Materials Science & Engineering C 121 (2021) 111772

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Hydrogen plasma treatment confers enhanced bioactivity to silicon carbide-based nanowires promoting osteoblast adhesion

Benedetta Ghezzi [a,b], Paola Lagonegro [c,*], Giovanni Attolini [d], Pasquale Mario Rotonda [e], Christine Cornelissen [e], Joice Sophia Ponraj [f], Ludovica Parisi [a,b,g], Giovanni Passeri [b], Francesca Rossi [d], Guido Maria Macaluso [a,b,d]

[a] Centro Universitario di Odontoiatria, Università di Parma, Via Gramsci 14, 43126 Parma, Italy
[b] Dipartimento di Medicina e Chirurgia, Università degli Studi di Parma, via Gramsci 14, 43126 Parma, Italy
[c] SCITEC-CNR, Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", Via Corti, 12, 20133 Milano, Italy
[d] IMEM-CNR Institute, Parco Area delle Scienze 37A, 43124 Parma, Italy
[e] Lintes Research Laboratory, via Isola 2, 64010 Colonnella (TE), Italy
[f] International Iberian Nanotechnology Laboratory, Avenida Mestre José Veiga s/n, 4715-330 Braga. Portugal
[g] Laboratory for Oral Molecular Biology, Department of Orthodontics and Dentofacial Orthopedics, School of Dental Medicine, University of Bern, Freiburgstrasse 3, 3010, Bern, Switzerland

ARTICLE INFO

Keywords:
Nanowires
Osteoblasts
Wettability
Hydrogen plasma treatment
Bone regeneration

ABSTRACT

Nanomaterials play a pivotal role in modern regenerative medicine and tissue engineering, due to their peculiar physical, optical and biological properties once they are used in the nanometric size.

Many evidences are showing the importance of biomaterial micro- and nano-topography on cellular adhesion, proliferation and differentiation, and hence, tissue regeneration. It is well known that nanowires (NWs) can mimic many different tissues as a result of their shape and their surface characteristics, and that surface hydrophilicity affects early protein adsorption and cellular adhesion.

Therefore a material able to induce bone regeneration might be obtained by combining optimal surface topography and hydrophilicity.

Based on these evidence, we designed silicon carbide (SiC) and core/shell silicon carbide/silicon dioxide (SiC/SiO_x) nanowires with modified wettability in order to analyze cell behavior, using an in-vitro osteoblastic model. First, we synthetized SiC NWs and SiC/SiO_x NWs through a chemical-vapour-deposition (CVD) process, and then we used hydrogen plasma to modify their hydrophilicity. Subsequently we evaluated the four types of NWs in terms of their morphology and contact angle, and we studied their behavior in the presence of MC3T3-E1 murine osteoblasts. Cell metabolic activity, viability, morphology and focal adhesions formation were considered.

Morphological data showed different dimensions between SiC and SiC/SiO_x NWs. SiC NWs before the hydrogen plasma treatment showed a very low contact angle, that was absent after the treatment.

Osteoblastic cells appeared healthy on all of the samples. Interestingly, both hydrophilic SiC NWs and SiC/SiO_x NWs generated a favorable distribution of focal adhesions around the cell body confirmed also by scanning electron microscopy images. Moreover, osteoblasts grown on hydrogen plasma treated SiC/SiO_x NWs showed an increased metabolic activity testified by a significantly higher cell number.

In conclusion, we are here demonstrating that hydrogen plasma treatment of SiC and SiC/SiO_x NWs induce a better osteoblastic cellular adhesion by increasing NWs wettability. We are therefore suggesting that hydrogen plasma treatment of SiC/SiO_x can offer a suitable method to develop scaffolds for bone tissue engineering applications.

* Corresponding author at: SCITEC-CNR, Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", Via Corti 12, 20133 Milano, Italy.
E-mail address: paola.lagonegro@scitec.cnr.it (P. Lagonegro).

https://doi.org/10.1016/j.msec.2020.111772
Received 29 June 2020; Received in revised form 30 October 2020; Accepted 26 November 2020
Available online 4 December 2020

1. Introduction

It has been widely shown that the physical and chemical characteristics of the implantable material affect host response, consequently influencing its own integration within the tissue [1].

In implant dentistry, one of the most important factors affecting osseointegration, the keystone for a useful scaffold, is the superficial micro-topography of the implantable device. Indeed, moderately rough titanium surfaces have been established to deliver a faster new bone formation and to confer greater stability during the healing process [2]. Additionally, it has also been proved that the surface free energy of the material, together with the resulting wettability, further affects the response of biological components [3]. Micro-rough surfaces with enhanced hydrophilicity are indeed known to accelerate the early stages of cell adhesion, proliferation and differentiation and to ameliorate matrix mineralization [4,5]. As such, hydrophilicity has lately gained considerable importance for bone-related biomaterials, with several studies focusing on the pre-clinical and clinical success of more hydrophilic surfaces [6–12].

The biological mechanism behind the effectiveness of the hydrophilic surfaces seems to involve different stages of the implant-bone intimate relationship. Hydrophilicity has been shown to affect the adsorption of proteins at the interface upon insertion, with consequent amelioration of cell response.

