A mechanical and computational investigation on the effects of conduit orientation on the strength of massive bone allografts

Brandon G. Santoni, Wesley J. Womack, Donna L. Wheeler, Christian M. Puttlitz

Abstract

Structural bone allografts are used to reconstruct large skeletal defects resulting from trauma, tumor resection, or revision arthroplasty. Though used for over a century, bone allografts suffer from a high rate of mechanical failure due to limited graft revitalization even after extended periods in vivo. The current study evaluated the mechanical properties of longitudinally perforated cortical bone allografts (LAP) that have been shown to promote accelerated graft incorporation in a large animal model. The compressive and tensile properties of longitudinally perforated allograft specimens, as determined through uniaxial compression and diametral compression tests, respectively, were not significantly affected by the presence of the conduit. However, transversely perforated grafts (TAP) demonstrated a marked decrease in tensile capacity ($p=0.04$). Finite element analysis demonstrated moderate increases in the maximum principal stresses in LAP specimens while TAP models indicated an 83.4% increase in maximum principle stress near the conduit on the endosteal surface of the graft. This research and the previous in vivo study suggest that LAP adequately serves as an internal template within the cortical bone allograft for osseous apposition and revitalization without adversely affecting the structural or mechanical integrity of the graft.

Keywords: Bone allograft; Perforation; Biomechanics; Diametral compression; Finite element analysis

Introduction

Massive allograft bone is the primary source of bone graft material for use in limb salvage procedures after tumor resection. However, allograft bone has been found to incorporate slowly into the host even after 10 years in vivo. The lack of graft revitalization, reported to be as little as 20% of the graft 5 years post-transplantation [8,9], has lead to a complication rate approaching 50% at 10 years [1,20,21,25,27,36], with the largest constituent being mechanical in nature. Non-union at the host–graft interface [14], allograft fracture at fixation points [1,36], and fatigue failure of the allograft due to microfracture accumulation [41] illustrate that accelerating allograft incorporation will reduce the frequency and severity of allograft-associated complications and improve the long-term clinical outcome of patients requiring structural allografts.

Prior in vivo research has investigated increasing the cortical porosity of structural bone allografts as a means to increase access to surrounding vascularity and accelerate healing [7,11,17,18,24]. These studies have demonstrated that perforating the graft cortex perpendicular to its long axis alone or in combination with cortical demineralization may improve revitalization and the clinical course of graft incorporation. Though reports of improved revitalization in animal models employing only transverse allograft perforations are mixed in the literature [7,19,24,38], studies combining transverse perforation with additional cortical demineralization have consistently enhanced graft integration. Despite histologic reports of accelerated revitalization of these grafts by Lewandrowski et al. [17,18] and O’Donnel et al. [24], more recent reports have indicated a significant deleterious effect.
of the combined modification on the flexural and compressive properties of the graft [16] resulting in accelerated in vivo mechanical failure. Subsequently, this combined modification has been abandoned clinically [31]. A recent in vivo study has demonstrated that graft incorporation can be expedited independent of cortical demineralization [35]. Specifically, longitudinal perforations (LAP), as opposed to those that penetrate radially into allograft cortex, were demonstrated to improve revitalization of 5 cm cortical bone allografts after 4 months in an intercalary defect in sheep. Though the biological usefulness of LAP has been demonstrated histologically, the implications, if any, on the mechanical properties of the transplanted tissue remain unknown. The objectives of this research were to determine the effect of LAP on the compressive and tensile properties of ovine cortical bone allografts prior to transplantation using axial and diametral biomechanical testing and finite element modeling. This novel form of graft modification was compared mechanically and computationally to non-perforated and transversely perforated specimens (TAP). It is hypothesized that the latter will demonstrate reduced structural and mechanical integrity in diametral compression than non-perforated specimens due to the orientation of the conduit and the presence of the stress riser in areas of high tensile stress. Graft integrity of LAP specimens is not expected to differ significantly from intact controls.

