日小整会誌（J Jpn Ped Orthop Ass）**23**(2)：433-440, 2014.
第 24 回日本小児整形外科学会学術集会　JPOA Asian Fellows

Efficacy of Antibiotic Impregnated Allogenic Bone Graft as Local Antibiotic Delivery System in Controlled Osteoarticular Infection

Lakhwani OP, Associate Professor Orthopaedics Surgery；
Kapoor S K, Professor Orthopaedics surgery and Dean

ESIC- Post Graduate Institute of Medical Sciences and Research
ESI Hospital, Basaidarapur, Ring road, New Delhi, India

Abstract：【Introduction】Osteomyelitis is dreaded infection necessitating prolonged and sustained antibiotic therapy which carries the disadvantages of systemic adverse effects and high cost of therapy. Current study aims to assess safety and efficacy of use antibiotic loaded allogenic cancellous bone as a carrier of antibiotics in controlled osteoarticular infection.

【Materials and Methods】Study involved case series of twelve patients with localized and controlled osteoarticular infection. Hydrophilic antibiotics were selected according to common organism responsible for the osteoarticular infection and culture and sensitivity. Allogenic cancellous antibiotics impregnated bone graft augmented with autologous bone graft or Bone marrow aspirate was used along with appropriate surgical procedure.

【Results】Patients were evaluated for infection eradication and osteointegration. Over all 6 cases were treated successfully thus obviating any subsequent procedure. Infection was eradicated in 10 cases. Osteointegration was achieved in all the cases in which autologous bone graft was used as adjuvant and in two out of four cases in which bone marrow aspirate was also used). Two cases were considered failure which required secondary procedures and change of fixation.

【Conclusion】Cancellous bone allograft as antibiotic carrier clinically provided safe, prolong and effective local antibiotic for treating osteoarticular Infection. It may be superior to surgical debridement and systemic antibiotic alone for infection eradication and may offer better osteointegration when used along with adjuvants like autologous bone graft or Bone marrow.

【Level of Evidence】level IV. Study can serve clinical basis of using the allogenic bone graft as carrier of antibiotics for safe and cost beneficial treatment of osteoarticular infection avoiding side effects of systemic antibiotics and simultaneously aiding bone healing by osteointregation.

INTRODUCTION

Bone and joint infections are known for chronicity, difficult eradication and osteolysis require prolonged systemic antibiotics for treatment. Surgical debridement alone may be ineffective

Key words：allogenic bone graft, local antibiotic, osteomyelitis
Correspondence duther：Dr. O P Lakhwani
Associate Professor Department of Orthopaedics Surgery
ESI Hospital, ESI PGIMSR
Basaidarapur, Ring road, New Delhi–110015, India.
E-mail – orth365@gmail.com, dr.omprakash.lakhwani@esic.in
Phone：+91 9555628578, Fax：+91 11–25440722

in eliminating micro-clusters from biofilms and small colony variant bacteria[3] that survive and replicate intracellularly in osteoblast, macrophages and other cells leading to recurrence of infection[2]. These pathogens are also difficult to retrieve and missed in culture. Hence high local concentration of antibiotic for a prolonged duration is desirable to eliminate such pathogens.

Antibiotics loaded synthetic material[5)6)] like Poly methyl metha acrylate bone cement (PMMA), Calcium hydroxy apetite, Calcium sulphate (Plaster of Paris) has been used as carrier of antibiotics for local delivery and filler of defect but have limitations of lack of osteointegration and second surgery for removal. Allograft bone material loaded with antibiotics may have advantage over them as it provides prolonged local antibiotic delivery, fills the defect and requires no second surgery for removal. Allograft bone loaded with antibiotics has been used in in-vitro study by Eivind Witso et al[17] using different antibiotics and found to be effective and safe. In an experimental animal study by Shyam K Saraf et al[13] using decal bone matrix for local antibiotic delivery in experimental animal study and found it to be effective. Clinical study by Heinz Winkler[16] have also found use of antibiotic impregnated bone graft to be effective and safe in one stage exchange of infected hip replacement. Current study evaluates further the efficacy and safety of Allogenic cancellous bone graft as local antibiotic carrier in treatment of controlled osteoarticular infection.

