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Assessment of Modified Knitted Hernia Implants after Implantation: I. Biological Effect on the Structural and Usable Properties

Abstract
The purpose of this article is to discuss the solution of the main clinical adverse events occurring in less invasive reconstruction of hernia. The study attempted to design the knitted implants, contributing to reduce the risk of adhesion to the viscera. Specially designed knitting structure of the hernia implant allowed to introduce the one-side enhancement of the surface in the form of loops stitched-out of the surface accelerated the connective tissue in-growth. The opposite side was modified the treatment in low-temperature plasma of low molecular weight fluorogenic compound resulted in the deposition of fluoropolymer nano-layer. The effect of the modification on the adhesion of implant to viscera was studied in vivo. Additionally, the influence of the implantation time on the structural properties, the connective tissue covering implants single monofilaments as well as the stability of the fluoropolymer layer formed during the implantation were detailed investigated.

Key words: *knitted hernia implants, low temperature plasma treatment, biological evaluation.*

■ Introduction

Hernia reconstructions are the main procedure performed in general surgery in the world. Less-invasive procedures are more frequently applied as they are relatively low time consuming and economical surgery. However, the implementation of man-made materials in sublay position causes the increase in the risk of uncovering internal organs in implant adhesion, being the main complication of intraperitoneal, laparoscopic hernia treatment.

On the other hand, the quick and effective fixation of hernia implants during surgery supports practitioners in the less-invasive hernia reconstruction. An appropriately designed structure of the hernia implant is able to prevent uncontrolled migration of the implant by connective tissue growth due to quick fixation.

There are several solutions supporting surgeons during intraperitoneal hernia treatment. The application of resorbable implants of polylactide (PLA) located between the internal organs and hernia mesh was the first method of visceral adhesion prevention. This procedure has significantly higher clinical effectiveness as compared with the standard technique, where a natural, abdominal structure – *omentum*, is used for preventing internal organs from direct implant contact. Presently three main types of man-made implants are used:

a) the hybrid hernia mesh, being the composition of a knitted mesh covered on a single or both sides by resorbable films made of PLA, cross-linked carboxymethylocellulose or collagen;

b) expandable implants made of polytetrafluoroethylene (PTFE);

c) knitted implants made of polyvinylidene fluoride (PVDF) or a composition of PVDF and PP.

The main disadvantage of the above medical devices is a relatively high surface mass inducing highly acute and chronic inflammatory reactions that lead to the formation of a thick scar resulting in hernia recurrence, loss of patient comfort, stiffness of the abdominal wall, etc. Moreover degradation products leaking out of the resorbable layer of types a. and b. intensify the inflammatory reaction effect.

The new idea for hernia implants was the subject of research described in [1 – 5]. The knitted technique specially elaborated [3] allows to fabricate an enhanced surface of the knitted mesh formed by loops stitched from the surface. The above design should improve the growth-in process of the connective tissue and finally cause the quick integration of the implant structure with surrounding fascia.

The opposite side of the hernia implants is modified in a relatively short time - low temperature plasma of fluoroorganic compounds polymerising during the process in the form a nano-layer located on the surface of the knitted implants. The modification proposed allows to design a hernia implant with significantly lower mass as compared with the a., b. or c. types of clinically used products. Moreover the nano-layer of the fluoropolymer prevents visceral adhesions.

The aim of the research was to verify:
■ potential changes in the microstructures of the explants and
■ the stability of the fluoropolymer layer formed during the implantation.

Moreover quantitative and qualitative evaluation of the connective tissue growth-in in the newly-elaborated structure of the knitted implants and of the risk of adhesion formation were also performed.

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◼ Materials and methods

Materials

Raw-materials

The properties of the raw materials – polypropylene monofilament have been described in [1].

Designing of the knitted implants

The process of fabrication of knitted implants has been described in detail in [2 - 3]. One-side modification of the surface of knitted implants using low temperature plasma in the presence of fluorine organic derivatives (tetradecafluorohexane/Fluka) is described in [4 - 5].

Knitted implants with a specially designed structure and one-side modification using low temperature plasma treatment were fabricated as test samples, and knitted implants with an identical structure without one-side modification were used as a reference.

