

The deposition of a lectin from *Oreochromis niloticus* on the surface of titanium dioxide nanotubes improve the cell adhesion, proliferation, and osteogenic activity of osteoblast-like cells

Keicyanne Fernanda Lessa dos Anjos^a, Cynarha Daysy Cardoso da Silva^a, Mary Angela Aranda de Souza^a, Janaina Viana de Melo^b, Alessandra Batista de Mattos^b, Luana Cassandra Breitenbach Barroso Coelho^c, Giovanna Machado^b, Regina Celia Bressan Queiroz de Figueiredo^{a,*}

^a Departamento de Microbiologia, Instituto Aggeu Magalhães (FIOCRUZ-PE), Av. Prof. Moraes Rego s/n Cidade Universitária, Campus da UFPE – CEP 50670-420, – Recife – PE, Brasil; ^b Centro de Tecnologias Estratégicas do Nordeste (CETENE), Av. Prof. Luiz Freire, 01. Cidade Universitária – CEP: 50740-540 – Recife – PE, Brasil. ^c Universidade Federal de Pernambuco (UFPE), Centro de Ciências Biológicas, Departamento de Bioquímica. Av. Prof. Moraes Rego s/n Cidade Universitária, Campus da UFPE – CEP 50670-420, – Recife – PE, Brasil. * Corresponding author: regina.bressan@fiocruz.br; rcbqf01@gmail.com

Abstract

Titanium and its alloys are used biomaterials for medical and dental applications, due to their mechanical and physical properties. The surface modifications of titanium with bioactive molecules can increase the osseointegration by improving the interface between the bone and implant. Titanium dioxide nanotubes (TiO₂NTs) have excellent bioactivity inducing cell adhesion, spreading, growth and differentiation. In this work, TiO₂NTs were functionalized with a lectin from the plasma of the fish *Oreochromis niloticus* aiming to favour the adhesion and proliferation of osteoblast-like cells, improving its biocompatibility. The TiO₂NTs were obtained by anodization of titanium and annealed at 400 °C for 3 h. The resulting TiO₂NTs were characterized by high-resolution scanning electron microscopy. The successfully incorporation of OniL on the surface of TiO₂NTs by spin coating was demonstrated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIE) and attenuated total reflection-Fourier transform infrared spectrum (ATR-FTIR). Our results showed that TiO₂-NTs were successfully synthesized in a regular and well-distributed way. The functionalization of TiO₂-NTs with OniL favoured adhesion, proliferation, and the osteogenic activity of osteoblast-like cells, suggesting its use to improve the quality and biocompatibility of titanium-based biomaterials.

Keywords: mannose-binding lectin, *Oreochromis niloticus* lectin, TiO₂ nanotubes, biocompatibility, osseointegration.

1. Introduction

Titanium (Ti) and its alloys have been extensively applied in the fabrication of implants and prosthesis to repair and/or replace hard tissues, due to their physical characteristics such as: high mechanical strength, corrosion resistance and good biocompatibility [1-2]. Although, TiO₂ presents several advantages, as low cost and improved biocompatibility over other biomaterials, therapeutic failure of TiO₂-based implants and other medical devices may occur due to ineffective bone formation and fixation, leading to bacterial infection and the implant loss. In this regard, the long-term success of titanium alloy implants is reliant on its stable fixation to the surrounding bone which, in turn, depends on the osseointegration, i.e., the formation of a direct interface between an implant and bone, without intervening soft tissue [3]. Furthermore, titanium *per se* lack biological activity and cannot significantly promote the cell adhesion and tissue healing. There is a consensus that the surface topography, morphology, chemical composition, and surface energy of titanium play a critical role on cell adhesion, proliferation, production, and maintenance of extracellular matrix during the osseointegration process [4]. Therefore, physical, and chemical modifications of the titanium surfaces have been developed to create more suitable interfacial microenvironment, promoting cell-material interactions and osseointegration [5,6]. Another way to improve the osseointegration can be achieved throughout the functionalization of implant material surfaces with biomolecules [7].

Lectins, a non-immune proteins, which bind reversibly and in a highly specific manner to simple or complex carbohydrates, have been widely investigated due to its prominent role in several physiological and pathological processes, including immune response, inflammation, cell-cell communication, recognition, and differentiation [8]. It has been demonstrated that lectins from plants and animals have mitogenic [9,10], antibacterial [11,12], immunomodulatory [13,14] antithrombotic [15] and healing [16] activities. Some of these activities are remarkably interesting from the point of view of osseointegration and tissue repair.

