ADVANCED COLLAGEN-INSULIN SYSTEMS FOR DIABETICS

NICOLETA MANOLACHE[1], MĂDĂLINA GEORGIANA ALBU KAYA[2], IZABELA-CRISTINA STANCU[1], ŞTEFANIA MARIN[2], CIPRIAN CHELARU[2], DIANA DRĂGUŞIN[1], VLAD CONSTANTIN[3], GEORGETA PĂUNICĂ-PANEA[3]

[1]*University Politehnica of Bucharest, Faculty of Medical Engineering, 1-7 Gheorghe Polizu Str., 011061, Bucharest, Romania*

[2]*INCDTP - Division Leather and Footwear Research Institute, 93 Ion Minulescu Str., 031215, Bucharest, Romania, albu_mada@yahoo.com*

[3]*"Carol Davila" University of Medicine and Pharmacy, Faculty of Medicine, 8 Bulevardul Eroilor Sanitari, 050474, Bucharest, Romania*

Diabetes is the most common disease in our world. In our country over 50.000 of people are diagnosed with diabetes every year. Diabetes is a metabolic disorder generated by the pancreas inability to produce insulin in order to complete glucose metabolic process. Therefore, the life of the patients is sustained by the insulin administration. Existing methods on the market for insulin administration are not too various and there are a lots of disadvantages. Insulin glargine is a long-action, man-made version of human insulin. It replaces the insulin that is not produced by the body and helps the metabolic process of glucose. To increase insulin stability and releasing time, zinc oxide (ZnO) can be used for insulin encapsulation. Collagen is a natural protein that can be used as a support for controlled releases system because of its high biocompatibility. The aim of this study was to develop and characterize a controlled released subdermal support, for diabetics patients, made of a composite material based on collagen, zinc oxide and insulin. Obtained matrices were characterized by FT-IR spectroscopy, optical and scanning electronic microscopy, water up-take, degradation in collagenase solution. The results showed that the combination between collagen, ZnO and insulin could be suitable active supports for subdermal support with controlled delivery of insulin for diabetics.

Keywords: diabetes, collagen, insulin, zinc oxide.

INTRODUCTION

Nowadays diabetes represents one of the most common metabolic diseases for our modern world. It is estimated that diabetes affects around 5% of the grown-up population around the world and annually provokes the death of 3.2 million people. At present about 246 million and by 2025, 380 million people will need medical support that means a percentage of 7.1% of the grown-up population. In Romania 50.000 people are diagnosed with diabetes and 5% of them have the disease confirmed (helpnet.ro).

Diabetes mellitus is a chronic metabolic disorder caused by the incapacity of the pancreas to produce enough insulin (a hormone who controls blood sugar) to complete the metabolic process of the glucose. This disease left untreated will have bad consequences causing kidney failure, retinopathy with potential blindness, cardio-vascular and cerebrovascular problems.

The absolute lack of insulin, also known as Type I Diabetes refers to an auto-immune action of the body that destroys his own β-cells, the producing cells of insulin. In this case, it is necessary to sustain patients' life by administrating a dietary intake of insulin (Dansinger, 2015).

For patients with Type I Diabetes, the main methods available on market to administrate insulin are: insulin injections (pre-filled pen systems); insulin pumps and inhaled insulin. The main disadvantages of those methods are: lumps or scars where patients had to many injections, problems with batteries and catheters for insulin pumps

and contraindications regarding inhaled insulin for smokers and people who have asthma or COPD (Alberti *et al*., 1998). Insulin glargine is a long-acting, man-made version of human insulin. Insulin glargine works by replacing the insulin that is normally produced by the body and by helping move sugar from the blood into other body tissues where it is used for energy. It also stops the liver from producing more sugar (medlineplus.gov). Zinc oxide is used to enhance insulin stability during release because studies showed that when insulin was encapsulated with a zinc salt, EE encapsulation efficiency increased significantly, secondary structure was unaltered, and no degradation or aggregation products were found. Initial burst release and release kinetics were markedly changed with the addition of zinc salts. More than 87% of the encapsulated insulin was released over a 2-week period with the addition of a zinc salt (Manoharan *et al*., 2009). Collagen is a natural protein found in skin, bones and tendons. Because of its high biocompatibility is widely use in tissue engineering, pharmaceutical and cosmetic industries which makes it a good candidate for controlled releases system.

