Received: 11 November 2019 | Revised: 22 February 2020 | Accepted: 7 April 2020

DOI: 10.1111/xen.12600

REVIEW ARTICLE

Xenotransplantation WILEY

Bone xenotransplantation: A review of the history, orthopedic clinical literature, and a single-center case series

Daniel N. Bracey[1] | Natalie E. Cignetti[1] | Alexander H. Jinnah[1] | Austin V. Stone[2] | Bettina M. Gyr[3] | Patrick W. Whitlock[4] | Aaron T. Scott[1]

[1]Department of Orthopaedic Surgery, Wake Forest School of Medicine, Winston-Salem, NC, USA

[2]Department of Orthopaedic Surgery and Sports Medicine, University of Kentucky, Lexington, KY, USA

[3]Department of Orthopedic Surgery and Sports Medicine, Children's Hospital of the King's Daughters, Norfolk, VA, USA

[4]Division of Orthopaedic Surgery, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Correspondence
Daniel N. Bracey, Department of Orthopaedic Surgery, Wake Forest School of Medicine, Winston-Salem, NC, 27157, USA.
Email: danielbracey@gmail.com

Abstract

Background: One-half of all orthopedic surgeries require bone grafting for successful outcomes in fusions, reconstructive procedures, and the treatment of osseous defects resulting from trauma, tumor, infection, or congenital deformity. Autologous bone grafts are taken from the patient's own body and remain the "gold standard" graft choice but are limited in supply and impart significant patient morbidity. Xenograft bone is an attractive alternative from donors with controlled biology, in large supply and at a theoretically lower cost. Clinical results with xenograft bone for orthopedic applications have been mixed in the limited clinical trials published.

Methods: In the current review, we introduce fundamental principles of bone grafting, systematically review all orthopedic clinical studies reporting outcomes on patients transplanted with xenograft bone, and we present our own clinical results from patients grafted with bovine bone in foot and ankle reconstructive procedures.

Results: Thirty-one clinical studies were identified for review and the majority (47%) were from spine surgery literature. Favorable results were reported in 44% of studies while 47% of studies reported poor outcomes and discouraged use of xenograft bone products. In our own clinical series, xenograft failed to integrate with host bone in 58% of cases and persistent pain was reported in 83% of cases.

Conclusions: This is the first systematic review of clinical results reported after bone xenotransplantation for orthopaedic surgery applications. Current literature does not support the use of xenograft bone products and our institution's results are consistent with this conclusion. Our laboratory has reported promising pre-clinical results with a xenograft product derived from porcine cancellous bone, but additional testing is required before considering clinical translation.

KEYWORDS

alpha-gal, bone graft, bone scaffold, bovine, xenograft, xenotransplantation

1 | INTRODUCTION TO BONE GRAFTING PRINCIPLES

An estimated 5.2 million musculoskeletal surgeries are performed every year in the United States,[1] and nearly half of these procedures utilize bone grafts.[2] Bone is the second most frequently transplanted tissue, with only blood transfusions being more common.[3] In 2005, the United States healthcare system spent over 1.2-2.5 billion US dollars on bone graft products,[4,5] which are frequently used in procedures such as spine fusions, foot reconstruction, revision total joint arthroplasty, and segmental bone defect grafting. Segmental bone defects may result from high-energy trauma with accompanying bone loss, tumor resection, fracture non-union, revision surgery, and infection.[2,6] These large areas of bone loss cause significant patient morbidity and are difficult to manage as the body is incapable of regenerating such a large defect. Successful surgical management requires bone grafting, and as a result, the economic and clinical implications of this technique are substantial.

Large defects in skeletal structures are commonly termed "critical defects," defined as "the smallest osseous defect in a particular bone and species of animal that will not heal spontaneously during the lifetime of the animal."[7-10] Critical defect management requires bone augmentation/grafting to fill the void. The ideal bone graft is biocompatible, osteoconductive, osteoinductive, osteogenic, and readily available without risk of disease transmission.[2] For the bone graft to be biocompatible, it must not elicit an adverse host reaction to the graft. This requires the graft to be cleared of any contaminating pathogens and devoid of any cytotoxic reagents used during tissue processing.[11] Osteoconduction refers to the graft's ability to serve as a "scaffold" that facilitates ingrowth of adjacent host bone capillaries, perivascular tissue, and osteoprogenitor cells, and this is dependent on the biomechanical support and micro-architecture provided by the graft.[12,13] Osteoinduction refers to the graft's ability to recruit host precursor cells to the graft site and stimulate cell differentiation into osteogenic lineage.[14] This is dependent on the presence of viable growth factors from the insulin-like growth factor (IGF) family and transforming growth factor-β (TGF-β) family, which includes bone morphogenic proteins (BMPs).[6,14-16] Osteogenic properties are imparted by the presence of living osteoprogenitor cells within the graft. This typically requires transplantation from a living host, and most commonly, these osteogenic bone grafts are taken from the patient's own body, termed an "autograft."

Autograft bone is the "gold standard" because it possesses all ideal bone graft properties. Autografts are commonly taken from a patient's pelvis where osteoconductive bone resides that possesses osteoinductive growth factors and osteogenic cells.[10] However, graft retrieval requires a separate surgical procedure with added risk. Persistent donor site pain is common one year after the procedure and often more severe than the index procedure itself.[8] Autograft supply is limited in quantity and quality depending on patient age and health.

Allograft is retrieved from a living or deceased human donor and imparts very little added morbidity to the patient.[17] After retrieval, allografts are kept fresh with sterile technique, fresh-frozen, or processed with various vendor-specific proprietary decellularization and sterilization protocols. Allograft tissue banking grew rapidly in the 1980s with tissue banks offering a variety of fresh-frozen allografts for clinical use.[3] Allografts are osteoconductive but have limited osteoinduction after sterilization processes inactivate surface proteins. Allografts have no osteogenic potential but still risk host rejection. Allograft use is limited by the risk of graft contamination during processing, donor disease transmission, the limited pool of healthy allograft donors, and the high cost of tissue processing and banking.[18]

Xenograft bone is available in large supply from healthy donors with controlled biology at lower cost.[19] Retrieved xenograft bone contains foreign cellular material which requires decellularization to minimize the risk of human-recipient rejection. Xenograft-derived bone products have attracted considerable attention in orthopedics but their use has historically produced poor outcomes. Adverse host reactions to the xenograft have been described with failed integration and delayed graft rejection requiring revision surgery. To the authors' knowledge, there is currently no commercial bone xenograft in routine use for orthopedic applications.

