Product Characterization Report: AmnioShot™

Cytokines and growth factors contribute to the effects that amniotic fluid have on promoting the healing process.

Key Terms

Amniotic Fluid
Cytokines
Growth Factors
Proteins
Mesenchymal Stem Cells (MSC)

ABSTRACT

Amniotic fluid (AF) possesses anti-inflammatory, anti-microbial and regenerative properties that make it attractive for use in clinical applications (Pierce et al. 2016).

INTRODUCTION

Early after conception and until the mother's water breaks for the delivery of their infant, the fetus is bathed in amniotic fluid (AF). AF functions as a supportive cushion to the fetus and provides a protective environment. AF is a rich source of nutrients, cytokines and growth factors that are required for fetal development and maturation (Underwood et al. 2005). AF also contains stem cells with the potential to differentiate along multiple cell lineages (In't Anker et al. 2003; Prusa et al. 2003; Bottai et al. 2012). The protective and regenerative properties of AF are achieved via the exchange of water and solutes with surrounding tissues. This is accomplished via the utilization of different pathways during the course of a pregnancy that likely contribute to changes in the composition of the AF with gestational age (Underwood et al. 2005).

BACKGROUND

Among some of the first evidence that AF has protective biological properties is a report describing that concentrates of AF inhibit the development of peritonitis (Johnson et al. 1936). This is followed by a report by Shimberg and co-workers that AF accelerates defense-repair mechanisms within damaged joints (Johnson et al. 1936; Shimberg 1938). Since these early publications, more sophisticated evaluations have revealed the presence of antimicrobial, immunomodulatory, and growth-promoting activities in AF (Underwood et al. 2005). Reports of antimicrobial activity in AF differ (Ismail et al. 1989) among investigators. Some studies show that AF is inhibitory, while others show no effect against the same microorganisms. Components with antimicrobial, antiviral and anti-fungal activity that are present in AF include lysozyme, peroxidase, transferrin, b -lysin, immunoglobulins and zinc-peptide complexes (Ismail et al. 1989). Immunomodulatory properties of AF are evident from studies showing that enteral feeding of AF suppresses the pro-inflammatory responses in preterm pigs with necrotizing enterocolitis (Siggers et al. 2013). While growth promoting activities of AF are supported by animal studies and by in vitro culture studies showing that AF can enhance neochondrogenesis (Ozgenel et al. 2004), regenerate peripheral nerves (Ozgenel and Filiz 2003) and bone (Karacal et al. 2005), accelerate reepithelialization in corneas (Castro-Combs et al. 2008), and promote healing of human skin wounds (Nyman et al. 2013). Some of the factors that are found in AF that may contribute to these activities include inflammatory mediators, but are not limited to TNF-a, IL-6, IL8, and IL-10 (Weissenbacher et al. 2012), trophic factors that include EGF, IGF-1, FGF, HGF and TGF-a (Merimee et al. 1984; Watanabe 1990; Lang and Searle 1994; Kurauchi et al.

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1995; Hirai et al. 2002), and HA, an important factor in promoting reepithelialization in human skin wounds (Nyman et al. 2013).

The observations presented in the data and supporting literature support the conclusion that the cytokines and growth factors found in AmnioShot™ are likely to contribute to the effects that amniotic fluid have on promoting the healing process (Shimberg 1938; Colombo et al. 1993; Ozgenel et al. 2001, 2004; Castro-Combs et al. 2008; Ghaderi et al. 2011; Nyman et al. 2013; Feizi et al. 2014).

METHODS & RESULTS

Amniotic Fluid Collection

Human AF is collected from consenting mothers (donors) who deliver live-birth C-sections within a sterile operating room. A physician typically performs an abdominal fenistil incision through the abdominal and uterine muscles without cutting into the amnion membrane. Using a sterile soft suction catheter connected to a sterile MediVac suction container, a blunt end insertion with a catheter is made into the amnion membrane and the AF is aseptically suctioned into the container. The container is then labelled, wrapped in frozen Insul-ice mats and placed in a temperature monitored shipping container that is validated for transport between 2° C and 8° C. Upon arrival at the manufacturing facility, the product is immediately placed in a refrigerator until processing occurs.

