

Development of Innovative technologies to detect long-term diabetes using Human toenail by Vibrational spectroscopy

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Aim and Justification

- The aim and objective of the study is to investigate the extracellular damage in diabetes and compared in normal subjects using toenail and its impact on human body is determined by various aspects of biochemical parameters to be correlated.
- The justification of the spectral studies is non invasive and we can predict before encountering (prone chances of diabetes) diabetes and metabolic syndromes.
- Found significant changes in prolong diabetic specimens.

Risk and Outcome

- There is no risk to patients during sampling and whole study. The name of patients and clinical data's are undisclosed to general public. After coding it will be utilized for publication and other purposes.
- The outcome benefits to the subjects about their health status and prone chances to diabetes are avoided by changing diet and physical exercise and counseling will be given to stressed individuals to avoid the SI-diabetes.
- The statistical data of this study will help the epidemiological and preventive measures of the diabetes nationwide.

Specific Objective

- Identification of structural symptoms in long-term diabetes.
- Investigation of the blood parameters and structural changes in toenails with reference to diabetes in multi system disorder.
- Management of diabetes in early stages by diet and physical exercise.

Materials and methods

- Source of data: The prospective study includes diabetic and nondiabetic patients of
 - ❖ ESI – Vaniyambadi Dispensary
 - ❖ ESI – Ambur Dispensary
 - ❖ ESI – Pernambut and Gudiyattam Dispensary.

Duration: May 2014 – May 2015.

However this work is permitted by Tamilnadu Govt. Letter No. 13577/F1/2014-1, health and Family Welfare Department, dt-28.05.2014.

DMS- ESI, Ref No. 167/ESI/P1/2014.

Recommended by IEC, CSIR-CLRI (IEC/2010-2015/001)

Sample size: Multicentre together with 400 cases and extended to get Statistical Significance of each group.

Methodology:

- The protocol is designed by gender wise with different age group with **three** categories.
- Male and female with age group between **18 - 60** and above.
- The toenail sample collection is quite simple by scraping the nail using sterile blades and It is utilized for spectroscopic analysis.
- The biochemical and structural parameters of the normal, diabetic and prone to diabetic to be correlated with spectroscopic revealed data.
- This presentation covers Diabetic and non-diabetic specimens with FBS, PPBS, HbA_{1c}, Urea, Creatinine, Albumin, Triglycerides and Total Cholesterol were studied.

Inclusion and Exclusion Criteria

➤ Inclusion Criteria:

- Diabetic Patients with different age group
- Acute and chronic Diabetic patients.
- Non-diabetic patients with all parameters within normal limits.

➤ Exclusion Criteria:

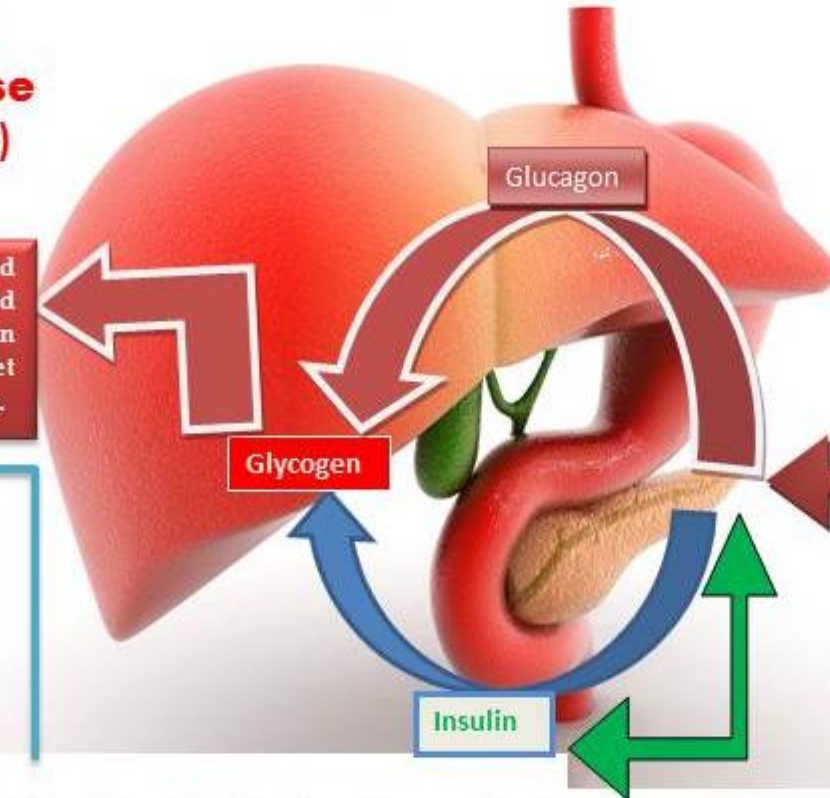
- Consent not interest to participate in this study.
- Patients age less than 18.
- Old age people unable to fit to give samples.

Introduction Diabetes

Blood Glucose Regulation Mechanism

Increased Blood Glucose (Hyperglycemia)

Glucose released for energy invaded via circulation reached to target organs and tissues.



Stimulates Insulin Secretion and act for glycogen synthesis and also transport Glucose to tissues.as a routine for energy.

Decreased Blood Glucose (Hypoglycemia)

Stimulates glucagon secretion and act for glycongelysis and also transport Glucose to energy needed tissues.

Background of study:

- What is diabetes?
- Types of diabetes?
- How To Determine Whether You Have Diabetes, Prediabetes or Neither.
- Normally, hemoglobin A1 (HbA1), glycosylated hemoglobin, has been clinically used as an indicator of long term control of blood glucose in diabetic patients.
- Significant changes in human fingers nails during diabetes
is possible to use long-term indication of diabetes mellitus.

Human Nails: Introduction

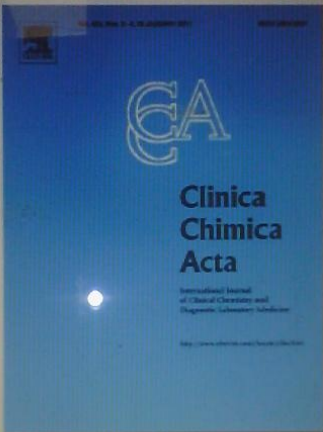
- In human anatomy, a nail is a hornlike envelope covering the dorsal aspect of the terminal phalanges of fingers and toes.
- Nails are similar to claws in mammals and birds.
- Fingernails and toenails are made of a tough protein called keratin, as are animals' hooves and horns.
- The human nail plate is one of the most impervious of biological structure and composed of hard α – keratin, which is the substance forming stratum corneum.
- Hard α -keratin has a high cystine content compared to soft α - keratin.
- The α -keratin contains α -helical polypeptides, which are organized into intermediate filaments (IFs).
- The IF polypeptides are richest in those amino acids favoring an α -helix formation, namely lysine, aspartic acid, glutamic acid and leucine, and comparatively poor in half-cystine and proline.

Nail in disease condition

- The condition of Human nail was used as a prediction of the probable course and outcome of a disease. i.e. Diagnostic tool.
- The condition of nails, such as yellow discoloration and side flutes, reflects systemic diseases of the
 - Kidney
 - Thyroid and liver
 - systemic lupus erythematosus (SLE)
 - Human immunodeficiency virus (HIV)-infection
 - Diabetes mellitus, which cause chronic fatigue, are known to induce abnormality of the nails.