Materials and methods

Uniaxial compression test

Uniaxial compression tests of twenty-eight 5-cm specimens of allograft bone harvested from an identical location in the mid-diaphysis of 14 matched pairs of cadaveric sheep tibiae were performed. At harvest, the skeletally mature (4–7 years old) Rambouillet–Columbia ewes weighed between 54 and 82 kg. These animals were humanely euthanized for inclusion in non-related studies. A pattern of 16...
points was marked on both end plates with the first four placed at the 12, 3, 6, and 9 o’clock positions and the remaining 90-degree increments filled with three equally spaced marks for a total of 16 points identified for drilled perforations. The 500-μm diameter conduits were drilled to a 10-mm depth in the proximal and distal cortex parallel to the long axis of the allograft (Fig. 1). The same author (B.G.S.) made all perforations, promoting repeatability in the patterns. Perforations were 5 mm apart and spanned the circumference of the cortex. The specimens were then wrapped in 0.9% saline (NaCl) soaked gauze, submerged in saline and refrigerated for approximately 12 h until testing. Bone specimens from control allograft (non-perforated) and longitudinally perforated allografts were tested to failure by applying an axial load with a servohydraulic materials testing machine (MTS, Eden Prairie, MN) at a loading rate of 0.5 mm/min [16]. Failure was defined as a sharp, observable decrease in the monotonically increasing force profile. Failure load and displacement data from the longitudinally perforated specimens were compared to the allograft control with a paired t-test at a significance level of α=0.05 with SigmaStat statistical software (Systat Software, Inc., San Jose, CA).

Diametral compression test

Diametral compression experiments have been used to determine the tensile strengths of brittle materials [10] with recent application to the field of biomaterial testing [6,28]. Application of a point force along the diameter of a cylinder generates tensile stresses perpendicular to the loading axis. In a closed cylindrical ring exposed to diametrally opposed loads, the circumferential cylinder generates tensile stresses perpendicular to the loading axis. In a closed biomaterial testing [6,28]. Application of a point force along the diameter of a cylindrical test specimens were cut from each 12-cm span. Specimens from the right limbs were designated to the perforation groups, either longitudinal (LAP) or transverse (TAP) to the axis of the bone, while specimens from the contralateral limbs served as the intact controls. The specimens were wrapped in 0.9% saline (NaCl) soaked gauze, submersed in saline and refrigerated for approximately 12 h until the time of testing. A total of 104 sections were tested in diametral compression using a materials testing machine (MTS) at a loading rate of 0.5 mm/min [12,13,39,40]. Failure was defined as a sharp, observable decrease in the monotonically increasing force profile. Soft cardboard insets [29] were oriented parallel to the original long axis, in accordance with the experimental approach. Each of the three solid models (intact, LAP, and TAP) was meshed at the 500-μm depth in the proximal and distal cortex were oriented perpendicular to the periosteal surface and longitudinal perforations modified to include 16 longitudinal or transverse perforations. Radial perforations were compared with a pooled-variance two-sample t-test using SigmaStat (α=0.05). Percent change from intact control for the two perforation groups was also compared using a pooled-variance two-sample t-test.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of specimens</th>
<th>Mean failure load (kN)</th>
<th>% Difference, p-value</th>
<th>Maximum displacement (mm)</th>
<th>% Difference, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCTL</td>
<td>14</td>
<td>18.77 ± 1.71</td>
<td>0.484 ± 0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAP</td>
<td>14</td>
<td>17.52 ± 1.45</td>
<td>6.70%, p=0.459</td>
<td>0.522 ± 0.026</td>
<td>8.29%, p=0.319</td>
</tr>
</tbody>
</table>

iCTL = internal, contralateral control; LAP = longitudinal allograft perforation. Results are presented as mean ± SEM.

Tensile strength of the non-perforated rings was calculated from measurements of cylindrical geometry, made with digital calipers (Mitutoyo, Aurora, IL), and ultimate force to failure data and compared to values reported in the literature. Assuming a perfectly cylindrical specimen, calculations of maximum circumferential stress (σθθ) at failure were completed using the Winkler-Bach Equation [2] as well as supplementary calculations including a cross-section shape factor (m), normal force in the cross-section (Nθ), and induced moment (Mθ) as follows:

\[
m = -1 + \frac{R}{A} \ln \left( \frac{R_i}{R_o} \right)
\]

\[
N_\theta = \frac{P}{2} \cos \theta
\]

\[
M_\theta = \frac{P+R}{2} \left( 2 - \cos \theta \right)
\]

\[
\sigma_{\theta\theta} = \frac{N_\theta}{A} + \frac{M_\theta}{A^*R} \left( 1 - \frac{1}{m} \frac{y}{R+y} \right)
\]

where \( P \) is the failure load, \( R \) is the cylinder radius (Fig. 2), \( A \) is the cross-sectional area, \( \theta \) is the cortical thickness, \( R_i \) and \( R_o \) are the outer and inner radii, respectively, of the specimen, and \( y \) is the distance from the centroidal axis of the ring to the endosteal surface of the specimen. The angle \( \theta \) is measured from the horizontal. An exact analysis of the stress distribution may include consideration of radial and circumferential stresses, together with the superimposed effect of shear; however, the maximum stresses in the circumferential direction considerably exceed the maximum radial stress [3] thus lending practical applicability to the Winkler-Bach Equation.