Materials and Methods

The study was approved by institutional ethical committee and all patients were fully informed and agreed to participate in the study. The inclusion criteria were patients of all age group with localized and controlled osteoarticular infection (in whom adequate surgical margins free from dead necrotic tissue could be achieved after surgical debridement) and wound coverage could be obtained primarily by suturing or flap coverage. Patient having minimum followup of six months were included in the study. Exclusion criteria were active osteoarticular infection and hypersensitivity to the antibiotics to be used in the study. Hydrophilic antibiotics among (Vancomycin, Ciprofloxacin, Clindamycin, Gentamicin, Tobramycin) were selected according to common organism responsible for the osteoarticular infection and culture & sensitivity. Twelve consecutive cases were treated. Surgical procedure and fixation was performed wherever required (Table – 1).

Graft Preparation

Allogenic bone graft was procured from consented patients having undergone total hip and knee arthroplasty. Donors were pre screened for diseases and infection and graft was gamma sterilized with a dose of 25 grays of radiation at an accredited centre and stored at (−76 degree Celsius) for minimum of three months before use in institutional bone bank.

During surgery allogenic cancellous graft was manually morcelized and washed with plenty of sterile saline. The bacterial culture of the allograft was taken before implantation to ascertain sterility. Morcellized bone graft was kept in 20 ml distilled water solution consisting of antibiotic in concentrations (Table 2) for a period of 45–60 minutes. Adjuvant autologous bone graft was used in four cases, bone marrow aspirate in another four cases and in rest four cases allogenic bone graft alone was used.

Postoperatively all patients were given usual course of systemic antibiotic for seven days. Pa-

Table 1. Case series study observation and out come

S n	Diagnosis	Surgical procedure	Antibiotic used	Adju-vant	Out come		
					Osteointegration	Infection	Over all outcome
1	Infected partial union radius/ulna Plate in situ	Plate removal debridement ALBG	Vancomycin	ABM	Healing of#ulna 5 months. Partial osteointegration in radius	Eradicated	Infection eradication with healing
2	Infected NU S/F#bone loss ILN in situ	Debridement ALBG	Vancomycin Tobramycin	Nil	Gradual lysis of bone graft 5 m.	Infection eradicated	Bone grafting ex-change nailing union at 6 m
3.	Segmental#Tibia	ILN rotation flap ALBG	Vancomycin	ABG	osteointegration at 7 months	eradicated pri-mary healing	Fracture union 6 months
4	Atrophic infected nonunion tibia#	ALBG Cortico-tomy ilizarov	Vancomycin	Nil	Graft resorption seen at 5 m	Infection healed	Second procedure (7 months later) with TENS + ABM
5	S/C femur#bone sinuses	ilizarov ALBG + ABG	Vancomycin Tobramycin	ABG	osteointegration at 8 months	Eradicated	healed fracture with infection eradication
6	Infected I/T#with PFN in situ	Debridement + AlBG	Vancomycin	Nil	Gradual lysis of graft at 4 m	Serous discharge culture (-)	Improved bone quality, required revised fixation
7	#Tibia with discharging sinus	Debridement ALBG	Vancomycin	ABM	Healing of fracture	No further sign of infection	Healing of fracture
8	Open#tibia proximal third	ALBG, plating, flap rotation	Vancomycin-Netlimycin	ABG	osteointegration seen at 5 months	eradicated pri-mary healing	Fracture healing with eradication of infection
9.	Osteomyelitis tibia	Debridement ALBG	Vancomycin	ABM	improved bone filling lesion	Eradicated	Healed infection and bone
10	Osteomyelitis Hu-merus	ALBG	Vancomycin	ABG	healing of defect	Eradicated	Eradication of infection with healing
11	Infected Open#tibia ext. fixater in situ	AlBG Flap rotation ilizarov	Vancomycin-Gentamycin	Nil	lysis of bone graft at 4 m	Infection Eradicated	Ilizarov removed fracture healed 8 months
12	#S/F discharging sinus	Debridement ALBG	Vancomycin	ABM	Lysis of graft, healing of defect	Sterile dis-charge at 5 day, healed there after	Eradication of infection with healing

