

# What Are The Active Components In Hilltop Bio's Products & How Do They Work?

Daniel J. Dreyfuss, DVM, MA

## Introduction

*What are the factors in orthobiologics that enable them to aid in the healing process of so many different tissues? Where do these factors come from? How exactly do they work?*

The short answer to the first question could be: “There are many factors in orthobiologic products that give them the ability to aid tissue healing.” Many biological tissues—birth tissues, platelet rich plasma (PRP), stem cells from various sites— can be the source of stem cells, extracellular vesicles (EV), and other mediators that may have widespread clinical application in the field of regenerative medicine. The honest answer to the last question is: “We don’t specifically know.”

Hilltop Bio has done extensive work assessing the proteomic constituents of the birth tissues used to source their products. Using cytokine analysis, and other characterization methods, Hilltop Bio has gained an understanding of how to optimize their products for veterinary applications. There are a few shared characteristics across the groups of factors found in amnion and other birth tissues. Many of the effects attributed to birth tissue-derived products come from the ability of the various components to exert paracrine effects— cell signaling or cellular communication that occurs when signaling molecules induce changes in nearby cells— and thus these factors stimulate the cells to undergo the reparative process. The effects of these factors are also pleiotropic— they can affect many different cell types and can exert different effects on different cell types or even different effects on the same cell type under differing conditions. These characteristics result in a tremendous range of potential effects, and a great deal of complexity in determining a “singular” mechanism or explanation by which they accomplish these effects.

The goal of this paper is to provide a brief overview of the diverse groups of mediators produced by birth tissues, and specifically selected by Hilltop Bio, which make their products capable of activating the intrinsic reparative processes of damaged tissues. By using specific characterization methods, Hilltop Bio can better identify the factors included in their products: and, just as importantly, the factors excluded from their products. As opposed to most PRP, stem cell, and conditioned serum derived products, the ability to influence the composition of their products sets Hilltop Bio apart from other orthobiologics. Over the next few months, we will select several different individual factors— some more familiar, others less familiar to veterinarians— and highlight their unique characteristics in more depth.

## Cytokines

Cytokines are small, non-structural proteins, or glycoproteins, < 30 kDa in weight (< 200 amino acids in length), which serve as intercellular signaling messengers. All the interleukins (IL), interferons (IFN), tumor necrosis factor (TNF), some growth factors, and several factors involved in hematopoiesis are all cytokines. Cytokines affect nearly every biological process.<sup>1</sup> Following release into the intercellular environment, cytokines exert their biological effects via binding to specific receptors on the membranes of target cells, causing intracellular signaling cascades to activate target cell genes. A hallmark of cytokines is their pleiotropism (Greek for “many effects”), one cytokine may exert both beneficial and deleterious effects simultaneously depending on its circulating concentration or which cells are targeted, among other variables.<sup>2,3</sup>

Interleukins are commonly mentioned when discussing the “active” molecules in biologically based products. Interleukin-1 (IL-1) and IL-1ra are a potent pro-inflammatory cytokine and its receptor antagonist that have been extensively studied. Tumor necrosis factor alpha (TNF $\alpha$ ) is another pro-inflammatory cytokine, commonly targeted by therapeutics to negate its effects in disease processes that have a significant inflammatory component. IL-10 is an anti-inflammatory cytokine that exerts its effects by inhibiting inflammatory genes, repressing inflammatory cytokines such as IL-1 and TNF $\alpha$ , increasing release of IL-1ra, and decreasing release of matrix metalloproteinase enzymes.<sup>4</sup>

## Growth Factors

Growth factors are any of a group of proteins that stimulate the growth of specific tissues, during both development and healing.<sup>5</sup> Growth factors play a significant role in promoting cellular differentiation and cell division and are produced by many different tissues. As seen with cytokines, the activity of growth factors is pleiotropic, so that one factor may have multiple, and even opposing effects, depending on the target tissue, the concentration, or the local environment.<sup>6</sup>

Amongst the growth factors known to be present in birth tissue-derived formulations are vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF). These, and other growth factors, are potent regulators of cellular function, including proliferation, migration, differentiation, and survival/apoptosis. Growth factor stimulation of cells is a complex and multistep action that transmits binding to a cell surface receptor into the target cell by using various signaling cascades.<sup>7</sup>

## Extracellular Vesicles

Extracellular vesicles (EVs) are membrane bound vesicles— tiny sacs— that are released by many different cells that contain cell signaling cargo that is exchanged between cells. EVs can be divided into ectosomes that branch off the exterior membrane of the cell and exosomes that originate inside the cells.

Exosomes contain a wide range of bioactive molecules such as nucleic acids, lipids, and proteins. These vesicles participate in intercellular communication and regulate various intracellular biological functions. The cell signaling molecules that they can carry between cells— cytokines, growth factors and other proteins, as well as messenger RNA (mRNA) and micro-RNA (miRNA)—can mediate many cellular functions. As we have seen with cytokines and growth factors, the effects are pleiotropic— they can have varied effects depending on the target tissue and other variables. It is widely accepted that the beneficial effects of mesenchymal stem cells are the result of the secretome they release, in large part extracellular vesicles.<sup>8,9,10</sup>

## Tissue Inhibitors of Metalloproteinases (TIMPs)

Tissue inhibitors of metalloproteinases (TIMPs) are a group of four endogenous inhibitors of metalloproteinases and are consequently important regulators of extracellular matrix turnover, tissue remodeling and cellular behavior.<sup>11</sup> TIMPs play a significant role in several biological activities including the modulation of cell growth and differentiation, and angiogenesis. These functions are an integral part of normal tissue remodeling and wound healing.<sup>12</sup> TIMPs (specifically TIMP-1) demonstrate cytokine-like behavior, may have anti-inflammatory effects, and consequently attenuate the development of inflammatory pain.<sup>13</sup>

## Summary

Amnion and amniotic fluid derived products rely on the wide array of cytokines, growth factors, extracellular vesicles, and TIMPs produced by amniotic cells acting in concert to provide a range of beneficial clinical effects as a means to augment tissue healing.<sup>14</sup> This multi-faceted approach to tissue repair results in the multiple characteristics of birth tissue-derived products that make them amenable to clinical applications. They have demonstrated the ability to reduce pain, scarring and inflammation, modulate angiogenesis, enhance tissue healing, possess antimicrobial properties, and the extracellular matrix can serve as a scaffold for cell proliferation and differentiation.<sup>15,16,17</sup>

## Summary cont'd.

The clinical effects seen following the use of birth tissue-derived products result not from the activity of one factor acting alone, but from the collaboration of the many factors. The results arise from the amniotic cell's ability to produce growth factors, cytokines, anti-bacterial peptides, and anti-inflammatory agents required for the reparative process.<sup>15</sup> Additionally, these tissues exhibit low immunogenicity<sup>15</sup>, allowing for the use of allogeneic tissue sources.

Hilltop Bio has performed extensive proteome analysis to identify and optimize the cytokines, growth factors, and extracellular vesicles in their birth tissue derived products. By then using a lyophilization technique for storage, the product is consistent, requires no processing, and is readily available for immediate off-the-shelf use.

## References

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