The lectin OniL, from the plasma of Nile tilapia *Oreochromis niloticus*, is a mannose recognition lectin with mitogenic and immunomodulatory activities already proven in the literature [17]. These features make OniL a promising functionalizer of TiO₂ surfaces. OniL is a C-type lectin that depends on Ca²⁺ for carbohydrate recognition. This class of lectins has demonstrated important roles on the bone biology and pathogenesis. Clec11a, also known as the stem cell growth factor (SCGF) or osteolectin 1, for example, has been identified as a growth factor able to promote the proliferation and differentiation of hematopoietic stem/progenitor cells. The messenger RNA for this protein is abundantly expressed in proliferating chondrocytes, the primary ossification centre, perichondrium and periosteum [18]. Furthermore, it has been shown that secreted blood mannose-binding lectins (MBLs) of fishes,

as well as other organisms, are important components of innate immunity, playing a crucial role in the body defence [17]. MBL is a constituent of the lectin pathway of complement system activation, one of main components of innate immunity. This lectin plays an essential role in the defence against infectious microorganisms and maintenance of bone the homeostasis [19] and participates in different stages of bone healing.

Previous studies have demonstrated that a lectin from seed of leguminous plant *Cratylia mollis* was efficiently immobilized on the nanotubular surface of TiO₂ nanotubes enhancing the adhesion of osteoblast-like cells [20]. In the present study we explore, for the first time, the potential of an animal-derived lectin with mannose-binding specificity, as a functionalizer agent of TiO₂ nanotubes. For this the TiO₂ nanotubes are produced by anodic oxidation followed by thermal treatment [21,22]. The TiO₂NTs were then negatively charged to improve the binding of OniL. The deposition of OniL on the surface of TiO₂NTs was performed by spin coating. This technique is a solution-based process developed for low-cost deposition of thin films of molecules over a substrate surface [23]. The functionalized TiO₂ nanotubes were characterized and investigated for adhesion and osteogenic activity in osteoblast-like cells.

2. Materials and methods

2.1 Sample preparation - Titanium samples (99.6% purity, Realum Ltd.) with 0.5 mm thickness were prepared as square sheets sized 1×1 cm. The metal surface was polished utilizing a 400-grit emery paper down to 1200-grit emery paper followed by wet polishing in a $15 \mu\text{m}$ alumina slurry. Next, the titanium samples were washed with distilled water, cleaned up with neutral detergent and sonicated for 10 min in isopropyl. Then, the samples were rinsed with deionized water and dried in a nitrogen stream. TiO_2 nanotubes (TiO_2NTs) were obtained by anodization of titanium samples by using a solution containing 89.3% ethylene glycol / 0.7% ammonium fluoride / 10% distilled water, as the electrolyte. The anodization was performed at a potential of 30V for 30 min at 2A. The time-dependent anodization current was recorded with a computer controlled Minipa ET-2076A multimeter. After the electrochemical treatment, the samples were rinsed with deionized water for acid replacement and dried in a nitrogen flux. To crystallize the amorphous anodized TiO_2NTs arrays into crystalline anatase phase, the samples were annealed in a furnace at 400°C for 3 h under air atmosphere.

2.2. Functionalization of TiO_2 with OniL- For the lectin adsorption, the TiO_2NTs were preincubated for 10 min in a 10% NaOH ethanolic solution to confer a negative charge to NTs surface (Neg- TiO_2NTs). The OniL lectin, was then diluted at 100 and 200 $\mu\text{g}/\text{mL}$ in PBS. Neg- TiO_2NTs were functionalized with the lectin by spin coating, using a WS-650 Mz-23 NPPPB Spin Coater (Laurell Technology Corporation, Holland), operating at initial spin speed of 500 rpm and final speed of 2000 rpm for 6 min.

2.3. Morphological Characterization of $\text{TiO}_2\text{-NTs}$ - The morphological characterization of $\text{TiO}_2\text{-NTs}$ was performed by high-resolution scanning electron microscopy, (FEG/EDS – Philips QUANTA 200F). The binding of OniL on the surface of $\text{TiO}_2\text{-NTs}$ was evaluated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIE) and attenuated total reflection-Fourier transform infrared spectrum (ATR-FTIR). The CV and EIE assays were performed using a potentiostat/galvanostat Autolab (Metrohm PGSTAT 128). The Fourier transform infrared spectrum (FTIR) was recorded using a FT-IV Bruker spectrometer.

