Effects of Diabetes and Anti-diabetic Compounds on Fracture Toughness, Nano-mechanical Properties, and Physicochemical Properties of Mouse Bone

A Thesis

Submitted

by

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Affectionately

dedicated

to my loving

Parents

Declaration by the Research Scholar

I hereby declare that the entire work embodied in this Thesis entitled **"Effects of Diabetes and Anti-diabetic Compounds on Fracture Toughness, Nano-mechanical Properties, and Physicochemical Properties of Mouse Bone"** is the result of investigations carried out by me in the ***School of Engineering***, Indian Institute of Technology Mandi, under the supervision of ***Dr. Rajesh Ghosh***, and that it has not been submitted elsewhere for any degree or diploma. In keeping with the general practice, due acknowledgments have been made wherever the work described is based on finding of other investigators.

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Declaration by the Research Advisor

I hereby certify that the entire work in this Thesis has been carried out by ***Pankaj Shitole,*** under my supervision in the ***School of Engineering,*** Indian Institute of Technology Mandi, and that no part of it has been submitted elsewhere for any Degree or Diploma. In keeping with the general practice, due acknowledgements have been made wherever the work described is based on finding of other investigators.

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Dr. Rajesh Ghosh

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Abstract

Diabetes is a metabolic disease in which the body is unable to produce enough adequate insulin. Hyperglycemia and poor glycemic control affect bone health and increase fracture risk in both types of diabetes, i.e., type-1 diabetes (T1DM) and type-2 diabetes (T2DM). There are different anti-diabetic drugs available to control the increased level of blood sugar. Recently, it was found that low-dose naltrexone (LDN) and PK2 improves glucose tolerance and insulin sensitivity in T2DM and T1DM, respectively. The effect of such drugs on bone health is not clearly understood. In the T2DM mouse model, 24 mice were divided into 4 groups according to diet and treatment (six animals in each group). The first group of animals was fed with normal diet and treated with saline water, the second group of animals was fed with a normal diet and treated with LDN, the third group of animals was fed with a high-fat diet (HFD) and treated with saline water, and the fourth group of animals was fed HFD and treated with LDN. The animals were humanely killed following the ethical protocols and extracted the femur bones to study the effect of LDN and T2DM at different hierarchical levels. At first, femurs were tested for fracture toughness (whole-bone level) using three-point bending. Thereafter, local level properties such as nano-Young's modulus and hardness (nano-level) were estimated via nano-indentation. The mineral crystal size and compositional properties, including the mineral-to-matrix ratio, were estimated using X-ray diffraction and thermogravimetric analysis. Additionally, the effect of LDN on compositional properties such as mineral-to-matrix ratio, carbonate substitution, crystallinity, collagen quality, and advanced glycation end-products (AGE) levels in T2DM bones was studied using Raman spectroscopy. It was observed that T2DM reduces the fracture toughness, nano-Young's modulus, and hardness; however, LDN protects these alterations in HFD-induced T2DM bones. Similarly, T2DM reduces the mineral crystal size and bulk mineral-to-matrix ratio, and LDN protects diabetic alterations in T2DM bone. Additionally, LDN controls the Raman properties and reduces the AGE level in T2DM bone. In the case of a T1DM mouse model, similar to T2DM, a total of 24 mice were divided into 4 groups (six in each) according to the treatment. The first group was normal control animals. The second group of animals was treated with streptozotocin (STZ) as it represents the model of T1DM, the third group of animals was treated with PK2 before the STZ, and the last group of animals was treated with PK2 after the STZ treatment. After treatment, femur bones were sacrificed and tested for

fracture toughness, mineral crystal size, and bulk compositional properties similar to T2DM bones. T1DM reduces fracture toughness, mineral crystal size, and mineral-to-matrix ratio. Interestingly, it was found that PK2 restores the properties at the whole bone level and the nano-level in T1DM bone. Therefore, it was concluded that LDN and PK2 control the T2DM and T1DM affected bone properties at the multiple hierarchical levels in mouse bone.

Keywords: Diabetes, mouse bone, bone properties, fracture toughness, Raman properties, nano-indentation, mineral-to-matrix ratio, nano-Young's modulus, hardness, mineral crystal size.

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