2 | XENOGRAFT BONE SCIENCE AND CLINICAL LIMITATIONS

Reports of xenotransplantation were documented as early as the 17th century when animal blood was transfused into humans.[20] The first bone xenotransplantation was reported in 1668 when bone from a canine skull was transplanted into a human patient in Russia.[21] Bovine bone transplantation was reported in 1957 by Maatz and Bauermeister.[3] Recent bone xenotransplantation literature has focused on bovine-derived bone graft substitutes for various orthopedic applications.[19,22,23] Although a promising theoretical alternative, the majority of clinical reports on bovine bone transplantation have produced unfavorable results highlighted by failure of graft integration with host bone, graft rejection, and adverse local tissue reactions.[24-27] The greatest clinical barrier to bone xenotransplantation has been the alpha-Gal epitope.[28-31] The alpha-Gal epitope is expressed on millions on glycolipids and glycoproteins on the cell membranes of non-primate animals and new world monkeys. Humans and old-world monkeys do not express the alpha-Gal epitope but do produce a natural antibody against it. This antibody represents 1% of all circulating antibodies in humans.[32] The interaction between the anti-alpha-Gal antibody and alpha-Gal epitopes on xenograft tissues leads to compliment activation, clotting, and acute rejection of tissues with vascular beds. Without compliment activation, grafts are still rejected by mechanisms of antibody-dependent cell-mediated cytotoxicity, which is a slower process that can result in graft degradation and subsequent failure.[33]

TABLE 1 Commercial xenograft bone products reported in orthopedic peer-reviewed literature

Product	Vendor	Species	Graft Form	Status
CANCELLO-PURE®	Wright Medical	Bovine	Bone wedge	Discontinued
Kiel—Surgibone®	Unilab	Bovine	Bone dowel, chips	Recalled
Tutoplast®	Tutogen Medical	Bovine	Bone block, granules	Merged with RTI Biologics, process adapted to allograft tissue
Tutobone®	Tutogen Medical	Bovine	Bone chips, dowels, wedges	Merged with RTI Biologics
Lubboc®	OST Developpement	Bovine	Cancellous bone, granules	unknown
BIO-GEN®	BiOTECK®	Equine	Bone block, wedge, granules, paste	unknown

To avoid alpha-Gal-mediated host rejection, xenografts are decellularized to reduce epitope levels and create a scaffold of the biologic tissue. Decellularization protocols should aggressively remove donor tissue without compromising the graft's structural properties important for osteoconduction or removing the osteoinductive growth factors embedded in the extracellular matrix.[12,34-36] While a number of commercial xenograft-derived bone graft products have been approved for orthopedic clinical use (Table 1), inconsistent, and often unsatisfactory clinical results have limited their use.

3 | SYSTEMATIC REVIEW OF ORTHOPEDIC BONE TRANSPLANTATION CLINICAL LITERATURE

A systematic review of the literature according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines was performed to capture all studies reporting clinical outcomes after xenograft bone transplantation for orthopedic surgery applications. All available PubMed/MEDLINE-indexed sources were searched using terms “xenograft bone” and/or “transplantation” and/or “bone xenotransplantation” and/or “bovine bone.” Inclusion criteria were original articles reporting clinical results after bone xenotransplantation. Exclusion criteria included the following: (a) non-English language publications, (b) animal-model studies, (c) periodontal/dental studies, (d) review articles, (e) absence of clinical outcomes, and (f) basic science and/or pre-clinical studies. All references from included studies were reviewed to ensure that additional relevant studies were captured. Thirty-eight studies were identified, and after exclusion criteria were applied, 32 studies were included for review (Table 2). Thirty-one studies (97%) reported on bovine products, and one study reported a proprietary porcine product. Grafts were used for spine surgery in 15 studies (47%), trauma in 5 studies (16%), foot and ankle reconstruction in 4 studies (13%), joint arthroplasty in 3 studies (9%), and sports/congenital/oncology/other in 5 studies (16%). Most studies had low sample size with 8 studies (25%) <10 patients, 14 studies (44%) 10-50 patients, 6 studies (19%) 50-100 patients, and only 4 studies (13%) with >100 patients. Overall, favorable clinical results were reported in 14 studies (44%), while 15 studies (47%) advised against the use of xenograft bone, and 3 studies (9%) were unable to make any recommendation. Studies discouraging use of xenograft bone frequently cited high rates of graft non-union, failure of the graft to integrate with host tissue, and failure of the graft to remodel over time.

Shibuya et al retrospectively reviewed 61 patients undergoing foot reconstruction with Cancello-Pure bovine xenograft and found that by 48 weeks post-operatively, 58% of the xenografts had failed to incorporate compared to only 5% of patients in a separate non-xenograft group.[37] Mean time to xenograft incorporation was 57 weeks in which the authors deemed unacceptable. Rawlinson et al reported the only prospective randomized study with 49 patients receiving Surgibone bovine xenograft for cervical spine fusions and found unacceptably low fusion rates compared to a control group receiving traditional autograft.[38] Patients also reported mechanical neck pain, and 3 required explantation of the xenograft with explant histology showing an adverse host inflammatory reaction to the graft. Studies by Saveland[39] and Charalambides[24] reported similar adverse outcomes with Surgibone and recommended against its use. Kiel Bone bovine xenograft was used in spinal fusion by McMurray et al[40] and in femoral grafting by Hallen et al[41] with poor results in a total of eight patients secondary to poor graft healing and failure to integrate. Revision surgery was required in spine patients with explanted histology showing invasion of fibrous tissue and failure to integrate with adjacent host tissue similar to the Surgibone histology results cited by Rawlinson et al[38] Tutobone bovine cancellous xenograft was used by Patil et al[42] for subtalar fusions in 9 patients and compared against 17 patients receiving autograft. All nine xenograft patients failed to integrate the bone graft with 8/9 reporting persistent pain compared to an autograft group where 17/17 patients went onto bony union without pain. Tutoplast is a similar bovine cancellous graft that was used in a prospective cohort study by Schultheiss et al[43] for spine fusions in 11 patients with thoracolumbar spine fractures. Results were compared against 11 similar patients treated with autograft. CT imaging showed 2/11 xenograft patients and achieved full osteointegration compared to 8/11 autograft patients. One of the 11 xenograft patients required revision surgery for graft failure. The authors advised against bovine cancellous blocks given the unacceptably high non-union rates reported.

Several large patient series have supported xenograft use, however. Use of Kiel bone was supported by Ramani et al,[44] Goran et al,[45] Taheri et al,[46] and Siqueira et al[47] who all reported equivalent outcomes with autograft or xenograft (Kiel) bone in patients undergoing

TABLE 2 Peer-reviewed, MEDLINE-indexed studies reporting orthopedic clinical results after bone xenotransplantation are presented

