Title: GFOGER Peptide Inhibits Vascular Calcification *In Vitro* By Blocking the Osteogenic Switch of Vascular Smooth Muscle Cells.

Cardiovascular Diseases (CVDs) are classified on top of the list among different death leading causes in the world. Calcification of the vessel wall accounts for high death rates in patients with many diseases like diabetes, atherosclerosis and Chronic Kidney Disease (CKD). Vascular Calcification (VC) is considered as an active multifaceted pathology that resembles bone physiology and regulated by inductive and inhibitory mechanisms. During the calcification process, Vascular Smooth Muscle Cells (VSMCs) release Extracellular Vesicles (EVs) which act as nucleating foci for crystallization through their interaction with type I collagen and also they undergo active osteogenic process to become osteoblast like cells. Using Surface Plasmon Resonance (SPR) in our previous studies, we successfully identified a specific collagen six amino acid sequence (GFOGER) involved in the recognition of integrin expressed on EVs. Recently, we chemically synthesized this GFOGER peptide and we hypothesized if it is able to prevent VC *in vitro* and *ex-vivo.* Our results showed that GFOGER peptide decreased Pi-induced calcification, by 66% and 60% in MOVAS-1 and primary human VSMCs respectively. Moreover, GFOGER sequence was effective against aortic rings cultured *ex-vivo* where it decreased Pi-induced calcification by 91%. At the molecular level, the expression of different osteogenic markers including Runt-related transcription factor 2 (Runx2), Matrix Gla-Protein (MGP), Osteocalcin (OCN) and Tissue Non-Specific Alkaline Phosphatase (TNAP) were downregulated in the presence of the GFOGER peptide. These results suggested that inhibition of VC by GFOGER peptide is in part mediated by a decrease of the osteogenic de-differentiation of vascular smooth muscle cells. Finally, we aim to expand our study in order to check the capacity of GFOGER peptide to inhibit VC on *in vivo* models.

Key Words: Vascular Calcification, Vascular Smooth Muscle Cells, Extracellular Vesicles, Osteoblast like cells, GFOGER Peptide.