Some of our early results

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Clinica Chimica Acta 412 (2012) 209–216

Journal homepage: www.elsevier.com/locate/clinchem

Short communication

Comparative study on secondary structural changes in diabetic and non-diabetic human finger nail specimen by using FTIR spectra

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ARTICLE INFO

Article history:
 Received 11 August 2012
 Received in revised form 2 November 2012
 Accepted 12 November 2012
 Available online 18 November 2012

Keywords:
 Diabetic patients
 Nails
 FTIR spectroscopy
 Amyloid formation
 Collagen
 α-Keratin

ABSTRACT

Background: In human anatomy, a nail is a keratinized outgrowth covering the distal aspect of the terminal phalanx of fingers and toes. Nail disorders are most common among the geriatric population. Diabetic patients are also supposed to affect the condition of nails. Acceptable differences in infrared (IR) spectra of chronic and acute diabetes in vitro and in vivo nail specimen compared to control normal specimens were investigated in this study.

Methods: Using a Nicolet 370 Fourier Transform Infrared (FTIR) spectrometer, the spectra of the nails of diabetic and normal specimens were recorded.

Results: In the case of non-diabetic patients, the amide I band was observed at 1640 cm⁻¹ (1626, 1632, and 1638 cm⁻¹). The bands around 1637 cm⁻¹ were attributable to amide I of different structures. Amide II bands were absent in all the non-diabetic patients. Amide II bands around 1570 cm⁻¹ were observed both in diabetic and non-diabetic patients. In all the diabetic patients, a peak at 1510 cm⁻¹ (particularly around 468 cm⁻¹) was observed.

Conclusion: The presence in the nails of diabetic patients contains helical structure, including the presence of amide II bands. Amyloid and collagen were observed both in non-diabetic patients and in the amide II structure.

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1. Introduction

The human nail plate is one of the most impressive biological structures and the penetration of chemical agents is low [1]. The nail plate is mainly composed of hard α-keratin, which is the substance forming the stratum corneum [2,3]. The change in mechanical and physical properties and the alteration in water content may be reflected in the molecular structure of nail proteins [4]. The nail keratins belong to the group of hard keratins, which also include hair and hoof keratins [5]. Hard α-keratins has a high cystine content compared to soft α-keratins. The α-keratins contain α-helical polypeptides, which are organized into bundles of filaments [6]. The β-polypeptides are related to those amino acids bearing an α-helix formation, namely tyrosine, aspartic acid, glutamic acid and leucine and comparatively poor in half-cystine and proline [7]. Cysteine-cysteine disulfide cross-links and secondary cross-linking prosthemes stabilize the matrix phase. The matrix adheres to the basement through secondary bonds, including hydrogen bonds, hydrogen bonds and ion interaction [8]. The hydration of nails is thought to be the most important factor influencing the physical properties of nail and possibly act through changes in keratin structure. Miao et al. described a quantitative method for the determination of all the seven amino acids in nail hydrolyzate and quantitatively gave the data [9]. NIR FT Raman spectroscopy has been shown to be an efficient method to detect structural changes of water, proteins and lipids in skin, hair and nails. Williams et al. [10] examined nails and the different structure of keratins. Ahsan et al. [9] compared the Raman spectra of different keratins: keratohyalin (stratum corneum, human nail, feather, and hoof horn). The study pointed out that the maximum keratin occurs mainly in the α-helical form, and that the C–S–C linkage shows the gauche-gauche-gauche conformation. Schröder et al. [11] and Grzesińska et al. [12] found that the several waves of the nail matrix exist in the basal form. Here, we therefore, investigate the time-dependent penetration of water into nails and its influence on protein and water structure [13]. Jones et al. [14] used NIR FT spectroscopy to examine the molecular structural changes of untreated nails. They found that protein-water interactions could lead to a slight change of the dihedral angle of the C–S–S–C bonds and to protein changes involving hydrogen of the α-helical protein [15]. Recently, Kozminski et al. [16,17], employed Raman spectroscopy, has been initially used to study collagen of the hoof horn of blood glucose in diabetic patients [18]. The research conducted in the human α-keratin

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doi:10.1016/j.clinchem.2012.11.012

Fig.1. IR Spectra of nails of normal subjects, there is no peak at 468 cm⁻¹.

Fig.2. IR Spectra of nails of Diabetic patients, appearance of peak at 468 cm⁻¹ is seen.

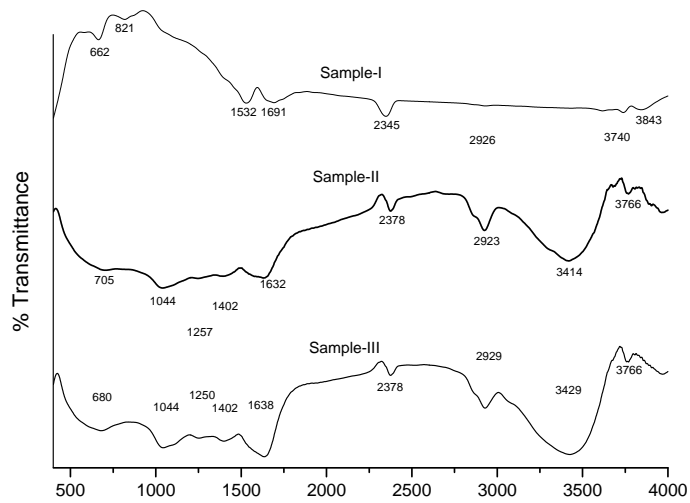


Figure 1.

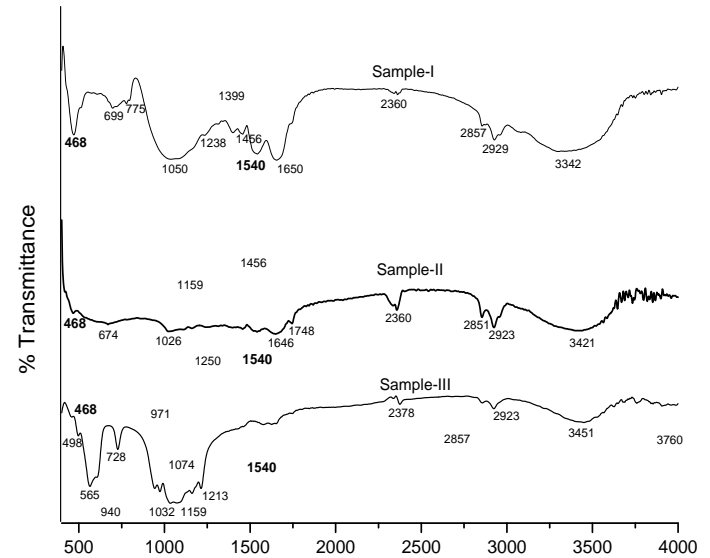


Figure 2.

DHN: Amide I band at 1645- 1659 cm⁻¹, where 1650 cm⁻¹ corresponds to amide I of α - helical structures. Amide II bands are observed around 1540 cm⁻¹. Amide III bands are observed around 1259cm⁻¹

NDHN: Amide I band is observed below 1640 cm⁻¹.the bands are observed like 1626, 1632 and 1638 cm⁻¹.The amide III band is observed as such at 1250 cm⁻¹.