Methods

Intra-abdominal implantation of the knitted implants

Intra-abdominal implantation of the test samples as well as the reference was performed based on the PN-EN ISO 10993-6:2007 standard.

The samples and reference tested were implanted in 15 rabbits in sublay (intraperitoneal) positions simulating the natural position of the hernia mesh during laparoscopic hernia reconstruction. The implantation periods were 2, 4 or 12 weeks, as described in Standard PN-EN ISO 10993-6:2007. In each period 5 animals were used. The sample tested (on left side) and reference (on right side) were implanted into the fascia of each rabbit.

Samples with a size of 2 × 4 cm (contact area - 8 cm²) with a surface enhanced by stitched loops were located in orientation toward muscle fascia and a planar surface (modified by low temperature plasma for the tested samples and without modification for the reference) in the same direction an uncovered viscera so that the highest area of the internal organs came in contact with the implants in a direct manner (without *omentum* covering).

During the research, risk estimation was carried out with respect to:
- the adhesion of the implants designed to the viscera in the case of direct contact of the implant with internal

organs. The adhesion was determined by quantitative methods – measurements of the adhesion force of the viscera from the knitted implants and qualitative methods – by microphotograph analysis of the adhesion place using the ESEM technique;
- change in the microstructural properties of the samples tested and reference during implantation by the DSC and FTIR techniques.

Estimation of the adhesion force of the knitted implants

After explanation, the samples and reference tested were frozen at -20 °C. After defrosting at room temperature, measurement of the adhesion force of the implant to the fascia or viscera was performed using an Instron 5944. The speed of the testing head of the Instron instrument was 6 mm/min.

ESEM investigation

The structure of the explants, incl. the tissue covering and adhesion place quality was estimated using ESEM microscopy (at a voltage of 25 kV and pressure of 6.67 hPa).

Estimation of the structural changes after implantation

The explanted implants were purified using hypochlorite solution (10 wt%) according to the procedure described in [6].

The knitted implants after removing the animal tissue were subjected to DSC and FTIR (ATR) testing.

DSC was elaborated using Q2000 Differential Scanning Calorimeter apparatus (AT Instruments/USA). Differential Scanning Calorimeters (DSC) measures temperatures and heat flows associated with thermal transitions in a material.

$$ICr = \frac{\Delta H_m}{\Delta H_{m100\%}} \times 100\% \qquad [1]$$

where:
ICr – index of crystallinity, %;
ΔH_m – melting enthalpy, J/g;
$\Delta H_{m100\%}$ – melting enthalpy of polypropylene with 100% crystallinity; 207 J/g [7].

ATR - FTIR spectra were recorded on an FTIR–Nicolet 6700 (THERMO Scientific) and analysed using OMNIC 8.0.380 software.

◼ Results and discussion

The implantation of the foreign body elicits several reactions of the surround-

ing tissue, affecting implant properties. The structure, chemical properties and leachability of the implant, in some cases, intensify the reaction of the surrounding tissue, provoking local reaction.

The research work involved quality and quantity estimation of changes occurring during the implantation of the new prototypes of hernia meshes in the intra-abdominal cavity. The ESEM investigation allowed to view the quality of integration of the connective tissue with the enhanced surface of the implant, whereas the adhesion determinations showed, in a quantitative manner, the effect of viscera adhesion as well as connective tissue integration with the knitted structure of the implants.

On the other hand, the DSC study is able to confirm potential post-implantation changes in the crystalline structure of the implants, whereas ATR - FTIR spectroscopy allows to identify changes in the chemical structure of the implants as well as in the newly-formed fluoroorganic layer on the surface of the implants.

ESEM evaluation of the explanted knitted implants

The first stage of the research was to estimate the quality of:
a) integration of the fascia with implants enhanced by loops stitched out of the surface
   as well as
b) the potential visceral adhesion of the non-modified (reference) or modified by low temperature plasma treatment of the opposite surface of the implant.

Figure 1 shows ESEM microphotographs of knitted implants modified by low temperature plasma treatment or without modification after explanation of 2, 4 or 12 weeks from the intra-peritoneal cavity of the rabbit's abdomen.