Finite element modeling

Computed tomography (CT) scans of a normal ovine tibia were imported into AMIRA image analysis software (Mercury Computer Systems, Chelmsford, MA) to develop a solid model of a 5-mm diaphyseal region. The solid model was modified to include 16 longitudinal or transverse perforations. Radial perforations were oriented perpendicular to the periosseal surface and longitudinal perforations were oriented parallel to the original long axis, in accordance with the experimental approach. Each of the three solid models (intact, LAP, and TAP) was meshed at high resolution (approximately 300,000 elements) with 8-node linear hexahedral elements in PATRAN (MSC Software Corporation, Santa Ana, CA). Given the static loading condition, the bone was modeled as linearly elastic using the transverse mechanical properties of compact mid-diaphyseal human femoral bone (elastic modulus=11 GPa, Poisson’s ratio=0.42) [33]. The models were oriented in the maximum stability, minimum width orientation, per the experimental technique. The central region of the bottom surface was kinematically constrained and a non-destructive 100 N load was distributed over a 5 x 5 mm² area on the top surface of each sample to mimic the experimental conditions. The resultant magnitude and distribution of maximum principal stress in each model permutation were computed in ABAQUS (ABAQUS, Inc., Providence, RI) to elucidate the effect of conduit presence and orientation on these mechanical parameters.
Results

In uniaxial compression (Table 1), longitudinally perforated specimens failed at 17.52±1.45 kN, representing a nonsignificant change in compressive force to failure relative to non-perforated controls (p=0.399). Failure typically occurred at the proximal or distal end plate in both the groups. In some longitudinally perforated grafts, fracture propagated between the 500-µm conduits and along the periosteal surface of the graft. Fracture of the intact controls also occurred along the circumference of the cortical endplate and extended along the periosteal surface.

Average maximum force at failure and stiffness for the longitudinally perforated cylindrical grafts in diametral compression was 301.14±48.36 N and 465.35±48.48 N/mm, respectively (Table 2). This difference relative to non-perforated specimens was not statistically significant (p>0.05). Transversely perforated grafts exhibited a 21.47% decrease in failure load relative to non-perforated controls (p=0.040), while TAP specimen stiffness was not affected by the presence of the radial conduit (p>0.05). Percent decrease in ultimate force to failure in the TAP group relative to the intact controls (21.47%) was significantly greater than the percent change of longitudinally perforated grafts relative to the intact controls (p=0.035). Failure consistently occurred in the vertical, diametral plane, and through the longitudinal or transverse perforations when present. The average ultimate tensile strength of the intact, non-perforated femoral allograft rings (mean±SEM) as determined with the Winkler-Bach Equation was 76.95±2.53 MPa. When subjected to non-destructive loading, tibial finite element models (Fig. 3) predicted the highest principal (tensile) stresses on the endosteal surface adjacent to the transverse perforations (σ_MAX =75.2 MPa) in the diametral plane, corresponding to an 83.4% increase in stress magnitude in the vicinity of the stress riser relative to the intact model. A modest 11.4% increase in maximum principal stress on the endosteal surface of LAP specimens was noted relative to the non-perforated model. Increases in stress magnitude were not noted in the vicinity of the longitudinal conduits (Fig 3B).

Discussion

Previous studies concluded that 500 µm by 10 mm longitudinal conduits created in 5 cm intercalary bone allografts promoted accelerated graft revitalization [35]. Despite any evidence of in vivo failure, it is imperative to quantify the effects of graft modification prior to transplantation. The objectives of the current study were to quantify the effect of conduit orientation on the biomechanical performance of massive bone allografts. This research demonstrates that longitudinal conduits have minimal effects on graft structural integrity while the presence of transverse/radial perforations significantly reduces the tensile properties of the allogeneic bone. Our findings demonstrate the negative effects of TAP on mechanical integrity, even without additional demineralization, thus supporting recent clinical findings [31] of accelerated graft failure in vivo.

