Nano-selenium activates Mucin gene expression in intestinal crypt cells

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Abstract
Selenium is known for its antioxidant activity and it reduces the effects of free radicals in cells. The role of selenium is an essential nutrient to prevent oxidative damage in cells. However the limitation on its wide application is due to the narrow range in between the nutritional and tolerable intake levels. The optimization between supplemental Se acquired through diet and its bioavailability depends mainly on the source of selenium which requires proper models to mimic in vivo understanding of food and drug molecules during absorption to excretion which is lacking in the present scenario. But recent knowledge on crypt cell culture approaches are promising in the designing of suitable models to explore in vitro drug and cellular interactions. In the present study to mimic intestinal effects, the primary cultured intestinal crypt cells, representing true stem cell compartment were applied to analyse the effect of selenium nano particle intake with reference to expression of Mucin gene which has active role in the nutrient digestion and absorption.

Keywords: Intestinal crypt cells-nano-selenium-Mucin gene expression

Introduction
Chicken has been used as a favourite model in embryology and developmental biology and it serves as a good model for nutrigenomic studies in other mammalian system including humans due to its closeness to the mammalian genome. Selenium forms an important component of seleno-proteins that prevent cell damage in many metabolic functions. Selenium is known for its antioxidant activity and it reduces the effects of free radicals in cells. The role of selenium as an essential nutrient to prevent oxidative damage in cells has been reported earlier [1]. How every the limitation on its wide application is due to the narrow range in between the nutritional and tolerable intake levels. The optimization between supplemental Se acquired through diet and its bioavailability depends mainly on the source of selenium. Hence the dietary intake for the improvement of Se bioavailability depends on the Se forms in Se-containing food/feed sources and selenium in nano form is preferred over other dietary forms due to low toxicity with high bioavailability [2]. Selenium in nano form has wide attention for its potential application in the prevention of oxidative damage in animal tissues through high surface activity with wide active centres, high catalytic efficiency and strong absorbing ability. However, the effect of nano-selenium activity in vivo in cellular metabolism is poorly understood. This may be due to the lack of proper in vitro models to mimic in vivo understanding of food and drug molecules during absorption to excretion. Many reports on the intestinal epithelial cell culture in vitro proved them as a reliable model of intestinal metabolism successfully applied in trace elements [3, 4]. While the intestinal epithelium represents the most vigorously renewing adult tissue in mammals, the stem cells that fuel this self-renewal process have been identified recently [5] and the unique epithelial anatomy makes the intestinal crypts one of the most accessible models for the study of adult stem cell biology. Intestinal crypts were shown to display a remarkable regenerative capacity following DNA and cytotoxic damage or surgical resection [6]. The absorption biology of food bio actives and drug molecules lack proper understanding due to the lack of proper in vitro models to depict in vivo. But recent knowledge on crypt cell culture approaches are promising in the designing of suitable models to explore in vivo drug and cellular interactions. In the present study to mimic intestinal effects, the primary cultured intestinal crypt cells, representing true stem cell compartment were applied to analyse the effect of selenium nano particle intake with reference to expression of much in gene which has active role in the nutrient digestion and absorption.
Materials and Methods

Intestinal crypt culture
Crypts isolated from duodenum regions of chicken small intestine were characterized morphometrically and through Lgr gene expression as reported earlier [7]. Cultured crypt cell population were subjected to morphometric image acquisition and mucin gene expression analysis in nano selenium supplementation. The nano selenium prepared from organic sources as a part of post graduate research by one of the author was in the range of 90 nm (Un published data) were used for analysis. The intestinal crypt analysed for sizes were picked up and approximately uniform sizes were grouped and rinsed with PBS twice and incubated for 24 hrs, 48 hrs and 72 hours in nano selenium supplemented cultures with a predefined concentration of 50μg/mL that showed better proliferation above which a decline in proliferation was recorded (Fig 1). The liveability of the crypt was analysed in culture by representative culture analysis with Fluorescent staining.