Abbreviations -#- fracture, I/T – intertrochanter, NU – non union, PFN – Proximal femoral nail, BG – bone graft, ILN - Interlocking nail, ABM autologous bone marrow, S/F Shaft femur, T/F tibia-fibula, TENs – titanium elastic nail, AlBG – Allogenic Bone graft, ABG – Autologous Bone graft, m - months

tients were followed regularly at appropriate interval. Particular attention was given to any sign of infection / inflammation. Radiographs at intervals were done till completion of follow up to determine the status of host bone and graft, Bone healing and osteointegration by Sloof et al crieteria[15] (Table 3). Infection was assessed by clinical examination, blood investigation and radiological examination.

Results

All the twelve patients could be followed for more than six months with a mean followup of 12 months (8 months – 17 months). None of the patients showed clinical or histological evidence of any immunological reaction.

Table 2. Eivind Witso' et al[3] Concentration of antibiotics solution used in the study

Antibiotic	Concentration
Vancomycin	50 mg/ml
Ciprofloxacin	2 mg/ml
Clindamycin	150 mg/ml
Tobramycin	75 mg/ml
Netlimicin	100 mg/ml
Teicoplanin	100 mg/ml

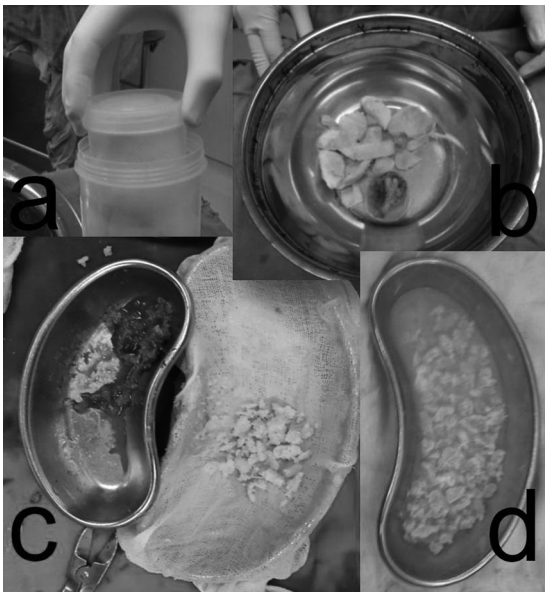

Fig. 1 (a) showing allograft stored in double jar, (b) Graft reconstituted (c) Morcelised graft before and after mixing with bone marrow (d) graft mixed with antibiotic solution.

Osteointegration - Evaluated as per sloof et al[15] criteria. In four cases (two infected tibia-fibula, one fracture shaft femur and one subtrochanter fracture) in which cancellous allograft with antibiotics alone was used showed gradual complete resorption of bone graft in 16-20 weeks. (Figure -) without any sequestrum with improved bone quality. Other four cases involving fractures of supra condylar femur, shaft femur, shaft Humerus and Tibia fibula in which autologous bone graft was used as adjuvant showed good healing and partial graft osteointegragion in immediate vicinity of the host bone while rest of the allograft gradually got resorbed in a mean time of 16.8 weeks (14-22 weeks). Among four cases with autologous bone marrow as adjuvant two (osteomyelitis and fracture tibia fibula) gradually showed improved bone quality, with allograft partial osteointegration. One case of fracture shaft femur and other of fracture Tibia showed gradual complete resorption of bone graft but with eradication of infection.

