THE EFFECTS OF 830 NM LASER RADIATION ON NANOSTRUCTURED COMPOSITE SUBSTRATES

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Keywords: *laser, irradiation, nanostructured, composite substrate, Raman spectroscopy*

Abstract: *Laser radiation produces different effects on composite substrates made of nanostructured materials according to the data from the literature. In this study, laser radiation with a wavelength of 830 nm was used for the irradiation of nanostructured substrates with hydroxyapatite (HA), gold nanoparticles (AuNPs) and graphene in the composition. The laser parameters used were: pulse mode – 50 Hz and the energy density of 4.4 J/cm^2, for 30 seconds. To evaluate the nanostructured substrates changes, Raman spectroscopy was performed before and after laser irradiation. The results of the performed investigations have shown that there are no changes produced by laser radiation on composite substrates. Also, no thermal alteration of substrates after laser irradiation was found. The wavelength of 830 nm used in this study should have effect mainly on cells and less on substrates, according to the study.*

INTRODUCTION

Nanostructured composites based on hydroxyapatite (HA), gold nanoparticles (AuNPs) and graphene are new nanomaterials which have great scientific and commercial advantages. Composites based on single layer and few-layer graphenes received great interest due to exceptional characteristics including high surface area, as well as optimal mechanical, thermal, electronic and chemical properties in various fields, such as physics, chemistry, materials science, biotechnology and nanomedicine.(1,2,3)

In situ synthesis of few-layer graphenes over an Au/HA catalyst by using the method of radio frequency chemical vapor deposition (RF-CVD) was previously presented in other studies.(4,5) More research works have been devoted to the fabrication of graphene or its derivatives, such as reinforced hydroxyapatite (HA) biocomposites.(6,7,8) It was reported that nanocomposites (Au/HA@graphene) can be synthesized in situ and present a high biocompatibility with bone cells in vitro.(9)

Influence of the laser on carbon composites was reported in the literature lately. Carbon composites presented much better mechanical properties after the laser treatment than untreated ones.(10)

Effect of the laser irradiation on carbon nanostructured composite mostly depends on laser parameters such as energy, power and pulse duration.(11) Also, in the last years, it has been shown that laser irradiation can modify graphene properties by reducing graphene layers, patterning and cleaning surface.(12,13,14)

Research on the effects of laser radiation on nanostructured composite substrates is not numerous in the literature. Most of these studies are related to the production of composite substrates using laser irradiation and to obtain new properties of these substrates. Because these nanostructured composite substrates are intended to be used as scaffolds for osteoblastic cells in bone regeneration and remodeling, we wanted to see how the properties of these substrates are influenced by their irradiation with a laser wavelength of 830 nanometers (nm).

The choice for a wavelength of 830 nm laser radiation is due to our previous research related to irradiation of fibroblasts. In these studies, by using lasers with different wavelengths, 830 nm laser radiation was found to be the most useful in stimulating cell proliferation.(15)

PURPOSE

The aim of this study was to evaluate changes produced by the laser radiation of 830 nm on different substrates made of graphene composites, gold nanoparticles (AuNPs) and hydroxyapatite (HA). It was also evaluated whether laser radiation produces thermal damage on the substrates used. To evaluate the nanostructured substrates changes, Raman spectroscopy was performed before and after laser irradiation.

MATERIALS AND METHODS

Preparation and characterization of the nanostructured composites

Nanostructured composites made of graphene, gold nanoparticles (AuNPs) and hydroxyapatite (HA) were synthesized by catalytic chemical vapor deposition using induction heating (CCVD-IH) method with acetylene as the carbon source and over a Au/HA catalyst, as it was previously described by Biris et al. in 2011.(9)

The powders of composites obtained were characterized by Raman spectroscopy (figure no.1).

The Raman spectra were collected at room temperature with a JASCO NRS 3 300 spectrophotometer in a black-scattering geometry and a Charge Coupled Device (CCD) detector with a 1 200 L/mm grid and a resolution of 7.58 cm^{-1}.

The incident excitation beam with a diameter of ~ 1 μm^2 was focused on the sample surface by using an Olympus microscope (Olympus 100X objective), while the calibration was done based on the 521 cm^{-1} Si peak. The excitation was

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Article received on 12.02.2015 and accepted for publication on 18.03.2015
ACTA MEDICA TRANSILVANICA June 2015;20(2):115-118

accomplished by using an Ar-ion 514 nm laser with a surface power of 1.5 milliwatts (mW).

Figure no. 1. Powders of nanostructured composites

Preparation of nanostructured composite substrates: culture plates with a diameter of 3 cm were used as support for making nanostructured composite substrates. The powders of nanostructured composite substrates have different elements in the composition as follows: nanostructured HA (S1), nanostructured HA+ 1% AuNPs (S2), nanostructured HA+ 1% AuNPs + 1.6% graphenes (S3), nanostructured HA+ 1% AuNPs + 3.15% graphenes (S4). Colloid suspensions of these powders were produced in PBS (Dulbecco's Phosphate Buffered Saline from Sigma Aldrich) with a final concentration of 0.6 mg/ml for each substrate (S1-S4). From those suspensions, 1 ml was added in four plates. The plates were let to dry for a few hours in a class II laminar flow hood.

