Cell-Based Therapies Viability Contributes to Regenerative Medicine

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........... the Future
... Accommodating Focus...

Innovation

Weighing Value......
Expense vs. Benefit
Balancing Focus
Market demand
and Moral resolve
Marrow Isolated Adult Multilineage Inducible (MIAMI)

**Characteristic Molecular Markers**

- Oct 4
- Nanog Sox2
- Rex1
- Bmi1
- SSEA-4
- CD29
- CD103
- CD122
- CD164

D’Ippolito et al., 2004 J Cell Sci 117:2971
Features and Benefits

- Human Cell Tissue Product (HCT/P)
- 3 essential components for bone growth
  - Osteoinductive, Osteoconductive, Osteogenic
- Supraphysiologic levels of MIAMI cells, OPCs, MSCs
- 100-300 µm bone particle size
- Novel cryoprotectant that is effective and safe to implant
- Convenient handling and preparation in OR
- 2 year shelf life from cell freeze date
The graft (scaffold and cells) is processed in accordance with AATB and FDA
Regulated under FDA CFR Title 21 Part 1271
Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P 361)
  - Minimal Manipulation
  - Homologous Use
  - Needs to be free of contamination
Donor Criteria and Screening

- Males and Females, Ages 15 – 55
- Extensive donor screening by licensed laboratories and physicians
- Follows process that meets FDA and AATB requirements for screening/testing

<table>
<thead>
<tr>
<th>TEST</th>
<th>SYMBOL</th>
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<tbody>
<tr>
<td>Human Immunodeficiency Virus (HIV)</td>
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<tr>
<td>HIV-1/2 Antibodies</td>
<td>HIV-1/2-Ab</td>
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<tr>
<td>Nucleic Acid Test for HIV-1 RNA</td>
<td>HIV-1 NAT</td>
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<tr>
<td>Hepatitis B Virus (HBV)</td>
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</tr>
<tr>
<td>HBV Surface Antigen</td>
<td>HBsAg</td>
</tr>
<tr>
<td>HBV Core Antibody (IgG &amp; IgM)</td>
<td>HBCab</td>
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<tr>
<td>Nucleic Acid Test for HBV DNA (if applicable)</td>
<td>HBV NAT</td>
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<tr>
<td>Hepatitis C Virus (HCV)</td>
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<tr>
<td>HCV Antibody</td>
<td>HCVAb</td>
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<td>Nucleic Acid Test for HCV RNA</td>
<td>HCV NAT</td>
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<td>Human T Cell Lymphotrophic Virus I/II (if applicable)</td>
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<td>HTLV-I/II Antibody</td>
<td>HTLV-I/II-Ab*</td>
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<td>Syphilis</td>
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<tr>
<td>Rapid Plasma Reagin Screen</td>
<td>RPR**</td>
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<tr>
<td>or</td>
<td></td>
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<tr>
<td>T. Pallidum IgG</td>
<td>T. pallidum IgG</td>
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<tr>
<td>Cytomegalovirus</td>
<td>CMV Ab (IgG &amp; IgM)</td>
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</table>
Safety

- Processed under aseptic conditions in class 100 clean rooms
- Microbial cultures performed throughout process according to industry standards
- MLR for HLA
Cell Component

- **Product:**
  - 150,000 viable cells/cc
  - 100,000 MSC/cc
- Viability approaching 92% in all donors
- MSCs > 70% of viable cells
- Supra-physiologic concentration of MIAMI cells
- Enriched population of osteogenic cells
Novel Cryoprotectant

- DMSO-Free
  - Not toxic to cells at room temperature
  - No need to decant!
- Sustains cell viability after freezing and thawing
- Sustains Phenotypic durability – cell identity
Small Animal Model - Athymic Rat