Infection Eradication - Six patients healed primarily without requiring any further intervention. Two patients required second time augmentation with antibiotic impregnated allogenic bone graft with adjuvant (autologous bone graft) to achieve infection eradication and bone healing. One patient of infected fracture tibia fibula developed serous discharge first observed on 5th postoperative day which on culture revealed Pseudomonas aeruginosa. The patient was put on appropriate systemic antibiotic therapy for three weeks and there was no further clinical sign of infection. Another patient of fracture subtrochanter femur with nail in situ further required debridement, secondary procedures and change of fixation. No other case developed any sign of infection.

Success rate of procedure in terms of infec-

Table 3. Sloof' s Criteria[13] for assessing osteointegration of allogenic bone grafts

i.	Presence/Absence of progressive lucent lines between the preexisting bone and the graft.
ii.	Absence of resorption of graft
iii.	Radiographic homogeneity of the graft.

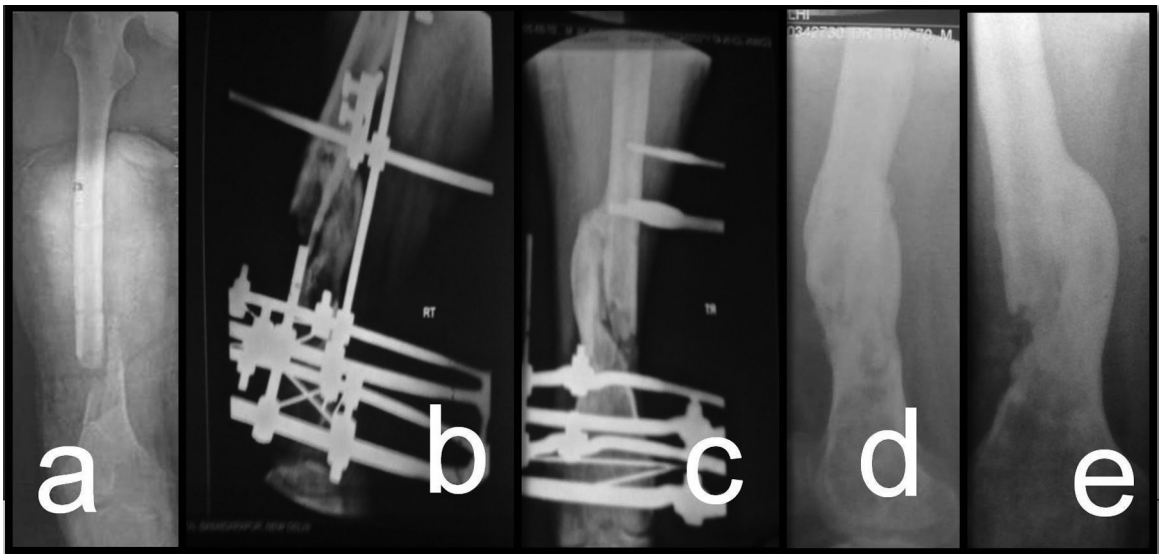

Fig. 2 (a) skiagram showing infected non union supracondylar femur (b) treated by ilizarov & allogenic antibiotic graft augmented with autograft (c) & (d) fracture healing osteointegration and infection eradication.

