

Cellular Bone Allografts – Scientific Basis and Clinical Applications

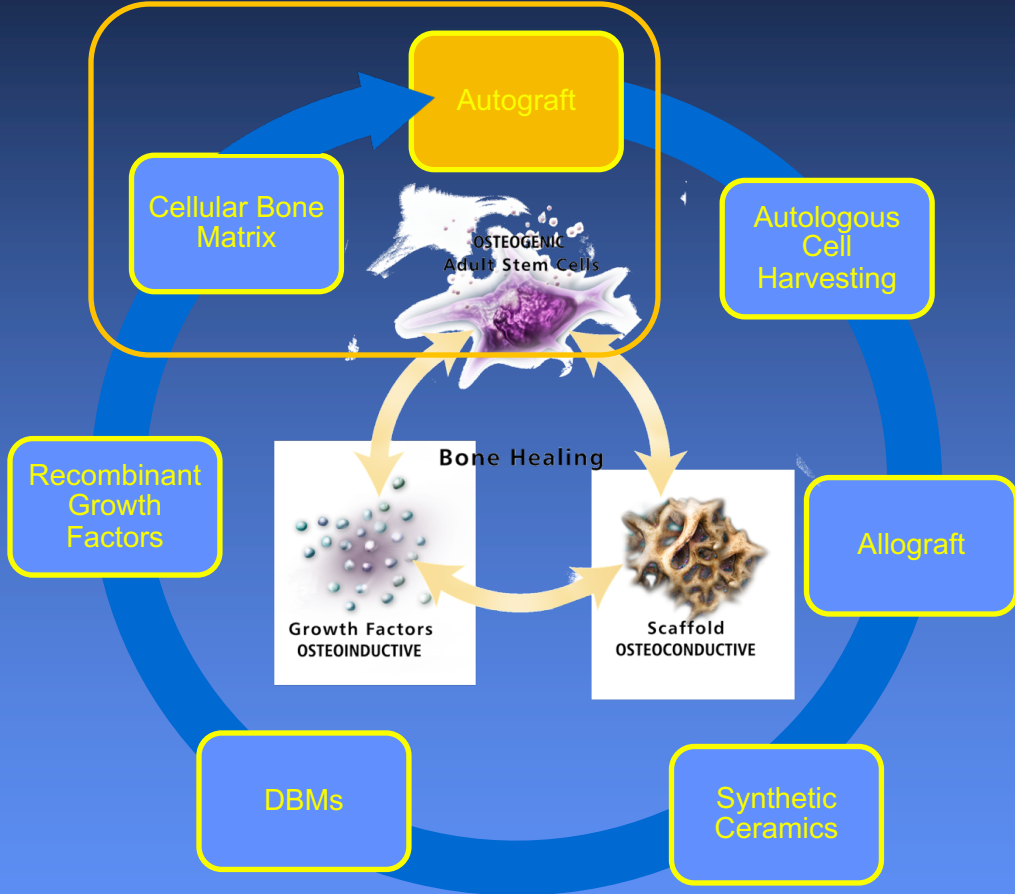
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Consultant

Disclosure

- Dr. Ryaby is a consultant to and has stock ownership/options in Orthofix Medical, Chrysalis BioTherapeutics, and consults for BRANY and Sante Ventures.