[13,14], and recently, a pro-healing phenotype of the host immune cells has been shown using hydrophilic surfaces, that were also capable to mitigate the secretion of pro-inflammatory cytokines [15,16].

Silicon carbide (SiC) materials represent a quite new category of materials, which could find applications in tissue engineering, owing to interesting features, such as excellent biocompatibility, hydrophilicity, high Young's modulus and high mechanical resistance, characteristics that make SiC-based implants suitable for bone tissue engineering. Accordingly, several biomedical devices have already been proposed in regenerative medicine [17–20].

We previously developed SiC-based nanowires and tested their biocompatibility in vitro to reproduce the microrough surfaces of clinically used titanium dental implants [21].

Most interestingly, we observed the establishment of a dynamic dialogue between osteoblastic cell and the NWs-based platform suggesting the possibility that SiC-based NWs are a promising platform to develop implantable devices [13].

In the present study, we aimed to enhance the hydrophilicity of SiC-based NWs platforms using a hydrogen plasma treatment and evaluated the biological response of osteoblastic cells.

2. Materials and methods

2.1. Materials preparation

2.1.1. Nanowires synthesis

Core/shell SiC/SiO_x nanowires (NWs) were grown through an Iron catalyzed CVD process on silicon substrates. 0,05 M iron nitrate in an ethyl alcohol solution was deposited by drop-casting on the silicon surface to cover it completely. After drying, substrates have been inserted into the growth chamber at a temperature of 1100 °C, and CO as precursor gas has been fluxed for about 30 min [22,23]. The complete characterization of the relative composition of core/shell SiC/SiO_x nanowires has been already investigated in some previous publications, which underlined the presence of a SiC internal core and an external SiO_x shell, constitutive the nanowires [22,23].

Cubic silicon carbide nanowires (SiC NWs) were obtained from the core/shell NWs by chemical etching, as described by Fabbri et al. 2014 [24].

2.1.2. Plasma treatment

A low pressure plasma enhanced (LPPE) treatment was made in hydrogen with plasma inductive radiofrequency (RF-ICP) using a self-made machine (Lintes Laboratories).

2.2. Materials characterization

2.2.1. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to evaluate substrate topography; samples images were taken through a dual beam Zeiss Auriga Compact system equipped with GEMINI Field-Emission SEM column (Carl Zeiss). SEM analysis was performed at 5 keV.

2.2.2. Contact angle measurements

The wettability of the samples, in the absence (W/O PT) and in the presence (PT) of hydrogen plasma treatment, has been investigated using contact angle measurements through a selfmade apparatus (Lintes Laboratories) consisting of a sample holder, a syringe to deposit the drop, a microscope in reflected white light episcope with 10× optical magnification and an image acquisition system with video. The experimental conditions were kept at a temperature of 27 °C and atmospheric pressure in a dehumidified room. The drops were bubbled freely from a micropipette and each drop had a volume of 12 μl. The drop fell in the center of the sample which had an area of about 1 cm^2. Once the images have been acquired, the contact angles have been measured with Autocad Software after 4 and 180 s the drop fell on the substrates, to verify the stability of the values.

2.3. Biological assays

2.3.1. Cell culture

MC3T3-E1 pre-osteoblastic cells isolated from neonatal murine calvaria were obtained from the American Type Culture Collection (LGC Standards S.r.l., Sesto S. Giovanni, MI, Italy).

Cells were cultured in complete Alpha-MEM (Thermo Fisher Scientific, Carlsbard, CA, USA) further additioned with 10% Fetal Bovine Serum (FBS, Thermo Fisher Scientific), 1% glutamine (Thermo Fisher Scientific) and 1% antibiotics (Penstrep, Thermo Fisher Scientific).

Cells were seeded at a final concentration of 80.000cells/cm^2 on NWs substrates (size 0,5 cm × 0,5 cm), which were previously sterilized in absolute ethanol (Sigma-Aldrich) for 15 min.

2.3.2. Metabolic activity assay

Cell metabolic activity was evaluated through a tetrazolium salts-based assay 24, 48 and 72 h after seeding. Briefly, cells were washed in Phosphate Buffer Saline (PBS, Thermo Fisher Scientific) and incubated for 4 h at 37 °C with a 0,15 mg/ml solution of Resazurin salt (Sigma-Aldrich) diluted in PBS. Supernatants were then collected, and fluorescence recovered with a Cary Eclipse spectrophotometer (Varian/Agilent, Santa Clara, CA, USA) at ex/em 560/585 nm. After supernatants collection, samples were gently rinsed in PBS and fresh medium added to keep the culture until the following time point.

Cells were seeded in duplicate and the experiment was repeated three times. Data are reported as the mean ± SD.

2.3.3. Live/dead fluorescent staining

To evaluate the amount of live and dead cells on different substrate, a binary fluorescent staining with Calcein AM (Thermo Fisher Scientific) and Propidium Iodide (Sigma-Aldrich) was developed 48 h after seeding. To this purpose, after discard of culturing medium, samples were washed twice in PBS and incubated with a solution of Calcein AM 4 μM and Propidium Iodide 1 μM in PBS for 15 min at 37 °C. Samples were thus rinsed in PBS and fixed with PFA 4% (Sigma-Aldrich) for 20 min.