2.3. Cell culture- Osteosarcoma HOS (ATCC® CRL1543™) cell lineage was used as a model for adhesion, proliferation, and osteogenesis assays. The cells were cultured in Minimum Essential Eagle Medium (MEM) supplemented with 10% of FBS/ 1% penicillin/streptomycin, at 37°C in 5% CO_2 atmosphere. For the experiments, the cells (10^5 cells/mL) were seeded in 24-well culture plates, containing bare TiO_2NTs or Neg $\text{TiO}_2\text{-NTs}$, coated or not with OniL (100

or 200 µg/mL). The cultures were maintained in MEM supplemented with 10% of FBS, under a 5% CO₂ atmosphere for up 72 hours.

2.4. Cell adhesion, proliferation, and viability assays- Cells cultured on the modified TiO₂NTs, coated or not with OniL, for 24 to 72 hours, were assayed for adhesion and proliferation. For this, the samples were washed twice with PBS, fixed in 3.7% formaldehyde in PBS for 10 min and permeabilized with 0.1% triton in PBS for 5 min, at room temperature. After permeabilization, the cells were washed in PBS and staining with rhodamine-phalloidin fluorescent probe for F-actin. After the incubation time, the cells were washed twice in PBS, and counterstained with 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI; Invitrogen Co., USA) for nucleus visualization, and observed in a ZEISS Observer Z.1.apoTome microscope (Zeiss Co., German). The number of adhered cells was estimated by counting the DAPI labelled cell nuclei in ten randomly chosen field/sample (10X objective), using ImageJ 1.4.v software. The cell viability was determined by CellTiter-Glo® (Promega, USA) luminescence cell viability assay (Promega, USA) in accordance with the manufacturer's instructions. Briefly, 250 µL of CellTiter-Glo reagent was added to each culture well and the plates were maintained under agitation for 2 min. After 10 min of incubation the luminescence signal was read on GloMax multiplate reader (Promega, USA.) ATP concentrations were calculated from the standard curve and expressed as µmol.

2.5. Osteogenic potential- Cells cultured on the TiO₂NTs were evaluated for its osteogenic potential. For this, after 24, 48 and 72 hours of cultivation, the samples were washed three times in PBS and transferred for a new 24-well culture plates. The cells were lysed in a 0.5% triton solution in PBS by three freezing/thawing cycles. The samples were collected, centrifuged at 10,000 x g for 5 min and tested for ALP activity assay. ALP activity was quantified by the colorimetric alkaline phosphatase kit (LabTest, Brazil) according to manufacturer's instructions using para-nitrophenol phosphate as substrate. For calcium quantification the cells were cultured for 72 hours, lysed as described above and submitted to calcium detection using Calcium liquiform kit (LabTest, Brazil) according to manufacturer's instructions. The absorbance of the samples was read at 590 nm for alkaline phosphatase and 570 nm for calcium detection, using a Multiskan GO spectrophotometer (Thermo Scientific, USA).

2.6. Statistical analysis- The data are expressed as mean ± SD of two independent experiments in triplicate and analysed using analysis of variance (ANOVA) test followed by Bonferroni post-test or t-Student test. $p < 0.05$ was considered statistically significant. The statistical analyses were performed using the GraphPad Prisma 5.0.

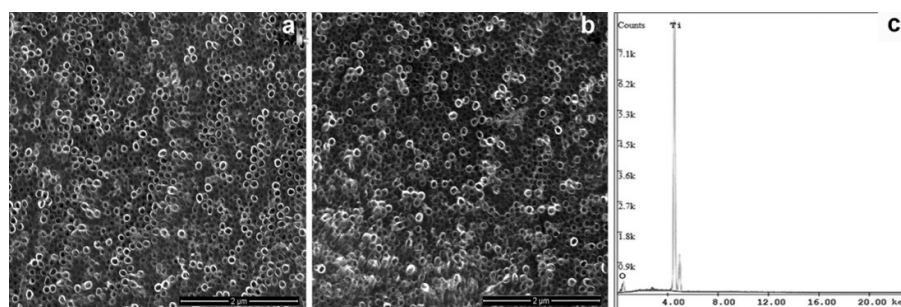
3. Results and discussion

Nowadays, modifications on the surface of titanium implants and its further functionalization with biomolecules have been investigated to improve the quality and biocompatibility of medical devices as prosthesis, and implants [6,7,21,22].

