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**REVIEW ARTICLE** **OPEN ACCESS**

# Osteopontin - Structure, Function and Nutritional Potential of Milk Protein

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## ABSTRACT

Osteopontin (OPN) multipurpose protein mostly present in bones. The designation of word 'osteo' specify that protein is exhibit in bone, addition 'pontin' is obtain from 'pons'the Latin word for bridge that revel osteopontin's part as a associate protein. OPN is available in most tissues and body fluids with the elevated concentrations found in milk which is an acidic as well as highly phosphorylated glycoprotein carrying the intergrin binding Arg-Gly-Asp (RGD) and SVVYGLR sequence. Specific structural domain of milk OPN is critical function. Proteolysis of milk OPN give rise to functional fragment and may be important for development of vascular disease. Milk OPN is component in variety of physiological processes such as inhibition of calcification, bone remodeling and immune modulatory functions. Role of OPN in milk directly bind to bacteria & enhance phogocytosis and other function such as reduce dental biofilm, immune defence, form complex with lactoferrin. Procedure for isolation of OPN from bovine milk for make use of in infant formula have been developed. Based on the functional properties of milk OPN used as infant formula, oral hygiene, wound healing.

**Keywords:** OPN, Protein Structure, Function, Nutritional importance

## Introduction

OPN (Figure 1) is the extracellular matrix proteins which is the group of extracellular particles produce by cells that gives structural and biochemical carry to the nearby cells. OPN is Sialic acidic-rich and mostly phosporylated glycoprotein. Containing intergrin binding Arg-Gly-Asp (RGD) carry αv intergrin sticky domain (Liaw et al., 1995). SVVYGLR carry α9 β1 binding site (Smith *et al.*,1996). Present in primarily whey fraction of milk protein and in milk at highest concentration upto 18 µg/ml (Schack *et al.*, 2009). Basically OPN is impervious to harsh environment like heat treatment at 90ºC no reaction on the post translation modifications (Sorensen *et al.*, 1995). Key component diversification of phylogical action like, inhibition of calcification, immune modulatory functions and bone remodelling (Anborgh *et al.*, 2011).

OPN is, a release phosphorylated glycoproteins that moderate various biological functions. Initially isolated from bone and known as protein is expressed in bone, OPN was later apper to have a vast distribution (Brown *et al.*, 1992). OPN is the organic component of bone and main role in linking of protein. In adults, OPN expression is normally limited to the bone, kidney, and epithelial linings, and is produce in bodily liquids including milk, blood and urine (Chen *et al.*, 1993). In difference to its minimal dispensation in common tissue, OPN is strikingly upregulated at sites of swelling and tissue modify (Liaw *et al.*, 1998; O'Brien *et al.*, 1994). OPN exists both as a constituents of the extracellular matrix and as a

dissolvable cytokine. Physiologically OPN is thought to manage bio-mineralization in bone tissue, and to decrease growth and aggregation of calcium crystals in epithelial tissues (Wesson *et al.,* 2003). OPN has also been involve in a diversity of disorder states, where it moderate various cellular consequence such as adhesion, migration, and survival of various distinct cell species, together with synchronize and generating inflammation reaction of macrophages, Tcells, and dendritic cells. The pleiotropic nature of OPN may give back the several isoforms, post translational moderation, and diverseness of cell variety which OPN can interconnect with it. Clinically, OPN plasma levels are corresponded with chronic inflammatory disorder such as Crohn's disease (Agnholt *et al.,* 2007), cancer (ElTanani *et al.,* 2006), atherosclerosis, aortic abdominal aneurysms (Golledge *et al.,* 2007), and autoimmune diseases together with lupus (Kariuki *et al.,* 2009), various sclerosis (Comabella *et al.,* 2005), and rheumatoid arthritis (Sennels *et al.,* 2008). In this review we will focus on the role of OPN in inflammation biology.

**Structure and Expression - Comparison of Human and Bovine Milk OPN**

General structure of OPN contain highly negatively charged, extracellular matrix protein so lack in secondary structure (Wang K, 2008) with molecular weight of 33 kDa (nascent protein) and 44 kDa (on posttranslational modification) (Kundu *et al.,* 2006). Coming towards amino acid of OPN. Human milk contains 298 amino acid and Bovine milk contains 262 amino acid. Bovine milk lacks series of 22 residues corresponding to residues 188 – 209 in human OPN (Figure 2.1) 182 AA identical (61%) in both milk OPN. Both open to proteolytic separating which is near to RGD and SVVYGLR series. Huge fraction of milk OPN put in place in fragmented form with uncovers integrin binding moties. Proteolytic cleavage closed to integrin binding moties and it enlarge the integrin binding properties of OPN (Yamashita *et al.,* 2005).

