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# PHARMACEUTICAL POTENTIALS FOR PHOSVITIN AND PHOSVITIN PHOSPHOPEPTIDES

## OPPORTUNITIES FOR FURTHER RESEARCH

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## Executive Summary

**Ecovatec's revolutionary technology has unlocked the amazing potential of phosvitin (PV) and phosvitin phosphopeptides (PVP)**. Ecovatec is the only known commercial producer of phosvitin in the world. Phosvitin is isolated using EcovaPure™ technology from commercial egg yolk and is sold as both native phosvitin or PVP, the bioactive peptide form of PV.

Both PV and PVP have been characterized by many scientific papers, and the literature consensus is that they have huge potential as bioactive molecules. Bioactivity is based on the ability of a compound to be "able to exert a biological effect at a physiological level, and it must be measurable and able to provide health benefits."[1]

The scientific community has done extensive research on the uses of PV and PVP but have not been able to perform clinical trials due to the difficulty and expenses of isolating phosvitin. *Samaraweera, et al* (2011)[2] and *Ren, et al* (2015)[4] both noted that the potential use of phosvitin (and PVP) as a functional ingredient is only hampered by lack of a scalable method of extraction. **Ecovatec Solutions Inc has eliminated this barrier for researchers in the biomedical, nutraceutical, and food industries.**

## Background

Phosvitin is the *most highly phosphorylated protein found in* nature and is derived from the egg yolks of vertebrates[2]. Proteins are made up of peptides, which are short chains of amino acids linked by peptide bonds. Bioactive peptides have shown the most promising potentials as therapeutic or health promoting agents[2].

Serine is the major amino acid present in phosvitin (50%)[1], and almost all the serine residues are phosphorylated. This phosphorylation gives phosvitin its unique properties, including its strong **antioxidant, antiviral, antibacterial, anticancer, and metal chelating** properties.[1-4] While full-protein phosvitin exhibits some of these properties, it is often only when it is hydrolyzed into smaller peptides (PVP) that it fully realizes its bioactive potential. EcovaPure™ processing isolates native "whole" phosvitin which, in turn, allows for commercial production of high-quality PVP. Both products can be purchased from Ecovatec Solutions Inc.

## Areas for Pharmaceutical Research into Clinical Applications for PV and PVP

### Inhibiting Melanogenesis

In 2012, Jung *et al.*[5] investigated phosvitin's ability to inhibit melanogenesis in melanoma cells. They noted that several compounds that were excellent metal chelators were also melanogenesis inhibitors. B16F10 melanoma cells were cultured in 96-well plates and then varying concentrations of phosvitin was added to the wells, with distilled water as the control.

A variety of tests were performed, and it was shown that phosvitin inhibited tyrosinase activity (the enzyme necessary for melanogenesis), though not as well as ascorbic acid (which has side effects when used on the skin). Melanoma cell viability decreased by 20% in the cells exposed to 1000 µg/ml phosvitin. There was also a 17% reduction in cellular melanin synthesis with 50µg/ml.

Phosvitin also inhibited other melanogenesis-related enzymes such as tyrosinase-related protein 1 and 2 (TRP-1 and TRP-2), and microphthalamia-associated transcription factor (MITF) which lead to a 39% reduction in cyclic adenosine monophosphate (cAMP) in the melanoma cells with just a 50 µg/ml dose.

The researchers suggested two ways the phosvitin might inhibit the tyrosinase activity is due to its hydrophobic-rich N and C terminals or due to its metal chelating properties. If the inhibition is due to the metal binding abilities of PV, the PVP derived from phosvitin might have the same or an enhanced effect on melanogenesis while also reducing oxidative stress on skin cells which lead to the appearance of ageing. This indicates a **research opportunity to investigate the use of PVP in skin creams and lotions** for its anti-aging effects and to reduce the appearance of age spots, freckles, and moles. Clinically, there is also a research opportunity to investigate phosvitin as a potential adjuvant therapy (treatment) for melanoma cancer.

