AmDrop™
Regenerative Therapy

Memo: Evaluation of Sterilized Amniotic Fluid Samples

Introduction

The purpose of this study was to evaluate the growth factor content present within amniotic fluid samples provided by Bone Bank Allografts (BBA). These fluid samples consist of Amniotic fluid that has been sterile filtered, sterilized, and then stored at ambient temperature.

Methods

Growth Factor and Cytokine Analysis

Growth factor and cytokine content from ambient sterile fluid samples from 3 donors was determined through the H18-1/2 S3 Raybiotech custom proteomics microarray kit according to the manufacturer's instructions (RayBiotech, Narcross GA). Each sample was assessed in duplicate, requiring 100 µL per well within the array.

Angiogenic Growth Factors	**Regenerative Growth Factors**	**Immune Modulating Cytokines**	**Osteogenic & Chondrogenic Growth Factors**
Acidic Fibroblast Growth Factor (aFGF)	Adiponectin (APN)	Tissue Necrosis Factor Alpha (TNFα)	Bone Morphogenetic Protein 6 (BMP-6)
Angiopoietin (ANG)	Epidermal Growth Factor (EGF)	Interleukin-4 (IL-4)	Bone Morphogenetic Protein 7 (BMP-7)
Angiopoietin-2 (ANG-2)	Galectin-7 (GAL)	Interleukin-6 (IL-6)	Fetuin A (FET)
Basic Fibroblast Growth Factor (bFGF)	Hepatocyte Growth Factor (HGF)	Interleukin-8 (IL-8)	Osteoprotegerin (OPG)
Endocrine gland-derived vascular endothelial growth factor (EG-VEGF)	Insulin-like growth factor-binding protein 1 (IGFBP-1)	Interleukin-1 family member 5/ Interleukin 36 receptor antagonist (IL-1F5)	*Osteopontin* (OPN)
Platelet Derived Growth Factor AA (PDGF-AA)	Insulin-like growth factor-binding protein 5 (IGFBP-5)	Interleukin 1 receptor antagonist (IL-1ra)	
Platelet Derived Growth Factor AA	Insulin-like Growth Factor-1 (IGF-I)	Interleukin 10 (IL-10)	

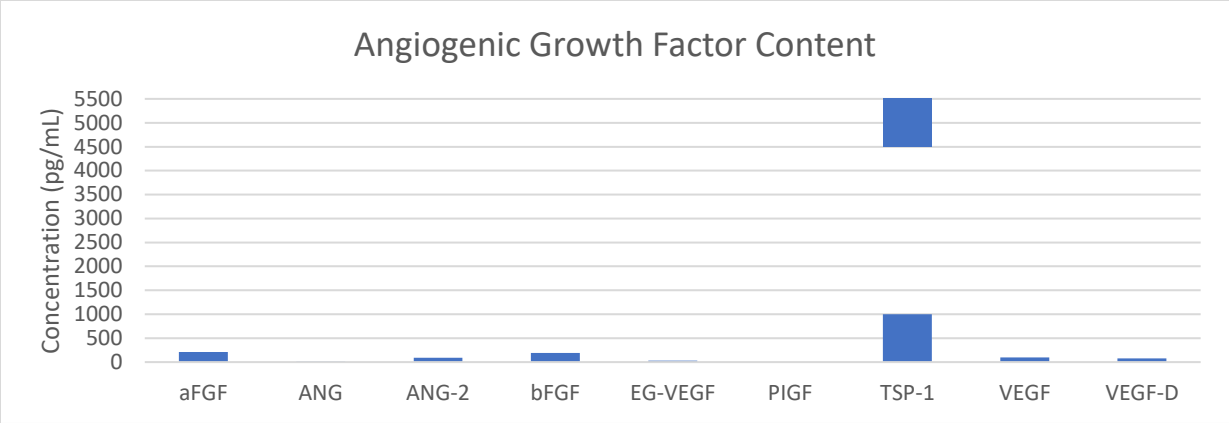
(PDGF-BB)			
Placenta Growth Factor (PlGF)	Insulin-like Growth Factor-2 (IGF-II)	Tissue Inhibitor of Metalloproteinase 1 (TIMP-1)	
stromal cell-derived factor 1 (SDF-1)	Transforming growth factor alpha (TGF-α)	Tissue Inhibitor of Metalloproteinase 2 (TIMP-2)	
Thrombospondin 1 (TSP-1)	Transforming growth factor beta 1 (TGF-β1)	Tissue Inhibitor of Metalloproteinase 4 (TIMP-4)	
Vascular Endothelial Growth Factor (VEGF)	Transforming growth factor beta 1 (TGF-β3)		
Vascular Endothelial Growth Factor D (VEGF-D)			
Angiopoietin-like 4 (APL4)			