Regulatory and Processing

The AmnioShot™ product is manufactured by the University of Utah Cell Therapy and Regenerative Medicine (Reference Exhibit B). It is classified as a human tissue allograft (HCT/P) and meets the requirements for "Homologous Use" as outlined in 21 CFR 1271 under Section 361 of the Public Health Service Act. The sterile filtered amniotic fluid has no live cells, cell debris, hair, vernix or other debris, however it does contain human proteins, hyaluronic acid, cytokines, proteins and chemokines which are known to promote healing and protect human cells and tissues.

AmnioShot™ is sterile filtered and tested under USP 71 guidelines. Each manufactured lot is tested to ensure there is no evidence of maternal blood contamination and that maternal blood samples test negative for infectious disease agents (i.e. human immunodeficiency virus, hepatitis B virus, Hepatitis C Virus, Syphilis).

Accreditations

AmnioShot™ is manufactured by the University of Utah Cell Therapy and Regenerative Medicine and is registered with the FDA (FEI 3004471729) and accredited or certified by the American Association of Tissue Banks (AATB), Foundation for Accredited Cellular Therapy, Clinical Laboratory Improvement Act (CLIA) and College of Pathologists (CAP).

Cell Characterizations & Viability

3rd party testing - A sample of AmnioShot™ was tested by an independent third party, Franciscan Institute for Science and Health, for concentration of exosomes found within the product. The exosomes were isolated using an SBI ExoQuick-TC product and were quantified using the SBI FluorCet Exosome Quantification kit. The results found over a billion exosomes within the tested 1mL samples (Reference Exhibit A).

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Figure 1 (From Exhibit A: Product Analysis Report)

Amniotic Fluid Product Analysis	Sample: 1	AmnioShot
Concentration of Exosomes (done in duplicate):		
1) 1.17 x 10^8/ml		
2) 1.23 x 10^8/ml		
Average Exosome Concentration: 1.20 x 10^8/ml		
Notes: Exosomes were isolated using SBI ExoQuick-TC product. Exosomes were quantified using the SBI FluorCet Exosome Quantification kit.		

DISCUSSION

The presented research, combined with the product characteristics data, identified within this report, demonstrate that nutrients, cytokines and growth factors contained in the non-cellular fraction of AF are useful for reparative and regenerative treatments in patients.

MANUFACTURER

AmnioShot™ is manufactured by the University of Utah Cell Therapy and Regenerative Medicine and is registered with the FDA (FEI 3004471729) and accredited or certified by the American Association of Tissue Banks (AATB), Foundation for Accredited Cellular Therapy, Clinical Laboratory Improvement Act (CLIA) and College of Pathologists (CAP).

DISTRIBUTOR

AmnioShot™ is distributed by Orca Biotech, LLC, located at: 126 Sego Lily Drive, #195, Sandy, UT 84070.

DISCALIMER

This product is exclusively intended for homologous use in humans. It may not be transferred to third parties, resold, modified or mixed for resale or used to manufacture commercial products for resale without written authorization from. There is no claim that treatment using this product is a cure for any condition, disease or injury.

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Exhibit A

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Franciscan Regenerative

Amniotic Fluid Product Analysis	Sample: 1	AmnioShot
Concentration of Exosomes (done in duplicate):		
1) 1.17 x 10^8/ml		
2) 1.23 x 10^8/ml		
Average Exosome Concentration: 1.20 x 10^8/ml		
Notes: Exosomes were isolated using SBI ExoQuick-TC product. Exosomes were quantified using the SBI FluorCet Exosome Quantification kit.		

Date: 12-21-18

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