The initial biologic response to cortical allografts is a transient period of accelerated resorption that can weaken the graft mechanically by 40% [4]. Thus any intervention employed to accelerate allograft integration should not compromise the initial structural and mechanical properties. Destructive uniaxial compression tests of longitudinally perforated allografts revealed no significant difference in ultimate force relative to non-perforated controls (p=0.339). Though not evaluated here, Lewandrowski et al. [16] reported similar results with transverse perforations. Therefore, the mechanical and structural influences of the longitudinal and transverse perforations in axial compression are minimal. However, LAP has been shown to improve graft integration [35] whereas TAP has been shown to have little beneficial biological effect [24,19]. Successful incorporation employing TAP was documented only when combined with cortical demineralization. However, the flexural and compressive properties of transversely perforated and demineralized grafts were reduced by 40% [16].

The diametral compression test has been utilized to evaluate the tensile strengths of brittle biomaterials [12,13,26,34,40] including cortical allograft [23,37]. Here, 5-mm thick cylindrical rings from sheep femora were compressed to failure along the cranial/caudal direction to evaluate the effect of the presence of the longitudinal and transverse conduits. Intact specimens failed at ultimate stress magnitudes of 76.95±2.53 MPa, slightly higher than the values of 52.0±8.0 and 55.3±8.3 MPa reported in the literature for bovine bone [5,30,32]. Our findings of a slightly increased tensile capacity of ovine bone are thought to be the result of small deviations in test specimen geometry from one that is perfectly cylindrical. Further, the Winkler-Bach Equation assumes a point load applied to the cylindrical ring, though in practice the load was distributed over a 5-mm×5-mm square area.

![Fig. 3. (A–C) FEA models of 5-mm-thick tibial rings subjected to non-destructive loading. FEA indicate highest principal stresses on the endosteal surface relative to the intact model.](image-url)
to reduce the large compressive stresses at the loading lines. A rigorous investigation by Fahad [10] comparing both loading cases, however, demonstrated near equivalency in maximum tensile stresses generated in cylindrical discs, thereby justifying our use of this experimental technique.

It was hypothesized that the presence of the longitudinal conduits would result in similar failure loads to non-perforated controls since the conduit is oriented near the neutral axis of the specimen (Fig. 2D). Uniaxial compression tests and finite element analyses confirm this hypothesis. Transversely perforated cylindrical specimens failed on average at 78% of the failure loads of the non-perforated controls (p = 0.040). Concurrent finite element analyses demonstrated an 83.4% increase in the principal stress magnitude near the stress riser on the endosteal surface of the cylinder, illustrating the negative effect of TAP on graft integrity, even without additional demineralization.

Prior studies examining the effects of allograft modification on material properties have employed four-point bending. Because LAP increases graft porosity only in the vicinity of the cortical endplates, it seems unlikely that this modification would have significant effects on the flexural properties of the graft. Thus, the diametral compression experiment served as a conservative (“worst case”) test to compare the effects of conduit orientation relative to intact specimens. Though the femora used to generate the diametral test specimens came from a relatively controlled animal population, it was noticed that the intact controls in each arm of the study were different from each other indicating that paired femora designated to the TAP group were derived from smaller sheep. This discrepancy is not thought to confound our findings. Future endeavors will ensure that specimens randomized to all treatment groups are from skeletally similar animals, perhaps through post-euthanasia radiography.

Fatigue loading of modified allograft is an important question that this study did not address. Cyclic loading of healthy bone in vivo generates microdamage in the form of microcracks [15,22], which have been reported as a necessary stimulus for remodeling [15]. In massive bone allografts, cyclic loading also leads to the accumulation of microfracture damage [41]. Because the graft is non-viable in the acute period before incorporation commences, these microcracks are unable to be repaired. Wheeler and Enneking [41] reported that it is partly the accumulation of these microcracks that leads to the deterioration of the mechanical strength of allograft in vivo and ultimately propagates graft fracture. Histological assessment of longitudinally perforated intercalary allografts in sheep revealed appositional bone formation within the conduits [35]. Principles of fracture mechanics predict that under cyclic loading, the perforations may promote crack formation and propagation within the allograft. A lack of osseous apposition within transverse perforations combined with the continual exposure to repetitive loading may lend such grafts to premature failure, as has been documented clinically [31]. In contrast, microfracture at the ends of the conduits within longitudinally perforated allografts may further stimulate graft revitalization given the newly viable tissue that has apposed onto the surface of the conduit.

In summary, modification of the cortical shell by introducing 500-μm diameter conduits parallel to the long axis of the allogeneic tissue has minimal effects on the compressive and tensile properties of the transplanted bone. This research and our previous animal study suggest that LAP may provide an option for increasing porosity, and thus potential in vivo osseous apposition and revitalization, without adversely affecting the structural integrity of the graft.

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References

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