

In Vitro Evaluation of Injectable, Placental Tissue-Derived Products for Interventional Orthopedics

Dustin R. Berger^{1,2}, Nicolette F. Lyons³, and Neven J. Steinmetz³

¹Interventional Orthopedics Foundation ²School of Biomedical Engineering, Colorado State University ³Regenerative Sciences

Background Information

Placental tissue-derived products, obtained from amniotic fluid and the amniotic/chorionic membrane, have been recently touted as effective orthobiologics in promoting the repair and regeneration of soft tissue injuries. The amniotic membrane is well known to have beneficial characteristics for homologous use, including anti-inflammatory, anti-microbial, anti-fibrotic, and low immunogenic properties¹. Freshly harvested membrane has been reported to function as a versatile, temporary wound covering, providing distinct advantages compared with other biological dressings². Moreover, when dehydrated, the amniotic/chorionic membrane retains biologically active growth factors, and promotes the migration and proliferation of human bone marrow-derived mesenchymal stem cells (hMSCs) in vitro^{3,4}. Placental tissues could provide an alternative source for stem cells, as those isolated from amniotic fluid and membranes have been reported to have many of the same qualities as hMSCs⁵.

A current trend in placental tissue-derived products is the micronization of the amniotic membrane to facilitate needle-based delivery. These “morselized”, flowable products exist in a dehydrated or cryopreserved state, and are classified as 361 Human Cellular and Tissue Based Products (HCT/PS), as defined by US FDA 21 CFR Part 1271. According to Part 1271.10(a), a 361 HCT/P must **not** be dependent the metabolic activity of **living** cells⁶. However, many of these products are currently marketed as a form of stem cell therapy, implying the existence of viable cells. Here, we test several placental tissue-derived products for the presence of stem cells and their ability to modulate in vitro cultures of hMSCs.

Many Placental Tissue-Derived Products are Currently Marketed as “Stem Cell Therapy”

Introducing BioDRestore™, a revolutionary way to enhance life through birth, and it's exclusive locally to Vista Medical Centers.

Unlocking the regenerative power of the human body. Using the latest advancement in stem cell and other cellular growth factors, BioDRestore is derived from the building blocks of life, so the types of cells that developed and formed a human life are available from donated placental tissue. This type of tissue use is legal and FDA sanctioned and arrives to our clinic cryopreserved after a stringent screening process. **These cells have amazing adaptogenic and healing properties which is why they work so well in a variety of applications and tissue repair.**

<http://vish.healthcenter.com/bo-d-treatment/>

Amniotic Fluid As The Suspension Mechanism

Amniotic fluid contains nutrients and growth factors that facilitate fetal growth and provides mechanical cushioning and antimicrobial properties that protect the fetus. Amniotic fluid also contains carbohydrates, proteins and peptides, lipids, lactate, pyruvate, electrolytes, enzymes, hormones, epidermal growth factor, amnion-derived stem cell, transforming growth factor (TGF)- α , TGF- β 1, and fibroblast growth factor (FGF), hyaluronic acid, amnion epithelial cells, derived from the embryonic ectoderm, and amnion mesenchymal cells. It should be noted that the presence of the referenced native organic components may provide an ancillary benefit to FloGraft's primary purpose as a soft tissue defect filler.

<https://www.appliedbiologics.com/product-showcase/flograft-freedom-sports-injury/>

PROMOTE SOFT TISSUE RECONSTRUCTION

Growth Factors found in amniotic/placental tissue such as PDGF, VEGF, EGF, FGF, and TGF- β promote cellular proliferation and new collagen formation. The mesenchymal stem cells attached to the amniotic tissue are at age 0. Their ability to proliferate and the robust nature of these neonatal cells make for an ongoing healing cascade that will last for many months after the initial injection.