### Antibiotics for Multi-Drug Resistant Bacteria and Sepsis

Antimicrobial peptides are being investigated by researchers, as they can kill microorganisms directly by disrupting their cell functions in **multiple pathways**, making drug resistance unlikely[6]. *Li et al* (2016)[6] have found that a specific phosvitin-derived peptide which was tested on 5 strains of **multi-drug resistant bacteria** and found to affect multiple cellular processes to lead to **bacteria cell death**. The growth of all 5 strains were inhibited in a dose-dependent manner causing cell death through 3 cellular processes: the membrane of the cells underwent a shift in electric charge distribution (making the membrane not function properly), intracellular reactive oxygen species

(ROS) levels increased, and the peptide bound directly to the membrane causing permeabilization. They note its promising therapeutic potential.

Sepsis or septic shock remains a serious disorder associated with high morbidity and over 50% mortality in critically ill patients. Sepsis often results in life-threatening attack on organs. Gram-negative bacterial infection and their toxic cell wall component lipopolysaccharides (LPSs) or endotoxin are the most-often cited cause of septic shock.

There is no current therapy to protect from endotoxin-mediated tissue damage and organ failure. Current treatment methods still have **over 50% mortality**.[7] *Ma et al* (2012)[7] note that antimicrobial peptides (AMPs) are the ideal candidates for future therapies as they have the potential to bind pathogen ligands such as LPS on endotoxin cells and prevent it from binding to body cells that cause the inflammation. When compared to an antibiotic called polymixin B (PMB), native **phosvitin was not only effective to kill the bacteria, but it showed no cell toxicity to human cells**. The other drug studied, PMB, was slightly more effective at reducing mortality in mice models, but it is severely toxic to human blood cells**.** The researchers concluded that hen-derived phosvitin is "an endotoxin neutralizing agent with therapeutic potential in clinical treatment of LPS-induced sepsis" as it is directly microbicidal, and binds to pathogen ligands such as LPS, lipoteichoic acid (LTA), and peptidoglycan (PGN). *Hu et al* (2013)[8] further corroborated these findings.

There is huge research potential here **to optimize the use of hen egg derived phosvitin to reduce the sepsis mortality rate.**

## Cancer Treatment Therapeutic Potential

*Moon et al* (2014)[9] investigated the effects of phosvitin on various **cancer cell lines** *in vitro*. For **cervical cancer, PVP was 95% effective** at killing the cancer cells. Phosvitin slowed the growth of breast cancer cells and inhibited the growth of stomach cancer cells by 94%. The effects of phosvitin were most pronounced in killing liver cancer cells. The effect of phosvitin on stomach cancer is also very encouraging especially in Asian countries where the incidence of the disease is very high. The researchers concluded that these results indicate phosvitin has antigenotoxicity and cytotoxic activity against several human cancer cell lines and **could be researched further as an adjuvant therapy for the types of cancer mentioned above (anticancer agent).**

