An Intrauterine device (IUD) is a T-shaped device made of plastic and copper [1]. IUD is inserted into the uterus with the role to release copper, thus preventing pregnancy [2]. First Copper IUD was marketed in the 1970’s [3].

Hormonal Intrauterine Devices (IUD) were developed based on copper IUD with the role to release a daily amount of hormones. From a structural point of view Levonorgestrel-IUD (L-IUD) is a small T-shaped piece of plastic, which contains levonorgestrel. When compared with the oral route of L-IUD administration, it provides some advantages because it releases levonorgestrel directly into the uterus. Our aims were: (1) to compare the thickness of uterine junctional zone (UJZ) in two groups of patients with and without inserted L-IUD, (2) to evaluate the changes in thickness of UJZ after the insertion of L-IUD in two patients with adenomyosis, and (3) to obtain and characterize a similar T-shaped polyurethane (PU) foam used as a levonorgestrel delivery system. On the one hand, the results confirm that L-IUD is effective in thinning the UJZ and in the treatment of adenomyosis, while on the other hand, the characterization of the PU materials (Zetasizer measurements, their thermal degradation, UV-Vis spectra and skin irritation tests) revealed that this polymer can be used as a safety and prolonged levonorgestrel carrier.

Keywords: adenomyosis, junctional zone, levonorgestrel-releasing IUD, mexametry, polymer structures, thermal behavior, Zeta potential.
a PU carrier used as brassinolide delivery system. The administration of hormones is a top domain in bio-nanotechnology due to their side-effects and due to the need for a controlled release.

The main aims of this research were: (1) to compare the UJZ thickness in two groups of patients with and without inserted L-IUD, (2) to evaluate the changes in the UJZ thickness after the insertion of L-IUD in patients with diagnosis of adenomyosis, and (3) to continue one of our previous studies in the domain of hormones delivery [16].

**Experimental part**

**Measurement of UJZ thickness**

UJZ thickness was measured using a Voluson E8 Expert ultrasound machine equipped with a R6-12 volumetric endovaginal probe (General Electric, Boston, USA). A 3D volume of the uterus was acquired and UJZ was measured at the maximum and minimum thickness (fig. 1). A mean was calculated for each patient.

**Patients with one UJZ measurement**

![Fig. 1. 3D transvaginal ultrasound images (a) without and (b) with L-IUD](image)

The thickness of UJZ was studied in accordance with the aforementioned methodology in two groups of patients. The first group consisted of 311 women with and without uterine pathology, which had no inserted L-IUD (Mirena). The second group of patients consisted of 10 women with uterine pathology and with inserted L-IUD (Mirena). In the second group, we measured the thickness of UJZ at a variable interval after insertion of Mirena.

**Patients with two measurements of the endometrial junctional zone (EJZ) thickness**

The effect of L-IUD on EJZ thickness was studied in two patients, with diagnosis of adenomyosis. The measurement was performed in the day of L-IUD insertion and a minimum of 6 months later.

**The synthesis and the characterization of PU materials**

In order to synthesize PU foams, Elastoflex W 5516/115 was achieved from Elastogran (Hungary), while diphenylmethane-diisocyanate, toluene-diisocyanate, 2-amino-2-methyl-1,3-propanediol and 2-hydroxymethyl-1,3-propanediol were obtained from Sigma-Aldrich (Germany) and crystalline 1,4-diazo-bicyclo[2,2,2]octane from Air Products and Chemicals (U.S.A). The obtaining of PU microstructures was based on levonorgestrel from Cayman Chem. (U.S.A.), the aliphatic diisocyanate, polyethylene glycol, M=200, and Tween® 20 were achieved from Merck (Germany), while ethylene glycol was obtained from Lach-Ner s.r.o. (Czech Rep.).

First of all, PU microstructures were obtained following a procedure already described in our previous papers [17-19]. This procedure was repeated twice to obtain two different samples: PU_0 (empty microstructures), PU_1 (structures containing levonorgestrel). The PU microstructures were included between the raw materials of two flexible foams, synthesized based on the protocol described by F. Borcan et al. [20].

The electric charge of the PU microstructures and their size were monitored with a Zetasizer equipment (Cordouan Technol., France) at 25 °C, with a time interval (~20 µs), channels’ no. (~450), laser power (~90%), a continuous acquisition, Padé-Laplace as analysis mode, medium resolution, and Smoluchowski model as Henry function.

The thermal behavior of PU microstructures was studied using a Mettler-Toledo DSC1 (Mettler-Toledo, Switzerland) between 35 and 300 °C in an inert atmosphere (Argon), using aluminum crucibles with perforated lids and a 10°/min heating rate.

Five healthy young volunteers (2 men and 3 women, between 26 and 33 years old) were involved in the study of the skin irritation potential of PU microstructures. The tests were done with a Courage&Khazaka Multiprobe Adapter (Germany) equipped with a Mexameter® MX18 probe by a short press on the forearm skin in the area previously treated with 0.5 mL PU microstructures’ diluted aqueous suspensions (1:60 w/v).

The levonorgestrel release rate from the PU foam was measured by its concentration in a medium as absorbance at 242 nm using an UVI Line 9400 Spectrophotometer (Si Analytics, Germany) and Beer-Lambert-Bouguer law at every fifth day for one month while the sample of PU foam containing microstructures with levonorgestrel were maintained in a Kokubo degradation medium.

**Statistical analysis**

Data are expressed in median±SEM. Comparisons were performed by the Mann-Whitney U rank sum test. We used Instat Software (GraphPad Software, San Diego, CA, USA) for analysis.