<https://thebertsc.com/services/stem-cell-amni/>

FloGraft™

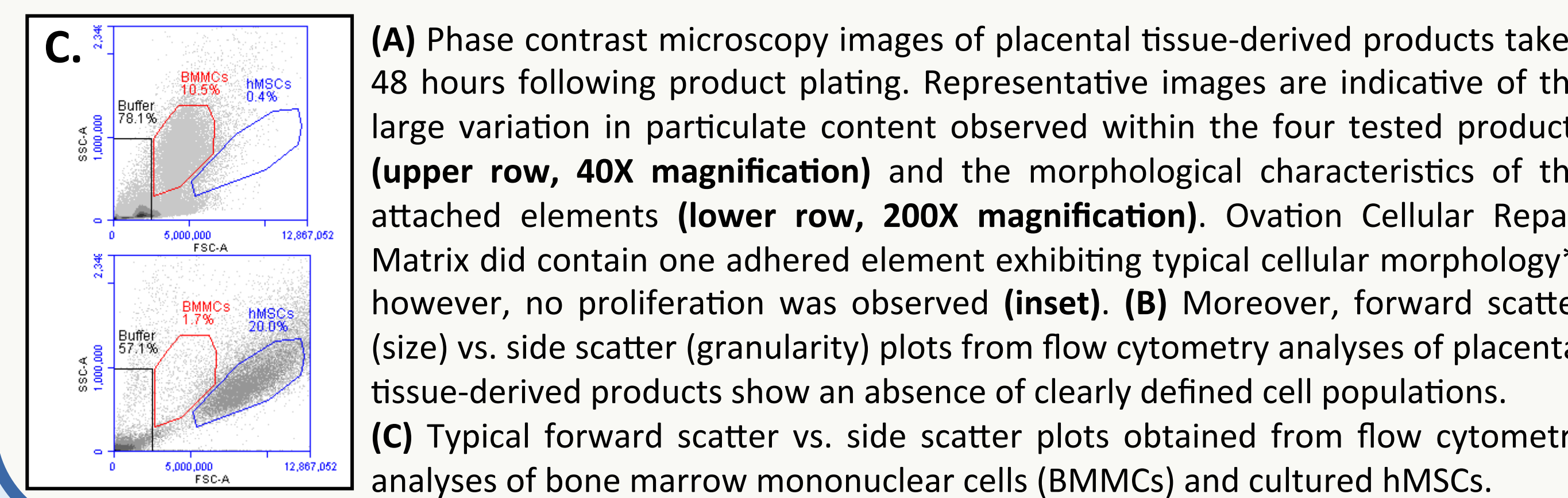
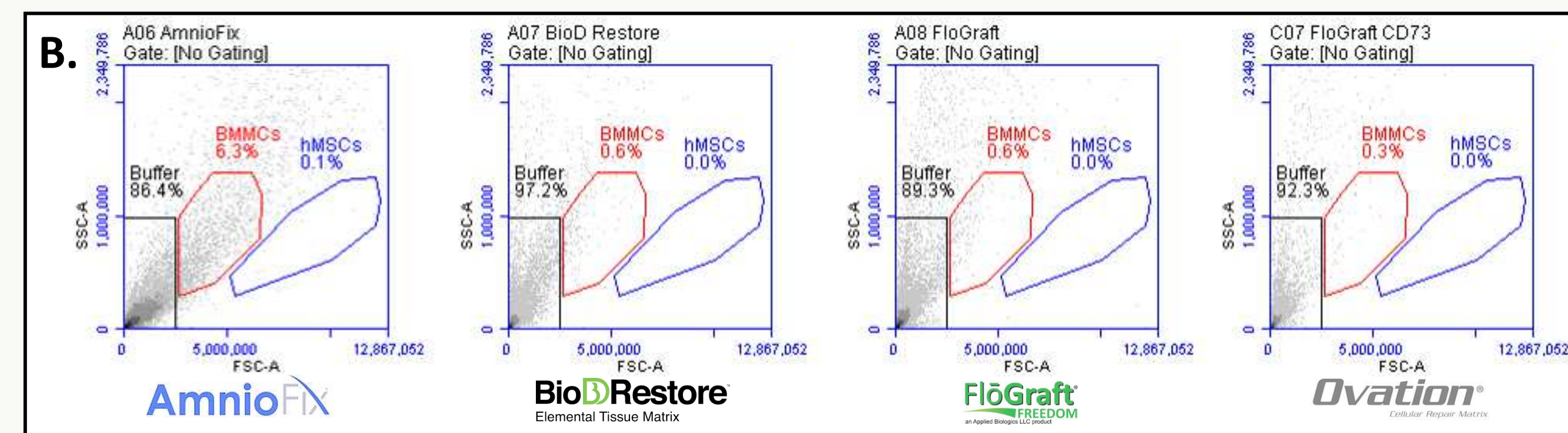
