Bactericidal activity of PDLLA/carbon nanotubes/nHAp scaffolds for orthopaedic applications

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Abstract: In this study, we evaluated the bactericidal effect of a polymeric scaffold with incorporation of carbon nanotubes (VAMWCNT-O_2) and hydroxyapatite (nHAp) nanoparticles. This biomaterial was developed for use in bone regeneration and the bactericidal activity was correlated with bacterial adhesion mechanism according to thermodynamic theory. The antibacterial tests were performed against *E. coli*. We investigated also the influence of VAMWCNT-O_2/nHAp nanoparticles content on composition, surface energy and surface roughness. The PDLLA/Carbon nanotube/nHAp had an increase of roughness and surface energy. In addition, the bactericidal activity and interfacial free energy of adhesion increases, which is consistent with the thermodynamic theory. PDLLA/VAMWCNT-O_2/nHAp membranes can be a successful as antibacterial bone implant.
Keywords: PDLLA, Carbon nanotube, Hydroxyapatite, Bone regeneration, Bactericidal activity.

Introduction

Infections in orthopedic implant surgery are related to biofilm formation in the implant site [1].

The biofilms formation on the biomaterials surface increases the bacterial resistance to conventional treatments [2]. A promising alternative in the field of bone regeneration is the development of medical devices with bactericidal action [3-4].

In this study, we evaluated the bactericidal effect of a polymeric scaffold with incorporated nanoparticles, specially developed for use in bone regeneration and its correlation with bacterial adhesion mechanism according to thermodynamic theory [5].

Materials and methods

1. Production of porous PDLLA/VAMWCNT-O_2/nHAp membranes

The vertically aligned multiwalled carbon nanotubes were produced using a microwave plasma chamber at 2.45 GHz (MWCVD) and a simple oxygen plasma treatment to obtain superhydrophylic (VAMWCNT) character [6]. We electrodeposited nHAp nanocrystals on VAMWCNT-O2 using a simple electrolyte and three-electrode electrochemistry cell in accordance to Lobo et al. [7].

We removed the nHAp/VAMWCNT-O2 nanoparticles from their respective subtracts and transferred to chloroform solution (20 mL). After we dispersed the nanoparticles under ultrasound irradiation for two min (1200 $J.m.L^{-1}$) maintained at a temperature less than 40°C. After that, we diluted the PDLLA, 9/04 m/m (Purasorb®; Puracbiochem, Holland) 1g under constant agitation by 120 min. We inserted the solutions into a square mold with 0.5 mm diameter and dried in ambient temperature for 12 h in slow evaporation in controlled relative humidity, 70-80% and at room temperature. We performed a simple and fast functionalization to obtain superhydrophilicity character due to incorporation of oxygen-containing groups, using a pulsed-DC plasma reactor with an oxygen flow rate of 1 sccm, at a pressure of 85 mTorr, -700 V, at a repetition rate of 20 kHz. We used PDLLA membranes without any incorporated nanoparticles or oxygen plasma treatment as control.

2. Sample characterization

We used two scanning electron microscopes (SEM): (i) model JEOL JSM 5610 VPI used for the magnifications ranging between 100 - 15.000 times and (ii) high resolution SEM (Field emission Gun, FEG-SEM JSM 6330F) to obtained magnifications ranging between 10.000 - 100.000 times.

We used an optical profilometer for topography and roughness measurements (WYKO NT 1100 series Optical Profiling System).

We performed the contact angle (CA) measurements using an Easy Drop contact angle Measuring Instrument model (Easy Drop DSA 20S) by the Sessile Drop Technique with deionized water (2µL) and diiodomethane (2µL) in temperature and pressure controlled atmosphere. After this, we calculated the surface energy of each sample group, by the methodology proposed by Owens [8].

Thermodynamically, the bacterial adhesion on a solid surface can be described through the balance of interfacial free energy by the following equation [5].

$$\Delta F_{Adh} = \gamma_{SB} - \gamma_{SL} - \gamma_{BL} \qquad (1)$$

Where, ΔF_{Adh} is the interfacial free energy of adhesion, γSB is the solid-bacteria interfacial free energy, and γSL is the solid-liquid interfacial free energy, and γBL is the bacteria-liquid interfacial energy

(equation 1). The adhesion force is favorable thermodynamically when the result is positive.

The interfacial free energy of adhesion is determined by following equation 2 [8-9]:

$$\Delta F_{Adh} = \left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_B^{LW}}\right)^2 - \left(\sqrt{\gamma_S^{LW}} - \sqrt{\gamma_L^{LW}}\right)^2 - \left(\sqrt{\gamma_B^{LW}} - \sqrt{\gamma_L^{LW}}\right)^2 + 2\left[\sqrt{\gamma_L^+}\left(\sqrt{\gamma_S^-} + \sqrt{\gamma_B^-} - \sqrt{\gamma_L^-}\right) + \sqrt{\gamma_L^-}\left(\sqrt{\gamma_S^+} + \sqrt{\gamma_B^+} - \sqrt{\gamma_L^+}\right) - \sqrt{\gamma_S^+\ \gamma_C^-} - \sqrt{\gamma_S^-\ \gamma_C^+}\right] \quad (2)$$

3. In vitro bactericidal activity

The antibacterial activity PDLLA/VAMWCNT-O_2/nHAp membranes were determined using *Escherichia coli* ATCC 25922 (E. coli, gram negative).