Bone Grafting Options



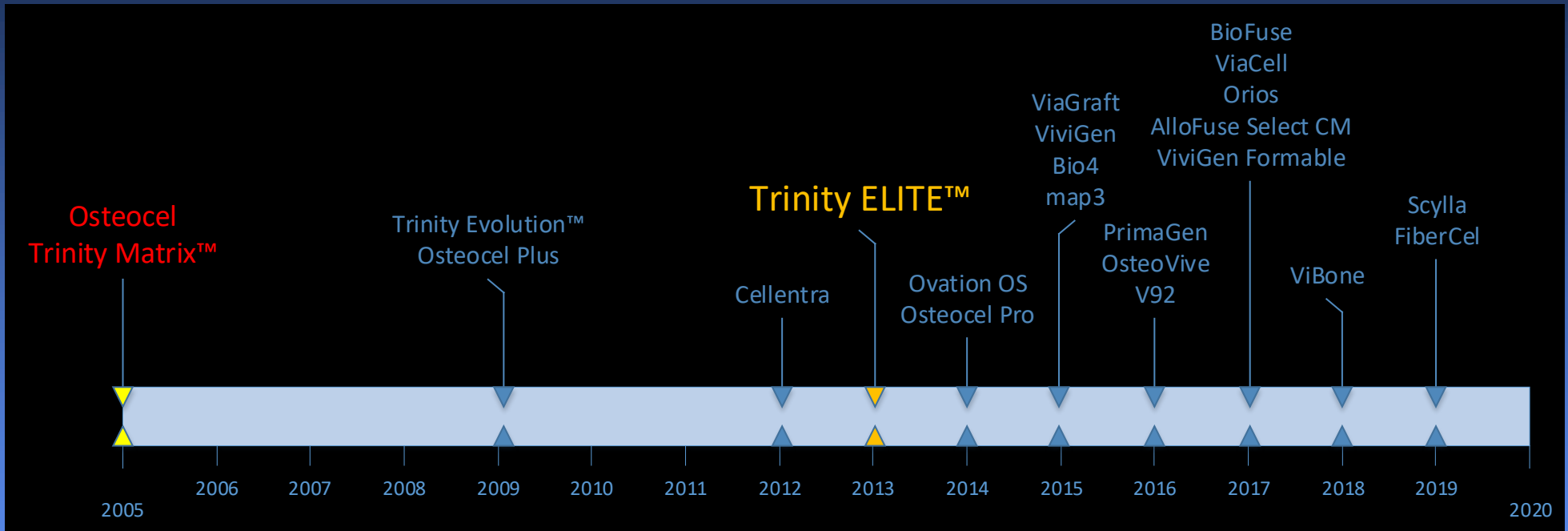
CBMs: The Value to Clinical Practice

- Attributes of autograft:
 - Osteoconductive matrix
 - Osteoinductive factors
 - Osteogenic cells
- Flexibility of surgery
 - Unlimited volume
- Consistent graft
 - Quality control testing

Variables That Play a Role in Graft Quality

- Donor Screening
- Processing Techniques
 - Treatment types
 - Time/temperature exposures
- Cryopreservation
- Storage
- Quality Testing
 - Assessment Method
 - Timing
- Shipment
- Handling at Hospital
- Thaw procedure

CBMs on the Market



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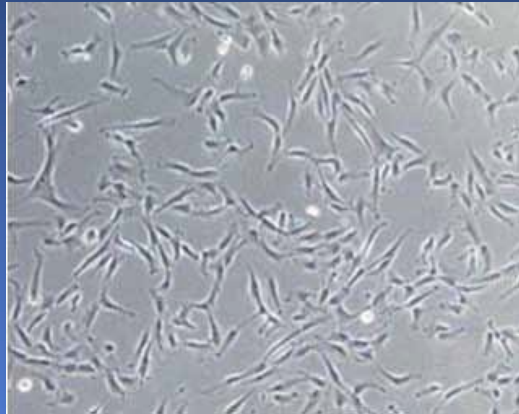
Preclinical Evaluation of CBMs

Characterization

Cell Presence



Cell Proliferation



Cell Differentiation



Alizarin Red



Von Kossa

Gene Expression Comparison of CBMs

Gene	BMSCs Sample A	BMSCs Sample B	Evolution Sample A	Evolution Sample B	ELITE Sample A	ELITE Sample B
Stem Cell Marker						
Oct-4	+	+	+++	+++	+++	+++
Osteocyte Marker						
SOST	-	-	++	++	++	++
Osteoblast Markers						
BSP	-	-	+	++	++	++
Col-I	+++	+++	++	++	++	++
OC	+	+	++	++	++	++
RUNX-2	++	++	++	++	+++	+++
Osx	-	-	++	++	++	++
Osteoclast Markers						
Cath K	++	+	-	-	-	-
TRAP	-	-	+	-	+	+
Monocyte/Macrophage Markers						
CD-11b	-	-	-	-	-	-
CD-14	+	-	-	-	-	+
MCP-1	+++	++	-	+	-	-
NFAT-2	+	-	-	-	-	-

+++: High expression or extremely high expression ($X \geq 0.1000$)

++: Intermediate expression ($0.1000 > X \geq 0.0100$)

+: Low expression ($0.0100 > X \geq 0.0010$)

A Comparative Evaluation of Commercially Available Cell-Based Allografts in a Rat Spinal Fusion Model

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ABSTRACT

Background: To evaluate the comparative abilities of commercially available, viable, cellular bone allografts to promote posterolateral spinal fusion.

Methods: Human allografts containing live cells were implanted in the athymic rat model of posterolateral spine fusion. Three commercially available allogeneic cellular bone matrices (Trinity Evolution, Trinity ELITE and Osteocel Plus) were compared with syngeneic iliac crest bone as the control. All spines underwent radiographs, manual palpation, and micro-computed tomography (CT) analysis after excision at 6 weeks. Histological sections of randomly selected spines were subjected to semiquantitative histopathological scoring for bone formation.