Study	Design	Surgery performed	Xenograft product	Number of xenograft patients	Outcomes assessed
Hallen (1966)[41]	Clinical Trial	Hip bone grafting	Kiel bone—bovine	4	Histology for creeping substitution, inflammation, callus formation
Taheri et al (1972)[46]	Retrospective case series	ACDF	Kiel Bone (Unilab, Inc, 30 Church Street, New York, New York)—bovine	200	Radiographic evidence of graft union, clinical outcome at <1 y to >2 y
Wilppula et al (1972)[68]	Retrospective cohort study	Tibia fracture ORIF with bone graft	Kiel bone—bovine	37	Radiographic evidence of fracture healing, graft incorporation, clinical recovery
Ramani et al (1975)[44]	Case series	ACDF	Kiel bone—bovine	65	Radiographic assessment of fusion, spine alignment, clinical outcomes
Goran et al (1978)[45]	Prospective cohort study	ACDF	Kiel bone—bovine	10	Radiographic assessment of fusion, spine alignment, clinical outcomes
McMurray (1982)[40]	Case series	Spine fusion	Kiel bone—bovine	4	Radiographic evidence of graft union, explant histology
Siqueira et al (1982)[47]	Case series	ACDF	Kiel bone—bovine	221	Radiographic assessment of fusion, spine alignment, clinical outcomes
Salama (1983)[22]	Case series	Tibial plateau ORIF, fusions, reconstruction	Kiel bone—bovine	98	Graft integration
Mosdal et al (1984)[69]	Retrospective case series	ACDF	Kiel bone—bovine	614	Clinical outcomes
Saveland et al (1994)[39]	Retrospective case series	Occipitocervical spinal fusion	Surgibone® bovine bone chips	9	X-ray and CT evaluation of graft preservation, incorporation 12-15 mo post-operative
Savolainen et al (1994)[48]	Retrospective cohort study, 6 surgeons	ACDF	Bovine Unilab Surgibone® (Unilab Inc USA)	101	Radiographic evidence of fusion, alignment, clinical outcomes. Average follow-up 6 mo
Rawlinson (1994)[38]	Prospective randomized study	ACDF	Bovine Unilab Surgibone® (Unilab Inc USA)	49	Radiographic evidence of union, fusion, graft migration, clinical outcomes, explanted bone pathology
Sutter et al (1995)[70]	Retrospective case series	ACDF	Unilab Surgibone® (Mississauga, Canada) cancellous bovine dowel	66	Radiographic evidence of union, graft incorporation at 1-4 y post-operative
Seite et al (1998)[71]	Case report	ACDF	Kiel bone—bovine	1	Radiographic assessment of fusion
Werber et al (2000)[72]	Prospective case series	Distal radius open reduction, grafting with internal vs external fixation	Bovine spongiosa hydroxyapatite ceramic blocks (Merck Darmstadt, Germany).	14	Radiographic (XR, MRI) evidence of graft union and incorporation. Graft site biopsy at time of plate removal
Hartl et al (2004)[73]	Retrospective cohort study	Bone tumor curettage and bone grafting	Lubboc, bovine xenograft (Ost Developpement, Clermont-Ferrand, France)	7	Radiographic evidence of graft union, integration out to 12 mo post-operative
Christodoulou et al (2004)[74]	Retrospective case series	ACDF	Lubboc, bovine xenograft (Ost Developpement, Clermont-Ferrand, France)	15	Radiographic assessment of fusion, spine alignment, clinical outcomes
Charalambides et al (2005)[24]	Prospective case series	Revision hip arthroplasty	Surgibone (Unilab Inc, NJ)—bovine cancellous	27	Radiographic evidence of bone graft incorporation, prosthetic component stability, clinical outcomes

Results	Study conclusion
No evidence of graft healing at 8-15 mo	Kiel bone cannot be recommended
97% excellent or good radiographic outcomes, 88% excellent or good clinical outcomes. No failures attributable to xenograft	Outcomes with Kiel bone equivalent to those with autograft but with less morbidity from graft harvest
Kiel bone associated with loss of reduction. No rejection of graft noted	Kiel bone connected with greater risk of impaired fracture reduction. Caution recommended in use
86% had acceptable spine alignment, 2 cases required surgical explantation, 7 had post-operative complications. No X-rays demonstrated graft fusion at 5 y post-op	No disadvantage to use of xenograft given it avoids invasive autologous bone graft harvest
All patients fused spine, no adverse events attributable to xenograft use. Xenograft preferred over autograft in osteoporotic patients to avoid complication and pain	No significant difference between autograft and xenograft product
Revision surgery required, biopsies showed Kiel bone graft was invaded by fibrous tissue without incorporation to adjacent host bone	Kiel grafts do not seem justified in current orthopedic practice.
Fusion obtained in every patient receiving xenograft product. No complications attributable to xenograft use	Xenograft is suitable for cervical spine fusion use
Excellent results with bone grafting. Mostly satisfactory results for pseudoarthrosis treatment	Deproteinized xenograft impregnated with autologous marrow serves as a viable bone bank implant
Xenograft complication seen in 2% of 958 interbody fusions	ACDF is a reliable surgery
Graft resorption in 1 patient, 1 patient revised, 8 with preserved graft morphology	Use of bovine chips in posterior occipitocervical fusion is unpredictable
Fusion rate 98% both groups, insignificantly increased angular deformity with xenograft (50%) vs autograft (43%)	Recommend bovine xenograft for cervical fusion
Significantly greater rates of bony fusion in autologous bone group, increased rates of persistent neck pain in xenograft group. Three xenografts required explant. Histology showed failed integration, inflammatory reaction	Surgibone is not an adequate substitute for autograft in ACDF due to high non-union rate, new onset mechanical neck pain, and adverse host reaction
Equivalent clinical outcomes with xenograft, good construct stability, limited evidence of union or remodeling of the graft	Inconclusive, cannot support use of xenograft, future study required
Non-union	Recommend against xenograft use. Autograft remains ideal bone graft choice
Fibrovascular ingrowth and osteointegration in 13/14 patients	Bovine HA is an acceptable alternative to autograft that is well tolerated and incorporated into host
Integration of allograft significantly faster than xenograft	Allograft bone preferred to xenograft
Fusion within 6 mo in 100% patients	Xenograft combined with human allograft is an acceptable alternative to autograft
Three had no graft incorporation, Three had graft rejection, One deep infection, six revised for graft loosening	Use of Surgibone xenograft in revision hip surgery, even in combination with autograft, resulted in failure and the need for re-revision in at least one quarter of the cases studied

(Continues)

TABLE 2 (Continued)