Spectral Wave numbers and its configurations

Infrared absorption bands from proteins and peptides:

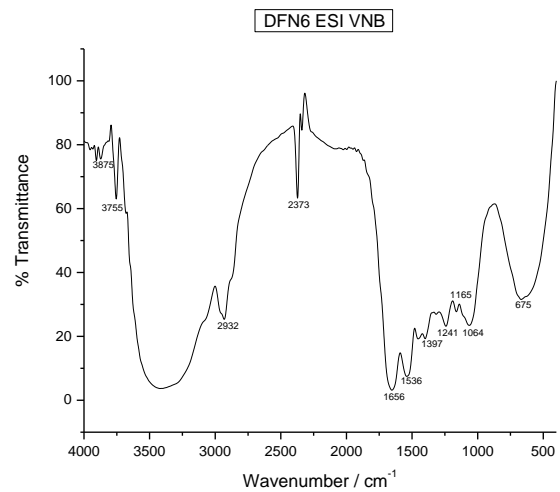
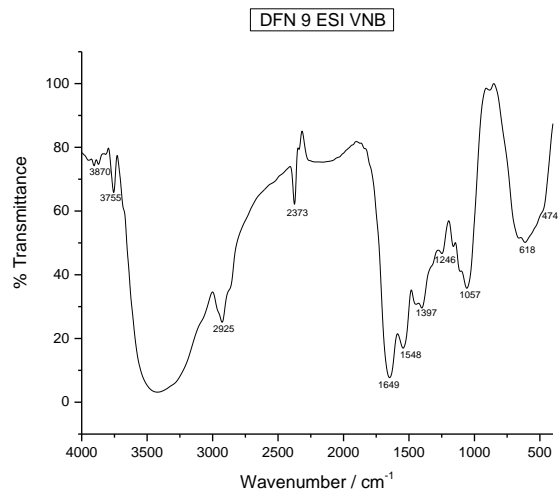
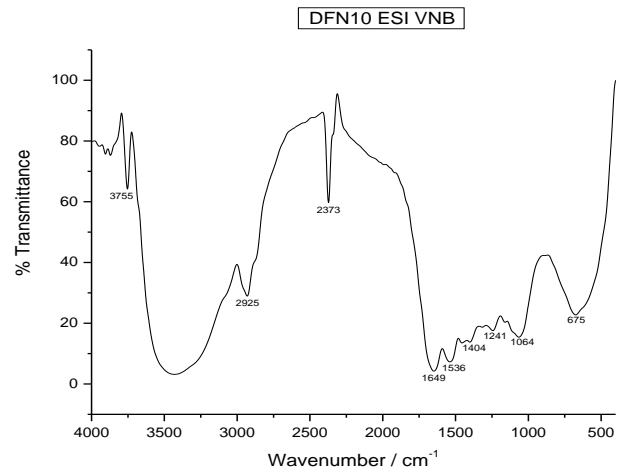
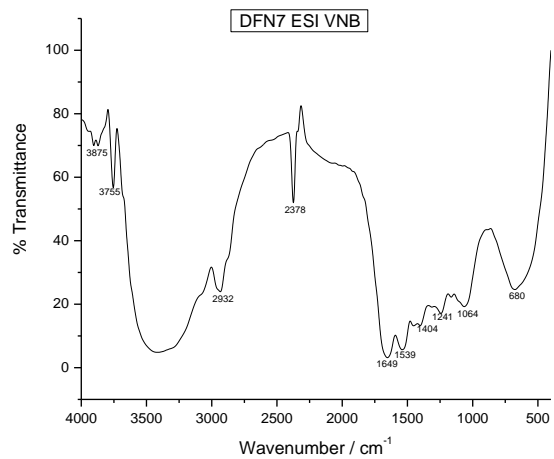
Amide A	3,300	NH stretching
Amide B	3,100	NH stretching
Amide I	1,600–1,690	C=O stretching
Amide II	1,480–1,575	CN stretching; NH bending
Amide III	1,229–1,301	CN stretching; NH bending
Amide IV	625–767	OCN bending
Amide V	640–800	Out-of-plane NH bending
Amide VI	537–606	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

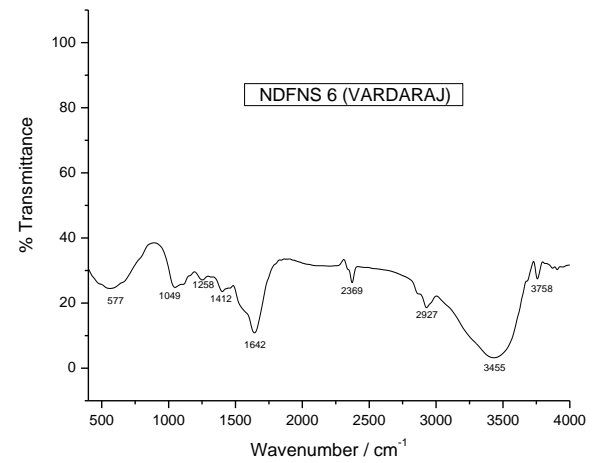
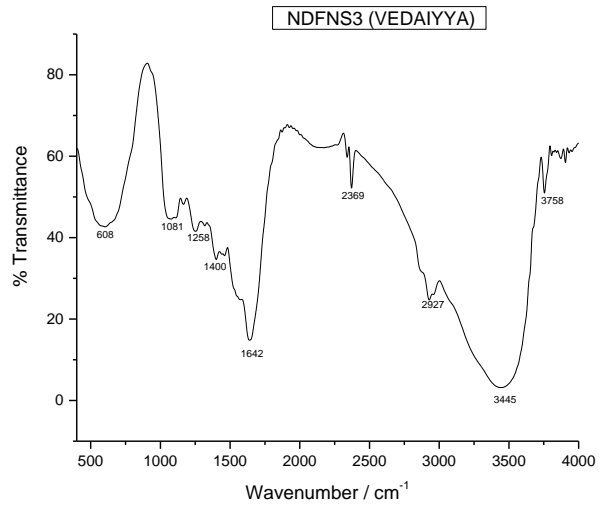
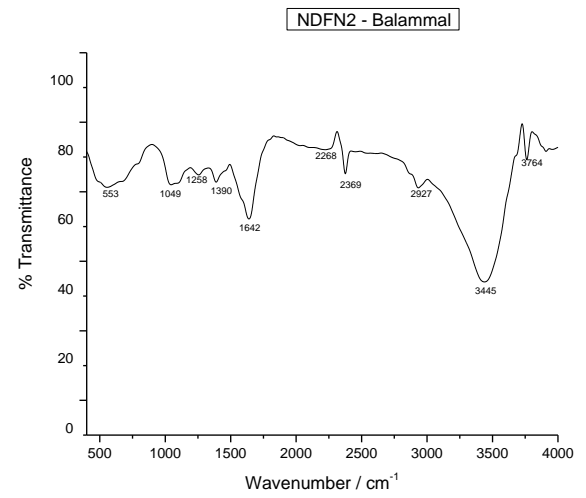
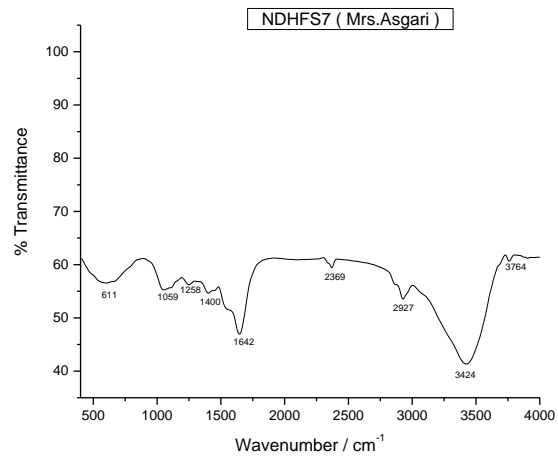
Amide I frequencies assigned to protein secondary structure