Implantation

3-weeks

6-weeks
Small Animal Model - Athymic Rat

Implantation 3-weeks 6-weeks
Small Animal Model - Athymic Rat

Implantation

3-weeks

6-weeks
Implantation – Bilateral; Ant/Post
### Animal 30

| 30 | | L1 | 6 | Via® Graft 2 with cells and gel | 6 weeks |
| 30 | | L2 | 7 | Via® Graft 3 (just particulate) | 6 weeks |
| 30 | | R1 | 8 | Via® Graft 3 with cells | 6 weeks |
| 30 | | R2 | 9 | Via® Graft 3 with cells and gel | 6 weeks |

**ViaForm for animal studies**

<table>
<thead>
<tr>
<th>Components</th>
<th>Particle Size</th>
<th>ViaForm 2.0 Percentage</th>
<th>Viaform 3.0 Percentage</th>
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</thead>
<tbody>
<tr>
<td>Mineralized Cortical Shavings</td>
<td>300um+</td>
<td>60%</td>
<td>50%</td>
</tr>
<tr>
<td>Demineralized Cortical Shavings</td>
<td>300um+</td>
<td>20%</td>
<td>20%</td>
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<tr>
<td>Mineralized Cort Shavings Powder</td>
<td>106-300um</td>
<td>10%</td>
<td>10%</td>
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<tr>
<td>Crushed Cancellous</td>
<td>1000-1700um</td>
<td>10%</td>
<td>20%</td>
</tr>
</tbody>
</table>

**3 weeks**

**6 weeks**
Micro-CT Analysis

Average Bone Volume

Bone volume ($mm^3$) ± s.d.

- Via Graft
- Via Graft with Cells
- Via Form
- Via Form with Cells

* Significant difference
Nude Rat Model Viagraft without Cells – 6 weeks

DBM – Blue Green
New Bone – Rose
Nude Rat Model Viagraft with Cells – 6 weeks

DBM – Blue Green
New Bone – Rose
Placement: Bone vs. Skeletal Muscle
Placement: Bone vs. Skeletal Muscle

- Bone
- Mesangioblasts
**CF** – Collagen Fleece
**SM** – Skeletal Muscle
**Vas** – Vascular
CF – Collagen Fleece
SM – Skeletal Muscle
Vas – Vascular
Adjacent Muscle Margin Maintained

Skeletal muscle clearly delineated (yellow arrows), no evidence of heterotopic calcification. (ABOVE)

Exacting and profuse bone formation, no suppression of peripheral nerve morphology (RIGHT)
Introduction

When conservative treatments fail to eliminate abnormal motion, spinal fusion has been shown to provide symptomatic treatment for spinal instability, stenosis, spondylolisthesis, and symptomatic degenerative disc disease. The trend and rates of fusion over the past few decades have been dramatic in the United States. Accompanying that higher incidence has been the shifting from traditional open surgery to minimally invasive techniques to reduce scar tissue formation, signification of muscle stripping, and muscle retraction which all have been shown to adversely affect outcomes. Other reasons supporting the widespread transition to minimally invasive spine (MIS) techniques include decreased postoperative pain, decreased intra-operative blood loss, shorter postoperative hospital stay, faster return to normal activity, and reduced reoperation rates.

Surgical fusion is a procedure that involves fusing two vertebrae together to stiffen the spine and prevent movement. While grafting options include allograft, autologous, and synthetic materials, recent interest in viable allograft material with living cells has shown attention and attraction for incorporating a biologic basis for regenerative consideration. A recent viable allograft, complete with cellular and denuded bone carrier (VivaGraft, VivaBiomedical, Marietta, GA) has been developed. This study represents a retrospective review of a single surgeon, single surgeon with the objective of evaluating the product in 50 consecutive patients for fusion by CT and radiographic evaluation at 3 months in conjunction with a minimally invasive surgical approach.

Methods

A retrospective review of patients treated for revision surgery who received VivaGraft cellular bone matrix material in minimally invasive transforminal lumbar interbody fusion (MITLIF) with a minimum of 12-month follow-up.