The main purpose of this article is to find an alternative of the already existing products on the market and to find a solution to overcome daily treatment by creating a controlled released subdermal support for diabetic patients. The drug release system is made of a composite material based on collagen, zinc oxide and insulin.

MATERIALS AND METHODS

Materials

The type I fibrillar collagen gel having a concentration of 2.84% (w/v) was extracted from calf hide as we previously described (Albu, 2011). Zinc oxide (ZnO) was purchased from Merck, Germany; the insulin glargine from LANTUS SoloStar (Germany). Sodium hydroxide and hydrochloric acid were of analytical grade. Type I collagenase obtained from *Clostridium histolyticum* was purchased from Sigma-Aldrich, Germany and glutaraldehyde (GA) from Sigma-Aldrich (Germany).

Preparation of Gel Composites and Their Corresponding Matrices

The concentration of each collagen gel was adjusted at 1% and 7.4 pH using 1M sodium hydroxide (the pH of the physiological medium in human body). ZnO and insulin glargine were added to collagen gel (w/v) and then the composite gels were cross-linked with 0.025% glutaraldehyde (reported to collagen dry substance) as Table 1 presents.

Table 1. Composition and name of collagen gels

Code of gels	Code of matrices, %	Coll, %	Insuline, %	ZnO, %
Coll	N1	1	0	0
Coll-Insuline	N2	1	0.25	0
Coll-ZnO	N3	1	0	0.25
Coll-insuline-ZnO	N4	1	0.25	0.25

The collagen gels were freeze-dried using Delta 2-24 LSC (Martin Christ, Germany) lyophilizer using the freeze-drying program presented in Figure 1.

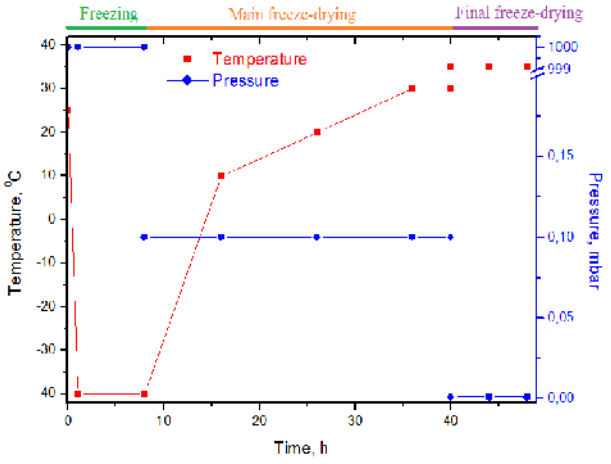


Figure 1.Graph chart of freeze-drying process (Marin *et al*., 2014)

The resulted matrices were named as shown in Table 1 and were characterized using the following methods.

Methods

FTIR-ATR Analysis

FT-IR spectral measurements were recorded by spectrophotometer Jasco FT/IR-4200. All the spectra were recorded at the following parameters: spectral range 4000-600 cm^{-1}, resolution 4 cm^{-1} with 30 acquisitions per each sample.

Water Up-take Capacity and Enzymatic Degradation

The water uptake capacity and enzymatic degradation were performed using the protocol as we previously described (Albu, 2012) on the obtained matrices with compositions according with Table 1.

Optical and Scanning Electron Microscopy (SEM)

All images were captured with a Leica Stereomicroscope model S8AP0, 20-160x magnification capacity. For better evaluation of the samples, a 20x magnification and incident external cold light were used and optical images were obtained.

