STIMULAN®

Usos en infección articular periprotésica

La única matriz de calcio aprobada para el transporte de vancomicina, gentamicina y tobramicina en huesos y tejidos blandos en presencia y en ausencia de infección

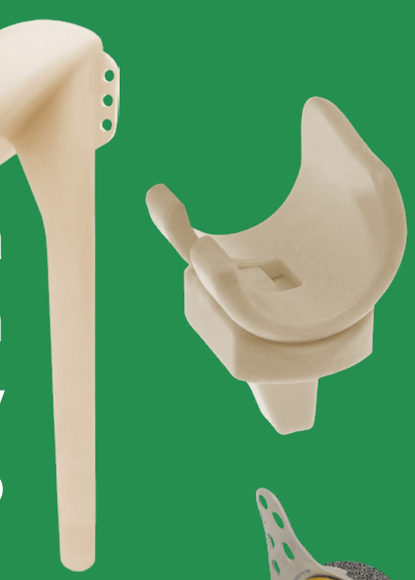
FLEXIBILIDAD
CASO POR CASO

REVISIONES
EN 1 TIEMPO

Absorción completa sin residuos

click

REVISIONES
EN 2 TIEMPOS



Mayor cobertura de liberación de antibiótico y durante más tiempo

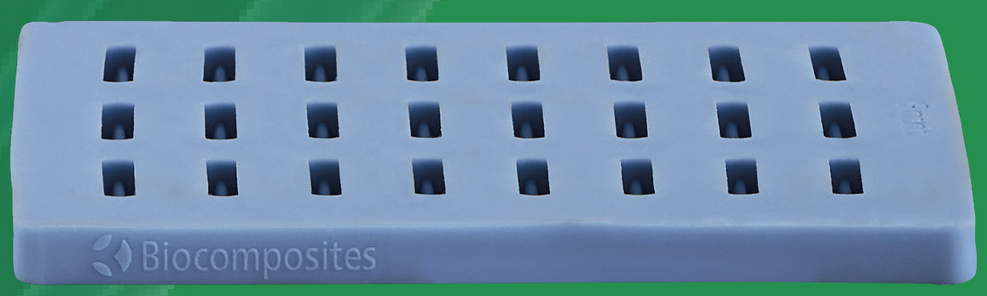
click

Reduce el riesgo de re-infección sin dañar las prótesis

click

Introductor

click



Fabricado por: Biocomposites®

MBA® SURGICAL EMPOWERMENT

OFICINAS CENTRALES
Avda. Jardín Botánico 1345, Silos del Intra. 33203 Gijón. Asturias T: +34 985 195 505 F: +34 985 373 452

www.mba.eu

B.155es V3/202005

STIMULAN®

PODER PARA TRANSFORMAR LOS RESULTADOS

Biocomposites®

Elegir un dispositivo innovador para trabajar junto con su estrategia de manejo de infecciones es clave para:

- ✔ minimizar las complicaciones evitables
- ✔ mejorar los resultados
- ✔ reducir los costes

"Los beneficios económicos son significativos... el coste de una infección recurrente es de cientos de miles en relación con un producto que es tan solo de unos pocos cientos"

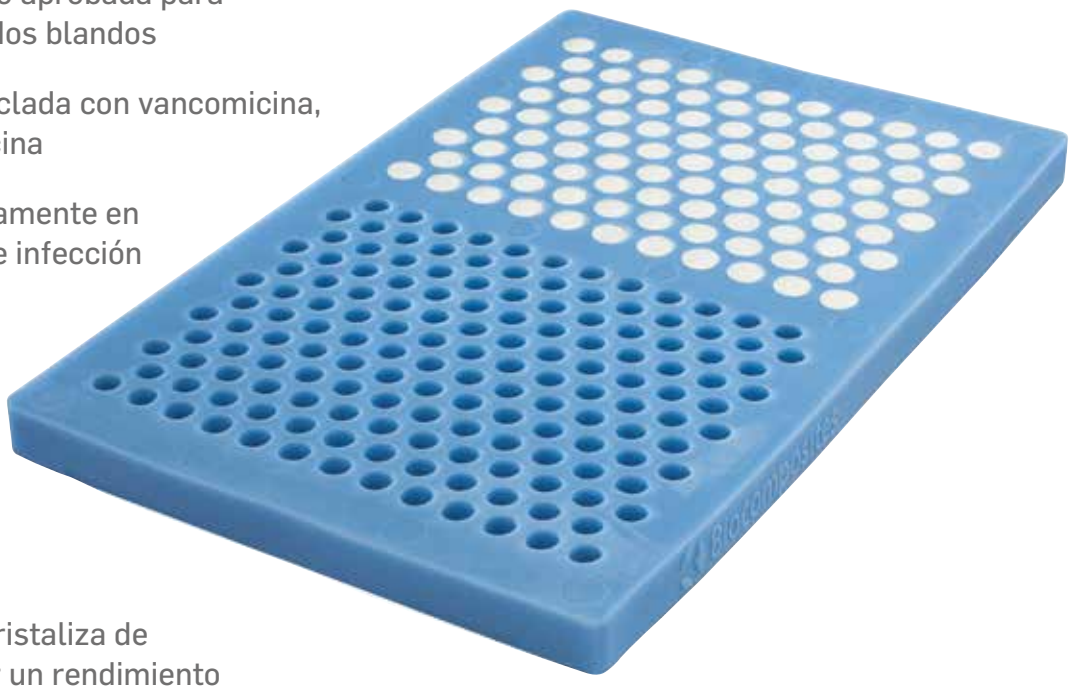
Dr. John Xenos

STIMULAN®

El complemento perfecto para su estrategia de manejo de infecciones

STIMULAN es un portador de antibióticos de sulfato de calcio completamente absorbible – especialmente diseñado para apoyar la gestión proactiva del espacio muerto y la infección en el sitio quirúrgico con una flexibilidad sin igual y la aplicación quirúrgica más amplia.[1]

- ✔ la única matriz de calcio aprobada para su uso en huesos y tejidos blandos
- ✔ aprobada para ser mezclada con vancomicina, gentamicina y tobramicina
- ✔ se puede aplicar directamente en presencia o ausencia de infección



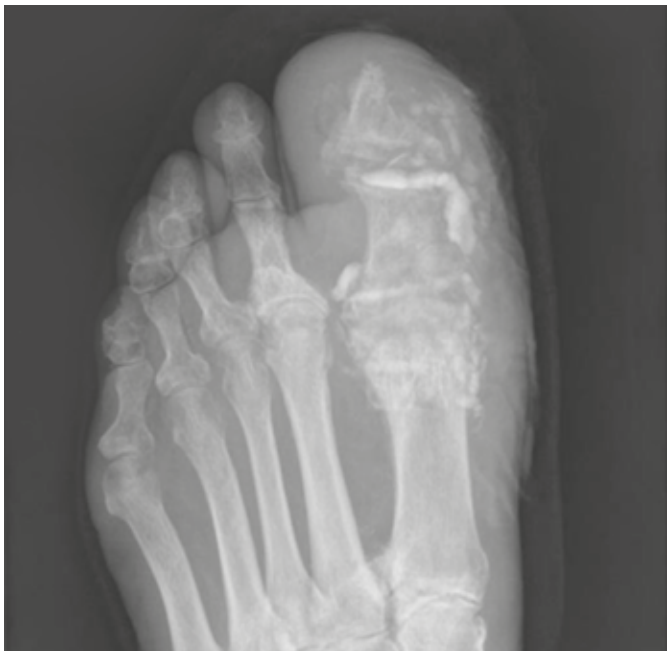
STIMULAN se recristaliza de forma única para obtener un rendimiento consistente y fiable en el transporte de antibióticos para el tratamiento de las infecciones musculoesqueléticas

Propiedades ideales de un portador de antibióticos

- Elección de antibiótico
- Concentración terapéutica
- Duración eficaz
- Implantación en el lugar de la infección

Mantenga el reto de la infección bajo su control

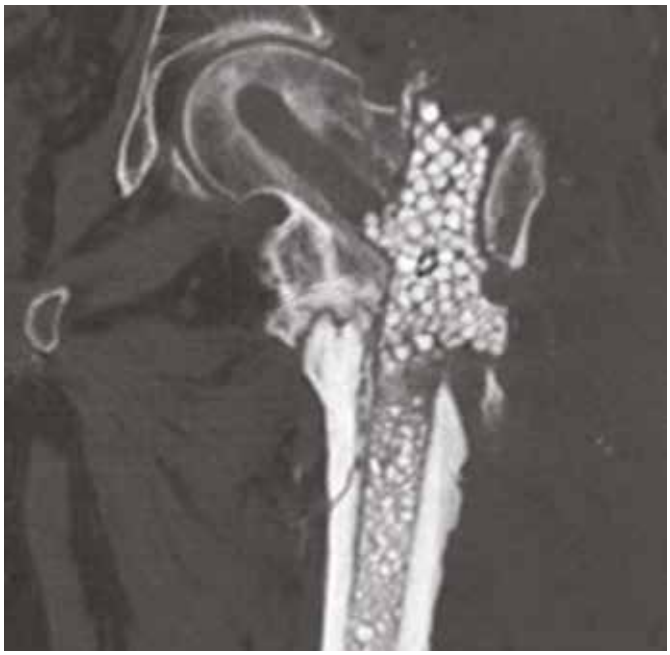
La administración local de antibiótico en espacios óseos y tejidos blandos con STIMULAN permite tratar de manera eficaz y racional un amplio espectro de riesgos de infección en distintos entornos – mediante concentraciones inalcanzables por vía sistémica.



Transformación de osteomielitis en pie diabético[2*]

Caso: osteomielitis persistente y destrucción de la primera articulación interfalángica en el pie izquierdo. El paciente ya había sido tratado por artropatía de Charcot en el pie derecho.

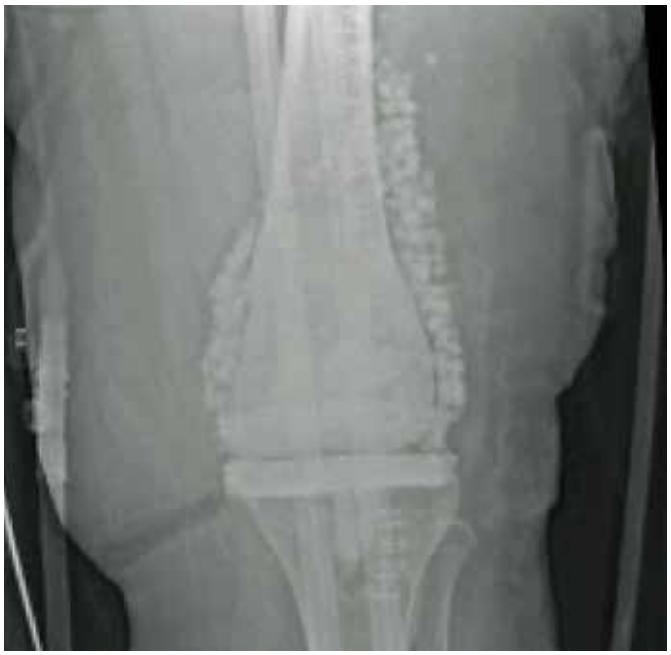
Resultado: a las dos semanas de la cirugía, el tamaño del dedo había disminuido. En la radiografía realizada a los 16 meses, el paciente no mostraba signos de infección y se había evitado la amputación. La absorción completa de STIMULAN se produjo a los 4 meses.



Transformación de traumas infectados por *Staphylococcus aureus*[3*]

Caso: infección del clavo femoral y pseudoartrosis del fémur izquierdo con heridas con secreción persistente a nivel proximal y distal.

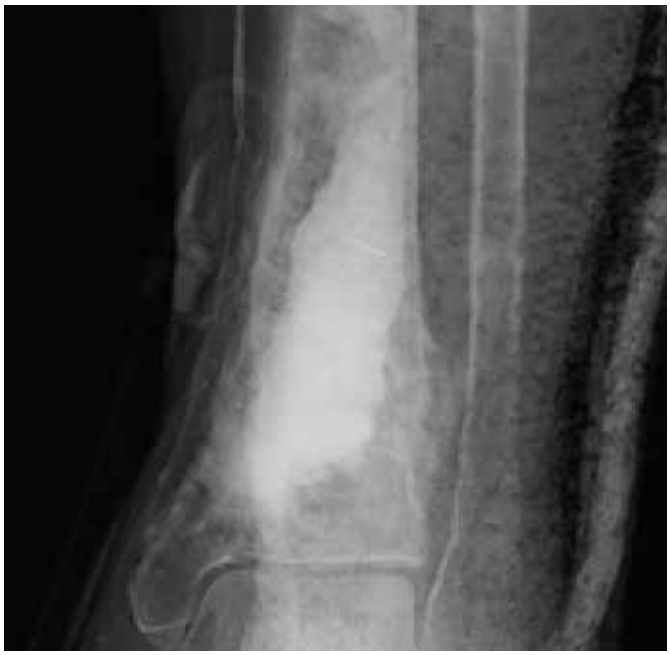
Resultado: a los 7 meses se observa resolución completa de la pseudoartrosis y al año el paciente sigue sin infección y camina sin dolor.



Transformación de artroplastias de revisión infectadas por *Streptococcus* del grupo B[4*]

Caso: artroplastia total de rodilla, infectada 2 años después de la primera intervención.

Resultado: en la visita de seguimiento realizada un año después, la paciente permanece libre de infección y continúa bajo seguimiento periódico.



Transformación de fractura del pilón tibial infectada por *Streptococcus* del grupo B y SARM[5*]

Caso: problemas de drenaje un año después de la reparación de una fractura del pilón tibial, 2 semanas después se procedía a la retirada del implante.

Resultado: a los 6 meses, la paciente podía soportar todo su peso sin restricciones de actividad – con absorción completa de STIMULAN.

"Ahorra dinero al hospital, ya que disminuye la tasa de readmisión hospitalaria"

Dr. Jorge Casas-Gánem

*Información adicional y estudios de casos disponibles previa solicitud.

Completamente absorbible, portador de antibióticos recristalizado para un mejor rendimiento clínico

STIMULAN es un sulfato de calcio de grado farmacéutico con una estructura cristalina y unas propiedades estrechamente controladas.[1]

- ✔ grado de pureza controlada
- ✔ pH fisiológico
- ✔ sin hidroxiapatita
- ✔ se mezcla fácilmente con antibióticos líquidos y en polvo

Sólo STIMULAN se somete a DRy26™, un método patentado de recristalización que comienza con reactivos de grado farmacéutico y resulta en un rendimiento consistente y fiable, adecuado para administrar antibióticos localmente en espacios infectados.[1,6-11]

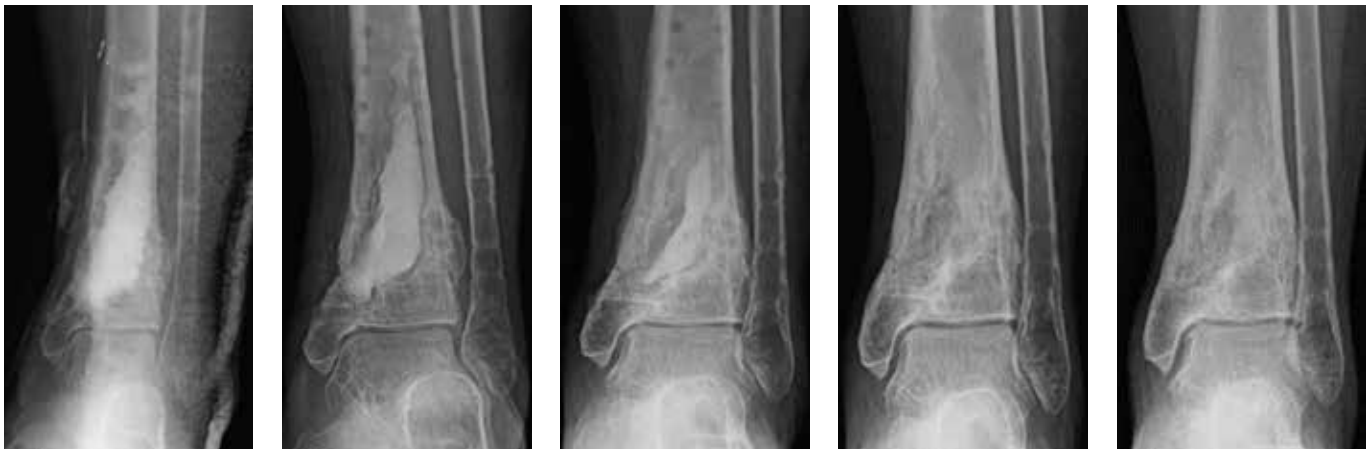
- ✔ absorción completa a un índice óptimo
- ✔ no daña las superficies articulares
- ✔ perfil de elución predecible
- ✔ acción demostrada contra el biofilm
- ✔ flexibilidad para ajustar el antibiótico a la necesidad clínica

"Es muy reproducible... Obtengo los mismos resultados una y otra vez"

Dr. Herrick Siegel

Absorción completa a un índice óptimo[1]

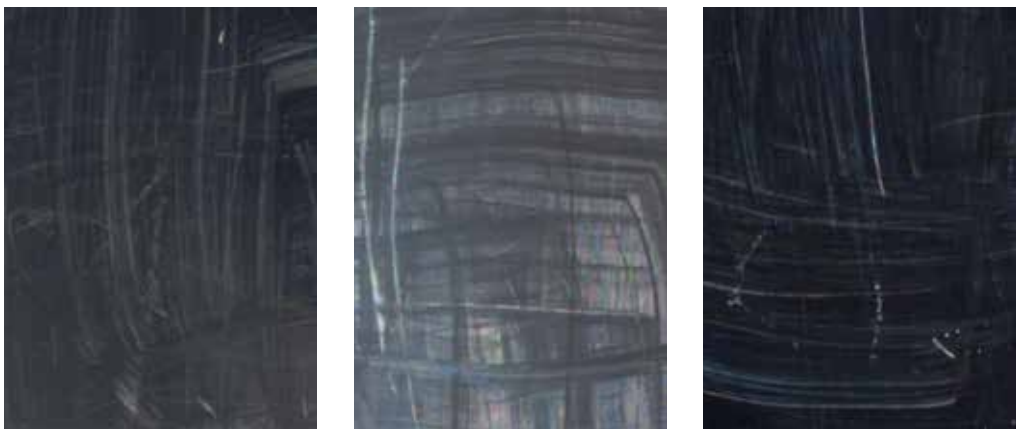
Sin hidroxiapatita, impurezas insolubles o residuos de PMMA – no deja nidos para la infección.[12-17]



Postoperatorio | 1 mes | 11 semanas | 6 meses | 15 meses

No daña las superficies articulares[7,8]

Menos rallado que otros productos con sulfato de calcio.



STIMULAN | Otro producto con Sulfato de calcio | Control

Imágenes microscópicas (x6,5) de simulación experimental del deterioro de una placa de cromo-cobalto (360.000 ciclos) con partículas de un tercer cuerpo atrapadas entre la placa y una superficie articulante de UHMWPE.

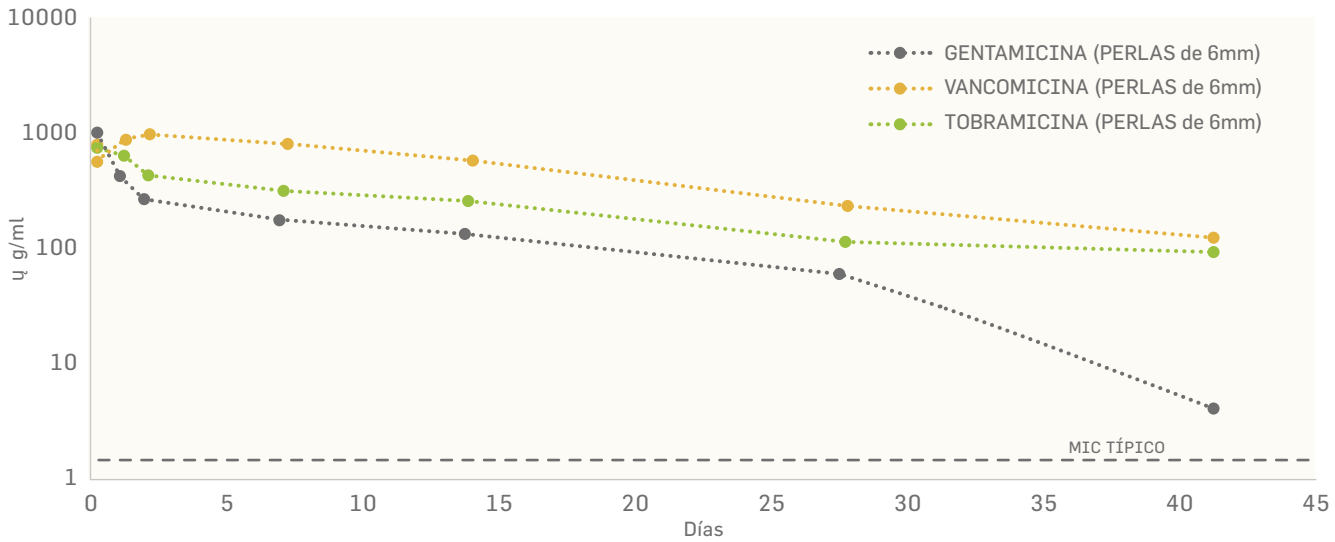
STIMULAN no daña los componentes de la artroplastia total de rodilla cuando se encuentra localizado entre las superficies articulares del implante.

Diseñado de forma única para la precisión y control demandado en cada ocasión

Con la capacidad de mezclar sustancias según las necesidades antimicrobianas específicas de cada infección, STIMULAN combina flexibilidad con la previsibilidad y consistencia necesarias para garantizar una cobertura antibiótica sostenida.

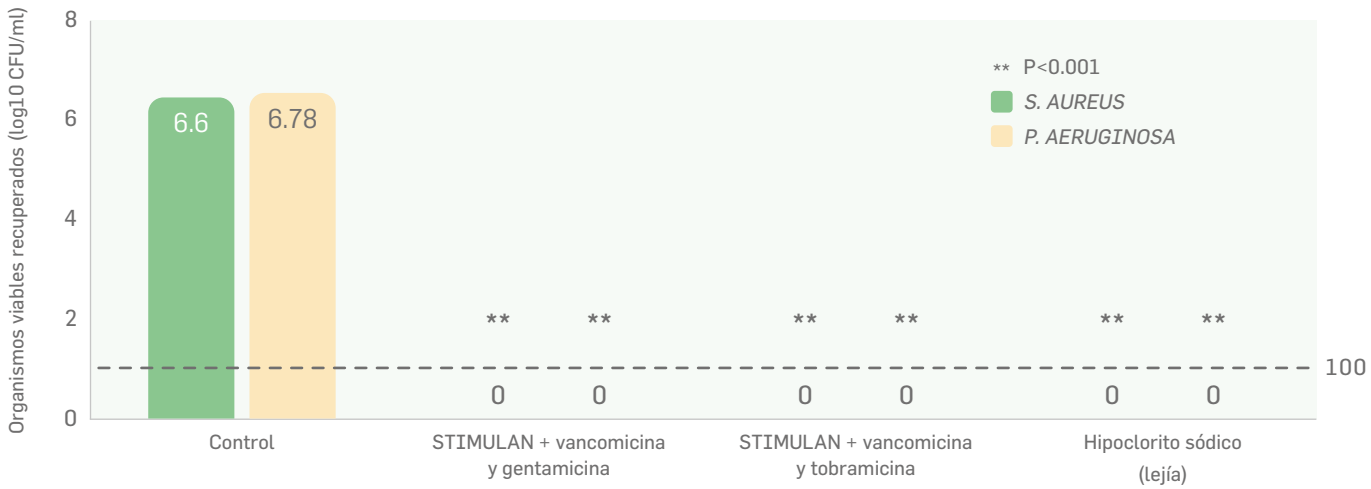
Perfil de elución supra terapéutico predecible[9]

Niveles de antibióticos mantenidos por encima de la concentración mínima inhibitoria (MIC por sus siglas en inglés) durante más de 40 días con STIMULAN Rapid Cure.



Acción demostrada contra el biofilm[10]

No se recuperaron organismos viables en el biofilm preestablecido.



Estudio *in vitro* para determinar la eficacia de las perlas STIMULAN cargadas con antibióticos contra el biofilm de *Pseudomonas aeruginosa* y *Staphylococcus aureus*.

Flexibilidad para adaptar los antibióticos a las necesidades clínicas[11]

Eficaz contra un amplio espectro de patógenos.

Staphylococcus aureus	*Staphylococcus epidermidis*
 Vancomicina	 Gentamicina
 Tobramicina	 Tobramicina

Test de Zona de inhibición (ZOI) utilizando el método de disco de difusión Kirby-Bauer. Perlas de 6mm después de 24 horas.

Flexibilidad al alcance de la mano

La formulación de STIMULAN está optimizada para trabajar de acuerdo con las demandas clínicas y quirúrgicas de cada paciente individualmente. Independientemente de las limitaciones de tiempo, forma, accesibilidad o tamaño, STIMULAN le ofrece un formato que se adapta a cada caso.

STIMULAN Rapid Cure[1]

Tiempos de fraguado más rápidos

+ antibiótico

Vancomicina
(1000mg polvo)

Gentamicina
(240mg en 6ml solución)

Tobramicina
(240mg en 6ml solucón)

STIMULAN Kit[1]

Más tiempo para moldear o inyectar

+ antibiótico

Vancomicina
(1000mg polvo)

Gentamicina
(240mg en 6ml solución)

Tobramicina
(240mg en 6ml solución)

STIMULAN está disponible en varios tamaños de envase que le permiten mezclar y combinar para cualquier tamaño de defecto.

Flexibilidad de formatos

Molde de perlas disponible con STIMULAN Rapid Cure y STIMULAN Kit

Jeringa disponible con STIMULAN Kit

Rellena rápida y fácilmente los canales medulares

STIMULAN Bullet Mat and Introducer

Diseño optimizado y flexible que simplifica la aplicación de STIMULAN, en el canal medular – de manera más eficaz y rentable que el empleo de pasta.

(Consulte la contraportada para más detalles y códigos de referencia del producto)

STIMULAN®

Evidencia y conocimientos técnicos inigualables que proporcionan confianza

Con nuestro conocimiento, dedicación y experiencia líderes en la industria, puede estar seguro de que se cumplirá el alto nivel de consistencia que exigen sus casos.

Documentos revisados por expertos, presentaciones y posters

Con más de 20 años de experiencia y utilizado en 50.000 casos nuevos cada año, STIMULAN continúa ampliando las posibilidades para los cirujanos y pacientes a través de un compromiso con la innovación y niveles de satisfacción consistentemente altos.

"...estoy más satisfecho con este producto que con cualquier otro que he utilizado en los últimos 30 años"

Dr. Richard Biama

Caso práctico

Por cortesía del Dr. Rajesh Jogia

Especialista en Cirugía Podológica, Leicester, Reino Unido

Datos clínicos

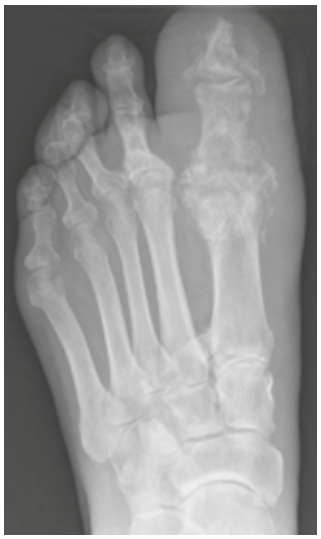
Varón de 67 años con diabetes tipo 2 y un IMC de 37, presentaba osteomielitis y destrucción de la primera articulación interfalángica en el pie izquierdo. El tratamiento convencional con antibióticos por vía oral y descarga no habían funcionado. El proceso quirúrgico consistía en amputar el dedo del pie izquierdo. Sin embargo, existía riesgo de re-ulceración ya que el paciente estaba recibiendo tratamiento para una artropatía de Charcot en el pie derecho.

Tratamiento

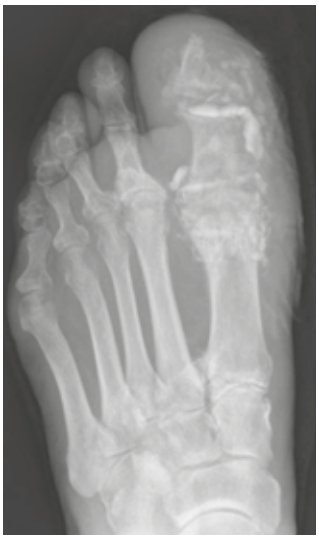
Como último recurso para evitar la amputación, se realizó un desbridamiento quirúrgico, se perforó el hueso y se utilizó STIMULAN para rellenar las cavidades. Se utilizó gentamicina y vancomicina para tratar la infección. La intervención se efectuó como un caso ambulatorio con anestesia local.

Resultado

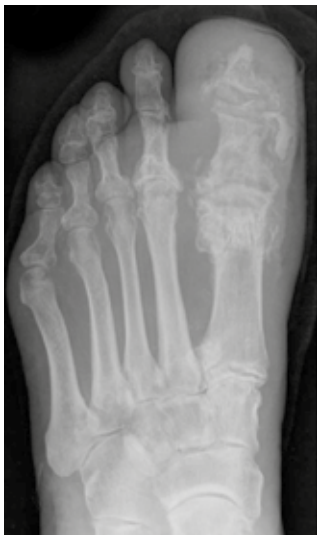
A las dos semanas tras la cirugía se había reducido el tamaño del dedo. En la radiografia realizada a los 16 meses, STIMULAN se había absorbido completamente y el paciente no mostraba signos de infección, evitando la amputacion del miembro.



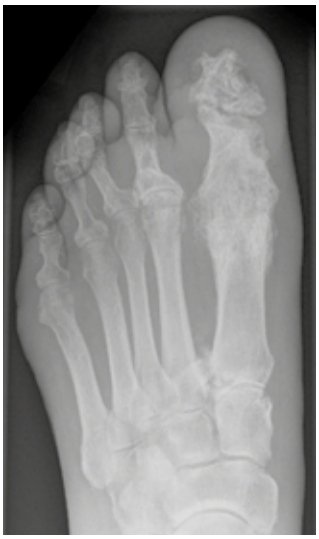
Presentación



Postoperatorio



2 semanas



16 meses

Caso práctico

Por cortesía del Dr. Hemant K Sharma

Especialista en Cirugía Ortopédica, Tutor Clínico Senior, Hull, Reino Unido

Datos clínicos

Varón de 35 años implicado en un accidente de tráfico, sufrió múltiples lesiones y una fractura sub-trocantérea de fémur izquierdo. Esta se trató con un clavo intramedular y posteriormente el paciente desarrolló una infección con secreción tanto del área proximal como distal del tornillo de fijación. Se sometió a múltiples intervenciones y desarrolló una herida de aproximadamente 15cm en la zona proximal lateral del muslo, que se trató con terapia VAC. Un año después, el paciente presentaba una herida con secreción proximal y distal.

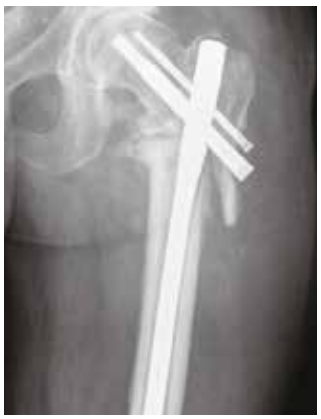
Tratamiento

Se extrajo el clavo femoral, se ensanchó el conducto femoral mediante perforación y se realizó un lavado estándar. Se utilizaron 40cc de perlas de STIMULAN para administrar el antibiótico en el canal intramedular. Los cultivos revelaron que había infección por *Staphylococcus aureus* que fue tratada con vancomicina y tobramicina.

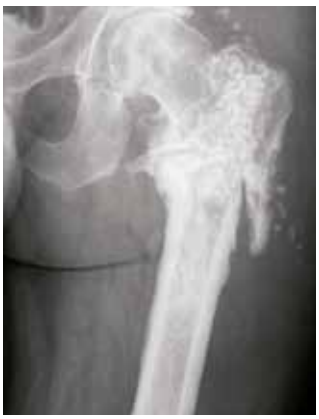
Resultado

A los 2,5 meses del postoperatorio, las radiografias mostraron una absorción casi total de las perlas STIMULAN y, a los 7 meses, la consolidación de la pseudoartrosis era total.

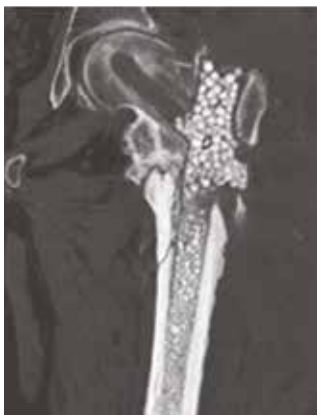
En una visita de seguimiento realizada un año después, el paciente sigue libre de infección y camina sin dolor.



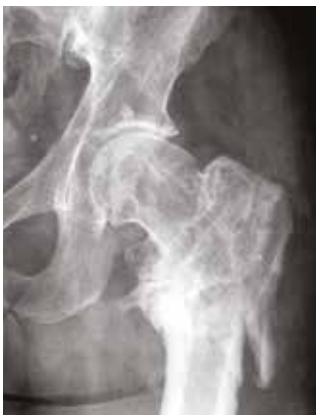
Radiografía preoperatoria mostrando pseudoartrosis



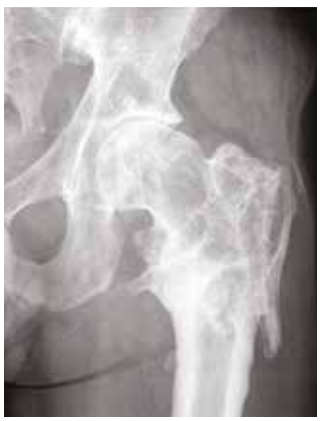
Radiografía postoperatoria – 2 meses



Tomografía Computarizada (TC) postoperatoria – 2 meses



Postoperatorio – 2.5 meses



Postoperatorio – 1 año

Caso práctico

Por cortesía del Dr. Ramasubramanian Dharmarajan

Especialista en Cirugía Ortopédica, Cumbria, Reino Unido

Datos clínicos

Mujer de 59 años, presentaba una artroplastia total de rodilla bien fijada, infectada dos años después de la primera operación. Se trata de un caso agudo, con características clínicas de infección, con muestras que daban positivo en *Streptococcus* del grupo B.

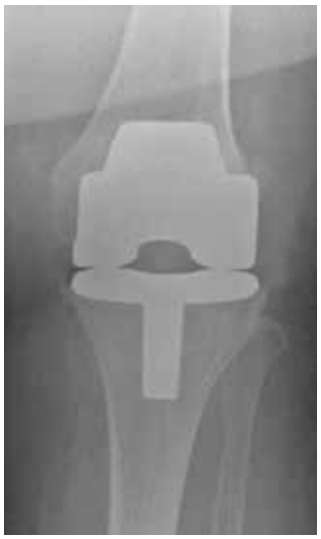
Tratamiento

Primera fase – desbridamiento radical, extracción del implante e inserción de un espaciador de cemento cargado con antibióticos y aplicación de perlas de STIMULAN mezclado con vancomicina para tratar la infección.

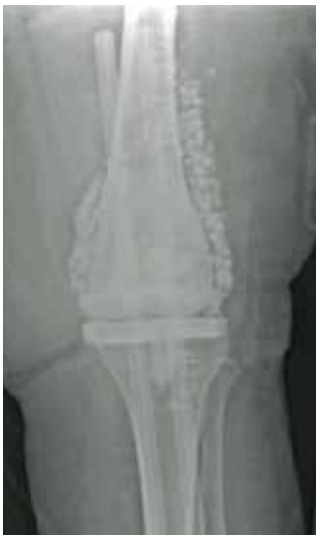
Segunda fase – a las 10 semanas, los tejidos blandos estaban sanos y los especímenes intraoperatorios estaban libres de organismos. Se le reimplantó una prótesis con bisagra rotatoria.

Resultado

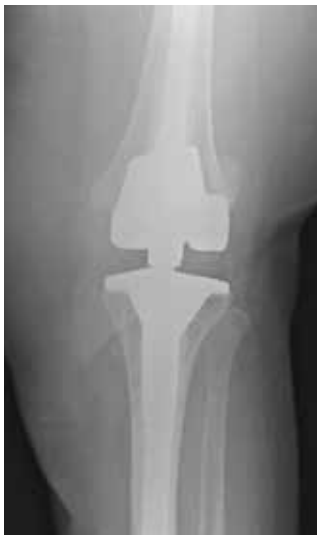
En la visita de seguimiento realizada un año después, la paciente permanece libre de infección y continua bajo seguimiento periodico.



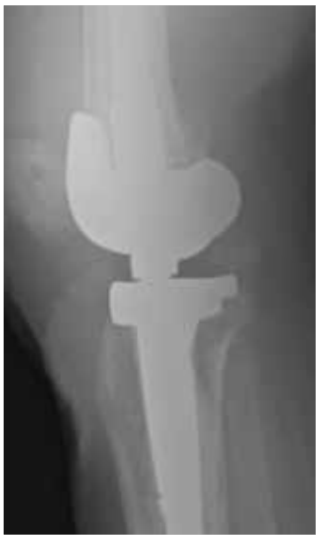
Presentación



Primera fase – Postoperatorio



Segunda fase – Postoperatorio



Segunda fase – Postoperatorio

Caso práctico

Por cortesía del Dr. Daniel Schlatterer

Cirujano Ortopédico, Atlanta, Georgia, USA

Datos clínicos

Mujer de 73 años con osteomielitis causada por *Streptococcus* del grupo B e infección por SARM (MRSA por sus siglas en inglés).

