Clinical Overview and Description of Amniotic Fluid and OrthoFlo

Introduction:

The historical use of various clinical applications of amniotic membranes began relatively early in the 20th century. The first applications were in wound healing, followed by a large variety of other applications, including use in musculoskeletal conditions. More recently, applications have been described for using umbilical cord and various other combinations of birth tissue. The use of amniotic fluid as a treatment for orthopedic conditions was recorded as early as 1938.[1]

Description:

Amniotic fluid has a variety of homologous uses as *in utero* it naturally functions to protect and cushion, reduce inflammation and enhance mobility.[2,6] It is a dynamically changing biological fluid that changes in both volume and composition throughout the course of gestation and the fluid normally ranges from 25 mL at 10 weeks to about 400 mL at the time of delivery.[2]

Composition of amniotic fluid. Key elements of amniotic fluid include growth factors, carbohydrates, proteins, lipids, electrolytes, and other nutrients, as well as hyaluronic acid (HA), a principle component of viscosity and lubrication in synovial fluid.[2,3]

Functions of the amniotic fluid *in utero* include:[2]

- An extension of the fetal extracellular compartment.
- A connection between the intracoelemic and extracoelemic components of the developing infant.
- A physiologic buffer for various extra-fetal compounds.
- Modulation of fluid and electrolyte transport between the mother and fetus across fetal and placental membranes.
- Nutritional support of the fetus.
- Provision of a supportive fluid cushion to the developing fetus, allowing fetal movement and growth.
- Protective functions provided by the inclusion of multiple growth factors and biological molecules.
- Provide antimicrobial effectors that protect the fetus

Concentrations. Concentrations of the various composition of amniotic fluid vary over the course of gestation, frequently changing near the time of delivery. Actual measurements of concentrations have been done for some of the components as follows:

Sozanskii measured the concentrations of various compounds in pregnancy and compared serum and AF concentrations. He found that "in 136 women at various terms of pregnancy…the biochemical composition of amniotic fluid changed as follows: there was a rise of urea, rest nitrogen, and of the total protein; sugar concentration dropped; chloride level remained unchanged."[4]

Campbell did a similar measurement set and found comparable results. "Levels of sodium, potassium and bicarbonate were significantly higher in amniotic fluid whilst chloride, urea, bilirubin, protein, albumin, glucose, creatinine, calcium and phosphate were present in higher concentrations in extraembryonic coelomic fluid. All differences in concentration were significant (P less than 0.05; unpaired t-test). No relation was demonstrated between electrolyte concentrations in amniotic fluid or coelomic fluid and stage of gestation."[5]

Biological activity. Many of the above compounds and substances have well characterized biological activities. While the exact mechanism of activity is known for individual compounds, the subtle interrelationship of how these agents interact is continuously being researched. Further, various pathologic states have been associated with dysregulation of the amounts and concentrations of these materials.

Studies with Amniotic Fluid:

Burns, et al., looked at the concentration of various inflammatory cytokines in amniotic fluid:[6]
"*In this study, we examined matched samples of term maternal blood, cord blood, and amniotic fluid obtained from 24 elective cesarean deliveries for both pro- and anti-inflammatory cytokines thought to be important in maintaining a balanced response leading to successful pregnancy outcome. These included interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), IL-10, and IL-1 receptor antagonist (IL- 1ra). Amniotic fluid levels for each of the cytokines examined were significantly higher than those for cord blood or maternal plasma."*

Karacal, et al., have noted that human amniotic fluid may have a positive effect on bone healing in an experiment done with New Zealand white rabbits.[7]

Ozganel, et al., noted a salutary effect of human amniotic fluid on nerve healing in rats. *"Preliminary data showed that human amniotic fluid enhances peripheral nerve regeneration. The preventive effect of human amniotic fluid on epineural scarring and the rich content of neurotrophic and neurite-promoting factors possibly contribute to this result."*[8]

Shimberg in 1938 reported on a series of 68 cases in which amniotic fluid was used in the treatment of patients with joint disease for various orthopedic conditions.[1] His paper concluded the following:

- *In sixty-eight cases in which amniotic-fluid concentrate has been employed in the treatment of various pathological conditions of joints, the use of the fluid concentrate has not been attended by a single unfavorable reaction.*
- *Its action is probably both biological and mechanical.*

- *It speeds up a defense-repair mechanism within the joints.*
- *The results obtained have been impressive in intra-articular fractures, and encouraging in selected cases of atrophic arthritis, as well as in persistent joint effusions.*
- *It successfully prevents the formation of new adhesions after closed manipulation of joints.*
- *It is a valuable prophylactic after arthrotomy of any type.*
- *Its use both in soft tissues and in other serous cavities is suggested.*

Demesmin, in unpublished research presented in an open forum in June, 2015, reviewed an "Interim Analysis of Prospective, Multi-Center Outcome Observational Cohort Registry of Amniotic Fluid Treatment for Osteoarthritis of the Knee."[9] With the caveats that this is an open label, non-randomized, uncontrolled trial, and that the full explanation for the N analyzed at the various time points was not presented, the data suggest a potential positive effect of AmnioVisc™, an allograft amniotic fluid viscosupplement, on osteoarthritis of the knee.[9]

This effect was achieved with one dose, while the HA data compared were from a study in which 3-4 doses were given. VAS and WOMAC scores were measured in the study. The positive impact possibly persists out to 90 days. While this potential efficacy signal needs to be confirmed in a controlled trial, the results are encouraging.