Specimens were finally observed with a fluorescence microscope Zeiss Axio Imager 2.0 (Carl Zeiss), while images taken with an AxioCam ICm1 (Carl Zeiss). Moreover, images were analyzed, and the number of viable cells was quantitated through the NIS-Element Br5.11 Software (Nikon, Tokyo, Japan).

2.3.4. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to better evaluate osteoblasts morphology. 24 h after seeding, culturing medium was discarded, and samples rinsed in PBS. Subsequently, cells were fixed in a 2,5% glutaraldehyde solution (Sigma-Aldrich) for 20 min and washed in sodium-cacodylate buffer (Sigma-Aldrich) for 5 min. Specimens were thus dehydrated in ethanol at increasing concentrations, dried to critical point with liquid carbon dioxide (CPD 030 Baltec, Wallruf, Germany) and covered with a nanometric thin gold layer.

Samples images were taken through a dual beam Zeiss Auriga Compact system equipped with GEMINI Field-Emission SEM column (Carl Zeiss). SEM analysis was performed at 5 keV.

2.3.5. Immunofluorescence

An immunostaining for vinculin was performed in combination with actin cytoskeleton labelling in order to study focal adhesions distribution within cell cytoplasm on treated and not NWs.

Culturing medium was removed 24 h after seeding, and cells fixed in 4% PFA for 10 min at room temperature (RT). Samples were then rinsed twice in PBS and cells permeabilized with 0.1% Triton X-100 for 5 min, then washed again in PBS. Subsequently, 1% bovine serum albumin solution (BSA, Sigma-Aldrich) in PBS was added for 30 min at RT, followed by 1 h of incubation with primary anti-Vinculin monoclonal antibody, clone 7F9 (FAK100, Merk Millipore) at a dilution of 1:100. After two rinses in PBS, samples were incubated with secondary anti-mouse antibody labelled with the Alexa-Fluor®488 chromophore (Thermo Fisher Scientific) at a dilution of 1:400, to reveal the primary anti-vinculin antibody, and co-incubated with a 1:200 solution of TRITC-conjugated phalloidin (FAK100, Merk Millipore) for 1 h. Afterwards, samples were rinsed three times in PBS and cell nuclei counterstained with DAPI solution (FAK100, Merk Millipore) for 10 min at RT. Samples were then washed and mounted on a microscope glass using a mounting medium to reduce photo-bleaching (Agilent Technologies, Saint Claire, CA, USA). Samples were observed, and images were taken with a stereomicroscope equipped for fluorescence (SMZ, Nikon, Tokyo, Japan).

3. Results

3.1. Hydrogen plasma treatment improved the hydrophilicity of SiC NWs

Nanostructures morphology was observed through SEM (Fig. 1 A-B). NWs samples presented a diameter of 60–80 nm for core/shell SiC/SiO_x NWs and of 20 nm for SiC NWs, with a length of several tens of microns. As shown in Fig. 1, substrates were homogeneously covered by nanowires, that appeared tangled together. The contact angle measurements of SiC NWs and SiC/SiO_x NWs showed that samples presented excellent hydrophilicity (Fig. 1 C-D-E-F). Interestingly, SiC NWs contact angle has been completely eliminated after the hydrogen plasma treatment, improving the hydrophilicity of the sample. The technique was not able to detect wettability changes on SiC/SiO_x NWs.

In order to proof the reliability and the stability of this procedure, pictures were taken 4 and 180 s after the fall of the drop on the surfaces, the measures were repeated three times and no differences were detected between the two time points.

3.2. Hydrogen plasma promoted cell proliferation and viability on core/shell SiC/SiO_x NWs

Hydrogen plasma treatment did not affect cellular metabolic activity of murine osteoblasts on both the nanowires surfaces (Fig. 2). Remarkably, at 48 h, osteoblasts grown on SiC/SiO_x nanowires showed a strong increase in cell proliferation (Fig. 2B), phenomenon not detected on SiC samples (Fig. 2A).

The viability of MC3T3-E1 cells on SiC and SiC/SiO_x NWs prior and after hydrogen plasma treatment has been qualitatively assessed by a live/dead fluorescent staining at 48 h (Fig. 3).

Cells appeared to be healthy, with intact cytoplasm and their typical polygonal shape on all the tested surfaces. Furthermore, very few dead cells were detected, and no considerable differences between treated and native samples. Of relevance, SiC/SiO_x NWs PT showed a significantly higher number of living cells after 48 h (Fig. 3F), thus confirming the difference reported in Fig. 2B.

3.3. Hydrogen plasma treatment promoted a dynamic and pro-active interaction between cells and SiC-based NWs

A SEM analysis was performed in order to better evaluate MC3T3-E1 morphology and interaction with the NWs substrate.

