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Biomechanical Effect of Nitrogen Plasma Treatment of Polyetheretherketone Dental Implant in Comparison to Commercially Pure Titanium

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ABSTRACT

Dental implants increase the quality of life for many patients with tooth loss, peek might replace titanium as dental implant, but its surface need to be modified in order to improve and accelerate osseointegration. To evaluate the effect of RF nitrogen plasma treatment of peek implant screws on the strength of implant-bone interface after 2 and 6 weeks after implantation in rabbit tibia bone. 6 Peek's discs and screws had been prepared to be treated with RF nitrogen plasma. Eighteen male New Zealand rabbits tibias were chosen as implantation sites, fifty four screws were implanted in tibia bone, three screws in each tibia, the rabbits were divided into two groups of two and six weeks according to healing intervals, twenty one screws were tested for torque removal of each group of implants in each periods, and six implants of each group of implants in each periods were used for histological examination and histomorphometric analysis. There was significant increase in mean values of torque removal for the nitrogen plasma treated implants in comparison with untreated peek implant for both periods of two and six weeks. Plasma treatment of peek implant by RF device had remarkable influence in increasing the osseointegration than untreated peek implant.

Key words: Peek, Radiofrequency, Plasma, Dental Implants

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INTRODUCTION

Many patients with tooth loss had been increased their quality of life by dental implants [1]. The commercially pure titanium is the material of choice for oral implants, first known by Branmark at the end of 1960's [2]. In spite of well evidence-based implants made from titanium and titanium alloy[3,4], it was observed that their usage can be associated with a variety of disadvantage, such as hypersensitivity to titanium, distortion of the image in CT scan, the stress caused by difference in elastic moduli between bone and titanium which will cause bone loss, and titanium can cause esthetics problem especially in aesthetic zone with high smile line, all of these reasons required to find new biomaterial serving as dental implants.

PEEK is a thermoplastic polymer with a high-performance capable of replacing components of metallic implant in the field of orthopedics [5, 6] and traumatology [7, 8]. Also, peek implants can be used for the constructions of calvarias bone [9]. With such findings that made suggestions for PEEK to be substitution for titanium as dental endosseous implants' material [10].

Many advantages for the use of PEEK such as radiolucency; peek had the ability to be imaged by X-ray, CT scan, or MRI with no distortion in the image when comparing to the titanium [11, 12]. PEEK is considered for long term use in a body without any breakdowns products because of its chemical stability and resistance [11, 12]. Another attractive property of PEEK in its young modulus which is nearly close to the young modulus of cortical bone [13].

An essential problem with PEEK, is their low-surface energy, and the hydrophobic property of the surface can reduce cellular adhesion making it categorized as bioinert [12, 14]. So surface energy of the peek must be changed to have direct bone interaction. Many methods have been used to alter the surface energy, one of them is plasma treatment for surface modification without altering bulk of the material and its properties [15]. Surface modification by plasma treatment is widely used on polymeric materials. Surface modification can be carried out with specific gases such as N2, O2, Ar and H2 [16]. Because of its low penetration level, plasma modification proposes a method of tailoring the surface while keeping the bulk material excellent physical and chemical properties [17].

In this study, RF nitrogen plasma treatment is used for peek screws, and it is implanted in rabbit tibia, to evaluate influence on osseointegration by torque removal test, histological examination and histomorphometric analysis after two and six weeks.

MATERIALS AND METHODS

Sample preparation

PEEK block was used as a substrate for nitrogen plasma treatment. By lathe machine, the PEEK was cut into discs (2 mm thickness and 20 mm diameter).