Study	Design	Surgery performed	Xenograft product	Number of xenograft patients	Outcomes assessed
Schultheiss et al (2005)[43]	Prospective cohort study	MIS spine fusion	Tutoplast bovine cancellous bone blocks (Tutogen, Erlangen, Germany)	11	Radiographic evidence of fusion based on 12-mo follow-up with CT imaging, explant histology
Xie et al (2006)[27]	Case report	ACDF	Bovine xenograft, generic	1	Radiographic evidence of graft union, clinical outcome, pathology of explant
Stone et al (2007)[49]	Clinical Trial	ACL reconstruction with porcine BTB grafts	Porcine BTB grafts treated with proprietary protocol including alpha-Galactosidase	10	Clinical results, graft stability, human anti-alpha-Gal antibody response, in vivo graft biopsy
Meyer et al (2008)[75]	Retrospective cohort study	High tibial osteotomy, revision hip arthroplasty	Tutobone® (Tutogen Medical GmbH, Neunkirchen a. Brand, Germany)—bovine	9	Radiographic evidence of graft union, biopsy histology, clinical outcomes
Rosito et a. (2008)[76]	Prospective cohort study	Revision hip arthroplasty	Bovine bone obtained from Brazilian cattle processed at the Tissue Bank (Hospital de Clínicas de Porto Alegre-TBHCPA)	25	Radiographic evidence of graft incorporation, acetabular component position, migration, graft histology
Bansal et al (2009)[77]	Prospective case series, single surgeon	Tibial fracture ORIF with bone graft	Tutoplast Bovine cancellous xenogenous bone granules	19	Radiographic evidence of fracture union, graft incorporation at 1.5, 3, 6, 12 mo
Elliot et al (2011)[78]	Retrospective case series	Clavicle reconstruction	Tutobone (Tutogen Medical Inc, Alachua, Florida, USA)—bovine	2	Radiographic evidence of graft union, clinical outcome, 3 mo
Patil et al (2011)[42]	Retrospective cohort study	Subtalar ankle fusion	Tutobone® Block (Tutogen Medical GmbH, RTI Biologics, Neunkirchen am Brand, Germany)—bovine	9	Radiographic evidence of graft union, incorporation using XR and CT. AOFAS scores. Explant histology
Shibuya et al (2012)[26]	Retrospective case series	Foot reconstruction	CANCELLO-PURE wedge (Wright Medical Technology, Inc, Arlington, TN)—bovine	22	Radiographic evidence of graft incorporation at 12, 24, 36, 48 wk post-operative
Makridis et al (2012)[79]	Prospective case series	ICBG donor site filled with xenograft	Tutobone®, (Tutogen Medical GmbH, Neunkirchen a. Brand, Germany)—bovine	16	Radiographic evidence of bone graft incorporation, clinical outcomes
Ledford et al (2013)[25]	Retrospective case series	Foot reconstruction	CANCELLO-PURE wedge (Wright Medical Technology, Inc, Arlington, TN)—bovine	10	Radiographic evidence of union, graft incorporation at >6 mo, explant pathology, n = 3 surgeons
Maffulli et al (2013)[80]	Prospective cohort study	High tibial osteotomy	Tutobone—bovine	52	Radiographic evidence of graft union and deformity correction at 3, 6, 12, 24 mo
Shibuya et al (2014)[37]	Retrospective cohort study	Foot reconstruction	CANCELLO-PURE wedge (Wright Medical Technology, Inc, Arlington, TN)—bovine	61	XR evaluation of graft incorporation at 12, 24, 36, 48 wk post-operative
Prakash et al (2017) Y[81]	Retrospective cohort study	ACDF	Tutobone (Tutogen Medical Inc, Alachua, Florida, USA)—bovine	95	Radiographic assessment of fusion at 3, 12 mo

Note: Xenograft products are reported exactly as described by authors.

Abbreviations: ACDF, anterior cervical discectomy and fusion; ACL, anterior cruciate ligament; AOFAS, American Orthopaedic Foot & Ankle Society; BTB, bone-tendon-bone; CT, computed tomography; HA, hydroxyapatite; ICBG, iliac crest bone graft; MIS, minimally invasive surgery; MRI, magnetic resonance imaging; ORIF, open reduction internal fixation; TCP, tricalcium phosphate; XR, X-ray.

Results	Study conclusion
Autograft 8/11 osseointegration, 3/11 partial, no failures. Xenograft 2/11 complete osseointegration, 3/11 partial integration, 4/11 no integration, 1/11 complete graft failure requiring revision surgery	Use of bovine cancellous blocks is not considered reliable with high non-union rates in the spine. Use of these products was discontinued
Symptomatic non-union requiring revision surgery with iliac crest allograft at 15 mo post-op. Graft with necrotic tissue, fibrous non-union	Low biocompatibility with xenograft, risk of failure
5/6 evaluable subjects had suitable graft function >2 y, 1/6 had bone plug loosening requiring explantation. 4/10 had complications unrelated to graft. Alpha-Gal antibody response present	Porcine BTB graft may be viable ACL graft alternative. Peak anti-alpha-Gal response at 2-8 wk possibly to marrow content in bone plugs
Equivalent patient outcomes with autograft or xenograft, Tutobone is excellent biocompatible scaffold, 100% autograft remodeling, remnant Tutobone in 47% specimens	Tutobone degradation and replacement may be slower in human than animal environment. Tutobone may represent viable alternative to autograft.
No clinical/radiographic difference found between the allograft and xenograft, both showed graft incorporation in 88.5% and 76% of patients respectively ($P = .424$)	Bovine bone is suitable for revision hip arthroplasty with results comparable to human freeze-dried allograft
Average time to union 20 wk, no infections, all patients with excellent graft incorporation, average subsidence 4 mm	Favorable outcomes can be achieved with xenograft bone and beneficial to elderly population
Both cases with symptomatic non-union requiring hardware removal and explantation of graft	Authors caution against use of Tutobone as graft material for clavicle pseudoarthrosis
Xenograft group: 8/9 with persistent pain and non-union, 9/9 showed no graft incorporation, 7/9 required revision surgery. Autograft group: 17/17 asymptomatic at 6 mo, 100% union rate at 12 mo	Advise against the use of bovine cancellous xenograft bone for subtalar fusion surgery
Median time to graft incorporation 54 wk. 61% failed to incorporate	Xenograft incorporation slower than other graft types with high failed incorporation rate. May not be advisable for foot and ankle use
Graft integration in 15/16 patients over 3 mo post-operative period. 1/16 had wound hematoma. 1/16 failed to incorporate	Bovine cancellous bone is a suitable graft source to fill ICBG donor sites
54% xenografts painful, failed to incorporate, all required revision with human iliac crest allograft. Failed explant histology showed necrotic bone with foreign body giant cell reaction	Xenografts resulted in unacceptable high rates of failure requiring revision surgery. Caution against bovine xenograft use in pediatric foot deformity surgery
TCP greater loss of correction compared to xenograft. No non-unions in either group	Xenograft with locking plate fixation was superior to TCP to prevent loss of surgical correction
At 48 wk, an estimated 58% and 5% of the xenografts and non-xenografts had not incorporated, respectively. Median incorporation period for the non-xenograft and xenograft group was 16 and 57 wk, respectively	Not advisable to use a bovine-based bone xenograft in foot and ankle surgery
Rates of fusion and time to fusion were lower with xenograft compared to autograft bone	Tutobone can be used but autograft remains superior

anterior cervical discectomy and fusion (ACDF). Siqueira et al[47] are one of the largest case series that reported 221 patients undergoing ACDF with xenograft. All patients were reported to have obtained bony fusion with no complications attributable to xenograft use. Similarly, Taheri et al[46] reported equivalent outcome with autograft or xenograft (n = 200) in ACDF patients with 97% of xenograft patients demonstrating good or excellent radiographic results and no patients failing with Kiel bone. Cervical spine fusions performed by Savolainen et al[48] using either iliac crest autograft (n = 149) or Surgibone bovine graft (n = 101) produced 98% fusion rates in both patient cohorts, leading the authors to support Surgibone use in cervical fusion surgery.

In the studies reporting poor outcomes with xenograft bone, few consider the etiology of graft failure. The most informative clinical trial published by Stone et al[49] followed a series of 10 patients for a minimum 24 months after undergoing anterior cruciate ligament (ACL) reconstruction with porcine bone-patellar tendon-bone xenografts that were decellularized with a proprietary technique that included recombinant alpha-galactosidase enzyme digestion. To the authors' knowledge, this is the only orthopedic clinical study reporting xenotransplantation with porcine bone tissue. In addition to reporting clinical outcomes, alpha-Gal epitope concentrations in the grafts were measured and host anti-alpha-Gal serum antibodies were measured at serial time points after transplantation. Despite removing >99% of the alpha-gal epitope from grafts, human recipients still displayed 2- to 8-fold increases in anti-Gal IgG activity 2 weeks post-transplantation. The authors hypothesized this antibody response was against residual alpha-Gal epitopes on porcine marrow cells enclosed in bone cavities which were not accessible to alpha-galactosidase enzyme digestion. When bone plugs were cut for graft implantation or remodeled in the host, undigested epitopes were exposed. At 24 months, 5 of the 10 patients returned to full sports activity. Of the five failures, the authors attributed only one to failure of the xenograft device which had tibial bone plug loosening and required explantation. It is unknown whether an alpha-Gal response directly contributed to tibial plug loosening or any of these failures. The anti-Gal antibody response is one of the few specific etiologies suggested in the orthopedic literature to explain host rejection of decellularized bone xenografts.