MC3T3-E1 on SiC NWs without hydrogen plasma treatment (Fig. 4 A-B-C) appeared to be equally distributed on the surface. Most of the cells presented several cytoplasmatic projections protruding from the cellular body and favouring their anchorage to the substrate. On the contrary, after hydrogen plasma treatment of the material (Fig. 4 D-E-F) cells were

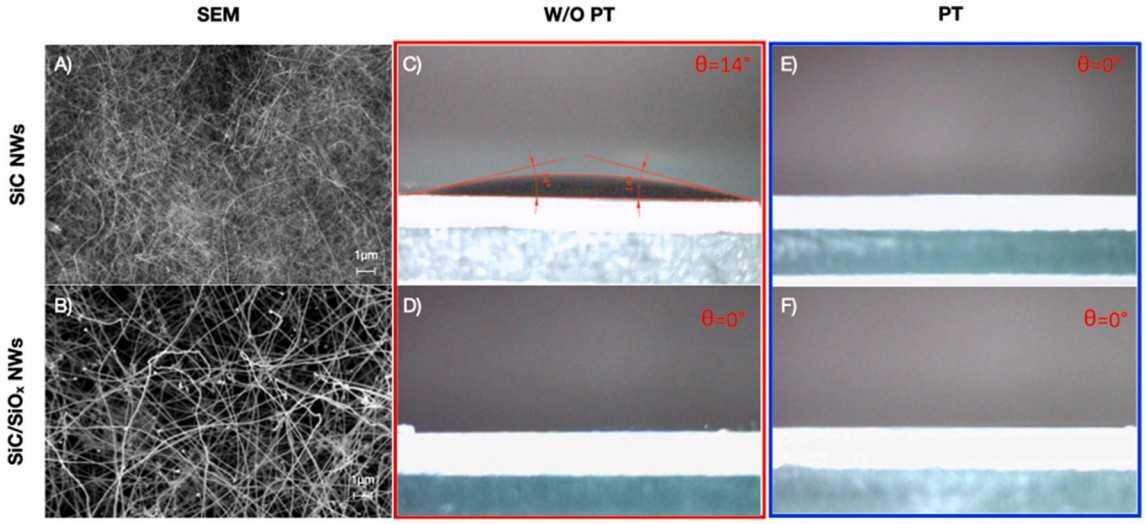

Fig. 1. SEM images of SiC NWs (A) and SiC/SiO_x NWs (B) morphology. Images of the contact angles before (W/O PT) and after (PT) plasma treatment on SiC (C-E) and SiC/SiO_x (D-F) nanowires. The median values of the contact angles of three repeated measures has been indicated in the figure.

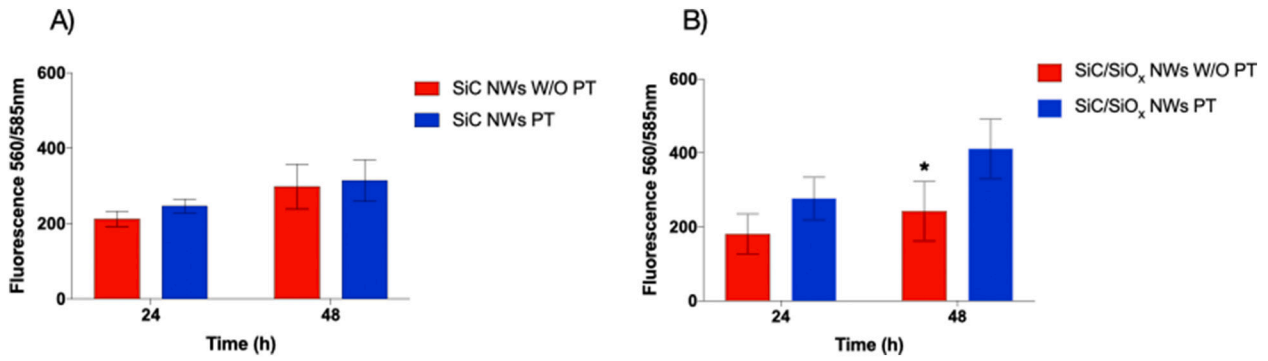

Fig. 2. Metabolic activity assay of MC3T3-E1 osteoblastic cells cultured on SiC (A) and SiC/SiO_x (B) nanowires for 24 and 48 h. *p = 0,025 PT vs. W/O PT.