Figure 1: Peek sample attached to the slides

6 discs of Peek were attached to microscope slides by adhesive tape, and the slides were fixed on the base of plasma device which is auto rotated, the distance between the source of plasma and the base of the device is fixed and the base was rotated at steady speed, so that all the surfaces of the discs were receiving equal amount of plasma treatment, then vacuum started to evacuate the chamber of the device from air, and the plasma surface modification was performed for 1800s. The nitrogen was introduced to the chamber with a gas flow of 150 cm^3/min, with a power of 45 W, a frequency of 13.5 MHz, and a pressure of 0.5 torr. Once modified, the discs were removed from the plasma reactor, and kept in dishes and ready for surface analysis. Surface analysis then done by Energy-dispersive X-ray spectroscopy (EDS), Scanning electron microscope (SEM), Contact angle (wettability).

Method

Thirty-six shaped implants were machined from peek block and eighteen shaped implant were machined from titanium rods using Lathe machine. The screw length was 8mm (3mm flat part and 5mm threaded part) and about 3 mm in diameter. The height and width of the pitch is 1mm to fit the screwdriver during insertion and removal [18]. The titanium screws were cleaned by solution of (3ml nitric acid, 1ml hydrofluoric acid and 6ml distal water). Then ultrasonic cleaning of all screws with ethanol was carried out to remove any debris.

2 groups of peek implants consist of 18 screws for each group, the first group was control (without nitrogen plasma treatment), and the second group was nitrogen plasma treated, the peek screws were attached by adhesive tape to microscopical slides which is fixed on the base of plasma device, the distance between the plasma source and the base of plasma device are fixed. The nitrogen was introduced to the chamber with a gas flow of 150 cm^3/min, with a power of 45 W, a frequency of 13.5 MHz, and a pressure of 0.5 torr. The screws were then sterilized with a physical mean of sterilization by gamma radiation at dose selected according (AECL, 1984).

Experimental animal description

18 adult male of New Zealand Albino rabbits (weight 1.5 - 1.75 kg) 12-14 months of age were used. Intramuscular injection of an antibiotic (ceftriaxone) was given once daily (0.5ml) for 3 days to avoid any infection [19].

Anesthesia was given by intramuscular injection of xylazine (0.7 ml/kg Body weight) and ketamine 10% (0.5 ml/kg Body weight). If the animal wakes up during the operation, Isoflurane anesthetic inhalation was used (Isoflurane 1 bar with oxygen 1.5 bar). Tibia bone was shaved using spray hair removal from inner side. Before placing the sterilized towel around the operation site, the skin was sterilized with alcohol and iodine. The incision on the medial side was made, then reflection of skin and fascia, and blind dissection was made to the muscle to expose the distal side of the tibia bone. A round bur of 1.3 mm in diameter was used for bone penetration. Three holes with 1cm distance between them was made. The penetration was done by intermittent pressure at a rotary speed of 1500 rpmand reduction ratio of 16:1, and continuous irrigation with normal saline for cooling. Then the holes were enlarged gradually with fissure burs to 2.31 mm [20].

Nitrogen plasma treated screw was implanted in the first upper hole via screw driver until the screw was introduced completely into the bone using torque meter (approximately 1N.cm). The titanium screw was introduced into the second hole, while the untreated screw (control) was placed in the third hole.

Mechanical testing (torque measurement)

A digital torque meter was used that has a measure strain range from 0.1 to 147.1 N.cm (TQ-8800/ Taiwan).

Histological sample preparation

Two legs of two animal from each group of healing interval were used for histological test. The animals were killed through overdose anesthetization of isoflurane general anesthesia. The bone around the implant was cut by a disc cutter with slow speed of rotation and normal saline irrigation. Bone-implant block was obtained by cutting about ½ cm away from the implant screw [21- 23].

Histomorphometric analysis method

The histomorphometric analysis (new bone formation percent measurement) after 2 and 6 weeks of healing period was performed using Fiji ImageJ program (version 1.50b).