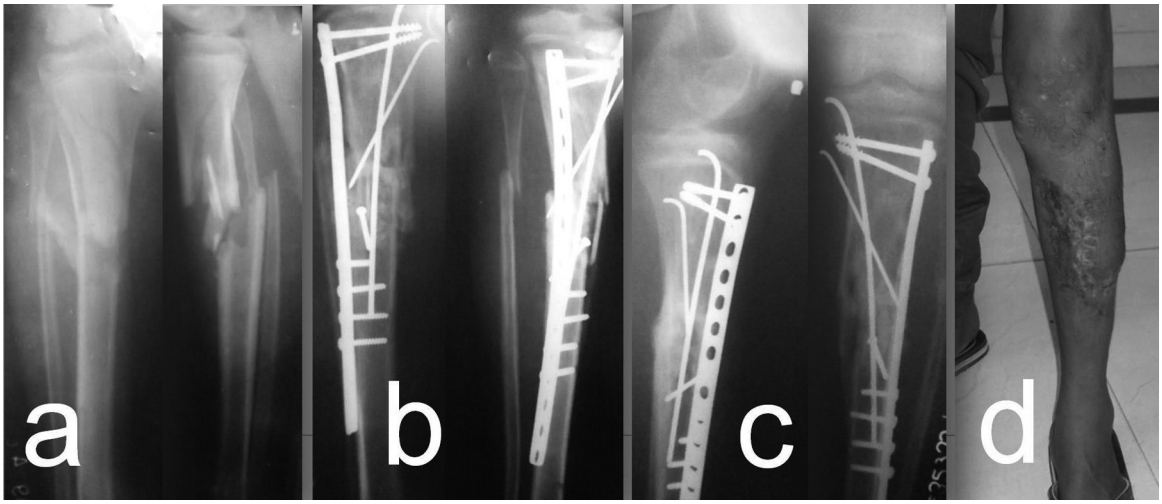

Fig. 3 (a) showing infected fracture proximal tibia (b) treated with fixation allogenic antibiotic graft augmented with autograft (c) & (d) fracture healing osteointegration and infection eradication.

tion eradication without requiring any subsequent procedure was 50 percent (six out of twelve cases). Overall infection eradication was achieved in 10 out of 12 cases. Osteointegration was achieved in all four cases in which autologous bone graft was used as adjuvant and in 50 % cases (two out of four) where autologous bone marrow aspirate was used. Two cases were considered failure in which allogenic bone graft alone was used (fracture subtrochanter and one fracture tibia fibula) which required secondary procedure and revised fixation.

Discussion

Earliest use of bone allograft for reconstruction has been practiced by Aubigne RM[1] since 1966. In most experimental models, fresh allografts are associated with a rigorous inflam-

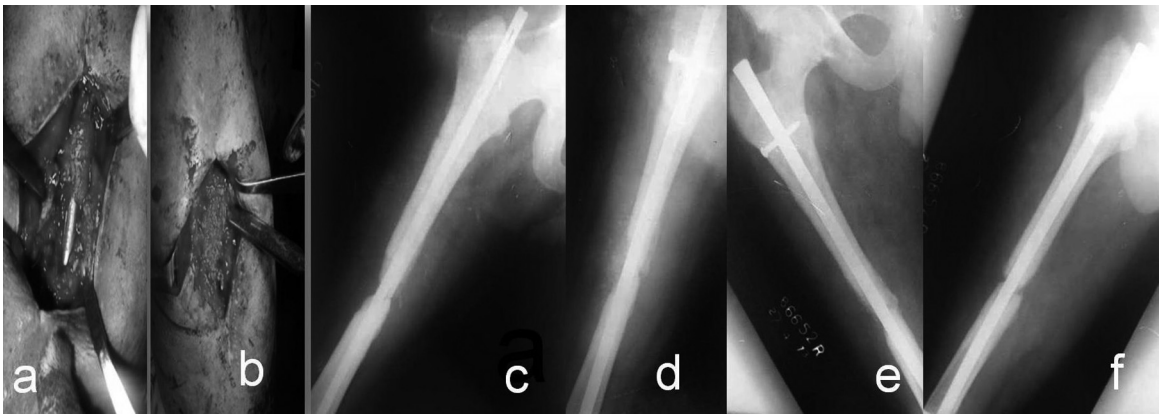

Fig. 4 (a) showing infected shaft femur fracture intraoperative after debridement with bone loss (b) grafted with allogenic antibiotic graft alone (c) & (d) gradual graft lysis (e) & (f) infection eradication, complete graft re-absorption without osteintegration.

matory response as well as a specific immune response. However, deep frozen allografts are associated with decreased immunogenicity and no demonstrable change in initial mechanical properties as reported by Friedlander G[4] in 1987.