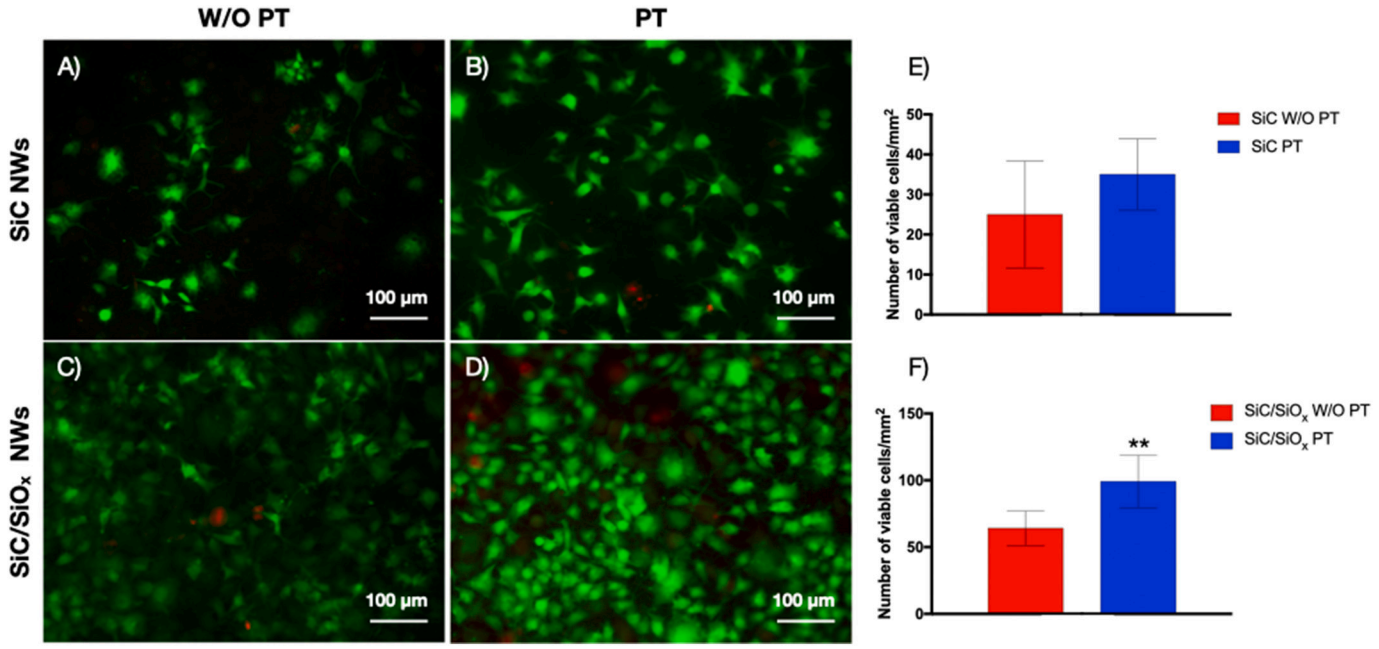

Fig. 3. Fluorescent staining with Calcein AM and Propidium Iodide on MC3T3-E1 osteoblasts cultured for 48 h on SiC and SiC/SiO_x NWs before (A-C) and after (B-D) hydrogen plasma treatment; *E*-F) histograms presenting the amount of viable cells on SiC and SiC/SiO_x NWs before (W/O PT) and after (PT) hydrogen plasma treatment, **p = 0,0049.

flattened with a more pancake-like shape. This behavior resembles a better recognition of the NWs substrate, which appear to be pulled by the cell during an active cytoplasm contraction (Fig. 4 F).

Interestingly, when cells were cultured on SiC/SiO_x NWs in the absence or in the presence of hydrogen plasma treatment (Fig. 5), they all showed their typical polygonal shape together with an intact cytoplasm. Hydrogen plasma treatment did not promote any visible change of cell morphology, suggesting that the hydrophilicity of the surface strongly affects the cellular response.

Finally, we evaluated the different interaction among MC3T3-E1 cells and four NWs tested, using a tri-fluorescent staining for actin cytoskeleton, focal adhesions and cells nuclei.

Data reported in Fig. 6 were in line with SEM observations. Cells cultured on SiC NWs W/O PT showed numerous and evident cytoplasmatic protrusions (Fig. 6A) and vinculin expression was detected only at the edge of the cells. On the contrary, both SiC and SiC/SiO_x NWs treated with plasma presented a more homogenous vinculin distribution along the entire cell body (Fig. 6B-D).

Furthermore, in both NWs W/O PT, focal adhesions shape was organized as dot-like labels (Fig. 6 yellow circles), while after plasma treatment, they appaired more elongated (Fig. 6 yellow squares).

This observation supports the hypothesis that plasma treatment over promoting the wettability of the surface, would also confer a higher degree of bioactivity to the substrate, which thus better interact with the cells.

4. Discussion

New implantable materials for orthopedic and dental surgery aim to regenerate bone through the use of engineered scaffolds allowing the synthesis of new extracellular matrix (ECM). Implants require a favorable tissue-material interface to properly function, thus their surface characteristics have been implemented to direct cell behavior. In particular, the main features leading to a proper osseointegration are surface chemistry, topography and energy. The majority of implantable materials present a roughened surface due to its ability to promote cell differentiation into mature osteoblasts able to deposit new bone [25,26]. It has been clearly shown that different macroscopic topography as well as micron- and nano-scale features of the surfaces are differently recognized by cells and can deeply affect their behavior [27–29]. In these regards, to favor tissue regeneration, the introduction of nanostructured surfaces paved the way to the construction of new biomimetic materials. The final goal was to recreate the in vivo microenvironment of cues and stimuli necessary for cell adhesion, proliferation, migration