The new bone formation percent (NBFP) was calculated according to the following formula: [24, 25]

NBFP% =

Area of newly formed bone

RESULTS

Energy-dispersive X-ray spectroscopy (EDS)

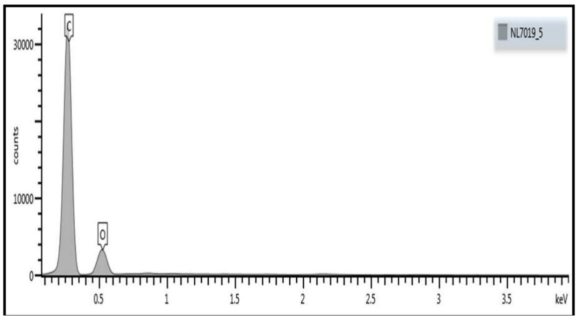
The EDS is showed the control group showed no nitrogen on the surface of the sample, while experimental group with nitrogen plasma showed 1.43 % by weight on the surface as shown in table (1) Figure (2).

Table 1: A: shows no nitrogen on peek surface before plasma treatment

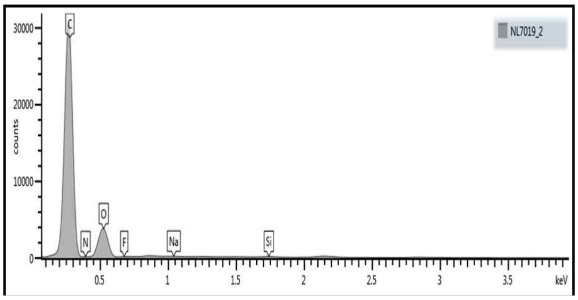
Result Type	Weight %		Total
Spectrum Label	C	O	
1) NL7019_5	81.47	18.53	100.00

Table 1: B: shows nitrogen on peek surface after plasma treatment

Result Type	Weight %						Total
Spectrum Label	C	N	O	F	Na	Si	
1) NL7019_2	77.41	1.43	20.65	0.17	0.10	0.24	100.00



(a)

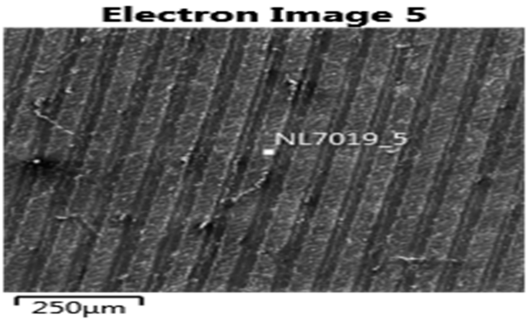


(b)

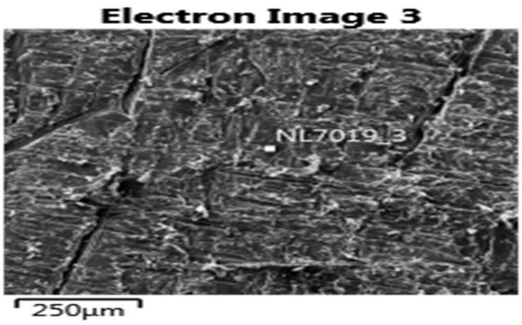
Figure 2: (a) shows no nitrogen on peek before plasma treatment (b): shows nitrogen on peek after plasma treatment.

Scanning electron microscope (SEM)

The SEM images of untreated PEEK sample (Figure 3) showed fiber-like textures and fissures with depressions. While the nitrogen plasma treated peek sample appear to have more obvious tiny fissures when compared to the untreated sample, some features of depressions was maintained from the original sample.



(a)

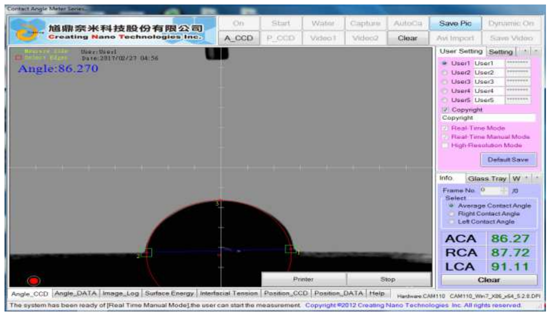


(b)