In this work, the anodization followed by thermal treatment of titanium surface efficiently produced a self-organized and homogeneous layer of TiO₂ nanotubes with a mean diameter 73.8 ± 8.2 nm, as confirmed by the EDS-SEM analysis (Fig 1a). Several studies reported that the viability, proliferation, migration, and differentiation of mesenchymal and hematopoietic stem cells, as well as the behaviour of osteoblasts and osteoclasts are strongly affected by the nanometre scale of TiO₂NTs [21,22]. The diameter of TiO₂-NTs obtained in our study was able to promote cell adhesion and proliferation, even without any functionalization of its surface. Our data corroborated previous studies that demonstrated an increase in the biocompatibility of TiO₂NTs in 60-80 nm size range [24,25]. The annealing treatment after anodization changed the structure of TiO₂-NTs to the anatase, as observed by XRD analysis, according to the JCPDS file No. 21-1272. This crystallographic form of TiO₂NTs has been reported as a highly organized structure that favours the nucleation of hydroxyapatite, the inorganic component of bone tissue, supporting the osseointegration process [7, 26-30]. Although the electrochemical anodization is a useful approach to improve the biocompatibility and osteogenesis, as it provides an appropriate microenvironment for the fixation of bone cells, previous studies have demonstrated the great advantages of TiO₂ surface functionalized with biocompatible and bioactive molecules [7,31-34]. This procedure has been shown to reduce the postoperative infection and improve the biocompatibility and osseointegration of the implant [7]. In this work, we used the lectin OniL to functionalize the surface of TiO₂NTs. This lectin is a 17 kDa protein consisting of two subunits of 11 and 6.6 kDa and presents a high affinity for methyl- α -D-mannopyranoside and D-mannose [14]. In mammals, mannose-binding lectins (MBLs) constitute a fundamental link between the innate immune system and other functions, such as coagulation, homeostasis after injury and defence against microorganisms [35]. As the success of the implant and prosthesis is intrinsically associated with the processes mentioned above, we hypothesized whether the functionalization of TiO₂ with OniL could improve the cell adhesion and proliferation compared to the bare TiO₂NTs. For this, we first incubated TiO₂NTs with NaOH solution (pH 13,0) to charge them negatively improving the adsorption of OniL on its surface. According to Bavykin et al. [36] negatively charged nanotubes (Neg-TiO₂NTs) promote electrostatic interactions with cations, presenting an excellent matrix for protein binding [37]. After being negatively charged, the samples were subjected to spin coating to immobilize OniL on the surface of Neg-TiO₂NTs. The spin coating is a useful technique to fast

and easily create a homogenous film with desired and well-controlled thickness [38]. As observed for the bare TiO₂NTs the SEM analysis showed that the deposition of OniL on the surface of these nanotubes did not alter its morphology. (Fig1b). The elucidation of the chemical composition of these TiO₂NTs by EDS showed the presence of Ti and O, demonstrating the successful anodization process, as well as the absence of sample contaminants (Fig.1c).

Figure 1. Ultrastructural assay of TiO₂ modified nanotubes by high resolution scanning microscopy (a) bare TiO₂NT, and (b) OniL-TiO₂NTs (200 µg/mL) (c) EDS-spectrum of TiO₂NTs.