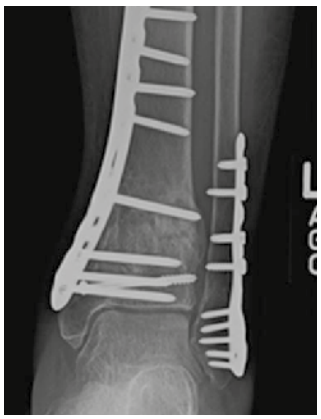
Un año después de la reparación de una fractura del pilón tibial, la paciente presentaba el implante al descubierto y problemas de drenaje, 2 semanas después se procedía a la retirada del implante.

Tratamiento

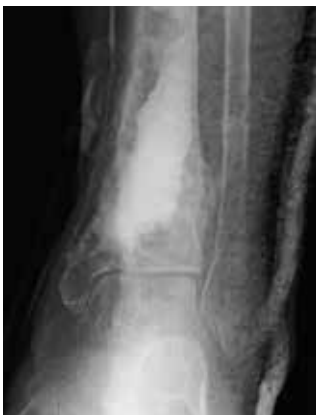
La retirada del implante y desbridamiento radical en el lado interno del tobillo dejaron un amplio espacio muerto que fue tratado con pasta STIMULAN mezclada con antibiótico. Para tratar la infección se usó una mezcla de vancomicina y tobramicina.

Resultado

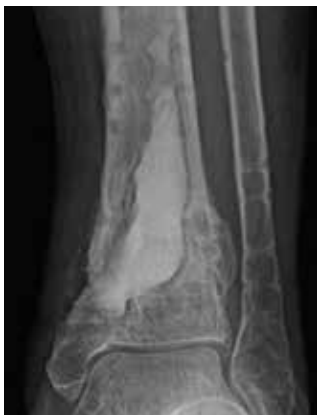
6 meses después del tratamiento, la paciente ya no presentaba infección y podía soportar todo su peso sin tener restricciones de actividad – con absorción completa de la pasta STIMULAN.



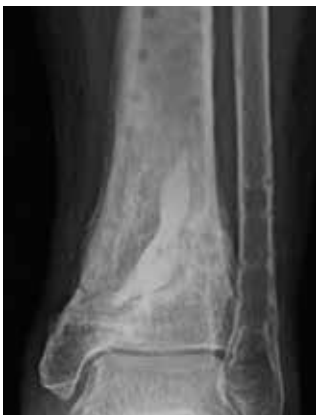
Presentación



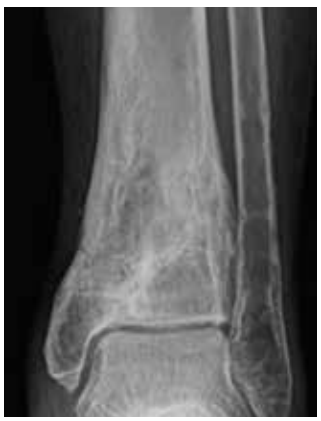
Postoperatorio



1 mes



11 semanas



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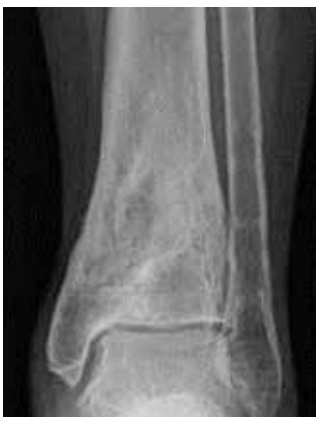


15 meses

Caso práctico

Por cortesía del Dr. Daniel Schlatterer

Cirujano Ortopédico, Atlanta, Georgia, USA

Datos clínicos

Varón de 40 años con una fractura de calcáneo abierta prolongada tras sufrir una caída desde 6 metros de altura. La operación inicial consistió en irrigación y desbridamiento con una fijación definitiva 10 días después de haber sufrido la lesión. 6 meses más tarde, el paciente presenta una pseudoartrosis infectada y el fracaso en la fijación del implante. Los cultivos dieron positivo para SARM.

Tratamiento – Etapa 1

Retirada del implante, desbridamiento del hueso y de los tejidos blandos afectados por la infección. Se utilizó STIMULAN para rellenar el espacio muerto resultante, y se administraron antibióticos por vía intravenosa durante 8 semanas.

Resultado – Etapa 1

Se erradicó la infección y se logró curar los tejidos blandos, con analíticas de estudio de infección normales (un mes después de completarse el tratamiento con antibióticos por vía intravenosa). El pie estaba listo para corregir la migración proximal del calcáneo (liberación del tejido blando) y artrodesis subastragalina.

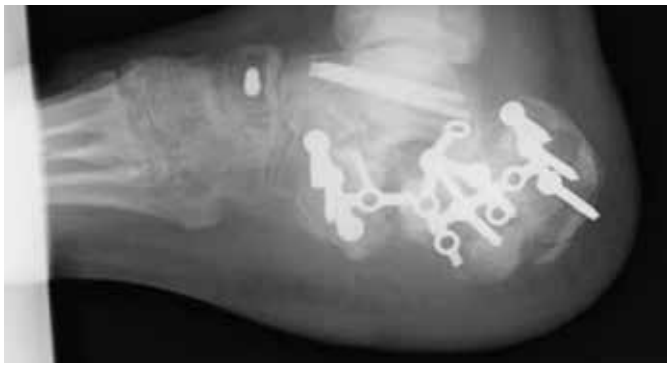
Tratamiento - Etapa 2

Se realizó una artrodesis subastragalina. Se utilizó STIMULAN de nuevo para rellenar el espacio muerto resultante, alrededor del astrágalo y del calcáneo (imagen de STIMULAN en la etapa de artrodesis no incluida). Se realizó el tratamiento de profilaxis antibiótica por vía intravenosa. 2 meses después, se retiró el implante debido a resultados positivos en los hemocultivos. Los cultivos del calcáneo fueron negativos. Sin embargo, la punta de la sonda del catéter central insertado periféricamente, CCIP, dio positivo. Se colocó una nueva sonda del CCIP después de 8 semanas de antibióticos por vía intravenosa.

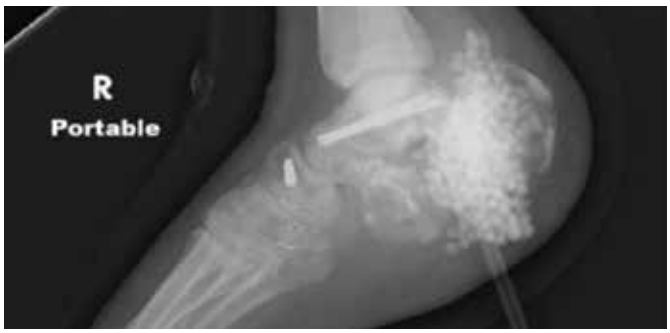
Resultado – Etapa 2

El paciente ya soporta la carga y no presenta signos de infección.

La infección en las fracturas de calcáneo abiertas es común y en algunas series la tasa de amputación supera el 50 %. A este paciente se le practicaron 2 intervenciones quirúrgicas para tratar la pseudoartrosis infectada. Retirada del implante y desbridamiento radical del hueso. El espacio muerto se trató con STIMULAN.

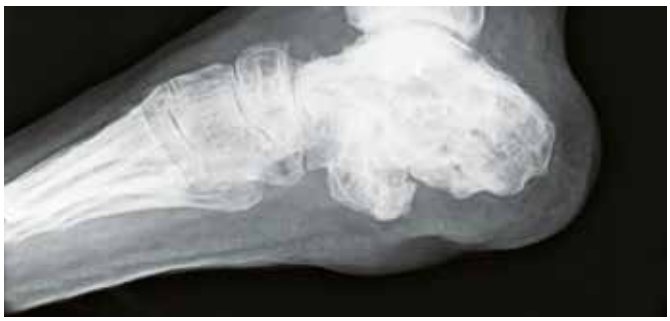


Presentación



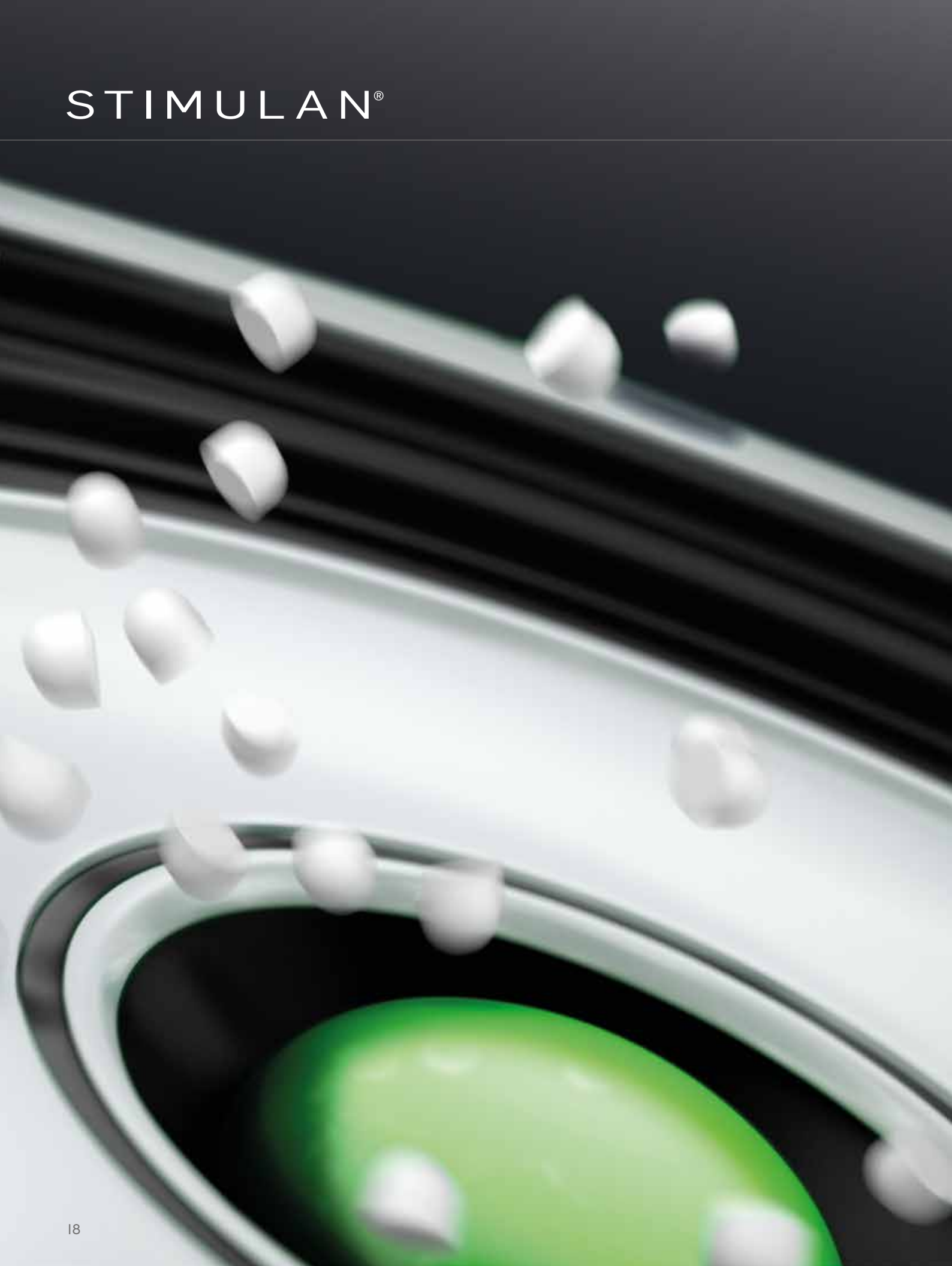
Postoperatorio – Etapa 1

Etapa 1 completa



13 meses después de la artrodesis subastragalina, 11 meses después de la retirada del implante

STIMULAN®



Resumen

STIMULAN Rapid Cure

Volumen en Pasta	Volumen en Perlas	Contenido del envase	Código de referencia
5cc	12cc	· Polvo y Solución · Espátula · Aplicador de pasta · Molde de perlas	620-005
10cc	25cc		620-010
20cc	50cc	· Polvo y Solución · Bol para realizar la mezcla · Espátula · Aplicador de pasta · 2 Moldes de perlas	620-020

STIMULAN Kit

Volumen en pasta	Volumen en Perlas	Contenido en el envase	Código de referencia
5cc	10cc	· Polvo y solucón · Espátula · Aplicador de pasta · Molde de perlas · Jeringa y cánula de aplicación	600-005
10cc	20cc		600-010

STIMULAN Bullet Mat and Introducer

Dimensiones de las balas	Diámetro de fresado	Contenido del envase	Código de referencia
7mm x 640mm	10mm diámetro de fresado (mínimo)	· Molde Bullet mat · aplicador de 7mm (negro) · aplicador de 9mm (plateado) · Obturador	660-001
9mm x 480mm	12mm diámetro de fresado (mínimo)		

Referencias: **1.** Biocomposites, STIMULAN Instructions for Use. **2.** Data on file, Mr. Rajesh Jogia. **3.** Data on file, Mr. Hemant K. Sharma. **4.** Data on file, Mr. Ramasubramanian Dharmarajan. **5.** Data on file, Dr. Daniel Schlatterer. **6.** Cooper, J.J., Method of producing surgical grade calcium sulphate; Patent. 1999. **7.** Analysis of the Wear Effect 3rd Body Particulate (Bone Cement) has on UHMWPE, Accutek Testing Laboratory, Fairfield OH, K13107732-1, 2014. **8.** Cowie, R.M., et al., The influence of a calcium sulphate bone void filler on the third-body damage and polyethylene wear of total knee arthroplasty. Bone Joint Res, 2019. 8(2): p. 65-72. **9.** Cooper, J.J., et al., Antibiotic stability in a synthetic calcium sulphate carrier for local delivery. Poster presented at European Bone and Joint infection Society Annual Meeting, Prague, Czech Republic, 2013. **10.** Delury, C., Aiken, S., Thomas, H., et al., Determining the Efficacy of Antibiotic-loaded Calcium Sulfate Beads against Pre-Formed Biofilms: An In Vitro Study. Poster presented at ASM Microbe 2019, 20-24 June 2019, Moscone Center, San Francisco, CA, USA. **11.** Laycock, P., et al., In Vitro Efficacy of Antibiotics Released from Calcium Sulfate Bone Void Filler Beads. Materials, 2018. 11(11): p. 2265. **12.** Somasundaram, K., Huber, C.P., Babu, V., et al., Proximal humeral fractures: the role of calcium sulphate augmentation and extended deltoid splitting approach in internal fixation using locking plates. Injury, 2013. 44(4): p. 481-7. **13.** Lei D., Zhanzhong, M., Huaikuo, Y., et al., Treatment of Distal Radius Bone Defects with Injectable Calcium Sulphate Cement. In: Bone Grafting, A., Zorzi, Editor. 2012, InTech. p. 125-134. **14.** Lei, D., Jing, L., Yang-yong, S., Calcium sulfate versus calcium phosphate in treating traumatic fractures. Journal of Clinical Rehabilitative Tissue Engineering Research, 2008. 12. **15.** Lei, D., Ma, Z., Jing, X., Treatment of bone defect with injectable calcium sulfate powder in distal fractures of radius. Chinese Journal of Bone Tumor and Bone Disease, 2007. **16.** Aiken, S.S., Cooper, J.J., Zhou, S., Osseointegration of a calcium sulphate bone substitute in a large animal model, in The 5th International Congress of Chinese Orthopaedic Association. 2010: Chengdu, China. **17.** Lazarou, S.A., Contodimos, G.B., Gkegkes, I.D., Correction of alveolar cleft with calcium-based bone substitutes. J Craniofac Surg, 2011. 22(3): p. 854-7.

Siga las indicaciones y consulte las contraindicaciones, advertencias y precauciones en las instrucciones de uso. El uso concomitante de antibiótico local puede afectar el tiempo de fraguado, las características de absorción y/o la formación de hueso. Los antibióticos pueden afectar al tiempo de fraguado como se ejemplificó anteriormente para 10cc de STIMULAN® Rapid Cure. Sólo se pueden añadir los antibióticos mostrados y se debe evitar la combinación con otros antibióticos. Es responsabilidad del cirujano / profesional sanitario tener debidamente en cuenta los datos contenidos en la autorización de comercialización del medicamento para decidir si es apropiado para el paciente bajo su cuidado. Debe consultarse el Resumen de las Características del Producto correspondiente. El tipo y la dosis del medicamento también deben evaluarse en función de las circunstancias clínicas individuales de cada paciente. Este catálogo puede contener el uso de STIMULAN y/o técnicas que van más allá de la autorización / aprobación actual concedida por la autoridad reguladora competente. Para obtener más información, póngase en contacto con su representante local.

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Patentes pendientes: GB1502655.2, US 15/040075, CN 201610089710.5, GB1704688.9, EP 18275044.8, US 15/933936, CN 108619579A

STIMULAN®

PODER PARA TRANSFORMAR LOS RESULTADOS

- El complemento perfecto para el transporte de antibióticos
- Sólo STIMULAN está aprobado para su uso en huesos y partes blandas[1]
- Método único de recristalización DRy26™ para un rendimiento consistente y fiable[6]
- Proporciona flexibilidad caso por caso

Todos los productos de Biocomposites son diseñados, producidos y distribuidos desde nuestras instalaciones en Keele, Reino Unido.

En Biocomposites, nos enorgullecemos de ser pioneros en la mejora de los resultados de una gran variedad de aplicaciones clínicas para cirujanos y pacientes. Con dedicación exclusiva de nuestro equipo de especialistas en el desarrollo de compuestos de calcio innovadores para uso quirúrgico.

Con más de 30 años de experiencia y dedicación a la calidad inigualables, los productos que investigamos, diseñamos y fabricamos están a la vanguardia de la tecnología basada en el calcio.

Más información en biocomposites.com

Biocomposites®

STIMULAN®
Bullet Mat and Introducer

Lanzamiento de un dispositivo innovador para su estrategia en el manejo de infecciones

STIMULAN es un verdadero sulfato de calcio absorbible, especialmente diseñado para complementar su estrategia de manejo de infecciones y espacios muertos.[1]

✓ **Aprobado para su aplicación directa en la zona de infección**

El nuevo dispositivo innovador STIMULAN Bullet Mat and Introducer facilita el suministro del producto en las zonas de difícil acceso. El dispositivo Bullet Mat moldea balas alargadas con una ranura a un lado para reducir el riesgo de presión en la zona del defecto.

La forma aerodinámica y flexible del Introducer facilita que se pueda insertar profundamente en el canal fresado o espacio vacío. El obturador permite empujar las balas en la zona quirúrgica. Las balas pueden prepararse en dos diámetros de 7mm y 9mm en función de cada necesidad.

Diseño aerodinámico y flexible que facilita el suministro de STIMULAN

Guía técnica y preparación de STIMULAN Rapid Cure con STIMULAN Bullet Mat and Introducer

Elija el tamaño de la bala y el Introducer para ajustarse a la zona quirúrgica

Diámetro de la Bala	Diámetro exterior del Introducer	Mínimo diámetro de fresado	Longitud máxima (de punta a punta)	Referencia
7mm	9mm (mango negro)	10mm	640mm	660-001
9mm	11mm (mango gris)	12mm	480mm	

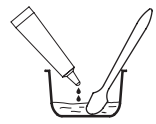
Preparar STIMULAN Rapid Cure 20cc

Utilice la solución de mezcla del tubo proporcionado en el envase. No añada ningún otro líquido. Reemplace el molde de perlas por el Bullet Mat.

1

Vacíe STIMULAN Rapid Cure en polvo en el bol de mezclar estéril suministrado

2



Añada la solución de mezcla y mezcle perfectamente hasta obtener una pasta homogénea (Aprox. 30 segundos)

3

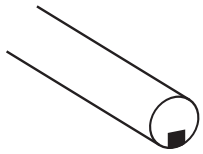
Aplique la mezcla en el molde inmediatamente

4

Permita que la pasta fragüe por lo menos 8 minutos después de mezclar. Flexione el molde Bullet Mat para liberar las balas

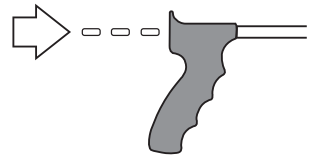
Técnica para cargar y usar el Introducer

1



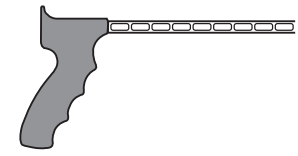
Asegúrese de que la pestaña de retención de la extremidad distal esté en posición, como se muestra en la imagen

2



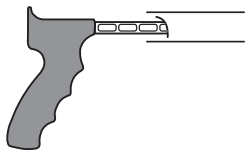
Saque el obturador e introduzca las balas al nivel requerido para llenar el espacio vacío

3



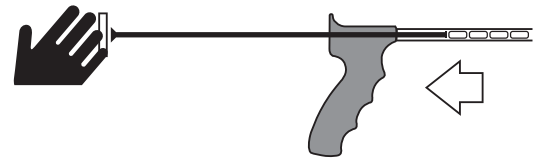
Transfiera el Introducer pre-cargado a la zona quirúrgica. Debe mantenerlo en posición horizontal

4



Inserte despacio el Introducer en la zona quirúrgica

5



Inserte el obturador y aplique una presión positiva. Mantenga la presión positiva y tire hacia atrás del Introducer, dejando las balas de STIMULAN dentro del espacio vacío

References: 1. Biocomposites Ltd, STIMULAN Instructions for Use.
Siga las indicaciones y consulte las contraindicaciones, advertencias y precauciones detalladas en las instrucciones de uso. Es responsabilidad del especialista decidir el tipo y la cantidad de antibiótico empleado. El uso simultáneo de antibióticos administrados localmente puede afectar el tiempo de fraguado.
La práctica de mezclado de antibióticos con el dispositivo STIMULAN Kit/STIMULAN Rapid Cure no ha sido evaluada por ninguna autoridad competente europea de medicamentos y está considerada como una indicación fuera de etiqueta (off-label en inglés). Realizar esta práctica corre por cuenta y riesgo del cirujano/profesional sanitario.
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Patentes pendientes: GB1502655.2, US 15/040075, CN 201610089710.5, US 15/288328, GB1704688.9

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Research Paper

Treatment of Cavitary Bone Defects in Chronic Osteomyelitis: Bioactive glass S53P4 vs. Calcium Sulphate Antibiotic Beads

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Abstract

Aim: To evaluate the efficacy of bioglass (BAG-S53P4) in the treatment of patients with chronic osteomyelitis and compare the results with calcium sulphate antibiotic beads in one medical centre.

Methods: Retrospective analysis of 25 cases. Inclusion criteria: patients diagnosed clinically and radiographically of osteomyelitis and treated surgically (Group 1: cavitary bone defects treated with bioglass and Group 2: cavitary bone defects treated with calcium sulphate antibiotic beads) during the period of 2014 and 2015 in one medical centre.

Results: Patients in group 1 (bioglass treatment): total of 12 patients (11 males and 1 female) with mean age: 50 years (30-86). Average length of hospital stay was 22 days and mean follow-up time: 23 months (16-33). Mean erythrocyte sedimentation rate (ESR) and mean c-reactive protein (CRP) before surgery: 55mm/hr and 54 mg/L, respectively. Mean ESR and mean CRP in last blood exam: 18 mm/hr and 8 mg/L, respectively. There were 2 postoperative complications: seroma formation and delayed wound healing. Only 1 patient had recurrence of infection.

Patients in group 2 (calcium sulphate antibiotic beads treatment): total of 13 patients (9 males and 4 females) with mean age: 48 years (17-67). Average length of hospital stay was 21 days and mean follow-up time 22 months (16-29). Mean ESR and mean CRP before surgery: 51mm/hr and 41 mg/L, respectively. Mean ESR and mean CRP in last blood test: 15 mm/hr and 11 mg/L. 2 postoperative complications were registered: chronic expanding hematoma of the muscle flap donor site and seroma formation. 1 patient had recurrence of infection. Overall, there were no differences in recurrence of infection, p=0.740 and in complication rate, p=0.672. 11 (91,7%) patients in group 1 and 12 (92,3%) patients in group 2 showed no signs of recurrence of infection both clinically and radiologically at final follow-up.

The most frequent cause of osteomyelitis in group 1 was post traumatic while a postsurgical aetiology was more frequent in group 2. The distal tibia was the most common location. The most frequent pathogen isolated in both groups was methicillin sensible staphylococcus aureus.

Conclusions: An advance in treatment of patients with cavitary bone defects in chronic osteomyelitis is the use of synthetic bone substitutes although current evidence is low. In this study, we demonstrate how bioglass without local antibiotics and calcium sulphate antibiotic beads are both equally effective treatment options. Overall, there were no differences between groups in mean hospital stay, complication rates and recurrence of infection.

Key words: bioactive glass, osteomyelitis, calcium sulphate, bone substitute, cavitary defect.

Background

Osteomyelitis represents one of the most challenging conditions in orthopedic surgery. It consists of a destructive inflammatory process in the bone caused by infectious microorganisms. *Staphylococcus aureus* and other Gram-positive pathogens are usually the most commonly involved.[1] The primary cause of infection has multiple etiologies; it can arise from haematogenous, post-traumatic or post-operative colonisation.[2,3]

Acute osteomyelitis is characterized by the cardinal features of inflammation along with soft tissue edema, locally decreased blood supply and pus formation. When this condition persists over time due to not-treatment or treatment failure, the infection can progress to a chronic phase, with formation of large areas of necrotic bone called sequestrum, providing the perfect place for infection recurrence.[4]

Given the limited ability of antibiotics to penetrate poorly vascularized or devitalized bone and surrounding tissues, treatment often requires aggressive surgical debridement, although unfortunately this often results in large bone defects.[3,5]

The management of this bone defect has been addressed in various ways, which includes local or soft-tissue flaps, bone transport or local administration of antibiotics, delivered via polymethylmethacrylate (PMMA), bone graft or bone substitutes.[6–8]

For several years, bone defects in osteomyelitis have been treated through debridement in combination with gentamicin-loaded PMMA. However this method isn´t free of drawbacks, such as thermal damage to the antibiotic, the need of a second intervention to remove PMMA and facilitation of biofilm formation by multiresistant colonies due to inadequate antibiotic concentration.[9,10]

With the development of synthetic bone substitutes, such as calcium sulphate (CS) and bioglass, new possibilities of adjuvant treatment in osteomyelitis have arisen. On the one hand, CS is a biodegradable ceramic used as bone graft material since 1892[11] and is a well-established antibiotic carrier[12]. It allows obliteration of the dead space and release of the associated antibiotic after its entire degradation. However, CS has intrinsic disadvantages. First, it cannot provide structural support due to its quick hydrolysis, 6-12 weeks in bone[13]. In addition it has been related to wound issues owing to serous exudate[14–16].

On the other hand, Bioactive glasses are synthetic materials that have been shown to have antibacterial, osteoconductive and angiogenic properties, making them ideal for treating bone defects in osteomyelitis.[9,17–21] Particularly S53P4, composed by SiO_2, Na_2O, CaO and P_2O_5, has been reported to facilitate tissue growth by chemically binding to the bone matrix, thereby promoting new bone formation.[22] Sodium and calcium ions are released from the surface and increase the local pH and osmotic pressure granting the antibacterial properties without the need of local antibiotics while calcium phosphates crystalize to natural hydroxyapatite, which starts the activation of osteoblasts for the formation of new bone.[23,24] At present, no induction of bacterial resistance to S53P4 has been reported.

Aim

Due to the different properties of these two synthetic bone substitutes, we conducted a study to evaluate their efficacy in the treatment of patients with cavitary defects due to chronic osteomyelitis.

Patients and methods

This was a retrospective study of 25 consecutive patients with chronic osteomyelitis operated during the period of 2014-2015 in one medical center specialized in bone and joint infections. Patients were diagnosed of osteomyelitis clinically and were confirmed by radiology and laboratory results. MRI and CT scans were ordered preoperatively only in specific patients for surgical planning (to measure bone void and sinus tract extension). Patients susceptible to bone void filling were allocated into the two treatment groups (group 1: bioglass without local antibiotics, group 2: antibiotic loaded calcium sulphate) by order of consultation at the outpatient clinic in a 1:1 fashion by senior surgeon (J.B). Patients that could require a concomitant plastic surgical procedure were consulted with the Plastic Surgery Division and surgery was planned (1 stage). All patients gave informed consent and the study was conducted in accordance with the ethics committee of our institution. The primary endpoint of the study was absence of recurrence of osteomyelitis based on clinical and radiological findings and no need for further surgery. The secondary endpoints were mean hospital stay and complication rates when using the two bone substitutes.

Group 1 consisted of 12 patients that were treated with surgical debridement, systemic antibiotics and local application of Bioactive glass S53P4 (BonAlive, Bone Alive Biomaterial Ltd, Turku,

Finland). Meanwhile, group 2 was composed of 13 patients treated with surgical debridement, systemic antibiotics and local application of calcium sulphate antibiotic beads (Stimulan, Biocomposite Ltd, Staffordshire, England).

Surgery was carried out through usual technique by senior surgeon (J.B). First, incision was centered at the focus of osteomyelitis/ sinus tract. A bone window was marked first with a drill guide. The drill holes were connected by osteotomes and lastly an oscillating saw was used to perform the finishing cut (Figure 2). A thorough debridement of the medullary canal was performed with different size curettes and 6-7 samples of the infected medullary site and soft tissues (sinus tract) were collected and sent to the Microbiology Department. Posteriorly, the medullary canal (if osteomyelitis of long bone) was reamed in its entirety with a motorized burr if suspicion of infection was present (preoperative images of cement in the canal or sequestrae). Lastly, a waterjet hydrosurgery system was used copiously at primary site of osteomyelitis. After re-gloving and a new sterile field set-up, cavities were filled with bone substitutes, group 1 with bioactive glass granules and no local antibiotics, while group 2 with calcium sulphate beads with a combination of local antibiotics: vancomycin and gentamycin. Special attention was taken to completely fill the defects with the bone substitutes. To prevent the biomaterial from being distributed outside the bone, a layer of bioglass in putty form (paste solution) was used to create a physical barrier to cover the bone window in the cases in group 1. No steps were taken in the cases of group 2 with calcium sulphate beads other than skin closure. Suction drains were not used in any group.

At discharge, patients received 6-12 weeks of systemic antibiotic therapy established by the Infectious Disease Department and according to culture results.

Patient follow-up at outpatient clinic took place at weeks 2 and 4 to check skin closure and at 3, 6 and 12 months. Radiographs were obtained in the immediate postoperative period and at 4 weeks, 6 months and yearly thereafter. CT scans were ordered in specific cases to evaluate bone substitute integration and bone void filling and were evaluated by the Musculoskeletal Radiology Section. Laboratory evaluation was conducted by the Infectious Disease Department at week 4, 6, 8, 12(depending on the microorganisms isolated and antibiotic therapy) and at 6 months and yearly thereafter. Only patients with more than one year follow-up were included in the study for comparison.

Statistical analysis

Comparison of preoperative and postoperative values was performed using the unpaired t-test. Comparison of qualitative data was performed using the chi-square test. The Kolmogorov-Smirnov test was used to assess the normality of the variables' distribution. A p-value less than 0.05 was considered significant. All statistical analyses were performed using IBM SPSS1 statistics software (Version 20.0; Chicago, IL, USA).

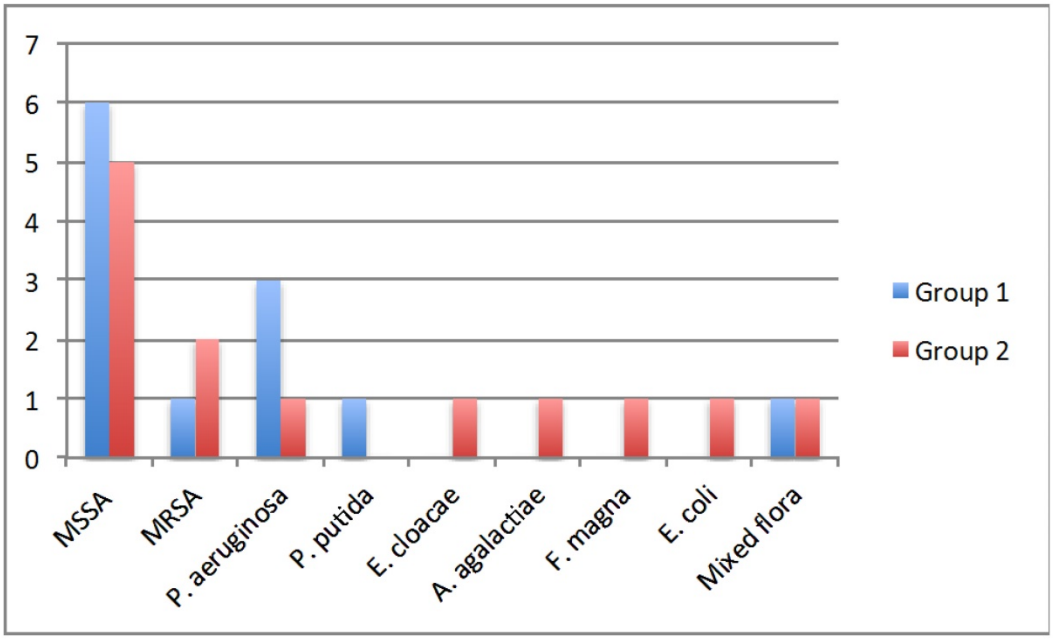

Figure 1. Microorganisms isolated in the two different treatment groups.

Results

Pre-operative data did not differ between groups compared (Table 1). Patients in group 1 (bioglass granules) had a mean age of 50 years (11 males, 1 female). Majority of patients were classified as ASA II (7 patients). The most frequent location of osteomyelitis was the tibia (7 patients), followed by the femur (4 patients) and calcaneus (1 patient). Regarding osteomyelitis etiology, six of the patients had sustained a traumatic event in the lower extremity, four patients had undergone previous operative treatments at their regional hospital and two cases had an hematogenous origin. Six patients presented an active draining sinus at index surgery (swab samples were sent for preoperative microbiological analysis). Mean erythrocyte sedimentation rate (ESR) and mean c-reactive protein (CRP) before surgery were: 55 mm/hr and 54 mg/L respectively.

Meanwhile, patients in group 2 (calcium sulphate antibiotic beads) had a mean age of 48 years (9 males and 4 females). Majority of patients were classified as ASA II (8 patients). The most frequent location was also the tibia (6 patients), followed by the calcaneus (4 patients), femur (2 patient) and humerus (1 patient). The most frequent etiology in this group was postsurgical (11 patients had undergone previous treatments including revisions and arthrodesis at their regional hospital) followed by posttraumatic (2 cases). Seven patients presented an active draining sinus at time of surgery and swab samples were sent for microbiological analysis. Mean ESR and mean CRP before surgery were: 51 mm/hr and 41 mg/L, respectively. The most frequent microorganism isolated for both groups was methicillin-sensitive *S. aureus*.

The estimated sizes of the bone defects were estimated preoperatively with CT images when available, but final amount was decided intraoperatively after adequate debridement. In the case of group 1, the mean amount of bioglass required to fill the bone defect was 17,7cc (granule size was always chosen medium,1-2mm, except in 1 patient where the bone window was very small and was mixed with size small 0,5-0,8mm for better filling) while the mean amount of calcium sulphate used was 9.4cc (pellet size 4.8mm, 10cc kit in 9 patients and 5cc kit in one patient) in combination with 1 gr of vancomycin and 240 mg of gentamycin with the 10cc kit and 500mg vancomycin and 160mg of gentamycin with the 5 cc kit. Patients in both groups had similar mean hospital stay, similar to published literature, which was not statistically significant: 22 days in group 1 and 21 days in group 2, p=0.831[10]. Also, patient mean follow-up at the outpatient clinic was similar in both groups, 23,3 months in group 1 (range 16-33 months) and 22 months in group 2 (range 16-29), p=0.578. In the last laboratory test, mean ESR and mean CRP in group 1 were: 18 mm/hr and 8 mg/L respectively. Meanwhile, mean ESR and mean CRP at final follow-up in group 2 were: 15 mm/hr and 11 mg/L.

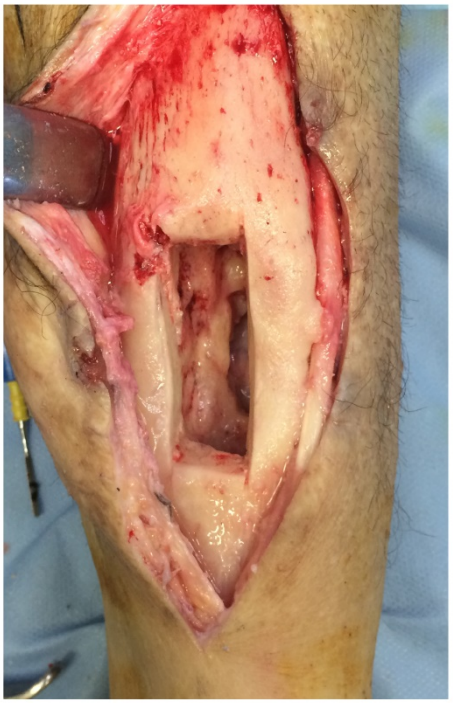

Figure 2. Rectangular bone window.

Table 1. Preoperative data.

