The influence of sterilization on properties of polyurethane/polylactide blend

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Abstract
The biodegradable polyurethane/polylactide blend was treated with low temperature hydrogen peroxide plasma, ethylene oxide and immersing in ethanol combined with ultraviolet radiation. The samples sterilized by hydrogen peroxide and ethylene oxide stood practically unaffected, while UV/EtOH caused distinct changes in their mechanical properties. For example the significant reduction of tensile strength occurred, elongation at break became twice lower, while the Young's modulus increased by 23%. The XPS measurements showed that after all types of treatment atomic carbon and nitrogen concentrations in the surface layer was slightly lower than in the bulk. Instead the surface layer was more enriched with oxygen. Ethylene oxide sterilization caused that both surfaces became more hydrophobic i.e. the contact angle increased about 15% for the top surface and 8% for the bottom surface, respectively. Sterilization with ethanol and UV radiation changed the nature of surface into more hydrophilic, the contact angle of the top surface was reduced about 6% and the bottom about 24%. The FT-IR spectra of all sterilized samples were recorded and discussed. From all used sterilization methods only hydrogen peroxide plasma is fully suitable for biodegradable PU/PLA blend.

Key words: sterilization, surface, biodegradable polymer, topography, morphology

Introduction

Sterilization of implantable medical devices made of polymers, in many cases, can cause chemical or physical changes in the material. This is especially often observed for implants made of polymers such as polyhydroxyacids [1].

The standard methods of sterilization of medical devices include the use of dry or moist heat, chemicals (ethylene oxide, ethanol, hydrogen peroxide) or radiation. Steam sterilization by autoclaving at 121°C is the most widely employed method nowadays but might induce hydrolysis and/or melting of the polymer matrix and, in effect, it is of limited use for most polymeric materials [2, 3]. Irradiation with gamma rays is a very common sterilization technique. It is a costly process, not only because of indispensability of employing the high qualified staff, but also because of the strict security precautions which have to be taken. Besides, the relatively high irradiation intensities affect not only the surface of the treated materials, but also in the bulk. As a result chain-scission or cross-linking of the polymer takes place. Because the polymer chain length can be affected by irradiation, the property scaffold most significantly altered by irradiation is the degradation rate, which in turn influence mechanical stability. Both bond cleavage and crosslinking are critically important to clinical success, because degradation rate should be synchronized with tissue regeneration and this may vary considerably, depending on the specific tissue engineering applications [4]. Sterilization of polylactide by γ-irradiation resulted in extensive decrease in molecular weight and thus lead to negative impact on thermal and mechanical proprieties [5–8].

Sterilization with ethylene oxide (EtO) is another common low temperature sterilization technique. It has no effect on the bulk characteristics of the material. The EtO residues absorbed on the implant or medical devices after the sterilization processes, may have carcinogenic properties, when being left inside and later reacting with tissue proteins upon implantation [9]. It is necessary to apply to the reactor a vent cycle, which may take more time than the sterilization. The safety of the operators is also a serious concern when using this technique. In many sterilization installations toxic formaldehyde and glutaraldehyde are also employed [10, 11].

Plasma is an alternative method, and seems to have advantages over the other sterilization methods currently in use. Advantages of plasma include the fact that the technique is effective in inactivating the required microbial load to safety assurance level, can be performed at room temperature, and no toxic gases are used. Plasma has the characteristics of an ideal sterilant,

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namely, a high degree of efficacy, fast action, penetrability, lack of toxicity, compatibility with different materials. There are several mechanisms which may be responsible for the sterilization involving interaction of UV radiation with the DNA of the spores and bacteria, reaction of radicals with the biological material and chemical etching i.e. creating CO_2 and other easily removable gaseous compounds [12–15].

The objective of the study was to investigate how various sterilization technique such as UV/EtOH, ethylene oxide and hydrogen peroxide cold plasma affected physicochemical properties of biodegradable polyurethane/polylactide (PU/PLA) blends. The composition has been successfully employed for implants supporting peripheral nerve regeneration.