Laser irradiation procedure

For irradiation, a semiconductor laser type BTL-10 (Beautyline, Ltd., Prague, Czech Republic) with a wavelength of 830 nm and a hand piece with a convergent radiation emission was used. The irradiation was performed in pulse mode (50 Hz) and the energy density was of 4.4 J/cm^2. The irradiation time was 30 seconds for each culture plate containing different nanostructured substrates. Laser irradiation was performed in a single session for all four culture plates at the density of energy and frequency determined in accordance with this protocol (table no. 1). The temperature of substrates irradiated with laser was measured during the experiment by using a digital multimeter UNI-T model UT33C (Uni-Trend Technology Limited, Dongguan City, China,). The temperature measured during irradiation with 830 nm laser BTL 10 on the substrates ranged between 24 and 26 degrees Celsius. After irradiation with laser wavelength of 830 nm, a new Raman spectroscopy analysis was performed in order to identify specific changes produced by laser radiation on nanostructured components.

Table no. 1. Laser irradiation parameters

Substrates / **Laser BTL-10 with a wavelength of 830 nm**	**S1** HA	**S2** HA + 1% AuNPs	**S3** HA + 1% AuNPs +1.6% graphene	**S4** HA + 1% AuNPs + 3.15 % graphene
A (irradiated area in cm^2) E (energy density in J/cm^2) P (power in mW) F (frequency in Hz) t (time in sec) T (temperature in ^{o}C)	A = 0.20 cm^2 E = 4.4 J/cm^2 P = 36 mW F = 50 Hz t = 30 sec T = 24-26 ^{o}C			

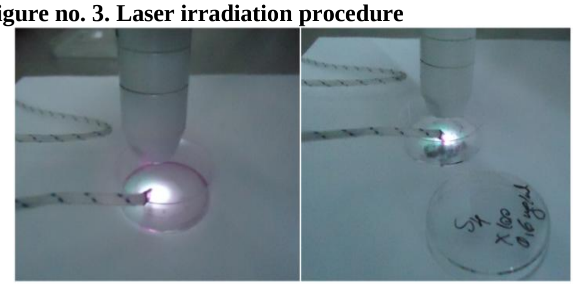
RESULTS

After the preparation of nanostructured composite substrates, four culture plates numbered S1, S2, S3 and S4 were obtained (figure no. 2).

Figure no. 2. Substrates with nanostructured composite

The irradiation was carried out with the laser hand piece at the level of the areas marked on the substrate. At the same time, temperature was measured in the irradiated area. During the experiment, temperature variations ranging from 1 to 2 degrees Celsius were recorded (figure no. 3).

Figure no. 3. Laser irradiation procedure



Analysis of the quality substrates prepared was performed by Raman spectroscopy to identify the components of the nanostructured composite substrates. After laser irradiation with a wavelength of 830 nm, a new analysis of the quality of the irradiated substrates was performed using Raman spectroscopy to identify changes produced by the laser radiation on nanostructured substrate composition.

Figure no. 4 shows the Raman spectra of the spectral range 0-4 000 cm^{-1}, obtained with an excitation of 514 nm, for the substrate S3, before (red) and after irradiation with 830 nm (blue).

Figure no. 4. Raman spectra before and after the irradiation of the substrate S3 with 830 nm (a)

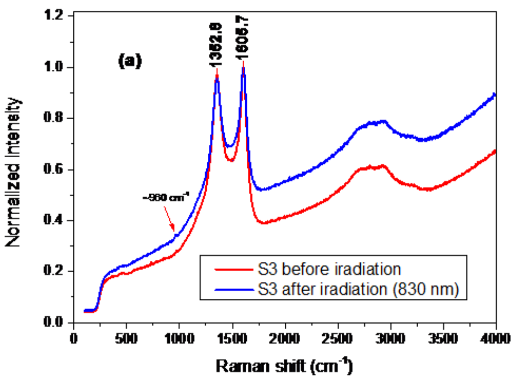
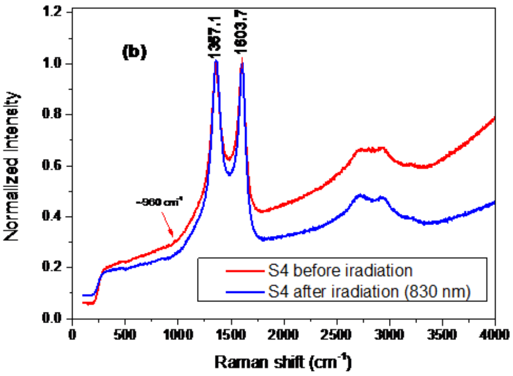


Figure no. 5 shows the Raman spectra of the substrate S4 before (red) and after irradiation with 830 nm (blue), obtained with a 514 nm excitation in spectral range of 0-4 000 cm^{-1}. The spectra are normalized to the amount of G band (1 600 cm^{-1}). In both figures, the peaks for the elements that enter into the composition of the substrates are presented.

