.0 mL of growth medium. This experimental template was placed in a humidified incubator at 37^0 C under an atmosphere of 5% CO_2 and 95 % air. Aliquots (1.0 mL) of the medium were taken from each well at 1, 3, 6 and 7 days and replaced with 1.0 mL of fresh growth medium. Samples collected were frozen at -20^0 C for analysis. The activity of ALP and the concentrations of Ca^{2+} and of PO_4^{2-} were obtained using Ortho Vitros dry slides Chemistry Analyzer (VITROS® 4600).

Electron Scanning Microscope Analysis

The composition and images of the surfaces of all discs were obtained before they were placed in the experimental template. After seven (7) days, each disc was removed from the experimental template with osteoblast cells and its image and surface composition recorded. All discs were vacuum dried then coated with 15-40 nm gold (Denton Vacuum Desk II). The surface

compositions and images were recorded using a Scanning Electron Microscope (Phillips SEM 515) set at 30 KV. The data collected was analyzed using Genesis (EDAX) software.

RESULTS

The mean levels of the markers, Ca^{2+}, PO_4^{2-}, and ALP activity, after various times (1, 3, 5, 7 days) are given for the different disc materials in Figure 1.

Electron Microscope Analysis

The primary cell cultures adhered to the base of the wells and to the surfaces of all discs. This occurred within two hours after the cells were introduced into the wells. Confluence was also seen in all wells after five days of culture. Chemical analysis showed the presence of Ca^{2+} after seven (7) days in all cell compartments. SEM surface analysis revealed (1) osteoblast cells growing on the surface of all disc materials, (2) no Ca^{2+} on the surface of any disc material, including wood, before it was placed in the cell cultures, and (3) the presence of Ca^{2+} on the surface of the wood disc but not on that of any of the Co-Cr, Ti and PMMA discs- Figure 2.

Impacts of 'Disc Material' and 'Time' on Levels of Ca^{2+}, $PO_4^{2+}$and ALP Activity:

Figure 1 illustrates these impacts graphically. The results of the two-factor ANOVA tests are summarized in Table I. These test (1) whether there are significant differences between the disc materials in terms of the amounts of Ca^{2+}, PO_4^{2-}, and ALP activity found in their cell culture compartments, (2) whether Time has an impact on the amounts of these same 3 markers, and (3) whether there is a Time-Material interaction in that the pattern observed over Time is different for the various disc materials. Tests which are significant are designated as '**S'** and those that are non-significant as **'NS'**. The **'p'** values are given in parentheses and matched next to the critical value ά =0.05.

The impacts of the disc material on the levels of Ca^{2+} and PO_4^{2-} were not significant (no real differences between the levels of $Ca^{2+}$and of PO_4^{2-}found in cells with discs of different material). With ALP activit**y,** on the other hand**,** there was a significant impact of the disc material on the levels found in the cell culture medium after 7 days**,** with the highest activity found in cells with the wood disc (Figure 1).

Culture time impacted significantly on the levels of all markers. This is expected. This research aimed not at establishing the obvious but at looking to see how differences between discs in the levels of all the markers (Ca^{2+}, PO_4^{2-} and ALP) changed with Time. This comes from the Time-

Material interaction effect. There is a significant interaction effect between the factors of 'Time' and 'Disc Material' for the levels observed in the cell cultures for each of the 3 markers (Table 1). The implication here is that the pattern of each marker's relative values with time changes from one disc material to the next. Hence, the impact of 'Time' on the levels of any marker is different depending on which disc Material is used in the cell culture.

Correlations among Dependent Variables at Different Culture Times

The data for each sampling day was subjected to correlation analysis to see how the relationships among the 3 outcomes- Ca^{2+}, PO_4^{2-}, and ALP activity- developed over time. The correlation coefficients for pairs of outcome variables are given in Table 2. These were estimated for each day's data using information from all of the 5 individual replicate cell cultures (instead of the mean values) for all 4 disc materials plus the control.

As the culture time increased there appeared to be an increasingly stronger correlation between the level of ALP activity in the cell and the concentrations of either PO_4^{2-} or Ca^{2+}, with the strongest correlations in each case (0.465 and 0.807 respectively) obtained for the Day 7 data. The relationship between Ca^{2+} and PO_4^{2+} was also more pronounced (r = 0.771) for the longer culture times.