The surface of single monofilaments forming the surface structure of the test implants, modified by low temperature plasma treatment, after 4 weeks of the implantation, is characterised by partial covering of the tissue. The reference – knitted implants without surface modification were characterised by a significantly larger surface covered by the tissue (*Figures 1.a – 1.h*). In a few places the viscera had adhered to the reference implants (*Figure 1.g*).
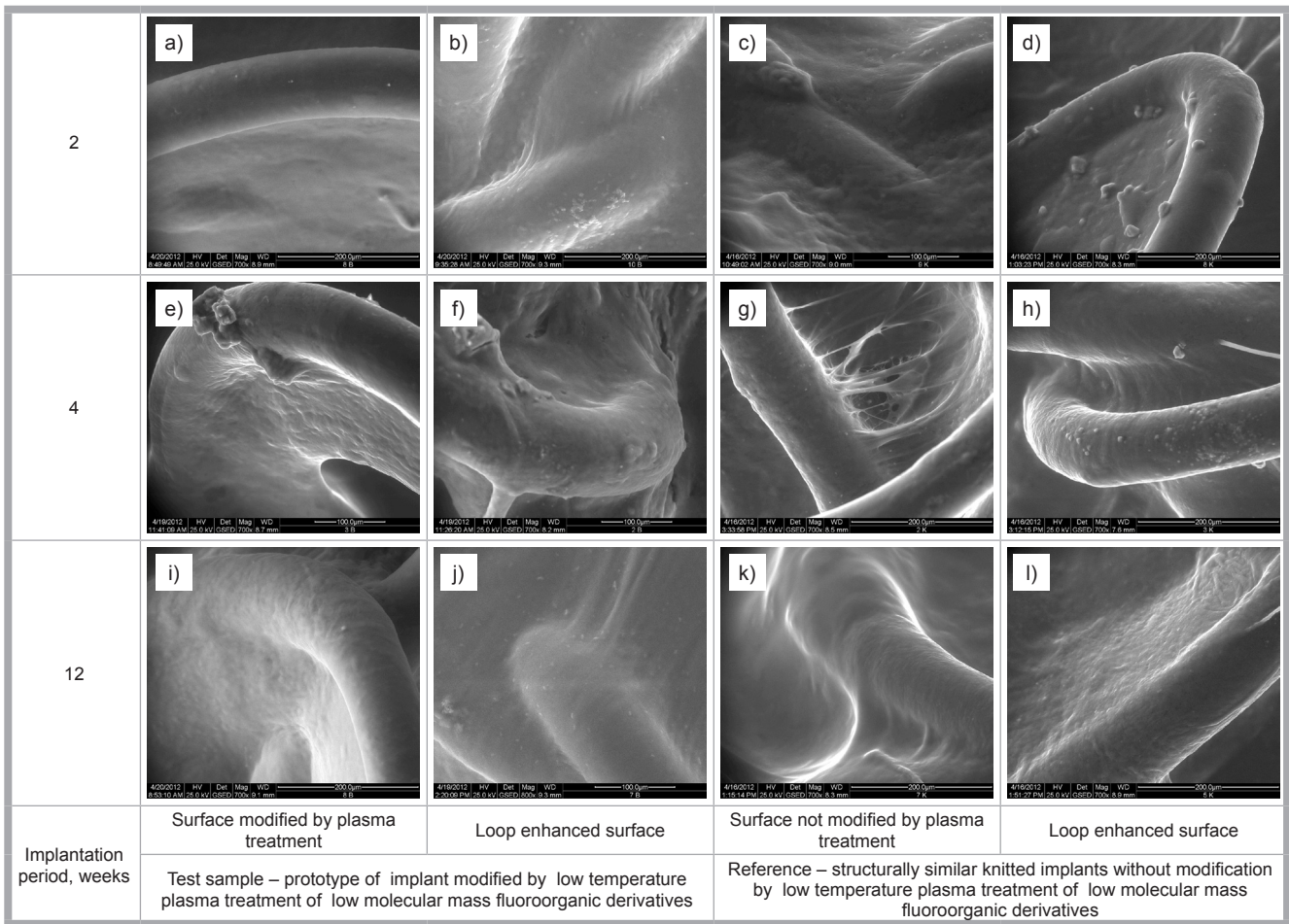
	a)	b)	c)	d)
2				
	e)	f)	g)	h)
4				
	i)	j)	k)	l)
12				
Implantation period, weeks	Surface modified by plasma treatment	Loop enhanced surface	Surface not modified by plasma treatment	Loop enhanced surface
	Test sample – prototype of implant modified by low temperature plasma treatment of low molecular mass fluoroorganic derivatives		Reference – structurally similar knitted implants without modification by low temperature plasma treatment of low molecular mass fluoroorganic derivatives	

Figure 1. ESEM microphotographs of knitted implants modified and not modified by low plasma treatment after explantation.