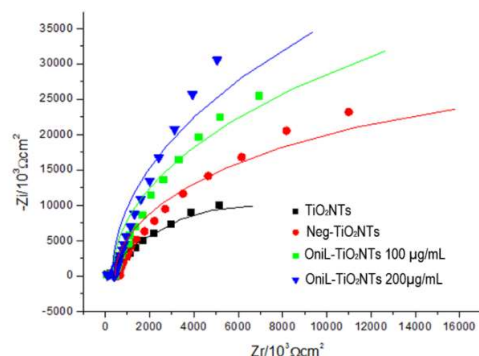
The adsorption of OniL on the TiO₂ surface was monitored by electrochemical impedance spectroscopy (EIS) using K₄ [Fe(CN)₆] / K₃[Fe(CN)₆] (1: 1) as redox pair. The Figure 2 shows that each step of lectin immobilization generates a blockage in the transfer of electrons, increasing the resistance value (R_{ct}). This result demonstrates that lectin was efficiently immobilized on the surface of the samples. The bare TiO₂NTs samples showed a R_{ct} value of 1.7 kΩ. For negatively charged nanotubes, this resistance increased to 3.2 kΩ. The adsorption of OniL to TiO₂NTs substantially increased the R_{ct} value to 24.2 kΩ, while causing a simultaneous decrease in C_{dl} to 6.89 µF. The impedance parameters, adjusted to the Randles equivalent circuit, are shown in Table 1.

Table 1. The impedance parameters for TiO₂NTs modified surfaces using the equivalent circuit, in 10mM ferri/ferrocyanide solution. The values were extracted from the parameters of the EIS equivalent circuit.

TiO ₂ NTs treatment	C _{dl} (µF)	R _{ct} (kΩ)
TiO ₂ NTs	38.89	1.71
Neg-TiO ₂ NTs	35.33	3.30
OniL-TiO ₂ NTs	6.89	24.20

C_{dl}, double layer capacitance; R_{ct}, load transfer resistance.

Figure 2. The Nyquist plots of TiO₂ modified surfaces. TiO₂NTs (black line); Neg-TiO₂NTs (red line), OniL-TiO₂NTs 100 µg/mL (blue line) and OniL-TiO₂NTs 200 µg/mL (green line).

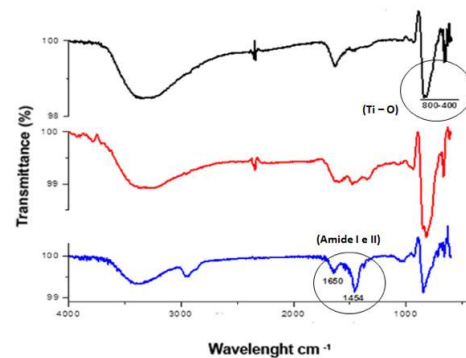


The capacitive behaviour observed in the Nyquist plots was demonstrated by the presence of a double electrochemical layer at the electrode-solution interface, and the dielectric nature of TiO₂ [40].

The ATR-FTIR analysis of TiO₂ and Neg-TiO₂NTs corroborated our electrochemical data. Our results revealed the presence of one absorption band peak characteristic of Ti-O vibration in the region of 400–800 cm⁻¹. The presence of OniL lectin can be confirmed by the appearance of the two main stretches, corresponding to the lectin amide groups in 1643 cm⁻¹ [41] / 1025 cm⁻¹ and 1456 cm⁻¹/1010 cm⁻¹ [42] (Fig. 3). Secondary protein structures are usually identified by analysing the vibration of amide I (1700 - 1600 cm⁻¹), mainly due to the C = O elongation and amide II (1600 and 1500 cm⁻¹), with minor contributions from C-N elongation and N-H [41-42].

In a previous work, the lectin Cramoll from seed of *Cratylia mollis* bean was efficiently immobilized on the surface of anodized TiO₂ nanotubes using Layer-by-Layer (LbL) technique [20]. This technique consists in the growth of alternated layers of poly (allylamine hydrochloride) (PAH) and poly(acrylic)acid (PAA). This self-assembling process occurs by adsorption of oppositely charged polyelectrolytes on the surface of TiO₂NTs [20]. In the present work the lectin OniL was directly bound onto Neg-TiO₂NTs without the need of any additional functionalization step, remaining strongly attached to the surface of negative charged TiO₂NTs.

Figure 3. The FTIR analysis of TiO₂ modified surfaces. General aspect of the FTIR spectra of bare TiO₂NTs (black line), Neg-TiO₂ (red line) and NegOniL-TiO₂NTs (blue line).