SEM analysis was performed using a Quanta 200 FEI microscope in order to obtain high-resolution images of collagen discs surfaces to study the structure of collagen fibers and the absorption of ZnO.

RESULTS AND DISCUSSION

After composite gels lyophilization, the 3D porous collagen sponges based on collagen, insulin and ZnO, cross-linked, were obtained, with the aspect presented in Figure 2.

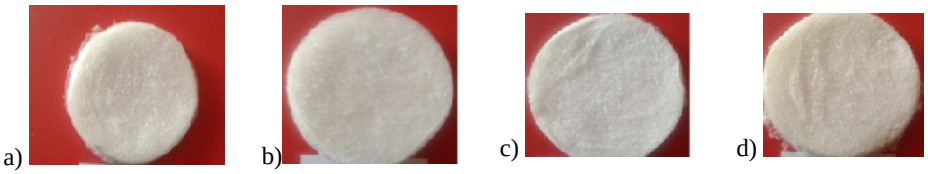


Figure 2. Collagen spongious forms: a) N1; b) N2; c) N3; d) N4

The samples from Table 1 were analysed by FT-IR spectroscopy, water up-take, optical and SEM microscopy, enzymatic degradation.

From the FT-IR spectra (Figure 3) the typical bands from collagen can be observed: amide A, B, I, II and III (Albu, 2011) at 3300 cm^{-1}, 2936 cm^{-1},1630 cm^{-1},1544 cm^{-1}, and 1238 cm^{-1}.

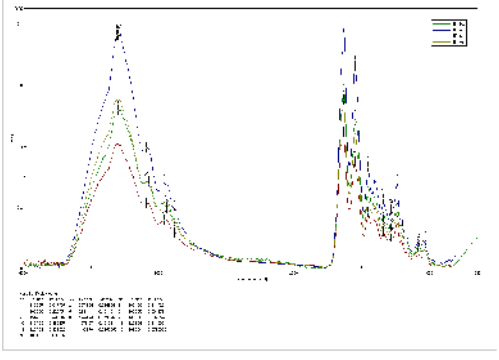


Figure 3. ATR-FTIR spectra of overlayed spectrum of collagen samples from Table 1

There are not significant changes when insulin and ZnO was added, possible because too less amount of them.

The water up-take for all the studied samples is presented as kinetics during 24 hours in Figure 4.

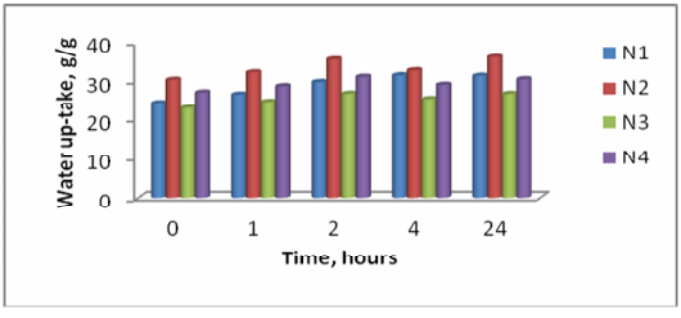


Figure 4. Water up-take during 24 hours for spongious forms

Figure 4 presents the water up-take during 24 hours for the studied samples. The samples with insulin absorbed the higher amount of water than the others, due to higher

content of protein. The samples with ZnO absorbed lower amount of water due to their more compact structure.

Optical images showed porous structures with interconnected pores. In Figure 5 is presented as example the Coll-Insulin-Zn.

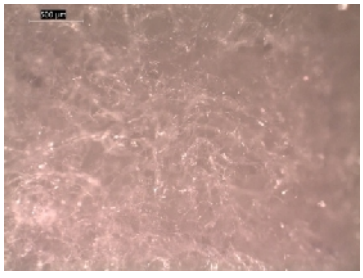


Figure 5. The optical microscopy image for Coll-Insulin-Zn

Figure 6 (a and b) presents SEM images of Coll and Coll-Insulin-ZnO samples and can be observed the fibrilar structure of collagen, the porosity of the samples and the adsorption of zinc salts on fibrilar structure.

