

## Brief Note on Nanofibers Led to Accelerated Healing for Rabbit

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### Description

As various studies have shown, nanofibers are able to partially substitute missing extracellular matrix and to stimulate cell proliferation and differentiation in sutures. Therefore, we tested nanofibrous membranes and cryogenically fractionalized nanofibers as potential materials for support of the healing of intestinal anastomoses in a rabbit model.

We compared cryogenically fractionalized chitosan and PVA nanofibers with chitosan and PVA nanofiber membranes designed for intestine anastomosis healing in a rabbit animal model. The anastomoses were biomechanically and histologically tested.

In strong contrast to nanofibrous membranes, the fractionalized nanofibers did show positive effects on the healing of intestinal anastomoses in rabbits. The fractionalized nanofibers were able to reach deep layers that are key to increased mechanical strength of the intestine. Moreover, fractionalized nanofibers led to the formation of collagen-rich 3D tissue significantly exceeding the healing effects of the 2D flat nanofiber membranes. In addition, the fractionalized chitosan nanofibers eliminated peritonitis, significantly stimulated anastomosis healing and led to a higher density of microvessels, in addition to a larger fraction of myofibroblasts and collagen type I and III. Biomechanical tests supported these histological findings.

We concluded that the fractionalized chitosan nanofibers led to accelerated healing for rabbit colorectal anastomoses by the targeted stimulation of collagen-producing cells in the intestine, the smooth muscle cells and the fibroblasts. We believe that the collagen-producing cells were stimulated both directly due to the presence of a biocompatible scaffold providing cell adhesion, and indirectly, by a proper stimulation of immunocytes in the suture.

### Hypothalamic Pituitary Adrenal

Magnesium (Mg) deficiency in serum is associated with METS through the concept that metabolic syndrome is conditioned by the formation and development of inflammation. Mg deficiency is associated with stress and activates the Hypothalamic Pituitary Adrenal (HPA) axis and the sympathetic nervous system. Activation of the renin-angiotensin-aldosterone system

is a factor in the development of insulin resistance by increasing oxidative stress. Copper (Cu), zinc (Zn), and iron (Fe) are involved in numerous antioxidant enzymes. Blood concentrations of Cu and Fe may be positively related to diabetes risk. In turn, a higher dietary intake of Zn may be associated with a reduced risk of METS. The relationship between serum Pb concentration and the diagnosis of obesity is unclear. Studies show different results, a positive correlation and a negative correlation, with the relationship between type 2 diabetes diagnosis and Pb concentration frequently described as positive.

Although literature describes a link between macronutrient concentrations and metabolic disorders, the data are often inconclusive, which may be related to the multiplicity of indices used to assess fat accumulation and metabolism. Among the anthropometric parameters used to assess body fat, the most common is Body Mass Index (BMI). However, BMI is not the most objective index for assessing adipose tissue function as it does not take into account factors that affect visceral fat mass and its function. That is why the Visceral Adiposity Index (VAI) is used as a marker of visceral adipose tissue dysfunction. The mathematical model for calculating VAI covers both anthropometric and biochemical parameters.

The relationship between the indicators of metabolic disorders and concentrations of bio elements in tissues is poorly described, and the available data are often discrepant. Earlier studies have shown that there may be a correlation between serum Zn and BMI and serum Fe in older adults. A study on an animal model has shown that chronic supplementation with high doses of Zn induces visceral adipose tissue hypertrophy and impairs protein kinase B signaling. A study analyzing the relationship between Cu concentrations and lipid parameters indicates that deficiencies of this element can result in increased lipid levels in the peripheral circulation; this concerns glycolipids, cholesterol, triglyceride, and phospholipids.

Comparisons of element concentrations in bone and serum can provide valuable information on the relationship between these tissues in the human body. The wide variety of diagnostic methods for detecting metabolic disorders makes it difficult to compare test results and analyze the relationship between metabolic disorders and element concentrations. It is worth noting that bone is a compartment in which the dynamics of changes in element concentrations is slower than in other tissues of the body.

## Analysis and Sterilization

Nanofibers were prepared by electrospinning. To obtain purified chitosan, chitosan isolated from shrimp shells was dissolved in a 10% aqueous acetic acid 1% w/v and filtered. The filtered chitosan solution was next precipitated using NaOH, and the precipitate washed with a mixture of distilled water and acetone and dried at room temperature. Dried purified chitosan was dissolved in a mixture of tri fluoroacetic acid and dichloromethane to obtain 5% w/v chitosan solution. From the chitosan solution, chitosan nanofibers were prepared with a spinner having a special glass needle electrode and a metal plate collector covered with polypropylene nonwoven textile; the electrical field intensity used was 25 kV/cm. To prevent dissolution of the chitosan nanofibers in the wet environment, the chitosan nanofibers were stabilized in a saturated solution of

NaOH in absolute ethanol. After 10 minutes in the NaOH solution, the chitosan nanofibers were washed in distilled water at neutral pH, and then dehydrated by immersion in increasing concentrations of ethanol. Dehydrated chitosan nanofibers were dried at room temperature.

Polyvinyl alcohols were dissolved in distilled water at 80°C solution was used for electrospinning with addition of phosphoric acid to lower the pH and glyoxal as a PVA cross linker. Electrospinning was performed using a spinner with a wire electrode and a wire collector covered with polypropylene non-woven textile; the electrical field intensity used was 25 kV/cm. To prevent dissolution of the chitosan nanofibers in the wet environment, the PVA nanofibers were cross linked using glyoxal for 72 hours at a temperature of 60°C.