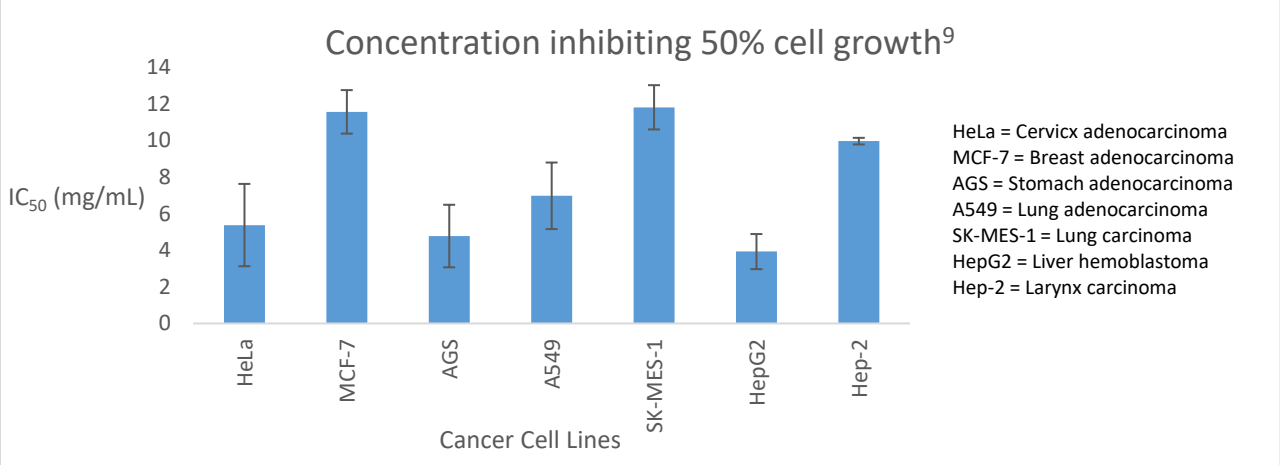


*Figure 1. Concentration of phosvitin inhibiting 50% of human cancer cell growth in vitro due to cytotoxic effects. Mg/mL refers to the milligrams of phosvitin present per mL of cell culture.* ***Lower concentrations*** *indicate that phosvitin is* ***more effective*** *at inhibiting the cancer growth. The most effective cytotoxic effects are seen in cervical adenocarcinoma, stomach adenocarcinoma, lung adenocarcinoma, and liver hemoblastoma.*

## Antiviral Pharmaceutical Drugs

The **antiviral properties** of phosvitin peptides are also being investigated. *Sun et al.* (2013)[10] showed that a peptide isolated from phosvitin was able to protect against a virus that affects many marine and freshwater fishes *in vitro.* While this cannot be extrapolated to human applications yet, it does illustrate that many antimicrobial peptides might also act as antiviral peptides and phosvitin should be researched for its potential as an antiviral pharmaceutical.

## Conclusion

Based on the research opportunities described in this paper, there is great potential for phosvitin and phosvitin phosphopeptides to be investigated as pharmaceutical drugs. The barrier that has prevented this research has been eliminated as phosvitin can now be obtained from Ecovatec Solutions in quantities sufficient for research at a fraction of its current cost. We look forward to partnering with pharmaceutical companies and biomedical, nutraceutical, and food research institutions to further investigate the properties of this "wonder protein".

## Sources


[1]Mudiyanselage and Himali. "Production and Characterization of Phosphopeptides from Egg Yolk Phosvitin." *Graduate Thesis and Dissertations.* 2012. Paper 12451.

[2]Samaraweera et al. "Egg Yolk Phosvitin and Functional Phosphopeptides-Review." *Journal of Food Science*, vol. 76, no. 7, Jan. 2011, doi:10.1111/j.1750-3841.2011.02291.x.

[3]Samaraweera et al. "Characterisation of phosvitin phosphopeptides using MALDI-TOF mass spectrometry." *Food Chemistry*, vol. 165, 2014, pp. 98–103., doi:10.1016/j.foodchem.2014.05.098.

[4]Ren, J., et al. "Preparation and characterization of phosphopeptides from egg yolk phosvitin." *Journal of Functional Foods*, vol. 18, 2015, pp. 190–197., doi:10.1016/j.jff.2015.07.007.

[5]Jung, S., et al. "The functional property of egg yolk phosvitin as a melanogenesis inhibitor." *Food Chemistry*, vol. 135, no. 3, 2012, pp. 993–998., doi:10.1016/j.foodchem.2012.05.113.

[6]Li, Z, et al. "Antibacterial activity and modes of action of phosvitin-derived peptide PT5e against clinical multi-drug resistance bacteria." Fish & Shellfish Immunology, vol. 58, 2016, pp. 370-379., DOI:10.1016.j.fsi.2016.09.044

[7]Ma, J., et al. "Endotoxin-neutralizing activity of hen egg phosvitin." Molecular Immunology, vol. 53, 2013, pp. 355-362., DOI:10.1016/j.molimm.2012.09.006

[8]Hu, L., et al. "Lipopolysaccharide neutraliziation by a novel peptide derived from phosvitin." *The International Journal of Biochemistry & Cell Biology,* vol. 45, 2013, pp. 2622-2631., DOI:10/1016/j.biocel.2013.09.002.

[9]Moon, S., et al. "Cytotoxic and antigenotoxic activities of phosvitin from egg yolk." *Poultry Science*, vol. 93, no. 8, 2014, pp. 2103–2107., doi:10.3382/ps.2013-03784.

[10]Sun, C., et al. "Antiviral activity of phosvitin from zebrafish Danio rerio." *Developmental & Comparative Immunology,* vol. 40, no. 1, 2013, pp. 28–34., doi:10.1016/j.dci.2012.12.009.