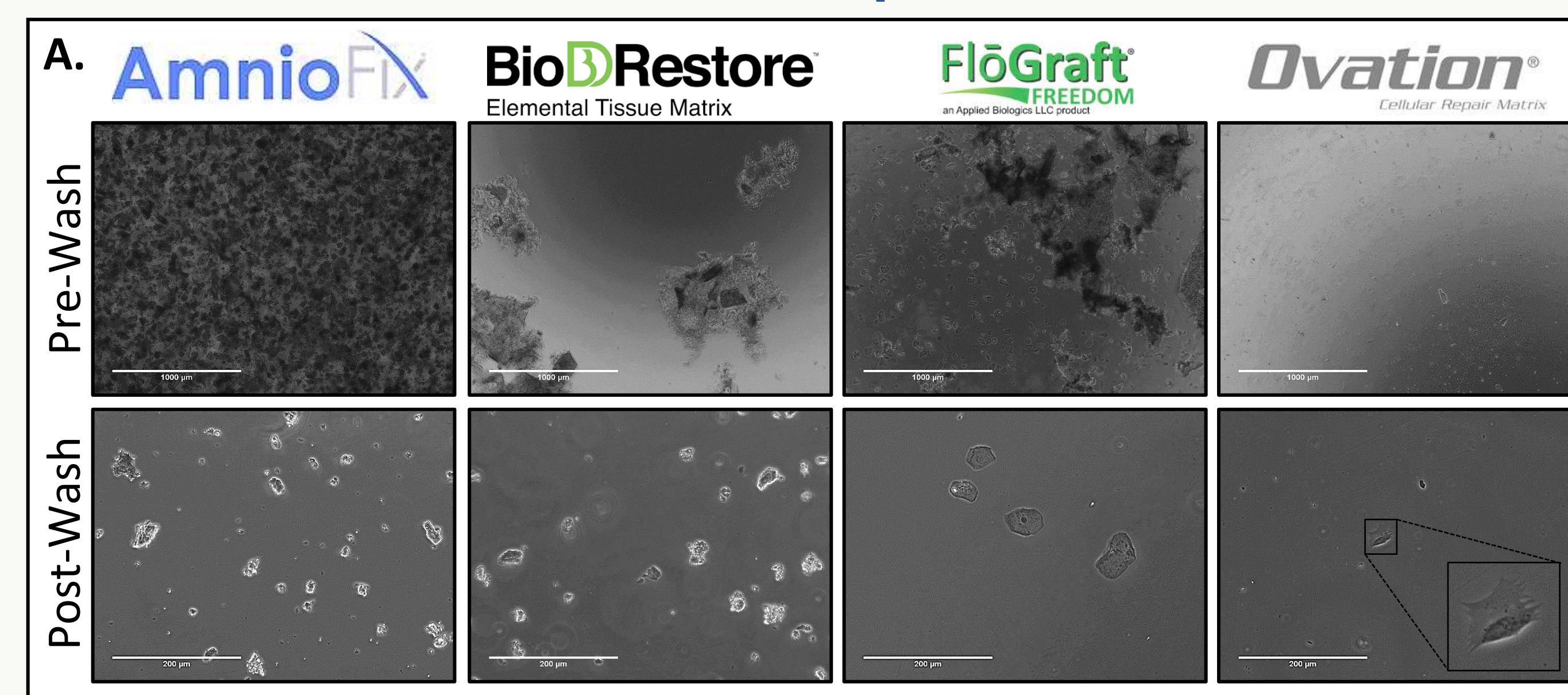
- Used to treat tendons, muscles, ligaments, cartilage, joints
- Uses amniotic epithelial stem cells to enhance recovery