The enhancement of the surface by stitched loops improved, in both cases – reference and test samples, the connective tissue in-growth. The stitched loops were completely covered by smooth connective tissue tightly adhered to the monofilament's surface (*Figures 1.b, 1.d, 1.f, 1.h, 1.j*).

After 12 weeks of the implantation, the structure of tissue integrated with the implants was similar for the test sample and reference. The connective tissue was grown in the implant's structure, forming a smooth surface onto the single monofilaments (*Figures 1.i – 1.l*).

No significant difference in the stitched loops in ESEM microphotographs was observed. They were relatively quickly covered by the connective tissue's growing-in. Dynamic integration of the enhanced implant's structure with the fascia improves the implant's stabilisation, prevents its migration, and increases the resistance of the implantation region against the dynamic action of intra-abdominal pressure.

Estimation of the adhesion force of the knitted implants

Results of the maximal adhesion force determination for the reference and test samples after implantation for 4 or 12 weeks are shown in *Figure 2*. (see page 82)

Implant adhesion to the fascia was studied for evaluation of the effect of the enhanced surface's integration with the connective tissue. Additionally the adhesion susceptibility of the surface of implants modified or not modified by low temperature plasma to the viscera was quantitatively estimated.

The first dynamic in-growth of the connective tissue into the enhanced surface of the implant over a period of between 2 to 4 weeks was observed. The maximal adhesion force of the implants to the fascia for both the reference and test samples amounted to approx. 10 N with an increase in implantation time. After 12 weeks of implantation in the abdominal cavity, the above-mentioned parameter increased for the reference by

an average of 15.7 N and for the tested samples by an insignificantly lower value of 13.6 N. The differences in parameters measured for the reference and test samples may be related to surface modification performed with low-temperature plasma, resulting in a reduction in the local inflammatory reaction and finally a decrease in the dynamic of the connective tissue in-growth into the knitted implants. However, the surface of the knitted fabric unfolding by means of the stitched loops guarantees the full integration of the implant with the fascia after 4 weeks of implantation. The value of the adhesion force of the implant to the fascia determined is much higher as compared with the anatomical requirements (> 10 N).

In the case of the adhesion force of the surface modified or unknot modified by low temperature plasma to the internal organs, a lower tendency to adhere of the samples tested was found as compared to the reference. The implants covering the adhered organs were not whole, and
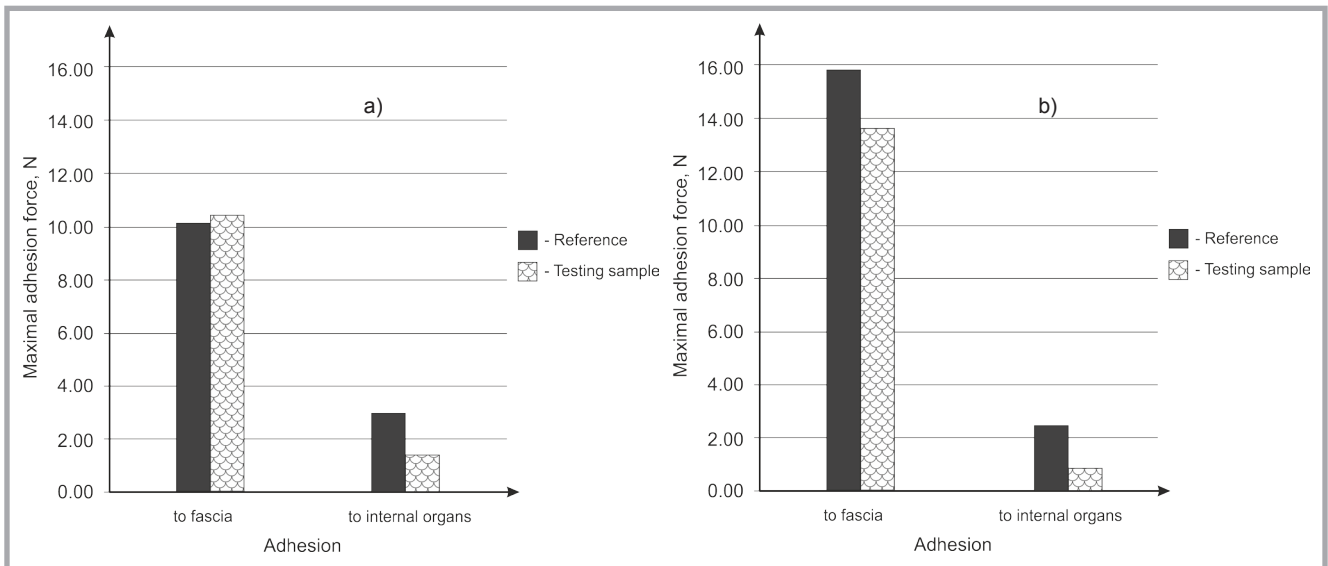
Figure 2. Maximal adhesion force of the implant (modified by plasma treatment – test sample or unmodified implant - reference) to the fascia or viscera after: (a) 4 weeks of implantation; (b) 12 weeks of implantation.

for that reason only the areas of adhesion identified were tested.

After 4 weeks of implantation, the adhesion force of the implant surface to the viscera ranged from 1.4 N (samples tested) to 3.1 N (reference), whereas after 12 weeks it was from 0.9 N (samples tested) to 2.5 N (reference).

The above measurements confirm the clinical effectiveness of implants whose surface is modified by low temperature plasma in the presence of a low molecular fluoroorganic derivative. Plasma polymerisation established a thick layer of the fluoropolymer of significantly lower affinity to adhesion formation. It should be remarked that the study was performed using samples in various degrees of covering, which influenced the final results. Moreover the contact of reference and test sample surfaces with the viscera was similar. In regard to the clinically used hernia implants of significantly higher surface contact, significantly lower complications of adhesion of the implants designed will be excepted.

Changes in the polymer structure during implantation

The living body acts on the implanted material as a whole, evoking changes in its surface and structural properties. The range of the alteration as well as their dynamic depends on the types of material implanted as well as on time and tissue in contact. The destruction of the materials occurring during implantation at the beginning yields an increase in crystallin-

ity due to the degradation of amorphous regions. Then the polymer crystallinity slowly decreases [8 - 9].

During the research presented, the change in the microstructure of the knitted implants as well as in the surface layer originated as a result of the low temperature plasma treatment during the intra-peritoneal implantation.

The alteration in the ICr of the samples tested as well as the reference during implantation in comparison to the initial, steam sterilised samples is presented in *Figure 3*. The ICr results of each implantation period were the average of the three explanted samples from various animals.

The intra-peritoneal implantation of the knitted implants modified by low temperature plasma for 4 weeks led to an insignificant change in ICr (47.3% in relation to initial value of 49.4%). Prolongation of the implantation resulted in an increase in ICr up to 51.1%. In the case of the reference samples, no significant difference in ICr was found, even after 12 weeks of implantation .Taking into account the above, no significant differences in ICr affecting the usable properties of implants elaborated were observed. It can be assumed that the first period after implantation (till 2 – 4 weeks) is a key time for effective hernia treatment. At a later period, the implant structure is ingrown by the connective tissue, which
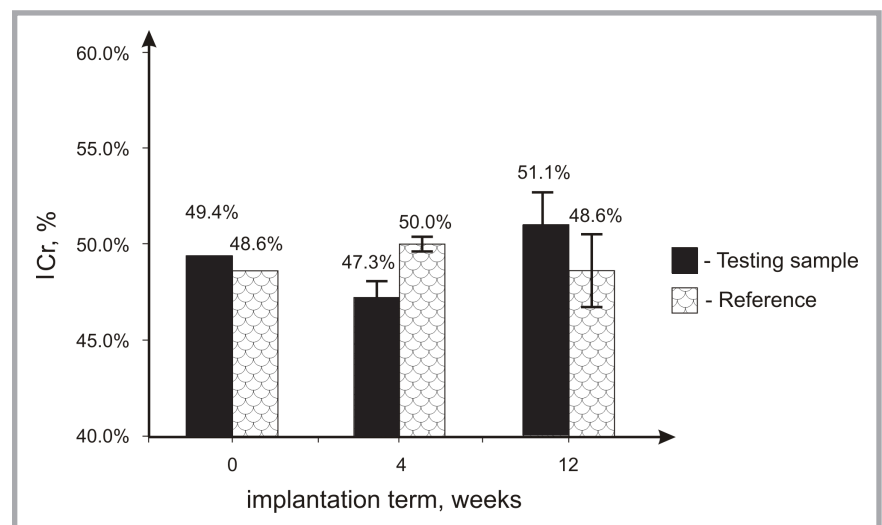

Figure 3. Changes in the crystallinity of the implants tested (modified by low temperature plasma treatment) as well as the reference during 4 or 12 weeks of intra-peritoneal implantation, compared to the ICr of initial implants.

Table 1. *Absorption bands separated from the ATR-FTIR spectra of raw – sources (PP monofilament and tetradecafluorohexane), surface of initial implant modified by low temperature plasma (steam sterilized) and its explants after 2, 4 or 12 weeks of implantation.*

ATR-FTIR absorption bands of standard PP, cm⁻¹	Identification of chemical bond type	ATR-FTIR separated absorption bands, cm⁻¹				
		Raw source – PP monofilament	Initial implant tested (modified by low temperature plasma and steam-sterilised)	Explants weeks of implantation		
				2	4	12
808	C-C	808	-	808	808	809
840	C-H	840	-	839	840	839
973	CH₃; C-C	972	972	971	972	971
996	CH₃	997	997	996	998	996
1166	C-C; C-H; CH₃	1166	1167	1165	1166	1165
1376	CH₃	1376	1376	1376	1376	1376
1456	CH₃	1453	1453	1456	1456	1455
2870	CH₃	2877	2878	2866	2866	2866
2920	CH₂	2917	2918	2921	2918	2921
2950	CH₃	2950	2949	2955	2950	2955
ATR – FTIR absorption band specific for tetradecafluorohexane						
C-F		-	1339	1331 / 1339	1332 / 1339	1329 / 1339
Additional absorption bands identified in ATR - FTIR spectra of low temperature plasma surface of the knitted implants related to protein residues						
				1744	1744	1744
				2836	2836	2836

consolidates and stabilises the hernia mesh.

The separation of the FTIR spectra of the explanted, test and reference samples is shown in **Table 1**.

ATR-FTIR spectra of the surface of explants modified by low temperature plasma after 2, 4 or 12 weeks of implantation, purified from the tissue according to [6], are characterised by a weak absorption band at a wavelength ranging from $\lambda = 1339$ cm⁻¹ to 1331 cm⁻¹, related to chemical bonds formed during the modification. The intensity of these bands is significantly lower as compared with the initial, steam-sterilised implants. The above phenomenon is related to the specific environment surrounding the implanted material and, more probably, to the procedure aimed at the purification of the implant from the connective tissue.

The additionally separated absorption bands in a wavelength of $\lambda = 1744$ cm⁻¹ and $\lambda = 2836$ cm⁻¹ are related to protein residues of the connective tissue permanently bonded to the implant surface. The insignificant shifting of the absorption bands specific for the polypropylene was also detected in ATR – FTIR spectra of the explants.

Conclusions

The investigation of the explanted materials allows to anticipate the behavior of the newly-designed implants after implantation. Changes in the microstructure, such as crystallinity, may lead to a reduction in the mechanical strength required, whereas surface verification of the stability of the newly-formed layer of fluoropolymer is essential for confirmation of the usable properties assumed.

The research presented confirms the absence of significant changes in the microstructure of the implanted materials, which did not affect the mechanical properties required during the early period of implantation. This period is related to the highest risk of potential hernia recurrence originating from the loss of the mechanical strength of the implant or its migration.

Modification of the surface by low temperature plasma in the presence of the tetradecafluorohexane is stable and functional after implantation in time, being crucial for preventing the adhesion of the implant to the uncovering viscera.

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