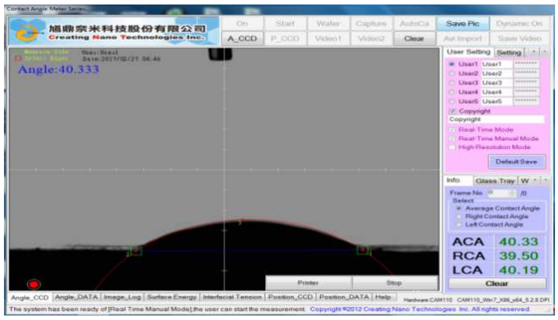
Figure 3: (a) SEM image of peek before plasma treatment shows its depressions from manufactures. (b): image of sem of peek after plasma treatment shows small cavities and irregularities.

Contact angle

The water contact angle was decreased to 40.33 for nitrogen plasma treated peek samples as compared to control group where the contact angle was 86.27 as shown in Figure (4).



(A)



(B)

Figure 4: (A) shows measure of contact angle before plasma treatment (B): shows how contact angle decreased after plasma treatment.

Mechanical testing

After 2 weeks, the nitrogen plasma treated peek implants needed higher torque value to be removed (3.971 N.cm), as compared to untreated peek implants, and for the titanium implants the torque removal were (2.5 N.cm).

After 6 weeks healing period, the nitrogen plasma treated peek implants needed higher torque removal value (9.1 N.cm) as compared to untreated peek, while for the titanium implants mean value for torque removal were (9.02 N.cm). Table (2) Figure (5).

Table 2: Descriptive statistics of torque removal among subgroups for 2 and 6 weeks healing periods

Periods		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
2 weeks	Peek N2 plasma	7	3.971	.795	.301	3.000	5.100
	Tit	7	2.500	.503	.190	1.900	3.300
	Peek	7	1.429	.150	.057	1.200	1.600
	Total	21	2.633	1.189	.259	1.200	5.100
6 weeks	Peek N2 plasma	7	9.157	.199	.075	8.900	9.400
	Tit	7	9.029	.350	.132	8.500	9.500
	Peek	7	5.871	.461	.174	5.500	6.800
	Total	21	8.019	1.593	.348	5.500	9.500

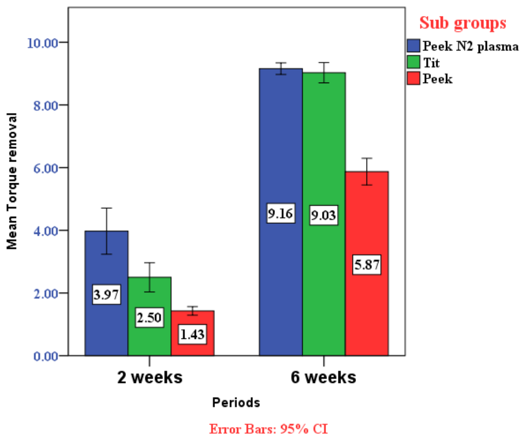


Figure 5: bar chart revealed the torque removal values for different healing periods of subgroups.

In table (3) the comparison between nitrogen plasma treated peek implants with untreated peek implants after 2 weeks, it showed that it's highly significance (p≤ 0.001), while the comparison between nitrogen plasma treated peek implants with titanium implants for same period, it showed that its significance (p≤ 0.05), and in comparison between titanium implants and untreated peek implants it showed highly significance difference (p≤ 0.01).

Table 3: Multiple Comparisons of torque removal among subgroups in 2 weeks using Dunnett's T3

Period	(I) Subgroup	(J) Subgroup	Mean Difference	Std. Error	Sig.
2 weeks	Peek N2 plasma	Tit	1.471	.356	.006
		Peek	2.543	.306	.000
	Tit	Peek	1.071	.198	.003

For the 6 weeks interval, table (4) comparing of the effect of nitrogen plasma treated peek and untreated peek implants revealed highly significance difference (p≤0.001), while in comparison between nitrogen plasma treated peek implants with titanium implants for same period, the difference is non-significance (p > 0.05), and in comparison between titanium implants and untreated peek implants the difference is highly significance (p≤0.001).