Lastly, the safety profile as presented showed only 4 treatment-related adverse events (2.2%) were reported. This was noted as transient pain and tenderness which, in all cases, resolved within days with no treatment.

MiMedx® OrthoFlo:

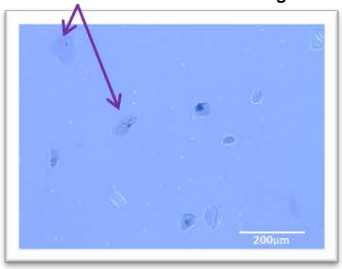
OrthoFlo contains an array of well–known regulatory proteins, growth factors, cytokines and chemokines that are naturally present in amniotic fluid as follows:[10]

A partial list of regulatory proteins, cytokines and chemokines in OrthoFlo	
Acronym	Name
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
CCL28	Chemokine (C-C motif) ligand 28
CXCL16	Chemokine (C-X-C motif) ligand 16
EGF	Epidermal growth factor
EG-VEGF	Endocrine gland-derived vascular endothelial growth factor
Eotaxin	Eotaxin
Eotaxin-2	Eotaxin-2
GDF-15	Growth differentiation factor 15
HCC-1	Chemokine (C-C motif) ligand 14
HGF	Hepatocyte growth factor
I-309	I-309 (a CC chemokine)
IGFBP-1	Insulin-like growth factor binding protein-1
IGFBP-2	Insulin-like growth factor binding protein-2
IGFBP-3	Insulin-like growth factor binding protein-3
IGFBP-4	Insulin-like growth factor binding protein-4
IGFBP-6	Insulin-like growth factor binding protein-6
IL-1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IL-8	Interleukin-8
MCP-1	Monocyte chemotactic protein-1
MCSF	Macrophage colony-stimulating factor
MIF	Macrophage inhibitory factor
OPG	Osteoprotegerin
OPN	Osteopontin
PARC	Pulmonary and activation-regulated chemokine
PDGF-AA	Platelet-derived growth factor-AA
PF4	Platelet factor 4
TGF-α	Transforming growth factor alpha
TGF-β1	Transforming growth factor beta 1
TIMP-1	Tissue inhibitor of metalloproteinase-1
TIMP-2	Tissue inhibitor of metalloproteinase-2

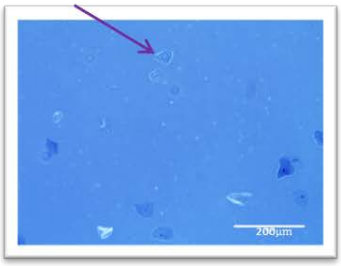
Comparison of purity, cell and particulate content in OrthoFlo vs other birth tissue fluids

OrthoFlo is a purified human amniotic fluid containing growth factors and live cells which are preserved as part of the processing. It should be noted that the product is not dependent upon these preserved cells as its primary function. There are no contaminating tissue fragments. Other amniotic fluid products contain live or dead cells plus tissue particulates of unknown origin. The light micrographs shown below illustrate the content in several other commercially available amniotic fluid products.

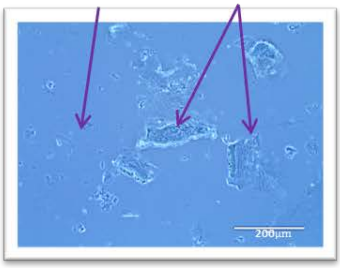
OrthoFlo
Purified human amniotic fluid
Viable cells **No tissue fragments**



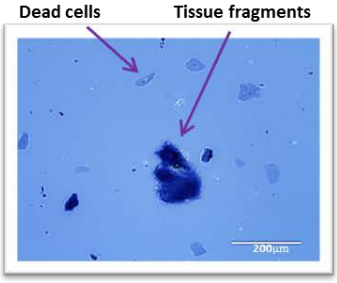
AmnioVisc™
Purified human amniotic fluid
Viable cells **No tissue fragments**