SPECIAL ISSUE ARTICLE

Comparing cellular bone matrices for posterolateral spinal fusion in a rat model

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Abstract

Introduction: Cellular bone matrices (CBM) are allograft products that provide three components essential to new bone formation: an osteoconductive scaffold, extracellular growth factors for cell proliferation and differentiation, and viable cells with osteogenic potential. This is an emerging technology being applied to augment spinal fusion procedures as an alternative to autografts.

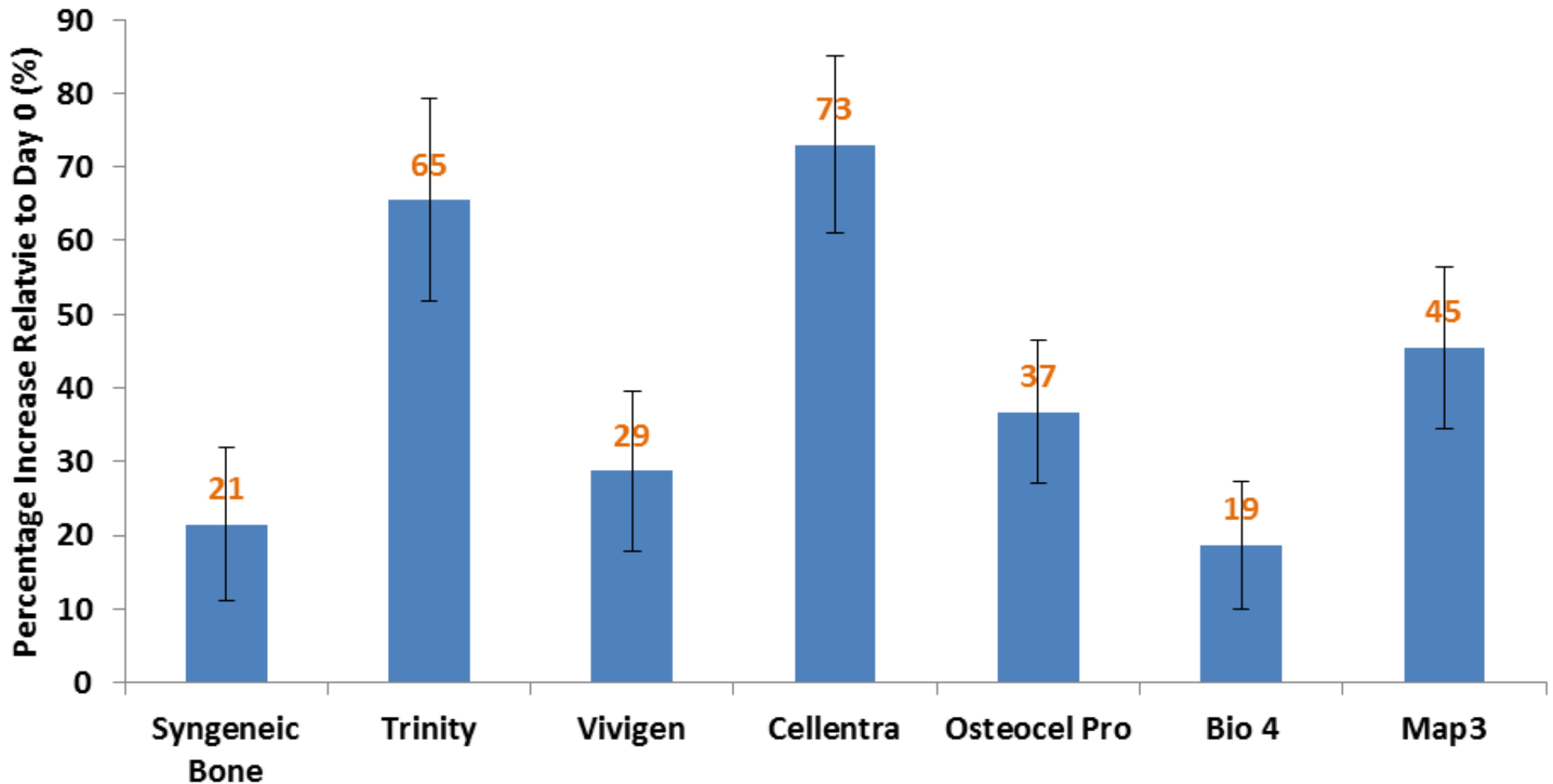
Methods: We aim to compare the ability of six commercially-available human CBMs (Trinity ELITE[®], ViviGen[®], Cellentra[®], Osteocel[®] Pro, Bio4[®] and Map3[®]) to form a stable spinal fusion using an athymic rat model of posterolateral fusion. Iliac crest bone from syngeneic rats was used as a control to approximate the human gold standard. The allografts were implanted at L4-5 according to vendor specifications in male athymic rats, with 15 rats in each group. MicroCT scans were performed at 48 hours and 6 weeks post-implantation. The rats were euthanized 6 weeks after surgery and the lumbar spines were harvested for X-ray, manual palpation and histology analysis by blinded reviewers.