<http://progressivepainandports.com/regenerative-treatments/>

Ovation® is a matrix of placental mesenchyme and of mesodermal origin. It includes extracellular matrix, mesenchymal stem cells (MSCs) and growth factors and is used to support bone repair, mainly spinal fusion. Ovation® provides a 3-dimensional architecture that has all of the necessary cellular and molecular components for wound healing. More specifically, Ovation® provides the three essential components of the periosteum—collagen matrix, viable endogenous MSCs and key growth factors, such as bone morphogenic proteins and vascular endothelial growth factor — to enable tissue regeneration.

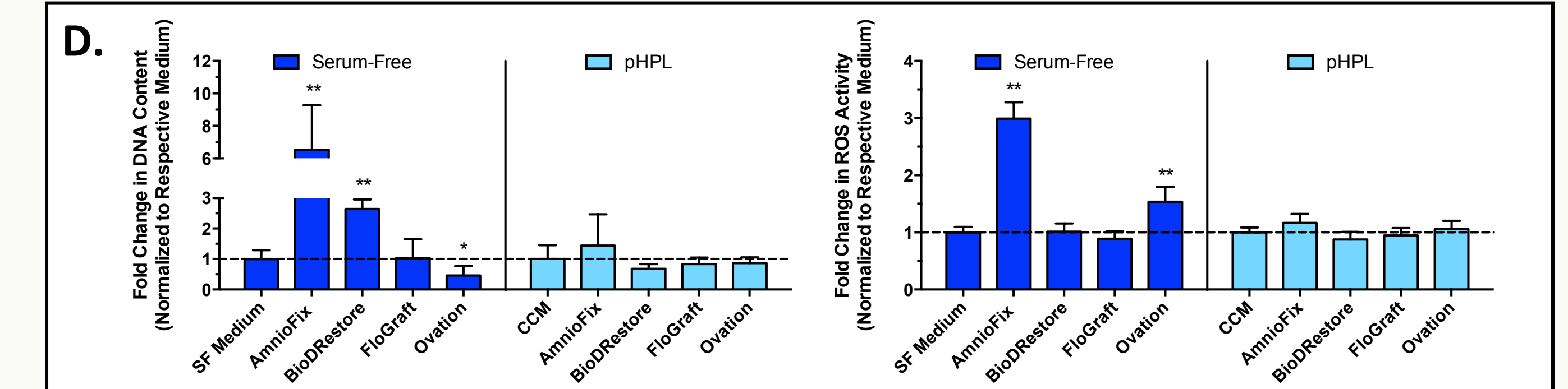
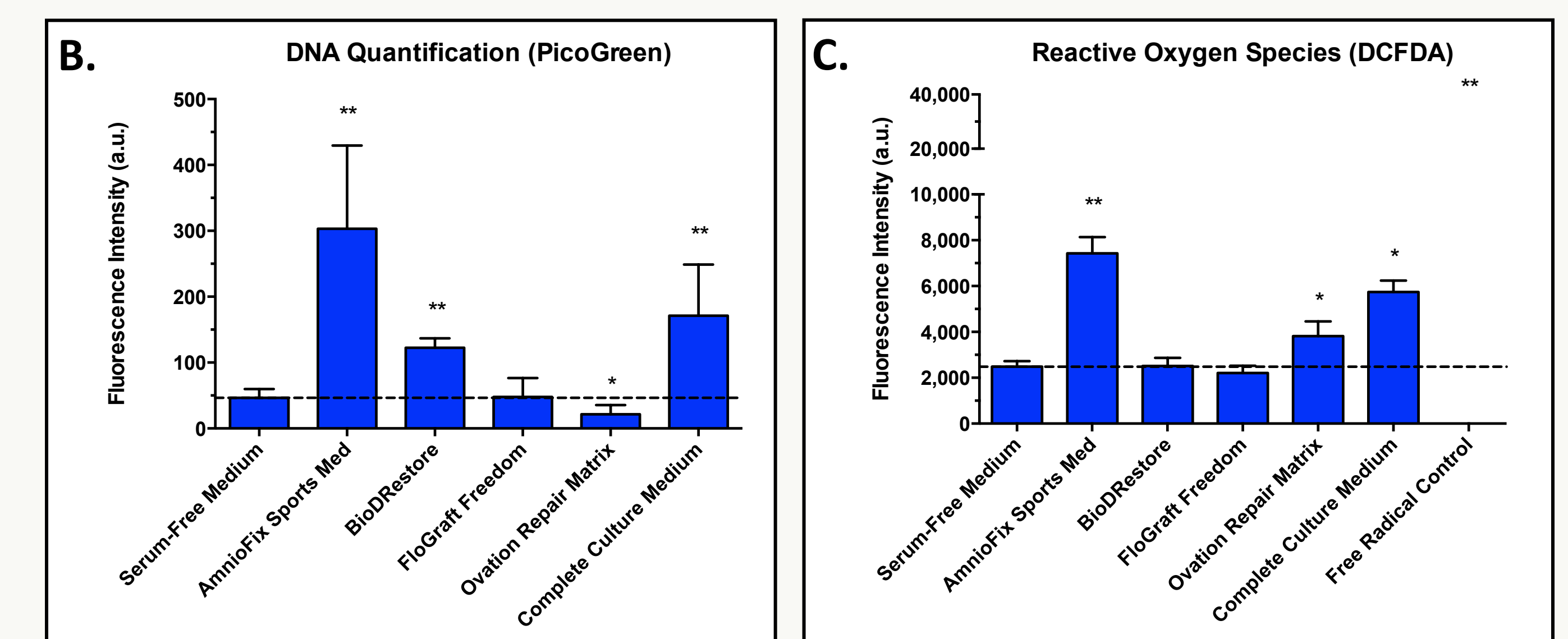
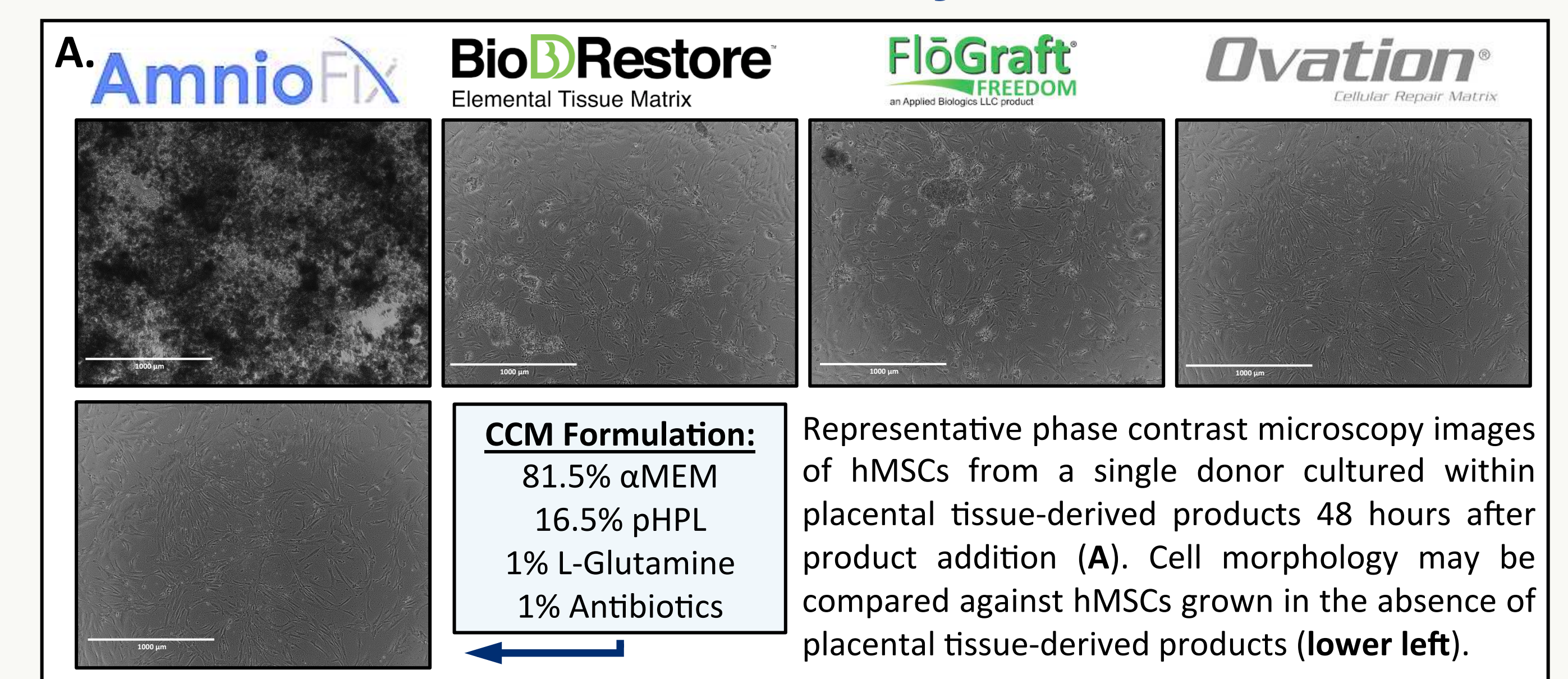
<http://discovery.lifeasp.com/regenerative-medicine/cell-therapy-applications/bone-ovation-mesenchymal-stem-cells-for-bone-repair>