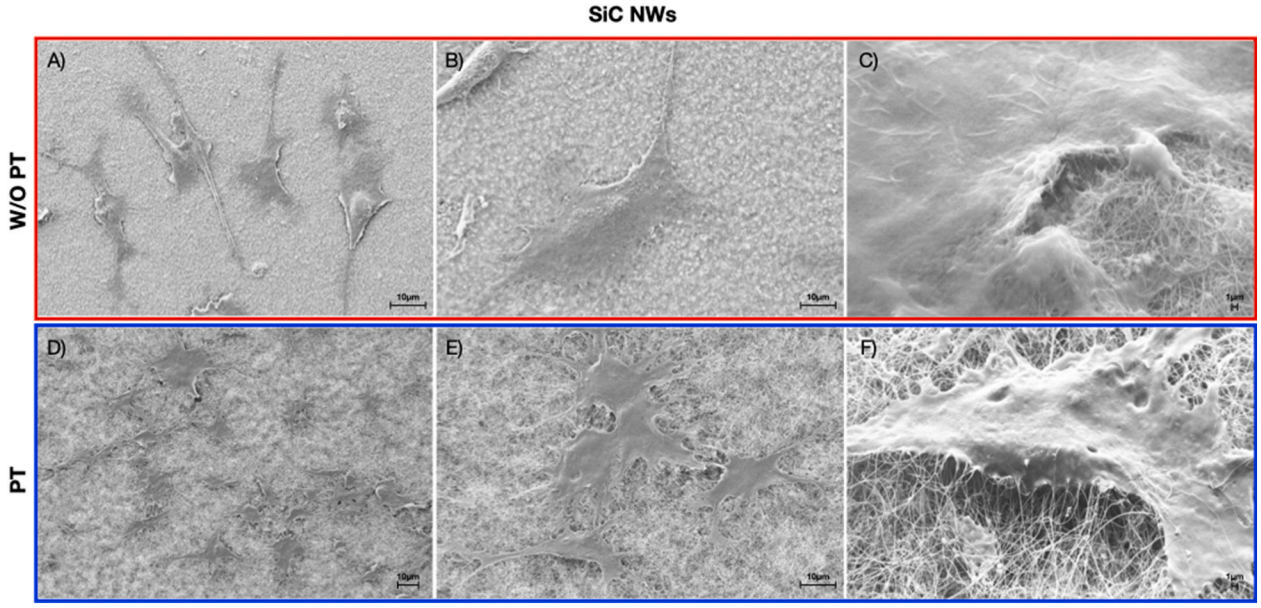

Fig. 4. MC3T3-E1 cells cultured for 24 h on SiC NWs before (A-B-C) or after (D-E-F) hydrogen plasma treatment.

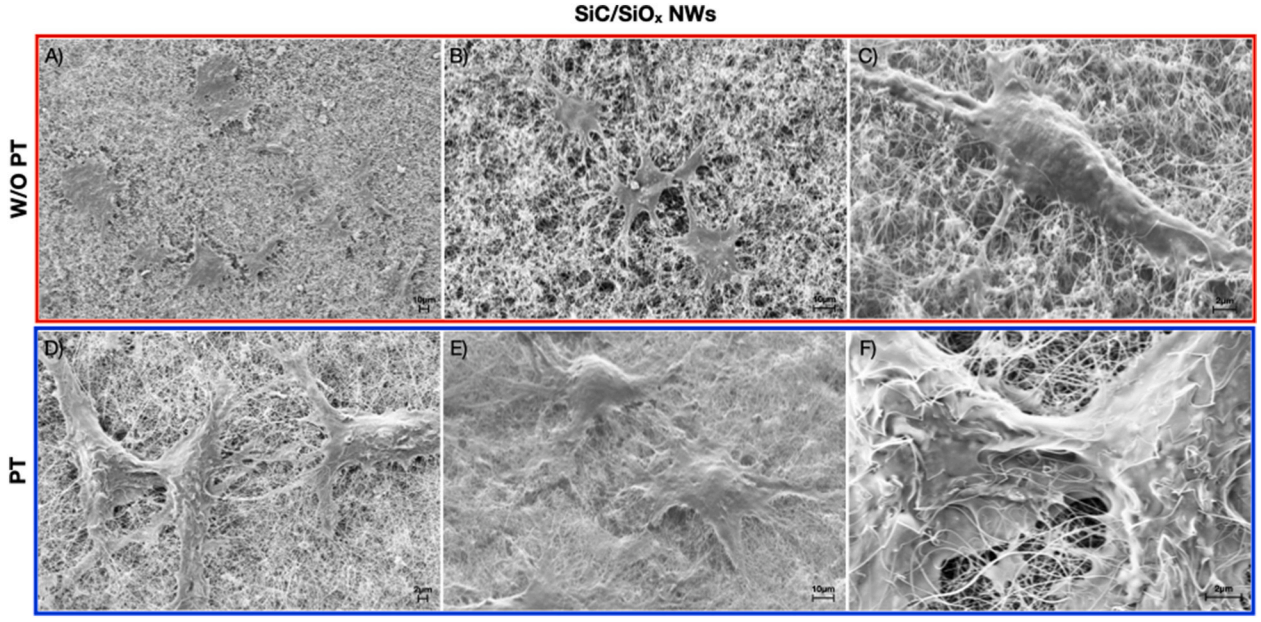

Fig. 5. MC3T3-E1 cells cultured for 24 h on SiC/SiO_x NWs before (A-B-C) or after (D-E-F) hydrogen plasma treatment.

and differentiation, in order to favor tissue regeneration [30].