Table 1: Description of growth factor categories.

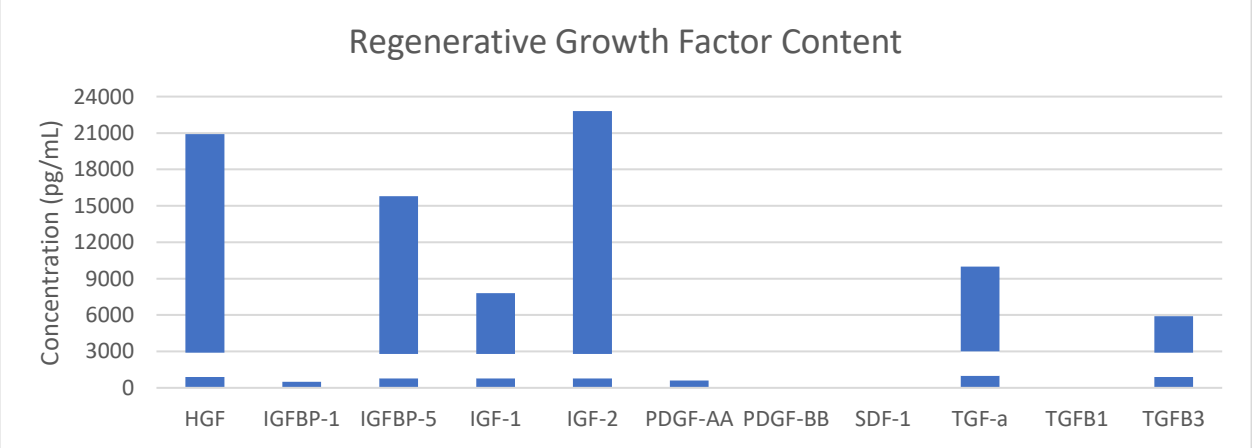
Results

Proteomic evaluation of fluid samples revealed high concentrations of multiple growth factors including TSP-1, HGF, IGFBP-5, IGF-1/2, TGFa, and TGF-β3, IL-1F5, TIMP-2, BMP-7, Fetuin A, and OPN (shown below).