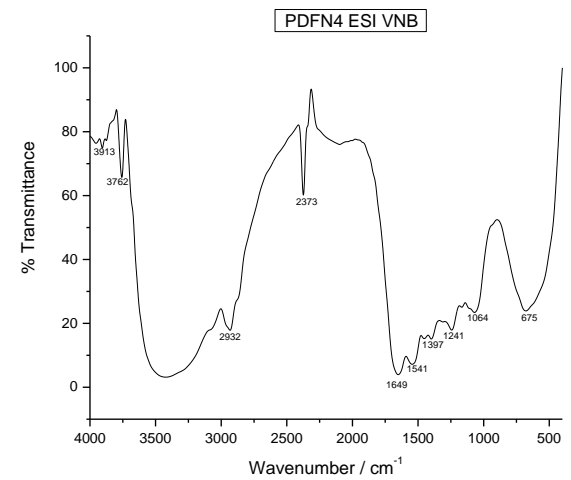
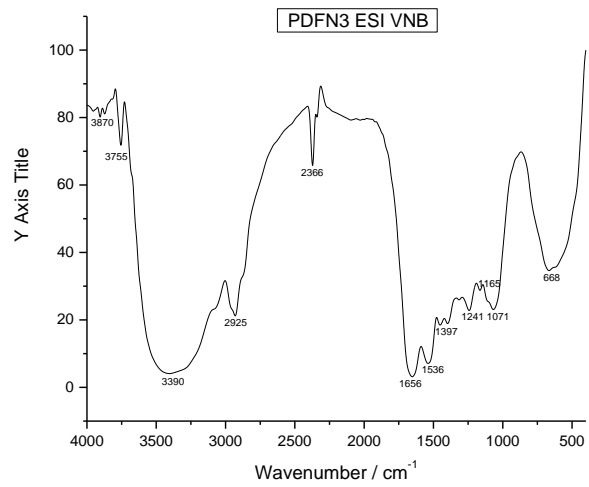
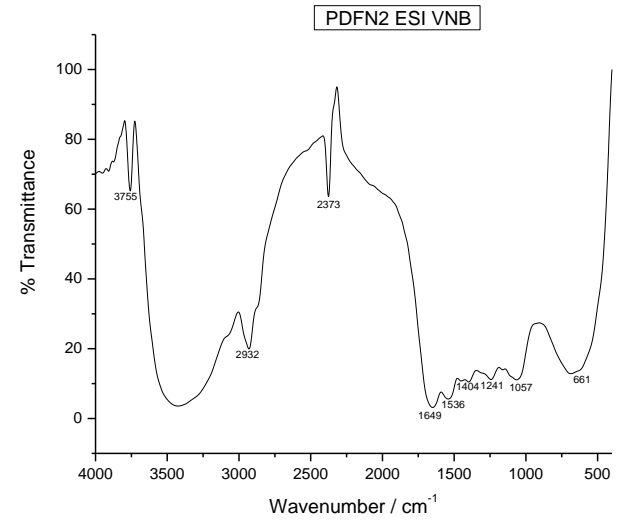
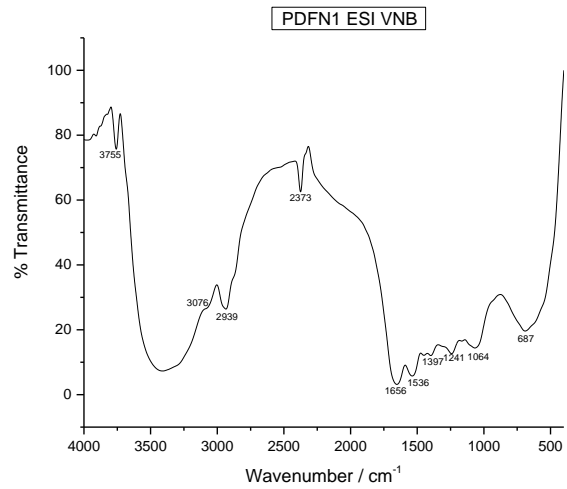
Secondary structure	Range	Average
A-helix	1,648–1,660	1,654
a α -helix turns	1,630	1,630
β α -sheet	1,612–1,641	1,625
	1,626–1,640	1,633
	1,670–1,694	1,682
Turns	1,662–1,684	1,673
Random coil	1,640–1,650	1,645

Based on experimental data and assignments available from the literature (Goormaghtigh et al. 1994; Mantsch and Chapman 1996; Pelton and McLean 2000)

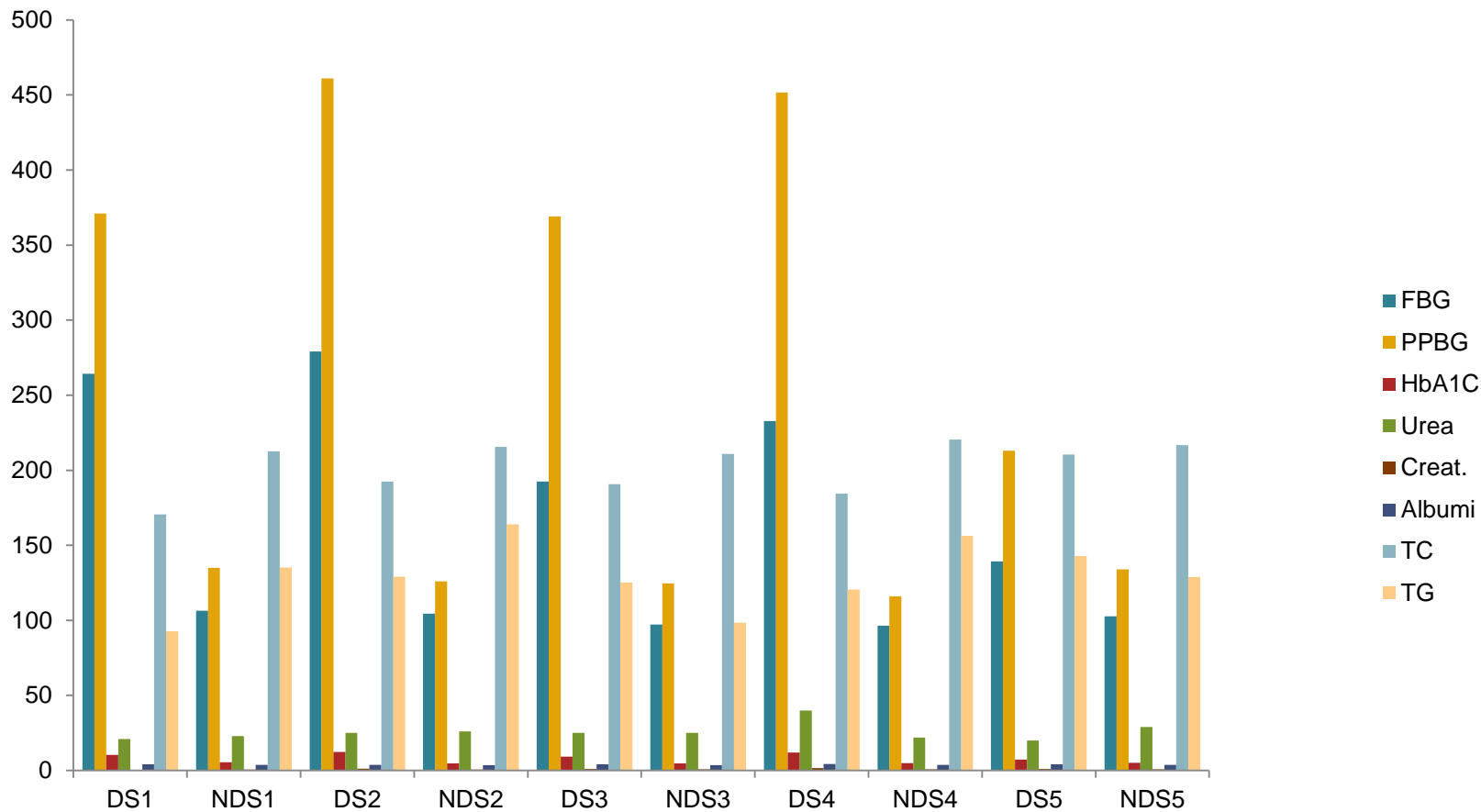
a According to Murayama and Tomida (2004)