There is a paucity of orthopedic clinical outcomes reported after bone xenotransplantation, and to the authors' knowledge, we present the first systematic review of the orthopedic literature. Most studies have reported unfavorable results and advised against xenograft use. Periodontal studies, although outside the scope of this review, have frequently reported porcine xenograft bone to impair bony healing of alveolar ridge defects in the mandible.[50] Currently, xenograft bone is not accepted as a viable bone graft alternative, and the majority of xenograft products have been removed from clinical markets (Table 1).

4 | A RETROSPECTIVE CASE SERIES AT A SINGLE ACADEMIC CENTER

With local institutional review board approval, all pediatric patients at a single academic center who underwent reconstructive foot and ankle procedures (Cotton osteotomy (Figure 1A), Evan's osteotomy) using a commercially available bovine xenograft wedge were retrospectively reviewed. Patients without clinical and radiographic follow-up were excluded from study. Clinical outcomes assessed included post-operative pain and correction of foot/ankle deformity. Radiographs were independently reviewed by two separate orthopedic surgeons to assess graft integrity, graft placement, and graft integration at 3 weeks, 6 weeks, 3 months and 6 months post-operatively.

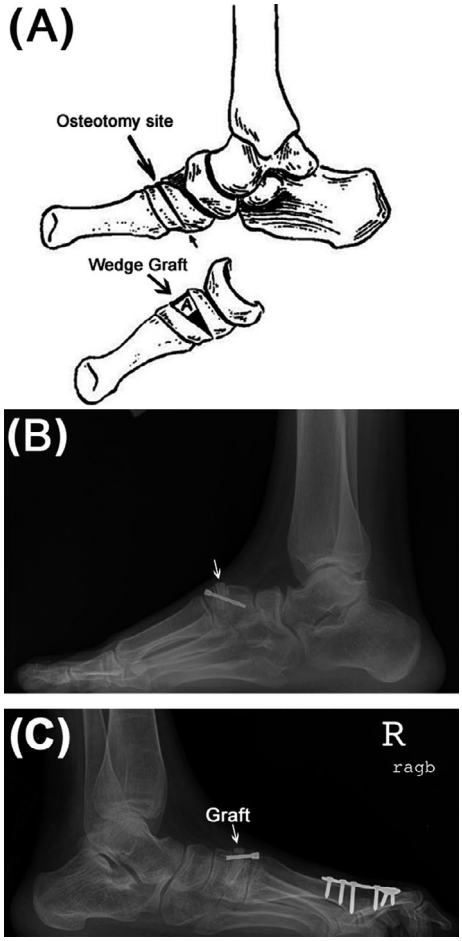

FIGURE 1 A, Cotton osteotomy concept. An opening wedge osteotomy on the dorsal aspect of the midfoot using structural bone graft ("A") is performed to restore the arch of the foot and correct clinical pes planus (flat foot). B, Radiographs obtained at 13 mo after Cotton osteotomy performed with CANCELLO-PURE® bovine xenograft show radiolucent lines surrounding the graft consistent with graft non-union and failure to integrate with surrounding host bone. C, Fracture of the superior aspect of the graft about the compression screw found at 20 mo post-operative with surrounding graft non-union

Ten patients (mean age 11.8 years, range 9-18 years, 5 males, 5 females) undergoing 12 separate foot and ankle reconstruction procedures utilizing bone xenograft performed by one pediatric fellowship-trained orthopedic surgeon and one foot and ankle fellowship-trained orthopedic surgeon over a 27-month period were identified for review (Table 3). All patients were grafted with CANCELLO-PURE® (Wright Medical) bovine bone wedges fixed with internal hardware. Mean clinic follow-up was 32.6 months

TABLE 3 Ten patients undergoing 12 separate foot reconstruction osteotomies using bovine bone xenografts were retrospectively reviewed

Patient	Age/ Gender	BMI	Surgery	Radiographic follow-up (mo)	Clinical follow-up (mo)	Xenograft wedge(s) implanted	Outcome
1a	11M	26.2	Cotton osteotomy	3	36	6 mm	Graft union at 6 wk post-op, deformity correction, no pain at midterm follow-up, persistent pain over hindfoot at long-term follow-up, graft incorporation at 3 mo
1b	11M	26.2	Cotton osteotomy	3	36	6 mm	Graft union at 6 wk post-op, deformity correction, no pain at midterm follow-up, persistent pain over lateral midfoot at long-term follow-up, graft failed to integrate
2	18F	20.5	Cotton osteotomy	20	20	6 mm	Graft non-union and failure to incorporate, graft fracture on superior aspect, no pain at midterm follow-up with good patient satisfaction, persistent forefoot pain at long-term follow-up
3	15F	19.1	Evans osteotomy	5	5	10 mm	Graft union at 6 wk with incorporation by 5 mo, no pain with patient satisfaction at midterm follow-up, required custom orthotics at long-term follow-up and had not returned to full activity
4	14F	16.6	Cotton osteotomy, Evans osteotomy	9	9	10, 6 mm	Graft union at 8 wk with incorporation by 9 mo, patient satisfied and reported no pain at both midterm and long-term follow-up
5	10M	26.0	Evans osteotomy	112	112	10 mm	Graft union at 5 wk post-op with early integration, patient satisfied, and no pain reported at midterm or long-term follow-up
6	10M	30.0	Evans osteotomy	5	5	10 mm	Graft union at 10 wk post-operative with integration at 5 mo. Patient satisfied with no pain at midterm follow-up. Reported pain and residual flatfoot deformity at long-term follow-up
7	12F	51.5	Cotton osteotomy, Evans osteotomy	48	48	10, 6 mm	Non-union of Cotton osteotomy with failure of graft to integrate. Evans osteotomy achieved union at 12 mo. Patient dissatisfied with persistent pain at midterm and long-term follow-up. Residual flatfoot deformity present
8	15M	30.9	Evans osteotomy	10	10	10 mm	Graft union at 11 wk post-op but failure to integrate. Patient reported no pain at midterm follow-up but persistent lateral ankle pain at long-term follow-up requiring orthotic shoe support
9a	10F	13.6	Evans osteotomy	25	45	10 mm	Graft union at 12 wk post-operative but failure of graft to integrate. Patient reported persistent pain, dissatisfaction. Experienced recurrence of foot deformity
9b	9F	13.6	Evans osteotomy	30	52	10 mm	Graft non-union and failure to incorporate. Patient reported persistent pain, dissatisfaction at midterm and long-term follow-up
10	16M	21.0	Cotton osteotomy	13	13	6 mm	Graft non-union with failure to incorporate. Patient reported pain at midterm and long-term follow-up

Note: Radiographic and clinical outcomes are reported.