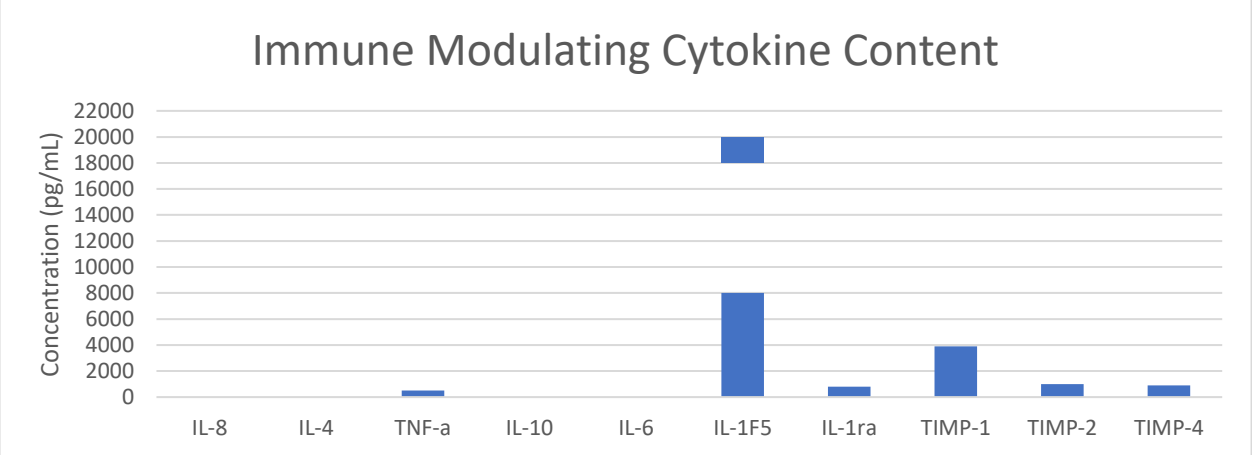
A


Angiogenic Growth Factor Content
Concentration (pg/mL)
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
aFGF
ANG
ANG-2
bFGF
EG-VEGF
PIGF
TSP-1
VEGF
VEGF-D

B


Regenerative Growth Factor Content
Concentration (pg/mL)
24000
21000
18000
15000
12000
9000
6000
3000
0
HGF
IGFBP-1
IGFBP-5
IGF-1
IGF-2
PDGF-AA
PDGF-BB
SDF-1
TGF-a
TGFB1
TGFB3

C


Immune Modulating Cytokine Content
Concentration (pg/mL)
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
IL-8
IL-4
TNF-a
IL-10
IL-6
IL-1F5
IL-1ra
TIMP-1
TIMP-2
TIMP-4

D

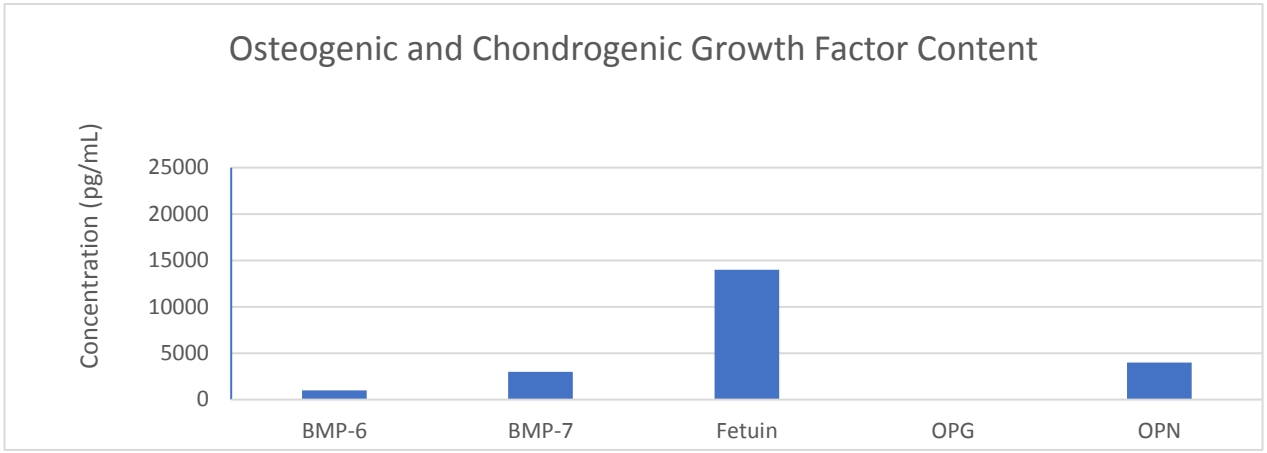


Figure 1: Multiplex ELISA proteomics microarray measuring growth factors and cytokines present within sterile fluid samples: A) angiogenic growth factors, B) regenerative growth factors, C) immune modulating cytokines, D) osteogenic and chondrogenic growth factors. For all growth factors and cytokines, results are shown as average ± standard deviation (n=6)

Conclusions

These results shown that amniotic fluid contains high levels of several growth factors, and that this growth factor content may be maintained for a period of time following sterilization and room temperature storage. As these samples were evaluated quickly after production and commercially a shelf life would be necessary. Further studies should be conducted to determine the bioactivity of the growth factors identified within these fluid samples and the effect of shelf life on growth factor content/bioactivity.

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