The behaviour of osteosarcoma cells in response to the OniL-decorated nanotubes was investigated (Fig.4). This osteoblastic cell-derived lineage is widely used as a cell model to investigate the osseointegration on the surface of nanomaterials *in vitro* [39-40]. To evaluate the human osteosarcoma cell attachment on the TiO₂ modified nanotubes, the cells were labelled with rhodamine-phalloidin, a fluorescent probe for actin, emitting fluorescence at the red channel. The quantification of the adhered cells was performed by counting the cell nuclei labelled with DAPI, which specifically binds to nucleic acids, emitting fluorescence at the blue channel. Our results showed that the deposition of OniL on the surface of TiO₂NTs did not exhibited cytotoxicity and were able to significantly improve the cell adhesion on the NTs. The osteosarcoma cells cultured on both the bare TiO₂NTs and OniL-TiO₂NTs (at 100 and 200 µg/mL) for 24 hours showed a typical distribution of the actin filaments with the formation of focal adhesion points, an essential characteristic to maintain the shape, migration, and proliferation of the cells on the substrate [41]. At this time, there is a predominance of cells with spindle morphology and cell-cell interactions could be easily observed. However, the existence of empty spaces in the bare TiO₂NT samples indicates a low rates of cell proliferation. After 48 and 72 hours a confluent monolayer was observed in all TiO₂NT preparations.

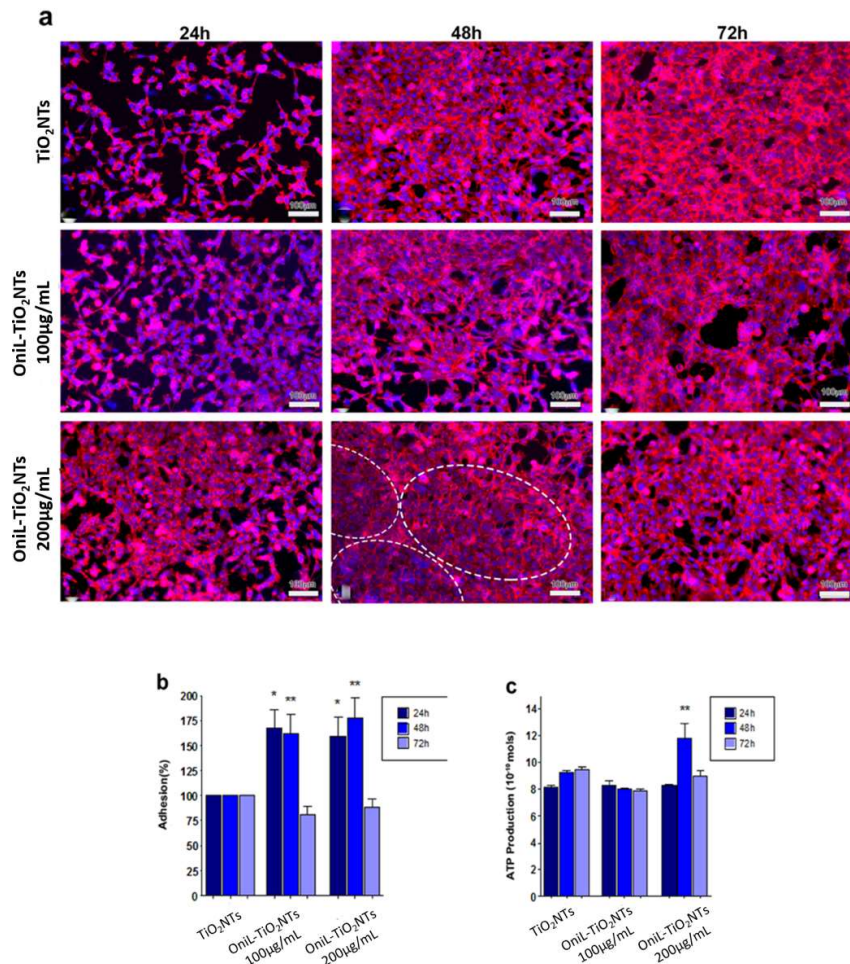
In the OniL-TiO₂NTs samples was possible to observe that cells presented a more flattened extended phenotype and an increased spread on the TiO₂ surface. Interestingly, by 48 hours of cultivation, TiO₂NTs decorated with 200 µg/mL of OniL begun to orient themselves in a more organized concentric manner in comparison to the bare TiO₂NTs (Fig. 4a). Violin et al [42], using the lectin histochemistry methodology, evaluated the differential expression of surface glycoconjugates in Rabbit's tibia implanted with microporous biphasic ceramic material. These authors showed that the lectin binding pattern during bone formation changed,

corroborating the role of differential expression of glycoconjugates and its putative recognition by lectins during the osseointegration process. After 72 hours of cultivation, the cell morphology remained preserved and strict cell-cell contacts could be observed in all modified TiO₂NTs.

The orientated organization of osteosarcoma cells observed on the OniL-treated surfaces, compared to bare TiO₂NTs, may reflect what happens *in vivo*, showing the importance of carbohydrate recognition during osteogenesis on the surface of implants and prosthesis. The quantification of DAPI-labelled nuclei demonstrated a significant increase in the cell adhesion in the samples treated with OniL for 24 and 48 hours compared to the bare TiO₂NTs (Fig. 4b). The functionalization of NTs with OniL was able not only to significantly increase the number of adhered cells by approximately 50%, but also stimulate their proliferation and differentiation on the TiO₂NTs, in both concentrations tested, compared to the control group (TiO₂NTs). After 72 hours of cultivation, the percentage of adhered cells decreases in all samples at the level compared to the TiO₂NTs. (Figure 4b) this behaviour is also observed in the human osteosarcoma cultures maintained in culture plates under standard conditions for up 48 hours (data not shown).

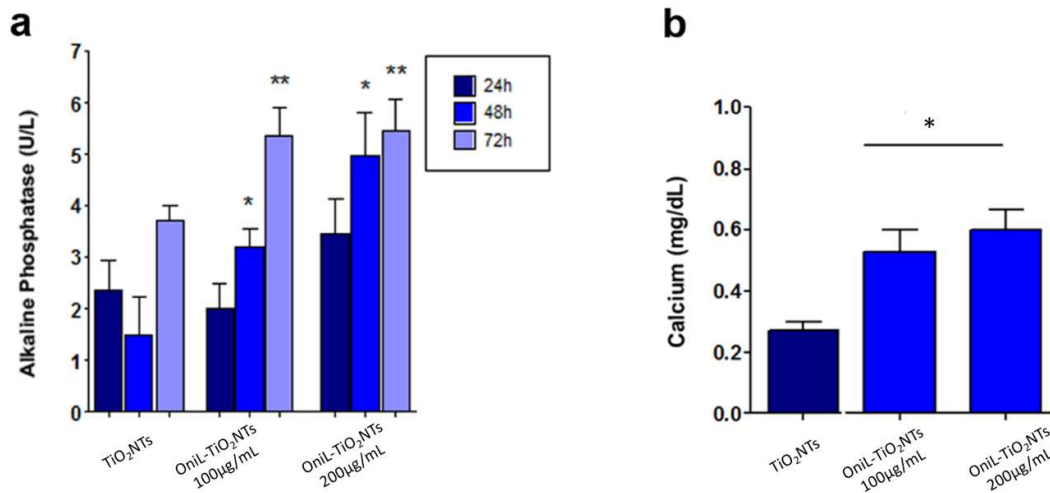
Previous studies showed that mannose-binding lectins as *Lens culinaris* (lentil) lectin (LCL) and α -d-mannose-binding *Narcissus pseudonarcissus* (daffodil) lectin (NPA) did not elicit potent cytotoxicity against osteosarcoma cells [43]. To show that the adhered cells were metabolically active and viable, we quantified the amount of ATP produced by them (Fig. 4c). All the samples actively produced ATP, indicating that the adhered cells were still viable over time. However, only in the cells cultured on OniL-TiO₂NTs at 200 μ g/mL there was a significant increase of cell viability compared to TiO₂NTs. These data corroborate the fluorescence microscopy analysis which showed a peak in the percentage of adhered cells at this concentration at 48 hours of cell cultivation (Figure 4c).

Figure 4. The biocompatibility analysis of OniL-TiO₂NTs. a) Representative fluorescence images of osteosarcoma cells adhered on the surface of TiO₂NT after 24, 48 and 72 hours of cultivation. The actin filaments and nuclei were showed in the red and blue channel, respectively. Note the concentric orientation of cells in the OniL-decorated nanotubes samples (dashed circle) (b) The quantification of cell nuclei in the DAPI-stained osteosarcoma cells, c) The cell viability assayed by ATP quantification in the osteosarcoma cells adhered on the TiO₂ surfaces. Significant differences compared to TiO₂NTs for (*) 24 hours and (**) 48 hours of incubation, $p < 0.05$. The data were obtained from two independent experiments in triplicate.



To investigate whether the functionalization of TiO₂ with OniL was able to induce the osteogenesis, we examined the activity of alkaline phosphatase (ALP) (Fig. 5a). Herein, we clearly demonstrated that the functionalization of NTs with OniL favoured the rapid colonization of the substrate, allowing cell proliferation and osteogenic activity. Osteosarcoma cells cultured on OniL decorated nanotubes, showed a significant increase in the ALP activity as compared with TiO₂NTs, mainly at 200µg/mL of OniL. The highest increase was observed for OniL group after 48 hours with values of ALP activity of 1.5 U/L (TiO₂NTs), 3.2 U/L (OniL100µg/mL) and 5.0 U/L (OniL200µg/mL) (Fig. 5a). Calcium, one of the main elements involved in the bone tissue remodelling was also quantified after 72 hours (Fig. 5b). All the samples from the OniL group presented a significant increase in the amount of calcium compared to the bare TiO₂NTs samples. No statically significant differences could be observed between the lectin treatments (Fig. 5b).

Figure 5. The osteogenic activity of osteosarcoma cells adhered to TiO₂-modified surfaces (a) ALP activity (b) Calcium quantification, after 72 hours of cultivation. The data were obtained from two independent experiments in triplicate. Significant differences compared TiO₂NTs, $p < 0.05$ for (*) 24 hours and (**) 48 hours of incubation.



The osteoblasts and osteoblastic cells are responsible for the synthesis, deposition, and mineralization of the bone extracellular matrix. In this process, the production of alkaline phosphatase is one of the parameters used to assess the effect of biomaterial on bone tissue activity [5]. The increased activity of this enzyme indicated the induction of the biomineralization process. In addition, during growth and remodelling of adult bone, osteoblasts secrete calcium-rich vesicles to the calcifying osteoid [20, 44-45]. As expected, the increase in ALP activity observed (Fig 5a) was followed by the simultaneous enhancement in the calcium deposition (Fig. 5b) [45-46]. Besides its role in the adhesion and differentiation of osteosarcoma cells, the deposition of OniL on the surface of TiO₂NTs may have other beneficial consequences. Due to its immunomodulatory role, this protein can regulate the local inflammatory response, assisting in the bone healing and regeneration. Lectins can bind to cell surface carbohydrates and trigger various cell events, such as stimulation of cell proliferation. In our study, we used an inexpensive and faster methodology to absorb OniL on TiO₂NTs without the need for intermediate polymers.

4. Conclusions

In this study we successfully functionalized TiO₂NTs with the lectin OniL, by using spin coating methodology. Osteosarcoma cells cultivated on the surface of OniL-decorated

TiO₂NTs presented an improved adhesion and proliferation. The OniL also promoted an increase in both the deposition of calcium and ALP activity, which is indicative of an enhanced osteogenic activity compared to the bare TiO₂NTs. Although further studies are still needed to better understand the nature of OniL-TiO₂NTs/osteoblast interactions, our results suggest that OniL could enhance the biocompatibility of medical devices based on TiO₂NTs, assisting in the osseointegration between the bone and TiO₂NTs surfaces.

5. Captions to figures

Figure 1. Ultrastructural assay of TiO₂ modified nanotubes by high resolution scanning microscopy (a) bare TiO₂NT, and (b) OniL-TiO₂NTs (200 µg/mL) (c) EDS-spectrum of TiO₂NTs.

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Figure 5. The osteogenic activity of osteosarcoma cells adhered to TiO₂ modified surfaces (a) ALP activity (b) Calcium quantification, after 72 hours of cultivation. The data were obtained from two independent experiments in triplicate. Significant differences compared TiO₂NTs, $p < 0.05$ for (*) 24 hours and (**) 48 hours of incubation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported herein.

Acknowledgment

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Author Contribution

K.F.L.A. conceived, planned, and carried out the experiments and wrote the manuscript with input of **R.C.B.Q.F.**, **J.V.M.** and **G.M. C.D.C.S.** and **L.C.B.B.C** isolated the lectin and contributed for sample preparation; **M.A.A.S.** and **A.B.M.** contributed for the analysis and interpretation of the results. **R.C.B.Q.F.**, **GM** and **JVM** conceived the study and were in charge of overall direction and planning. **R.C.B.Q.F** reviewed the manuscript and supervised the project.

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