Discussion

In this population, 94% of the patients treated achieved the surgical objective in 94% of the levels treated were fused. The high rate of fusion, the lack of secondary morbidity with autologous bone harvest, and the clinical success account the benefits of viable allograft matrix for MITLIF use. Limitations to the finding in this study exist because of single surgeon, single site, and although all patients were treated by MITLIF, the study was not randomized or compared with patients undergoing open TLIF. Clinical data will be added to the radiographic assessment to attend the considerations of early relief, more efficient gains in quality of life, and in cost analysis regarding patient morbidity, return to work, facility cost, and the overall economics of advanced biologics in patient care.

Results

Patient outcomes were assessed at 12 months. In total, 46 levels in 35 patients were evaluated. This study population consisted of 11 males and 4 females with 20 and 26 levels respectively treated. Of the 25 patients, 23 demonstrated fusion at all levels treated at the 12-month follow-up, and both patients who had not achieved complete fusion had undergone second procedures where one of the two levels treated was fused.
TLIF Fusion with Viable Allograft - 50 Consecutive Cases at 12 month Follow-up

• 50 Patients – 75 Levels
• 12-month clinical
• 47/50 Fuse (94% of Patients; 96% of Levels)
• 3 patients not fused
  – all single level procedures
  – all failure occurred at L5-S1.
  – None of the patients who did not fuse were assigned to co-morbidities of smoking or diabetic complications.

• ANOVA demonstrated no correlation in the success of the spinal fusion between age, BMI, or number of levels treated

-Will Tally, MD, Athens Orthopaedic Clinic, Athens, GA, USA
LLIF Fusion with Viable Allograft - 25 Consecutive Cases at 12 month Follow-up

• 25 Patients – 46 Levels
• 12-month clinical
• 23/25 Fuse (92% of Patients; 96% of Levels)
• 2 patients not fused
  – Both 2-level procedures
  – Despite 1 level unfused, adjacent segment fused.
  – Neither of the patients who did not fuse were assigned to co-morbidities of smoking or diabetic complications.
• ANOVA demonstrated no correlation in the success of the spinal fusion between age, BMI, or number of levels treated.

-Will Tally, MD, Athens Orthopaedic Clinic, Athens, GA, USA
Substantiation of Biologic Activity

Pre-clinical data
• 30 animal OI study (42-day)
  Radiographs 3 weeks and 6 weeks
• 8 animal OI study (8-weeks)
• 8 animal 8-week, 2-level fusion study
  Micro-CT, histology,
  Radiography, histology,
  quantitative assay
  Radiography, histology,
  biomechanical testing

Clinical Study
• Viagraft Fusion I
  96%
• Viagraft Fusion II
  94%
  LLIF 25 patient, 46 levels,
  TLIF 50 patient, 75 levels,

Near Future Pipeline
• ViaDisc Launch
One relatively smooth curve

We kept this part

But this is new
The evolutionary increments over the last 4000 years are probably far smaller than we might suppose. In fact, the pathology attendant to our species is more likely better scaled in technology than in disease. – John C. Baird
Table 2: Age-specific prevalence estimates of degenerative spine imaging findings in asymptomatic patients

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<tr>
<th>Imaging Finding</th>
<th>Age (yr) 20</th>
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<th>Age (yr) 40</th>
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<th>Age (yr) 60</th>
<th>Age (yr) 70</th>
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<tr>
<td>Disk degeneration</td>
<td>37%</td>
<td>52%</td>
<td>68%</td>
<td>80%</td>
<td>88%</td>
<td>93%</td>
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<tr>
<td>Disk signal loss</td>
<td>17%</td>
<td>33%</td>
<td>54%</td>
<td>73%</td>
<td>86%</td>
<td>94%</td>
<td>97%</td>
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<tr>
<td>Disk height loss</td>
<td>24%</td>
<td>34%</td>
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<td>67%</td>
<td>76%</td>
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<tr>
<td>Disk bulge</td>
<td>30%</td>
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<td>Disk protrusion</td>
<td>29%</td>
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<td>38%</td>
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<td>43%</td>
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<td>Annular fissure</td>
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<td>27%</td>
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<td>Facet degeneration</td>
<td>4%</td>
<td>9%</td>
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# Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations


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## Table 2: Age-specific prevalence estimates of degenerative spine imaging findings in asymptomatic patients

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*AJNR Am J Neuroradiol ●:● ● 2015*
Cell-Mediated Therapy

- Focus of therapy – new tissue
- Component of tissue – cells
- Growth factor intent – bring cells
- Cells maintain *in situ* autocrine capacity
Cell-Based Therapy

| Chondrocyte-like Cells | Mesenchymal Stem Cells | Notochordal-like Cells |

**Growth Factors**

**Gene Therapy**
Cell Replacement Strategy

• Cells responsible for disc metabolism
• Correct matrix insufficiency
  – Inhibit further tissue breakdown
  – Enhance structural proteins
• Disc Cell Population
  – Augment
  – Re-orient
  – Replace
• Restore segment biomechanics
Directed Regeneration Strategy

Directed Regeneration Strategy

Directed Regeneration Strategy

Directed Regeneration Strategy

MICRONIZED NUCLEUS PULPOUS

Directed Regeneration Strategy

Directed Regeneration Strategy

Directed Regeneration Strategy

MICRONIZED NUCLEUS PULPOSUS

Cells delivered:
Viability
Durability
Nucleus Morphology

Nucleus Pulposus
Following MSC Implantation

Intervertebral Disc
12 months following
Adipose derived cell implantation

Stem Cell Viability
12 months following implantation
Development

• Composition
  – Nucleus Pulposus Graft
  – Flowable through 22-gauge cannula

• Cell contribution ViaCells
  – Cell Toxicity
  – Inductivity to disc phenotype
  – Matrix production

• Mechanically integrated
Volume = \( \frac{(4\pi r^3)}{3} \)

\(~ 25\% \) increase in radius doubles volume
Motion Segment Compression
After 0.7ml of ViaDisc supplementation achieves same load with less excursion **
Loading Device/Cryo-Planed Filling
Summary

• Supplementation with ViaDisc Allograft augments mechanical support for intervertebral disc.
• The same force can be reached with a lesser excursion.
• Cell biology has shown that disc and disc cells are both frequency and amplitude dependent.
• Disc augmentation reduces amplitude of loading which has been shown to stimulate matrix production.
• Force was achieved at 85% of displacement.
• Average intervertebral disc thickness of 1 cm, 1.5-mm difference might protect nerve root compression, subsequently reduction in pain, and ultimately in functional recovery.
Prevalence of Spine Pain

Self-Reported Prevalence of Musculoskeletal Pain in Past Three Months by Sex and Age, United States 2012

Prevalence of Surgical Fusion

• Increase of 137% from 1998-2008
  – 174,223 in 1998
  – 413,131 in 2008

• Cost difference
  – $4.3B in 1998
  – $33.9B in 2008

• “Stalled innovation in non-fusion technologies....”

**Efficacy Evaluation**

<table>
<thead>
<tr>
<th>CONFIDENTIAL</th>
<th>Clinical Protocol</th>
<th>DATE</th>
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Viable Allograft Supplemented Disc Regeneration in the Treatment of Patient with Acute Spinal Disc Herniation – **VAST Trial**

**Sponsor:**
Vivex Biomedical, Inc.
VAST Trial Studyclinical

**VAS**

**Oswestry**

**MRI – 12 months**
Early Intervention....Preventative

How long do you wait
Value of intervention

- Fusion represents approximately 1% of spine care.
- 400,000 fusions account for $33.9B ($82,500 each).
- Assuming a rate of 10% successful intervention:
  - Savings of 40,000 fusions per year of prevention:
    - $3.4B
    - 87.7% reduction in cost of care for that 10%:
      - Cutting the $82,500 cost by 12.1% - $10,000
    - $3B saved in 40,000 treatments ($83,333 on 360,000)
  - System saves $3B; Vivex revenues $400M