Moreover, surface wettability is known to play a pivotal role in the acceleration of osteogenesis and in the increase of bone-to-implant contact both in clinical and preclinical settings [10,31,32].

Surface energy and wettability are also fundamental for the early stage of protein adsorption to the implant surface, which could consequently alter the long-term biological response [33,34]. Protein adsorption to the surface is one of the first event of implant positioning and, even if the biological mechanisms are not completely understood, surface conditioning seems to deeply influence the clinical success of dental implants [35]. Many modification has been tested to enhance implant hydrophilicity, as the use of thermal treatments, known to enhance surface wettability and consequently leading to a better cellular response [14,36].

We have previously shown that treating a nanowires surface with 3-mercaptopropyltrimethoxysilane enhanced surface hydrophilicity. This procedure altered cell response in terms of adhesion and differentiation, probably due to the improved capability to bind proteins and subsequently cells [13].

Moreover, also on titanium implants, we recently demonstrated the importance of surface hydrophilicity for cellular adhesion and differentiation, underlining the pro-differentiative stimuli of enhanced wettability on murine cells, leading to an osteoblastic commitment [37].

Silicon carbide-based NWs strongly resemble the structure of bone ECM, and the morphological aspect of NWs is close to that of titanium implants used in dentistry. Therefore, SiC-based NWs seem to be a viable option to design materials for dental and orthopedic applications [38–40].

In the present study, we aimed to enhance the hydrophilicity of SiC-based nanowires by hydrogen plasma treatment, thus, to analyzed their

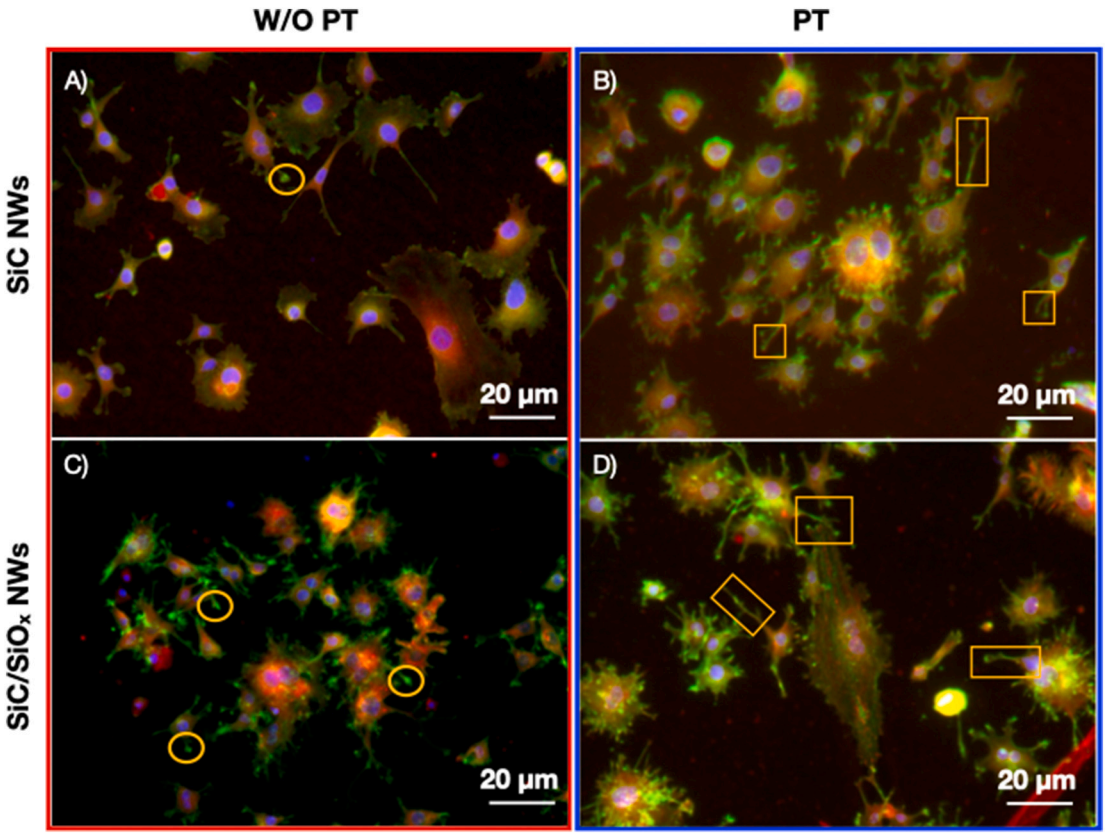

Fig. 6. Immunofluorescence for focal adhesions (green), cytoskeleton (red) and nuclei (blu), after 24 h of culture on A) SiC NWs W/O PT, B) SiC NWs PT, C) SiC/SiO_x NWs W/O PT and D) SiC/SiO_x NWs PT. Dot-like discrete focal adhesion on W/O PT samples are indicated by the yellow circles, while more elongated focal adhesions on PT samples are indicated by the yellow squares. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effectiveness for adhesion and proliferation of murine pre-osteoblastic cells.