Abbreviations: BMI, body mass index; F, female; M, male; mm, millimeter.

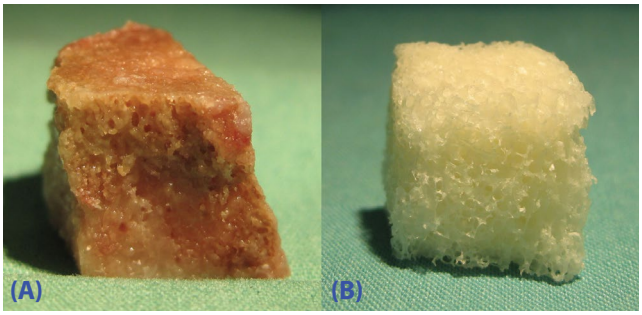

FIGURE 2 A, Cancellous bone retrieved from the distal metaphysis of a porcine femur is filled with marrow contents that include a dense cellular network embedded in lipid-rich extracellular matrix. B, Bone scaffolds are derived from the host cancellous bone using a decellularization and oxidation protocol. Cell and lipid content have been removed, isolating the osseous extra cellular matrix that serves as a bone scaffold, intended for use as a bone graft substitute

(range 5-112 months), and mean radiographic follow-up was 23.6 months (range 3-112 months). At intermediate follow-up, patients reported pain in 2/12 (17%) of cases, while at final/long-term follow-up, patients reported pain in 10/12 (83%) of cases. Patients failed to achieve bony union with obvious radiolucent lines (Figure 1B) at the osteotomy site grafted with bovine xenograft in 5/12 (42%) cases. Grafts failed to integrate with surrounding host bone in 7/12 (58%) of cases. One patient sustained a fracture of their xenograft (Figure 1C), but no patients required revision surgery or graft explantation. No patients experienced infection or overt rejection of the graft material.

Early results from our patients collected over a 27-month period demonstrated an unacceptably high rate of graft non-union and failure to integrate with host bone. The majority of patients experienced persistent pain at the graft site or midfoot at long-term follow-up. While we cannot directly attribute persistent pain to xenograft use, concomitant graft non-union and recurrence of foot deformity in some cases is concerning for graft etiology. Our experience with allograft bone use for the same foot reconstructive osteotomy procedures has been much more favorable with predictable rates of graft union, integration, and patient outcomes. Considering these findings, bovine xenograft use was discontinued at our hospital, and since that time, CANCELLO-PURE® has been removed from commercial markets. Our unfavorable results are similar to those reported by Shibuya et al[37] and Ledford et al,[25] who both found unacceptably high non-union rates in similar foot reconstruction procedures using CANCELLO-PURE® bovine wedges (Table 2).

Our case series has several limitations. Patients were reviewed retrospectively by chart review. Our results with xenograft are limited without direct comparison against allograft use by the same surgeons in a similar patient population. We report a small number of patients with variable follow-up but we believe these results are still valuable considering the limited clinical results published in the orthopedic literature. Finally, we cannot propose any specific etiology contributing to graft non-union beyond what has been suggested in the literature.

5 | CONCLUSIONS AND FUTURE DIRECTIONS

Bone grafting continues to play a critical role in orthopedic surgery practice with significant clinical and financial implications for our healthcare system. Autograft bone remains the gold standard with unsurpassed clinical results, but its limited supply and added patient morbidity demands further consideration of allograft bone and bone graft substitutes. Xenografts will always be an attractive alternative because of their large supply from healthy donors with controlled biology at lower cost. The majority of available clinical results have reported unfavorable outcomes with xenograft bone, including our own, which has ultimately led to the recall of most commercial xenograft bone products. Xenograft-derived bone products are currently not FDA approved for use in any orthopedic surgery application. Nearly, all clinical results have been reported with bovine-derived bone, but our laboratory is currently considering use of porcine-derived bone[11,51] because porcine species share similar anatomy, organ size, physiology, and genetic makeup with human species and have a successful history with porcine cardiac tissue transplantation into humans.[20,52-55] We used a published decellularization and tissue oxidation protocol[56-61] to derive bone scaffolds from the cancellous bone found in the distal metaphyseal region of porcine femurs (Figure 2).[51] Using this decellularization technique, we were able to remove 98% of host DNA content and 98.5% of the alpha-Gal epitope from donor bone in pre-clinical studies.[11,51] We are encouraged by the significant reduction of alpha-Gal epitope but results published by Stone et al,[49] as referenced above, suggest that we still have not identified the reduction threshold required to prevent human hosts from mounting the immune responses believed to contribute to chronic rejection of bone xenografts. Various enzymes have been used to reduce alpha-Gal expression in transgenic pigs, and complete elimination of the alpha-Gal epitope was achieved by knockout of the α1,3GT gene using nuclear transfer. Transplantation of these pig organs did not result in the hyperacute rejection seen with anti-α-Gal antibody response, but we learned that humans develop anti-non-α-Gal antibodies that lead to delayed tissue rejection.[33,62-64] Other groups have also considered bone graft substitutes derived from porcine cancellous bone,[55,65-67] but there are currently no clinical results reported with these products. Future work will be required to investigate porcine bone graft products in animal and human models before considering their commercial use in orthopedic applications.

ACKNOWLEDGMENTS

No extramural funding was received to conduct this research.