Do Placental Tissue-Derived Products Contain Plastic-Adherent, Multipotent Stem Cells?



(A) Phase contrast microscopy images of placental tissue-derived products taken 48 hours following product plating. Representative images are indicative of the large variation in particulate content observed within the four tested products (upper row, 40X magnification) and the morphological characteristics of the attached elements (lower row, 200X magnification). Ovation Cellular Repair Matrix did contain one adhered element exhibiting typical cellular morphology*; however, no proliferation was observed (inset). (B) Moreover, forward scatter (size) vs. side scatter (granularity) plots from flow cytometry analyses of placental tissue-derived products show an absence of clearly defined cell populations. (C) Typical forward scatter vs. side scatter plots obtained from flow cytometry analyses of bone marrow mononuclear cells (BMSCs) and cultured hMSCs.

Can Placental Tissue-Derived Products Help the Culture of Human Mesenchymal Stem Cells?



(B) hMSC proliferation as assessed by double-stranded DNA Quantification via PicoGreen following 72 hours of culture. (C) The generation of reactive oxygen species as assessed by DCFDA oxidation following 24 hours of culture. (D) The effect of medium composition (serum-free vs pHPL containing medium) on hMSC proliferation and reactive oxygen species generation. Significant differences between experimental conditions were only observed when serum-free medium was used. (* P<0.05, ** P<0.001).

Materials and Methods

Product Plating: Placental tissue-derived products (AmnioFix Sports Med, BioDRestore, FloGraft Freedom, and Ovation Cellular Repair Matrix) were reconstituted or thawed following the supplied manufacturer's recommended protocols and diluted 1:10 (v/v) in **Complete Culture Medium (CCM)**. Products were plated in 24-well plates and were allowed 48 hours of incubation for cell attachment. Images were taken via phase contrast microscopy before and after washing wells with phosphate buffered saline (PBS). Attached particles were subsequently monitored for cellular outgrowth.

Flow Cytometry: Products were treated with 10% collagenase and hyaluronidase for two hours to help eliminate extracellular matrix proteins from the analysis. Following enzymatic digestion, the contents of each product were pelleted and resuspended in phosphate buffered saline. Forward scatter vs. side scatter plots were generated from 200,000 events collected using a BD Accuri C6 flow cytometer.