	Group 1 (Bio active glass)	Group 2 (calcium sulphate)	P-value
Total number of patients (n)	12	13	
Mean age (years), SD	50± 18	48± 17	0.845
Male/female	11/1	9/4	0.186
ASA Classification (I/II/III/IV)	4/7/1/0	3/8/2/0	0.852
Pathogenesis (haematogenous, post-traumatic, post-operative)	2/6/4	0/2/11	0.027
Location (tibia/femur/calcaneus/other)	7/4/1/0	6/2/4/1	0.320
Draining fistula at time of surgery	6	7	0.848
Mean pre-op ESR (mm/h), SD	55± 26	51± 29	0.717
Mean pre-op serum C-reactive protein (mg/l), SD	54± 52	41± 31	0.473

Table 2. Postoperative data.

	Group 1 (Bio active glass)	Group 2 (calcium sulphate)	P-value
Mean follow-up (months), SD	23,3 ± 6,6	22± 4,4	0.578
Mean hospital stay (days), SD	22,5 ± 10,6	21,3 ± 16,1	0.831
Mean systemic antibiotic treatment duration (weeks), SD	8,7 ± 1,6	8,9 ± 1,7	0.704
Mean post-op ESR (mm/h), SD	18± 15	15± 9	0.576
Mean post-op serum C-reactive protein (mg/l), SD	8 ± 6	11± 18	0.547
Microorganisms isolated (*MSSA, MRSA, P. aeruginosa, P. putida, E. cloacae, S. agalactiae, F. magna, E. coli, mixed flora*)	6/1/3/1/0/0/0/1	5/2/1/0/1/1/1/1/1	0.576
Intramedullary 0 of long bones	11	8	0.160
Single stage muscle flap reconstruction	2	1	0.593
Complications	2	2	0.672
Infection recurrence	1	1	0.740

Intramedullary reaming of long bones (tibia and femur) using the Reamer-Irrigator-Aspirator system was performed in 11 patients in group 1 and 8 patients in group 2, p=0.160. Planned single stage muscle flap reconstruction was performed in 2 patients in group 1 and 1 patient in group 2, p=0.593. After aggressive debridement, in all three cases, Plastic Surgery Division designed an anterolateral thigh flap for soft tissue coverage in the tibia.

Postoperative complications were similar in both groups, p=0.672. In group 1 there were two postoperative complications. In one patient, with calcaneal osteomyelitis, the surgical wound re-opened (1cm x 1cm) in the immediate postoperative period and bioglass granules extruded from the skin upon pressure. Wound was treated during hospital stay by applying pressure and eliminating excessive granules and serous fluid. At discharge, after 10 days, wound closed. However, at the 6-month follow-up visit, patient had developed a new sinus tract. Patient is currently in the waiting list to re-operate. Another patient in group 1 with distal tibia osteomyelitis presented a delay in wound closure and bone void filling. Patient had to be re-operated by the plastic surgery department and wound was covered with lateral thigh free flap. Currently, at last follow-up, patient is asymptomatic.

In group 2, there were also 2 postoperative complications. One patient developed a chronic expanding hematoma of the muscle flap donor site which required surgical drainage. The other patient with distal tibia osteomyelitis developed a mild seroma which was treated by applying pressure and wound healed in 7 days during hospital stay. However, last hospital visit at 1 year follow-up, patient is symptomatic with pain and altered laboratory parameters.

There were no differences between groups in microorganisms isolated from osteomyelitis site, p=0.576. Methicillin-sensitive *Staphylococcus aureus* (MSSA) was the most frequent pathogen isolated in both groups (6/12 cases in group 1 vs 5/13 cases in group 2). Methicillin- resistant *Staphylococcus aureus* was the second most frequent isolated pathogen in group 2 (2 cases) while *P. Aeruginosa* was the second most frequent in group 1 (3 cases). Other micoorganisms isolated in individual cases included *P. putida, E.cloacae, S. agalactiae, F. magna, E.coli* and one case of mixed flora.

Overall, there were no differences in recurrence of infection, p=0.740 and in complication rate, p=0.672. 11 (91,7%) patients in group 1 and 12 (92,3%) patients in group 2 showed no signs of recurrence of infection both clinically and radiologically at final follow-up.

Discussion

This comparative study demonstrates that both bioglass and calcium sulphate are equally effective bone substitutes useful for filling bone defects. Our primary endpoint was absence of recurrence at minimum one year follow-up, and we obtained results similar to those published in literature (around 90% absence of infection in both groups)[10].

Another key aspect is that we found no differences in hospital stay or complication rate when comparing both groups. We do take note, that our mean hospital stay is longer than previous studies but this can be attributed to the fact that we did not exclude patients that had local plastic surgery procedures (1 stage)[10]. Nevertheless, since bone substitute treatment was assigned in a 1:1 fashion between the two groups, authors assumed that patients that would require skin or muscle flaps would be distributed equally among both groups. Also, like mentioned before, wound complication rate was similar in both groups. Seroma formation appeared in both groups which resolved in a similar fashion with repeated drainage of serum fluid in alternative days in a period of one to two weeks. It is interesting to note, that both cases at last follow-up had recurrence of infection. Due to the small sample size, we cannot attribute with certainty seroma formation to recurrence of infection. Authors believe that the key for successful treatment lies in aggressive surgical debridement and correct soft tissue coverage.

Probably in these two cases, surgical debridement was inadequate and a focus of osteomyelitis was not addressed.

This leads us to present one case to emphasize the importance of surgical debridement of all focus. A 68-year-old patient with posttraumatic osteomyelitis of the tibia was transferred from his regional hospital to our Bone and Joint Infection Department for treatment decision. He referred several previous surgeries at his regional hospital but all had failed. On examination, patient presented an active draining sinus on medial aspect of distal tibia. Preoperative CRP and ESR were 65,3mg/L and 36mm/h respectively. X ray images and CT scan were ordered for surgical planning and bone void filling. After image analysis, patient was consented for surgery and treatment with bioglass. As evidenced with the CT scan, large areas of sequestrae were present in the whole medullary canal (Figure 3). 30cc of bioglass was estimated that would be necessary to fill the cavity. Surgery was carried out in regular fashion as described above. After debridement and medullary canal reaming, 45 cc of medium size bioglass was used to fill the cavity. Upon closure, surgical team was able to close skin but with excessive tension. Plastic surgery department had not been consulted for this case since senior surgeon had believed that a primary closure would be possible. In the immediate postoperative period, there was a delay in wound healing with border necrosis (Figure 4). In addition, post-op X ray and CT scan showed a cavity which had not been addressed during the surgical procedure even though fluoroscopy had been used (Figure 5). Consequently, the plastic surgery department was contacted, patient was consented and taken back to the surgery room. Intraoperatively, we noticed that the not-addressed cavity was independent of the primary cavity. We performed a new bone window, aggressive surgical debridement, irrigation and filled the defect with 5cc of bone glass. Then, the Plastic Surgery Team performed a lateral thigh free flap to cover the surgical wound. Postoperatively, x ray images showed adequate filling (Figure 6) of the cavity and the free muscle flap healed correctly. Patient was discharged. At 2 year follow-up patient is asymptomatic with no active draining sinus (Figure 7). In addition, similar to other studies[4,10,25,26], bioglass can still be seen on the plain radiographs at 2 year post-operatively unlike calcium sulphate which has complete incorporation in all of our cases at the 6 month follow-up. We believe that this case is particularly important to demonstrate the importance of aggressive surgical debridement of all osteomyelitis focus and adequate skin coverage without tension to prevent infection recurrence. If correct filling of the second cavity had not been performed, infection recurrence would have been inevitable.

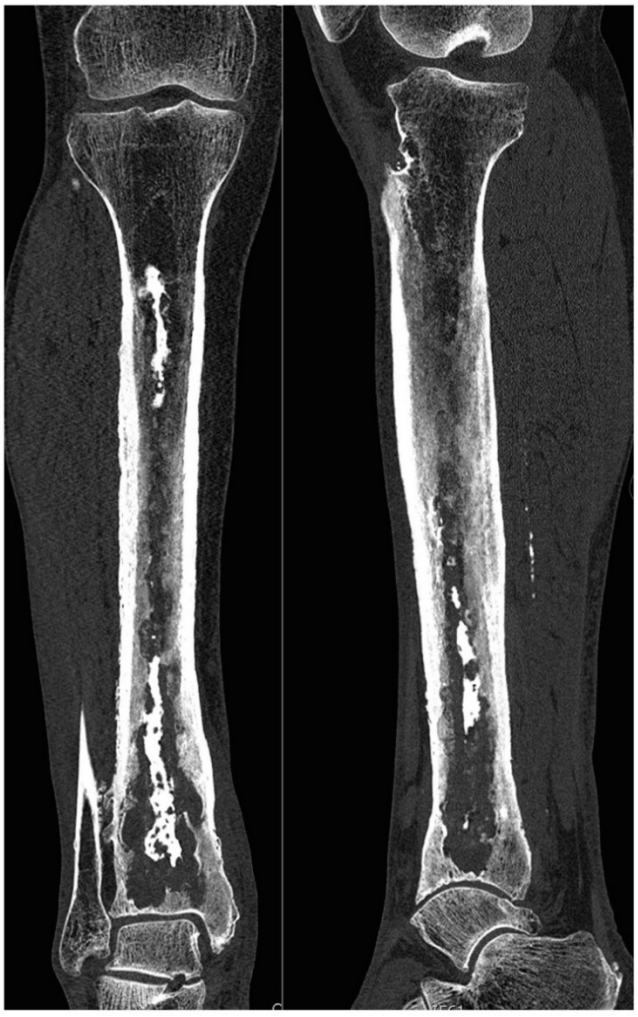

Figure 3. Visible sequestrae along the whole medullary canal.

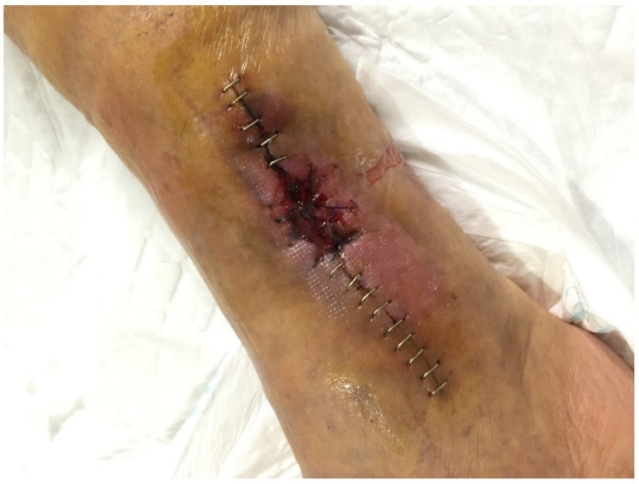

Figure 4. Skin necrosis and wound dehiscence probably due to excessive tension.

Limitations of this study consist primarily of the small sample size and retrospective nature. However, this is the second largest study[4,10,25] to compare two bone substitutes in one centre by same surgical team with an adequate follow-up (at least one year, mean 23 months for both groups). Also, we compared only two bone substitutes when there are many more commercially available with different efficacy profiles. Larger studies, with longer follow-up and different substitutes are desirable to establish conclusions.

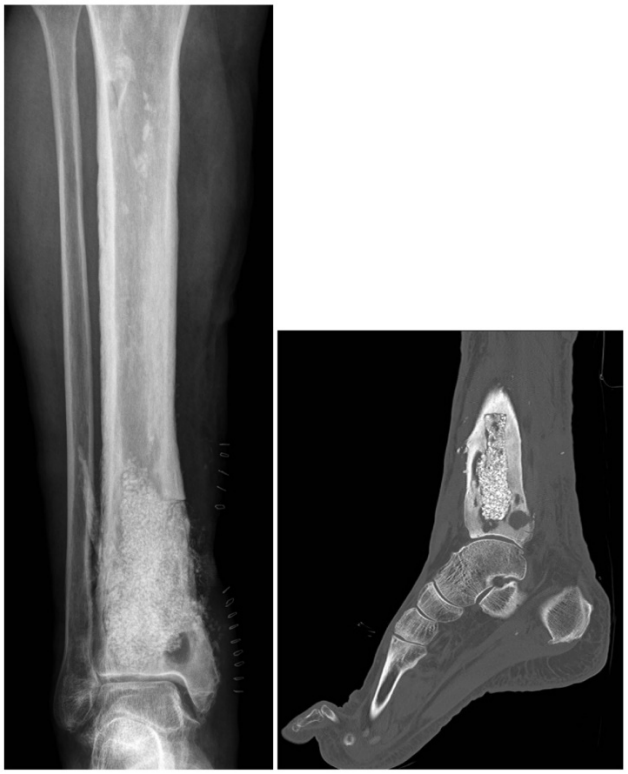

Figure 5. A. Small posteromedial cavity not filled with bioglass B. Second cavity is independent of main cavity.

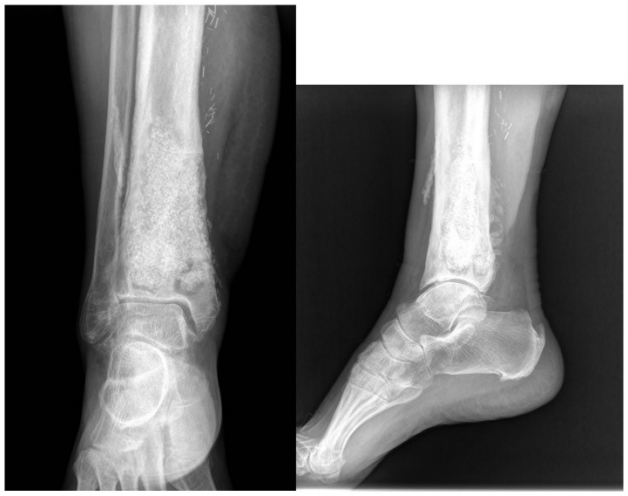

Figure 6. Correct bone void filling of all the cavities with bioglass.

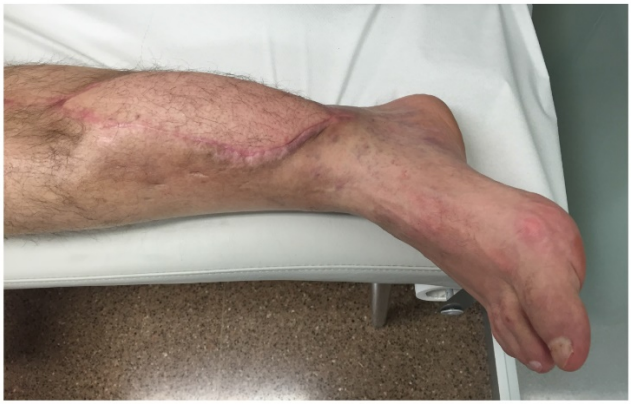

Figure 7. Last follow-up, lateral thigh free muscle flap has survived completely with absence of recurrence of infection.

Conclusions

In the antibiotic era, chronic osteomyelitis remains difficult to treat. Aggressive surgical debridement and pathogen-specific antibiotics is key to eradicate infection. An advance in treatment is the use of synthetic bone substitutes although current evidence is low. In this study, we demonstrate how bioglass and calcium sulphate antibiotic beads are both equally effective treatment options for cavitary bone defects in osteomyelitis. Both treatment groups showed similar absence of recurrence, mean hospital stay and complication rates.

Competing Interests

The authors have declared that no competing interest exists.

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Review Article

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Debridement, antibiotic pearls, and retention of the implant (DAPRI): A modified technique for implant retention in total knee arthroplasty PJI treatment

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Abstract

We describe a modified surgical technique developed to enhance the classical irrigation and debridement procedure to improve the possibilities of retaining an infected total knee arthroplasty. This technique, debridement antibiotic pearls and retention of the implant (DAPRI), aims to remove the intra-articular biofilm allowing a higher and prolonged local antibiotic concentration using calcium sulfate beads. The combination of three different surgical techniques (methylene blue staining, argon beam electrical stimulation, and chlorhexidine gluconate brushing) might enhance the identification, disruption, and finally removal of the bacterial biofilm, which is the main responsible of antibiotics and antibodies resistance. The DAPRI technique might represent a safe and more conservative treatment for acute and early hematogenous periprosthetic joint infection.

Keywords

DAIR, DAPRI, infection, PJI, TKA

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Introduction

Total knee arthroplasty (TKA) is one of the most successful procedures in orthopedic surgery, yet nearly 20% of patients remain unsatisfied by their clinical outcomes. Principal causes of TKA failures include periprosthetic joint infection (PJI), instability, failure of osteointegration, and polyethylene wear.[1] TKA failures can occur early (<2 years) or late (>2 years), and PJI represents one of the main causes of an early complication.[2] PJI can have a devastating effect locally as well as on the general health of the patient, often leading to a decrease in daily activity level and an increase in mortality. While the incidence of PJI following primary TKA may only be approximately 1–2% over the lifetime of a prosthetic joint,[3] nearly 25% of all TKA failures today are due to infection.[2]

PJI represents a significant economic burden. The annual incidence of PJIs in the United States is predicted to be between 38,000 and 270,000 by 2030.[4] The average cost per hospitalization of a PJI has been estimated to be US$24,200.[5] In the United States, US$566 million was

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spent treating PJI in 2006, and this is projected to increase to US$1.62 billion by 2020.[5]

The current evidence supports several surgical treatments depending on timing.[6,7] Exchange arthroplasty is indicated in chronic and delayed PJI; whether this occurs best as an immediate or staged replant may depend on the identification and virulence of the microorganism and patient factors.[8–11] However, in the case of an acute or early hematogenous infection (within 4–6 weeks from the original surgery or 7 days from symptom onset), debridement, antibiotics, and implant retention (DAIR) procedure is often employed as a reasonable treatment choice.

The current authors here describe a novel surgical technique developed to enhance the classical DAIR procedure to improve the possibilities of retaining an infected implant. The following surgical technique, debridement, antibiotic pearls, and retention of the implant (DAPRI), aggressively improves upon the conservative DAIR procedure in several aspects. First, by incorporating bioabsorbable antibiotic pearls, we believe that we can prolong the local antibiotic concentration. In addition, we describe a targeted and focused removal of biofilm on implant and soft tissue surfaces by incorporating an initial intra-articular staining injection.

Surgical technique

At present, we applied DAPRI procedure in settings that would otherwise be amenable to DAIR—that is, with acute (within 4–6 weeks from the original surgery) or early hematogenous (within 7 days from clinical onset) infections with a known microorganism. At our institution, we have not performed the DAPRI procedure outside of these indications.

The DAPRI procedure has a stepwise approach. Following the administration of regional and neuraxial anesthesia, (an adductor canal block and spinal anesthesia at our institution),[12] preoperative antibiotic therapy is intentionally held to improve the sensitivity of intraoperative cultures.

Methylene blue-guided debridement

Prior to skin incision and arthrotomy, a large bore needle is introduced into the knee and as much fluid as possible is aspirated from the knee joint. This is sent for culture. Fifty milliliters of dilute 0.1% methylene blue (40 cc normal saline and 10 cc of 0.5% methylene blue solution) is then injected into the knee joint. This technique varies from the technique described by Shaw et al.[13] as it allows for range of motion of the knee while the dilute methylene blue is in the joint. Methylene blue is known to stain bacterial biofilm. We have observed that this method provides a reliable and complete staining of all tissues in the effective joint space with minimal spill of dye outside of the effective joint space. After injection, the knee undergoes at least 1 min of flexion and extension to allow for intra-articular distribution of the staining dye. An arthrocentesis is performed under sterile conditions to aspirate as much dye as possible from the joint prior to arthrotomy, and then immediately after arthrotomy, suction is used to aspirate the remaining dye from the joint. This prevents overflow of dye to the surrounding tissues.

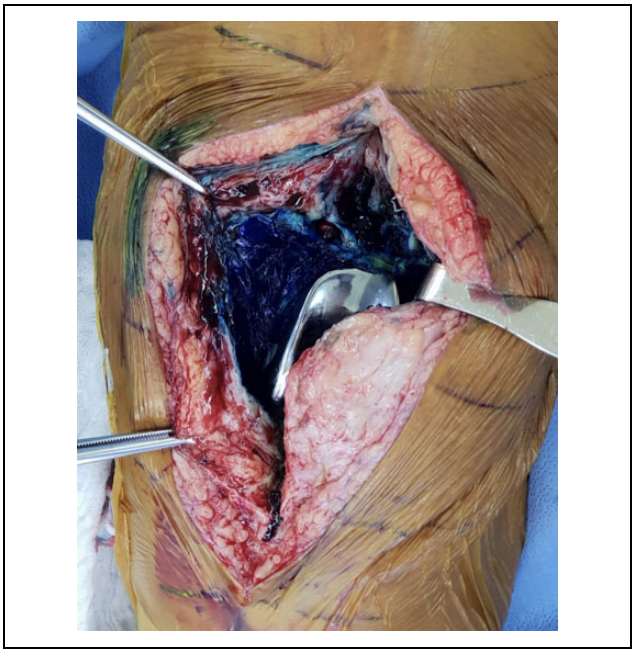

Figure 1. Left knee: Dyed intra-articular space.

Following a standard medial parapatellar approach and capsulotomy, blue staining of all intra-articular surfaces is noted (Figure 1). Five soft tissue culture samples are obtained from different stained intra-articular areas for standard microbiological studies (aerobic, anaerobic, and fungal exams). The suprapatellar pouch and the patellar tendon are then freed of scar and adhesions, the knee is exposed, and the polyethylene insert is removed.

After obtaining wide exposure, an aggressive and radical "tumor-like" synovectomy is performed including the synovial layer on the posterior capsule. The objective is to remove all stained soft tissue. These tissues had been in contact with the infected intra-articular space.

Argon beam

Next, the biofilm on retained surfaces is addressed by mechanical disruption with an argon beam coagulator, (ConMED, USA), as electrical stimulation has been shown to enhance detachment of biofilm from orthopedic implant surfaces.[14] The argon beam coagulator is set to 120 watts and had a probe that applied the beam in a painting brush-like fashion for all visible surfaces on the femoral and tibial components (Figure 2). This technique seems to work best on conductive metal surfaces, but more recently we have approached this step with caution as it is still unclear whether this part of the procedure can alter the surface of the metal.[15]

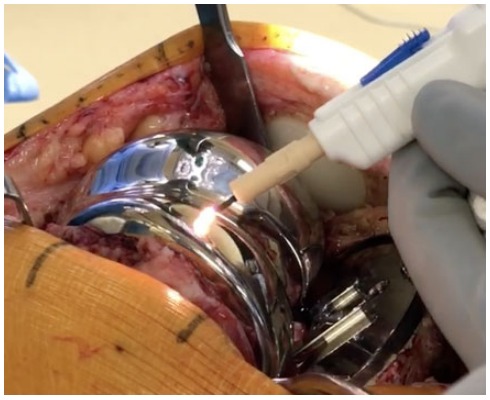

Figure 2. Argon bean application on the femoral component.

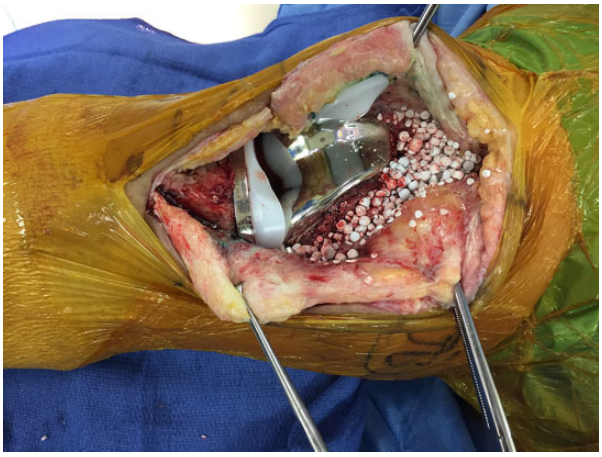

Figure 3. Right knee: Calcium sulfate beads applied in the suprapatellar pouches at the end of the procedure.

Chlorhexidine gluconate scrub

At this point, a 4% chlorhexadine gluconate-added brush is used to scrub all visible surfaces of the femoral, tibial, and patellar components: this is performed to mechanically remove biofilm as suggested by Tria et al.[16]

Last, abundant pulse irrigation with 9 L of bacitracin-added saline is performed.

At this point, we place a povidone-iodine-soaked gauze within the wound and an antimicrobial incision drape is placed on the wound. The surgical team removes their used gowns and gloves and the back table with contaminated instruments is no longer used. The patient is then re-draped with clean drapes, the surgical team uses new gowns and gloves, and a new back table with new instruments is used. The gauze is removed from the wound. A further irrigation of the joint is undertaken using 1 L of bacitracin-added 0.9% sodium chloride prior to inserting the new, implant specific, polyethylene insert.

Calcium sulfate antibiotic-added beads application

Calcium sulfate antibiotic-impregnated beads are then prepared. A 10-mL kit of PG-CSH (Stimulan; Biocomposites Ltd, UK) is mixed with 1000 mg of vancomycin hydrochloride powder, 6 mL of a 40 mg/mL tobramycin solution, and a third antibiotic powder according to the preoperative antibiogram. A smooth paste is formed by mixing all components for 60 s and is pressed into 4.8-mm diameter hemispherical cavities in a flexible mold.[11] The beads harden and are ready for implantation after resting for at least 15 min. These calcium sulfate beads are a biocompatible and resorbable antibiotic-loaded intra-articular delivery system, which allow for a continuous local elution of the appropriate antibiotic. The hydrophilic crystalline structure is normally reabsorbed over 4–6 weeks, and their presence is visible on radiographs.

Ordinarily, the antibiotic beads are inserted in the suprapatellar pouch (Figure 3) and in proximity of the proximal tibia and distal femur to allow for a high concentration of antibiotics in the intra-articular space. After placing an intra-articular drain, the capsule is closed with Stratafix size-1 (Ethicon, Johnson & Johnson, Bridgewater, New Jersey, USA) suture, the subcutaneous tissue with 2-0 PDS suture, and the skin with staples.

All patients follow an identical, standard postoperative rehabilitation protocol, including weight-bearing as tolerated with crutches on the first postoperative day. Discharge from the hospital occurs when the patient achieves independence in activities of daily living, including walking 20 steps, climbing and descending stairs, and independent toileting.

Postoperative antibiotic treatment is routinely established in accordance with our institutional infectious disease service based on preoperative and intraoperative findings. A DAPRI procedure is usually followed by a 6-week course of intravenous antibiotic therapy: after this, another 6-week course of oral antibiotic therapy follows. The procedure is usually considered successful when clinical presentation and serologic tests (erythrocyte sedimentation rate, C-reactive protein, and D-dimer) normalize.

Results

The authors were able to report the initial outcome of 10 patients treated with DAPRI following a PJI in the knee joint. The original procedure was primary TKA in all patients.

Most patients undergoing DAPRI were male in our series (90%). The mean age of the patients was 69 years (range 63–92 years) and the mean BMI was 35 (min 30–max 48).

No patient was lost to follow-up at a minimum of 24 months (range 24–31 months): all the infections were classified as acute (within 6 weeks from the original surgery) or early hematogenous (within 7 days from clinical onset) infections with a known microorganism. All DAPRI

procedures were performed on average 2 days after onset of symptoms (range 1–4 days). At the final follow-up, the procedure was considered a failure in 2 of 10 (20%) patients who underwent resection arthroplasty at 2 and 3 months, respectively, from the DAPRI procedure and a two-stage revision TKA afterward.

Most infections were caused by *Staphylococcus aureus* species (60%) and 20% of them were methicillin-resistant *S. aureus* (MRSA). The rest of the patients developed streptococcal infection (30%) and *Granulicatella adiacens* infection (10%). The procedures considered a failure after DAPRI were in two patients with MRSA infection. Overall infection could be controlled in eight patients, thus the success rate of infection eradication was 80%. The current data did not have sufficient power for statistical analysis.

Discussion

DAPRI augments the standard DAIR technique in several respects. Added interventions include intra-articular use of methylene blue to stain the intra-articular tissues, use of an argon beam coagulator as a biofilm disrupter, use of chlorhexidine gluconate brush as a biofilm remover, and use of calcium sulfate antibiotic-impregnated beads to prolong the intra-articular antibiotic concentration.[17] Combined, these techniques may lead to an increase in the overall success rate in implant-retention revision surgery.

This article presents a novel surgical technique DAPRI with the objective of improving the results of the standard DAIR procedure by allowing for a high and prolonged local antibiotic concentration using a calcium sulfate beads and eradicating the bacterial biofilm both on the intra-articular soft tissues and on the implant surfaces through the use of a multimodal approach. The goal of these added steps to DAIR is to disrupt the bacterial biofilm, which is the main culprit of antibiotic resistance and immune evasion.

Historically, the DAIR procedure showed a reasonable infection eradication rate between 50% and 80% when used in appropriately selected patients. Several factors have been associated with treatment failure: patient's own immune deficiency and a high ASA score, MRSA, and *Pseudomonas aeruginosa* species, poor local tissues viability, the presence of rheumatic disease, delayed presentation from the original surgery (i.e. more than 4 weeks after TKA at the authors Institution), and erythrocyte sedimentation rate above 60 mm/h at presentation.[18,19] Success rates between 28% and 62% have been shown in a review article when DAIR is applied to chronic infections, as compared between 31% and 100% for acute infections.[20] Furthermore, DAIR has shown a higher success rate for acute postoperative infections when compared to a hematogenous spread in the late postoperative setting.[18,21,22] These reports highlight the importance of performing any implant retention technique in a timely fashion to achieve clinical success.

We believe that, in combination with timely intervention, our technique of targeted antibiotics mixed in calcium sulfate beads should provide a better outcome.

The calcium sulfate antibiotic-impregnated beads have the peculiar characteristic of being able to reach a prolonged and appropriate intra-articular minimal inhibitory concentration without reaching toxic systemic levels, even at their maximal local concentrations.[23] Furthermore, they are usually absorbed over 6 weeks from insertion, leaving no foreign body substrate ("nidus") for successive bacteria adhesion.[23]

Polymethylmethacrylate (PMMA) antibiotic-added beads have been historically used for local antibiotic delivery in the treatment of PJI, as shown by Buchholz and Engelbrecht.[24] However, the use of nonabsorbable PMMA beads has several limitations, including the need for a subsequent surgery to remove them from the articular space, and the risk of acting as a potential foreign body for bacterial colonization. Furthermore, when compared, to PMMA, calcium sulfate beads have showed a better "in vitro" elution profile[17] especially when cefazolin was used as a mixed antibiotic and a better "in vitro" inhibition of bacterial growth.[25–28]

The use of calcium sulfate beads is not free from complications. McPherson et al.[25] reported the presence of a copious exudate from the wound requiring surgical intervention in 3.2% of patients following the use of calcium sulfate beads to treat PJI. The formation of heterotopic ossifications is another complication reported in 1.2% of patients following calcium sulfate beads use.

Several studies have shown that the biofilm is the main culprit responsible for antibiotic resistance[29] in a PJI scenario. Biofilm is composed of an exopolysaccharide matrix produced by the microorganism when in contact with an implanted foreign material. It protects bacteria from harmful conditions in the host, sequesters in a nutrient-rich area, and provides an environment for the exchange of genetic material between cells.[29] Biofilm removal is mandatory for success with any implant retention procedure[30]: the current authors suggest the combination of three surgical techniques to identify, disrupt, and remove as much biofilm as possible. The current authors performed a methylene blue guided debridement technique, modified from Shaw et al.[13]: this approach allows the surgeon to adequately perform a "tumor-like" synovectomy, removing the proper amount of nonviable, infected tissue while avoiding healthy tissue. Another advantage of methylene blue is its antibacterial activity which might enhance the effectiveness of the DAPRI procedure itself.[31]

The use of argon beam coagulation is well-described in the general and gynecologic surgery literature[32] but its use as an adjuvant in biofilm eradication is currently evolving. This device delivers unipolar electrical current of inert argon gas that may disrupt bacterial biofilm. The use of 4% chlorhexidine gluconate has been previously reported as effective to treat MRSA biofilm.[33] The current authors

use the chlorhexidine gluconate brush on the femoral, tibial, and patellar component once the argon beam phase has been completed to mechanically remove the thermally disrupted biofilm: it must kept into consideration the potential cytotoxicity secondary to the use of chlorhexidine.[34]

The DAPRI technique proposed in this article has several major limitations. First, inclusion criteria are very strict and the authors do not suggest applying this surgical approach outside of those criteria, as preoperative microorganism identification and accurate timing are fundamental. Second, this technique is the step-wise synthesis of several surgical techniques previously reported by different authors. Third, clinical outcomes are not reported here: the authors are following their consecutive series and intend to present their results at 2 years minimum follow-up.

Conclusion

The DAPRI is a standard DAIR technique augmented by the intra-articular use of methylene blue to stain biofilm, the use of the argon beam coagulator to burn and disrupt the biofilm, the chlorhexidine gluconate brush to further kill and remove biofilm, and calcium sulfate antibiotic-impregnated beads to prolong and elevate the intra-articular antibiotic concentration in the setting of TKA PJI.

We believe that the DAPRI technique might represent a reasonable treatment for acute and early hematogenous PJI. We expect that this approach will improve upon the DAIR success rate, although more studies are necessary to evaluate the clinical efficacy of this surgical procedure.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: This study complies with the Declaration of Helsinki: however, no patient data are included in this study.

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Dissolvable Antibiotic Beads in Treatment of Periprosthetic Joint Infection and Revision Arthroplasty

The Use of Synthetic Pure Calcium Sulfate (Stimulan®) Impregnated with Vancomycin & Tobramycin

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Abstract:

This study reviews the clinical results using commercially pure, synthetic antibiotic-loaded Calcium Sulfate dissolvable beads (Stimulan, Biocomposites, Ltd., Keele, UK) in 250 cases of aseptic and septic revision total hip and total knee arthroplasty. A set protocol of Vancomycin and Tobramycin antibiotic was used in all cases. The rate of wound drainage in this series was 3.2%. Wound drainage was generally seen in cases using higher bead volumes. The incidence of heterotopic bone formation was 1.2%. There were nine failures in this study, six of which were due to infection. We feel that commercially pure, synthetic antibiotic-loaded dissolvable beads are an acceptable delivery tool for local antibiotic delivery in aseptic and septic revision joint arthroplasty of the hip and knee. Further studies are needed to examine the potential of improving outcomes of periprosthetic joint infection with this particular local antibiotic delivery system.

Key words: *Stimulan, Calcium Sulfate, Antibiotic Beads, Periprosthetic Infection, Revision Arthroplasty.*
Level of Evidence: *AAOS Therapeutic Study Level IV.*

Introduction

Periprosthetic joint infection (PJI) is a devastating complication that is potentially a limb and life threatening condition.[18,19] The extent of the infection is related to many factors including the health of the host patient, the condition of the local soft tissues, and the length of time the infection has been present within the joint. Treatment of periprosthetic infection currently follows established algorithms that have proven successful.[26] Treatment depends upon the presence of the bacterial biofilm which envelops the joint prosthesis and adjacent bone. In an acute infection, the biofilm is not established. Treatment is focused on preservation of the implant, with radical debridement surgery, modular bearing exchange, copious lavage, and perioperative antibiotic therapy. When a biofilm is present, the infection is considered chronic. In this scenario, the biofilm prevents eradication of bacteria and thus implants must be removed along with a radical debridement of bone and soft tissue. Resection of implants most commonly is performed in a two stage protocol. At some centers that focus on PJI, single stage protocols are utilized. With either

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protocol, success is particularly dependent upon the quality of joint debridement.[42,43,47]

Antibiotic therapy in the surgical treatment of a PJI is an important adjuvant therapy. Antibiotic penetration into the local infected area can be problematic. Specifically, local devascularization of infected tissues can prevent local antibiotic delivery. Additionally, any residual biofilm can shield the area from antibiotics.[4] Local delivery systems offer a solution to this problem. Antibiotic impregnated cement spacers are a useful tool, although a majority of the antibiotic placed into the cement does not elute into the host environment.[23] Non-dissolvable antibiotic polymethylmethacrylate (PMMA) beads can provide higher antibiotic concentrations, but fabrication is tedious. Additionally, it is often difficult to locate and remove all beads at reconstruction.

A local delivery system with dissolvable Calcium Sulfate has been developed to assist in the targeted delivery of antibiotics into the host joint.[5,6,10] Stimulan (Biocomposites Ltd., Keele, UK) is a synthetic hemihydrate form of Calcium Sulfate. It is produced using a synthetic process resulting in 100% purity with no traces of potentially toxic impurities which has been associated with naturally occurring mineral sources of Calcium Sulfate.[3,22] It is biocompatible, composed of hydrophilic crystals, soft after hydration, and disappears on X-rays after two to three weeks when placed within a joint compartment.

Stimulan also has the advantage of delivering a wider spectrum of antibiotic combinations into the joint. It cures at a low temperature, thus allowing heat-sensitive antibiotics to be mixed with Stimulan. This is in contrast to PMMA in which only heat-stable antibiotics can be used. Even with these advantages, there has been concern with using dissolvable antibiotic-loaded Calcium Sulfate.[3,22] The main concern has been with postoperative wound drainage. Prior to Stimulan, dissolvable Calcium Sulfate products were derived from gypsum, a natural product mined and processed into Calcium Sulfate. The processing of gypsum creates a product that has a relatively low pH and contains residual by-products that may illicit an inflammatory reaction when the product is placed into a joint wound. The inflammatory reaction in turn impedes wound healing and causes a wound to drain.[22,40]

The purpose of this study is to examine the initial review of the use of commercially pure, synthetic antibiotic-loaded dissolvable Calcium Sulfate beads (Stimulan) in their application in treating two groups of patients. One group contains patients with periprosthetic infection. The other contains patients undergoing revision joint arthroplasty. Historically this latter group has a higher known risk of periprosthetic infection.[11,14,15,39,43,47] We review outcomes and complications and compare our findings to previous studies employing processed calcium sulfate derived from gypsum product. To our knowledge, this is the first study reporting on the use of commercially pure, synthetic antibiotic-loaded Calcium Sulfate in the treatment of two such groups.

