INTRODUCTION:
Bone allograft is used extensively in more than 2.2 million orthopedic cases per year. An important problem related to this use of allograft is infection. Allograft impregnation with antibiotics has been used to prevent infection, but such elution systems usually release high, sometimes toxic, initial doses and the incorporated antibiotic is quickly depleted. We have described a covalently tethered antibiotic allograft (VAN-allograft) that effectively prevents bacterial colonization and biofilm formation providing protection of the graft for far longer time than can be achieved with impregnation techniques, without altering the attachment or expression profile of relevant bone-derived cell lines.

METHODS:
Allograft modification: Morselized human bone was washed, and coupled twice with excess Fmoc-aminooxyethoxyethylocte (Fmoc-AEEA); deprotected with 20% piperidine in DMF; and coupled with a 4X molar excess of vancomycin (VAN). Bacterial Challenge: Sterilized control and VAN-allograft cortical bone 1x1cm squares, were incubated with S. aureus (Ci=10^4 cfu) in trypticase soy broth (TSB), 37°C, for 12 hrs. Antibiotic treatment: Sterilized control and VAN-allograft were incubated with S. aureus (Ci=10^4 cfu) in TSB for 12 h with or without 10µg/ml VAN, washed 3x with PBS, moved to a new well, washed 3 more times and incubated in TSB with or without 10µg/ml VAN for another 4 h. Bacterial colonization was assessed by direct counts. SEM: After washing off non-adherent bacteria, samples were fixed with 4% paraformaldehyde, dehydrated in ethanol and gold sputter-coated before visualization. Actin cytoskeletal visualization: Human Fetal Osteoblasts (hFOBs) were fixed in 4% paraformaldehyde, 10 min, RT, permeabilized with 0.1% Triton X-100 in PBS, 5 min, RT, and after incubation with blocking buffer, 30min, stained with Alexa488-phalloidin (1:1000, Invitrogen) and propidium iodide (1:100, Invitrogen), 30 min, RT. Stained cells were visualized using confocal laser scanning microscopy. Expression profile: Preosteocyte-like cells (MLO-A5) were seeded on sterilized control and VAN-modified cortical bone surfaces (2x2 cm). At 6 days, cells were lysed, RNA was harvested, purified and quantitated. Using the GE Healthcare Ready-To-Go PCR kit Laminin, Runx2, Osteopontin, Osteocalcin, Alkaline Phosphatase and Osterix were amplified.

RESULTS:
VAN-allograft and solution antibiotics: We evaluated VAN-allograft in a system modeling systemic antibiotic therapy or antibiotic prophylaxis. After exposure to S. aureus for 12 h (data not shown), VAN-allograft showed significantly decreased bacterial colonization compared to controls. When 10 µg/ml exogenous VAN was added for the last 4 h of incubation (modeling systemic antibiotic therapy), colonization of control surfaces was reduced, while colonization of VAN-allograft was almost eliminated (Fig. 1). When 10 µg/ml VAN was added concomitantly with S. aureus, colonization of control surfaces still occurred, while VAN-allograft colonization was undetectable.

DISCUSSION:
We have previously described an antibiotic-tethered allograft that resists bacterial colonization. We now assess this technology with an in vitro model of bone implantation in the presence of solution antibiotics. In these models, solution antibiotics failed to prevent infection of control bone while completely clearing the bacteria on VAN-bone. SEM reveals abundant impervious biofilm formation on controls (which was absent from VAN-bone) explaining the failure of the solution antibiotics in clearing such infections. These allografts not only resist colonization and increase the effectiveness of prophylactic antibiotics but also do not show any signs of toxicity or osteoblastic genotype alterations circumventing concerns of side effects associated with current elution systems.

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