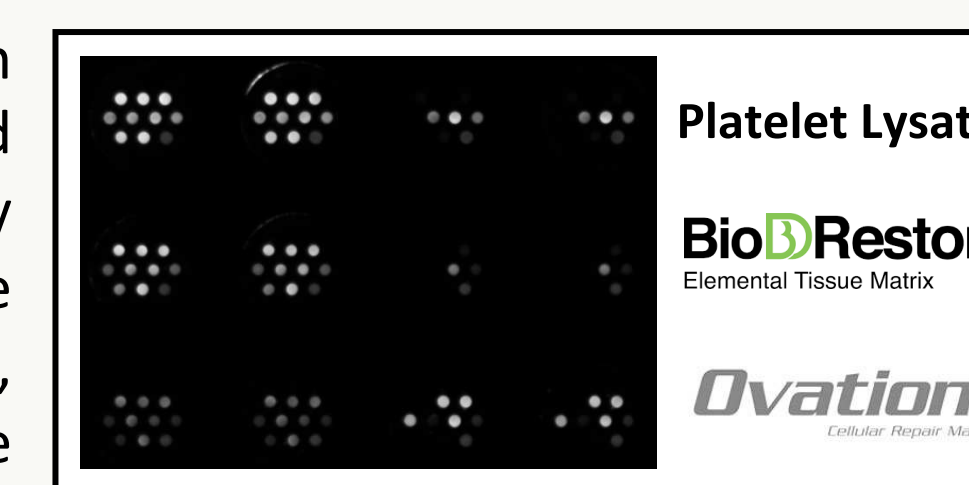
Cell Culture: Passage 2 bone marrow-derived human mesenchymal stem cells (hMSCs) from three donors, two females (ages 47 and 73) and one male (age 53), were plated in CCM within 96-well plates and were allowed 24 hours for cell attachment. Plating medium was aspirated, and placental tissue-derived products, diluted 1:10 (v/v) in CCM or Serum-Free medium, were added to the cultures. Quantification of reactive oxygen species (DCFDA) was performed 24 hours after introduction of the placental tissue-derived products, while quantification of DNA (PicoGreen) was performed after 72 hours.

Growth Factor Content of Two Placental Tissue-Derived Products Vs. Pooled Human Platelet Lysate