Materials & Methods

Between January 2010 and September 2012, 342 revision THA and TKA procedures were performed. This included aseptic revisions, two stage septic revisions, and one stage DECRA (Debridement, modular Exchange, Component Retention, IV Antibiotic) procedures for acute PJI. During this time we used dissolvable antibiotic beads in 250 of these cases. The antibiotic combination used in this series was a preset protocol consisting of one (1) gram of Vancomycin powder and 240mg of liquid Tobramycin mixed with 10cc of Stimulan powder (see technique below). For two-stage procedures for infected TKA and THA, Stimulan antibiotic beads were inserted both at the time of resection arthroplasty and reimplantation.

Preoperatively, all patients were staged for periprosthetic infection risk according to the Musculoskeletal Infection Society – Americas (MSIS-A) staging system.[26] The integrity of each patient's immune defense system was assessed and all compromising factors were documented.[26,27,8] Aseptic revisions in the MSIS-A classification were considered a Stage Zero. All revision procedures were preoperatively aspirated by the surgeon (ejm) with cell count and culture analysis. Pre-operative Westergren Sedimentation Rates and Quantitative CRP were also obtained on all patients. Clinical scoring was performed on all patients including

Harris Hip and Oxford scores for hips and Knee Society and Oxford scores for knees. Perioperative and post-operative complications were recorded. Radiographs were obtained at 3 months and 1 year post-operatively.

At the time of knee resection arthroplasty, the knee was stabilized with an articulated antibiotic-loaded PMMA spacer. When the knee was unstable, the leg was stabilized with an antibiotic-loaded PMMA endofusion device with medullary rods inserted into the femur and tibia. Cobalt cement (Biomet, Warsaw, IN) was used in resection and reimplantation/revision procedures. For resections, 5 grams of Vancomycin powder and 3.6 grams of Tobramycin powder were mixed into each 40 gram bag of Cobalt cement. Typically 3-5 bags of cement were used at resection. For revision or reimplantation procedures, 2-3 bags of Cobalt cement were typically used. One gram of Vancomycin powder was placed in each 40 gram bag of cement.

For knee cases, the Stimulan beads were delivered along the medial and lateral gutters of the knee, just before closure. A 10 french silicone Blake drain (Ethicon, Inc., San Angelo, TX) was placed along the lateral gutter and the arthrotomy closed in midflexion over a bump. No beads were placed in the subcutaneous layer. Superficial subcutaneous drains were placed as indicated. The deep drain was always removed between 24 and 36 hours. The superficial drain(s) was removed between 48 and 72 hours. In the two stage septic revision group, a compressive Robert-Jones dressing was placed on the leg for 5-7 days, both at resection and reimplantation. Figures 1 and 2 (see Appendices) demonstrate surgical technique and placement of the Stimulan beads.

Figures 1a - 1e: Radiographs of 65-year-old male who underwent a two-stage revision protocol for a chronic periprosthetic infection of his left TKA. The patient suffers from diabetes.

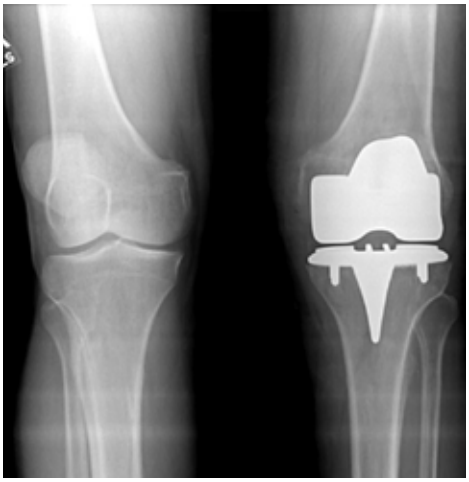

Figure 1a: Preoperative standing AP radiograph of left TKA. Aspiration cultures grew Staphylococcus warneri and Stenotrophomonas maltophilia.

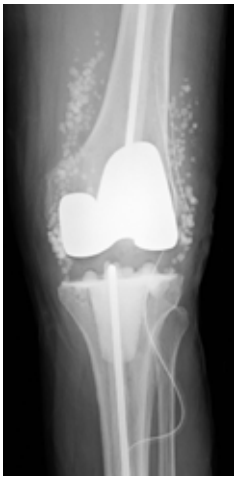

Figure 1b: Postoperative radiograph of resection arthroplasty. The knee was stabilized with a Vanguard (Biomet, Warsaw, IN) prostalac arthroplasty. 10cc of Stimulan antibiotic beads were placed into the medial and lateral gutters at closure. Note deep and superficial drains placed at closure. This case was one of the initial members of this study, thus accounting for the modest use of beads.

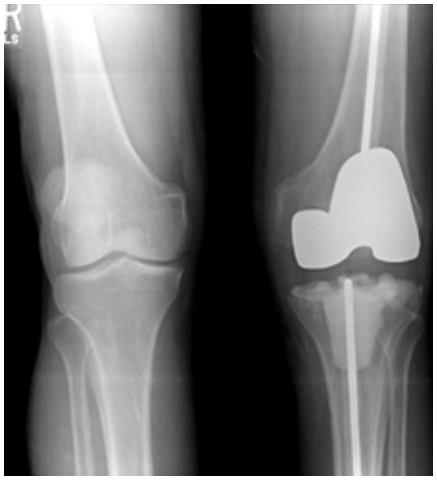

Figure 1c: Preoperative AP radiograph of knees prior to reimplantation. This radiograph was taken 9 weeks after resection arthroplasty. All Calcium Sulfate beads have dissolved. Preoperative aspiration of the knee was negative.

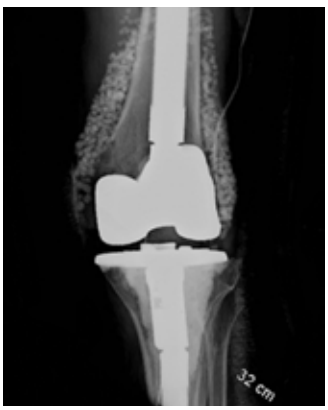

Figure 1d: Postoperative AP radiograph at reimplantation surgery. The knee was reconstructed using the Vanguard revision knee system (Biomet, Warsaw, IN). At closure 20cc of Stimulan antibiotic beads were placed into the knee joint. Again note deep drain placed into knee.

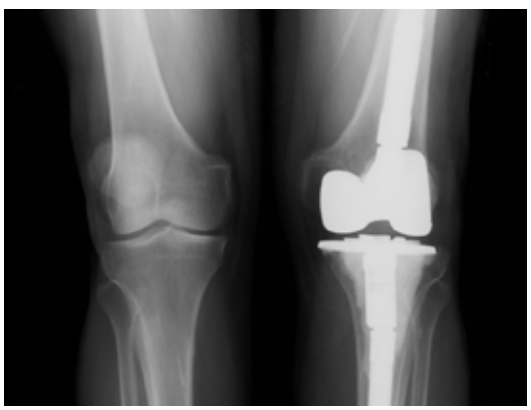

Figure 1e: Standing AP radiograph taken 18 months after reimplantion surgery. All Calcium Sulfate beads have dissolved. The patient remains infection free with knee range of 0-125 degrees.

Figures 2a – 2d: Radiographs of a 57-year-old male who underwent a single-stage revision protocol for Nickel hypersensitivity and clinical pain of his left TKA.

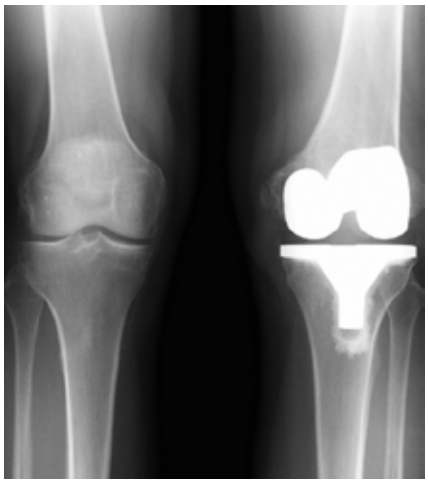

Figure 2a: Preoperative standing AP radiograph. Note well-fixed TKA implants. The patient had knee range of 0-130 degrees, but had a persistently large knee effusion and chronic pain.

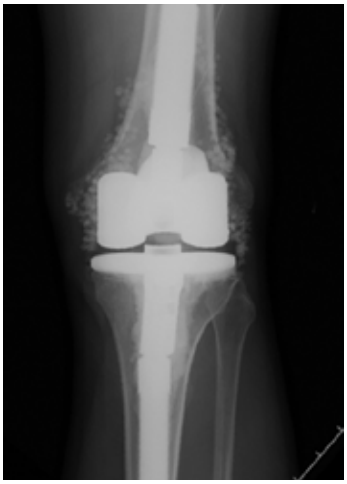

Figure 2b: Postoperative AP radiograph of revision TKA. 10cc of Stimulan antibiotic beads were placed into the medial and lateral gutters of the knee. Note drain placed into knee joint at closure.

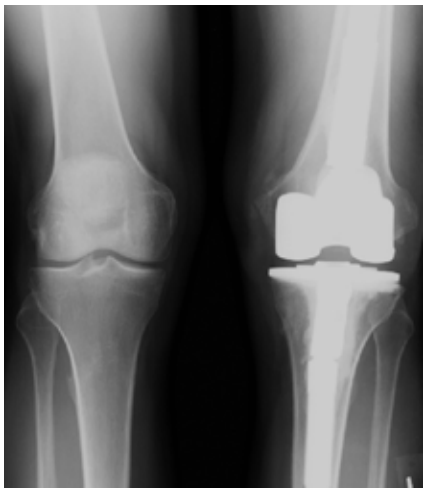

Figure 2c: Postoperative standing AP radiograph taken 2 months after revision surgery. All Calcium Sulfate beads have dissolved. Also note that the knee effusion has abated.

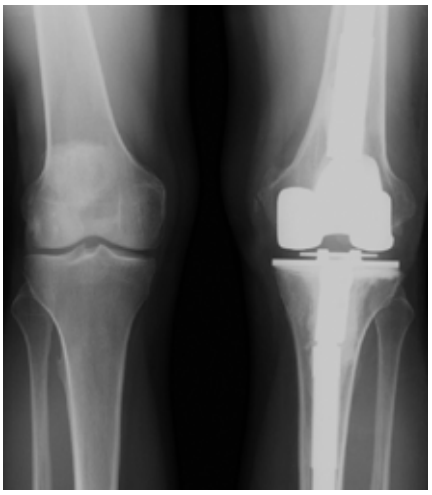

Figure 2d: Postoperative standing AP radiograph taken 8 months after revision surgery. The knee has regained knee range of 0-135 degrees. Knee effusion remains minimal.

At the time of hip resection, the hip was stabilized with an articulated antibiotic-loaded PMMA hip spacer. The Modular Stage One hip spacer system was used (Biomet, Warsaw, IN). When segmental deficiencies were present in the acetabulum, an antibiotic-loaded PMMA spacer was formed in-situ in the pelvis/acetabulum using a large monopolar head trial as a mold. The cement was secured with two to four 6.5mm titanium cancellous screws placed partly into bone to serve as rebar posts; this prevented spacer displacement. The screws were covered entirely with cement (screwdriver holes were filled with bone wax to allow removal at reconstruction). Cobalt cement was used at resection arthroplasty with the same antibiotic combination as the knee. For revision or reimplantation procedures almost all cases were implanted with cementless reconstruction systems. When a reconstruction cage was used for acetabular reconstruction, the acetabular socket was cemented into the cage with Cobalt cement. One gram of Vancomycin powder was mixed into each 40 gram bag of cement.

For hip cases, the Stimulan antibiotics beads were delivered into the deep hip space inferior to the acetabulum and around the proximal femur. A 10 french Blake Drain was placed just under the Tensor Fascia layer. Additional subcutaneous drains were placed as indicated. No beads were placed in the subcutaneous layer. The Tensor Fascia drain was always removed between 24 and 36 hours. The superficial drains were pulled between 48 and 72 hours. In the two stage septic revision group, a spica brace (set between 20-70 degrees) was used both at explantation (until reimplantation) and reimplantation (4-6 weeks). Figures 3 and 4 (see Appendices) demonstrate surgical technique and placement of Stimulan beads.

Figures 3a – 3c: Radiographs of a 72-year-old male who underwent a single-stage revision protocol for prosthetic femoral-acetabular impingement and clinical pain of his right THA.

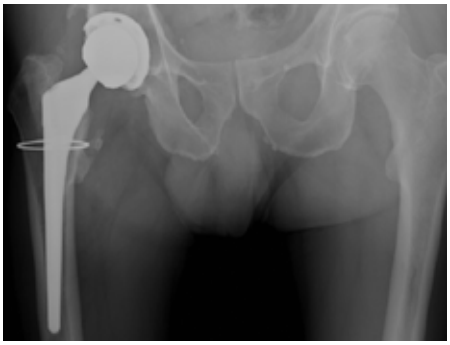

Figure 3a: Preoperative AP radiograph of pelvis. Note small amount of heterotopic bone near lateral acetabulum and lesser trochanter.

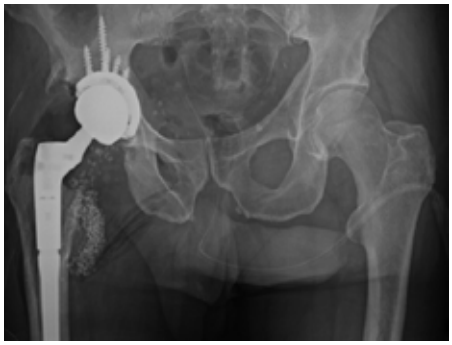

Figure 3b: Postoperative AP radiograph of revision THA. 10cc of Stimulan antibiotic beads were placed within the hip joint, mainly inferiorly. The beads gravitated to this region as this area was dissected to remove the heterotopic bone and scar tissue from the proximal femur.

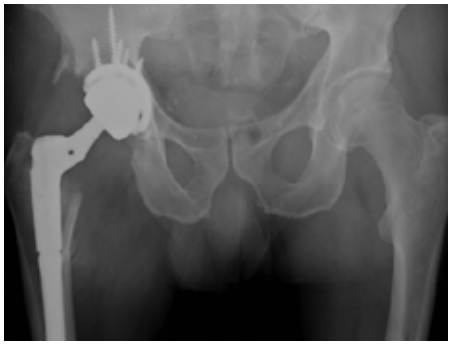

Figure 3c: Postoperative AP radiograph taken 3 months after revision surgery. All Calcium Sulfate beads have dissolved. Note no new heterotopic bone has formed. This patient did not receive any perioperative treatment to prevent heterotopic bone formation (i.e., no radiation or Indocin).

Figures 4a – 4f: Radiographs of a 64-year-old male who underwent a two-stage revision protocol for a chronic periprosthetic infection of his right THA. The patient suffers from hypertrophic osteoarthritis and DISH.

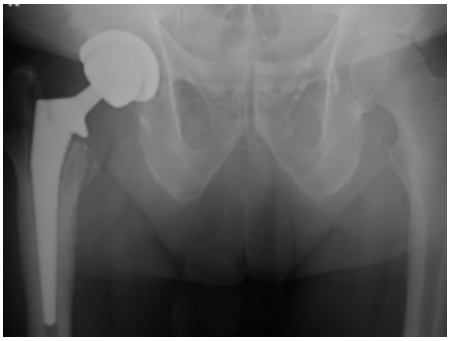

Figure 4a: Preoperative AP radiograph of pelvis showing infected right THA. Preoperative aspiration grew Staphylococcus epidermidis. Note endosteal resorption of bone around proximal femoral stem.

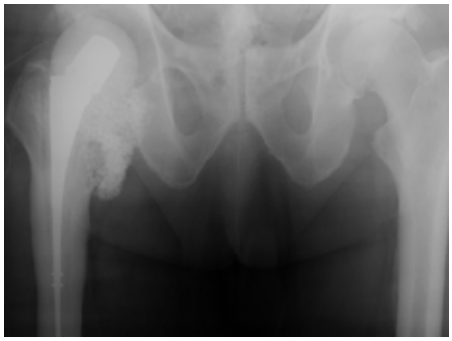

Figure 4b: Postoperative radiograph of resection arthroplasty. The hip was stabilized with a Modular Stage One (Biomet, Warsaw, IN) antibiotic loaded methyl methacrylate articulated spacer. 40cc of Stimulan antibiotic beads were placed into the hip joint. Note drain that was placed underneath the tensor fascia layer at closure.

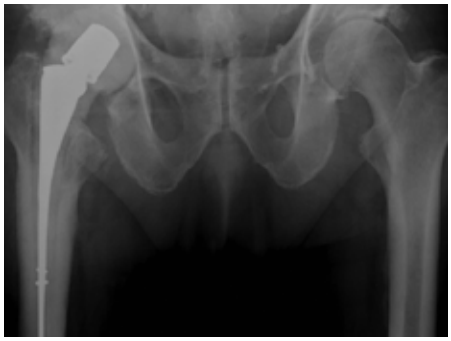

Figure 4c: Preoperative AP radiograph of pelvis prior to reimplantation surgery. This radiograph was taken 8 weeks after resection arthroplasty. All Calcium Sulfate beads have dissolved. Preoperative aspiration of the hip was negative.

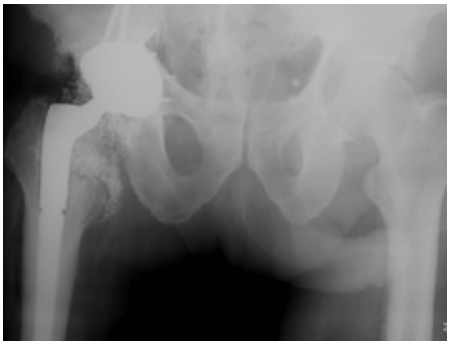

Figure 4d: Postoperative AP radiograph of pelvis at reimplantation surgery. The acetabulum was reconstructed with a porous cup cage (Signature Orthopaedics, Chatsville, AU) with screws. A Magnum cup (Biomet, Warsaw, IN) was cemented into the cup cage. A dual articulating bearing was utilized (Biomet, Warsaw, IN). The femur was reimplanted with an Arcos modular stem (Biomet, Warsaw, IN). At closure, 40cc of Stimulan antibiotic beads were placed into the hip joint.

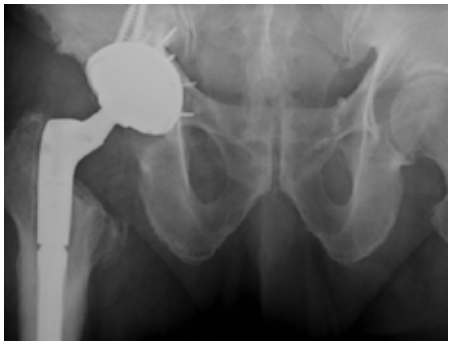

Figure 4e: AP radiograph of pelvis taken 5 weeks after reimplantation surgery. All Calcium Sulfate beads have dissolved. Implants show stable initial fixation.

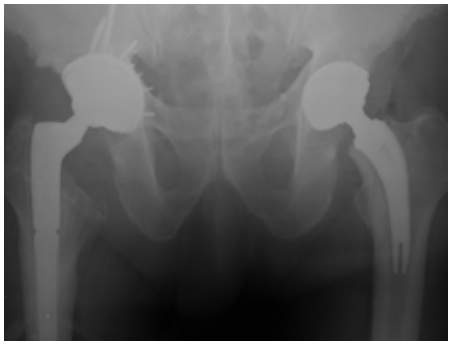

Figure 4f: AP radiograph of pelvis taken 3.5 months after reimplantation surgery. No heterotopic bone has formed. Implants maintain initial biologic integration.

Antibiotic Bead Preparation

This study utilized commercially pure, synthetic neutral pH balanced Calcium Sulfate (Stimulan). The rapid cure kit was used which includes 10cc (20gm) of Calcium Sulfate, 2 pre-measured mixing solution bulbs, 1 syringe, 1 pellet mold, and 1 spatula. The mold produces three different sizes for the beads (3, 4.8, and 6mm) as demonstrated in Figure 5 (See Appendices).

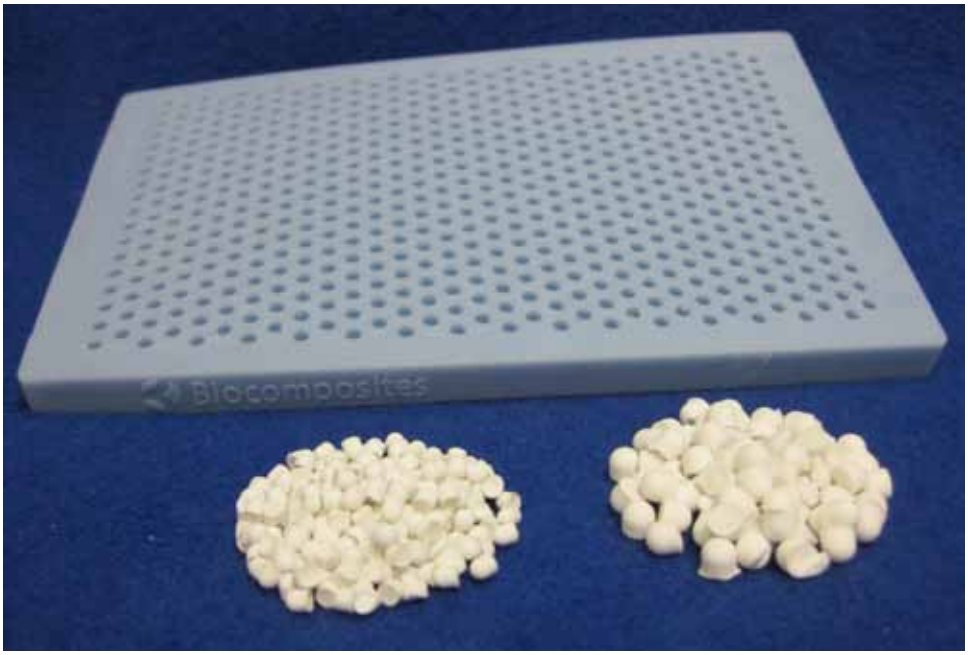

Figure 5: Photograph showing Stimulan Bead Set. The blue plate is a sterile, silicone mat that can make 3 different sized beads (3, 4.8, and 6mm). In this series we primarily used 3 and 4.8mm beads to increase overall surface area and allow for faster dissolution of Calcium Sulfate. The beads shown here are from a 10cc batch of Stimulan. The smaller beads are 3mm diameter beads. The larger beads are 4.8mm diameter beads.

For this study, one gram of Vancomycin powder is added to 10cc (20gm) of Calcium Sulfate and the two powders are well mixed. The mixture is then added to 240mg (40mg/cc) of liquid Tobramycin in a plastic mixing bowl provided in the kit. Ingredients are mixed for 30 seconds until "doughy." The paste is then applied with a spatula into a silicone bead mold and left to set for 10 to 15 minutes with a typical OR room temperature of 60-62° farenheit.[20,21,22] Once set, the beads are harvested and kept in a sterile container until used.

All patients were followed up for a minimum of 3 months. Failure was recognized as the need for component removal for any reason. Monitoring for infection included clinical exam with C-reactive protein tests at 3 months, 6 months, and one year post-operatively. A suspicion of infection prompted a joint aspiration. For patients undergoing reimplantation procedures, a pre-operative negative culture from joint aspiration was mandatory.

Results

The volume of Stimulan antibiotic-loaded beads used for each procedure ranged from 5cc to 70cc in hip cases and 5cc to 50cc in knee cases. As early cases showed no significant clinical problems, the volume of Stimulan beads was gradually increased. The upper limit of bead volume was dependant upon the ability to close the deep soft tissue envelope with a tension free closure. The average volume was different for each of the four different categories and all are listed in Table 1 (see Appendices).

The incidence of wound drainage in this study was relatively low considering the overall complexity of the cases. There were eight cases (3.2%) of post-operative wound drainage requiring intervention. Intervention included lavage and debridement, wound vac placement, and/or application of a compressive dressing on the wound. When the surgical wound began to drain, the post-operative thromboembolic prophylaxis regimen was modified, usually by using mechanical foot pumps, until wound drainage resolved. At the time of debridement surgery, the old Stimulan beads were removed and new beads were inserted into the wound. There were five cases (3.5%) of knee wound drainage, with two cases requiring surgical wound lavage and debridement. There were three cases (2.8%) of hip wound drainage, with two cases requiring surgical wound lavage and debridement.

Heterotopic bone formation was identified in three cases (1.2%). Heterotopic bone formation occurred in one knee case (resection arthroplasty with static spacer) and two hip cases (one resection arthroplasty and one reimplantation procedure).

Heterotopic ossification was seen generally when a large volume of Stimulan was used (average 33cc per case). In all cases, the heterotopic bone was considered mild, rated Brooker I-II class. The character of the heterotopic bone in the two cases that were re-operated (for reimplantation) was considered thin and "wispy." It was easily removed from the surrounding tissues. In review of the post-operative radiographs, the heterotopic bone formed in areas where the Stimulan beads were densely packed.

In two-stage hip and knee procedures, we were able to inspect the surgical wounds at reimplantation. The time between resection and reimplantation ranged from 9 to 15 weeks, with an average of 12 weeks. In all cases, there were no observable beads remaining. In twenty percent of the cases we noticed that the synovium was coated with a thin white layer of material that could not be rubbed away. This white material was typically located within the medial and lateral gutters of knee cases and in the infra-acetabular areas of hips. Transection of synovial specimens showed that the white material was only located on the superficial surface of the synovial tissue. The white coating was generally observed when bead volumes of 20cc or more were used.

Out of our 250 procedures there were 29 complications (11 hips and 18 knees) for a complication rate of 11.6%. All complications are listed in Tables 2 & 3 (see Appendices) along with their MSIS-A host grade. A majority of complications occurred in patients with a grade B or C (MSIS-A) medical host. Eight of the 29 complications had wound related complications (3 hips and 5 knees). There were nine failures (3.6%) in this study. All failures are listed in Tables 4 & 5 along with their medical host grade. Six failures were a result of infection. Excluding the above infection failures, all remaining patients had a normal C-reactive protein when tested between 6 and 12 months post-operatively.

Discussion

In this series we used Stimulan as a vehicle to deliver a localized dose of antibiotics to an area at risk for infection (i.e. operative wound). This is a preliminary study to gauge the effectiveness of utilizing this particular carrier in septic and aseptic revision joint arthroplasty of the hip and knee. The strategy of using a localized antibiotic delivery system is that it avoids the potential toxicity of intravenous antibiotics. The side effects of even short courses of IV antibiotics are well documented.[1] Localized delivery via Stimulan into a joint replacement has already been shown to deliver antibiotics up to 50 times greater than MIC levels for many pathogenic bacteria found in orthopaedic infections.[15,16,23,24,33] A local antibiotic delivery system is appealing, as it offers a high local concentration of the antibiotics with low serum levels. In contrast, antibiotic-loaded bone cement (PMMA) has historically been an alternative system used for local antibiotic delivery, but there are problems with this method. Firstly, the antibiotic is released by surface bleaching, not elution. This results in relatively low local drug concentrations.[23] There is also the need for a second surgery to remove the cement beads in single stage procedures. Furthermore, only heat stable antibiotics can be utilized with PMMA. Biodegradable delivery systems are more attractive because they provide solutions for these issues encountered with the PMMA method of antibiotic delivery.[1,2,9,13,28,31,32,46]

Calcium Sulfate has been employed as a bone void filler for a long time and its popularity as a local antibiotic delivery system is growing in the treatment of musculoskeletal infections.[7,12,17,29,34,36,37,38] Antibiotic-loaded dissolvable Calcium Sulfate beads have previously been used in clinical trials, but the results have not been favorable. Among the main problems encountered are post-operative wound drainage and a toxic reactive synovitis that occurs when beads are placed within a joint. Wound complication rates were reported to be between 25-30% with several explanations existing for such regular occurrence.[22,3,30,40] The predominant thinking attributes the cause of wound drainage to the purification processes of "traditional" Calcium Sulfate products. Prior to Stimulan, all Calcium Sulfate products were derived from gypsum harvested from the earth. Various proprietary filtering and wash processes were developed to produce medical grade Calcium Sulfate products. Despite arduous attempts to derive pure Calcium Sulfate products, however, impurities still exist.[22,35] Additionally, the chemicals used to wash the

gypsum product still remain within the Calcium Sulfate. The result is that the product, once delivered into the human body, is non-physiologic and potentially inflammatory when exposed to the synovial fluid environment. In contrast, Stimulan is derived from commercially pure, synthetic Calcium Sulfate which is blended via a proprietary process to create a product that is considered less "harsh" to the synovial joint environment. It was for this reason that this study was undertaken.

In our study, the incidence of wound drainage in revision joint arthroplasty was found to be low. Overall 3.2% of cases experienced wound drainage. A majority of the 8 occurrences were found in medically compromised hosts (MSIS-A Grade B or C hosts). Furthermore, wound drainage tended to present in cases where the volume of beads used was ≥30cc. There are several possible explanations for this occurrence. One explanation is that the large volume of beads caused excessive mechanical stretching of the deep soft tissue envelope with joint range, causing the wound to leak. Another possibility is a chemical effect, as large volumes of beads could potentially cause a hyperosmotic effect resulting in joint distension and wound leakage. A third possible factor is the quality of the local tissues and the health of the patient. In the revision scenario, soft tissues are often attenuated from previous surgery and mechanical damage to the local tissues is commonly encountered. This, combined with poor systemic health (e.g. diabetes, smoking, prednisone treatment), leads to wound drainage. We believe that wound drainage can be mitigated by employing modest bead volumes (<30cc) combined with surgical techniques which encourage a water-tight deep soft tissue envelope.

Heterotopic ossification is another potential concern with the use of Calcium Sulfate as a dissolvable pellet. Calcium Sulfate, when used in the intra-osseous environment, is an osteoconductive agent. Its application as a bone void filler is well established.[25,41,44,45] When it is placed within the intra-articular environment, the beads are dissolved within the synovial fluid and eventually resorbed. However, if there is a reduced synovial fluid environment (i.e. arthrofibrosis) and exposed intra-articular bone (from periosteal stripping during surgery) the Calcium Sulfate may have sufficient osteoconductive influence to form new periarticular bone. This is especially so when endoprosthetic hinge devices about the knee are used. Our overall incidence of heterotopic bone in this series was 1.2%. The type of heterotopic bone tended to be thin and laminate. In most cases the heterotopic bone did not dramatically affect joint function. In cases of resection arthroplasty where heterotopic bone formed, it was easily removed at the time of reimplantation. We feel that heterotopic bone formation is not a major prohibitive complication for using commercially pure, synthetic antibiotic-loaded Calcium Sulfate dissolvable beads.

A potential drawback to using Calcium Sulfate in revision joint arthroplasty is the potential for mechanical abrasion of the prosthetic articular surfaces. The beads within the joint envelope can migrate and get in between the articular surfaces. With weight bearing, the beads can get crushed and can potentially cause scratching of the articular surfaces. Current work is ongoing in 6 retrieval Prostalac spacers to look at the articular surfaces for pitting and scratching (Clarke I, McPherson EJ, Peterson Tribology Laboratory, Loma Linda, CA). Maale et al reported using Stimulan beads loaded with Vancomycin and Tobramycin in single-stage septic revision total knee arthroplasty. They found that the Stimulan beads were soft after hydration and do not scratch the joint surface.[23,24] Even if Calcium Sulfate beads did create scratches or polyethylene pitting, their use for localized antibiotic delivery in periprosthetic infection or total joints at risk for infection still may be preferable. Their use will depend on a case by case risk assessment. Long term clinical follow-up is needed to answer this question more definitively.

In summary, we find the use of commercially pure, synthetic antibiotic-loaded Calcium Sulfate is an acceptable adjuvant treatment tool in revision total hip and total knee arthroplasty. We noted low rates of postoperative wound drainage and heterotopic bone formation. In contrast, prior Calcium Sulfate products derived from processed gypsum have shown significant problems with wound healing and wound drainage.[1,2] The Stimulan-antibiotic construct is adaptable, whereby various antibiotic formulas can be utilized.[3] Furthermore, this localized antibiotic delivery method is relatively inexpensive and obviates the need for a second surgery (i.e. removal of PMMA antibiotic beads).

Initial observations with Stimulan antibiotic beads are encouraging. We will continue to explore and research the efficacy of antibiotic-loaded Stimulan beads. Our next phase is to measure local antibiotic concentrations in-vivo in revision joint arthroplasty. We strive to document and corroborate prior findings by Maale and Eager who showed high local antibiotic concentrations within prosthetic knee cases. Additionally, we will continue to review the mechanical effects that Calcium Sulfate beads have upon the articular surfaces of prosthetic implants. Finally, we would like to conduct a study to examine the potential of improving the results of PJI with Stimulan beads via randomized multicenter trials.

Table 1 - Results

Aseptic Revision TKA		***Aseptic Revision THA***	
N=66		N=58	
Avg Stimulan/case	16cc	Avg Stimulan/case	20cc
Complications	6	Complications	4
Failures	2	Failures	2
Deceased	1	Deceased	1
Drainage	1	Drainage	2
DECRA TKA		***DECRA THA***	
N=16		N=8	
Avg Stimulan/case	21cc	Avg Stimulan/case	35cc
Complications	1	Complications	1
Failures	2	Failures	1
Deceased	0	Deceased	2
Drainage	0	Drainage	1
Resection TKA		***Resection THA***	
N=35		N=24	
Avg Stimulan/case	27cc	Avg Stimulan/case	33cc
Complications	7	Complications	4
Failures	0	Failures	1
Deceased	0	Deceased	0
Drainage	2	Drainage	0
Reimplantation TKA		***Reimplantation THA***	
N=25		N=18	
Avg Stimulan/case	24cc	Avg Stimulan/case	34cc
Complications	4	Complications	2
Failures	0	Failures	1
Deceased	0	Deceased	0
Drainage	2	Drainage	0

Table 2 – Knee Complications

Procedure	***Case #***	***Volume***	***MSIS-A Host Grade***	***Complication***
Aseptic Knee Revisions	Knee 1	10 cc	B	Dynamic rotational instability with buckling.
	Knee 2	10 cc	B	Wound drainage, cellulitis, periprosthetic infection with wound drainage. I&D with modular bearing exchange. No infection at 2-year follow-up.
	Knee 3	10cc	C	Acute knee infection from dental abscess. Failed DECRA. Implants resected 5 months post-op.
	Knee 4	10cc	A	Arthrofibrosis – knee manipulation.
	Knee 5	20cc	A	Extensor lag.
	Knee 6	10cc	A	Fall with traumatic arthrotomy. I&D and reclosure. No infection at 1-year follow-up.
Aseptic Knee Revisions	Knee 1	10 cc	B	Dynamic rotational instability with buckling.
Aseptic Knee Revisions	Knee 1	10 cc	B	Dynamic rotational instability with buckling.
	Knee 9	20cc	B	Heterotopic bone formation in medial and lateral gutters. Removed at reimplantation.
	Knee 10	50cc	A	Secondary infection with Candida Albicans. Repeat debridement and spacer exchange.
	Knee 11	30cc	A	Wound drainage. Wound vac applied. Drainage resolved.
	Knee 12	30cc	B	Superficial wound dehiscence with drainage. Wound revised and closed.
	Knee 13	40cc	B	Acute renal failure, Creatinine 3.6. No dialysis.
	Knee 14	30cc	B	Acute on chronic renal failure. Dialysis for 3 weeks. Resolved to baseline.
Aseptic Knee Revisions	Knee 1	10 cc	B	Dynamic rotational instability with buckling.
	Knee 16	20cc	C	Wound drainage, I&D. Recurrent infection. AKA.
	Knee 17	30cc	C	Wound drainage at 2 weeks post-op. I&D with lavage. Stable.
	Knee 18	10cc	B	Partial small bowel obstruction. Readmitted at 3 weeks post-op for 5 days. No surgery required.

Table 3 – Hip Complications

Procedure	*Case #*	*Volume*	*MSIS-A Host Grade*	*Complication*
Aseptic Hip Revisions	Hip 1	10cc	B	Wound drainage at 3 weeks post-op. I&D with additional antibiotics beads. Wound infection at 2 months post-op. I&D with antibiotics beads. Stable at 1 year.
	Hip 2	30cc	B	Implant Bow Mismatch. Distal Femoral crack. No additional surgery.
	Hip 3	10cc	B	Hematoma with drainage. I&D with evacuation of the hematoma at 2 weeks post-op.
	Hip 4	10cc	B	Infection. I&D at 4 weeks post-op. Negative aspiration culture at 6 months post-op.
Hip DECRAs	Hip 5	40cc	B	Wound drainage post-op. Malnutrition, albumin 2.1. Recurrent infection at 3 months. Hip Resected at 6 months.
Hip Resections	Hip 6	40cc	A	DVT Rt. Arm from PICC line at 6 weeks post-op. Coumadin therapy.
	Hip 7	40cc	A	Heterotopic bone formation. Removed at reimplantation.
	Hip 8	40cc	B	Heterotopic bone formation. Removed at reimplantation.
	Hip 9	20cc	B	Intra-operative hypotension, sepsis.
Hip Reimplants	Hip 10	40cc	B	Recurrent dislocation. Revision to constrained socket.
	Hip 11	70cc	B	Wound drainage. Clear serous fluid. Wound Vac applied for 5 days.

Table 4 – Knee Failures

Procedure	*Case #*	*Volume*	*MSIS-A Host Grade*	*Reason for failure*
Aseptic Knee Revisions	Knee 20	20cc	B	MRSA infection, extensor allograft removal, lavage debridement. Implant infection free at 1 year.
	Knee 21	20cc	A	Infection -Staph A. Implants resected for 2 stage protocol.
Knee DECRAs	Knee 22	30cc	C	Failed DECRA. Recurrent infection. AKA.
	Knee 23	10cc	B	Recurrent patellar subluxation. VMO Advancement procedure at 4 months. Stable at 1 year. No infection.