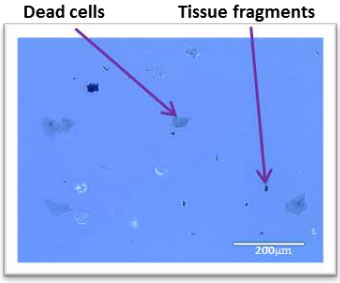
BioDFactor®
Human amniotic fluid with tissue
Few viable cells **Tissue fragments**



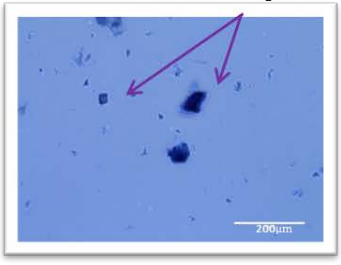
PalinGen® Flow
Human amniotic fluid with tissue
Dead cells **Tissue fragments**



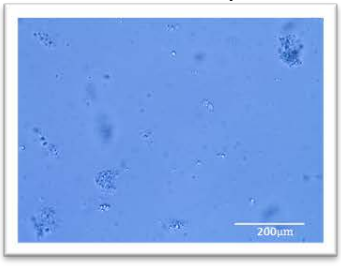
PalinGen® SportFlow
Human amniotic fluid with tissue
Dead cells **Tissue fragments**



FlowerFlo™
Human amniotic fluid with tissue
No visible cells **Tissue fragments**



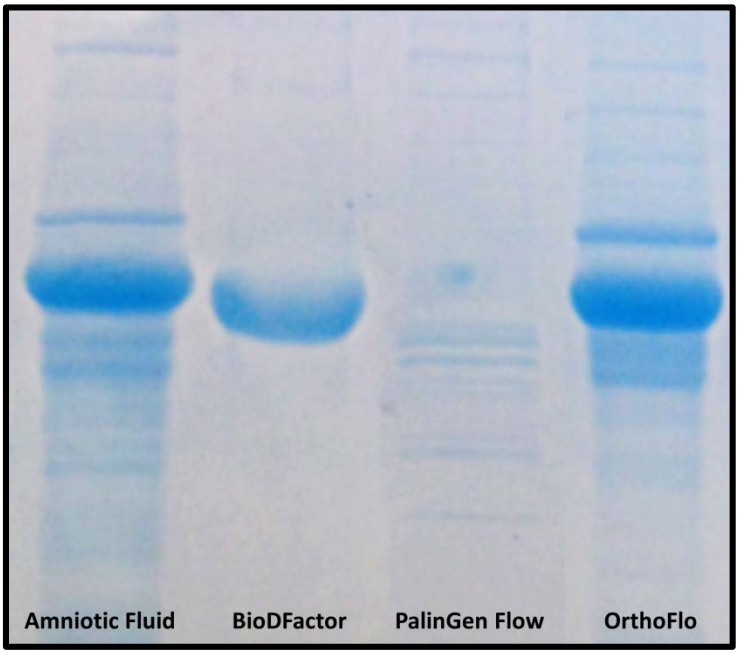
AlloGen®
Human amniotic fluid with tissue
No intact cells **Small particulate**



Comparison of protein composition of OrthoFlo vs. other birth tissue fluids

OrthoFlo is purified human amniotic fluid containing growth factors, cytokines, and other various regulatory proteins. The SDS-PAGE gel below illustrates the composition and relative abundance of proteins, separated by molecular weight, for OrthoFlo and other commercially available products, compared to fresh amniotic fluid. The protein composition of OrthoFlo (4th column) appears very similar to fresh amniotic fluid (1st column) with a nearly identical pattern of protein bands. Other amniotic fluid products do not possess the protein composition of fresh amniotic fluid, suggesting that they are either not amniotic fluid derived or are highly processed to remove essential amniotic fluid components.

Protein composition of OrthoFlo and other amniotic fluid products, compared to fresh amniotic fluid



OrthoFlo Information and Application

- OrthoFlo is a human amniotic fluid derived allograft, donated by mothers delivering healthy babies by scheduled Caesarean section
- OrthoFlo is intended for single use only
- OrthoFlo may be administered as an injection by an authorized medical professional
- OrthoFlo should be maintained at -80 ˚C or colder until ready for use
- Refer to “Instructions for Use” enclosed within the product packaging for full application instructions

OrthoFlo is procured and processed in the United States according to standards and/or regulations established by the American Association of Tissue Banks (AATB) and the United States Food & Drug Administration (FDA).

Product Offering

OrthoFlo is available in several product sizes:

Item Number	Size
LQ-0050	0.5 mL
LQ-0100	1 mL
LQ-0200	2 mL

References

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9. Demesmin D. Interim Analysis of Prospective, Multicenter Outcome Observational Cohort Registry of Amniotic Fluid Treatment for Osteoarthritis of the Knee. TOBI Orthobiologic Institute Conference, June 13, 2015.
10. Data on file at MiMedx.

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