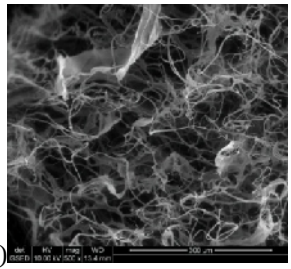
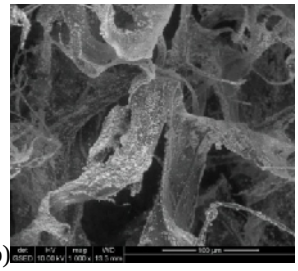
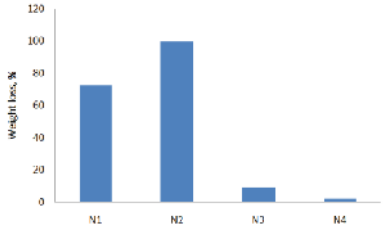
a)  b) 

Figure 6. SEM image for: a) Coll (x500) and b) Coll-Insulin-ZnO (x1000)

It is clearly evidenced that ZnO deposited on collagen fibers crosslinked them in a more compact structure. The pore sizes are between 50 and 250 µm.

The *in vitro* behaviour of samples in collagenase solution during 24 hours for all samples and during 2 weeks for samples with ZnO is presented in Figure 7 a and b.

a) 

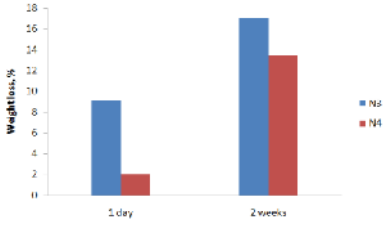
b) 

Figure 7. Weight loss of samples in collagenase solution: a) during 24 h and b) comparative study for 1 day and 2 weeks for samples N3 and N4

The degradation results are in correlation with water up-take ones. The sample N2, which contain collagen and insulin and which had the higher absorbance capacity is totally degraded during 24 hours, meanwhile the collagen as alone is degraded in 80%. The samples with ZnO are very slowly degraded, 9.12% N3 and 2.05% N4 during 24 hours. They kept stability also after 2 weeks, the weight loss being of 17.01% for N3 and 13.43% for N4.

CONCLUSIONS

Insulin was incorporated in collagen and collagen with ZnO in order to obtain advanced delivery systems for diabetics. Spongious lyophilized forms based on collagen, ZnO and insulin and their combination (together and two by two) were obtained and physical-chemical characterized. The results showed that the combination between collagen, ZnO and insulin could be suitable active supports for subdermal support with controlled delivery of insulin for diabetics.

Acknowledgements

The authors acknowledge the financial support from the project PN 201/2014 (Zettaskin).

REFERENCES

Alberti, K.G. and Zimmet, P.Z. (1998), "Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation", *Diabetic Medicine*, 15, 539-553.

Albu, M.G. (2011), *Collagen Gels and Matrices for Biomedical Applications*, Lambert Academic Publishing, Saarbrücken, 23-24.

Dansinger, M. (2015), "The Facts About Insulin for Diabetes", WebMD, available at http://www.webmd.com/diabetes/guide/overview.

Manoharan, C. and Singh, J. (2009), "Insulin Loaded PLGA Microspheres: Effect of Zinc Salts on Encapsulation, Release, and Stability", *Journal of Pharmaceutical Science*, 98, 529-542.

Marin, S., Marin, M., Ene, A-M., Kilic, T.I., Chelaru, C., Albu, M. and Ghica, M.V. (2014), "Collagen-Doxycycline Spongious Forms For Infected Tissue Treatment", *Proceedings of the 4th International Conference on Advanced Materials and Systems (ICAMS)*, Bucharest, Romania, 249-254.

*** http://www.helpnet.ro/metabolice-nutritie.

*** https://medlineplus.gov/druginfo/meds/a600027.html.