Table 5 – Hip Failures

Procedure	*Case #*	*Volume*	*MSIS-A Host Grade*	*Reason for failure*
Aseptic Hip Revisions	Hip 12	10cc	A	Aseptic loosening cup. Revision to triflange cage.
	Hip 13	30cc	A	Aseptic loosening cup. Revision to custom triflange cage.
Hip DECRAs	Hip 14	30cc	C	Recurrent infection. Patient died of concomitant bowel perforation.
Hip Resections	Hip 15	30cc	C	New infection hip at 6 months post-operative. Dental infection Strept Viridans. DECRA. Implant stable at 18 months. Normal CRP.
Hip Reimplants	Hip 16	20cc	C	Reinfection at 3 months. DECRA procedure. Patient with CLL. Died from blast crisis 6 months after DECRA procedure.

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Eradication of wound-relevant pre-formed biofilms following release of combination antibiotics from absorbable beads *in-vitro*

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Introduction: A key stage in the pathogenesis of periprosthetic joint infection (PJI) is biofilm formation. It is believed that approximately 80% of all PJIs are associated with bacteria which form a biofilm[1]. Once a biofilm has been established in a periprosthetic joint, it is difficult to diagnose and eradicate(**Figure 1**). Successful treatment of periprosthetic joint infection requires surgical intervention alongside antimicrobial therapy targeting surface-adhering microorganisms. The objective of the study was to assess the ability of synthetic recrystallised calcium sulfate beads* (SRCS) or β-tricalcium phosphate/calcium sulfate bi-phasic beads** (TPCS) containing a mixture of vancomycin and gentamicin (VG) or vancomycin and tobramycin (VT) to effectively eradicate pre-formed biofilms *in-vitro*.

Methodology: Single species *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms were established on polycarbonate coupons within a CDC biofilm reactor(**Figure 2**). Biofilms were established in a batch model for 72 hours prior to processing. Biofilms were exposed to a challenge plate containing suspended SRCS beads or TPCS beads containing a mixture of VG or VT.Positive and negative controls were tested concurrently. All testing was performed in triplicate. The challenge plates were incubated for 24 hours at 37°C ± 2°C. Students T-Tests were performed on the raw data to determine the significant effect of the test items.

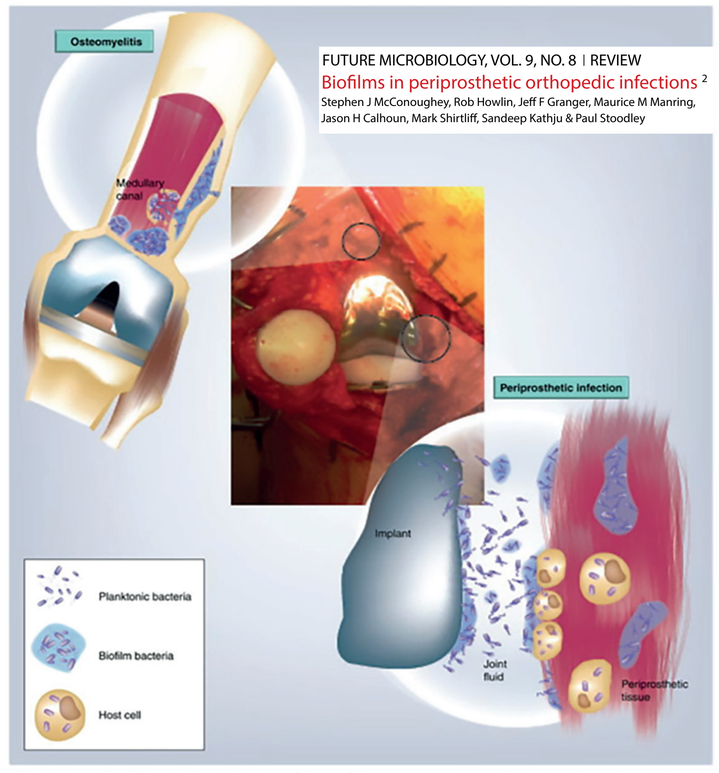
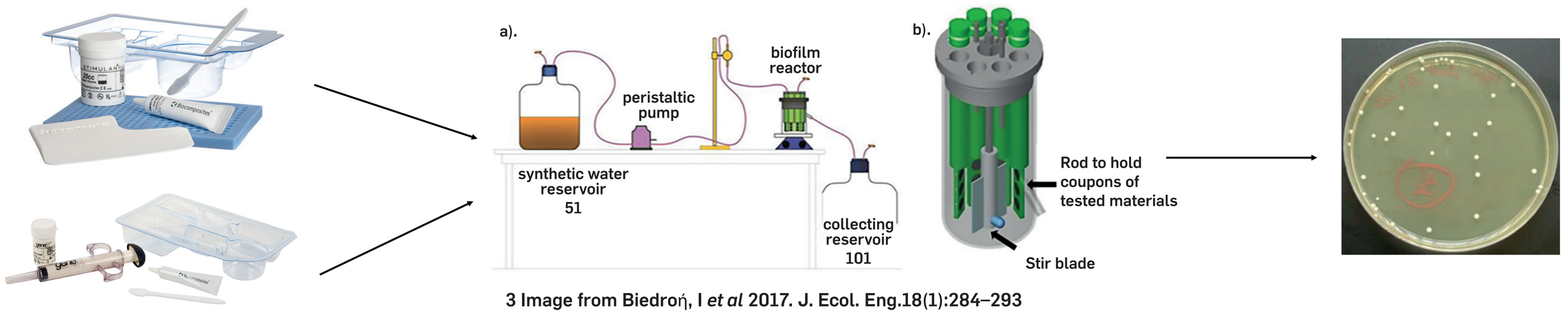


Figure 1. Distribution of biofilms in a periprosthetic joint

Figure 2. CDC reactor model demonstrating formation of biofilms onto polycarbonate coupons



3 Image from Biedroń, I *et al* 2017. J. Ecol. Eng.18(1):284–293

Results: Negative controls retained 6.78 ± 0.23 Log10CFUmL^{-1}/6.94 ± 0.11 Log10CFUmL^{-1} and 6.60 ± 0.23 Log10CFUmL^{-1}/5.08 ± 0.09 Log10CFUmL^{-1} from *P. aeruginosa* and *S. aureus* biofilms respectively. No viable organisms were recovered from biofilms exposed to the positive control or those exposed to SRCS(**Figure 3a**) or TPCS beads(**Figure 3b**) containing a mixture of VG/VT within detection limits. This equated to an average log reduction in *P. aeruginosa* of >5.78 Log10CFUmL^{-1} and an average log reduction in *S. aureus* of >5.60 Log10CFUmL^{-1} ($p < 0.001$) against SRCS beads(**Figure 3a**) and an average log reduction in *P. aeruginosa* of >5.94 Log10CFUmL^{-1} and an average log reduction in *S. aureus* of >4.08 Log10CFUmL^{-1} ($p < 0.001$) against TPCS beads(**Figure 3b**).

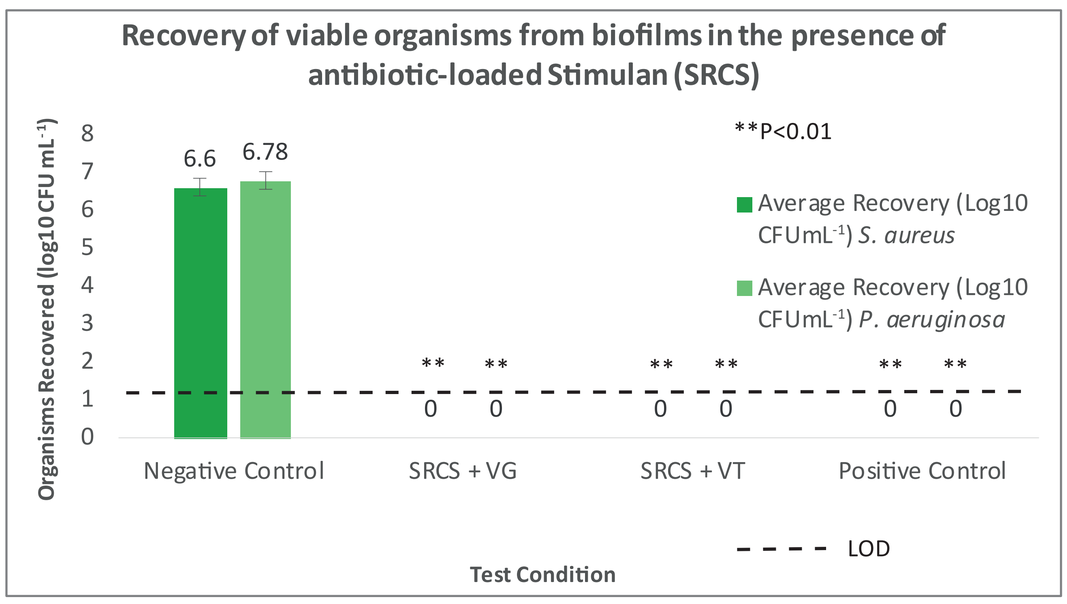


Figure 3a. Recovery of viable organisms from biofilms in the presence of SRCS beads

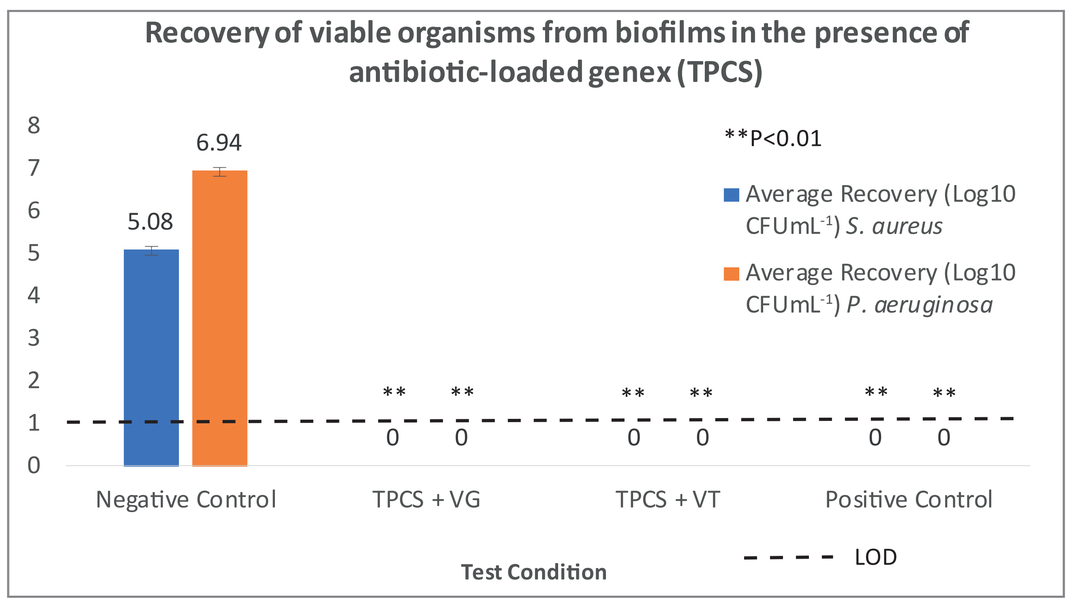


Figure 3b. Recovery of viable organisms from biofilms in the presence of TPCS beads

Conclusions: Exposure of the biofilm to SRCS and TPCS beads containing a mixture of VG or VT resulted in eradication of pre-formed biofilms in the test method described. Further assessment is required to confirm clinical performance.

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Complete Killing of Agar Lawn Biofilms by Systematic Spacing of Antibiotic-Loaded Calcium Sulfate Beads

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Abstract: Background: *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* (SA) are the major causative agents of acute and chronic infections. Antibiotic-loaded calcium sulfate beads (ALCSB) are used in the management of musculoskeletal infections such as periprosthetic joint infections (PJI). **Methods:** To determine whether the number and spatial distribution of ALCSB are important factors to totally eradicate biofilms, ALCSBs containing vancomycin and tobramycin were placed on 24 h agar lawn biofilms as a single bead in the center, or as 16 beads placed as four clusters of four, a ring around the edge and as a group in the center or 19 beads evenly across the plate. Bioluminescence was used to assess spatial metabolic activity in real time. Replica plating was used to assess viability. **Results:** For both strains antibiotics released from the beads completely killed biofilm bacteria in a zone immediately adjacent to each bead. However, for PA extended incubation revealed the emergence of resistant colony phenotypes between the zone of eradication and the background lawn. The rate of biofilm clearing was greater when the beads were distributed evenly over the plate. **Conclusions:** Both number and distribution pattern of ALCSB are important to ensure adequate coverage of antibiotics required to eradicate biofilms.

Keywords: antibiotic tolerance; biofilm; antibiotic-loaded bone cement; *pseudomonas*; *staphylococcus*; persister; periprosthetic joint infection

1. Introduction

Pseudomonas aeruginosa (PA) is a Gram-negative, opportunistic bacterium associated with periprosthetic joint infections (PJI) [1]. *Staphylococcus aureus* (SA), a Gram-positive coccus, is a leading cause of skin [2,3], soft tissue [4,5], bloodstream [6], pneumonia [7], and bone and joint infections [8,9]. PA and SA have developed diverse strategies to respond and adapt to antibiotic stress, including the formation of antibiotic-tolerant biofilms [10,11].

Biofilms are microbial communities adhering to biotic or abiotic surfaces. In vitro studies and anecdotal clinical evidence suggests that bacteria within biofilms can resist killing at high antibiotic concentrations and often require a prolonged or repeated courses of antibiotics [12,13] at concentrations that are often outside the therapeutic window by systemic administration [14]. Bacteria within a biofilm become tolerant and can survive antibiotic treatments without necessarily having an acquired, heritable, resistance phenotype [15,16].

There are a number of mechanisms for biofilm antibiotic tolerance including restricted penetration of antibiotics, the physiological state of the cells (which can exhibit dormancy or slow growth due to nutrient limitation within the biofilm), and the formation of sub-populations of small colony phenotypes and persister cells (which are dormant even in areas of the biofilm where nutrients are available [17,18]). Repeated cycles of antibiotic exposure and resuscitation of persister cells on removal of antibiotics can lead to resistance [16]. Persistence of bacterial infection due to antibiotic tolerance is a huge problem in the treatment of chronic infections, with last resort antibiotics often becoming ineffective [19,20], leading to serious implications for patient outcome as well as economic loss [20,21].

Diverse mechanisms that confer resistance by PA and SA to antibiotics have been described [22,23]. The common example reported to confer antibiotic tolerance/resistance is inactivation of the antibiotic through the activity of enzymes produced by the bacterial cells [24,25]. The other mechanisms include decreased drug accumulation inside the bacterial cell via active efflux or diminished cell wall permeability [26–29]. Besides these strategies, growth within biofilms [10,11] or the emergence of persister cells [17,18] can contribute to survival in the presence of antibiotics. In addition, slow growing phenotypes are observed in both PA and SA. Reports suggests that small colony variants (SCVs) have been observed after antibiotic exposure, both in vitro and in vivo [30–32]. Hoffman et al. (2006) also showed the co-isolation of *P. aeruginosa* and *S. aureus* in infections such as cystic fibrosis. It has been reported that prolonged growth of *P. aeruginosa* or its exoproduct, 4-hydroxy-2-heptylquinoline-*N*-oxide (HQNO) with *S. aureus* would select for typical *S. aureus* SCV production. The *P. aeruginosa* exoproduct (HQNO) also protects *S. aureus* during coculture from being killed by tobramycin [31]. A recent study has shown the mechanism of antibiotic tolerance of biofilms during agar diffusion antimicrobial susceptibility testing was due to a switch from planktonic to biofilm mode of growth [33]. Generation of antibiotic tolerance and resistance has been associated with the failure of antibiotic treatment and relapse of bacterial infections; therefore, treatment strategies are necessary.

To treat these infections, in the case of patients with PJI, prevention strategies following total joint arthroplasty includes the use of antibiotic-loaded poly(methyl methacrylate) [34,35] or calcium sulfate ($CaSO_4$) beads [36,37], facilitating the achievement of higher local antibiotic concentrations. Absorbable mineral-based materials are not as mechanically strong as acrylic cements, but they provide some advantages for antibiotic release and infection control. They release the full antibiotic load and achieve high levels of antibiotics locally over sustained periods, leaving no residual foreign body that could act as a nidus for biofilm formation. We have previously studied the killing of biofilms of bioluminescent strains of *P. aeruginosa* (PA-Xen41) and *S. aureus* (SA-SAP231) grown on agar surfaces by a combination of vancomycin and tobramycin impregnated calcium sulfate beads for three days [38]. In this present study, we extended the incubation period to determine whether we could achieve complete eradication of biofilm bacteria adjacent to the beads, including resistant phenotypes that develop in the presence of antibiotics. We used the replica plating technique onto non-antibiotic agar to allow persister cells, if present, to grow. In addition, we assessed whether the spatial pattern of the beads was important to completely eradicate the biofilms grown on agar surfaces by systematic

arrangement of antibiotic-loaded calcium sulfate beads (ALCSBs) since clinically beads sprinkled into the surgical site show different densities and patterns of clustering [36,39]. The overall goal of this study was to determine whether the arrangement of antibiotic loaded beads was an important factor in completely eradicating biofilms of PA and SA in a simple in vitro assay.

2. Methods

2.1. Bacterial Strain and Culture Conditions

Bioluminescent strains of *P. aeruginosa* PA-Xen41 [40] (Perkin-Elmer, Waltham, MA, USA) and USA300 *S. aureus* SA-SAP231 [40] were used. The glycerol stock cultures were stored at −80 °C and streaked onto fresh tryptic soy agar (TSA) and brain heart infusion (BHI) agar for PA and SA, respectively, and incubated for 24 h. The isolated colonies from the respective plates were transferred aseptically to 20 mL of TSA and BHI broth using a sterile inoculating loop and incubated overnight on a shaker incubator set at a temperature of 37 °C and a speed of 200 rpm. These bioluminescent strains have been genetically engineered to constitutively give off light when they are metabolically active and as such are useful tools to non-invasively track temporal and spatial changes in biofilm activity.

2.2. Preparing Lawn Biofilms

Lawn biofilms of PA-Xen41 and SA-SAP231 were prepared by spreading the overnight cultures onto TSA and BHI agar respectively, unless otherwise stated. Briefly, 100 µL of the overnight culture was mixed with 9.9 mL of liquid medium to make a 1:100 dilution. 200 µL of the diluted culture was spread onto 90 mm diameter polystyrene Petri dishes (Thermo-Fisher Scientific, Waltham, MA, USA) containing TSA or BHI agar. The plates were incubated at 37 °C for 24 h to develop lawn biofilms [41].

2.3. Preparation of Antibiotic-Loaded Calcium Sulfate Beads (ALCSB)

ALCSBs were prepared using 10 cc Stimulan® Rapid Cure as per directions from Biocomposites Ltd. (Keele, Staffordshire, UK) and in previously described methods [38]. Tobramycin (240 mg, Sigma-Aldrich, St. Louis, MO, USA) and vancomycin (1000 mg, Sigma-Aldrich, St. Louis, MO, USA) combinations were used to prepare the beads [36,38]. ALCSBs of 4.8 mm diameter were prepared using standard mold mats (Biocomposites Ltd, Keele, UK). The beads were left to cure for 1 h and removed from the mold mats by manual twisting. ALCSB were stored at 4 °C until use in the experiments. Vancomycin and tobramycin are commonly incorporated into bone cement and Stimulan absorbable bone filler to provide broad coverage of Gram-positive and -negative pathogens commonly associated with PJI [37,42].

2.4. Killing of Lawn Biofilms Using Antibiotic Beads

A combination of vancomycin and tobramycin was used to examine the killing of biofilms of PA-Xen41 and SA-SAP231. Briefly, after generation of a 24 h lawn biofilm on agar using the method described above, the ALCSB (containing vancomycin and tobramycin) were placed in the center of the plates containing pre-grown 24 h lawn biofilms of PA-Xen41 or SA-SAP231 using sterile forceps. ALCSBs were gently pushed into the agar to allow uniform diffusion of antibiotics throughout the medium. Plates were incubated at 37 °C with 5% CO_2 for seven days and images (both normal digital photographs and in vivo imaging system [IVIS]) were captured every day. The IVIS system has a very sensitive photon detector that can capture the low levels of light produced by the bioluminescent strains of bacteria. The relative brightness in an image is captured as a grey scale ranging from white (very bright indicating relatively high metabolic activity) to black indicating no detection of metabolic activity. To enhance visual detection of features, the greyscale image is false colored to convert to a "heat map" in which red is mapped to white showing the highest level of metabolic activity with blue being close to black indicating low relative metabolic activity and black indicating no detected

light. Since lack of metabolic activity does not necessarily mean that the cells in the biofilm are dead, a culturing technique (replica plating in the present study) is required to assess viability.

2.5. Killing of Lawn Biofilms by Antibiotics Impregnated on Filter Paper Discs

To determine whether the antibiotic carrier material had a role in the growth of the antibiotic resistant phenotypes seen with PA-Xen41, filter paper discs (6 mm, Sigma Aldrich, St. Louis, MO, USA) were impregnated with tobramycin and used instead of ALCSB. Tobramycin alone at a concentration of 100 μg/disc was used. Since PA-Xen41 was not susceptible to vancomycin, thus only tobramycin was used. The discs were placed on the pre-grown lawns of PA-Xen41 and incubated for seven days, and IVIS images were captured every day. For comparative purposes we also used ALCSB loaded with tobramycin alone (240 mg/10 cc pack).

2.6. Effect of ALCSB Arrangements on Killing of Lawn Biofilms

ALCSB containing vancomycin and tobramycin were placed on 24 h biofilms of PA-Xen41 and SA-SAP231 as: (i) a single bead in the center, sixteen beads placed as (ii) sets of four beads (iii) circularly, close to the edges of the plates, (iv) in the center, and (v) 19 beads placed hexagonally equidistant. For the hexagonal bead placement patterns, we used 19 beads, since previously we found that the area of the zone of biofilm killing (ZOB-K) for a single ALCSB was 1.8 cm^2, with the intention that zones of biofilm killing would overlap. These patterns were chosen to represent a range of different possibilities of clustering patterns that might occur from the manual sprinkling of beads into the surgical site; in this situation, unless there are enough beads to completely fill the site, inevitably, there will be some clustering while some beads remain more isolated [36,39]. The ZOB-K and the appearance of antibiotic-resistant phenotypes were monitored daily using IVIS and white light camera imaging.

2.7. Determination of Killing of Biofilms Using Replica Plating Technique

Spatial killing of lawn biofilms of PA-Xen41 and SA-SAP231 was determined using a traditional replica plating technique with minor modifications [43]. Briefly, a sterile cotton velveteen square cloth (150 mm by 150 mm) was aseptically draped over a PVC Science-ware replica plater (Sigma-Aldrich, St. Louis, MO, USA) and locked in place with an aluminum ring. ALCSBs were removed from the plate at day seven using sterile forceps. Plates were inverted over the velveteen cloth and tapped gently to ensure complete contact of the lawn biofilm with the cloth. A sterile plate containing TSA with tobramycin (5 μg/mL) was also inverted onto the velveteen cloth containing the previously stamped cells. Secondly, a fresh TSA containing plate without antibiotics was placed on the velveteen cloth and tapped gently to ensure complete surface contact. Both replica plated plates were incubated for five days at 37 °C in an incubator with 5% CO_2 to allow for the appearance of slow growing colonies. Colonies that grew out from the lawn and on the replica-plates were presumed to be resistant phenotypes. Colonies that appeared on the replica plates (without antibiotic) but not on the original tobramycin-containing plates were presumed to be persister cells [44–48].

2.8. Image Analysis to Determine the Influence of Bead Number on Killing of Lawn Biofilms

IVIS images of lawn biofilms of PA-Xen41 and SA-SAP231 subjected to ALCSB containing vancomycin and tobramycin at 24 h were subjected to image analysis using ImageJ (Version 1.51h) [49]. The plot profile function was used to measure the distance between the cleared edge and the peripheral bead. A circular region of interest (ROI) was used to measure the cleared area and the area of a single bead (20.1 ± 2.3 mm^2, n = 4, equivalent to a diameter of 4.94 mm, very close to mold size of 4.8 mm). Distance cleared from the bead, area cleared, and area cleared per bead of single and clusters of four and 16 ALCSB were compared.

2.9. Antibiotic Carryover During Replica Plating

To determine whether there was a carryover of antibiotic by velveteen cloth during replica plating, the ALCSBs were placed on a TSA plate for five days and replica plated onto fresh TSA plates. The replica plates were then spread with PA-Xen41 and were incubated at 37 °C in an incubator with 5% CO_2.

2.10. Estimation of Antibiotic Concentration Eluted

Tobramycin-loaded beads were placed in the sterile agar plates. Plates were incubated at 37 °C with 5% CO_2 and at various time points, plates were removed and marked with 5 mm by 5 mm squares from the edge of the bead to the edge of the plate. Each of these squares was excised using a sterile razor blade and forceps. The excised plugs were melted at 80 °C in a water bath. An overnight culture of *P. aeruginosa* PAO1 was diluted to an optical density measured at a wavelength of 600 nm OD_{600}) of 0.1 and spread onto sterile TSA plates. A sterile filter paper disc was then placed in the center of the plate and 10 µL of the melted plug was placed onto the paper disc. The plates were incubated for 24 h at 37 °C with 5% CO_2. After incubation, the zones of inhibition were measured and compared to a standard curve for tobramycin prepared in TSA to determine the concentration of antibiotic in the melted plug. This process was repeated at the various time points, using different plates each time.

3. Statistical Analysis

All experiments were performed in triplicate. Control and treated samples were compared by *t*-test, assuming equal variance. Student's *t*-test was used for all other comparison of differences between means, whereby $p < 0.05$ was considered significant. Data represented are plotted as mean ± SD.

4. Results

4.1. Killing of Lawn Biofilms by ALCSB

Bioluminescent strains of PA-Xen41 and SA-SAP231 were used in this study. The use of bioluminescent strains has the advantage of enabling identification of the zone of biofilm killing and the growth of antibiotic-resistant phenotypes on the lawn biofilms, which are otherwise difficult to differentiate with normal photographs (Figure 1). The previously established method for lawn biofilm formation was used, which considers an immobile community of bacteria attached to agar surface, embedded in an exo-polymeric substance (EPS), with high cell number and exhibiting tolerance towards antibiotics [41]. The initial cell concentrations of the 24 h lawn biofilms were in the range of $1 \pm 3 \times 10^9$ CFU/mL. The minimum inhibitory concentration (MIC) of both vancomycin and tobramycin against SA-SAP231 was 2.0 µg/mL and for PA-Xen41, the MIC of tobramycin was 1.5 µg/mL. Previous reporting has shown that vancomycin had no effect on the growth of PA-Xen41 [38]. Clinically used combinations of antibiotics (vancomycin and tobramycin) eluting from the ALCSB showed an increase in the zone of killing of biofilms over time (Figure 1). The zone of killing was observed in the case of both PA-Xen41 and SA-SAP231 biofilms, with rapid killing of PA-Xen41 as compared to SA-SAP231 biofilms (Figure 1). PA-Xen41 is not sensitive to vancomycin; therefore, the zone of biofilm killing with vancomycin was not determined against PA-Xen41.

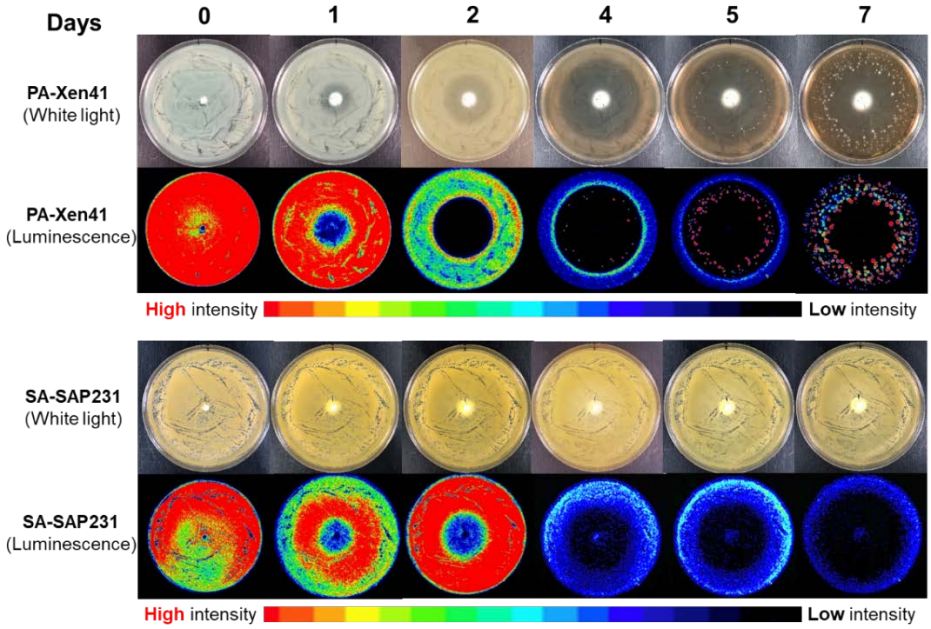

Figure 1. Killing of lawn biofilms of *P. aeruginosa* (PA-Xen41) and *S. aureus* (SAP231) with vancomycin and tobramycin loaded beads at the center. Zone of biofilm killing (ZOB-K) was monitored every day for seven days, and antibiotic-resistant phenotypes were observed in PA-Xen41 beginning at day four.

4.2. Evidence of Antibiotic-Resistant Phenotypes

Antibiotic-resistant phenotypes were observed with vancomycin and tobramycin against PA-Xen41 (Figures 1 and 2A). These phenotypes were not evident in SA-SAP231 when treated with vancomycin and tobramycin (Figures 1 and 2B). Three different zones were evident on biofilms of PA-Xen41 treated with ALCSB; (i) the zone of the edge of the still active biofilm lawn (ZOB-L), (ii) zone of biofilm resistance (ZOB-R), and (iii) the zone of biofilm killing (ZOB-K), (Figure 2A). SA-SAP231 biofilms treated with vancomycin and tobramycin had two zones (Figure 2B), the ZOB-L, ZOB-K but no ZOB-R. Antibiotics, vancomycin and tobramycin containing ALCSB showed the killing of PA-Xen41 for three days. Antibiotic resistant colonies emerged beginning at day four, that were clearly visible thereafter (Figure 1). The generation of antibiotic resistant colonies was evident away from the ALCSBs with the zone of killing towards the area surrounding the ALCSB. The ZOB-R followed the ZOB-K which showed the presence of antibiotic resistant colonies and the ZOB-L that diminished over time (Figure 2A). ZOB-R increased over time suggesting the rapid growth of antibiotic-resistant cells within the area of ZOB-K, where antibiotics gets depleted. Tobramycin alone showed similar tolerant zones on PA-Xen41 lawn biofilms at day four as observed with vancomycin and tobramycin (Figure S1). In the case of SA-SAP231, vancomycin and tobramycin had no tolerant phenotypes; however, with tobramycin alone antibiotic-resistant colonies were evident at day six (data not shown). Using the filter paper discs as antibiotic carrier, antibiotic-resistant phenotypes were also evident as with ALCSB. The generation of antibiotic resistant phenotypes were independent of the carrier material, whether tobramycin-impregnated filter paper discs or ALCSBs (Figure S1). From the IVIS images, we estimated that the concentration of these residual viable phenotypes in the PA zone of lawn clearing was approximately 10 CFU/cm^2. Since the initial 24 h lawn contained approximately 1.2×10^6 CFU/cm^2 [40], these represent a very low frequency of approximately 1:100,000 and may easily be missed by conventional clinical methods or a conventional Kirby-Bauer assay, which has a much lower initial concentration of cells on a plate (i.e., 100 µL of approximately 10^5 CFU/mL spread on a normal 100 mm diameter plate would be approximately 1000 CFU/cm^2).

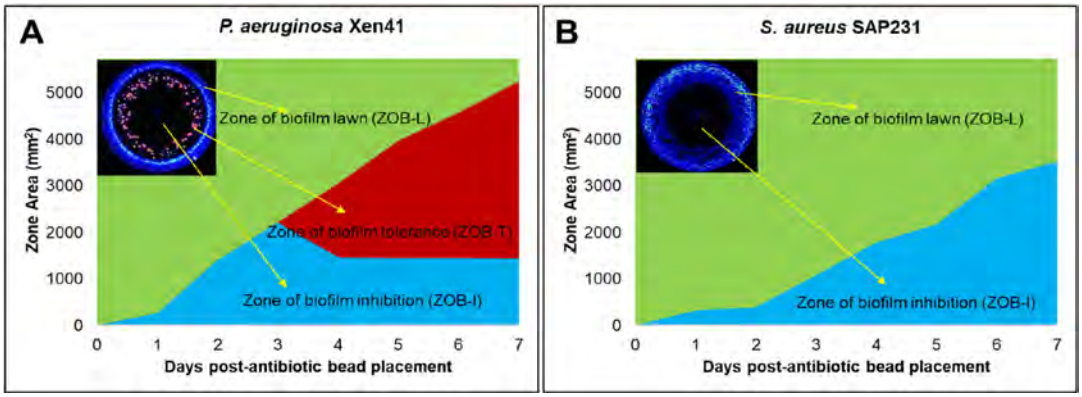

Figure 2. Different zones were developed after placement of vancomycin and tobramycin loaded beads on lawn biofilms of (**A**) PA-Xen41 and (**B**) SA-SAP231. Zone of biofilm killing (ZOB-K), zone of biofilm resistance (ZOB-R), and zone of biofilm lawn (ZOB-L) were evident over time after antibiotic bead placement. Images in insets show vancomycin and tobramycin treated day five lawn biofilms of Xen41 (**A**) and day six lawn biofilms of SAP231 (**B**).

4.3. Killing of PA-Xen41 and SA-SAP231 Lawn Biofilms by Systematic Arrangement of ALCSB

The ALCSBs containing vancomycin and tobramycin were arranged on the lawn biofilms of PA-Xen41 and SA-SAP231 as: (i) a single bead in the center, (ii) 16 beads placed as four clusters of four, (iii) 16 beads placed in a ring, (iv) 16 beads placed as a group in the center, or (v) 19 beads placed evenly across the Petri dishes (Figure 3). Compared to a single bead with vancomycin and tobramycin, all four bead arrangements were more effective in killing biofilms of PA-Xen41 (Figure 3A,B) and SA-SAP231 (Figure 3C,D). The PA-Xen41 lawn biofilms were rapidly killed at day three, except for the 16 beads when placed in the center, which showed loss of activity by IVIS at day four (Figure 4A). With all the bead placements, the killing of biofilms of PA-Xen41 at days one and two and SA-SAP231 at days two, three, and four were significantly different than the beads in the center ($p < 0.05$, Figure 4A,B). In the case of SA-SAP231, the lawns were killed at day five with all the ALCSB arrangements (Figure 4B), except the lawn with 16 beads placed in the center, which showed loss of activity under IVIS at day six. The arrangements of beads closer to the edges, all beads in the center, and the beads placed in hexagonal patterns rapidly eradicated the lawn biofilms within five days for both strains (Figure 4A,B). There could be a possibility of an edge effect, since the beads are very close to the edges of the petri plates, thereby limiting the diffusion of antibiotics. When all the beads were placed in the center, they did not effectively eradicate biofilms in the case of SA-SAP231 (Figures 3D and 4B). The bead placement showed significant killing of biofilms as compared to a single bead. The pattern of ALCSB placement was important in complete killing of lawn biofilms with no generation of antibiotic-resistant colonies (Figure 3A–D).

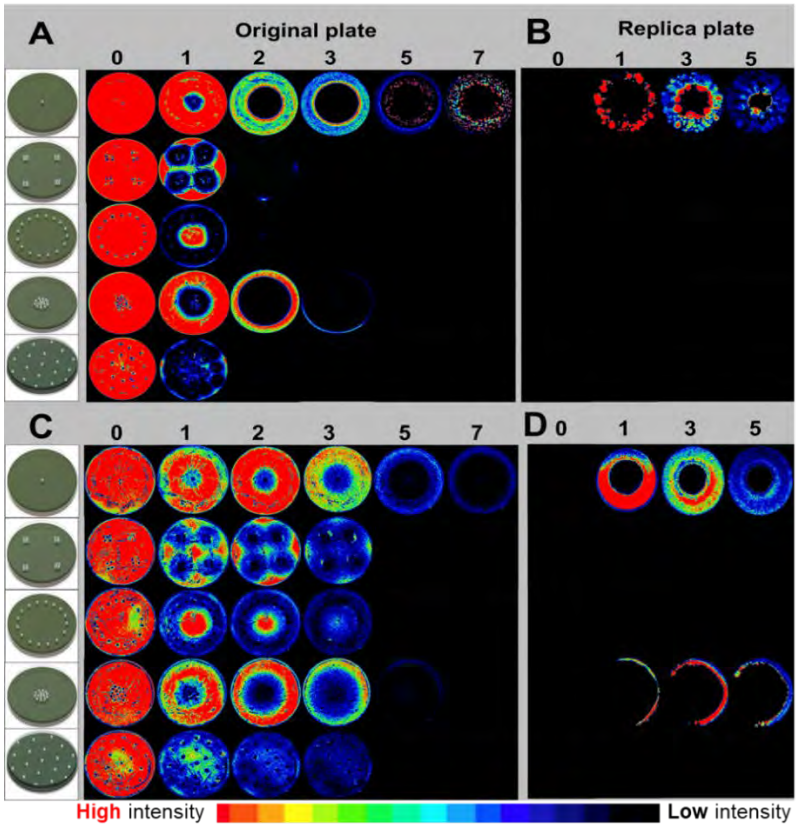

Figure 3. Effect of bead placement on killing of PA-Xen41 (**A**,**B**) and SA-SAP231 (**C**,**D**) biofilms treated with vancomycin and tobramycin, where (**A**,**C**) are original plates and (**B**,**D**) are replica plates of original petri-plates at day seven.