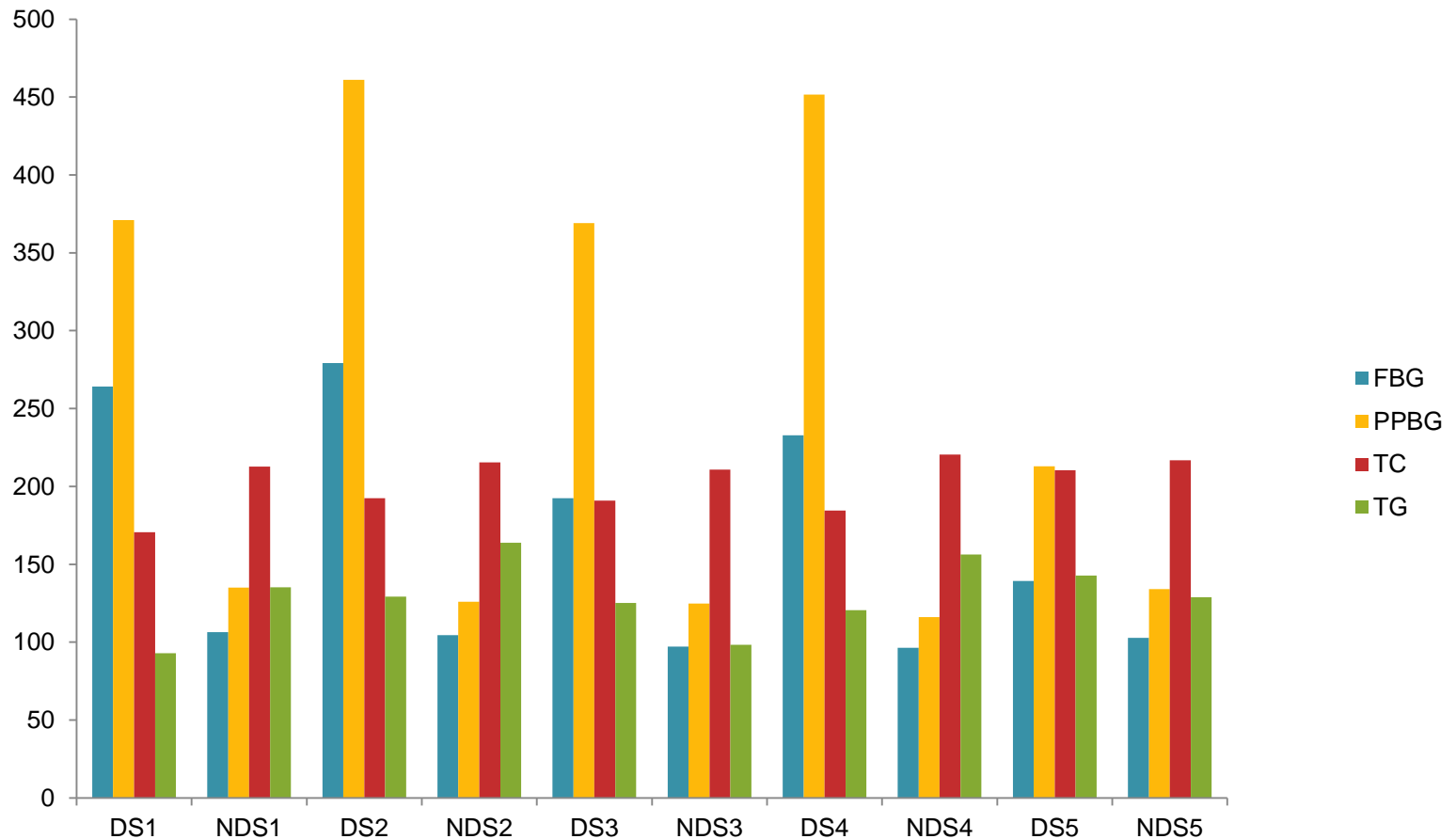




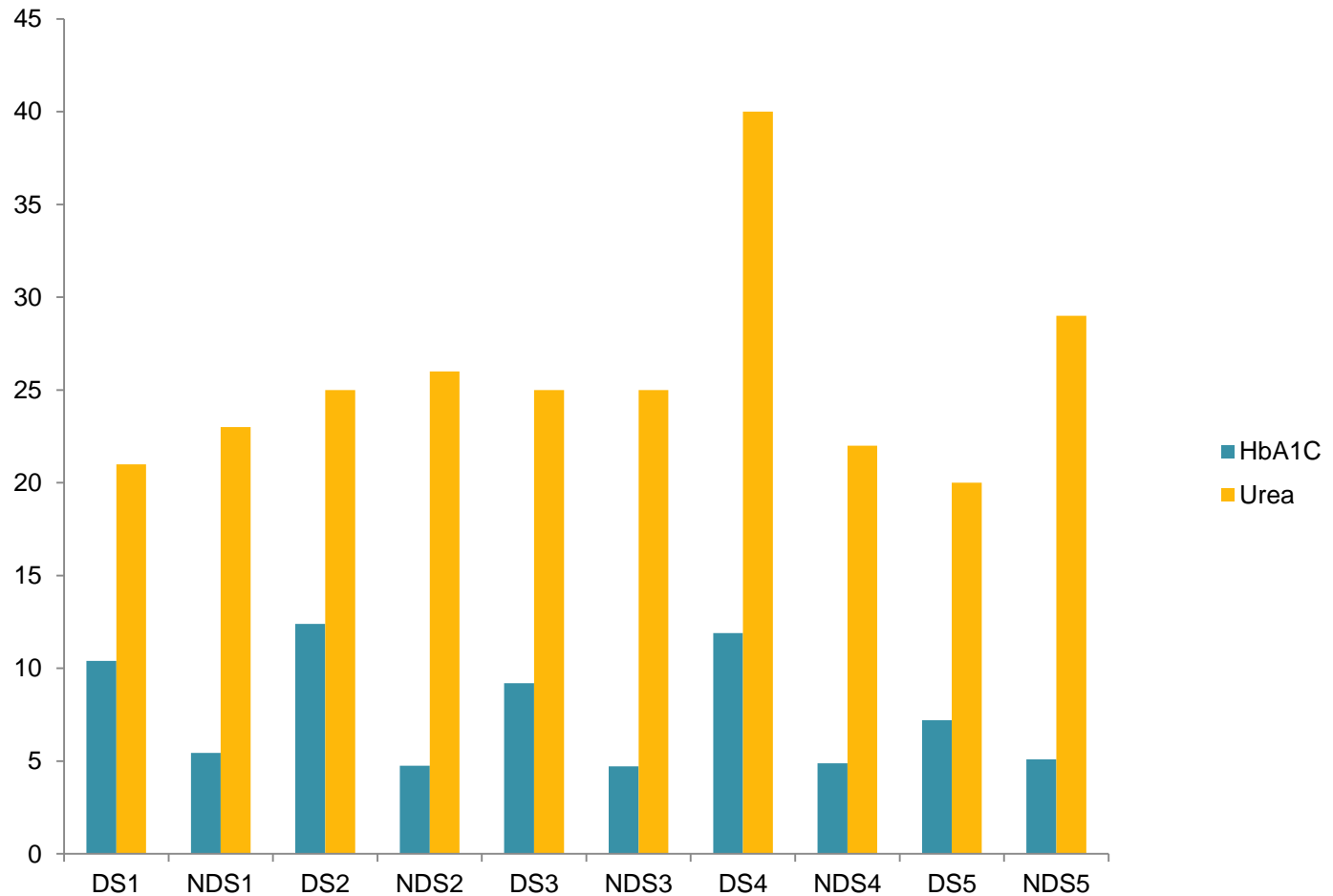
Graph shows over all Data of Blood Glucose Fasting & Postprandial, HbA_{1c}, Urea, Creatinine, Albumin, Total Cholesterol and Triglycerides for Both Diabetic and Normal Subjects:



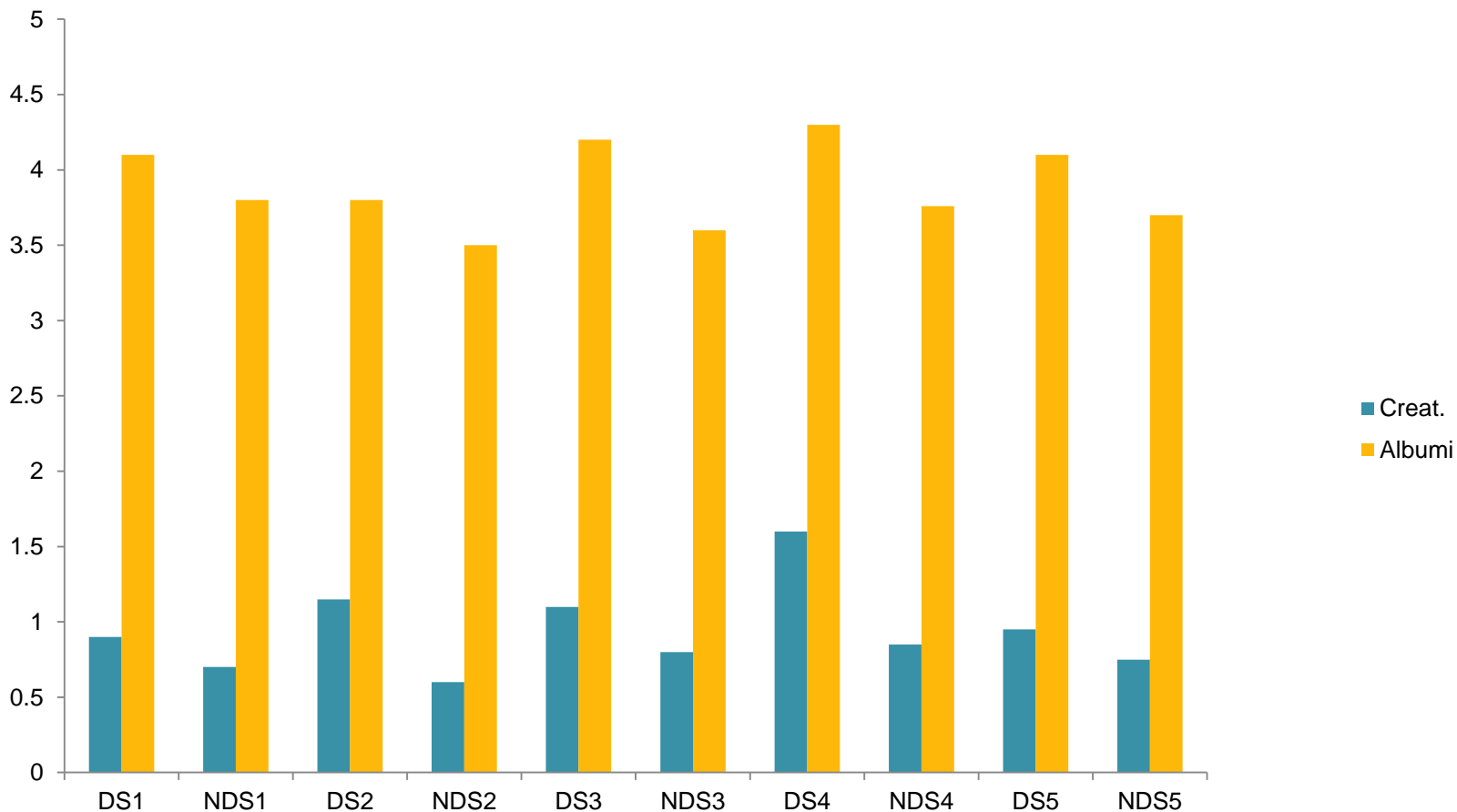
Graph shows Data of Blood Glucose Fasting & Postprandial, Total Cholesterol and Triglycerides for Both Diabetic and Normal Subjects:



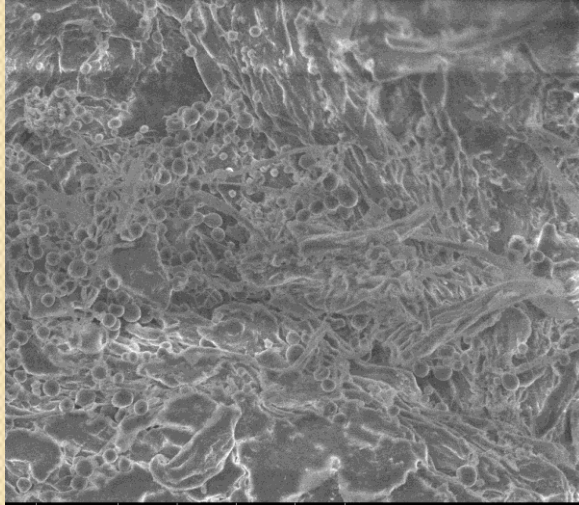
Graph shows Data of HbA_{1c} and Urea for Both Diabetic and Normal Subjects:



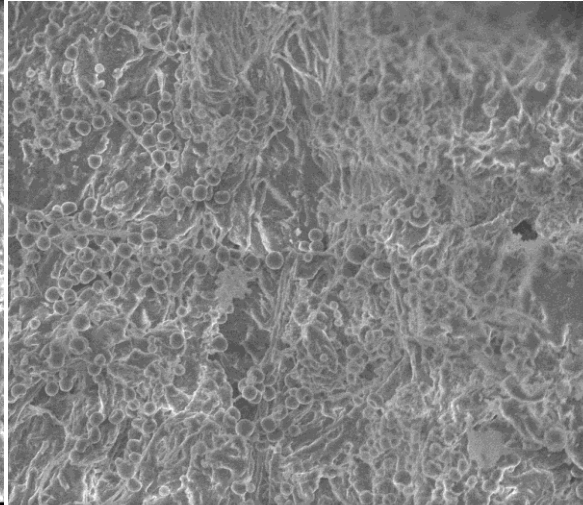
Graph shows Data of Creatinine and Albumin, for Both Diabetic and Normal Subjects:



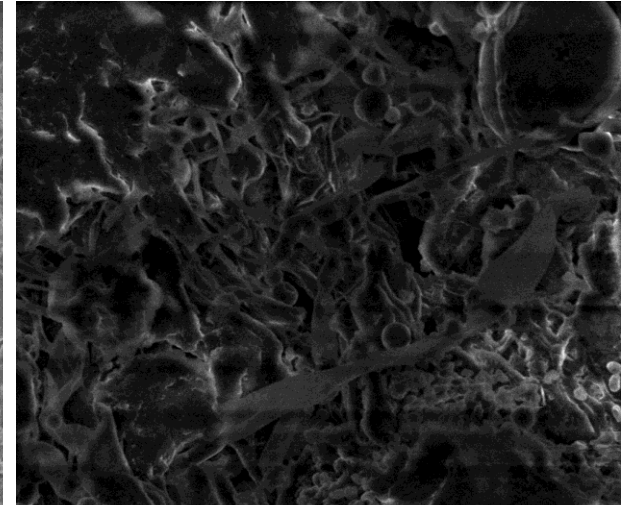
SEM Images of DHN and NDHN



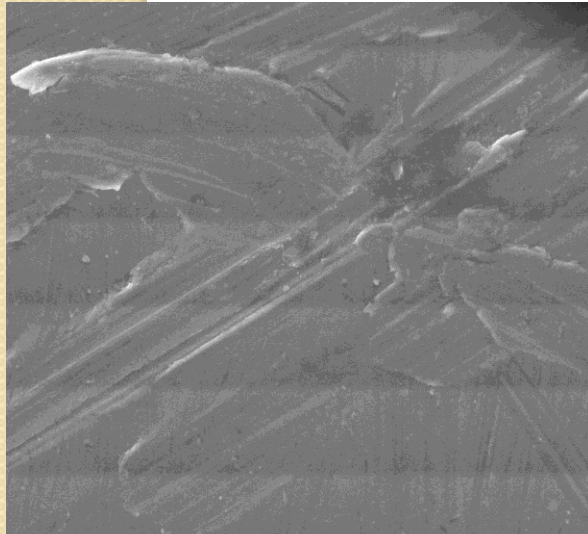
2/24/2011 HV mag WD det
6:07:01 PM 10.0 kV 800 x 9.7 mm GSED
50 μm
Quanta 3D FEG



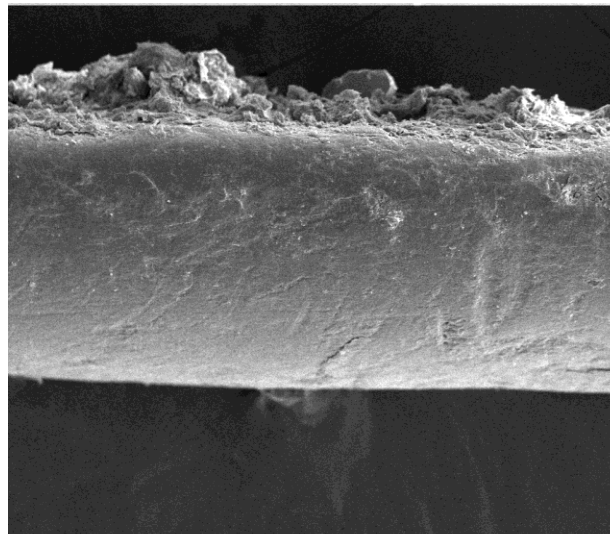
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50 μm
Quanta 3D FEG



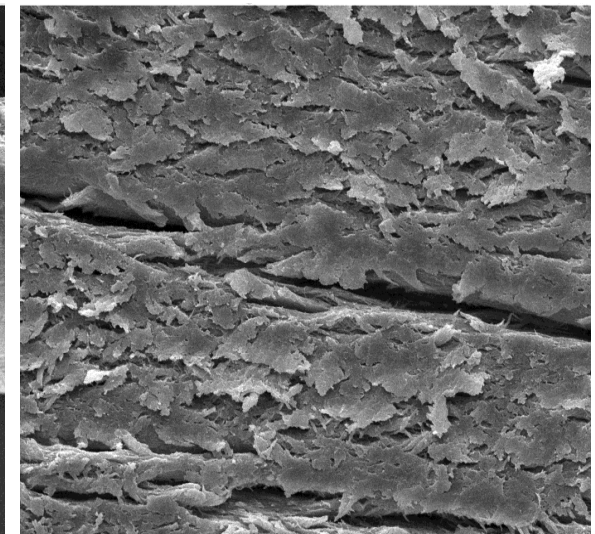
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30 μm
Quanta 3D FEG



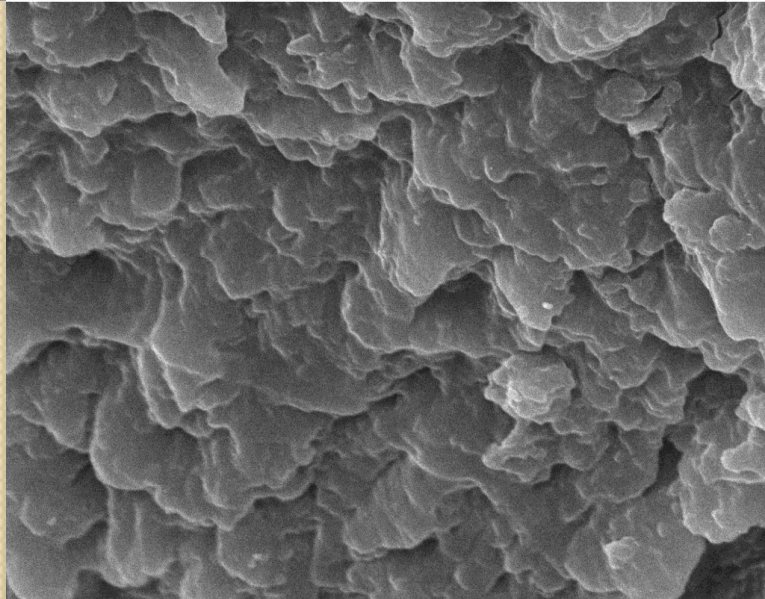
2/24/2011 HV mag WD det
5:37:08 PM 20.0 kV 700 x 9.8 mm GSED
50 μm
Quanta 3D FEG