ORCID

Daniel N. Bracey https://orcid.org/0000-0001-7159-9186

REFERENCES

1. CDC. Number of all-listed procedures from discharges from short-stay hospitals by procedure category and age: United States. 2009.
2. Calori GM, Mazza E, Colombo M, Ripamonti C. The use of bone-graft substitutes in large bone defects: any specific needs? *Injury*. 2011;42(Suppl 2):S56-63.
3. Campana V, Milano G, Pagano E, et al. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. *J Mater Sci*. 2014;25(10):2445-2461.
4. Jahangir AA. Bone-graft substitutes in orthopaedic surgery. *AAOS Now*. 2008;2:35-37.
5. Amini AR, Adams DJ, Laurencin CT, Nukavarapu SP. Optimally porous and biomechanically compatible scaffolds for large-area bone regeneration. *Tissue Eng Part A*. 2012;18:1376-1388.
6. Pneumaticos SG, Triantafyllopoulos GK, Basdra EK, Papavassiliou AG. Segmental bone defects: from cellular and molecular pathways to the development of novel biological treatments. *J Cell Mol Med*. 2010;14:2561-2569.
7. Bullough PG. Normal skeletal structure and development. In: Bullough PG, ed. *Orthopaedic Pathology*, 5th edn. New York, NY: Elsevier; 2009:1-39.
8. Mauffrey C, Barlow BT, Smith W. Management of segmental bone defects. *J Am Acad Orthop Surg*. 2015;23:143-153.
9. Sela JJ, Bab IA. Part I: physiology of bone healing. In: Sela JJ, Bab IA, eds. *Principles of Bone Regeneration*. New York: Springer; 2012:10-48.
10. Watson JT. Biology and enhancement of skeletal repair. In: Browner BD, ed. *Skeletal Trauma: Basic Science, Management, and Reconstruction*, 5th edn. Philadelphia, PA: Elsevier; 2015:69-93.
11. Bracey DN, Seyler TM, Jinnah AH, et al. A porcine xenograft-derived bone scaffold is a biocompatible bone graft substitute: an assessment of cytocompatibility and the alpha-Gal epitope. *Xenotransplantation*. 2019;26(5):e12534.
12. Sutherland D, Bostrom M. Grafts and bone graft substitutes. In: Lieberman JR, Friedlaender GE, eds. *Bone Regeneration and Repair: Biology and Clinical Applications*. Totowa, NJ: Humana Press; 2007:133-156.
13. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials*. 2005;26:5474-5491.
14. Bormann N, Pruss A, Schmidmaier G, Wildemann B. In vitro testing of the osteoinductive potential of different bony allograft preparations. *Arch Orthop Trauma Surg*. 2010;130:143-149.
15. Awad HA, Zhang X, Reynolds DG, Guldberg RE, O'Keefe RJ, Schwarz EM. Recent advances in gene delivery for structural bone allografts. *Tissue Eng*. 2007;13:1973-1985.
16. Termaat MF, Den Boer FC, Bakker FC, Patka P, Haarman HJ. Bone morphogenetic proteins. Development and clinical efficacy in the treatment of fractures and bone defects. *J Bone Joint Surg*. 2005;87:1367-1378.
17. Khan SN, Cammisa FP Jr, Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology of bone grafting. *J Am Acad Orthop Surg*. 2005;13:77-86.
18. De Long WG, Einhorn TA, Koval K, et al. Bone grafts and bone graft substitutes in orthopaedic trauma surgery. A critical analysis. *J Bone Joint Surg*. 2007;89:649-658.
19. Laurencin CT, El-Amin SF. Xenotransplantation in orthopaedic surgery. *J Am Acad Orthop Surg*. 2008;16:4-8.
20. Cooper DK, Ekser B, Tector AJ. A brief history of clinical xenotransplantation. *Int J Surg*. 2015;23:205-210.
21. Deschamps JY, Roux FA, Sai P, Gouin E. History of xenotransplantation. *Xenotransplantation*. 2005;12:91-109.
22. Salama R. Xenogeneic bone grafting in humans. *Clin Orthop Relat Res*. 1983;113–121.
23. Shibuya N, Jupiter DC. Bone graft substitute: allograft and xenograft. *Clin Podiatr Med Surg*. 2015;32:21-34.
24. Charalambides C, Beer M, Cobb AG. Poor results after augmenting autograft with xenograft (Surgibone) in hip revision surgery: a report of 27 cases. *Acta Orthop*. 2005;76:544-549.
25. Ledford CK, Nunley JA 2nd, Viens NA, Lark RK. Bovine xenograft failures in pediatric foot reconstructive surgery. *J Pediatr Orthop*. 2013;33:458-463.
26. Shibuya N, Jupiter DC, Clawson LD, La Fontaine J. Incorporation of bovine-based structural bone grafts used in reconstructive foot surgery. *J Foot Ankle Surg*. 2012;51:30-33.
27. Xie Y, Chopin D, Hardouin P, Lu J. Clinical, radiological and histological study of the failure of cervical interbody fusions with bone substitutes. *Eur Spine J*. 2006;15:1196-1203.
28. LaTemple DC, Galili U. Adult and neonatal anti-Gal response in knockout mice for alpha1,3galactosyltransferase. *Xenotransplantation*. 1998;5:191-196.
29. Feng W, Li D, Zang J, Fu L. Biomechanical comparison of xenogeneic bone material treated with different methods. *Xenotransplantation*. 2017;24:e12343.
30. Long B, Dan L, Jian L, Yunyu H, Shu H, Zhi Y. Evaluation of a novel reconstituted bone xenograft using processed bovine cancellous bone in combination with purified bovine bone morphogenetic protein. *Xenotransplantation*. 2012;19:122-132.
31. Galili U. Evolution of alpha 1,3galactosyltransferase and of the alpha-Gal epitope. *Subcell Biochem*. 1999;32:1-23.
32. Galili U. Xenotransplantation and ABO incompatible transplantation: the similarities they share. *Transfus Apher Sci*. 2006;35:45-58.
33. Galili U. The alpha-Gal epitope (Galalpha1-3Galbeta1-4GlcNAc-R) in xenotransplantation. *Biochimie*. 2001;83:557-563.
34. Urist MR. Bone: formation by autoinduction. *Science*. 1965;150:893-899.
35. Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials*. 2011;32:3233-3243.
36. Kawecki M, Łabuś W, Klama-Baryla A, et al. A review of decellurization methods caused by an urgent need for quality control of cell-free extracellular matrix' scaffolds and their role in regenerative medicine. *J Biomed Mater Res B Appl Biomater*. 2018;106:909-923.
37. Shibuya N, Holloway BK, Jupiter DC. A comparative study of incorporation rates between non-xenograft and bovine-based structural bone graft in foot and ankle surgery. *J Foot Ankle Surg*. 2014;53:164-167.
38. Rawlinson JN. Morbidity after anterior cervical decompression and fusion. The influence of the donor site on recovery, and the results of a trial of surgibone compared to autologous bone. *Acta Neurochir*. 1994;131:106-118.
39. Saveland H, Aspenberg P, Zygmunt S, Herrlin K, Christensson D, Rydholm U. Bovine bone grafting in occipito-cervical fusion for atlanto-axial instability in rheumatoid arthritis. *Acta Neurochir*. 1994;127:186-190.
40. McMurray GN. The evaluation of Kiel bone in spinal fusions. *J Bone Joint Surg*. 1982;64:101-104.
41. Hallen LG. Heterologous transplantation with Kiel bone. An experimental and clinical study. *Acta Orthop Scand*. 1966;37:1-19.
42. Patil S, Auyeung J, Gower A. Outcome of subtalar fusion using bovine cancellous bone graft: a retrospective case series. *J Foot Ankle Res*. 2011;50:388-390.
43. Schultheiss M, Sarkar M, Arand M, et al. Solvent-preserved, bovine cancellous bone blocks used for reconstruction of thoracolumbar fractures in minimally invasive spinal surgery-first clinical results. *Eur Spine J*. 2005;14:192-196.
44. Ramani PS, Kalbag RM, Sengupta RP. Cervical spinal interbody fusion with Kiel bone. *Br J Surg*. 1975;62:147-150.
45. Goran A, Murthy KK. Fracture dislocation of the cervical spine. Value of anterior approach with bovine bone interbody fusion. *Spine*. 1978;3:95-102.