Real-Time PCR
Real-time quantification of the expression of mucin gene MUC2 were done by normalizing with endogenous reference gene glyceraldehyde 3-phosphate dehydrogenase; GAPDH. Gene-specific primers were used for SYBR Green detection of cDNA sequences of the MUC2 genes. Primers used for the real-time PCR were MUC2-F: CCT GTG CAG ACC AAG CAG AAA, MUC2-R: CCT CTG AGT TTT TCA GCA AAC AC. The composition of PCR master mixture consisted of diluted cDNA (3 μL), each primer (200 nM), SYBR green qPCR mix10 μL (Takara) in a final volume of 20 μL. Amplification was carried out in CFX 96 Real time system, (Bio-Rad). PCR was performed in Hard Shell PCR plates, 96 well thin wall sealed with qPCR seals under the following conditions: 50 °C for 2 min, 95 °C for 2 min, and 40 cycles of 95 °C for 30 s and 60 °C for 1 min. Melting curve was determined and gene amplification was done in triplicate within a single instrument run. Non template control was run for each template and primer pair to avoid false positives. Fold change was calculated relative to the control using the relative quantification for the experimental genes and GAPDH (internal control). The mRNA abundance was determined by analyzing the resultant Cq values for each sample (gene of interest), normalized to the level of GAPDH mRNA abundance for the same RNA sample. Relative expression of mucin2 mRNA was determined using the (2-ΔΔCt) method.

Results and Discussion
Nano selenium supplementation in in-vitro cultured crypt cells (Fig 1) increased the mucin gene expression compared to control without supplementation. Relative expression of mucin2 mRNA was determined using the (2-ΔΔCt) methods showed a two fold increase (Fig2a and b).

Fig 1: Intestinal crypt cells in in vitro culture

Fig 2a: Melt curve analysis for Mucin gene in real time PCR analysis Melt Peak
There is a progressive increase in mucin gene mRNA expression in nano-selenium supplementation compared with unsupplemented crypt cell cultures in vitro and supports the earlier reports that mucins play an important role in uptake of nano molecules in diet through digestion through goblet cells. The mucins are the main component of the mucus layer, which are produced and secreted by goblet cells. The mucus layer is part of the innate host response preventing gastrointestinal pathologies and participating in the processes of nutrient digestion and absorption. The epithelium of the adult mammal an intestine is in a constant dialog with its underlying mesenchyme to direct progenitor proliferation, lineage commitment, terminal differentiation and ultimately cell death. The epithelium is shaped into spatially distinct compartments that are dedicated to each of these events. The entire surface of the chicken gastrointestinal tract is covered by a layer of mucus that functions as a diffusive barrier between the intestinal lumen and absorptive cells. Mucin2 is the major intestinal mucin gene that was initially isolated from a human jejunum cDNA library. The in vitro culture of intestinal epithelial cells has been developed to evaluate nutrient safety and to define the specific mechanisms of nutrient metabolism, because it was difficult to control various in vivo environmental parameters of intestine. As the mucous layer that covers the intestinal absorptive surface acts as a diffusive barrier between the intestinal lumen and Absorptive cells, results indicate that nano-selenium supplementation has a significant role in the processes of mucin biosynthesis via changes in the intestinal mucin 2 gene expression. These response in mucin dynamics influence gut function and health and may change nutrient uptake. The crypt to villus hierarchical migratory pattern of cell proliferation and differentiation is well established and it is therefore assumed that the stem cells are located at the origin of this system, the intestinal crypt. It is thought that stem cells reside within a stem cell compartment or niche. Those stem cells were thought to reside within a niche- stem cell compartment and were maintained by production versus cell deletion. The production was reported to be mainly based on asymmetrical division i.e., from one stem cell, a stem cell and a daughter cell. The strongest evidence for the stochastic nature of the division process has been shown to be leading to monoclonal conversation, and concludes that the cells in a crypt become descendant of a single stem cell. The present reports coincides with the reports on expression pattern of the mucin 2 gene in chickens fed antibiotic growth promoter or a probiotic product that were greater than the observation in controls. Also the present findings supports the documentation on supplement of turmeric, thyme and cinnamon to both basal diets that increased the expression of mucin 2 mRNA in jejunum of chickens. Hence it could be stated that any component, dietary or environmental, that induces changes in mucin gene expression has the potential to affect the integrity of the mucus layer and nutrient absorption.

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References
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