Mag HV 3/16/2011 VacMode WD
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NDHNS1 Cr

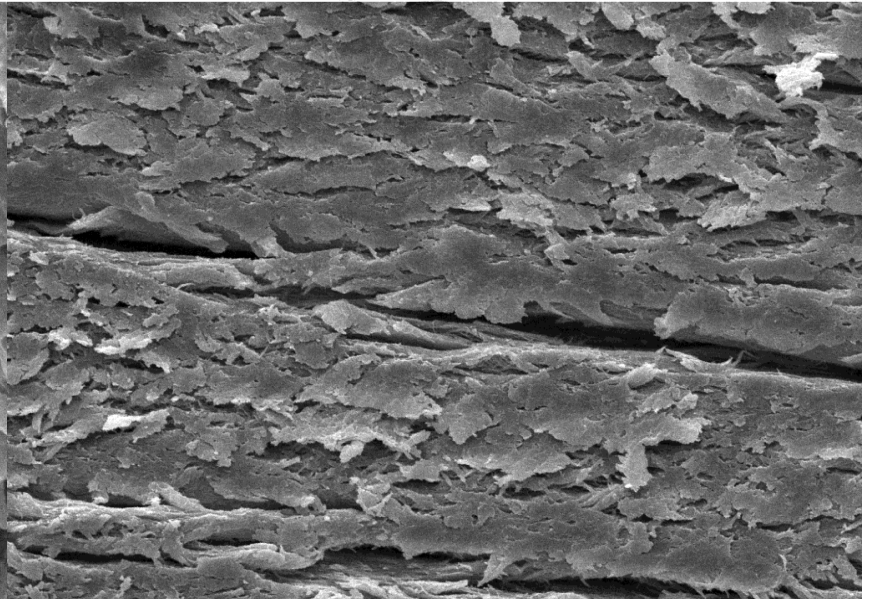


Mag HV 3/16/2011 VacMode WD
1000x 15.0 kV 1:11:08 PM High vacuum 10.2 mm
50.0 μm
DFNS1 Cr



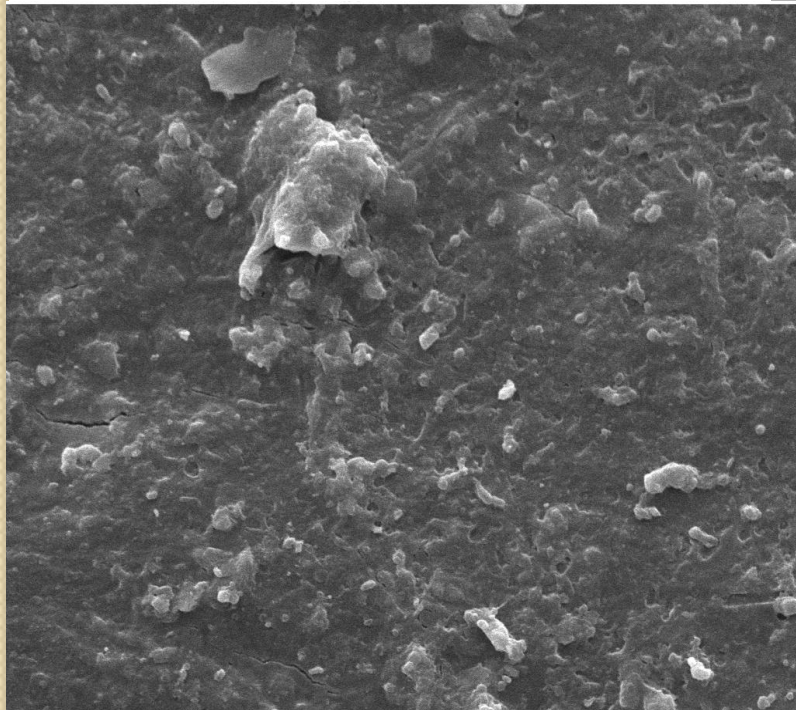
Mag 5000x HV 15.0 kV 3/16/2011 1:06:29 PM VacMode High vacuum WD 10.1 mm

10.0µm
DFNS1



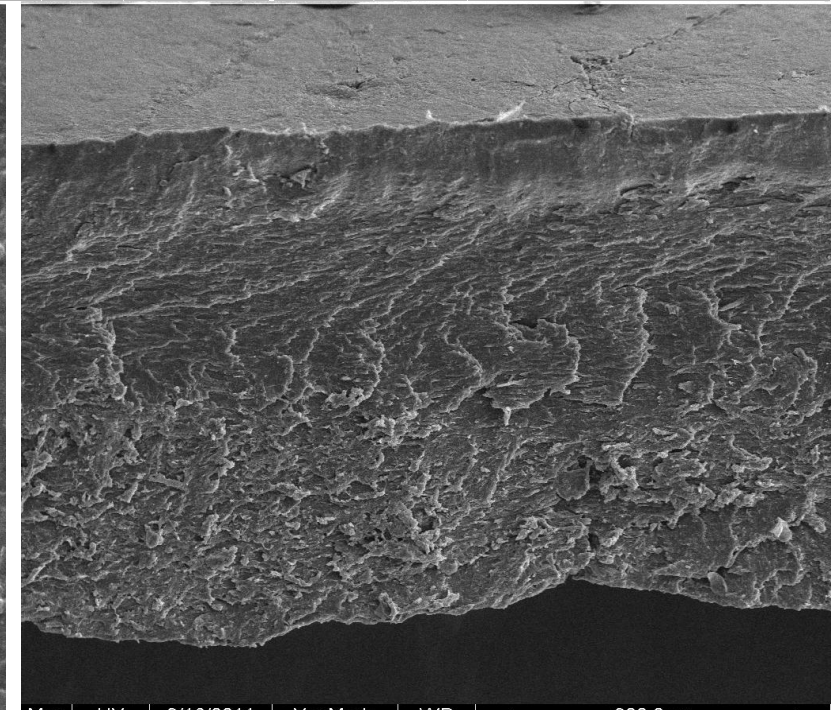
Mag 1000x HV 15.0 kV 3/16/2011 1:11:08 PM VacMode High vacuum WD 10.2 mm

50.0µm
DFNS1 Cr



Mag 2000x HV 15.0 kV 3/16/2011 12:24:08 PM VacMode High vacuum WD 9.4 mm

20.0µm
NDFNS1



Mag 150x HV 15.0 kV 3/16/2011 12:34:25 PM VacMode High vacuum WD 9.9 mm

300.0µm
NDFNS1 CR

Conclusion

- The significant change in nail is assessed by spectroscopically and confirmed by number of multiple and duplicated samples.
- This can be further confirmed with patients of diabetes with multi system disorders.
- In future we correlate the spectral changes of nails of non-diabetic, diabetic, prone to diabetic human specimen with biochemical, structural and functional changes in diabetic nephropathy, cardiopathy, and retinopathy.

Thank you