Aim and Experimental groups

- To assess the rates of fusion in cell-based allografts with syngeneic live bone as the 'gold standard' control; blinded CT evaluation by ImagenIQ, Cleveland, OH

Group	Graft Name	Graft Type	Vendor	Rats
1	Live bone	Syngeneic rat iliac crest bone (incl. 15 syngeneic bone donors)	-	30
2	Trinity Elite	Human cryopreserved cancellous bone containing viable cells, with demineralized cortical bone matrix (>500,000/cc)	MTF/Orthofix	15
3	Vivigen	Human cryopreserved viable corticocancellous bone and demineralized bone matrix (? cells/cc)	DePuy Synthes	15
4	Cellentra	Human cryopreserved cancellous bone combined with demineralized cortical bone matrix (>250,000 cells/cc)	Zimmer/Biomet	15
5	Osteocel Pro	Human cryopreserved cancellous bone containing viable cells, with demineralized bone matrix added (>3,000,000 cells/cc)	NuVasive	15
6	Bio4	Human bone allograft that contains both viable cells and growth factors (>600,000 cells/cc)	Stryker	15
7	Map3	Human cortical bone chips and demineralized bone matrix, with multipotent adult progenitor cells (MAPC) cryogenically preserved cells added at time of implantation (>50,000 MAPC/cc)	RTI Surgical	15
Total animals:				130

Results – New Bone Formation by Micro CT



Clinical Studies

Selected Peer Reviewed Data on CBMs – Spine

Publication	Technology	Procedure	# of Subjects	Endpoint	Assessment	Fusion Rate
Caputo, AM.	Osteocel Plus	Lumbar Fusion (XLIF)	30	12 months	Radiograph	96.4%
Tohmeh, AG	Osteocel Plus	Lumbar Fusion (XLIF)	50	12 months	Radiograph	90.2%
Ammerman, JM	Osteocel Plus	MIS TLIF	23	12 months	Radiograph	91.3%
Kerr, EJ	Osteocel Plus	ALIF/TLIF	52	24 months	Radiograph/CT	92.3%
McAnany, SJ, et al.	Osteocel Plus	ACDF	57	12 months	Radiograph	87.7%
Eastlack, RK et al.	Osteocel Plus	ACDF	182	24 months	Radiograph/CT	87%
Divi & Mikhael	ViviGen	ACDF, PCF	21	6 months	Radiograph	100%
Lee, D.	Map3/rh-BMP2	ALIF	20 map3 21 rh-BMP-2	12 months	Radiograph/CT	91% 91%

CBM Clinical Study Pipeline - Selected

CBM	Trial ID	Status	Start Date	Completion Date	# of Sites	Patient Enrollment	Study Type	Length of Study	Type of Surgery
Vivigen	NCT02814825	Active, not recruiting	June 2016	July 2020	Multi-Center (no info)	100	Prospective single-arm	Primary: 12 months post-op Secondary: 0-12 months	Cervical
Vivigen	NCT03733626	Recruiting	October 2018	November 2022	1	40	Prospective, randomized	Primary: 12 months post-op Secondary: 12 months	Lumbar
Vivigen	NCT03527966	Recruiting	July 2017	July 2020	1	60	Prospective, randomized	Primary: 12 months post-op Secondary: 12 months	Lumbar
Bio4	NCT03077204	Active, not recruiting	April 2017	December 2018	Single Center	20	Prospective single-arm	Primary: 12 months post-op Secondary: Pre-op-12months post-op	Cervical
Cellentra	NCT02182843	Completed	July 2014	January 2018	Multi-Center (Currently 6; up to 8)	81	Prospective single-arm	24-month post-op	Cervical
ViBone	NCT03425682	Recruiting	February 2018	December 2020	Multi-Center (Currently 1)	Total 200. Min 25 for ACDF, min of 100 for lumbar (TLIF or PLIF)	Prospective	12-month post-op	Cervical or Lumbar
ViBone	NCT03896347	Recruiting	July 1, 2019	June 2021		120	Randomized	12-month post-op	3 level OLIF
Nucel	NCT02808234	Recruiting	December 2015	February 2020	Multi-Center (3)	200	Prospective, non-randomized	24-month post-op	Lumbar
Nucel	NCT02023372	Enrolling by invitation	December 2013	June 2020	1	57	Prospective	12 months	Lumbar
Map3	NCT02628210	Active, not recruiting	December 2015	March 2020	Multi-Center (Currently 3)	30	Prospective single-arm	Primary: Baseline, 6 weeks, 3-, 6-, 12-, 24-months Secondary: Same unless indicated otherwise	Lumbar