***RGD domain -***

Cell attachment site of a large number of adhesive matrix protein (Hynes,1992). Extracellular matrix proteins is a group of extracellular particles produce by cells that gives structural and biochemical support to the nearby cells. Bind to intergrin receptor and to additional extracellular matrix molecules for example collagen (Chen *et al.,* 1992). Through this interaction, OPN act as adaptor protein, which bridge the cell surfaces with the surrounding matrix environment.

***Thrombin cleavage site***

Site closed to RGD domain and has physiological significance. The pair of OPN and thrombin are expected to be restricted in conjunction at sites of injury, inflammation, angiogenesis and in tumors. Potential role of OPN and its receptors in vascular diseases is specific structural domain of OPN are critical function and another one is proteolysis of OPN generate functional fragments and may be important for development of vascular diseases.

***Presence of OPN in Bovine and Human Milk***

Post translation moderation of bovine and human milk OPN. Phosphorylation and glycosylation sites are highlighted in black and grey (Christensen *et al.,* 2005). The region carrying the recognize splitting sites in bovine and human milk are boxed as shown in figure 2.2 (Sorensen *et al.,* 2014). Exons 4 and 5 misplaced in OPN – c and OPN – b are designate. The integrin binding RGD and SVVYGLR moities are underlined and found gaps are designate by dashed lines. Bovine milk OPN contain 22 phosphates allocate over 28 potential sites (Boskey *et al.,* 2012) and 3 O- glycosylated threonine closed to intergrin binding (Sorensen et al.,1995). Human milk OPN contain 25 phosphates allocated over 36 potential sites and 2 further O-glycosylated threonine (Christensen *et al.,* 2012). The effective role of OPN glycosylation in milk is not understandable, but their position near to intergin binding proposes a preventative role of carbohydrate opposed to splitting by endogenous milk proteases. Post-relocation modification of bovine and human milk OPN (Figure 2.2). Phosphorylation and glycosylation sites are highlighted in black and grey, separately (Christensen *et al.,* 2005; Sorensen *et al.,* 1995). The area carry the recognize splitting at a sites in bovine and human milk are boxed (Christensen *et al.,* 2010; Christensen & Sorensen, 2014). Exons 4 and 5 misplaced in OPN-c and OPN-b are specify. The integrin binding RGD and SVVYGLR-motifs are underlined and introduced gaps are indicated by dashed lines.

**Role of OPN in Milk**

***Inhibition of Calcification***

OPN show the intrinsic property with very acidic protein and phosphorylation of protein. Let OPN to attached and form soluble compound with calcium ions, which in conjunction with caseins. Inhibit intentional precipitation of amorphous calcium phosphate in milk (Holt *et al.,* 2014).

***Immune response***

OPN show cytokine like properties and Cytokine are group of protein. Bring communication between different cells types involved in immunity and to represent cytokine used interleukin (IL). Induce expression of Th 1 cytokine interleukin-12 and essential for clearance of intercellular pathogens. Inhibit production of Th 2 cytokine interleukin-10. Hence OPN key cytokine in the regulation of Th1 /Th2 balance immune response (Ashkar *et al.,* 2000).

***Enhance Phogocytosis***

OPN bind directly to bacteria (Schack *et al.,* 2009).

***Reduce dental biofilm formation***

OPN bind to surface of bacterial cells (Schlafer *et al.,* 2012).

***Immune defense***

OPN interact directly with invading pathogens.

***Form complex's***

OPN form complex's with lactoferrin, lactoperoxidase and IgM through electrostatic or affinity interactions (Azuma *et al.,* 2006).

***Transporter***

Immunomodulation and antimicrobial proteins to their site of action & protect them from proteolysis (Yamniuk *et al.,* 2009).

***Vascular disease***

A class of diseases of the blood vessel – arteries and veins of circulatory system of the body. OPN important regulator of arterial mineral deposition under conditions of injury and disease (Scetana M.& Guachelli C., 2007). Aeriental decalcification that favour plaque rupture. OPN responsible for changes within the arteosclerotic plaque and promote plaque instability (Huang *et al.,* 2001).