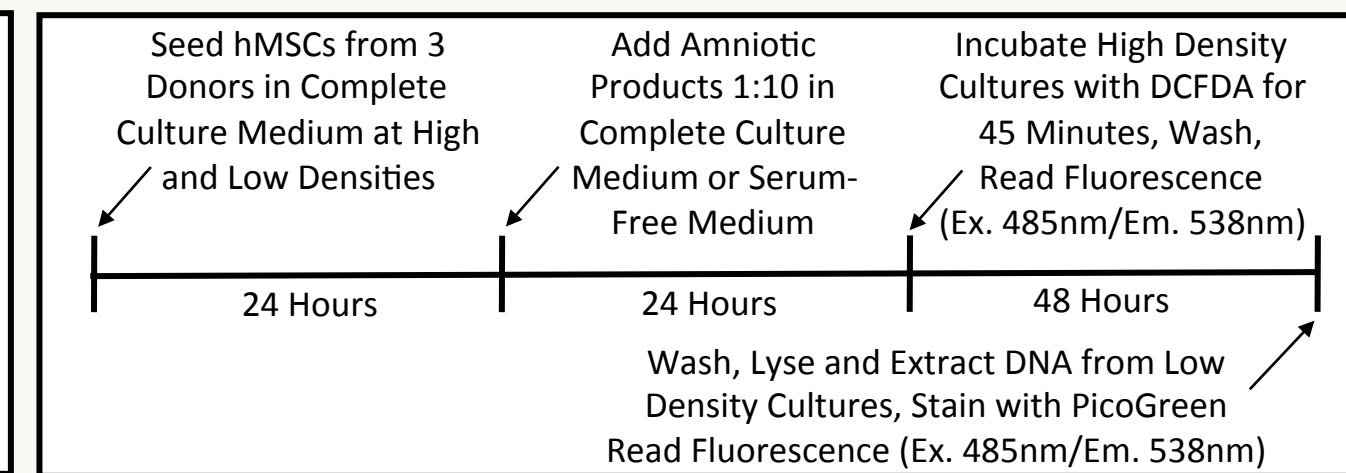
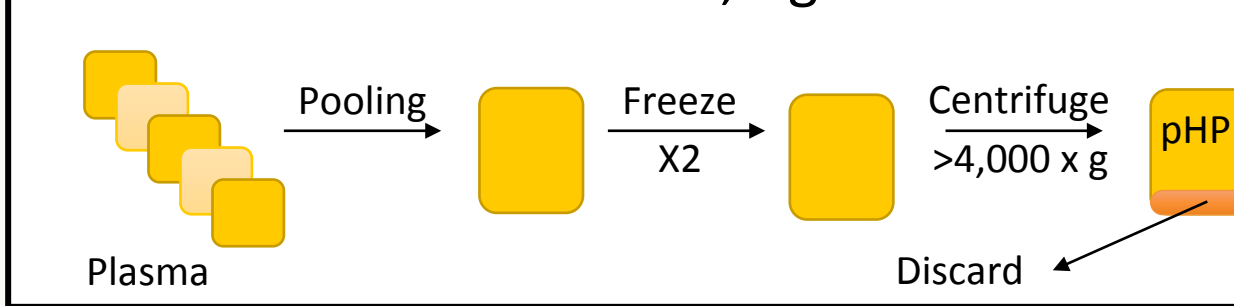
Growth Factor	BioDRestore* (pg/mL)	Ovation Matrix (pg/mL)	pHPL (pg/mL)
Angiopoietin-2 (Ang-2)	<13.3	<13.3	143.2
Fibroblast Growth Factor Basic (bFGF)	<61.4	2,880.1	<61.4
Hepatocyte Growth Factor (HGF)	476.2	8,681.3	197.8
Interleukin-8 (IL-8)	<9.3	>4,400.0	<9.3
Platelet Derived Growth Factor-BB (PDGF-BB)	<5.5	133.3	1,099.2
Tissue Inhibitor of Metalloproteinase-1 (TIMP-1)	4,533.2	>24,000.0	>24,000.0
Tissue Inhibitor of Metalloproteinase-2 (TIMP-2)	3,210.5	4,673.3	22,776.2
Transforming Growth Factor Beta (TGF β)	10,063.4	16,539.9	30,687.6
Vascular Endothelial Growth Factor (VEGF)	<3.0	11.4	56.0

*Note: BioDRestore was subjected to an additional freeze/thaw cycle prior to quantifying growth factors

Quantification of a subset of growth factors present in BioDRestore, Ovation Cellular Repair Matrix, and pooled human platelet lysate (pHPL) using a multiplexed ELISA array (Quansys Biosciences). pHPL had the highest detectable amounts for five of nine growth factors assessed (Ang-2, PDGF, TIMP-2, TGF β , and VEGF), while Ovation Matrix had the highest amounts in three of nine (bFGF, HGF, and IL-8).



Pooled Human Platelet Lysate (pHPL): Pooled from n = 5 Donors, Ages 26 – 68 Years



Significance and Conclusions

The placental tissue-derived products tested:

- Do not likely contain viable stem cells once they are thawed per the manufacturer's recommendations.
- Do likely contain some active growth factors that may have positive effects on hMSC proliferation (AmnioFix Sports Med and BioDRestore) in serum-free media conditions. However, when cultured with pHPL, there were no significant differences between any of the products tested.
- Do not generally help hMSCs show less oxidative stress. In fact, two tested products were shown to increase the presence of reactive oxygen species (AmnioFix Sports Med and Ovation Cellular Repair Matrix) when cultured in serum-free media conditions. Again, there were no significant differences between conditions when cultured with pHPL.

Placental Tissue-Derived Product	Adherent Cells Present?	Culture Expandable?	Increased Cellular Proliferation?	Increased ROS Levels?
AmnioFix Injectable	No	No	Yes	Yes
BioDRestore	No	No	Yes	No
FloGraft Freedom	No	No	No	No
Ovation Cellular Repair Matrix	Yes*	No	No	Yes