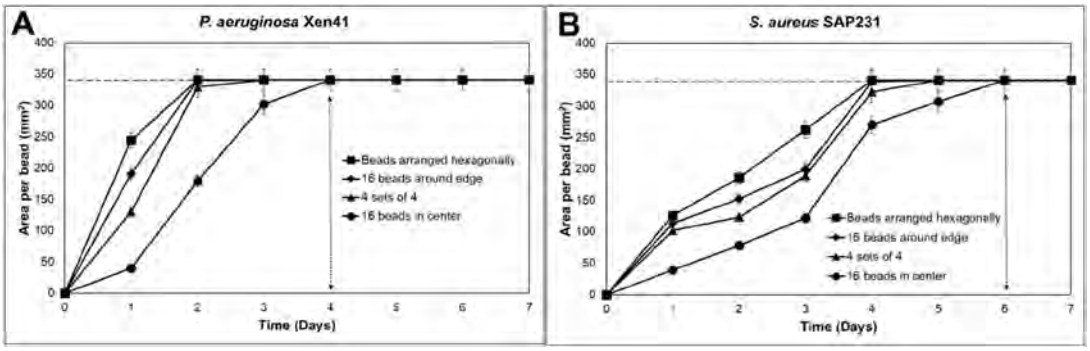

Figure 4. Killing of lawn biofilms of (**A**) PA-Xen41 and (**B**) SA-SAP231 treated with vancomycin and tobramycin. Dotted horizontal lines in the graphs represents edges of the Petri dishes and vertical lines represents days of complete lawn biofilm killing.

4.4. Replica Plating to Determine Complete Killing or Regrowth after ALCSB Treatment

Replica plating was used to determine whether all the biofilm cells were killed after treatment with ALCSB or if antibiotic-resistant cells would regrow when transferred to fresh plates. When PA-Xen41 and SA-SAP231 treated with vancomycin and tobramycin for seven days were replica-plated onto fresh TSA or BHI agar, growth of antibiotic-tolerant phenotypes was observed with single bead placement in the center (Figure 3B,D). No growth was observed in the area closest to the ALCSB suggesting that this area was completely sterile. All the bacterial cells present in the lawn biofilms close to the ALCSB were killed. For all the bead arrangements, replica plates incubated for five days showed complete killing and sterility due to the high concentration of antibiotics achieved (Figure 3B,D). No carryover of

antibiotics was observed during replica plating from ALCSB-containing plates onto fresh agar plates (Figure S2). Lawn biofilms of SA-SAP231 treated with vancomycin and tobramycin for seven days, when replica plated onto BHI agar revealed incomplete killing with a single bead and when 16 beads were placed in the center (Figure 3D). All other bead arrangements were effective in complete killing of the biofilms from the BHI agar containing plates (Figure 3D), as observed with the replica plating technique. This suggests that systematic arrangement of ALCSB, i.e., minimizing the bead separation and maximizing the coverage, is necessary to completely eradicate lawn biofilms as well as prevent growth of antibiotic-resistant cells.

4.5. Image Analysis to Determine the Influence of Bead Number on Killing of Lawn Biofilms

The influence of bead number on clearing of biofilms was determined using IVIS images of PA-Xen41 and SA-SAP231 treated with ALCSBs containing vancomycin and tobramycin at 24 h (Figure 5A,B). The plot profile across the single, four, or 16 beads clustered together suggested that the distance cleared from the edges of the bead clusters is relatively independent of the number of beads. More beads cleared a greater area, but the area cleared per bead was significantly reduced when more beads were clustered together (Figure 5B). Taken together, beads should be distributed densely and as evenly as possible over the whole biofilm (or at surgical sites) to make sure there are overlapping zones of biofilm killing.

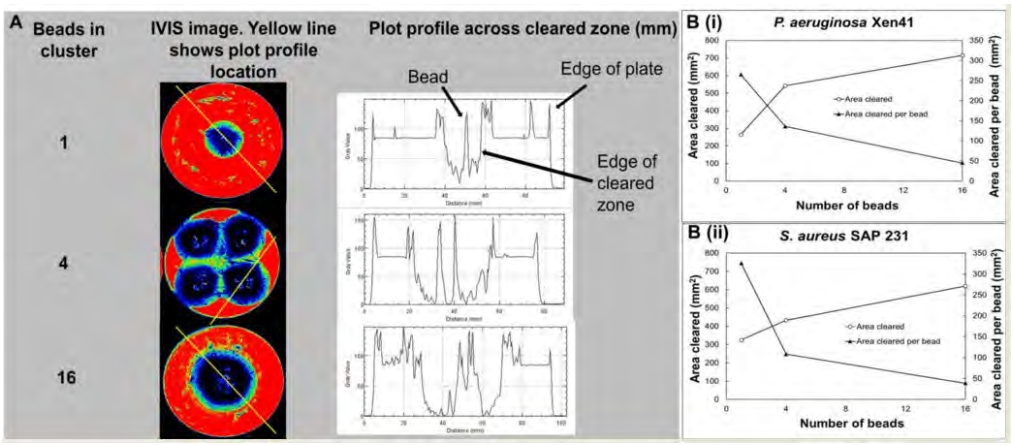

Figure 5. (**A**) Influence of bead number on clearing of lawn biofilms of PA-Xen41 after 24 h of treatment with antibiotic-loaded calcium sulfate beads (ALCSB) containing vancomycin and tobramycin. Image analysis (Image J) of beads in clusters showing profile plot across cleared zone (mm); (**B**) Influence of bead number on total area and area cleared per bead of lawn biofilms of PA-Xen41 (i) and SA-SAP231 (ii) after 24 h of treatment with beads containing vancomycin and tobramycin. Distance cleared from edge of bead cluster was relatively independent of the number of beads. More beads cleared a greater area; however, the area cleared per bead was dramatically reduced when more beads were clustered together.

5. Discussion

S. aureus and *P. aeruginosa* have been implicated in serious infections due to the formation of biofilms that are associated with resistance towards antibiotics [10,11]. Bacterial biofilms are a major concern in patients with PJI, and beads incorporated with antibiotics are routinely used at the site of infection during orthopedic surgeries to provide sustained release and local treatment to prevent infections [34–37]. While this therapy may be effective in killing a large proportion of bacteria, it may be ineffective against bacteria that are protected within biofilms, or at places where antibiotic concentrations could be low, and at areas away from the beads, potentially resulting in repeated and chronic infections.

We have previously demonstrated the short-term killing of lawn biofilms of PA and SA using ALCSB with combinations of vancomycin and tobramycin for up to three days [38]. In this present study, we were interested in understanding the long-term effect of ALCSB on killing of lawn biofilms; therefore, we extended the exposure time of killing of biofilms by ALCSBs to achieve complete eradication of biofilms grown on agar surfaces. Using the lawn biofilm killing method by ALCSB [38], we observed that PA-Xen41 biofilms were rapidly cleared with vancomycin and tobramycin compared to biofilms of SA-SAP231. Interestingly, in the presence of vancomycin and tobramycin, antibiotic-resistant phenotypes were evident in PA-Xen41 but not in SA-SAP231. To investigate which antibiotic from the combination is responsible for eliciting the generation of these antibiotic-resistant phenotypes, vancomycin and tobramycin were used independently against PA-Xen41. We found that antibiotic-resistant phenotypes were observed growing out of the PA-Xen41 biofilm lawn killed after exposure to tobramycin where the concentrations are much higher than the MIC (Figure S3). Notably, these colonies did not reveal themselves immediately but only after three days when the cells in the background gets cleared; thus, this small population could go undetected without extended incubation.

Tobramycin belongs to the aminoglycoside group of antibiotics. Antibiotic resistance towards aminoglycosides has been observed previously in PA and SA [15,17,18,47]. Different mechanisms have been proposed, as mentioned earlier, for the development of resistance towards antibiotics such as the role of efflux pumps, cell wall permeability, development of robust biofilms, and SCVs. In a recent study, Hoiby et al. (2019) showed that when planktonic bacteria are seeded, they start to grow and form aggregates. After 5 h these aggregates become tolerant to tobramycin and continue to grow and form a mature biofilm after 7 h of incubation, which are completely resistant to tobramycin [33]. These mechanisms contribute to the survival of bacteria to antibiotic treatment and might lead to the treatment failures. Therefore, novel strategies to eradicate biofilms and prevent the development of antibiotic resistance are needed. To achieve complete killing of biofilm lawns and to prevent the resistant phenotypes, we strategically arranged ALCSB containing vancomycin and tobramycin in four different patterns. On the agar lawn biofilms of PA and SA, we compared a single bead against the arrangements with sixteen beads (i) in groups of four, (ii) close to the edge of the plates, (iii) all the beads in the center, and (iv) 19 beads distributed hexagonally. All the arrangement of beads was found effective in killing of biofilms of PA (Figures 3A,B and 4A) and SA (Figures 3C,D and 4B). However, 16 beads clustered together in the center were not effective in completely eradicating the lawn biofilms of SA (Figure 3D). Importantly, in PA no colonies grew out of the zone of biofilm killing (Figure 3A,B). Replica plating suggested that all the cells were killed (Figure 3A–D).

These antibiotic bead placement strategies could be effective in cases where infections are not cleared efficiently because of difficulty in accessing the site of infection, such as in the case of device-associated joint infections. Furthermore, replica plating of these biofilms treated with ALCSB demonstrated complete killing of lawns and revealed that the inner concentration of antibiotics was high enough to completely kill the biofilms present in the areas close to the ALCSB (Figures 3A–D and 4A,B).

It is important to note that high concentration of antibiotics is also able to prevent the growth of resistant phenotypes. This is indicated by the lack of growth in the zone of biofilm killing on replica plates as well as by the lack of growth in cultures taken from the zones immediately surrounding the beads, where high concentration of antibiotics was present (Figure 2A,B and Figure S3). Total eradication of biofilms in orthopedic infections by systemic administration of antibiotics is extremely challenging, since the concentrations required to eradicate biofilms of clinical strains are often greater than 1000 μg/mL for vancomycin as well as a wide range of antibiotic classes [14,48], in part due to the presence of persister cells [49]. However, concentrations of vancomycin achieved in the serum and knee joint fluid by IV administration are much lower, approximately 25 and 7 μg/mL, respectively [50]. Castaneda et al. [51] has shown that biofilms can be eradicated, presumably including the killing of persister populations, using high concentrations over extended incubation periods [51]. Our data are in agreement, however; we did find differences in the rate of clearing, with the 16 beads clustered in the center taking longer to clear the biofilms than those that were distributed around the Petri dishes.

The hexagonally placed beads showed the most rapid killing compared to other combinations. Similar results were seen with SA, although the beads clustered in the center did not killed all the biofilms at the periphery of the plate as is evidenced by growth on the replica plates (Figure 3D). Our data suggest that in diffusion-dominated areas, it is not only the number of beads placed at the site that is important for controlling biofilm, but also that the beads have enough density and are distributed such that the concentration and duration of antibiotic exposure are sufficient to completely kill the biofilm cells in the vicinity of the beads. Our observations do not only apply to beads but to any carrier that is releasing antibiotics, since spatial and temporal concentration gradients will always develop, although the scales of these gradients will strongly depend on the carrier and local mass transfer conditions. The proximity of the beads to each other and to the infected material should be based not just on the area of the zone of biofilm killing, which is seen in the first 24 h, but also at later times in this zone in which bacterial resistant colonies only reveal themselves after extended incubations. Therefore, closely packed ALCSBs with enough coverage of areas suspected of infection might be effective in completely eradicating PA and SA biofilms at the surgical site, overcoming the problem of generation of antibiotic-resistant bacteria that could otherwise escape antibiotic treatment and regrow to establish biofilm-related infections.

In future studies, we will focus on the specific mechanisms underlying the generation of antibiotic-resistant phenotypes and determine whether these phenotypes are observed within a particular class of antibiotics and whether different combination of antibiotics could prevent the development of these antibiotic-resistant phenotypes that could potentially regrow and cause infection in the clinical settings.

6. Conclusions

We have shown that: (1) the antibiotics released from ALCSB can totally eradicate in vitro biofilms, including resistant population growing within the zones of biofilm killing around each bead; (2) in PA, antibiotic resistant phenotypes may survive outside the zone of biofilm killing; and (3) the distribution and the number of beads are important to ensure adequate coverage in order to eradicate in vitro biofilms from a given area.

Supplementary Materials: The following are available online at http://www.mdpi.com/1996-1944/12/24/4052/s1, Figure S1: Killing of lawn biofilms of *P. aeruginosa* (PA-Xen41) with antibiotic-loaded calcium sulfate beads (ALCSB) and paper discs loaded with tobramycin. The ALCSB loaded with 240 mg/10 cc tobramycin and filter paper discs with 100 µg/disc tobramycin were placed in the center on 24 h lawn biofilms. Zone of biofilm killing (ZOB-K) and resistant colonies were observed with both ALCSB and paper discs loaded with tobramycin, Figure S2: Carryover of antibiotic during replica plating. ALCSB containing tobramycin placed on 24 h grown lawn biofilms at day five was replica plated onto fresh tryptic soy agar (TSA) plates and spread with PA-Xen41. No inhibition of growth of PA-Xen41 was observed, suggesting no carryover of tobramycin during replica plating, Figure S3: Tobramycin concentration remains above MIC during resistant phenotype development. Tobramycin-loaded bead was placed in sterile TSA, and at various time points, agar plugs were extracted at various radii to examine the concentration of tobramycin in them by plating for MIC (n = 3). MIC zones were compared to a standard curve to calculate the tobramycin concentrations in the agar plugs. Data is reported as mean ± SD.

Author Contributions: Conceptualization, D.H.D., C.D., S.S.A., P.A.L., A.S. and P.S.; data curation, D.H.D., J.R.B., D.S., C.W.P., A.L., N.R.F., S.M.D., C.S.K., J.F.G. and P.S.; formal analysis, D.H.D., D.S., C.W.P., S.M.D. and R.D.P.; investigation, D.H.D., J.R.B., D.S., C.W.P., A.L., N.R.F., S.M.D., C.S.K., R.D.P. and P.S.; methodology, D.H.D., R.D.P., C.D., S.S.A., P.A.L., A.S. and P.S.; project administration, P.S.; resources, D.H.D., C.D. and P.A.L.; supervision, P.S.; writing–original draft, D.H.D., J.R.B. and C.S.K.; writing–review and editing, A.S., J.F.G. and P.S.

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In a Laboratory Model of Diabetic Foot Infection, Vancomycin and Gentamicin Loaded Calcium Sulfate Beads Were More Effective Than Systemically Achievable Concentrations of Antibiotics in Reducing Polymicrobial Biofilms Grown From Clinical Isolates.

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Introduction

- Diabetic foot infections (DFIs) are a common complication of diabetes, half become infected chronically and a sixth require surgery.
- Poor healing is associated with biofilm growth and a compromised immune system.
- Treatment can involve systemic or topical antibiotic administration, however the relative effectiveness of each remains uncertain.
- We grew biofilms from bacteria isolated from each debrided tissue and tested the relative efficacy of vancomycin and gentamicin released from calcium sulfate beads (CSB) (Stimulan Rapid Cure®) and antibiotics at concentrations relevant to those *in vivo* on biofilm eradication.

Methods

- We prepared soft tissue models comprising type 1 collagen, extracellular matrix (ECM) protein extract, hyaluronic acid and human primary fibroblasts.
- The model was inoculated with bacteria isolated from an individual's tissue sample and incubated for 72 hours.
- Vancomycin and gentamicin was introduced in loaded CSB (500/240 mg per 10 cc beads resp.) or antibiotics were added directly at concentrations in Figure 2 to the media at the base of the model.
- After 72 hours, bacterial viability within the model was assessed.

Methods

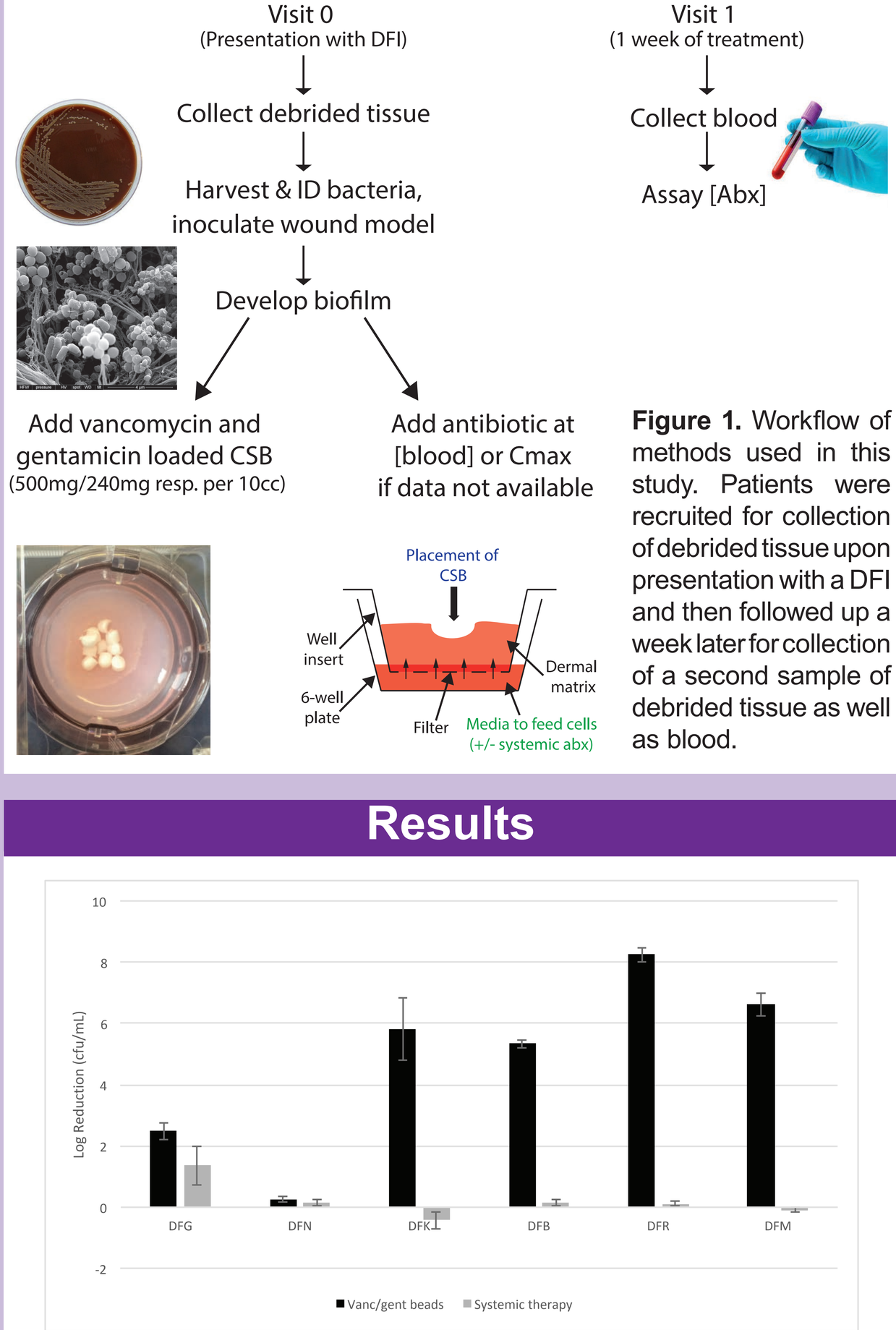

Figure 1. Workflow of methods used in this study. Patients were recruited for collection of debrided tissue upon presentation with a DFI and then followed up a week later for collection of a second sample of debrided tissue as well as blood.

Results

Patient	Oral antibiotic therapy	Topical Antibiotic Treatment within Wound Model	Systemic Antibiotic Treatment within Wound Model
DFG	Ciprofloxacin 500 mg BD Clindamycin 450 mg QDS	Vancomycin (500 mg)/gentamcin (240mg) per 10 cc calcium sulfate	Ciprofloxacin 2.4 mg/L* Clindamycin 4.2 mg/L*
DFN	Co-amoxiclav 625 mg TDS		Amoxicillin 7.19 mg/L* Clavulanic acid 2.4 mg/L*
DFK	Flucloxacillin 1g QDS		Flucloxacillin 11.5 mg/L
DFB	Flucloxacillin 1g QDS		Flucloxacillin 0.4 mg/L
DFR	Co-trimoxazole 960 mg BD		Trimethoprim 1.1 mg/L Sulphamethoxazole 6.9 mg/L
DFM	Flucloxacillin 1g QDS		Flucloxacillin 7.84 mg/L

Figure 2. Topical and systemic concentrations of antibiotics used in the collagen wound models based on concentrations of antibiotics assayed in blood. Where patient data was not available Cmax values from the literature were used, these data are denoted by *.

- Oral antibiotic therapy prescribed for each patient is indicated in Figure 2.
- Blood samples were assayed for these antibiotics, in some cases no assay was available for one or both antibiotics used therapeutically.
- CSB loaded with Vancomycin and Gentamicin were added to the model or systemic antibiotics at concentrations indicated in Figure 2.

Results

Figure 3. Mean (± SEM) log reductions of biofilms after exposure to loaded CSB or antibiotics at systemic concentrations.

- There were no significant decreases in biofilm after addition of systemic concentrations of antibiotics to models.
- Biofilms DFK, DFB, DFR and DFM all had large significant decreases in biofilm after exposure to loaded CSB (5-8 logs).
- A smaller (2.5 log) but significant reduction in sample DFG was observed upon exposure to loaded CSB despite MICs of isolates showing high tolerance to vancomycin combined with gentamcin (32 µg/ml total).
- There was no significant reduction in biofilm for DFN whose microbiome included yeast and so was antibiotic resistant.

Conclusions

- Our data demonstrate that even the highest likely levels of systemic antibiotics have little inhibitory effect on biofilm in a collagen wound model comprising matrix comparable to that within human soft tissues.
- Topical delivery resulted in increased log reductions compared to systemic antibiotics in 5/6 patients tested.
- These data support the assertion that systemic antibiotics may be less effective than topical antibiotics at treating infected DFUs.

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Evaluation of comparative soft tissue response to bone void fillers with antibiotics in a rabbit intramuscular model

Rema A Oliver, Vedran Lovric, Chris Christou and William R Walsh

Abstract

Management of osseous and soft tissue dead space can be a significant challenge in the clinical setting. Calcium sulphate and calcium phosphate-based biomaterials are increasingly being used as alternatives to PMMA for local release of antibiotics, in particular to fill dead space following surgical debridement. This study aims to observe the in-vivo absorption characteristics and tissue response of three commercially available calcium sulphate-based materials combined with gentamicin in an established soft tissue rabbit model. The implant materials (1cc) were placed into four intramuscular sites in 18 New Zealand White rabbits ($n = 6$). In-life blood samples and radiographs were taken from each animal following implantation. Animals were sacrificed at 0, 1, 7, 21, 42 and 63 days post-operatively ($n = 3$) and implant sites analysed by micro-computed tomography and histology. Radiographically and histologically, recrystallized calcium sulphate (RCS) absorbed the fastest with complete absorption by day 21. Calcium sulphate/HA composite (CSHA) and Calcium sulphate/calcium carbonate (CSCC) absorbed slower and were detectable at day 63. Residual bead analysis revealed the presence of detectable gentamicin at 24 h and 7 days for CSHA and RCS but none in CSCC. Systemic levels of gentamicin were only detected between 1 h and 24 h. Serological inflammatory cytokine expression for IL-6, TNF-α and IL-1β indicated no unusual inflammatory response to the implanted materials. Calcium sulphate materials loaded with gentamicin are effective in resolving a surgically created dead space without eliciting any adverse host response.

Keywords

Bone void filler, gentamicin, soft tissue reaction, dead space management

Introduction

Calcium sulphate and calcium phosphate-based biomaterials are increasingly being used as alternatives to polymethylmethacrylate (PMMA) for local release of antibiotics[1–3] in particular to fill dead space following surgical debridement in the management of infection. These materials are biocompatible and are absorbed by the body negating the need for removal while resorption rates can vary based on chemistry. Due to the low temperatures achieved during setting, they can be mixed with heat sensitive antibiotics.[4,5] Clinical reports of composite materials incorporating calcium phosphates such as hydroxyapatite (HA) with calcium sulphate were found to demonstrate soft tissue healing in the treatment of the infected diabetic foot[6] with no foreign body or immune host response. Published absorption rates of these materials are inconsistent with both complete absorption being reported as in the data above and partial dissolution and bony incorporation of the HA particles in other literature.[7]

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PMMA, however, is not absorbed in the body and therefore is required to be removed in a second procedure to decrease the possibility of the material becoming a nidus for infection.[8–10] Local implantation of gentamicin impregnated PMMA beads can provide high local levels of antibiotic, many times the minimum inhibitory concentration (MIC) while reducing the risk of systemic toxicity.[11] Gentamicin is stable when exposed to the high temperatures generated during the polymerisation reaction as the PMMA sets hard, therefore it is commonly used in the local treatment of osteomyelitis and soft tissue infections.[12]

The purpose of this study was to observe the in-vivo absorption characteristics and tissue response of three commercially available calcium sulphate-based materials combined with gentamicin based on a previously described novel soft tissue animal model[13] where the materials were implanted in four non-adjacent intramuscular sites in adult rabbits.

Methods

Preparation of implant materials

Three commercially available materials were used for this study as shown in Table 1.

The hemihydrate powder from 10cc kits for the recrystallized calcium sulphate (RCS) beads was mixed with 6 ml (240 mg) gentamicin solution (40 mg/ml, Hospira, UK) and was prepared under sterile conditions. The mix was thoroughly blended for 30 s to form a smooth paste which was then pressed into 6 mm diameter, 4.8 mm length, hemispherical cavities in a flexible mould. The beads were left undisturbed and allowed to set. The level of gentamicin combined with the RCS corresponded to the clinical ratio combined with RSC reported in literature.[14–16]

The 10cc kits of CSHA were mixed according to the manufacturer's Instructions For Use (IFU) with the gentamicin included in the pack at a concentration of 175 mg per 10cc. The mixed paste was then pressed into 6 mm diameter, 4.8 mm length, hemispherical cavities and allowed to set as described above. When all the beads had set hard, they were removed by flexing the mould. The level of gentamicin combined with CSHA was supplied co-packaged with the product, for combination with the product according to the manufacturer's instructions.

The CSCC beads were supplied as pre-formed white to light grey beads of biconvex rounded cylindrical shape. Each bead weighed 250 mg containing gentamicin at a concentration of 1%, equivalent to 2.5 mg gentamicin per bead. The CSCC beads were supplied preloaded with gentamicin.

Surgery

Following approval of the Animal Care and Ethics Committee of the University of New South Wales (ACEC#:15/85A), implant materials (1cc per side, five beads of material) were placed into intramuscular sites in 18 female New Zealand white rabbits (average weight 3.5 kg, aged 7–9 months old), 6 rabbits per material. For all 18 animals in the study, four implant sites were used per animal, two sites each side of the spine, in non-adjacent intramuscular sites (longissimus muscles) above the spine at the levels L1–L2, L2–L3, L3–L4 and L4–L5. Under gaseous anaesthesia of isoflurane and oxygen, the intermuscular plane between the multifidus and longissimus muscles was retracted to create a 1 cm $\times$ 2 cm void. Each void was filled with 1cc of sterile beads. The beads were counted at the time of surgery and were allocated in a sterile fashion into sterile syringes with the tip removed to facilitate implantation.

Each of the facial incisions was closed with an individual single strand non-absorbable suture. Closure was achieved with equidistant adjacent stitches at approximately 3 mm intervals. The skin of the incision was closed with an individual single strand absorbable suture. Post-operative radiographs in the posteroanterior and lateral planes were taken immediately following surgery using a mobile X-ray machine (Poskom Co., Ltd, Korea) and digital cassettes (AGFA, Sydney, Australia). Radiographs were used to visualise the appearance of the beads in the soft tissue post-

Table 1. Materials tested.

Material	Commercial name	Manufacturer
Recrystallized calcium sulphate (RCS)	Stimulan Rapid Cure	Biocomposites Ltd, UK
60% Calcium sulphate 40% Hydroxyapatite (CSHA)	Cerament G	Bone Support AB, Sweden
72% Calcium sulphate 18% Calcium carbonate 9% Hydrogenated triglyceride (CSCC)	Herafil Beads G	Heraeus Medical GmbH

operatively. These images were used as a baseline for examination at later time points.

Peripheral blood analysis and blood serum gentamicin levels

Peripheral blood was taken preoperatively and 1, 6, 12 and 24 h following surgery and prior to sacrifice at each time point for standard blood panel haematology/biochemistry (IDEXX Laboratories, Sydney, Australia) and serum cytokine levels for IL-1β, IL-6 and TNF-α and to determine systemic gentamicin levels.

Peripheral blood (approx. 3 ml) was taken from the saphenous vein and collected into a Vacutainer Plain Clot 4 ml tube and allowed to clot at room temperature for 30 min before centrifugation for 10 min at 2000×g. The serum was carefully removed and stored at −80°C until needed.

Serum IL-1β (E04I0010), IL-6 (CSB-E06903Rb) and TNF-α (CSB-E06998Rb) levels were analysed by enzyme-linked immunosorbent assay (ELISA) testing using ELISA kits (Cusabio Biotech, Beijing, China) according to the manufacturer's instructions. Each sample was measured in duplicate and standards were also run on each 96-well plate.

Blood serum gentamicin levels – Method validation in-vitro

Rabbit serum was harvested from other studies at the time of sacrifice to perform a dose response curve for rabbit serum with a known concentration of gentamicin. Concentrations were determined based on the assay range and also included concentrations lower and higher than the detectable assay range. The study was completed with a standardised assay. This was performed in duplicate. The antibiotic used was Gentam 100 (100 mg/ml gentamicin as gentamicin sulphate, Troy Laboratories, NSW, Australia).

The assay was based on the kinetic interaction of microparticles in a solution (KIMS) where the gentamicin antibody is covalently coupled to microparticles and the drug derivative is linked to a macromolecule. The kinetic interaction of microparticles in solutions is induced by binding of drug-conjugate to the antibody on the microparticles and is inhibited by the presence of gentamicin in the sample. A competitive reaction takes place between the drug conjugate and gentamicin in the serum sample for binding to the gentamicin antibody on the microparticles. The resulting kinetic interaction of microparticles is indirectly proportional to the amount of drug present in the sample. The assay range was 0.4–10 μg/ml. Several data points were also run outside of the range to challenge the assay. The serum samples collected post-implantation were analysed using this assay.

Euthanasia and necropsy

Time points for sacrifice were time 0, and days 1, 7, 21, 42 and 63. Each time point had three animals with four implantation sites per animal. Time points were chosen to examine the in-vivo release kinetics of gentamicin in local tissues as well as serum levels.

Implant sites were reviewed for general integrity of the skin incision along with the macroscopic reaction of the underlying subcutaneous tissues as normal or abnormal. The abnormal was further assessed as evidence of infection or macroscopic signs of inflammation/foreign body reaction. At the time of harvest, all organs were examined and any abnormalities noted. A portion of the distant organs was processed for routine paraffin histology and evaluated in a blinded fashion for any abnormalities.

Radiography and micro-computed tomography

Post-operative radiographs in the posteroanterior plane were taken using a mobile X-ray machine and digital cassettes. These images were used to determine radiographic absorption by comparison to radiographs obtained immediately after implantation.

Micro-computed tomography (microCT) was performed for all animals using an Inveon in-vivo micro-computer tomography scanner (Siemens Medical, PA, USA) in order to obtain high resolution images of the implant absorption. The surgical sites were scanned and the raw images reconstructed resulting in effective pixel size of 53.12 μm. Images were examined in the axial, sagittal and coronal planes and 3D models were created using Siemens image analysis software (Inveon Research Workplace 3.0, Siemens Medical, PA, USA).

Gentamicin levels in residual materials and adjacent muscle tissues

The surgical sites were carefully dissected and examined for the presence of any residual beads. A portion of any residual beads present at the surgical sites was harvested. This material was allowed to air dry and placed in a desiccator for 24 h. Following this, they were then morselized using a mortar and pestle. 0.1 g of the powder was immersed in 1 ml of serum for 24 h. Gentamicin levels in the samples per gram of material were determined using the assay described above.

A muscle sample (1 cm × 1 cm) at the implantation site was harvested and then minced. The local concentration of gentamicin in the muscle sample was

measured using the KIMS standard antibody assay already described.

Histology

Harvested implant sites were immediately fixed in phosphate buffered formalin for a minimum of 48 h followed by decalcification in 10% formic acid – phosphate buffered formalin at room temperature. The decalcified samples were placed into embedding blocks for paraffin processing. Paraffin blocks were sectioned using a microtome (Leica, Germany) to 5 microns and placed onto slides for routine haematoxylin and eosin (H&E) staining.

Stained sections were reviewed and photographed using an Olympus Microscope (Olympus, Japan) and Olympus DP72 Camera. In-vivo response and biocompatibility to the materials was assessed at the implant site/host tissue boundary in a blinded manner.

Results

Surgery was completed without any adverse events. All animals recovered following surgery.

On harvest, macroscopic observations revealed the skin, subcutaneous tissue and organs to all be normal.

Blood serum gentamicin levels

Results of measured gentamicin levels for each rabbit at each allocated blood sampling time point for each material are shown in Figure 1. Systemic levels of gentamicin were only detected at the time points between 1 h and 24 h. The maximum detected level was 9 μg/ml at the 6 h time point.

Blood serum inflammatory cytokine level (ELISA) analysis

The levels of systemic cytokines were quantified preoperatively (baseline) n = 6 then postoperatively at 1 h, 6 h and 12 h (n = 5). Further levels were taken prior to sacrifice for each animal at 1 day, 7 days, 21 days, 42 days and 63 days (n = 1). The levels were determined in comparison to standard curves for IL-6, IL-1β and TNF-α.

Inflammatory marker IL-1β was detected in all animals at each time point including those in the RCS group where no further material remained. IL-6 was only detected up to and including the 12 h time point. The TNF-α results were inconclusive and not detected in all animals in all groups. The data presented in Figures 2 to 4 represented the mean values where the cytokines were detected. In days 1 to 63 only single samples were reported.

Tissue sample harvesting and gentamicin levels

Analysis of the residual beads revealed the presence of detectable gentamicin at 24 h and 7 days for CSHA and RCS. No gentamicin was detected in the CSCC samples at seven days. Gentamicin was not detectable in the residual beads in the CSHA and CSCC groups at

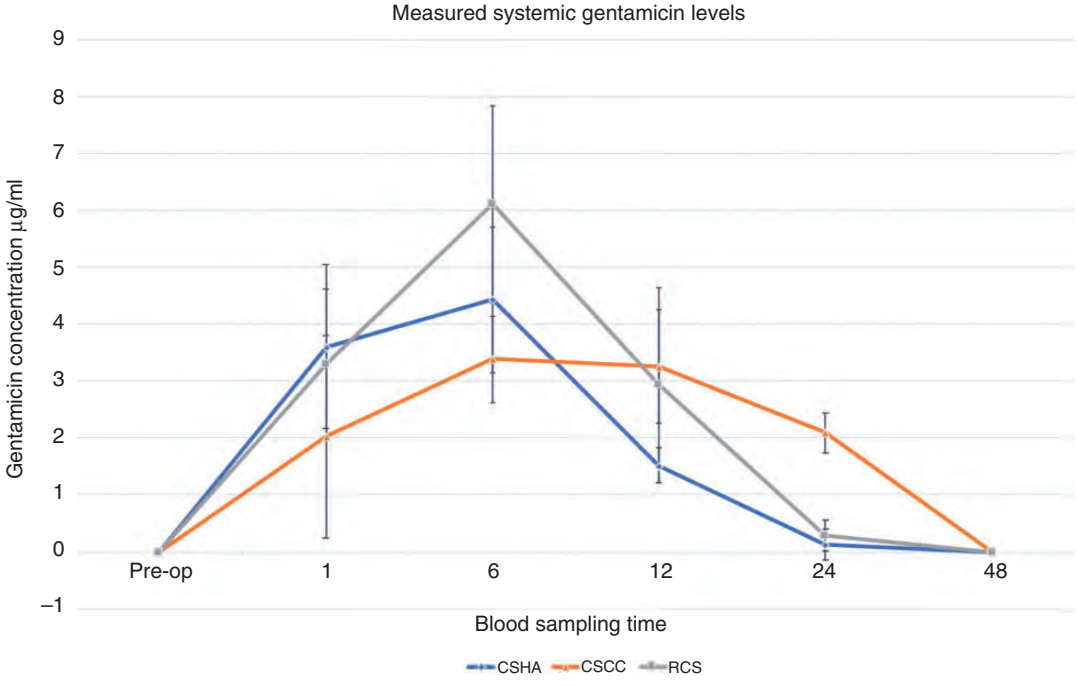

Figure 1. Systemic gentamicin levels. Mean values shown. n = 3. Error bars show standard deviation.