**Application of Milk Osteopontin**

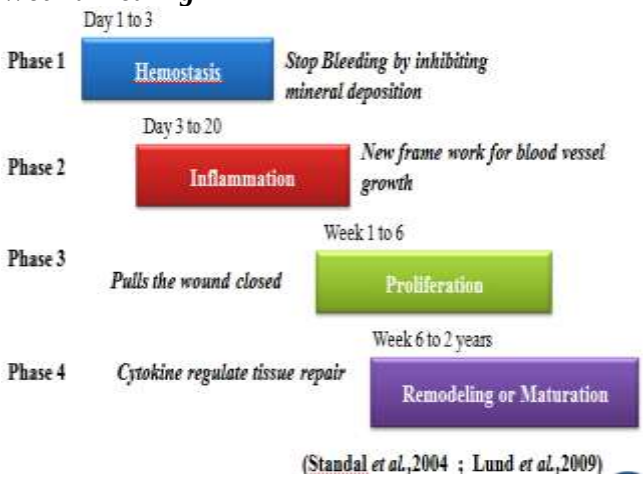
***Infant formula (IF)***

Humanization of IF's. Bovine milk and human milk OPN structurally similar in amino acid sequence and phosphorylation pattern suggest to use bovine OPN in infant formula (Boskey *et al.,* 2012). Human milk contains OPN concentration at level of 138 mg/L of total protein 2.1%. Bovine milk contains OPN concentration at level of 18 mg/L of total protein 0.05 %. Standard IF: OPN concentration at level of 9 mg/L of total protein 0.03% (Schack *et al.,* 2009). Oral intake of OPN not harmful. Whey based protein product (Lacprodan OPN -10) contain 95% bovine milk OPN and found not be genotoxic in vitro and in vivo (Kvistgaard *et al.,* 2014). Phogocytosis – bind to bacteria by macrophases (Schack *et al.,* 2009) OPN resistant to proteolysis by neonatal gastric juices at pH 3.0 for 1 hr at 37°C Contribute to polarize (Chatterton *et al.,* 2004).

***Oral Hygiene***

Microbes in dental biofilm produce organic acid upon exposure to fermentable dietary carbohydrate. Repeated reduction in pH at biofilm tooth interface lead to slow demineralization of dental hard tissues. Development of dental caries. Dental caries prevent by mechanical removal of dental biofilm such as cleaning using tooth brush but does not remove full biofilm (Prasad *et al.,* 2011). Bovine milk OPN may reduce dental biofilm formation by binding to surface of bacterial cells (Schlafer *et al.,* 2012). Without affecting cell viability and impact on amount of biofilm formed in flow cells. OPN protein used in mechanical tooth cleaning procedure. OPN provided by eg. a mouth rinse or a chewing gum, it reduce amount of biofilm formed on tooth surfaces. OPN might be valuable adjunct to professional and self-performed oral hygiene procedures with dental caries control (Sodek *et al.,* 2000).

***Wound Healing***



***Bone remodeling***

A regulated process in which removal of old bone by osteoclasts and formation of bone by osteoblasts (Standal *et al*., 2004).