Gentamicin (10 mg/mL) and sterile Luria Broth (LB) medium were the positive and negative control, respectively. All the samples with 0.25 cm^2 of area were sterilized by UV light exposition overnight. A suspension of the bacteria ($\sim 10^7$ colony-forming units) of volume 7 µL was dropped on tube containing 6 ml of Luria Broth medium (Sigma) to spread the bacteria uniformly for 3 h at 37 °C.

After that, the samples were placed in a 96 well plate and added to the LB medium with the bacteria solution (1:100 v/v), and the samples were incubated for 3 h at 37ºC. We measured the absorbance of each well content using a spectrophotometer (Spectra Count, Packard).

The bactericidal activity of the bacteria was determined as follows:

$$BA(\%) = 100\left(1 - \left(\frac{B}{C}\right)\right) \quad (3)$$

Where BA is bactericidal activity (%), B is the mean number of bacteria on the analyzed sample, and C is the mean number of bacteria on the control. Data were analyzed using software Origin 8.5® by one-way Anova and Tukey ($p<0.05$).

Results

Figure 1 shows micrographs of the produced PDLLA (Fig. 1a), PDLLA/VAMWCNT-O_2/nHAp membrane (Fig. 1b). Micrograph 1a illustrates the smaller pores on the surface of PDLLA. We observed after incorporated nHAp1 nanoparticles, larger pores with diameter between 12-31µm on the surface (Fig.1b).

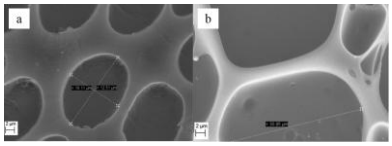

Figure 1: Micrographs of (a) PDLLA and (b) PDLLA/VAMWCNT-O2/nHAp membranes.

We observed a significant roughness difference between PDLLA (control) compared to PDLLA/VAMWCNT-O2/nHAp, 4.11 µm to 4.66 µm, respectively. The PDLLA/VAMWCNT-O2/nHAp presented a roughness increase of 13.38%.

The PDLLA membranes had a contact angle at 112°. After nHAp incorporated nanoparticles the membranes presented values at 89º (decreased of ~23%).

The surface energy components obtained to the Owens method [7] are also listed in table 1. The total surface energy was calculated by the sum of the polar (γp) and dispersive (γd) components.

We used thermodynamic expressions to estimate the dispersive and polar components, as well as the interfacial adhesion energy of membranes. We used for this purpose, the values of surface energy *E. coli* [10]. The data shows that the PDLLA/VAMWCNT-O_2/nHAp exhibited unfavorable results thermodynamically, respectively with ΔF_{adh} more positive values than control (Table 1).

Table 1: Surface energy components and interfacial free energy of adhesion of PDLLA and PDLLA/VAMWCNT-O_2/nHAp membranes.

Samples	Surface free energy (m.N.m^{-1})			ΔFAdh (mJ.m^{-2})
	γ_d	γ_p	Total	
PDLLA	10.47	1.4	11.85	6.64
PDLLA/VAMWCNT-O_2/nHAp	30.42	2.7	33.12	18.67

Figure 2 shows the antibacterial activity of the PDLLA and PDLLA/VAMWCNT-O_2/nHAp membranes. The incorporation of nanoparticles in polymeric matrix besides to change the surface properties and showed the 39.72% of bactericidal effect on *E. coli* culture.

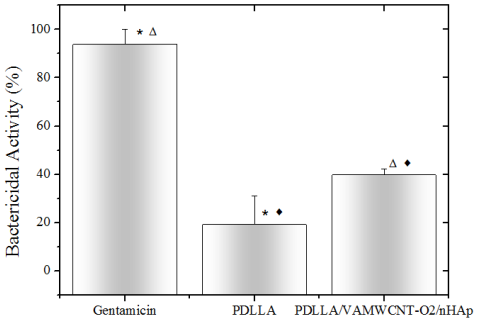

Figure 2: Antibacterial activity of PDLLA and PDLLA/VAMWCNT-O2/nHAp membranes ($p<0.05$).

Discussion

Several authors have reported the use of nanoparticles and polymers for antibiotics controlled delivery [11-12]. Another aspect is the combination of hydroxyapatite and carbon nanotube with silver and gold nanoparticles as biomaterials to prevent bacterial infections [13-14]. However, the mechanism of interaction with nanoparticles and bactericidal properties has been few reported.

In this study, the bactericidal effect can be assigned to the physical changes on the polymer surface due to the incorporation of the nanoparticles.

Some studies have shown that hydrophilic and roughness characteristic are related to the formation of biofilm. The bacterial adhesion increased occurs when the valleys has dimensions similar to the bacteria [15]. In addition, other researchers have reported that the hydrophilicity may be responsible for the decrease in biofilm formation [16].

Conclusion

The incorporation of carbon nanotube/nHAp increased the roughness and the surface energy. The *E. coli* suspension showed a decrease after 3 hours in contact with PDLLA/VAMWCNT-O_2/nHAp membranes showed a considerable bactericidal activity (36.9%). The thermodynamic approach also shows the decrease in bacterial adhesion. PDLLA/VAMWCNT-O_2/nHAp can be a successful as antibacterial bone implant.

Achnowledgment

The authors thank the Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (2011/17877-7), (2011/20345-7), (2012/02159-4), CAPES and FVE for financial support. Thanks to Purac that kindly provide the PDLLA. Thanks to José Evaldo Corat and João Paulo Machado by collaboration. Special thanks to Priscila Leite for scanning electron microscopy images.

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