DISCUSSION

The experimental method employed in this research followed a standard cell culture technique. The results indicate that ALP was produced by the osteoblast cultures grown in the presence of all of these materials. Cells were observed covering all test materials after five days.

The use of cultured osteoblast cells *in vitro* for experimental research has been carried out for over 40 years. Osteoblast cell lines, for example MG 63, Saos-2, U-2, have been used in many studies involving implants [21]. When these cells were compared to primary cultures of animal osteoblasts, osteoscarcoma cell lines showed distinct differences in behavior [22]. Researchers have expressed the view that experimental templates using primary osteoblast cultures reflect more phenotypic properties of normal osteoblasts than osteoblastic cell lines [23]. However, to acquire osteoblasts for primary cell culture experiments, tissue is usually obtained from prenatal and newly born animals.

Osteoblasts can be isolated by migration [24], by mechanical techniques [25], by cloning [26], and by enzymatic digestion, as is done here. It has been argued that cells harvested using enzyme digestion have several drawbacks. One of the main concerns is that when collagenase is used for

over one hour, damage to antigen receptors on cell membrane occurs. Also the population of cells collected is adulterated by fibroblasts [27]. Several studies reported that, in addition to this contamination, there is the problem that osteoblasts isolated by enzymatic digestion fail to show any mineralization *in vitro* [28,29].

Distinctly different results have been reported by numerous researchers [18]. It has been demonstrated clearly that bone cell purity is not a requirement to obtain bone formation *in vitro* [30].Although generalized osteogenesis is evident in fetal rats, a differential growth pattern is usually observed at nineteen days *in utero*. Cells isolated from calvaria are at different stages of differentiation. The cellular composition of the whole calvaria in rats shows that one fifth is composed of osteoblasts. The frontal and parietal bones, otherwise known as the skull cap, are used. The main advantage in using enzyme digestion in this study is to harvest a large amount of cells in a short time frame. Researchers have reported that incubating one calvarium in crude collagenase for two hours releases about 40,000 cells consisting of 85-90% osteoblasts [18].

The results show that Ca^{2+}, PO_4^{2-}, and ALP activity are produced in cells with discs of all materials tested, even wood. The statistical analysis shows further that there is no significant impact on the amount of Ca^{2+} and PO_4^{2-}produced by changing the disc material. The type of disc material does affect significantly the amount of ALP activity within the cell, with the wood disc showing the highest ALP activity after 7 days (Figure 1). Time caused significant increases in the amount of all markers for all disc materials. The rate at which each marker was produced, however, changed from one disc material to the next (the Time-Disc Material interactions were significant for all markers). These results are informative because they show that wood stacks up well against the known and approved materials, such as Ti, Co-Cr and PMMA.

After the seventh day, spectral analysis revealed the presence of Ca^{2+} on wood only. None of the other materials gave similar findings. This may or may not be significant. In contrast to the implant materials that are used today, wood is completely natural. The channel structure of wood creates a porous scaffold that could possibly allow favorable bone growth. Heat treated wood has reactive hydroxyl chemical groups at its interface with bone. Reactive hydroxyl groups on the cellulose chains are available for covalent bonding and for hydrogen bonding. At the level of the bone there are reactive Ca^{2+}, PO_4^{2-}, proteins and collagen available for interaction with the implant at the ionic level [31].

These results suggest that wood should be explored further as an option to the more standard implant materials in use today.

Acknowledgment:

This research was funded by the University of the West Indies Campus Research Fund, grant number CRP.4FEB07.6.

Disclosures:

This is original research carried out by the authors alone and there are no conflicts of interest.