Table 4: Multiple Comparisons of torque removal among subgroups in 6 weeks using Tukey HSD

Period	(I) Subgroup	(J) Subgroup	Mean Difference	Std. Error	Sig.
6 weeks	Peek N2 plasma	Tit	.129	.189	.777
		Peek	3.286	.189	.000
	Tit	Peek	3.157	.189	.000

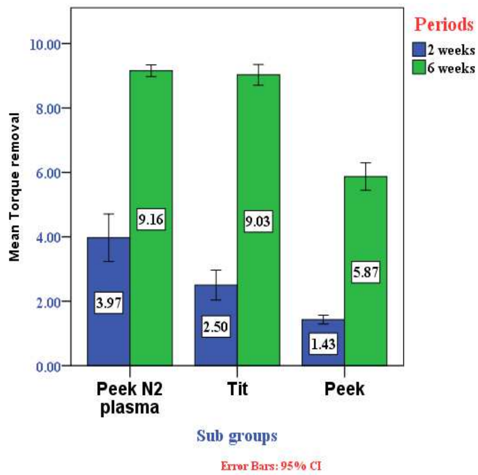


Figure 6: Bar chart reveal difference torque removal value of each group according to time.

Histological features of implanted areas in different intervals

A. Two weeks after implantation

Histological findings of nitrogen plasma treated peek implants after 2 weeks duration showed new bone formation surrounding the screw space**.** New bone trabeculae in the thread area are filled with new osteocytes (OS) and lined by osteoblast cells (OB)**.** The reversal line separate between new and old bone. The new bone area shows woven bone (immature bone) filled with large number of osteocytes Figure (7)**.**

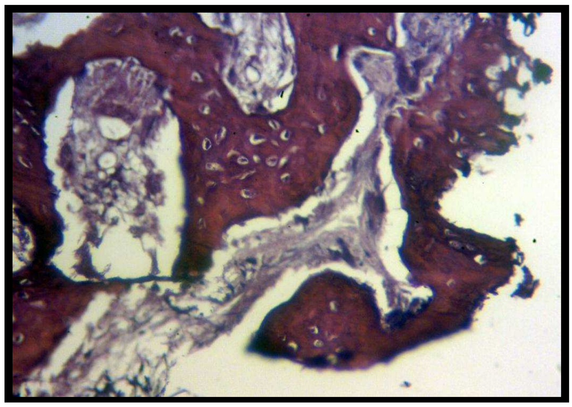


Figure 7: Thread bone formation surrounds the screw space. X10.

B. Six weeks after implantation intervals

The section of bone surrounding nitrogen plasma treated peek in rabbit tibia after six weeks showed bone development process actively, and it's indication by the active osteocytes arranged in circular pattern around Haversian canal (osteon formation) Figure (8).

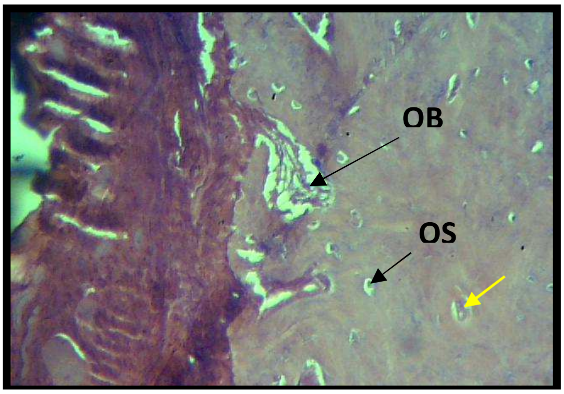


Figure 8: Microscopic photograph view of thread nitrogen plasma treated peek implant after 6 weeks of implantation, shows Haversian system (osteon formation) (yellow arrow), mature bone that filled by osteocytes (OS) and lined by osteoblast (OB) X20

Histomorphometric analysis

The new bone formation percent (NBFP) of the nitrogen plasma treated peek implants in rabbit tibia was greater than that of untreated peek implants after 2 weeks of implantation. The mean of NBFP of nitrogen plasma treated peek implants was 3.45% and for untreated peek was 1.382%, while for the titanium implants was 2.871 as shown table (6) figure (9).