The incorporation of antibiotic loaded bone graft was first studied by C. Gudmundson[7] 1971 who showed that the presence of tetracycline locally markedly inhibited bone graft incorporation. Use of one as a carrier for vancomycin has been studied in vitro by Witso E et al[17)18)] vancomycin is favoured because of its physiochemical properties of hydrophilicity, heat stability and efficacy against Staphylococus species which is most common cause of osteoarticular infection. Buttaro MA et al[2] in 2003 showed in an in-vitro study that vancomycin if used in appropriate concentration (1 gram dry vancomycin powder for 300 grams of bone graft.) will not affect osteointegration of allogenic bone graft. Peak levels attained locally by such a use were higher than Minimum Inhibitory Concentration (MIC) value of vancomycin for most susceptible organisms, but were 2000 times lower than the levels (10,000 μg/ml) associated with death of osteoblasts. Japaul S Gogia et al[9] studied the effect of local antibiotic therapy in osteomyelitis. Kakiuchi M et al[10] also used the human bone matrix as alloimplant in local control of orthopedic infection and found effective.

In our clinical study use of the allogenic morcelized cancellous bone as a carrier of antibiotic for prolonged elution in targeted area and facilitation of osteointegration was evaluated. Further risk of allograft related graft infection as described by Ketonis C et al[11] can be obviated by combing the antibiotic with allograft. Use of cancellous bone graft has advantage that it provides good substrate to absorb the antibiotic and adjuvant, it also get reabsorbed easily if not in cooperated without risk of further perpetuation of infection. Use of the adjuvant (Autologous bone graft and bone marrow) augment the healing potential of allograft as was observed in eight cases where adjuvants were used and achieved favourable outcome in terms of osteointegration. Extraossoues placed allogenic bone graft besides the fracture was inevitably got resorped in all the cases in a time period of 14–18 weeks.

Synthetic bone graft substitute such as calci-

um phosphate, calcium sulphate etc has been also used in various studies by Siegel HJ[14], El-Adl G, Mostafa MF[4]and Shibuya K etal[12]and showed better results when used in conjunction with bone marrow which provide the osteo-inductive property to scaffold. Recent trends show increasing use of the synthetic bone graft substitute[8]due to wide commercial availability but, do not demonstrated superiority of one over other. However synthetic substitute take long time to reabsorbed or incorporated in host bed hence may act as foreign body and after elution of antibiotic and may lead to persistence of infection. Canellous allobone graft if not integrated in host bed usually reabsorbed and prevent perpetuation of infection.

In the present series no adverse effect of antibiotic used in was observed. The two failure cases in the study group comprised of one established non union of mid diaphysial tibial fracture and Sub Trochanter fracture belonged to non union group which needed larger osteogenic potential which allogenic bone graft alone may have lacked and required subsequent surgery with change of fixation device. It appears that in such situations use of allogenic bone alone does not suffice and some osteogenic potentiator is also required to boost the already sluggish healing process. Intraoperative findings were suggestive of increased vascularity at the fracture site with complete resorption of allograft and presence of bone defect. These findings are suggestive of allogenic bone graft being able to induce an inflammatory reaction at the fracture site which may aid in healing process but is not sufficient to bring about fracture union as graft resorption occurred.

Conclusion

In present case series use of antibiotic augmented allogenic bone graft as carrier in controlled osteoarticular infections appears to be novel and effective step in treating these dreaded infections. However graft integration occurred partially and may be attributed to infection related lyses as well as limited osteogenic potential of allogenic bone graft, hence its augmentation with adjuvant such as autologous bone graft and or bone marrow is necessary to improve outcome. However further studies and larger case series are required for further validation.