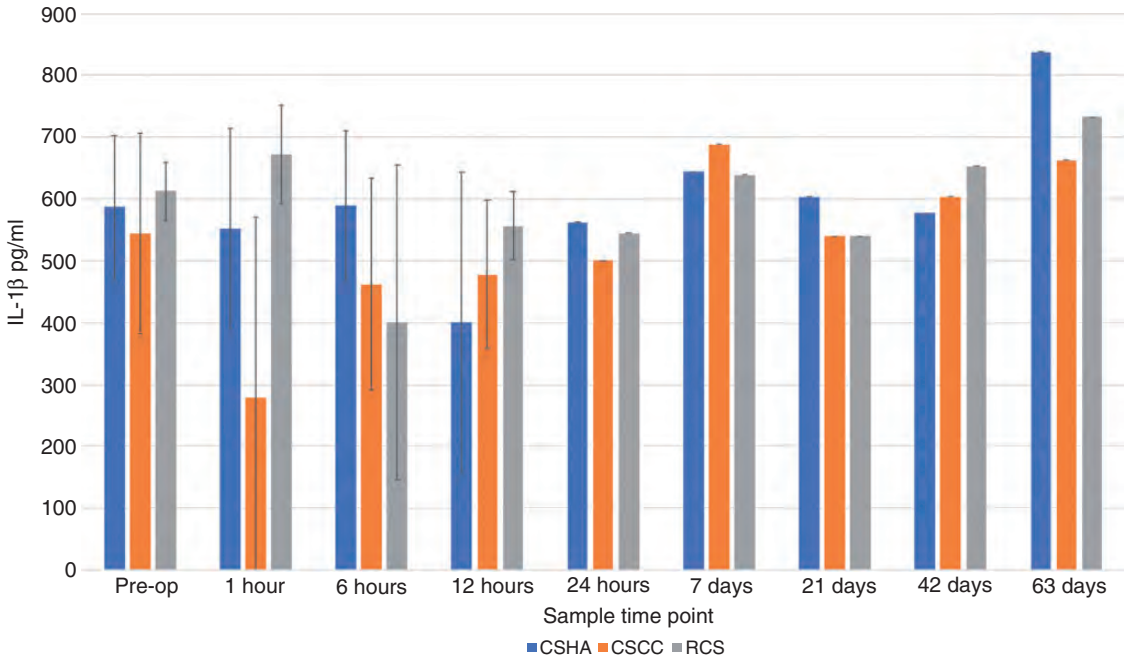

Figure 2. Serological cytokine expression for IL-1β. IL-1β levels were detected at each time point in all groups. The detection range of the assay was 78–5000 pg/ml. Error bars are only shown up to the 12 h time point as only single samples were obtained for the later time points.

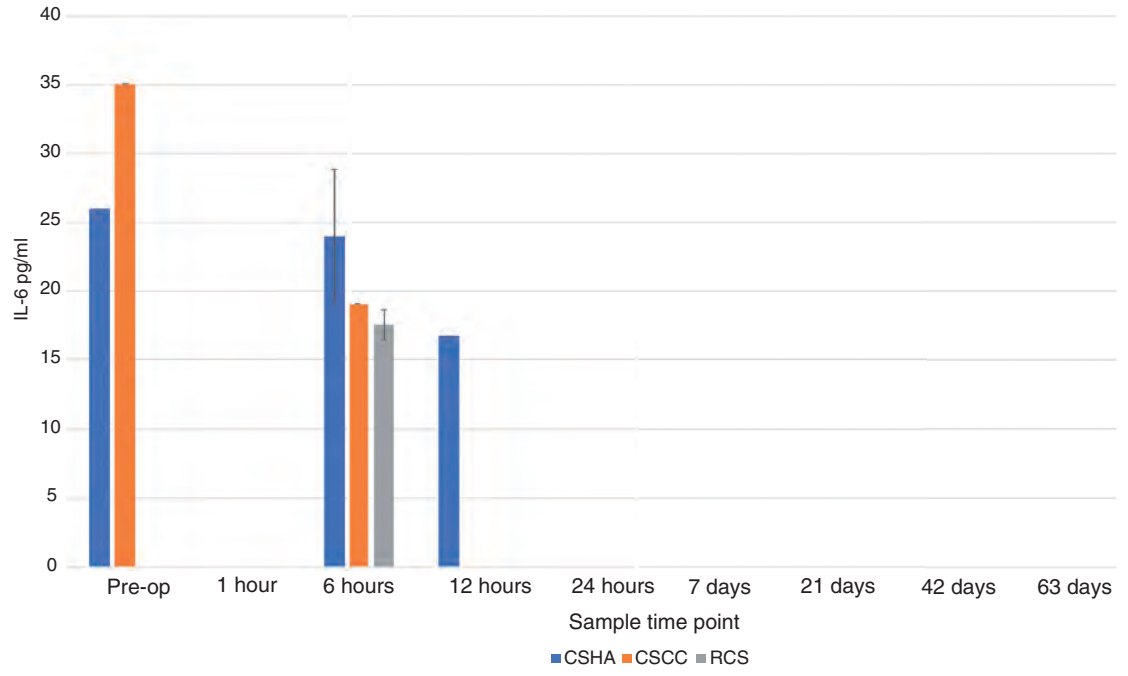

Figure 3. Serological cytokine expression for IL-6. IL-6 levels only detected up to the 12 h time point. The detection range of the assay was 15.6–1000 pg/ml.

day 21 or day 42 despite material remaining. As there was no presence of RCS beyond day 21 this was not evaluated (Table 2). No gentamicin was detected in the muscle samples.

Radiography

RCS demonstrated the most rapid absorption profile with changes observed by day 7 and the material radiographically almost completely absorbed by day 21.

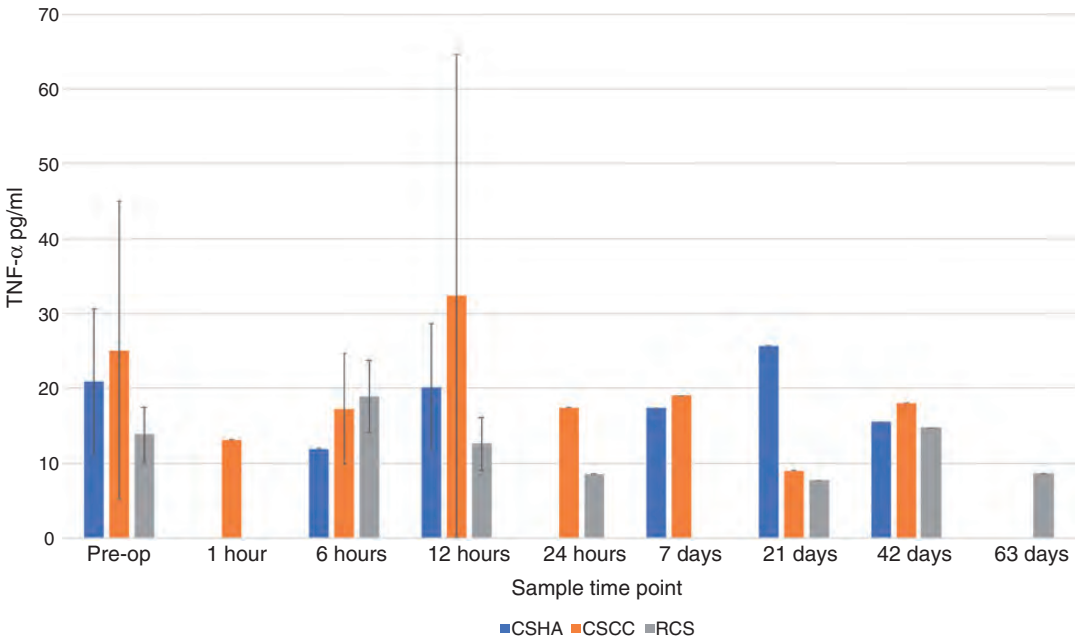

Figure 4. Serological cytokine expression for TNF-α. The detection range of the assay was 7–1000 pg/ml. Error bars are only shown up to the 12 h time point as only single samples were obtained for the later time points.

CSHA and CSCC absorbed slower based on radiographs and were detectable in all four implantation sites at day 63. The materials also did not appear to migrate based on the serial radiographs (Figures 5 to 7).

Micro-computed tomography

Imaging by microCT provided a clear assessment of bead absorption and confirmed the radiographic findings. Representative microCT images are shown in Figures 8 to 10. There was a clear progression of absorption over time with all implanted beads. This absorption appeared to be occurring from the outside in. In some cases at the latter time points, a 'halo' was observed surrounding the remaining beads, extending out into the surrounding soft tissue. There was also a clear difference in the rate of absorption between each type of implanted bead.

MicroCT indicated that RCS had the most rapid absorption profile with changes observed by day 7 and the material almost fully absorbed by day 21. CSHA and CSCC absorbed slower and were detectable in all four implantation sites at day 63.

Histology

The histological reaction demonstrated a subtle inflammatory response for all materials at the host interface versus time that included some lymphocytes and the occasional multinucleated cell (Figure 11). The overall intensity of the reaction was minor and resolved with time for all materials.

Table 2. Mean residual gentamicin levels in μg/ml.

	Post-op	24 h	7 Days	21 Days	42 Days
RCS	10.66	4.215	4.405	N/A	N/A
CSHA	8.29	6.37	4.39	0	0
CSCC	1.115	0.65	0	0	0

Two samples per group.

The histology beyond day 21 for RCS was unremarkable and by day 63 was normal in the implantation sites. The CSHA and CSCC samples presented slower absorption profiles and a longer presence of material in-vivo. This was paralleled with the presence of a few lymphocytes and multinucleated cells while the material remained. Complete absorption of these two materials by day 63 was not achieved (Figure 12).

Discussion

The animal model used in this study provided a robust means to evaluate intramuscular implantation of calcium sulphate materials combined with antibiotics in a pre-clinical setting. All animals recovered well following surgery with no in-life adverse events. Inspection of the wounds at the time of harvest revealed no adverse effects for the skin incision. All implanted beads could be clearly visualised on the radiographs and microCT images. There was a clear progression of absorption over time with all beads with no radiographic signs of migration. This absorption appeared to be occurring from the outside in. In some cases at the latter time

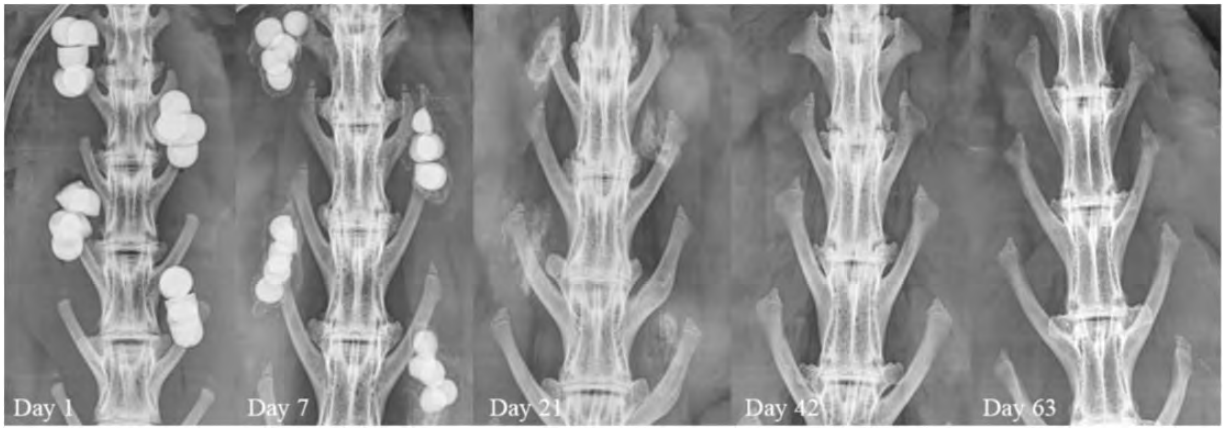

Figure 5. Representative RCS Radiographs demonstrating bead absorption. RCS had almost completely absorbed by day 21 and resorbed via surface absorption.

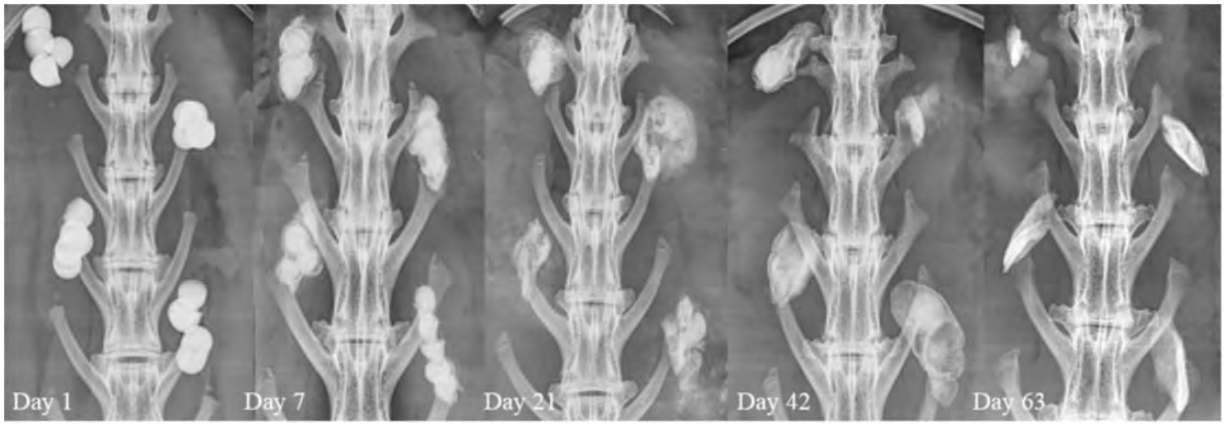

Figure 6. Representative CSHA Radiographs demonstrating bead absorption. Residual material was still present at day 63 and resorbed via surface absorption.

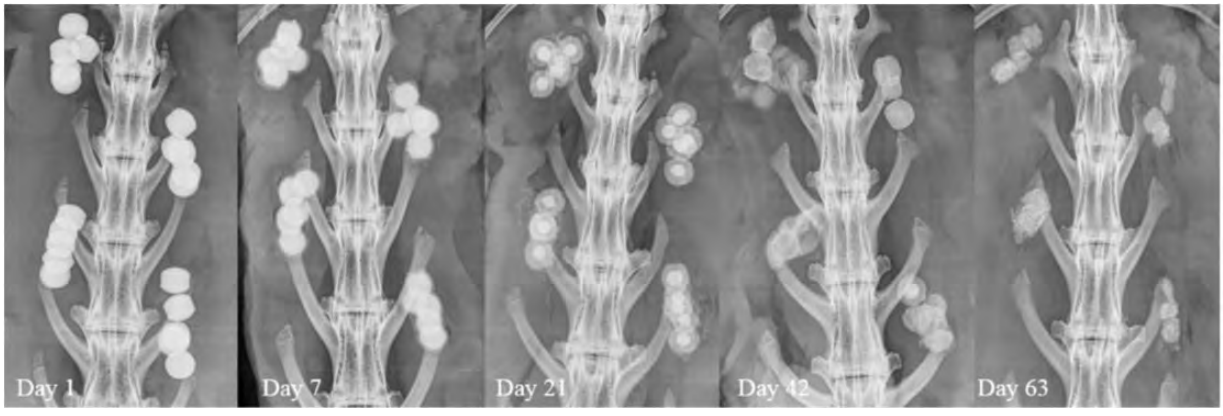

Figure 7. Representative CSCC Radiographs demonstrating bead absorption. Residual beads were still present at day 63 and resorbed via surface absorption.

points, a 'halo' was observed surrounding the remaining beads. It is suspected that this is a temporary carbonated apatite precipitation due to the release of high levels of calcium into the surrounding tissue which combine with ions *in situ* and precipitate on to the surface of the residual beads as has been observed in-vitro.[17,18] There was a clear difference in the rate of absorption between each type of implanted bead.

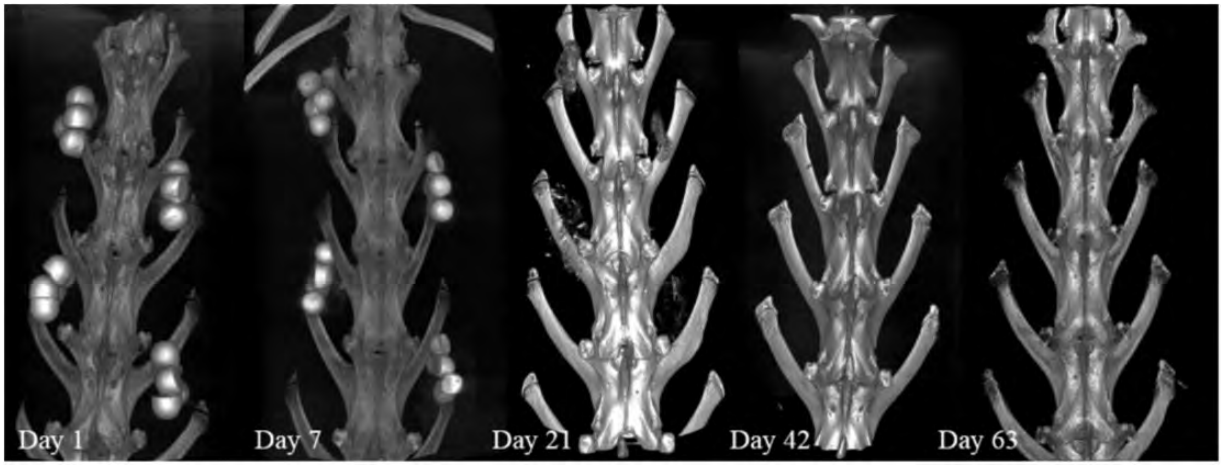

Figure 8. Representative RCS microCT images demonstrating bead absorption. The material was almost completely absorbed by day 21.

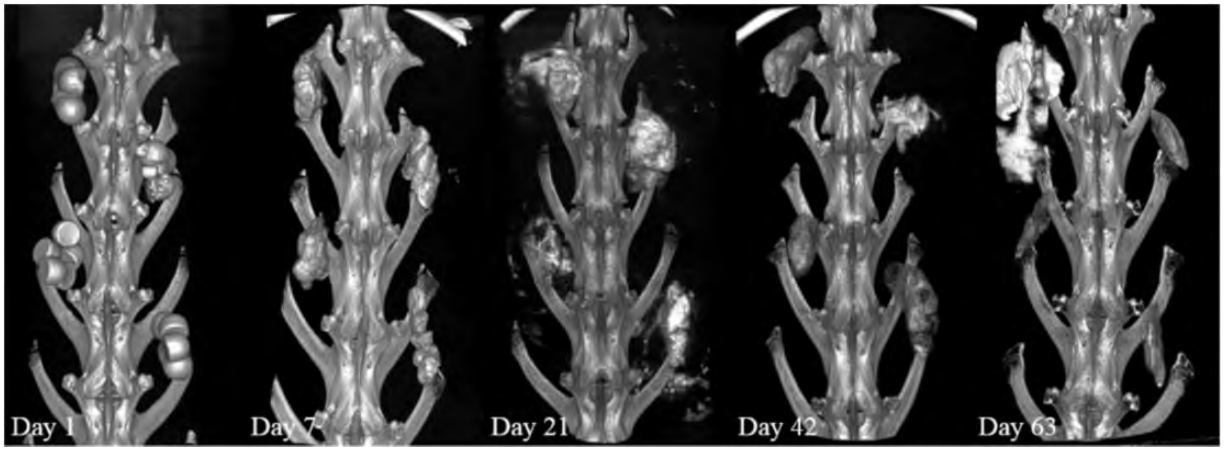

Figure 9. Representative CSHA microCT images demonstrating bead absorption. Residual material was still present at day 63.

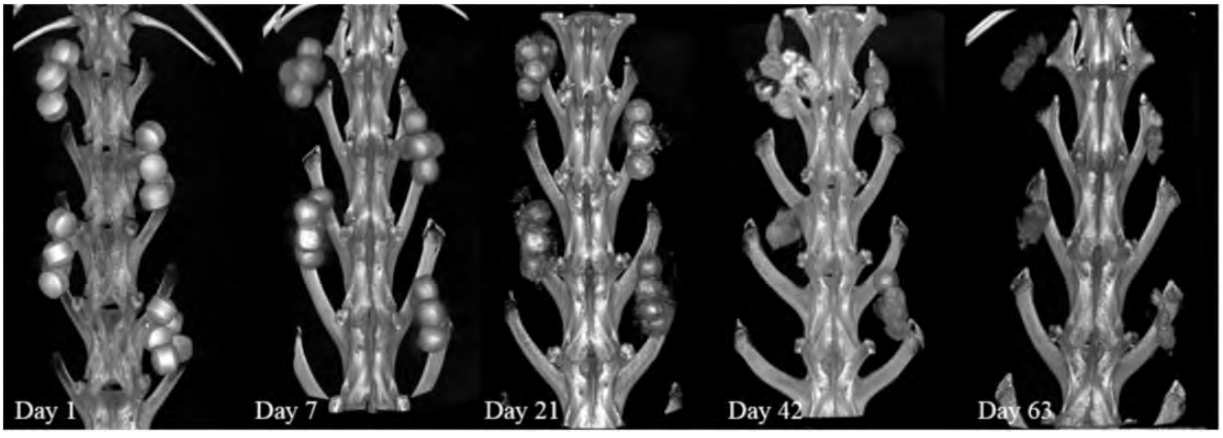

Figure 10. Representative CSCC microCT images demonstrating bead absorption. Residual material was still present at day 63.

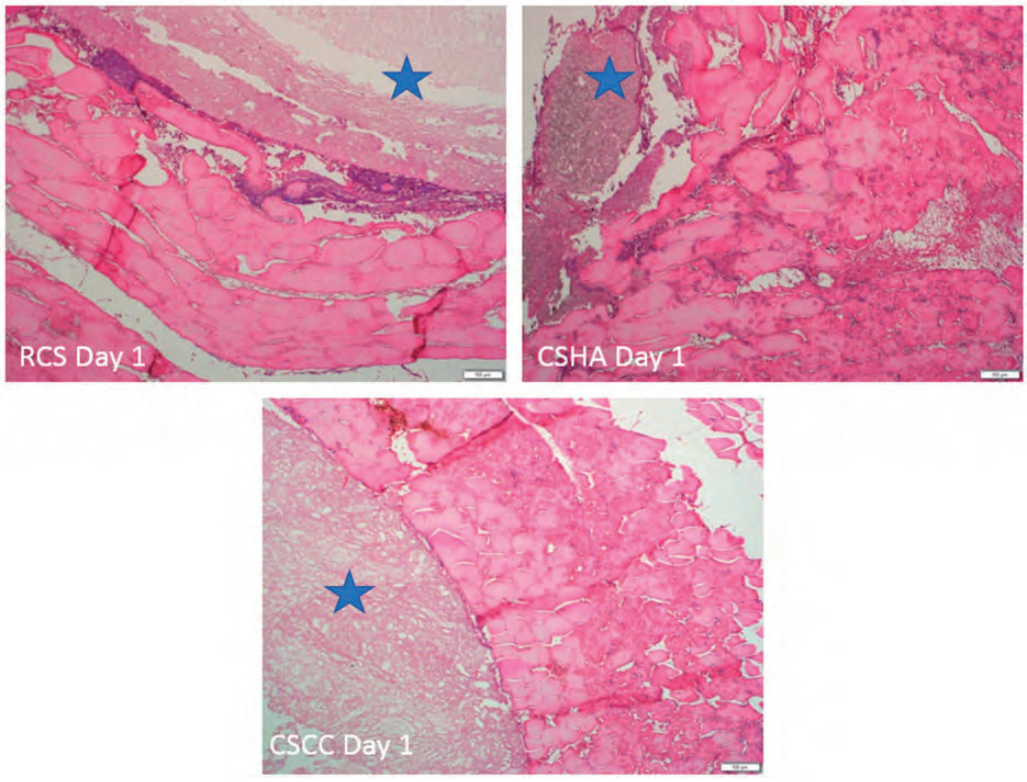

Figure 11. Haematoxylin and eosin (H&E) staining. Histology at day 1 for the RCS and the CSHA groups revealed the start of an initial inflammatory response at the margin with the host muscle. The initial cellular population included lymphocytes. Histology at day 1 for CSCC showed an overall lack of any initial inflammatory reaction. The materials are labelled with a star.

RCS demonstrated the most rapid absorption profile with changes observed by day 7 and the material radiographically almost completely absorbed by day 21. CSHA and CSCC absorbed slower and were detectable in all four implantation sites at day 63. Residual material was also apparent on explantation. Analysis of the residual beads revealed the presence of detectable gentamicin at 24 h and 7 days for CSHA and RCS. No gentamicin was detected in the residual CSCC samples at seven days. Furthermore, gentamicin was not detectable in the residual beads in the CSHA and CSCC groups at days 21 or 42, despite material remaining.

As seen with PMMA beads, once levels of released antibiotics are below MIC, any residual material may present a nidus for infection.[9,19,20] In this case any gentamicin-resistant bacteria could colonise unabsorbed materials.

The clinical use of calcium sulphate and calcium phosphate-based materials for the local release of antibiotics has been reported in indications where infection is present, ranging from diabetic foot infections to orthopaedic indication such as periprosthetic joint infections and trauma.[21–23] The antibiotic dosing[24–26] and release characteristics[27–29] for antibiotic-loaded PMMA has been widely reported, with clinicians frequently trying to achieve optimal antibiotic loading without compromising mechanical properties of the cement,[30] particularly when the cement is being used a spacer in two stage revision surgery or for prosthesis fixation. Unlike PMMA where the majority of antibiotic remains locked within the polymer, the combination of absorbable materials with antibiotics has the potential advantage of being able to release the entire antibiotic dose with which it is combined.

In-vitro studies measuring antibiotic release from absorbable materials have reported antibiotic elution being maintained at concentrations more than 500 μg/ml at 42 days,[31] but there is a large variation in the in-vitro experimental methods used to determine antibiotic elution from absorbable materials. Methodologies vary with respect to a number of parameters, including the volume removed for analysis and the eluent sampling intervals,[32] and the quantity of material tested.[31] In addition the nature by which the sample is presented to the solution can have an effect, with elution from a single small bead[33–35] will typically have lower antibiotic concentrations with elution for a shorter duration than if a larger cast cylinder of material is used.[36,37]

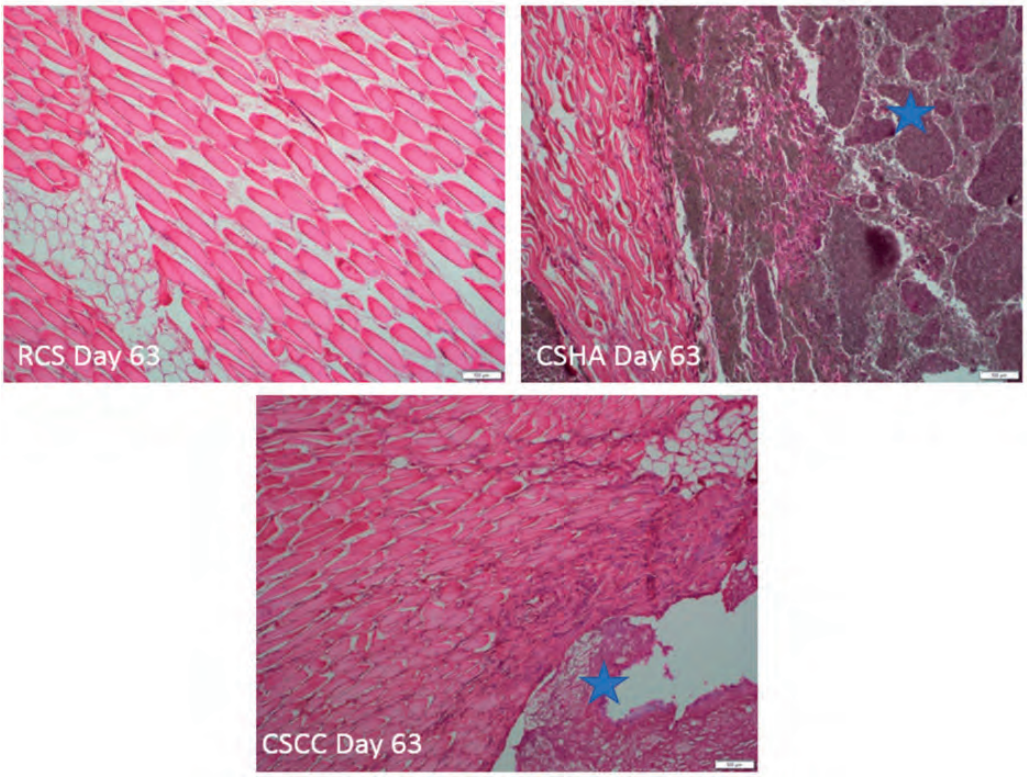

Figure 12. Histology at day 63 for the RCS group appeared normal. Histology at day 63 for the CSHA group demonstrated a similar appearance to days 21 and 42 with some fibrous tissue at the interface with the host muscle and a presence of a few lymphocytes. Histology at day 63 for the CSCC group was similar to days 21 and 42 with some fibrous tissue at the interface with the host muscle and a presence of a few lymphocytes. The materials are labelled with a star.

The clinical evaluation of antibiotic release from hydroxyapatite/calcium sulphate composite has reported levels of gentamicin were still present in urine 60 days post-surgery.[38] Another study has evaluated the local and systemic antibiotic levels in patients implanted with vancomycin-loaded calcium sulphate, reporting local concentrations were approximately ten times higher than with polymethylmethacrylate (PMMA) as a carrier, whilst serum levels typically remained less than 10 mg/l in the first days following implantation, decreasing rapidly.[2]

Upon implantation of a biomaterial into a surgical site, various reactions take place including foreign body response and inflammatory reactions. No adverse reactions to the implanted materials were noted. Histologically, all materials were well tolerated versus time. The inflammatory response included some lymphocytes and multinucleated cells that resolved with time for all materials.

The use of calcium sulphate in soft tissue sites suggest good tissue compatibility and complete absorption with minimal complications. Kallala et al.[39] reported on the use of calcium sulphate beads in 755 cases of revision total hip and total knee arthroplasty with 4.2% drainage and 1.7% heterotopic ossification. Swords et al.[40] details the use of calcium sulphate as an intracorporal cast for the treatment of infected penile implants acting as a filler, preventing fibrosis and loss of space with full absorption in four to six weeks and uneventful postoperative follow-up. Sherif et al.[41] carried out a retrospective analysis on the use of antibiotic-loaded calcium sulphate beads to salvage infected breast implants with positive outcomes and Kenna et al.[42] presented data using absorbable antibiotic beads for prophylaxis in immediate breast reconstruction in 68 patients, reducing the risk of periprosthetic implant infection with no complications. Healy et al.[43] reported on the direct placement of antibiotic-loaded calcium sulphate beads in the management of prosthetic vascular graft infections with dissolution in approximately six weeks.

Raina et al.[44] observed bone formation in the overlaying muscle covering surgically created bone defects when a calcium sulphate/HA composite material was used, implying that the combination of inductive proteins released from a defect in apposition to an osteoconductive material can enhance the process of ectopic ossification. Pre-clinical work by Wang et al.[45] investigated the reaction to the implantation of a calcium sulphate/HA composite material with and without the

addition of autologous bone marrow in rat muscle, evaluating the absorption and soft tissue reaction. Signs of an inflammatory reaction were noted and material remained at 12 weeks and no muscle necrosis was observed.

In contrast to these data, the release of HA particles by bone substitutes or as a coating on implants has previously been shown to induce an inflammatory response.[46] The cellular response to three types of calcium phosphate was investigated by van der Meulen et al.[47] and found that all materials produced a short mild inflammatory reaction. Mestres et al.[48] reported that hydroxyapatite substances can influence the growth and proliferation of macrophage-like cells. Recorded cases of implanted materials leaking from filled cavities causing tissue reactions have been reported and recorded by the FDA on the MAUDE database.[49]

RCS produced a reliable and reproducible in vivo resorption based on radiographs and microCT and was not detected on day 21. While some heterotopic ossification has been reported with calcium sulphate with vancomycin and tobramycin[50,51] this was not noted in the present study. The histology results for RCS versus time revealed the material to be very well tolerated in-vivo in this model. The local inflammatory cells present at the interface with the muscle in this model, while the material was absorbing, resolved with time and normal tissue was present in the implantation sites from 42 days.

Differences were noted for the in-vivo absorption of the three materials. The CSHA and CSCC beads both demonstrated material remaining at days 42 and 63 with evidence of persistent lymphocytic activity. For the CSHA beads, the remaining material was suspected to be the HA component of the material. The remaining material in the CSCC group warrants further investigation, as does, the persistent "halo" of extending into the surrounding soft tissue at the final follow-up for both the CSHA and CSCC. For both these groups, the presence of gentamicin was not detected in the residual material.

The in-vivo absorption of calcium sulphate has been investigated since the early 1960s. In the study of materials used to fill osseous defects, Bell demonstrated that plaster of Paris was absorbed twice as fast as autogenous bone and many times faster than homologous and heterogeneous bone when implanted in well vascularized gastrocnemius muscles. He reported complete absorption of plaster in approximately 33 days.[52,53]

Research has also studied the subcutaneous implantation of calcium sulphate into 12 Sprague-Dawley rats to determine the rate of material degradation in-vivo and the reaction of the surrounding tissues. Results indicated a localized reaction to the material as it degraded in the tissue, yet there was proliferation of granulation tissue which matured into a dense scar with little surrounding tissue reaction. The majority of material was absorbed within eight weeks.[54] Conclusions from this study were that when implanted into subcutaneous sites, calcium sulphate resorbed too rapidly to be effective in inducing bone replacement.

The in-vivo mechanism of absorption for calcium sulphate has been investigated. Ricci[55] observed that calcium sulphate materials were absorbed by rapid dissolution, both in-vitro and in-vivo, noting absorption from the outer surface inwards, at up to 1 mm per week. The absorption from outside in is observed in our study with RCS which supports Ricci's observations.

Based on the data presented here and reported clinical use, RCS when implanted into soft tissue is completely absorbed and inflicts a minor inflammatory response which resolves as the material is absorbed. CSHA and CSCC also demonstrate a minor inflammatory response but the presence of residual material demonstrates a slower absorption profile and the complete absorption of both these materials was not achieved at the time points evaluated here.

This study had a number of limitations. No sham control animals were employed in the model to determine the surgical site response of an unfilled intramuscular site. It was felt, that this would have provided limited information, as the intramuscular site would have closed with a dead space formation if no implant material was placed. A control group that included PMMA mixed with gentamicin was also not employed. In this study we were primarily focusing on calcium sulphate-based biomaterials. Lastly, the model as described is not an infection model. Hence it was not possible to determine the in-vivo efficacy of the antibiotic in the biomaterials on protecting the material from bacterial colonisation.

Conclusion

All materials were well tolerated, with no adverse host responses observed. All materials released gentamicin on implantation and were effective in resolving surgical dead space in this animal model. RCS demonstrated the most rapid absorption profile, showing almost complete absorption by day 21. CSHA and CSCC absorbed slower and were detectable in all four implantation sites at day 63. Analysis of the residual beads revealed the presence of detectable gentamicin at 24 h and seven days for CSHA and RCS. No gentamicin was detected in the residual CSCC samples at seven days. No gentamicin was detectable in residual beads of CSHA and CSCC groups at days 21 or 42. If implanted into an infected surgical site, unabsorbed

materials without residual antibiotic, or containing antibiotic at subtherapeutic levels, are at risk of colonisation by any gentamicin-resistant bacteria.

Declaration of Conflicting Interests

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Management of Traumatic Bone Defects in Tibial Plateau Fractures with Antibiotic-Impregnated Biodegradable Calcium Sulfate Beads: A Prospective Clinical Trial

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Purpose: There has been widespread interest in utilizing local antibiotics in fractures involving significant soft- tissue trauma where postoperative infections are a major concern. STIMULAN Rapid Cure (Biocomposites) is a resorbable calcium sulfate that can be mixed with a variety of antibiotics and formed into beads to create a nonstructural void filler. The primary goals of this study were to assess resorption of the device (STIMULAN Rapid Cure with antibiotics) and identify device-related adverse events in tibial plateau fractures.

Methods: This was a multicenter, prospective study of 30 patients with a tibial plateau fracture (AO-OTA type 41B and 41C), recruited from 5 trauma centers. The articular surface was reduced, the fracture was fixed with plates and screws, and the subchondral void was filled with the device. We assessed the local wound reaction to the device by recording redness, swelling, and serous drainage. We also measured the resorption rate of the calcium sulfate on serial radiographs. Follow-up was done at 6 weeks, 12 weeks, 6 months, and 1 year. Secondary outcomes were time to union and postoperative depression of the subchondral surface. Descriptive statistics were used for analysis.

Results: 13 male and 17 female patients were included. Patients had a mean age of 53 years (range, 29 to 78) and a mean body mass index of 29 (standard deviation [SD] = 8.7). Postoperative swelling and redness were within normal limits. Two patients reported serous drainage: 1 resolved without treatment, the other required oral antibiotics for superficial infection of a stitch abscess. There were no other infections at the operative site and no local or systemic allergic reactions. There was 1 report of intra-articular heterotopic ossification requiring debridement. Three patients experienced hardware irritation: 1 was revised to a smaller plate and 2 required implant removal. One patient was revised to a total knee replacement after loss of fixation. None of these were deemed related to the study device. 76.7% of fractures were healed by 3 months with 100% healed at 1 year with no significant subchondral collapse. Resorption of the material averaged 70% by 12 weeks and 87% had no visible calcium sulfate beads on radiographs at 6 months.

Conclusion: This device appears to perform well when mixed with antibiotics for fractures with a high risk of infection. An additional advantage is being able to choose from a range of antibiotics. Surgical site drainage was very low, and 100% union rate was achieved. There were no remaining beads visible at 1 year. This should be a safe surgical augment for local release of a chosen antibiotic into the subchondral void of a tibial plateau fracture.