46. Taheri ZE, Gueramy M. Experience with calf bone in cervical interbody spinal fusion. *J Neurosurg*. 1972;36:67-71.
47. Siqueira EB, Kranzler LI. Cervical Interbody fusion using calf bone. *Surg Neurol*. 1982;18:37-39.
48. Savolainen S, Usenius JP, Hernesniemi J. Iliac crest versus artificial bone grafts in 250 cervical fusions. *Acta Neurochir*. 1994;129:54-57.
49. Stone KR, Abdel-Motal UM, Walgenbach AW, Turek TJ, Galili U. Replacement of human anterior cruciate ligaments with pig ligaments: a model for anti-non-gal antibody response in long-term xenotransplantation. *Transplantation*. 2007;83:211-219.
50. Kivovics M, Szabó BT, Németh O, et al. Microarchitectural study of the augmented bone following ridge preservation with a porcine xenograft and a collagen membrane: preliminary report of a prospective clinical, histological, and micro-computed tomography analysis. *Int J Oral Maxillofac Surg*. 2017;46:250-260.
51. Bracey DN, Seyler TM, Jinnah AH, et al. A decellularized porcine xenograft-derived bone scaffold for clinical use as a bone graft substitute: a critical evaluation of processing and structure. *J Funct Biomater*. 2018;9(3):45.
52. Manji RA, Lee W, Cooper DK. Xenograft bioprosthetic heart valves: past, present and future. *Int J Surg*. 2015;23:280-284.
53. De Salvatore S, Segreto A, Chiusaroli A, Congiu S, Bizzarri F. Role of xenotransplantation in cardiac transplantation. *J Card Surg*. 2015;30:111-116.
54. Calvo-Guirado JL, Gómez-Moreno G, Guardia J, et al. Biological response to porcine xenograft implants: an experimental study in rabbits. *Implant Dent*. 2012;21:112-117.
55. Feng W, Fu L, Liu J, Li D. The expression and distribution of xenogeneic targeted antigens on porcine bone tissue. *Transplant Proc*. 2012;44:1419-1422.
56. Seyler TM, Bracey DN, Plate JF, et al. The development of a xenograft-derived scaffold for tendon and ligament reconstruction using a decellularization and oxidation protocol. *Arthroscopy*. 2017;33:374-386.
57. Whitlock PW, Seyler TM, Northam CN, et al. Effect of cyclic strain on tensile properties of a naturally derived, decellularized tendon scaffold seeded with allogeneic tenocytes and associated messenger RNA expression. *J Surg Orthop Adv*. 2013;22:224-232.
58. Whitlock PW, Seyler TM, Parks GD, et al. A novel process for optimizing musculoskeletal allograft tissue to improve safety, ultrastructural properties, and cell infiltration. *J Bone Joint Surg Am Vol*. 2012;94(16):1458-1467
59. Whitlock PW, Smith TL, Poehling GG, Shilt JS, Van Dyke M. A naturally derived, cytocompatible, and architecturally optimized scaffold for tendon and ligament regeneration. *Biomaterials*. 2007;28:4321-4329.
60. Whitlock PW, Van Dyke ME, Christ GJ, Inventors; *Structurally Modified Acellular Tissue Engineering Scaffolds and Methods of Production*. US patent US Patent Number 8,221,777 B2(17). Winston-Salem, NC: Wake Forest Health Sciences, assignee pp. 2012, 2012.
61. Stabile KJ, Odom D, Smith TL, et al. An acellular, allograft-derived meniscus scaffold in an ovine model. *Arthroscopy*. 2010;26:936-948.
62. Galili U. Induced anti-non gal antibodies in human xenograft recipients. *Transplantation*. 2012;93:11-16.
63. Galili U, Wang L, LaTemple DC, Radic MZ. The natural anti-Gal antibody. *Subcell Biochem*. 1999;32:79-106.
64. Macher BA, Galili U. The Galalpha 1,3Galbeta1,4GlcNAc-R (alpha-Gal) epitope: a carbohydrate of unique evolution and clinical relevance. *Biochim Biophys Acta*. 2008;1780:75-88.
65. Arca T, Proffitt J, Genever P. Generating 3D tissue constructs with mesenchymal stem cells and a cancellous bone graft for orthopaedic applications. *Biomed Mater*. 2011;6:025006.
66. Feng W, Lian Y, Zhou Z, et al. Distribution of the alpha-gal epitope on adult porcine bone tissue. *Transplant Proc*. 2006;38:2247-2251.
67. Li ST, Chen HC, Yuen D. Isolation and characterization of a porous carbonate apatite from porcine cancellous bone. *Sci Technol Innovation*. 2014;1-14.
68. Wilppula E, Bakalim G. Kiel bone in the surgical treatment of tibial condylar fractures. *Acta Orthop Scand*. 1972;43:62-67.
69. Mosdal C. Cervical osteochondrosis and disc herniation. Eighteen years' use of interbody fusion by Cloward's technique in 755 cases. *Acta Neurochir*. 1984;70:207-225.
70. Sutter B, Friehs G, Pendl G, Tolly E. Bovine dowels for anterior cervical fusion: experience in 66 patients with a note on postoperative CT and MRI appearance. *Acta Neurochir*. 1995;137:192-198.
71. Seite P, Otten P, Kurt AM. Non-union after a two-level anterior cervical decompression and fusion with Surgibonetrade mark. *J Clin Neurosci*. 1998;5:444-447.
72. Werber KD, Brauer RB, Weiss W, Becker K. Osseous integration of bovine hydroxyapatite ceramic in metaphyseal bone defects of the distal radius. *J Hand Surg*. 2000;25:833-841.
73. Hartl A, Bitzan P, Wanivenhaus A, Kotz R. Faster integration of human allograft bone than of the bovine substitute Lubboc: non-randomized evaluation of 20 cases with benign tumors or tumor-like conditions. *Acta Orthop Scand*. 2004;75:217-220.
74. Christodoulou A, Ploumis A, Terzidis I, Hantzidis P, Tapsis K, Pournaras J. Combined interbody cage and anterior plating in the surgical treatment of cervical disc disease. *Acta Orthop Belg*. 2004;70:461-465.
75. Meyer S, Floerkemeier T, Windhagen H. Histological osseointegration of Tutobone: first results in human. *Arch Orthop Trauma Surg*. 2008;128:539-544.
76. Rosito R, Galia CR, Macedo CA, Moreira LF, Quaresma LM, Palma HM. Acetabular reconstruction with human and bovine freeze-dried bone grafts and a reinforcement device. *Clinics*. 2008;63:509-514.
77. Bansal MR, Bhagat SB, Shukla DD. Bovine cancellous xenograft in the treatment of tibial plateau fractures in elderly patients. *Int Orthop*. 2009;33:779-784.
78. Elliot RR, Richards RH. Failed operative treatment in two cases of pseudarthrosis of the clavicle using internal fixation and bovine cancellous xenograft (Tutobone). *J Pediatr Orthop B*. 2011;20:349-353.
79. Makridis KG, Ahmad MA, Kanakaris NK, Fragkakis EM, Giannoudis PV. Reconstruction of iliac crest with bovine cancellous allograft after bone graft harvest for symphysis pubis arthrodesis. *Int Orthop*. 2012;36:1701-1707.
80. Maffulli N, Loppini M, Longo UG, Denaro V, Oliva F. Bovine xenograft locking Puddu plate versus tricalcium phosphate spacer non-locking Puddu plate in opening-wedge high tibial osteotomy: a prospective double-cohort study. *Int Orthop*. 2013;37:819-826.
81. Prakash SK, Mukerji N, Nath FP. Is tutobone an efficient alternative to other implants used in anterior cervical discectomy and fusion surgeries? *Br J Neurosurg*. 2017;31:340-344.

How to cite this article: Bracey DN, Cignetti NE, Jinnah AH, et al. Bone xenotransplantation: A review of the history, orthopedic clinical literature, and a single-center case series. *Xenotransplantation*. 2020;27:e12600. https://doi.org/10.1111/xen.12600