TE Lumbar Fusion Clinical Trial

NCT02969616

Prospective, Multicenter, Open Label

- 300 Subjects

Investigators

- Ammermans/Wind – DC
- Hassandazeh – Charlottesville, VA
- Lansford – Charleston, SC
- Nunley – Shreveport, LA
- Park – Royal Oak, MI
- Peppers – San Diego/Encinitas
- Russo – Butte, MT
- Sales – Portland, OR
- Techy – Denver
- Vokshoor – Los Angeles
- Yoo – Portland, OR

Study Design

Protocol:

- Assess safety and effectiveness of a Trinity ELITE in patients undergoing lumbar arthrodesis at 12 and 24 months
- Interbody or posterolateral fusion at 1 or 2 levels
- 75 of 300 patients have completed 12-month follow-up

Outcome Measures:

- Fusion
 - » Independent core lab (MMI) review of angular and translational motion ($<3^\circ$ and $<3\text{mm}$, respectively) from flex/ext X-rays
 - » Presence of bridging bone across the adjacent endplates on thin-cut CT scans
- Clinical pain, function and quality of life
 - » VAS back and leg pain, the Oswestry Disability Index (ODI) and EQ-5D

The current clinical study is ongoing, the slides therein are a snap shot of data currently available and is not conclusive on how the study may end

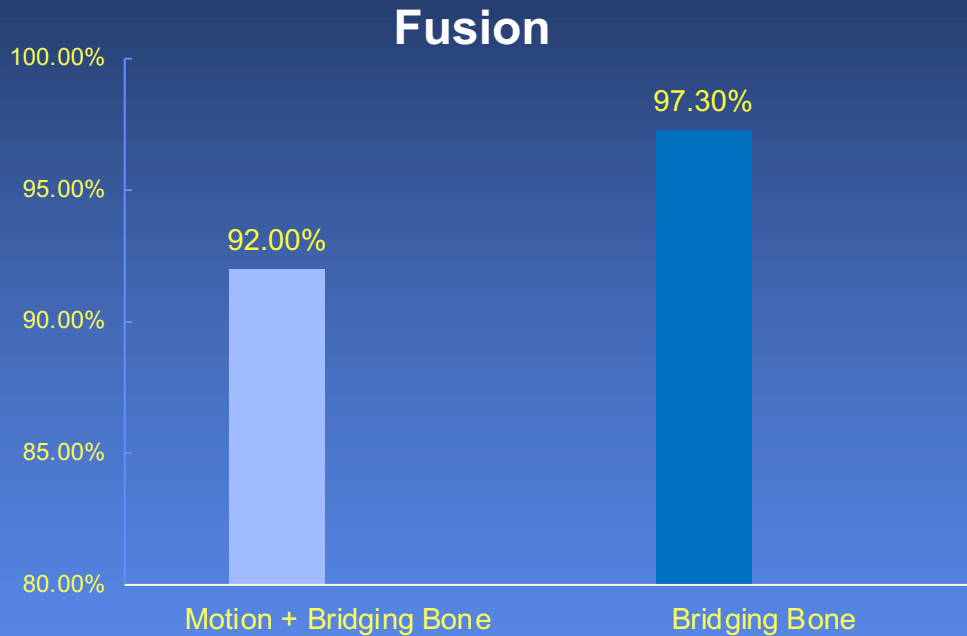
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Patient Demographics (n=75)

Gender:	69% Female
Mean age:	59.6 ± 1.4 years
Mean BMI:	29.7 ± 0.7 (40% obese or extremely obese)
Smokers (%):	9.3%
Diabetics (%):	21.3%

*Study is ongoing
Sponsored by Orthofix

Clinical Assessment (n=75)*



Secondary Clinical Endpoints

Assessment	Results
ODI	24.2 pt. decrease (P<0.001)
VAS Back Pain	33.8 mm decrease (P<0.001)

No serious adverse events occurred related to the allograft

*Study is ongoing
Sponsored by Orthofix

Summary

- Cell based allografts are a valid bone grafting solution that fulfills a clinical need
- Processing methods and storage need to be carefully followed to allow for maximum shelf/freezer life
- Additional clinical investigation is required to highlight safety and efficacy of CBMs in surgical procedures