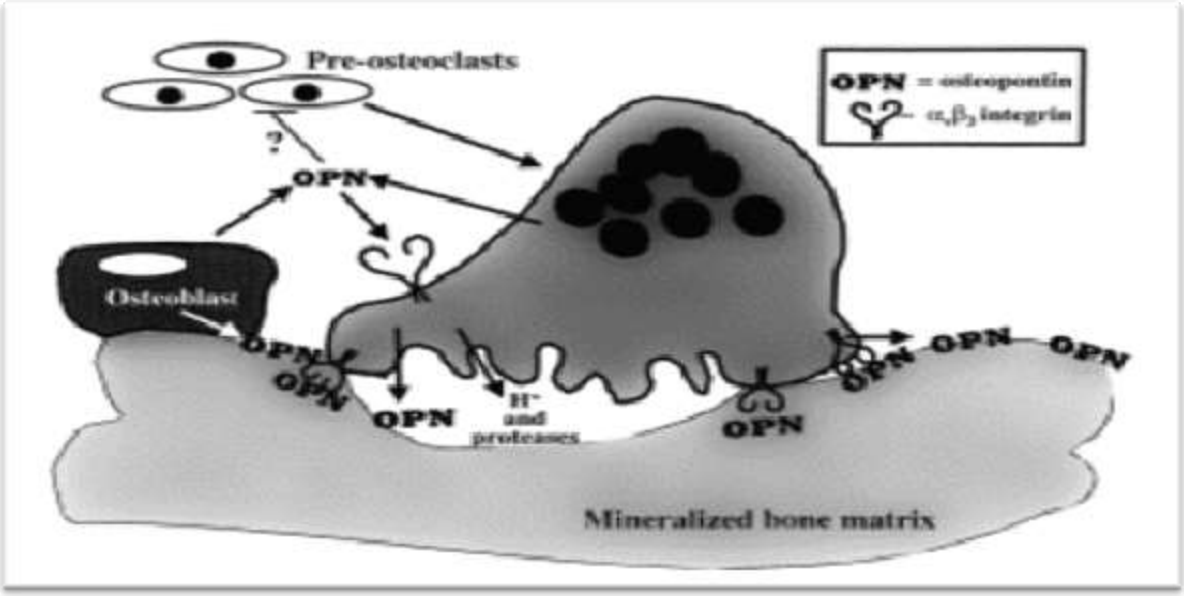



**Figure 1.1** OPN **(Giachelli & Steitz, 2000)**

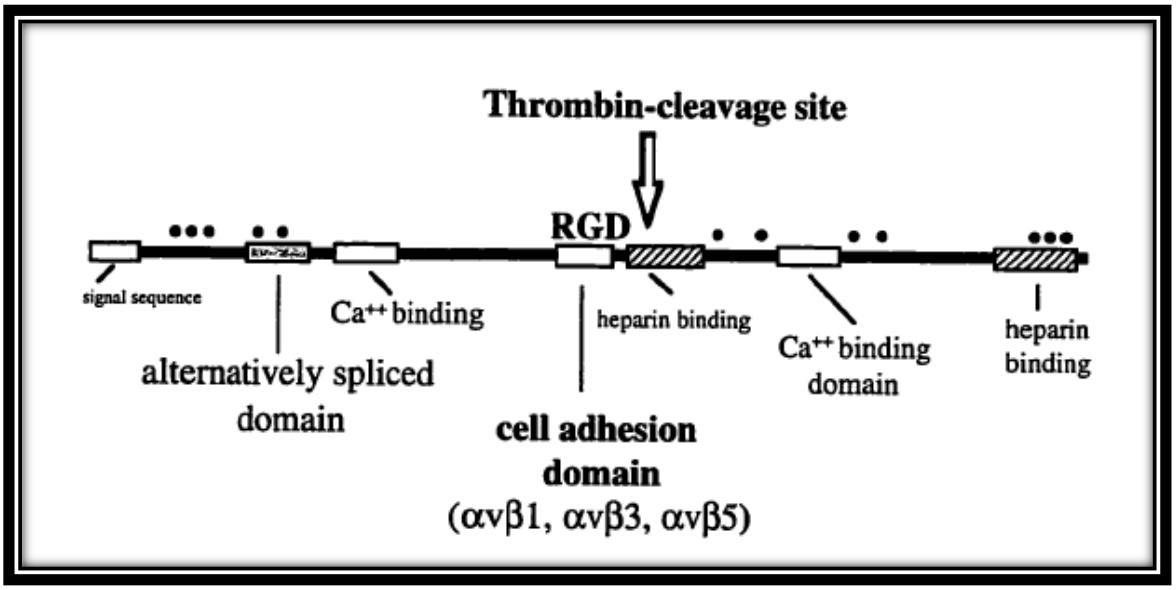



**Figure 2.1** OPN structure with Thrombin cleavage site.


**Figure 2.2** Presence of OPN in Bovine and Human Milk

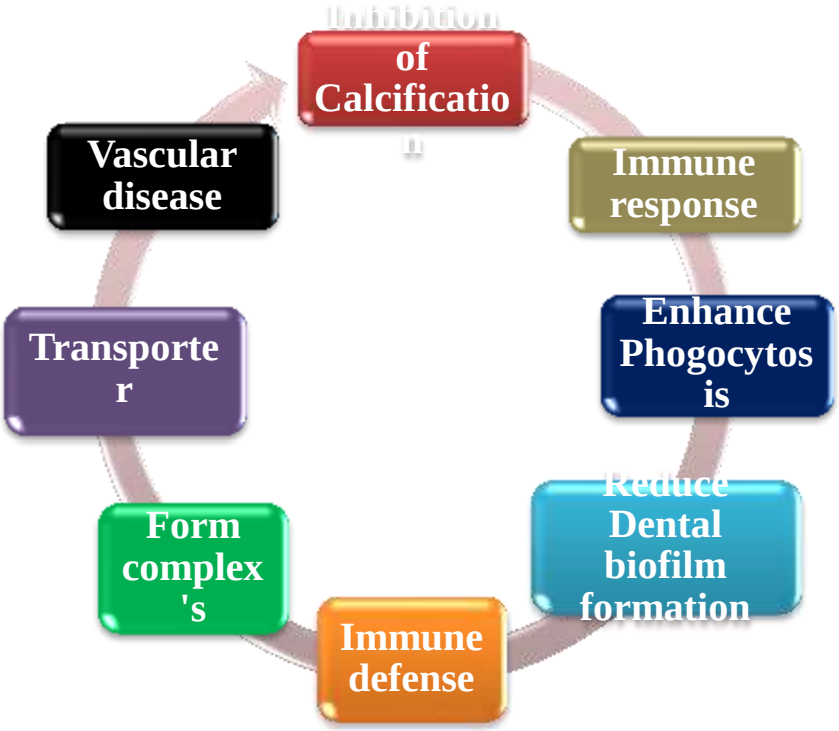



**Figure 3.1** Role of OPN in Milk

**Table 4.1** Composition of Infant formula

| | **Osteopontin (% )** |
|---|---|
| Protein (N*6.38) | 78 % |
| Osteopontin of total protein (N) | 95 % |
| Protein bound carbohydrate | 7 % |
| (Sialic acid) | |