Acknowledgements

We sincerely acknowledge valuable suggestion and help by my collegue inder pawar, Rajat Gupta, Lalchandani R, Ramavtar, Kuldeep, Dogra S, Jindal M and Agarwal S. Administrative and research help from my institution ESI Hospital, Anestesiologist and radiologist for their valuable contribution.

References

1) Aubigne RM, Meary R,Thomine JM in Rev: Resection in the treatment of bone tumours. Chir Orthop **52**：305, 1966.
2) Buttaro MA, González Della Valle AM, Piñeiro L et al: Incorporation of vancomycin-supplemented bone incorporation of vancomycin-supplemented bone allografts: radiographical, histopathological and immunohistochemical study in pigs.Acta Orthop Scand **74**(5)：505-513, 2003.
3) Easmon CS: The effect of antibiotics on the intracellular survival of Staphylococcus aureus in vitro. Br J Exp Pathol **60**(1)：24-28., 52：305, 1979.
4) El-Adl G, Mostafa MF, Enan A et al: Biphasic ceramic bone substitute mixed with autogenous bone marrow in the treatment of cavitary benign bone lesions. Acta Orthop Belg **75**：110-118, 2009.
5) Finkemeier CG: Bone grafting and bone graft substitutes, J Bone Joint Surg Am. **84-A**(3)：

454-464, 2002.
6) Friedlander G.E: Bone banking in support of reconstructive surgery of the hip, current concepts review – bone grafts: J Bone and Joint Surg **69**(5) : 786-790, 1987.
7) Gudmundson C: Oxytetracycline induced fragility of growing bones. An experimental study in rats, Clin Orthop **77** : 284-289, 1971.
8) Hagen A, Gorenoi V, Schönermark MP: Bone graft substitutes for the treatment of traumatic fractures of the extremities, GMS Health Technol Assess 8: Doc04. doi: 10.3205/hta000102. Epub 2012 Jun 14.
9) Jaspaul S G, John P M, Paul E et al: Local antibiotic therapy in osteomyelitis Semin Plast Surg **23**(2): 100-107, 2009.
10) Kakiuchi M, Hosoya T, Takaoka K et al: Human bone matrix gelatin as a clinical alloimplant, Int Orthop **13** : 13-24: 9 : 181-188, 1985.
11) Ketonis C, Barr S, Adams CS et al: Bacterial colonization of bone allograft: establishment and effect of antibiotics. Clin Orthp Rel Res 468(8): 2113-2121, 2010.
12) Shibuya K, Kurosawa H, Takeuchi H et al: The medium-term results of treatment with hydroxyapatite implants. J Biomed Mater Res B Appl Biomater **75** : 405-413, 2005.
13) Shyam K S, Awadhesh Y S N, MR Sen Decal: bone matrix as a local antibiotic delivery vehicle in a MRSA-infected bone model: an experimental study Indian J Orthop **44**(3): 246-251, 2010.
14) Siegel HJ, Baird RC 3rd, Hall J et al: The outcome of composite bone graft substitute used to treat cavitary bone defects. Orthopedics **31** : 754-4, 2008.
15) Slooff TJ, Huiskes R, van Horn J, Lemmens AJ: Bone grafting in total hip replacement for acetabular protrusion. Acta Orthop Scand **55** (6) : 593-596, 1984.
16) Winkler H: Rationale for one stage exchange of infected hip replacement using uncemented implants and antibiotic impregnated bone graft, Int J Med Sci **6**(5) : 247-252, 2009.
17) Wits E, Persen L, Lnrseth K et al: Adsorption and release of Antibiotics from morselized cancellous bone In vitro studies of 8 antibiotics, Acfa Orthop Scand **70**(3): 298-304, 1999.
18) Witsø E, Persen L, Benum P et al: Local concentrations without systemic adverse effects after impaction of netilmicin-impregnated bone. Acta Orthop Scand **75**(3) : 339-346, 2004.