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BIOMATERIALS

Use of Stimulan absorbable calcium sulphate beads in revision lower limb arthroplasty

SAFETY PROFILE AND COMPLICATION RATES

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Aims

Calcium sulphate has traditionally been used as a filler of dead space arising during surgery. Various complications have been described following the use of Stimulan bio-absorbable calcium sulphate beads. This study is a prospective observational study to assess the safety profile of these beads when used in revision arthroplasty, comparing the complication rates with those reported in the literature.

Methods

A total of 755 patients who underwent 456 revision total knee arthroplasties (TKA) and 299 revision total hip arthroplasties (THA), with a mean follow-up of 35 months (0 to 78) were included in the study.

Results

A total of 32 patients (4.2%) had wound drainage, and this was higher with higher bead volumes and in McPherson grade C patients. There was also a significantly higher bead volume in the 41 patients who developed hypercalcaemia, two of which were symptomatic ($p < 0.0001$). A total of 13 patients (1.7%) had heterotopic ossification (HO). There was no statistically significant relationship between the development of HO and bead volume ($p > 0.05$).

Conclusion

The strength of this study lies in the large number of patients and the detailed data collection, making it the most comprehensive report available in the literature on the use of calcium sulphate-based bone substitutes.

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Keywords: Biomaterials, Synthetic bone substitutes, Calcium Sulphate

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Article focus

- Biomaterials
- Orthobiologics
- Complications

Strengths and limitations

- Large patient case-series with extensive analysis of underlying factors.
- Focuses on one product, difficult to make like-for-like comparisons.

Introduction

The use of calcium sulphate to fill bone defects was first described in 1892 by Dreesmann, in Bonn. He reported the treatment of eight patients with bone voids filled with a mixture of calcium sulphate and phenol.[1,2] Peltier further popularised its use in 1959,[3] and amongst his conclusions were firstly that the implantation of calcium sulphate into bone or soft tissue does not produce a foreign body reaction; secondly, it stimulates new bone formation when periosteum or bone is also present, and thirdly it is usually absorbed and removed from the site of implantation.[3] Today, calcium sulphate beads may be used as an alternative void filler to poly(methyl methacrylate) (PMMA) in the presence of infection, nonunion or bone loss.[4,5] Traditional antibiotic-loaded PMMA

beads require subsequent removal and may develop biofilm on their surface if left *in situ* for long periods of time.[6] Some authors have shown a relatively short period of antibiotic release with a decrease in local concentrations to 10% of the initial levels within 24 hours.[5,7] In contrast, as it is absorbed, calcium sulphate releases 100% of its antibiotic load, resulting in superior elution characteristics and higher sustained antibiotic concentrations over a period of several weeks.[5,7-10] This results in concentrations of antibiotic locally that can be many times higher than the minimum inhibitory concentration for the relevant pathogen, while also ensuring that systemic levels and associated toxicity remain low.[8,11] The use of calcium sulphate in orthopaedics has therefore been increasing, both as a bone void filler and as an "off-label" delivery agent for antibiotics in arthroplasty, chronic osteomyelitis, open fractures and combat injuries.[4,5,12] As this practice has increased, so has the understanding of the associated benefits and complications, which include transient hypercalcaemia, wound drainage and heterotopic ossification (HO).[9,11] Anecdotally, there has been an increase in complications when higher volumes of beads are used, especially in subcutaneous structures and in patients with comorbidities such as diabetes and long-term steroid use.

This study aims to assess the safety profile of calcium sulphate bio-absorbable beads in revision arthroplasty, comparing complication rates in our patients with those reported in the literature.

Patients and Methods

Data were collected prospectively from patients undergoing revision arthroplasty of the knee and hip attending an arthroplasty centre in the United States (Los Angeles Orthopaedic Institute) between September 2010 and June 2016. All procedures were undertaken by a single surgeon (EM). Patients who had a manipulation under anaesthesia or arthroscopic washout and debridement were excluded. Those who underwent conversion of unicompartmental knee arthroplasty (UKA) to total knee arthroplasty (TKA) were included.

Demographic data including age and gender, and clinical data including patient staging, indication for revision surgery, procedure-specific information, follow-up, mortality and complications were recorded. Staging was based on the system described by McPherson et al (Tables I and II).[13] The indications were divided into infection, aseptic loosening, instability, periprosthetic fracture, metal allergy, implant failure and clinical need (pain or stiffness). The diagnosis of periprosthetic infection was confirmed using the Musculoskeletal Infection Society criteria (Table III).[14,15] Procedure-specific data included the type and volume (cc) of Calcium Sulphate (Stimulan, Biocomposites Ltd, Keele, United Kingdom) which were used. Types of procedure included single-stage revision, the first or second stage of a two-stage revision, and debridement, antibiotics, exchange of liner and implant retention (DAIR). The latter procedure included the implantation of Stimulan beads and intravenous antibiotics. The first of a two-stage revision

Table I. Staging system for prosthetic joint infection risk (part I)

Category	Grading	Description
Infection type	0	No active infection
	I	Early postoperative infection (< 3 wks postoperatively)
	II	Hematogenous infection (< 3 wks' duration)
	III	Late chronic infection (> 3 wks' duration)
Systemic host grade (Medical/immune status)	A	Uncompromised (no compromising factors)
	B	Compromised (1 to 2 compromising factors)
	C	Significant compromise (> 2 compromising factors) **or** one of the following: Absolute neutrophil count <1000 CD4 T cell count < 100 Intravenous drug abuse Chronic active infection at other site Dysplasia/neoplasm of immune system (e.g. Myelodysplasia, CLL)
Local Extremity Grade	1	Uncompromised (no compromising factors)
	2	Compromised (1-2 compromising factors)
	3	Significant compromise (> 2 compromising factors) **or** one of the following: Soft-tissue loss requiring muscle transposition or Free flap transfer Bone loss requiring structural allograft or Substituting megaprosthesis Local wound irradiation ≥ 4000 rads

Stage = infection type + systemic host grade + local extremity grade; e.g I-A-1, III-B-2

Table II. Staging system for prosthetic joint infection risk (part ii)

Systemic host (medical/immune) compromising factors
Age ≥ 80 yrs
Alcoholism
Chronic active dermatitis/cellulitis
Chronic indwelling catheter
Chronic malnutrition (albumin < 3.0gm/dL)
Current nicotine use (inhalational or oral)
Diabetes (requiring oral agents and/or insulin)
Hepatitic insufficiency (cirrhosis)
Immunosuppressive drugs (e.g. methotrexate, prednisone, cyclosporine)
Malignancy (history of, or active)
Renal failure requiring dialysis
Systemic inflammatory disease (e.g., RA, SLE)
Systemic immune compromise from infection or disease e.g., HIV, acquired immunodeficiency
Local extremity (wound) compromising factors
Infection of a revision arthroplasty
Recurrent infection after joint debridement with prosthesis retention
Recurrent infection after prosthetic exchange protocol
Recurrent open foot sores (neuropathic or structural)
Multiple incisions (creating skin bridges)
Sinus tract
Vascular insufficiency to extremity: absent extremity pulses, calicific aterial disease, venous insufficiency with skin plaques or intermittent sores

SLE, systemic lupus erythematosus; HIV, human immunodeficiency virus; ABG, arterial blood gases

Table III. Musculoskeletal Infection Society definition of peri-prosthetic infection[14]

Presence of a sinus tract communicating with the prosthesis
OR
Isolation by culture of a pathogen from ≥ two separate tissue or fluid samples from the affected joint.
OR
Any four of the following criteria are present:
Raised ESR and CRP.
Raised synovial leukocyte count.
Raised synovial neutrophil percentage.
Purulence in the affected joint.
Isolation of a micro-organism in one culture of joint tissue or fluid.
> five neutrophils per high-power field in five high power fields observed from histologic analysis of peri-prosthetic tissue at 9400 magnification.

Table IV. The classification of heterotopic ossification around the knee according to Harwin et al[18]

Grade	Radiographic findings
I	Sessile attached to the periosteum of the anterior femur and limited to the suprapatellar pouch
II	Amorphous or globular pattern limited to the quadriceps expansion
IIIa	Combination of sessile and globular with less than 75% of the height of the soft tissues on lateral radiograph involved.
IIIb	Combination of I and II with greater than 75% of the height of the soft tissues on lateral radiograph involved.

Table V. The classification of heterotopic ossification around the hip according to Brooker et al[19]

Grade	Classification
Grade I	Ossification islands around the hip
Grade II	Bone projection of pelvis or proximal femur at least 1 cm away from the opposite surface
Grade III	Bone projection of pelvis or proximal femur reducing space between opposite surface < 1 cm
Grade IV	Hip ankylosis

included washout, debridement, removal the components and the implantation of PMMA spacer and Stimulan beads. The second stage included the removal of the spacer and implantation of revision components and further beads. The diagnosis of metal allergy was made using a lymphocyte transformation test from venous blood (Orthopedic Analysis, Chicago, Illinois).

Routine intravenous antibiotics and 1g tranexamic acid were administered at induction, and approximately 40 minutes prior to closure. An additional dose was given during particularly long procedures. We used commercially pure, synthetic physiological pH calcium sulphate powder - Stimulan (Biocomposites Ltd, Keele, United Kingdom) with the RapidCure kit which includes 10 cc (20 g) of calcium sulphate hemihydrate powder, a pre-mixing solution bulb, pellet mould and spatula. The mould produces three sizes of bead (3, 4.8 and 6 mm in diameter). One gram of vancomycin powder was mixed with each 10 cc of calcium sulphate in the mixing bowl and 240 mg of liquid tobramycin (40 mg/ml) was added. The ingredients were mixed for 30 seconds until "doughy" and the resulting paste was applied to the moulds using the spatula and allowed to set for ten to 15 minutes in a typical theatre temperature of 16°C to 17°C.[16,17] In patients with fungal infection, 50 mg of amphotericin B was also added. The beads loaded with antibiotics were implanted around the components or the spacer before the wound was closed.

For patients undergoing knee surgery, the beads were placed in the medial and lateral gutters. For those undergoing hip surgery, they were placed deeply, inferior to the acetabulum and around the proximal femur.

PMMA, loaded with vancomycin and/or gentamicin, was used in all cemented procedures and in the first of two-stage procedures requiring the use of spacers. No other cement or absorbable beads were used. Routine postoperative blood tests included a full blood count, CRP, ESR, and bone profile. Routine radiographs were also undertaken prior to discharge from hospital. The patients were reviewed at six weeks, three, six and 12 months postoperatively and bi-annually thereafter. HO around the knee was categorized according to the classification of Harwin et al (Table IV).[18] HO around the hip was categorized according to the classification of Brooker et al (Table V).[19] The levels of calcium in the blood were recorded on the first postoperative day, and at routine review. Hypercalcaemia was defined as a level of > 10.7 mg/dL (equivalent to 2.6 mmol/L).

The complications arising from revision surgery and from the use of the beads were recorded. Any reoperation was classified as a surgical complication and divided into aseptic loosening, fracture, instability and those due to infection. Stimulan-specific complications included wound drainage, HO and hypercalcaemia.

Statistical analysis. Statistical analysis of all Stimulan-related complications was undertaken. Our primary aim was to relate the presence or absence of a complication to the volume of the beads, the grade of the patient and the location using logistic generalised linear model (GLM). The location, the presence of a complication and the relationship between the grade of the patient and type of complication were tested using the chi-squared test. All analyses were performed in R (R Foundation for Statistical Computing, R foundation, Vienna, Austria). Statistical significance was set at $p < 0.05$.

Results

A total of 755 patients with a mean age of 63 years (30 to 94), of whom 456 had revision TKA (RevTKA) and 299 had revision THA (RevTHA) were included in the study. Two patients in the RevTKA group underwent conversion of UKA to TKA. There were 381 women and 374 men. The mean follow-up was 35 months (0 to

Table VI. The demographics of the patients and outcomes (part i)

n = 755				**Total (n)**	**Hip (n)**	**Knee (n)**	**Total (%)**	**Hip (%)**	**Knee (%)**	**Patient host grade**
Knees	**n = 456**		Complications	100	34	66	13%	11%	14%	
	Age (yrs)	63.87 (29 to 96)	Aseptic failures	25	9	16	7%	6%	8%	
	Gender	243 F, 243 M	Loosening	5	3	2	5%	5%	6%	
	Avg F/U	35.08 mths (0 to 78)	Instability	13	4	9	11%	9%	12%	
	Stim avg	21.51 (5 to 80)	Periprosthetic	1	0	1	2%	0%	4%	
			Metal allergy	2	0	2	11%	0%	11%	
Hips	**n = 299**		Implant failure	2	2	0	29%	29%	0%	
	Age (yrs)	62.09 (31 to 92)	Pain/stiffness	2	0	2	3%	0%	4%	
	Gender	188 F, 160 M								
	Avg F/U	35.28 mths (0 to 78)	**Infection**	34	15	19	5%	5%	4%	blank
	Stim avg	25.27 (5 to 70)	Original indication infection	27	12	15	7%	9%	6%	
Indication (combined)										
Infection	387	Hip 140; Knee 247								
Loosening	95	Hip 61; Knee 34	Drainage	32	11	21	4%	4%	5%	3%/5%/5%
Instability	118	Hip 43; Knee 75	Hypercalcemia	41	19	22	5%	6%	5%	1%/7%/13%
Periprosthetic	50	Hip 22; Knee 28	Heterotopic Ossification	13	8	5	2%	3%	1%	1%/3%/1%
Metal allergy	19	Hip 1; Knee 18	Deceased	14	6	8	2%	2%	2%	blank
Implant failure	7	Hip 7; Knee 0								
Pain/stiffness	79	Hip 25; Knee 54								

Table VII. The demographics of the patients and outcomes (part ii)

Knees			**Hips**		
Single stage	n = 209		**Single stage**	n = 159	
	Age (yrs)	62.98 (29 to 96)		Age (yrs)	62.78 (32 to 92)
	Gender	127 F, 105 M		Gender	109 F, 91 M
	Avg F/U	34.81 months (0 to 78)		Avg F/U	36.00 mths (0 to 78)
	Stim avg	13.78		Stim avg	18.7
	Stim range	5 to 40		Stim range	5 to 50
	Complications	33		Complications	12
	Failures	14		Failures	9
	Septic	4		Septic	3
	Drainage	8		Drainage	3
DAIR	n = 49		**DAIR**	n = 19	
	Age (yrs)	61.29 (43 to 77)		Age (yrs)	57.77 (32 to 76)
	Gender	23 F, 30 M		Gender	14 F, 7 M
	Avg F/U	34.13 mths (0 to 77)		Avg F/U	35.18 mths (2 to 57)
	Stim avg	20.75		Stim avg	33.00
	Stim range	5 to 40		Stim range	10 to 60
	Complications	4		Complications	2
	Failures	6		Failures	1
	Septic	5		Septic	1
	Drainage	2		Drainage	2
First of 2 stage	n = 108		**First of 2 stage**	n = 68	
	Age (yrs)	65.24 (28 to 86)		Age (yrs)	61.39 (31 to 88)
	Gender =	53 F, 59 M		Gender =	38 F, 35 M
	Avg F/U =	35.57 mths (1 to 75)		Avg F/U =	33.22 mths (0 to 73)
	Stim avg =	34.15		Stim avg =	36.58
	Stim Range =	10 to 80		Stim Range =	10 to 60
	Complications =	10		Complications =	13
	Failures =	2		Failures =	4
	Septic =	1		Septic =	4
	Drainage =	2		Drainage =	2
Second of 2 stage	n = 90		**Second of 2 stage**	n = 53	
	Age (yrs)	65.70 (28 to 87)		Age (yrs)	62.04 (32 to 88)
	Gender	40 F, 49 M		Gender	27 F, 27 M
	Avg F/U	35.34 mths (3 to 73)		Avg F/U	35.67 mths (1 to 72)
	Stim avg	26.12		Stim avg	32.96
	Stim range	10 to 40		Stim range	10 to 70
	Complications	19		Complications	8
	Failures	12		Failures	8
	Septic	9		Septic	7
	Drainage	11		Drainage	4

DAIR, exchange of liner and implant retention

Table VIII. Summary of results for patients with hypercalcaemia

Procedure	Bead volume (cc)	Ca Peak (mg/dL)	Duration (days)	Host grade
Revision	50	14.9	10	B
Revision	20	14.2	6	B
Reimplantation	40	12.9	3	B
Resection	50	12.4	7	C
DAIR	40	11.9	5	C
Revision	40	11.8	5	C
Revision	20	11.5	4	B
Resection	20	11.5	8	C
Revision	40	11.3	2	B
Reimplantation	40	11.2	2	B
Revision	30	11.1	2	C
DAIR	40	11.1	5	C
Reimplantation	40	10.9	1	B
Reimplantation	30	10.9	1	C
Revision	40	10.9	4	B
Revision	20	10.9	7	B
Resection	40	10.9	7	B
Revision	30	10.8	2	B
Resection	20	10.8	1	A

DAIR, exchange of liner and implant retention

Table IX. Descriptive statistics for bead volume (cc) and calcium-related complications

Complication	n	Mean	Median	SD
Drainage	31	24.4	20.0	10.9
Hypercalcemia	41	32.3	40.0	10.7
Heterotopic ossification	13	27.7	30.0	12.8

Table X. Generalised linear model (GLM) results relating the presence or absence of a complication to bead volume, location and patient grade. GLM results relating to bead volume to the type of complication, location and patient grade

Dependent variable	Main effect	Chi-squared	Degrees of freedom	p-value
Complication presence			(n = 755)	
	Bead volume	10.2	1	**0.0014**
	Patient grade	12.3	2	**0.0021**
	Body location	0.13	1	0.72
Bead volume			(n = 86)	
	Complication type	4.38	2	**0.027**
	Patient grade	0.09	2	0.87
	Body location	8.98	1	**0.0077**

values in bold indicate statistical significance

78). The indications are shown in Tables VI and VII. Most (387) underwent revision for infection, followed by 118 who underwent revision for instability. All seven patients whose initial indication was implant failure had failure of the acetabular component due to fracture of the ceramic liner. A total of 209 in the RevTKA group had a single-stage revision, 19 using DAIR; 108 had the first of a two-stage revision, and 90 had the second of a two-stage revision. A total of 159 in the RevTHA group had a single-stage revision, 19 using DAIR; 68 had the first of a two-stage revision and 53 had the second of a two-stage revision. A total of 59 (7.8%) were reoperations, 34 were for infection (27 recurrent) and 25 for aseptic causes: loosening (5), instability (13), acetabular failure (2), periprosthetic fracture (1), metal allergy (2) and pain and stiffness (2).

A mean of 23.39 cc (5 to 80) of Stimulan was used per procedure. Stimulan-related complications occurred in 86 operations (11.4%), drainage in 32 (4.2%), hypercalcaemia in 41 (5.4%) and HO in 13 (1.7%) (Table VI).

Drainage occurred in 32 patients (4.2%), 21 knees and 11 hips. If this occurred within five days postoperatively and involved serous or serosanguinous fluid with or without calcium deposits, anti-coagulants were stopped and the wounds re-dressed. If drainage persisted for more than five days postoperatively, or was sanguinous in

nature, (as in 15 knees and eight hips in our series) a washout under general anaesthesia was undertaken.

Transient hypercalcaemia was detected in 41 patients (5.4%), 22 knees and 19 hips (Table VIII. The mean levels were 11.7 mg/dL (10.8 to 14.9); the levels returned to normal at a maximum of five days postoperatively. Two patients in the RevTHA group developed symptoms and were treated with one IV dose of bisphosphonate and 0.9% saline.

HO occurred in 13 patients (1.7%), five knees and eight hips. Knees (Harwin): Mode – 1; Range – 1-3 Hips (Brooker): Mode – 1; Range – 1-2.

One patient in the RevTKA group with Harwin II HO required manipulation. Two patients in the RevTHA group with HO (Brooker II and III respectively) were treated operatively at time of reimplantation. The remaining four knees and six hips with HO required no treatment. The volume of the beads for the grades of the patients are shown in Table IX.

Stimulan-related complications. Analysis of the factors relating to the presence or absence of a complication and the volume of the beads are shown in Table X. Overall, both bead volume ($p = 0.0014$) and patient grade ($p = 0.0021$) had a significant effect on the presence or absence of a complication. The location had no significant effect ($p = 0.72$; Fig. 1). The volume of the beads was significantly different for types of complication ($p = 0.027$; Fig. 2a). In *post hoc* comparisons looking at pairwise differences in bead volume between complication type, there was a significant difference between the hypercalcaemia and drain groups (Hypercalcaemia, HyCal – Drain Tukey adjusted $p = 0.045$), while the other pairwise difference were not significantly different (HO – Drain $p = 0.88$; HyCal – HO $P = 0.46$).The volume of the beads was significantly different for body location ($p = 0.0077$; Fig 2c). There was no significant relationship between volume and patient grade ($p = 0.87$; Fig. 2b).

Discussion

Clinical outcomes and patient-reported outcome measures were not the focus of this study and therefore were not reported. However, the demographics of the patients were comparable with those reported in the literature. With a mean age of 63 years, 57% being women, these demographics are similar to the data reported in both the National Joint Registry of England and Wales (NJR) (mean age 69.9, women 51.5%) and the American Joint Replacement Registry (AJRR) (mean age 66.5, women 59.2%) for patients undergoing revision TKA and THA.[20,21] Infection was the main indication for revision in our series, with 387 patients (51%), 247 RevTKAs and 140 RevTHAs, having proven infection preoperatively. The rate of reoperation was 7.8% at a mean follow-up of 35.2 months, comparable with that of 8.45% reported in the NJR.[20]

Wound drainage. The incidence of wound drainage, of 4.2%, is low, with rates ranging between 3.2% and 51% in the literature.[9,22] A previous study by the senior author (EM) analysed wound drainage rates following the use of antibiotic impregnated calcium sulphate in a series of 250 revision arthroplasties, reporting a rate of 3.2%.[9] Drainage tended to occur in patients in whom a higher volume of bead had been used, with more subcutaneous placement and in those with a poor host grade, such

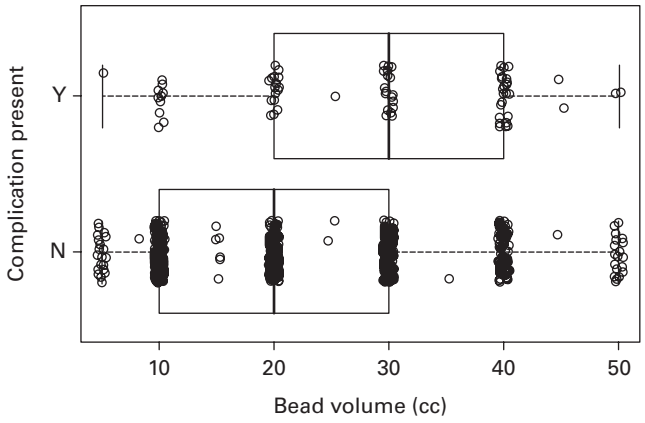

Fig. 1a

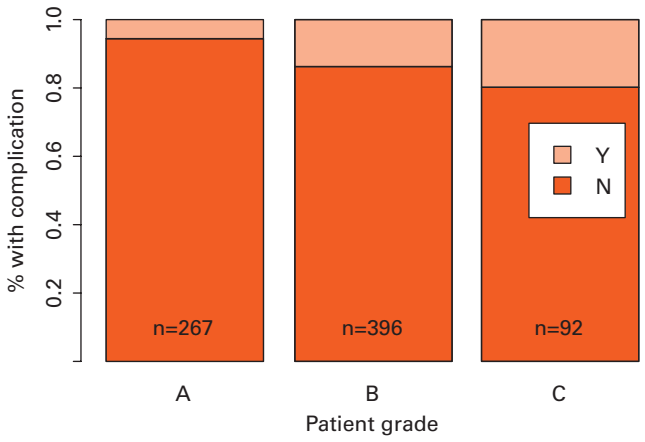

Fig. 1b

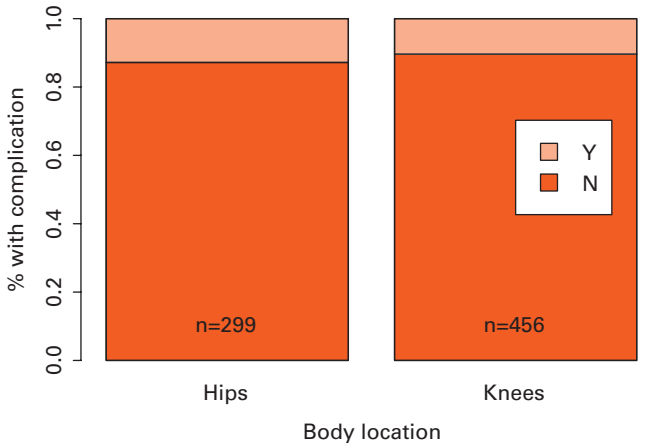

Fig. 1c

Graphs showing a) how bead volume changes for different combinations of complication (ComPresent); b) how the percentage with complications changes with patient grade; and c) how percentage with complications changes with body location. In graphs b) and c), total sample sizes are indicated at the bottom of the bars.

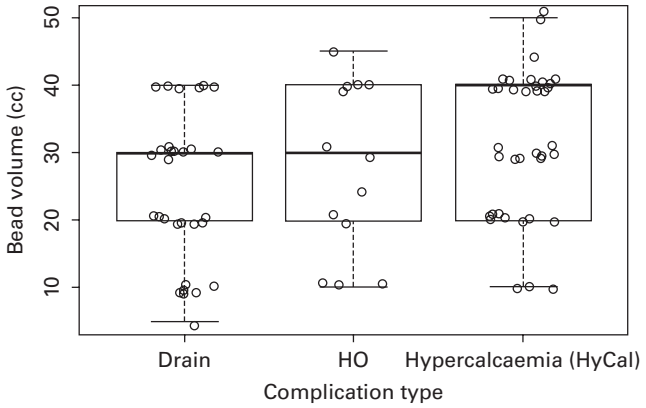

Fig. 2a

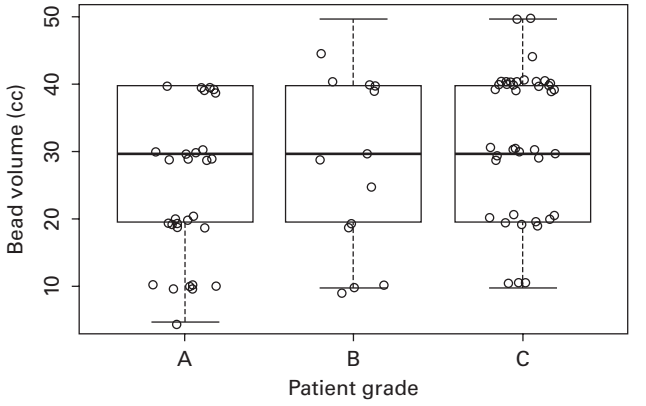

Fig. 2b

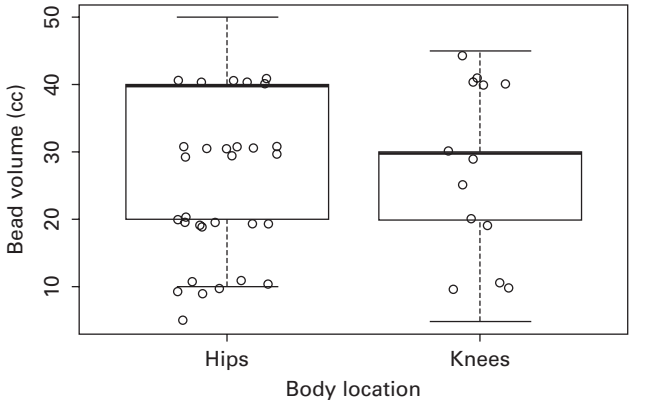

Fig. 2c

Graphs showing the subset of patients with a complication: a) bead volume changes for different types of complication; b) bead volume changes with patient grade and c) the percentage with complications changes with body location.

as McPherson grade C.[9,13] Kelly et al[23] reported a rate of drainage of 3.6% in 109 patients treated with calcium sulphate for bone defects using Osteoset (Wright Medical Technology Inc., Arlington, Tennessee). Ferguson et al[24] reported a rate of 15.4% when used to treat 195 patients with chronic osteomyelitis. The relative subcutaneous placement of beads and rate of drainage reported in this paper supports a role for location of beads in the development of drainage. Borelli et al[25] reported a rate of drainage of 23% (five patients) in the treatment of 26 patients with a nonunion and an osseous defect, using bone graft mixed with calcium sulphate. The highest rate, of 51%, was reported by Ziran et al[22] using calcium sulphate combined with demineralized bone matrix in the treatment of nonunions.

We found no significant difference in bead volume between patient drainage groups. There was also no significant difference in the rate of complications (the number of patients with drainage) and location between the RevTKA (22) and RevTHA (19) groups ($p > 0.05$). Therefore, the observation that drainage is increased in large bead volumes remains anecdotal.

Drainage within five days of surgery was managed conservatively but drainage beyond five days was treated surgically with a washout and closure. Therefore, the longer-term implications on infection and clinical outcome are not available for our dataset. However, in a case series reported by Ferguson et al,[24] there were no recurrent infections in the drainage group treated non-surgically, all of whom had drainage beyond 14 days postoperatively. This may reflect a high concentration of antibiotic draining from the wound, rather than exudate alone. It is of concern when a carefully undertaken revision joint leaks exudate that is similar to the discharge seen in infection. Wound drainage is a recognized complication after the use of calcium sulphate beads, notably when volumes of > 20 cc are used, particularly in subcutaneous bones, as around the knee.[4,26] As well as physical factors such as volume and anatomical placement, the grade of the patient and the dissolution of calcium sulphate are also thought to play a role.

Calcium sulphate ($CaSO_4$) is inorganic and has three principle forms: anhydrous, known as anhydrite, with the formula $CaSO_4$; hemihydrated with the formula $CaSO_4 \cdot 0.5H_2O$ and dihydrated with the formula $CaSO_4 \cdot 2H_2O$. Once water is added it converts to the dihydrate form, and this is the basis for the setting reaction. Stimulan is the synthetically pure hemihydrated form. It is hypothesized that the placement of calcium sulphate in the operating field alters the osmolality of the wound, leading to the movement of water out of cells with the accumulation of fluid and wound drainage.[5,11,27,28] Precautions taken by the senior author include reduced volume of Stimulan in subcutaneous tissues and fastidious wound closure (Fig. 3).

Transient hypercalcaemia. We found an overall difference in the volume of the beads in the different types of complication, with a larger volume in the group with hypercalcaemia compared to patients without a complication. This corroborates our previous observations[9,11] about the excessive use of calcium sulphate-based products and the associated risk of hypercalcaemia.

Peltier[3] initially described transient hypercalcaemia after implantation of calcium sulphate in his experiments in the 1950s. There were raised serum calcium levels after the implantation of calcium sulphate beads in a canine

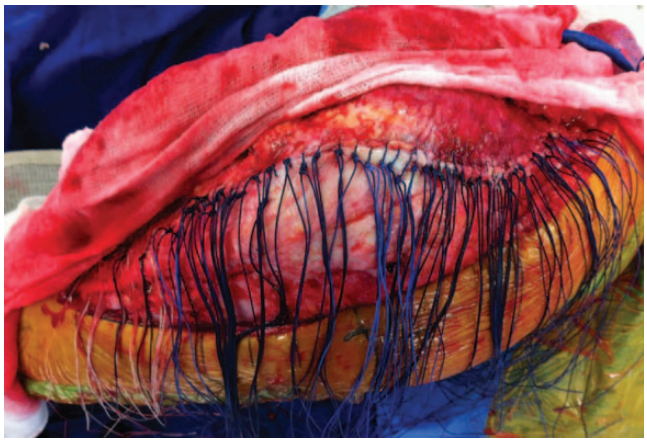

Fig. 3

Photograph showing wound closure of deep layer using interrupted absorbable sutures.

model, but this elevation was not sustained nor were systemic effects noted. A later study using calcium sulphate to fill osseous defects in five patients did not reproduce this transient hypercalcaemia.[29]

A previous study reported by the lead author involved 15 patients of whom three developed hypercalcaemia, one with symptoms requiring treatment. This patient had undergone a two-stage revision THA and had received 40 cc of Stimulan with antibiotics implanted around the revision components.

A case report by Carlson et al[30] described transient hypercalcaemia in a patient with a peak serum calcium of 14.5 mg/dL on the fifth postoperative day after revision of an infected THA, who was treated with a single subcutaneous dose of calcitonin 200 IU and IV fluids. The calcium level returned to normal four days later. A further case report described a 69-year-old woman who developed convulsions and coma after calcium sulphate beads were implanted during lumbar fusion.[31] The authors concluded that these were caused by leakage of calcium sulphate into the cerebrospinal fluid through a dural tear.[31] The serum calcium levels remained normal.

There is an active United States Food and Drug Administration adverse reaction report regarding transient hypercalcaemia following the use of vancomycin-infused calcium sulphate beads (Osteoset; Wright Medical Technology Inc.) in a patient undergoing revision THA for infection.[32]

Precautions should be taken to avoid hypercalcaemia which may cause convulsions, coma and cardiac arrest. All patients should be screened for contraindications and have their calcium levels monitored pre- and postoperatively. In our opinion, the volume of calcium sulphate should be limited to a maximum of 40 cc per operation, increasing to 80 cc if it is placed within the medulla of the bone. FDA guidelines on the use of materials, which include calcium to fill voids in bone, warn of the risks of transient hypercalcaemia, and cautions against their use in the presence of pre-existing disorders of calcium metabolism, as well as warning against "inappropriate material composition which may lead to substantially more rapid resorption of the implant".[33] It is hypothesized that premature breakdown within a short period of time results in the 'dumping' of calcium ions in a small area, and rapid absorption by local capillaries. The reasons for this are poorly understood but probably reflect a combination of host factors such as the grade of the patient, and surgical variables, such as excessive saline used to mix the calcium sulphate paste and implantation before complete setting of the beads. It should be noted that adding different antibiotics and combinations of antibiotics has very different effects on setting time.[16]

Heterotopic ossification (HO). There is little information about HO after revision arthroplasty and less about its incidence after the use of calcium sulphate. The combined rate of HO for revision knees and hips in our series, of 1.7% is low, with rates of up to 56% following revision TKA being reported.[34] We found that the volume of the beads had no statistically significant effect on the incidence of HO compared with patients without complication.

To our knowledge, there are no other reports regarding the incidence of HO following the use of calcium sulphate beads in revision surgery apart from those of the senior author. In that series, mild HO (Brooker I – II) occurred in eight of 250 revision TKA and THAs (3.2%) and there was a suggested link between high bead volumes and HO, as well as reduced intra-articular synovial volume, as occurs in osteoarthritic joints and exposed intra-articular bone following periosteal stripping during surgery. HO is the formation of mature lamellar bone in extraskeletal soft tissues.[35] Acquired HO is strongly associated with brain injury and other central nervous system conditions including tumours, spinal cord lesions and infection, as well as soft-tissue trauma as occurs in burns, polytrauma and arthroplasty.[35,36] Although not fully understood, its aetiology is known to involve the induction of osteoprogenitor differentiation in response to injury, and osteoblast formation with bone deposition.[35] Raina et al[37] reported that skeletal muscle acts as an osteoinductive niche for bone formation with the use of a biphasic calcium sulphate/hydroxyapatite biomaterial. Murine skeletal muscle cells were seeded onto hydroxyapatite/calcium sulphate and the phenotype of the cells analyzed after exposure to secretions harvested from rat bone cells to mimic the extracellular matrix proteins and growth factors present in an orthopaedic surgical site. The cells differentiated into osteoblast-like cells, expressing prominent bone markers after seeding on the biomaterial. The media of the cells contained bone morphogenetic protein-2 (BMP-2) (8.4 ng/mg, SD 0.8), and BMP-7 (50.6 ng/mg, SD 2.2). *In vitro*, this model, similar to that found in the surgical sites of our patients, induced differentiation of skeletal muscle cells towards an osteogenic lineage. In our series, no

intraoperative histological samples were taken and therefore it is not possible to say whether the HO was truly ectopic bone or ectopic calcification of soft tissue following chemical (calcium sulphate) and physical trauma (dystrophic soft-tissue calcification).[36] In either case, it is possible that the deposits represented both a complication of revision surgery and of implanting calcium sulphate, with clustering of beads leading to locally raised concentrations of calcium, increasing the risk of HO. Overall, the effect of HO in our patients was low, and it was brittle and easily removed during second-stage procedures. It did not cause symptoms when left in patients undergoing single-stage procedures. Therefore, it is not felt that HO is a significant issue when using absorbable calcium sulphate beads.

Patient grade. We found an overall effect of patient grade on the rate of complications. However, there was no tendency to use a higher volume of beads in grade B and C patients, who subsequently developed complications, suggesting that factors other than volume contribute to the complications.

The study has strengths, including the large number of patients and the detailed data collection, making it the most comprehensive report in the literature, to date, on the use of calcium sulphate-based bone substitutes. The main limitation is the overall design. Although detailed and prospective, the study lacks a control group. Furthermore, although the causes of Stimulan-related complications are likely to be multifactorial, we only assessed the effects of patient grade and bead volume. Also, although bead volume was shown to be a statistically significant factor in the development of hypercalcaemia, we were not able to support anecdotal observations in the literature about drainage and HO, nor did patient grade have a statistically significant effect on the rate of complications.

Overall, these findings contribute to the literature and will help inform surgeons about the risks and benefits of using calcium sulphate. Future studies should focus on multivariate analysis and incorporate control groups to elucidate the risks better.

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Author Contributions

- R. Kallala: Project concept, study design, data collection, data analysis, manuscript preparation.
- W. Edwin Harris: Data analysis, manuscript preparation.
- M. Ibrahim: Data analysis, manuscript preparation.
- M. Dipane: Data collection, data analysis.
- E. McPherson: Project concept, data collection, data analysis, manuscript preparation.

ICMJE COI Statement

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