**Ethical issues**

We confirm that all the research meets the ethical guidelines, including adherence to the legal requirements of the study country.

**Results and discussions**

**Comparison of UJZ thickness**

The results showed that although patients with uterine pathologies and inserted L-IUD (n=10) still showed higher values of UJZ than patients without L-IUD (n=311); the difference did not reach a significant threshold (5 ± 0.73 vs. 4 ± 0.12, p = 0.051) (fig. 2).

**Thinning of UJZ thickness after treatment with Mirena**

![Fig. 2. UJZ thickness in patients with and without inserted L-IUD](image)
It was observed in this research the thinning of UJZ (fig. 1) in the case of two patients monitored by UJZ thickness measurements during the L-IUD therapy. The results are observational and further study is needed to evaluate the measurements before and after the L-IUD insertion.

Hormonal IUD are used as a contraceptive method [21] or in the therapy of several conditions such as: heavy bleeding [22], women at risk for thrombocytopenia [23], endometrial hyperplasia [24], abnormal uterine bleeding in premenopause [25], endometriosis [26], and adenomyosis [27]. Before inserting an IUD, the patient should be investigated thoroughly because the symptoms caused by the IUD insertion may delay the diagnosis of serious illnesses. Some reports showed that for instance irregular bleeding caused by L-IUD could delay diagnosis of cervical cancer [28].

Insertion of hormonal IUD is mostly facile but attention should be paid to ultrasound assessment because the L-IUD has a different ultrasound appearance compared to copper devices. Diagnosis of misplaced L-IUD is difficult [29].

Adenomyosis is defined as the presence of benign endometrial glands and stroma in the myometrium [30]. However, there is no agreement on the classification of adenomyotic lesions from both the histopathological and the imaging point of view [31]. Adenomyosis is associated with pain, pregnancy loss [32], uterine bleeding or infertility, but sometimes no symptoms are present [31]. New MRI and ultrasonography techniques allowed development of a scoring system for uterine adenomyosis [33,34].

Management of adenomyosis must take into account the patient's clinical situation and their desire for further fertility preservation [35]. The spectrum of adenomyosis therapy include removal of adenomyoma by minimal invasive or open surgery, hysterectomy, uterine artery embolization or L-IUD insertion [35,36].

Since previous studies showed a good correlation between UJZ and histopathological features, we focused our research on the evaluation of the thickness of the UJZ in patients with adenomyosis [37]. Our results showed that there is no statistically significant difference between the thickness of UJZ in the two groups of women: those with uterine morbidities and Inserted L-IUD, and those without an L-IUD. We formulate the hypothesis that L-IUD contributed to thinning UJZ in patients with uterine morbidities and L-IUD.

A first weak point of our study is that we do not have values of measurement of UJZ before L-IUD insertion, but we suppose that these values are increased due to the fact that these patients have uterine pathologies. A second weak point is that the group of patients without L-IUD is heterogeneous, made up of women with and without uterine pathologies [38]. This is why we cannot be sure that UJZ values in this group are not elevated due to the presence of women with uterine morbidities which had a thickened UJZ. To overcome these deficiencies, further studies must be performed on control groups that include only women without uterine pathologies. However, our results show the thinning of UJZ in two patients with adenomyosis in which we measured the UJZ before and after L-IUD insertion. These observations advocate for the thinning effect of L-IUD on UJZ thickness.

**PU characteristics**

Diluted suspensions of the two samples containing PU microstructures (1:60, w/v) were used in Zetasizer characterization, while dried samples were heated during the thermal behavior investigation. Figure 2 and Table 1 present the size, homogeneity, and the surface charge of the obtained PU microstructures. The obtained DSC curves did not reveal any interesting point in the studied temperature interval, because the polyurethanes Tg is below 0 °C, while their degradation start over 300-320 °C. The absence of levonorgestrel melting point around 240 °C is probably due to its good encapsulation and/or to the wash of the free compound in the synthesis products' purification step.

The big size of these PU structures is confirmed by the low values of Zeta potential; M.F. Munteanu et al. [39] described that colloidal structures with Zeta potential values between 20 and 30 mV present a medium stability against the tendency to form agglomerations. The absorption and reflection of light by the skin represent the principle of erythema level observation (fig. 3). Very slow growing trends were observed ~20 arbitrary units / month and based on a 1,000 units scale, these low modifications indicate that our samples can be considered safe for administration to humans. Probably the most important determination of PU foams that were synthesized in this research is the evaluation of the material efficacy expressed as the profile of the bioactive substance release. Figure 4 reveals the levonorgestrel concentration inside the degradation medium (a simulated body fluid prepared after T. Kokubo’s recipe from 2006) and it can be observed that polyethers-based PU microstructures represent a carrier with a slow release.
Conclusions

The results of this research confirm that L-IUD is effective in thinning the UJZ and in the treatment of adenomyosis. UJZ could be monitored by 3D ultrasound. Measurement of the junctional zone can be proposed to monitor the course of treatment in adenomyosis patients. On the other hand, the polyethers-based PU microstructures present a very slow degradation and a prolonged release of the encapsulated bioactive agent, which is a very strong advantage in the hormones’ administration.

References

27. STANEKOVA V, WOODMAN RJ, TREMELLEN K. The rate of euploid miscarriage is increased in the setting of adenomyosis. Hum Reprod Open. 2018 Jul 4;2018(3): hoy011.

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