Table 6: Descriptive statistics of percentage of bone formed among groups in the 2 weeks

Periods		N	Mean	±SD	±SE	Min	Max
2 Weeks	N2Peek	20	3.450	.396	.089	2.430	3.920
	Peek	20	1.382	.346	.077	1.020	2.110
	Tit	20	2.871	.179	.040	2.410	3.120
	Total	60	2.568	.934	.121	1.020	3.920

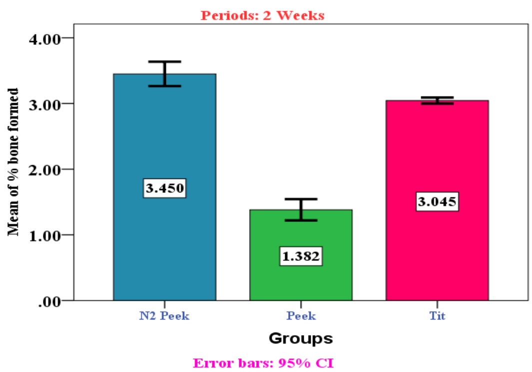


Figure 9: Bar chart shows NBFP value of nitrogen plasma treated peek, untreated peek, and titanium implants after 2 weeks of healing period

In the 6 weeks period of implantation, the new bone formation percent (NBFP) of the nitrogen plasma treated peek implants in rabbit tibia was greater than that of untreated peek implants, The mean of NBFP of nitrogen plasma treated peek implants was 4.777% and for untreated peek was 2.699%, while for the titanium implants was 4.586% as shown in table (7) figure (10).

Table 7: Descriptive statistics of percentage of bone formed among groups in the 6 weeks

Period	Group	N	Mean	SD	SE	Min	Max
6 weeks	N2 Peek	20	4.777	.506	.113	4.010	5.730
	Peek	20	2.699	.432	.097	2.040	3.650
	Tit	20	4.586	.479	.107	4.020	5.220
	Total	60	4.020	1.054	.136	2.040	5.730

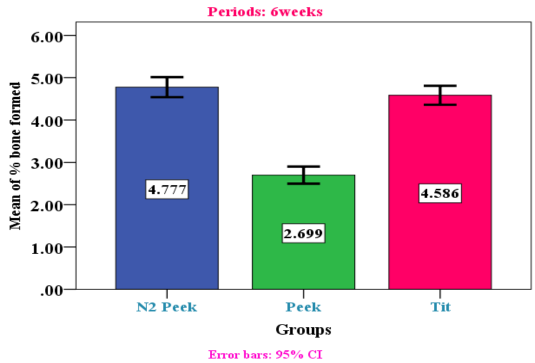


Figure 10: Bar chart shows NBFP value of nitrogen plasma treated peek, untreated peek, and titanium implants after 6 weeks of healing period

In comparison of means of NBFP values between nitrogen plasma treated peek implants and untreated peek implants after 2 weeks of healing period showed a highly significant differences table (8), also for nitrogen plasma treated peek with titanium implants it's highly significant, while in between titanium implants and untreated peek implants it's highly significant.

Table 8: Multiple comparisons of percentage of bone formed between groups in the 2 weeks using Dunnett's T3

	Groups	Groups	Mean difference	SE	P-value
2 weeks	N2 Peek	Peek	2.069	0.118	0.000
		tit	.406	.091	.001
	Peek	Tit	-1.663	.080	.000

HS: Highly significant at p≤0.001

For 6 weeks period of healing after implantation between NBFP values table (9), the nitrogen plasma treated peek implant is highly significant to the untreated peek implants, while between nitrogen plasma treated peek implants and titanium implants its non-significant, also for the titanium implants and untreated peek implants it's highly significant.