Materials and methods

Preparation of experimental blends

Blends of polylactide and polyurethane were tested in this research. Polyurethane (PU) was purchased from Bayer (Germany). PU molecules are built up of hexamethylenediisocyanate (HMDI) rigid segments, polycaprolactone (PCL) flexible segments and isosorbitol (ISO) as a chain extender (Figure 1). Polylactide (PLA), consisting of 80% poly-L-lactide and 20% poly-DL-lactide was purchased from Purac (Netherlands). Both used polymers were of biomedical grade and were used without further purification The N,N-dimethylformamide (DMF) of analytical grade was purchased from POCh (Poland).

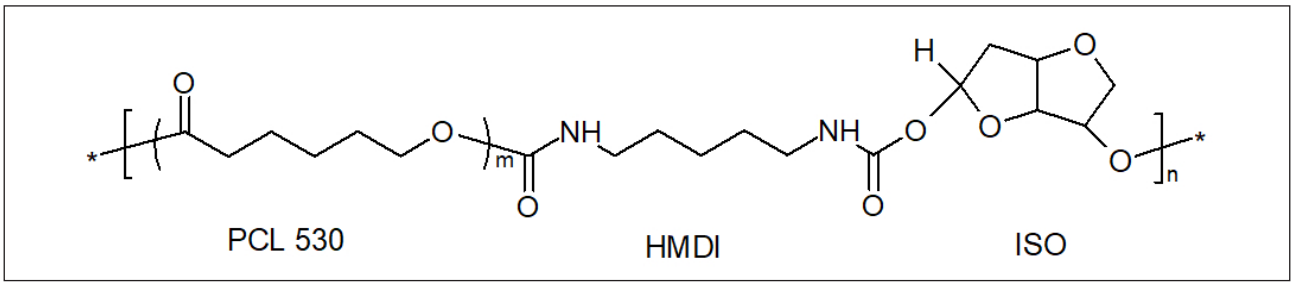

Figure 1. Structure of investigated polyurethane

PU/PLA blend was prepared by dissolving both polymers (weight ratio of PU to PLA was 80/20) in dimethylformamide to obtain a 10 wt. % solution. The mixture was stirred with magnetic stirrer for at least 48h at ~50°C, and then cast on glass Petri dishes. The films were dried at 50°C under vacuum for 48 hours. Porosity of blend was obtained by salt leaching method. Sodium chloride with the granulation of 50–100 µm has been applied as a water leachable porogen.

Evaluation of physical and mechanical properties of the films

Tensile strengths (TS), Young's modulus (E) and elongation at break ($\varepsilon_{F\,max}$) of the samples were measured using a universal testing machine (Zwick 1465, Germany) equipped with 5kN load cell. The sample length between the clamps was 45 mm and clamps' speed was 50 mm/min. The obtained results correspond to the average of six measurements (± standard deviation).

Infrared spectra in the attenuated total reflection mode (FT-IR ATR) were recorded on Digilab FTS 60v spectrometer from BioRad. The blends (before and after sterilization) were analysed in 600–4000 cm^{-1} range with 4 cm^{-1} resolution.

The water contact angle of the obtained PU/PLA films was measured using sessile drop method on Drop Shape Analysis System (DSA Mk2, Krüss, Germany). Ten measurements per one sample were accomplished and an average of ten records (± standard deviation) has been calculated.

Surface roughness of the film was measured using a profilometer (Hommelwerke, Germany), equipped with cone shaped diamond tip (radius of 5 µm) and the velocity of cone moving was 0.50 mm/s. The profiles were recorded for 10 different points of each side of the film. Prior to taking roughness measurements a glass slide with the thin PU/PLA films was fixed onto a mount with double-sided adhesive tape to prevent the samples from moving during the test. All the given values are presented as average of ten measurements (± standard deviation).

X-ray photoelectron spectroscopic (XPS) studies were performed on PHI Versaprobe II Scaning XPS Micriprobe (USA). The gas cluster ions beam source providing $Ar_{2,500}^{+}$ cluster ions was also used to obtain depth profile of samples before and after sterilization.

The morphology of elaborated materials was evaluated using scanning electron microscopy (Nova Nano SEM 200, FEJ EUROPE Company, USA). Prior to observations, materials were dried and sputtered with carbon.

The materials were sterilized by a hydrogen peroxide low temperature plasma (Sterrad 100S of Johnson & Johnson), ethylene oxide (sterilizer Getinge) and immersed in ethanol combined with ultraviolet radiation in a laminar chamber (Alpine).

Results and discussion

The mechanical parameters of porous films both before and after sterilization by hydrogen peroxide, ethylene oxide, and EtOH/UV radiation are given in Table 1. Low temperature H_2O_2 plas-

ma sterilization and ethylene oxide does not significantly affect mechanical properties of the material such as tensile strength, elongation at break, Young modulus and work of destruction. However, the tensile strength becomes slightly higher in both cases, and elongation at break is lower, all together suggesting increase of rigidity of the PU/PLA blend. It has been reported that H_2O_2 plasma treatment on PLA can result in diminishing of amorphous regions and vaporizing of low molecular compounds [16]. The Young's modulus and work of destruction are also only slightly lower. Changes of all four parameters are consistent. The most distinct changes can be observed after EtOH/UV treatment. The tensile strength is reduced by 7%, elongation at break becomes twice lower, the Young's modulus increased by 23% and the value of work of destruction decreased by almost four times. PLA is stable in the presence of ultraviolet irradiation, but it releases the cyclic oligomers while additionally treated with 96% ethanol. It clearly suggests that the UV radiation causes chain cleavage of PU and not crosslinking and the ethanol may partly dissolve the polylactide [17].

Table 1. Mechanical parameters of before and after sterilization 80/20 PU/PLA

Method of sterilization	TS [MPa]	ε_{Fmax} [%]	E [MPa]	W [Nmm]
before sterilization	0.66±0.22	91±33	1.70±0.9	19±10
H_2O_2 plasma	0.70±0.21	87±22	1.60±0.4	17±7
EtO	0.69±0.24	86±22	1.60±0.6	17±7
EtOH/UV	0.62± 0.10	45±11	2.2±0.8	5±1

TS – tensile strength, $\varepsilon_{F\ max}$ – elongation at break, E – Young's modulus, W – work of destruction

The XPS C1s spectra of PU/PLA before as well as after sterilization show four distinctive peaks. The main peak at 284.8 eV is attributed to hydrocarbons (C-C), the peak at 286.4 eV to ether carbon (C-O), the peak at 288.8 eV to carbonyl carbon (C=O) and the small peak at 290.4 eV to ester carbon (O-C=O). The O1s spectra of before and after sterilization consists of two peaks at 532.2 eV (carbonyl oxygen atoms) and at 533.7 eV (ester oxygen atoms). The N1s spectra of polyurethane hard segments demonstrate one typical peak at 399.6 eV (amine) and 401.0 eV (urethane nitrogen). There is one additional peak at 102.3 eV, which may be assigned to SiO_x compound. Such bond can directly come from glass substrate or can be potentially created when polymeric films were casted on glass substrate. The results of XPS analysis of PU/PLA before and after sterilization are presented in Table 2.

Table 2. XPS of 80/20 PU/PLA before and after sterilization

Method of sterilization	Treatment dependent	Atomic concentration [%]			
		C	O	N	Si
before sterilization	Surface	62.5	31.2	1.8	4.4
	Bulk	68.0	28.8	2.1	0.9
H_2O_2 plasma	Surface	67.6	29.9	1.8	0.6
	Bulk	69.2	28.1	2.7	0.0
EtO	Surface	65.6	32.3	1.6	0.4
	Bulk	69.0	28.4	2.5	0.1
EtOH/UV	Surface	67.4	29.1	2.1	1.3
	Bulk	70.8	25.4	3.3	0.1

Atomic carbon concentration of C-C, C-O and O-C=O bonds are lower about 3%, 21%, and 35% respectively at the surface layer than in the bulk of the film for untreated PU/PLA. Only atomic carbon concentration of C=O bond at the surface is slightly higher about 9%. It is noticeable that before as well as after all applied sterilization methods atomic carbon and nitrogen concentration in the surface layer of PU/PLA blend is slightly lower at the surface than in the bulk. It clearly suggests that the bulk of tested blend is enriched in nitrogen coming from hexametylene diisocyanate segments. In contrast to atomic oxygen concentration, which is narrowly higher at the surface than in the bulk of the blend connected with oxidation.

The surface atomic composition of PU/PLA film before and after various sterilization methods are listed in Table 3.

Atomic carbon concentration after H_2O_2 plasma, EtO and EtOH/UV sterilization has increased only partly. However, the molar ratio between carbon groups has changed significantly. For instance, the concentration of C-C bonds on the surface only after EtO sterilization has decreased by 14%. There was an increase of C-O-C and O-C=O groups concentration after all sterilization methods. Treatment of samples with H_2O_2 plasma and EtOH/UV caused the decrease of carbonyl groups concentration, while EtO treatment lead to its increase. The nitrogen bonds are strictly connected with the presence of hard segments of polyurethane, which are built up of hexamethylene diisocyanate. After sterilization with H_2O_2 plasma and EtOH/UV the concentration of NH bonds has slightly increased, but after EtO sterilization has significantly decreased by 14%. The atomic oxygen concentration before and after applied sterilization has not changed substantially. The O-C concentration after H_2O_2 plasma, EtO and EtOH/UV sterilization has decreased by ~20%, while the O=C concentration has increased. However, the ratio between O-C and O=C group concentration is different.

The results of wettability measurements, the porous film 80/20 PU/PLA before and after sterilization by hydrogen peroxide, ethylene oxide and EtOH/UV radiation are listed in Table 4.

Table 3. Chemical composition of the surface 80/20 PU/PLA before and after sterilization

Method of sterilization	Atomic concentration [%]				C1s				N1s	O1s		Si2p
	C	O	N	Si	C-C	C-O	C=O	O-C=O	N-H	O-C	O=C	SiO_x
before sterilization	62.5	31.2	1.8	4.4	33.6	16.4	11.3	1.3	1.5	18.6	12.6	4.4
H_2O_2 plasma	67.6	29.9	1.8	0.6	34.1	20.0	9.9	3.7	1.6	14.1	15.9	0.6
EtO	65.6	32.3	1.6	0.4	29.0	20.5	14.3	1.8	1.3	15.4	16.8	0.4
EtOH/UV	67.4	29.1	2.1	1.3	33.0	22.0	9.4	2.9	1.6	14.5	14.6	1.3

Table 4. Wettability and average roughness of 80/20 PU/PLA before and after sterilization

Method of sterilization	Θ [°]		R_a[μm]	
	Top surface	Bottom surface	Top surface	Bottom surface
before sterilization	83±7	75±5	7.3±1.3	9.2±0.2
H_2O_2 plasma	79±8	77±10	6.7±0.5	9.8±0.7
EtO	98±6	82±7	8.2±0.7	12.2±2.9
EtOH/UV	78±5	57±6	7.2±0.6	7.3±0.5

Θ - contact angle, R_a – average roughness

Only after sterilization with hydrogen peroxide low temperature plasma, the value of the contact angle of the top and bottom surfaces remains almost unchanged comparing to the initial sample. That slight increase in wettability was likely a result of the addition of polar groups such as hydroxyl radicals on the PLA surface [18]. Whereas lowering of contact angle on polyurethane part of surface might result from surface degradation and/or oxidation, which can cause creation of low molecular weight surfactant-like species on the surface [19]. In contrast, ethylene oxide sterilization caused that both surfaces became more hydrophobic i.e. the contact angle increased about 15% for the top surface and 8% for the bottom surface, respectively. The EtO is highly reactive molecule, because it consists of short and strained C-C bond, which makes its ring easy to open. Therefore PU/PLA chain ends can be easily modified by radical reaction of EtO molecule leading to surface changes [18]. Sterilization with ethanol and UV radiation changed the nature of the surface into more hydrophilic, the contact angle of the top surface was reduced about 6% and the bottom about 24%.

Average roughness of the top surface is substantially lower than of the bottom surface of untreated blend as well as after all methods of sterilization. None of used sterilization methods caused significant changes in the roughness of the top surfaces. After hydrogen peroxide plasma sterilization the Ra parameter of the bottom surface remained the same, while the ethylene oxide treatment increased the surface roughness by 25% and EtOH/UV reduces by 11%. Hydrogen peroxide plasma etched and substantially reduced the surface [18]. However, recent published research have shown that EtO may be able to alter the topographical properties of electrospun polyurethane scaffold, resulting in surface roughness [20].

All applied sterilization methods practically did not significantly change the surface texture except EtOH combined with UV radiation, which smoothed out the surface. It is clearly visible in SEM photographs in the Figure 2. This fact may be connected with partially dissolving of PLA units in 96% ethanol. Evaluation of samples' surfaces shows that hydrogen peroxide plasma minimally etched the surface by reducing the roughness of the bottom side, which was more hydrophilic than the top one.

The XPS tests give the information on the surface structure up to the depth of about 5 nm; while, ATR FT-IR spectra supplied the averaged information on the chemical composition in the entire bulk of the sample. The typical infrared spectra of the porous film 80/20 PU/PLA before and after sterilization by H_2O_2 plasma, ethylene oxide, and EtOH/UV radiation are showed in Figure 3. The absorption band at 3323 cm^{-1} corresponds to hydrogen-bonded –NH group. The absorption band at 2941 cm^{-1} and 2866 cm^{-1} are associated with stretching symmetric CH_3 side group and CH_2 group of the main chain of polymer. Symmetric and asymmetric stretching modes of C=O and C-O-C groups (ν C=O, ν_{sym} C-O-C and ν_{asym} C-O-C) were present at 1740, 1191and 1095 cm^{-1}, respectively. The stretching of the C-C bond in C-CH_3 group (ν C-CH_3) could be observed at 1045 cm^{-1}. Rocking asymmetric CH_3 absorption band was positioned at 1136 cm^{-1}. In the bending region a signals due to the -CH_3 asymmetric deformation modes (δ_{asym} CH_3) were positioned at 1463 cm^{-1} and 1404 cm^{-1}, while duplet at 1380-1365 cm^{-1} was assigned to δ_{sym} CH_3 and δ_i CH+ δ_{sym} CH_3 signals, respectively. The

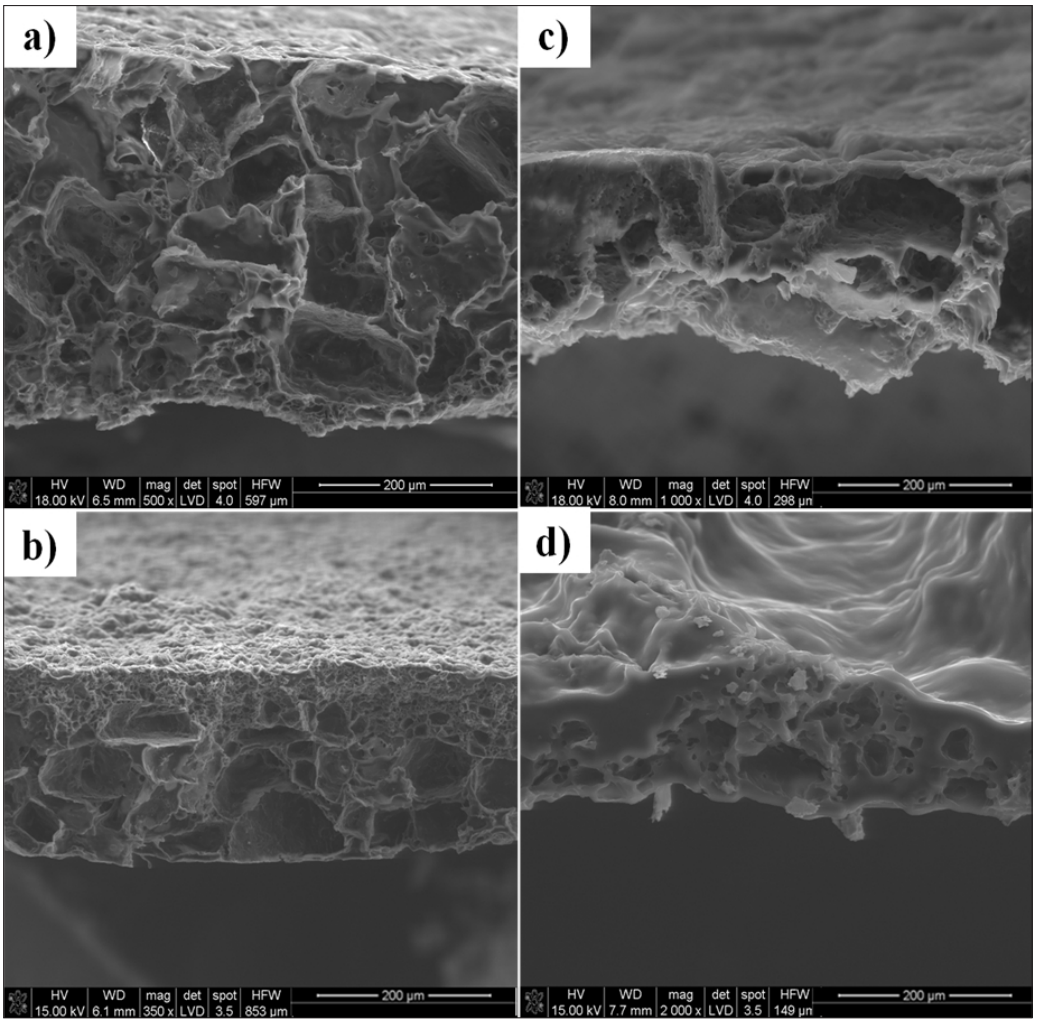

Figure 2. SEM photographs of cross-section of PU/PLA a) before, b) after H2O2, c) after EtO and d) after EtOH/UV

amide II absorption asymmetric bending band (ν_{asym} N-H) appears at 1541 cm^{-1} for poly(ester-urethane). The band at 1247 cm^{-1} corresponds to amide III aliphatic R-NH-COO-group [21, 22].

The spectra of all sterilized samples were found to match to the untreated PU/PLA spectrum, confirming that applied sterilization techniques are not harmful to the blend structure.

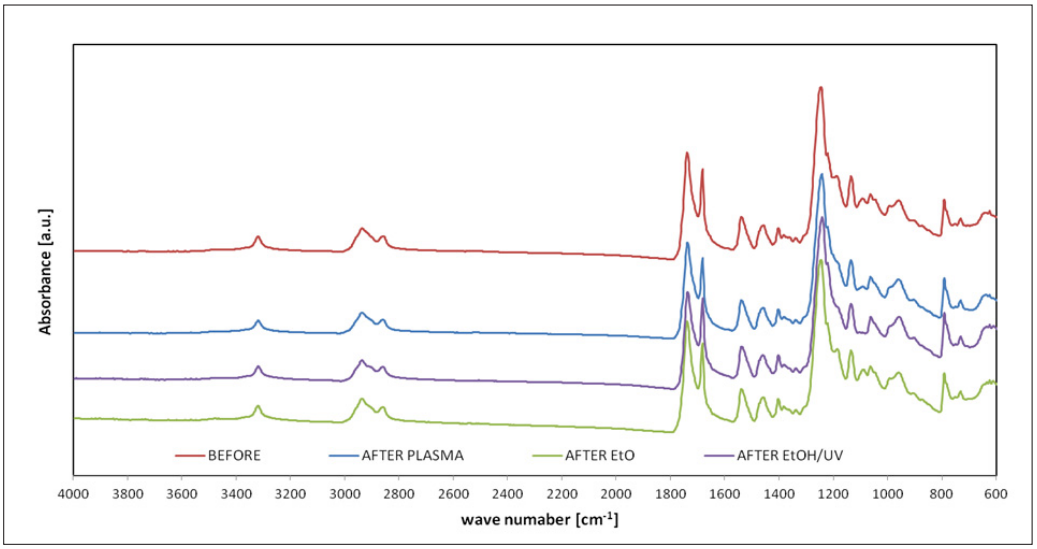

Figure 3. FT-IR ATR of 80/20 PU/PLA before and after sterilization

Summary

Stability of PU/PLA blends under sterilization conditions were studied. Three methods of strillization were used: hydrogen peroxide plasma, ethylene oxide plasma and ethanol combined with UV radiation. Discolouring, yellowing and any visible loss of clarity of the PU/PLA films was not observed after sterilization. All applied methods caused oxidation of the surface resulting in the increase of C=O group concentration. In addition, sputtering the surface with the gas cluster ion beam proved that only surface underwent oxidation while the deeper atomic layers did not. Surface chemistry analysis by ATR-FTIR indicated no other significant chemical modification in the polymers. Only after sterilization with low temperature hydrogen peroxide plasma the biodegradable PU/PLA surface topography remained unaffected as well as its morphology. From all used sterilization methods only hydrogen peroxide plasma is fully suitable for biodegradable PU/PLA blend.

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