| Lactose & fat | < 1 % |
| Ash (max.) | 9 % |
| Moisture | 5 % |

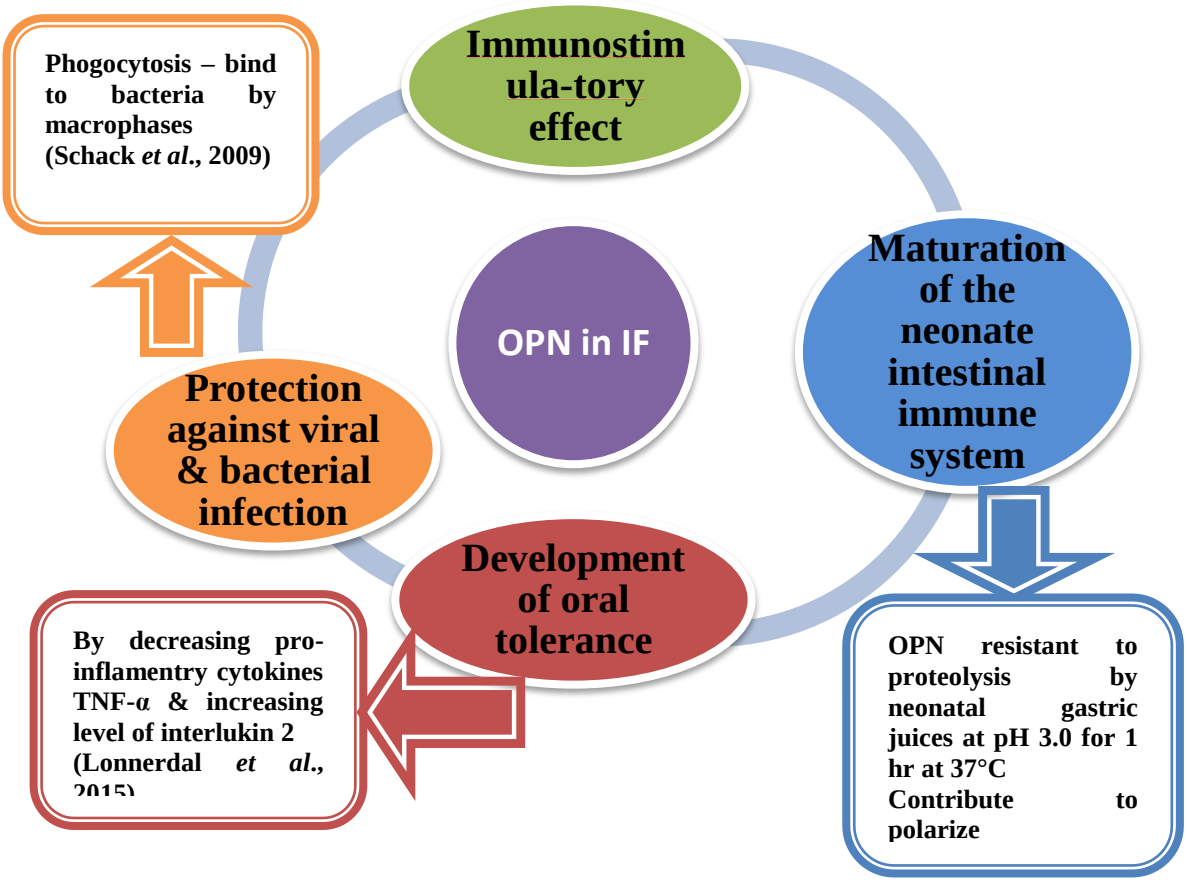



**Figure 4.1** OPN in Infant Formula

## Conclusion

Structure of OPN contain different binding site for calcium, heparin, receptors. Proteolysis of OPN generates functional fragments & may be important for development of vascular diseases. Multifunctional protein present in milk and highly phosphorylated which shown promising results in infant nutrition. Use for oral hygiene & wound healing.

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