SiC and SiC/SiO_x NWs have been grown by CVD as previously reported [22–24], These NW were further treated with hydrogen plasma to increase their wettability, that has been measured before and after plasma treatment. Plasma treatment showed considerably effects in ameliorating surface hydrophilicity only for SiC NWs. Consistently, the amelioration of cell adhesion on hydrophilic SiC NWs was evident, with cells showing a flattened and stellate shape (Fig. 4 and Fig. 6). Of relevance, cells cultured on SiC NWs in the absence of PT, resembled the typical morphology of mesenchymal cells when cultured on the commercially available SLA microrough dental implant surfaces [41]. On the other hands, cells cultured on SiC NWs with plasma treatment and on both SiC/SiO_x NWs in the absence or in the presence of plasma treatment, showed similar pancake-like shape, typical of a mature osteoblastic phenotype [27]. Normally, cell adhesion to the substrate is a fundamental part of cell cycle, it is the base of the control of cell growth, proliferation and differentiation. Cells can flatten out on a surface only after they have firmly adhered to it, their cytoskeleton can contract and cell body can spread becoming thinner and larger [42]. This mechanism of adhesion is mediated by peculiar structures characterized by multiproteic complexes involved in the clustering of ligated trans membrane receptors of integrins. It has been demonstrated that spacing between the integrin ligands is crucial for focal adhesion formation, and it should be comprised in the 50-70 nm, while a nanopatterned material with spacing larger than 100 nm tends to inhibit cell adhesion [43–46].

The size of our SiC and SiC/SiO_x NWs, with a mean diameter of 20 and 60 nm, respectively, may justify the high integrin clusterization observed for SiC PT and for both SiC/SiO_x NWs type. In this context, the enhanced hydrophilicity might led to an increased protein adsorption, a key step for an effective cellular adhesion on the material [37]. It was evident that the amelioration of surface wettability occurred on SiC NWs led to a better cell adhesion 24 h after seeding. Surprisingly, the use of plasma treatment on core/shell nanowires did not altered surface hydrophilicity (ie. contact angle), but caused a different cellular response, particularly evident in the immunofluorescent staining (Fig. 6). This result has been confirmed by a significative increase in cellular proliferation on core/shell nanowires after 48 h of culture, and this is probably due to the positive effect of the treatment itself or also to its ability to eliminate contaminants potentially present on the surface.

In the complex, our results underlined the importance of a hydrogen plasma treatment on nanostructured surface that has been shown to ameliorate surface wettability, enhance biological response and confer bioactivity to a scaffold potentially capable to support bone tissue regeneration.

5. Conclusion

We have here shown that SiC and SiC/SiO_x NWs hydrophilicity is a key factor to promote biological response of murine osteoblasts in terms of adhesion and spreading.

Taken together, these data suggest that hydrogen plasma treatment on silicon carbide-based nanowires may be an excellent technique to enhance scaffold wettability, bioactivity and consequently driving a better cellular response.

Our findings might provide new information in the field of implantable materials for regenerative medicine purpose.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to acknowledge the help of dr. Davide Calestani (IMEM-CNR, Parma, Italy) for his assistance with SEM-FIB analysis.

References

[1] K.J. Burg, et al., Comparative study of seeding methods for three-dimensional polymeric scaffolds, J. Biomed. Mater. Res. 51 (4) (2000) 642–649.
[2] A. Wennerberg, T. Albrektsson, Effects of titanium surface topography on bone integration: a systematic review, Clin. Oral Implants Res. 20 (Suppl. 4) (2009) 172–184.
[3] C. Massaro, et al., Comparative investigation of the surface properties of commercial titanium dental implants. Part I: chemical composition, J Mater Sci Mater Med 13 (6) (2002) 535–548.
[4] C. Eriksson, H. Nygren, K. Ohlson, Implantation of hydrophilic and hydrophobic titanium discs in rat tibia: cellular reactions on the surfaces during the first 3 weeks in bone, Biomaterials 25 (19) (2004) 4759–4766.
[5] M.M. Bornstein, et al., Bone apposition around two different sandblasted and acid-etched titanium implant surfaces: a histomorphometric study in canine mandibles, Clin. Oral Implants Res. 19 (3) (2008) 233–241.
[6] D.V. Kilpadi, J.E. Lemons, Surface energy characterization of unalloyed titanium implants, J. Biomed. Mater. Res. 28 (12) (1994) 1419–1425.
[7] D. Kohavi, et al., Wettability versus electrostatic forces in fibronectin and albumin adsorption to titanium surfaces, Clin. Oral Implants Res. 24 (9) (2013) 1002–1008.
[8] F. Rupp, et al., Roughness induced dynamic changes of wettability of acid etched titanium implant modifications, Biomaterials 25 (7–8) (2004) 1429–1438.
[9] F. