DECEMBER 14, 2017

# PHOSVITIN AND HYDROXYLAPATITE

## THE HUMAN BONE MINERAL PRECURSOR –
## USING PHOSVITIN IN BONE AND DENTAL TISSUE ENGINEERING

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## Executive Summary

**Ecovatec's revolutionary technology has unlocked the amazing potential of Phosvitin**. Ecovatec is the first and only commercial producer of phosvitin in the world. Phosvitin has been shown to be incredibly useful in the production of hydroxyapatite (HAP), leading to the biomineralization of bone and can be used in tissue/bone engineering and biomimicry to increase the reactions leading to bone and dental replacement products and perhaps even bone regeneration in the human body. However, in the past, phosvitin has not been easy to obtain for labs and companies, due to the difficulty and cost in isolating it from egg yolk. **Ecovatec has eliminated this barrier**, and offers pure phosvitin in commercial quantities and with commercial pricing to customers interested pursuing research and product development in hydroxyapatite-based tissue engineering, bone and teeth replacements, or bone regeneration.

Our related white paper on phosvitin and calcium uptake further explores the relationship between phosvitin and the creation of HAP as a nutritional supplement to promote calcium absorption and increasing bone density.

Ecovatec White Paper - Phosvitin and Calcium

## Background

Many research labs around the world are working to develop materials that can mimic both function and structure of natural bone tissues. Conventional methods of designing biomaterial, such as autogenic bone grafts are thought to be inferior to materials derived from natural materials with different mechanical properties and pore architecture.[10] For example, *Nandi et al.* (2015) looked at hydroxyapatite derived from marine coral. Other researchers have investigated how phosvitin can aid the creation of biomaterial from mammal-derived hydroxyapatite.

Phosvitin is a highly phosphorylated protein derived from the egg yolks of vertebrates. 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[1].

Serine is the major amino acid present in phosvitin (50%)[1], and almost all the serine residues are phosphorylated. This phosphorylation gives phosvitin its extremely strong metal binding capacity and inhibits the bioavailability of metal ions[1-6]. When phosvitin is hydrolyzed into smaller peptides, it increases the bioavailability of calcium and iron as it inhibits the forming of insoluble calcium or iron phosphates.

Phosvitin is usually de-phosphorylated to open the tertiary protein structure before it is hydrolyzed to give it its nutraceutical applications as an antioxidant or pharmaceutical and food industry uses as a metal ion carrier or anti-inflammatory protein[1-6]. Phosvitin's phosphopeptides are also known to improve bone and dental health. [1-6]

### HAP In Vivo

When the concentration of calcium ions in the blood is low, parathyroid hormone (PTH) is released and starts a biological reaction which leads to the resorption process where calcium is released from bone into the blood (decreasing bone density). When calcium ion concentrations in the blood are high, the opposite occurs, PTH is inhibited, and calcium ions transformed into HAP, leading to bone mineralization. Therefore, if calcium is more bioavailable and can be absorbed into the blood, the concentration of blood calcium should increase, promoting mineralization and increased bone density.

Calcium can be converted to hydroxyapatite (HAP) which is a precursor to the formation of bone mineral. This reaction can occur internally in the gut, or it can be created in the lab. This compound can then be ingested as a supplement or it can be used in its crystalline form as a filling for bones and teeth. It can also promote bone ingrowth in prosthetic implants. HAP is more easily absorbed by the gut than calcium and can be used instead of traditional calcium carbonate supplements.

### Relationship of HAP to Phosvitin

Many studies have investigated the effect of phosvitin on the creation of HAP.[4] When calcium (usually present as calcium phosphate) transforms into HAP, the reaction can be quite slow, often due to the lack of stabilizing molecules to bind to calcium in the intermediate reaction steps. *Zhang et al.* (2017)[6] found that the presence of phosvitin speeds this reaction by 6.5x by forming stable intermediate complexes with the calcium ions, providing nucleation sites for the crystal structure to form. This reaction can occur in the gut in the presence of ingested phosvitin, or it can occur in the lab to create HAP supplements or bone and teeth fillers.

## Bioactivity of Phosvitin in Bone Organ Culture Models[7]

*Liu, et al* (2013) used mouse skull bone organ cultures to look at the effects of PTH, phosvitin, and ascorbic acid (vitamin C) on bone resorption (shrinking) and growth. Vitamin C has been known to have the effects of promoting bone grown and calcium absorption, so the researchers wanted to determine if phosvitin was able to act in a similar way to vitamin C. As in the body, the PTH had the effect of promoting bone resorption, increasing calcium in the culture and showing increased osteoclast formation. It was also observed that **phosvitin inhibited this reaction** and caused less calcium release and significantly reduced bone resorption, just like vitamin C did. The greatest results were seen where vitamin C and phosvitin were used together to promote bone grown in the cultures. The data revealed that the bioactivity of phosvitin mirrors that of vitamin C during collagen synthesis and the formation of new bone. Many animals, including humans, cannot produce vitamin C in their body, so when producing bone cultures, phosvitin may be extremely important. They note that their evidence shows "the capacity of phosvitin to stimulate differentiation of osteoblasts, collagen synthesis, hydroxyproline formation, and biomineralization (**formation of HAP**)". *These findings could lead to a wide range of applications in tissue engineering and regenerative medicine/dentistry.*

## HAP Nucleation and Growth in the Lab[8]

In a 2017 study to explore the effects of phosvitin, mineralization and hydroxyapatite nucleation and growth, *Jie, et al.* concluded that growing HAP on collagen scaffolds may play an important role in future bone tissue engineering. They also evidenced a new system for mineralization by using **phosvitin to aid the growth of HAP**, which was proven to be very rapid and effective..

It is well known that phosvitin possesses the highest level of phosphorylation among egg proteins and it has large affects on mineralization in chicken embryo development. They used a type of collagen scaffold covered with a "film" for bone mineral to adhere to as it grew in the trials. Other researchers had reported that phosvitin allowed HAP to form on the scaffold more quickly than other films[8].

## In Vivo Bone Regeneration[10]

*Nandi et al* (2015) investigated in-vivo bone regeneration in rabbits with hydroxyapatite derived from marine corals. It is well known that "bone-derived growth factors… accelerate many biological activities leading to enhanced bone regeneration". Therefore, if growth factors could be delivered to a local fracture or diseased site, healing and recovery may be faster. However, there is a lack of available research on in vivo use of lab-created biomineral scaffolds (artificial bone grafts) loaded with growth factors, so Nandi et al used scaffolds of coral hydroxyapatite to investigate this in rabbits. Their results showed that growth factor loading onto the converted coral significantly accelerated early-stage bone formation. These results may show applications for bone healing in humans.

Since *Jie et al* (2017)[8] showed that growing HAP on collagen scaffolds could be aided by phosvitin, it follows that research could be done as to whether mats of phosvitin-aided collagen-HAP could be loaded with growth factor, and might have greater biocompatibility with humans than coralline HAP, as it more closely mimics bone systems in humans. Further research could have extensive medical applications for humans.

## Practical Applications

The most promising applications for the synthesis of HAP in the lab is for its biomimetic uses (mimicry of biological systems) and good biocompatibility as it has been used widely in bone replacement systems[9]. *Liang et al.* (2016)[9] noted that their experimental results show that phosvitin could be used in nanofibrous mats to induce HAP formation for tissue engineering. They also noted the good antioxidant activity in the nanofibrous mats which had potential to be used for wound dressings to promote wound healing.

## Sources

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