REFERENCES

1. Hayes JS, Richards RG. The use of titanium and stainless steel in fracture fixation. Expert Rev Med Devices 2010;7:843-853.
2. Taksali S, Grauer JN,Vaccaro AR. Material considerations for intervertebral disc replacement implants. Spine J 2004;4:Suppl:231S-238S.
3. Nayayanan R, Seshadri SK, Kwon TY,Kim KH. Calcium phosphate coating on titanium and its alloys. J Biomed Mater Res B Appl Biomaterial. 2008;85:279-99.
4. Rodella LF, Favero G, Sala L, Buffoli B, Paganelli C,Rezzani R. A comparative pilot study of two dental implant metals in pig mode. Implant Dent 2010;19:532-538.
5. Unwin PS, Cannon SR, Grimer RJ, Kemp HBS, Sneath RS,Walker PS. Aseptic loosening in cemented custom-made prosthetic replacements for bone tumors of the lower limb. J Bone Joint Surg Br 1996;78:5-13.
6. Catelas I, Jacobs JJ.Biologic activity of wear particles. Instr Course Lect 2010;59:3-16.
7. Cadosch D, Chan E, Gautschi OP,Filgueira L. Metal is not inert: role of metal ions released by biocorrosion in aseptic loosening-current concepts. J Biomed Mater Res 2009;91:1252-1262.
8. Maccauro G, Iommetti PR, Muratori F, Raffaelli L, Manicone PF,Fabbriciani C. An overview about the biomedical applications of micron and nano size tantalum. Recent PatBiotechnol 2009;3:157-165.
9. Andreiotelli M, Wenz HJ,Kohal RJ.Are ceramic implants a viable alternative to titanium implants? A systematic literature review. Clin Oral Implants Res 2009;20 Suppl:32-47.
10. Scotchford CA, Shataheri M, Chen PS, Evans M, Parsons AJ, Aitchison GA, Efeoglu C, Burke JL, Vikram A, Fisher SE,Rudd CD. Repair of calvarial defects in rats by prefabricated degradable long fibre composite implants. J Biomed Mater Res 2011;96:230-238.
11. Oliscovicz NF, Shimano AC, Marcantonio Junior E, Lepri CP, Dos Reis AC.Analysis of primary stability of dental implants inserted in different substrates using the pullout test and insertion torque,Int J Dent. 2013;2013:194987. doi: 10.1155/2013/194987. Epub 2013 Jan 22.

12. Murdoch AH, Mathias KJ, Shepherd DE, Investigation into the material properties of beech wood and cortical bone. Biomed Mater Eng 2004;14(1):1-4.
13. Rekola J, Aho AJ, Gunn J, Mathinlinna J, Hirvonen J, Vitaniemi P, Vallittu PK. The effect of heat treatment of wood on osteoconductivity. Acta Biomaterials 2009;5:1596-1604.
14. Gross KA, Ezerietis E.Juniper wood as a possible implant material. J Biomed Mater Res A. 2003 Mar 15;64(4):672-83.
15. Ingber DE. Cellular tensegrity : defining new rules of biological design that govern the cytoskeleton. J Science 1993;104:613-627.
16. Tampieri A, Sprio S, Ruffini A, Celotti G, Lesci I.G, Roveri N. From wood to bone: a multi step process to convert wood hierarchical structure into biomimetic hydroxyapatite scaffolds for bone tissue engineering. J Mater Chem 2009;19:4973-4980.
17. Clarke B. Normal bone anatomy and physiology. Clin AmSoc Nephrol 2008;3:131-139.
18. Harper E J. Bioactive bone cements. Proc Inst Mech Eng H 1998;212:113-120.
19. Landis WJ. The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. Bone 1995;16:533-544.
20. Yagiela JA, Woodbury DM. Enzymatic isolation of osteoblasts from fetal rat calvaria. Anat Rec 1977;188:287-306.
21. Shapira L, Halabi A. Behavior of two osteoblast-like cell lines cultured on machined or rough titanium surfaces. Clin. Oral Implants Res 2009;20:50-55.
22. Saldaña L, Bensiamar F, Boré A,Vilaboa N. In search of representative models of human bone-forming cells for cytocompatibility studies. ActaBiomater 2011;7:4210-4221.
23. Ho ML, Chang HT, Chuang LY, Hsu HK,Wang GJ. Characteristics of primary osteoblast culture derived from rat fetal calvaria. Kaohsiung J Med Sci 1999;15:248-255.
24. Jones SJ,Boyde A. The migration of osteoblast. Cell Tissue Res 1977;184:179-193.
25. Smith DM, Johnston CC,Severson AR. Studies of the metabolism of separated bone cells I. Techniques of separation and identification. Calcif Tissue Res 1973;11:56-69.
26. Kodama H, Amagai Y, Sudo H, Kasai S, Yamamoto S. Establishment of a clonal osteogenic cell line from newborn mouse calvaria. Jpn J Oral Biol 1981;184:899-901.
27. Xian-Ying C, Mei-Zhen Y, Li-Na Z, Shi-Pu L,Yang C. Establishment of a new model for culturing rabbit osteoblasts in vitro. Biomed Mater 2006;1:16-19.
28. Aubin JE, Heersche JNM, MerrileesMJ,Sodek J. Isolation of bone cell clones with differences in growth, hormone responses and extracellular matrix production. J Cell Biol 1982;92:452-461.
29. Simmons DJ, Kent GN, Jilka RL, Scott DM, Fallon M,Cohn D.V. Formation of bones by isolated, cultured osteoblasts in Millipore diffusion chambers. Calcif Tissue Int 1982;34:291-294.
30. Nefussi J, Boy-Lefeve ML, Boulekbache H,Forest N. Mineralization in vitro of matrix formed by osteoblasts isolated by enzyme digestion. Differentiation 1985;29:160-168.

31. Aho A, Rekola J, Matinlinna J, Gunn J, Tirri T, Viitaniemi P, and Vallittu P. Natural composite of wood as a replacement material for osteochondral bone defects. J Biomed Mater Res Part B: Applied Biomater2007;83:64-71.

Table 1: Results of Two-Factor (Disc Material and Time) ANOVA with replicates

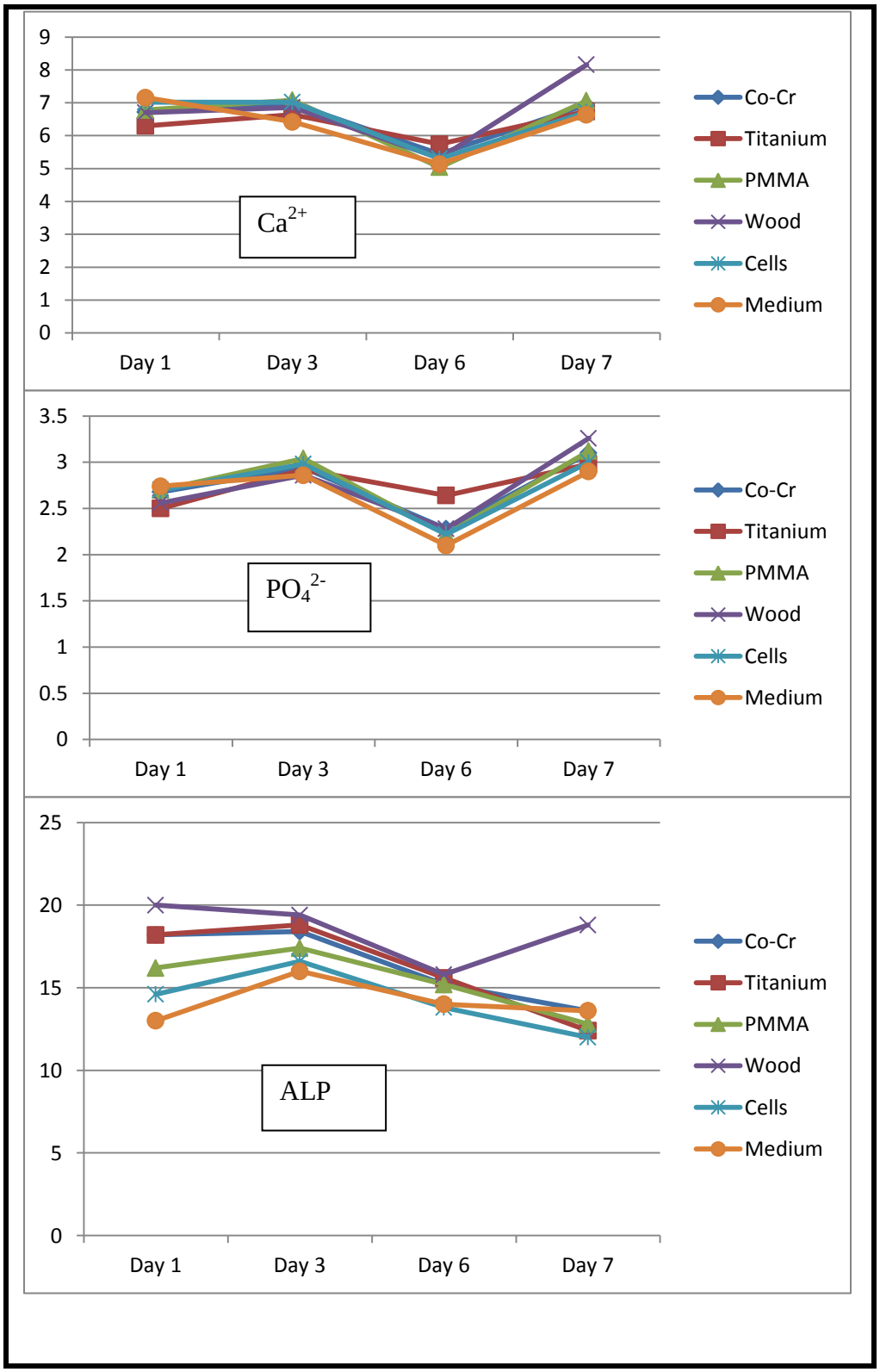
Outcome Measured	**Effect**	**Significance (p value)**
Ca^{2+}	Disc Material	**NS (p = 0.25 >0.05)**
	Time	**S (p = 0.000<<0.05)**
	Interaction	**S (p =0.012 <0.05)**
PO_4^{2+}	Disc Material	**NS (p =0.44 >0.05)**
	Time	**S (p = 0.000 < 0.05)**
	Interaction	**S (p = 0.0002 <0.05)**
ALP	Disc Material	**S(p = 0.0000<<0.05)**
	Time	**S(p = 0.0000 <<0.05)**
	Interaction	**S(p = 0.0000<<0.05)**

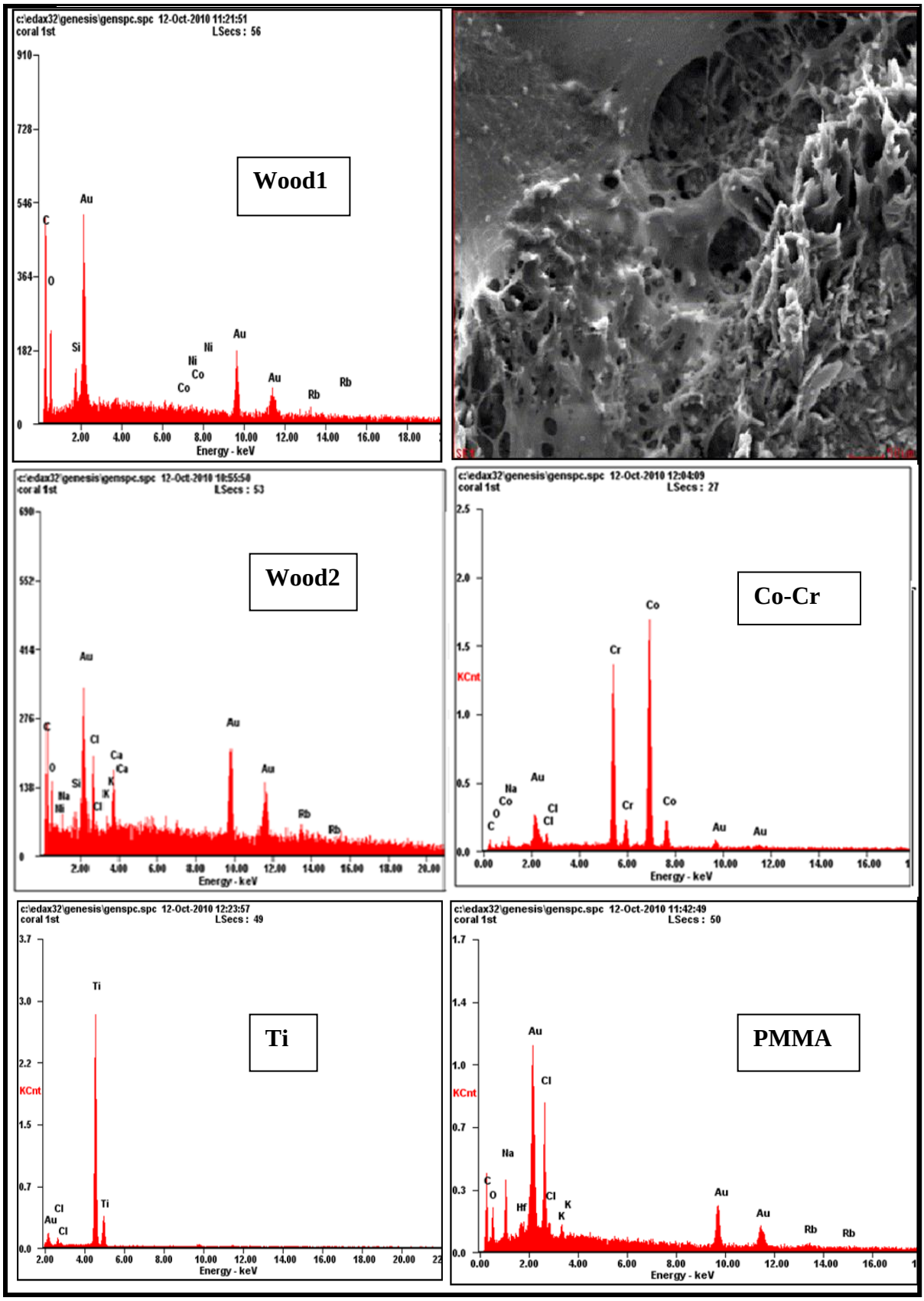
Table 2: Pearson Product Moment Correlation Coefficients for Pairs of Markers at Different Culture Times.

Variable 1	Variable 2	Pearson Product Moment Correlation Coefficients			
		Day 1	Day 3	Day 6	Day 7
Ca^{2+}	PO_4^{2-}	0.253	0.648	**0.771**	0.685
Ca^{2-}	ALP	0.007	0.229	0.391	**0.80**7
PO_4^{2-}	ALP	-0.127	-0.042	0.352	**0.465**

Figure 1: Change in Calcium (Ca^{2+}), Phosphate (PO_4^{2-}), and ALP Activity levels with Time for different disc materials.

Figure 2: Scanning Electron Microscope (SEM) surface analyses of different discs before (Wood1) and after (Wood2, Co-Cr, Ti, and PMMA) 7 days immersion in culture medium with osteoblast cells. The presence of Ca^{2+}on the disc surface was detected only with Wood2. (The top right SEM image is of osteoblast cells growing on a wood disc.)


9
8
7
6
5
4
3
2
1
0
Day 1
Day 3
Day 6
Day 7
Ca2+
Co-Cr
Titanium
PMMA
Wood
Cells
Medium
3.5
3
2.5
2
1.5
1
0.5
0
Day 1
Day 3
Day 6
Day 7
PO4 2-
Co-Cr
Titanium
PMMA
Wood
Cells
Medium
25
20
15
10
5
0
Day 1
Day 3
Day 6
Day 7
ALP
Co-Cr
Titanium
PMMA
Wood
Cells
Medium


c:\edax32\genesis\genspc.spc 12-Oct-2010 11:21:51
coral 1st LSecs : 56
Wood1
Energy - keV
c:\edax32\genesis\genspc.spc 12-Oct-2010 18:55:50
coral 1st LSecs : 53
Wood2
Energy - keV
c:\edax32\genesis\genspc.spc 12-Oct-2010 12:04:09
coral 1st LSecs : 27
Co-Cr
KCnt
Energy - keV
c:\edax32\genesis\genspc.spc 12-Oct-2010 12:23:57
coral 1st LSecs : 49
Ti
KCnt
Energy - keV
c:\edax32\genesis\genspc.spc 12-Oct-2010 11:42:49
coral 1st LSecs : 50
PMMA
KCnt
Energy - keV