Table 9: Multiple comparisons of percentage of bone formed between groups in the 6weeks using Tukey Honestly significant difference (Tukey HSD)

	Group	Group	Mean difference	SE	P-value
6 weeks	N2	Peek	2.079	0.150	0.000
	Peek	tit	0.192	0.150	0.413
	Peek	Tit	-1.887	0.150	0.000

DISCUSSION

There is an increased interest in the use of polyether ether ketone (PEEK) for orthopedic and dental implant applications because of its modulus of elasticity close to that of bone, biocompatible property, and its properties of radiolucency [15]. Polyetheretherketone (PEEK) has created much interest as a convenient polymer substituting metal components in the applications of biomedically [26]. PEEK with physical and chemical properties so excellent, like strength and stiffness is high, properties of fracture toughness and fatigue are good, compatibility with all common methods of sterilization, corrosion resistant, forming easy by molding or machining and a compared density to tissue of human [26,27]. Although with such superior properties, PEEK is still classified as hydrophobic because of its much decreased reaction with the tissue surrounding it, which restricts its applications potentially [27]. This hydrophobic surface property can decrease adhesion of cells. The absence of response from the biological environment made PEEK to be classified as bioinert [12, 14]. The bioinert properties of PEEK mean soft tissues growth around the PEEK implant rather than of bone growth [28]. On polymeric materials plasma treatment is used widely for Surfacemodification. Surface modification can be carried out with nitrogen gas [16]. Because of its low penetration level, plasma modification proposes a method of tailoring the surface while keeping the bulk material excellent physical and chemical properties [17].

In vitro experiment

Radiofrequency nitrogen plasma treatment (RF)

In generating RF plasma, a voltage of radio frequency applied between the two electrodes gives rise free electrons to pulsate and collide with molecules of the gas resulting a sustainable plasma. RF-excited discharges can be sustained without relying on the electrons secondary emission from the cathode. [29].When samples of PEEK is exposed to plasmas of gas, two important chemical processes are expected to occur on the surfaces during the plasma treatment [30, 31]. One is chain scission/etching where bombarding of plasma ions on the surfaces polymer chains on the PEEK, yielding in small volatile degradation products formation [15, 32].

Energy-dispersive x-ray spectroscopy (EDS)

The EDS used for surface analysis showed 1.43% of nitrogen on the surface. The total free energy of the surface of the samples of the plasma treated was greatly more than that of the untreated samples. Thus, the increase in total energy of the surface after plasma treatment is fundamentally because of the increase in the component of polarity [33], which is came from the implantation of nitrogen functional group resulted from interaction.with.radicals.in the plasma.

Scanning Electron Microscopy (SEM)

The nitrogen plasma treated peek showed more obvious tiny fissures on the surface in comparison to untreated peek, the microirregularities could be a factor of effectiveness in encouraging osseointegration through increasing of adherent of osteoblasts, boneformationand attachment to the surface of the implant [34].

Contact angle (Wettability)

After the nitrogen plasma treatment to peek samples the water contact angle showed a great decrease, so it changed the surface characteristics from hydrophobic to hydrophilic. Activating the polymer surface by nitrogen causes hydrophilization of surface increasing roughness and introducing the polar groups,Surface activation with plasma causes increase in the apparent free energy value of the surface,for the nitrogen-modified PEEK surface [35].

RF nitrogen plasma treatment of peek showed increased wettability and increased cell growth, higher rate of cell proliferation, and better cell adhesion [36].

Mechanical test

Several studies showed that implant resistance to removal torque has been related to the amount of osseointegration (degree of bone in contact with the implant) [37].

The removal torque was used to estimate the presence and degree of osseointegration and used for testing the mechanical property of bone-implant interface [38, 39]. The torsional force required for unscrewing the implant is exerted by torque meter. Although the results could be influenced by geometry and topography of implant, this technique basically concentrates on the properties of interfacial shear [40].