Figure no. 5. Raman spectra of the substrate S4 before and after the irradiation with 830 nm (b)



The results obtained after irradiation of substrates S1 and S2 were similar to those of the substrates S3 and S4, by Raman spectroscopic analysis. No changes were observed on peaks for hydroxyapatite and gold nanoparticles before and after laser irradiation with a wavelength of 830 nm.

DISCUSSIONS

Irradiation with a wavelength of 830 nm produced no changes in the irradiated substrates. Laser parameters used in this study like energy density, power, time of irradiation or frequency did not produce alterations of structures substrates. Changing the temperature in the irradiated area with 1 or 2 degrees produced no thermal damage to substrates.

Raman spectra analysis showed the existence of characteristic Raman bands for graphenes with fewer layers (D band at around 1 355 cm^{-1} and G band at around 1 600 cm^{-1}).(16,17) Because the irradiation of substrates with 830 nm produces no change in the position and intensity of the Raman bands of the two substrates, it can be asserted that there are no morphological changes in the substrate after irradiation. Also, the appearance of a peak can be observed (with a very low intensity) around 960 cm^{-1}. That peak could be assigned to the most intense band of Raman spectrum for hydroxyapatite.(18)

In a study of Celiesiute et al. (2014), the results showed that laser irradiation, especially with higher mean power than 150 mW (irradiation dose 3.34 J cm^{-2}), caused an increase in capacity to form nanocrystalline variant of graphene and significant thinning of the graphene-chitosan film. In that case, the laser activation of the films was performed with the picosecond laser Atlantic (Ekspla, Lithuania) with the pulse duration of 10 ps, pulse repetition rate of 100 kHz and the wavelength of the radiation of 1 064 nm. The Raman spectroscopy showed that the laser irradiation cut out the graphene flakes into smaller pieces inducing more edge defects, and the higher graphene load facilitated formation of a larger amount of side defects after the laser treatment.(19) The use of high frequencies and short pulse durations in the range of picoseconds had different effects on graphenes.

Other study demonstrated that the Raman spectrum of graphene was affected by laser irradiation with a power density of more than 3 MW/cm^2, indicating a defect generation by laser irradiation. The electrical resistance of graphenes increased at the laser power density of 1.4 megawatts/centimeter^2 (MW/cm^2), which means that the electrical resistance of graphenes is more sensitive to laser irradiation than the Raman spectrum. In this case, a KrF excimer laser was used with a wavelength of 248 nm in the ultra-violet (UV) laser spectrum.(20) In our study, the power was only 36 mW, for this reason we did not expect significant changes to occur at the level of graphenes in the substrate.

In our study, G band and D band of the Raman spectrum remained unchanged after irradiation with a wavelength of 830 nm. Similar results were recorded in another study in which it was observed that the G peak remained the same, the 2D peak grew and the D peak decreased only by 10% after irradiation. In that case, such a short spectrum alteration cannot be attributed to the change of graphene layers number. The number of graphene layers after irradiation with Nd:YAG laser with a wavelength of 532 nm remained constant.(21)

The use of other types of lasers, such as carbon dioxide laser (CO_2 laser) demonstrated that graphene structure can be modified by irradiation at a low power density (2–60 W/cm^2). Under CO_2 laser irradiation, when the power density was low, crystalline graphene disassembled into nanocrystalline structure and when the power density increased over a certain threshold, hydrogenated amorphous carbon formed on the surface. Also, the thermal effect played an important part in long-wavelength-laser irradiation process. A CO_2 laser device can produce such effects on substrates when using continuous wave (CW) for 5 minutes and power densities between 2 W/cm^2 and 60 W/cm^2.(22)

In comparison with other studies on the effects of laser radiation on graphene, in the present study, a low-power laser in a pulsed mode and with a low energy density was used for a limited time. The wavelength of 830 nm used in our study is close to laser wavelengths used for Raman analysis. For these reasons, we believe that the effects of laser radiation with such a wavelength on substrates are insignificant.

CONCLUSIONS

Laser with 830 nm wavelength produced no morphological changes in the irradiated substrates. No thermal alteration was identified on the substrates after using such laser parameters. These results suggest the use of such substrates as bioactive coatings for cell therapies.

The wavelength of 830 nm used in this study should have effect mainly on cells and less on substrates according to the survey.

The results of this study are limited, however, further studies are needed to evaluate several wavelengths and different parameters of irradiation.

Acknowledgement:

This paper was published under the frame of the European Social Found, Human Resources Development Operational Programme 2007-2013, Project no. POSDRU/159/1.5/136893. Grant with the title: "Strategic partnership to improve the quality of medical research in universities through doctoral and postdoctoral scholarships – DocMed.Net_2.0".

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