A Effect of nitrogen plasma treatment on torque removal value after 2 weeks of implantation

The nitrogen plasma treated PEEK screws implanted in bone of rabbit's tibia registered a higher value of torque of removal this attributed to the positive effect of nitrogen plasma on PEEK is by increasing wettability (decreasing contact angle), creating micro irregularities on the surface (rough surface), and increasing the bond strength, with no disadvantage on cell viability, increasing cell growth, higher rate of cell proliferation, better cell adhesion, improvement of adhesion of osteoblast, spreading, proliferation, and early differentiation of osteogensis, higher roughness of the surface and altering the chemistry of the surface of the treated PEEK, prominent increases in terms of adhesion of the cell, proliferation, and activity of metabolism, an improvement of bioactivity [28,33,36,41,42].

B. Effect of nitrogen plasma treatment on torque removal value after 6 weeks of implantation

Torque removal value was increased for nitrogen plasma treated peek implants than untreated peek implants, with nearly almost the same value for titanium implants. The more amount of the formation of new bone by nitrogen plasma treatment at 2 weeks which was transformed to mature bone at 6 weeks with continuous effect of the local factors like increase wettability, increase roughness altogether with the higher amount of formation of new bone could reflect the higher strength of bond at the interface of implant bone and higher torque removal resistant than the untreated peek implant.

Histological findings

Histological analysis is a highly trusted method to evaluate implant stability that can be done at any time of the implantation [43]. New bone trabeculae formation was showed by histological analysis for both groups with active osteoblast and osteocytes. Also, it was obvious from the results that there was no inflammatory reaction noticed during the periods of experiment. This agreed with the results of [44]. Two weeks after the implantation, the thread area revealed primitive new bone trabeculae formation in treated, untreated, and titanium implants which indicated the starting of bone formation. However, the nitrogen plasma treated implant showed thicker bone trabeculae than untreated implant one, which indicated early bone stimulation. Microscopical observation after 6 weeks of implantation revealed that the thread area of untreated implant showed immature bone with continuous bone remodeling, while for the nitrogen plasma treated implant showed mature bone with numerous osteon formation filled the thread area.

Histomorphometric analysis

Histomorphometric measurement is an invasive method used to test the nature of the implant-tissue surface. It is used for many studies to assess the interface of bone implant [45].

Bone formation percent after 2 weeks of implantation was higher in nitrogen plasma treated peek implants than untreated peek implants, and for titanium implants it is less than the nitrogen plasma treated peek but higher than the untreated peek, which could be attributed to activation of osteoblast by nitrogen plasma layer. The more number of osteoblast and more trabecule of bone and active osteoid tissue notice in the nitrogen plasma group may be evidence to the action of nitrogen plasma layer and effect of wettability and roughness.

Formation of bone ratio at 6 weeks was higher in nitrogen plasma treated peek implant than untreated implants, while for titanium was nearly the same as nitrogen plasma treated peek, Osteoblasts and osteocyte were more around nitrogen plasma treated peek implant which mean more bone formation and maturation and more osteoblast transformation.to osteocyte [46,47].

Hydrophilic surfaces are better for coagulation of blood than hydrophobic surfaces; therefore dental implants have been modified with high hydrophilic and rough surfaces of implant exhibit better osseointegration than conventional ones. Adsorption of proteins such as fibronectin and vitronectin on the dental implants surfaces could stimulate adhesion of cell and osseointegration. [48].

CONCLUSION

1. Radio frequency nitrogen plasma treatment is a successful method for changing peek surface topography and increase wettability.
2. Plasma nitrogen treated peek implant significantly increase the torque removal value at two weeks and six weeks compared to untreated peek implant and titanium implant.
3. Histologically showed that nitrogen plasma treated peek implant had early bone formation at two weeks, and it showed mature bone at six weeks in comparison with untreated peek.
4. Significantly new bone formation ratio increased for nitrogen plasma treated peek implants than untreated peek in both periods.

Conflict of interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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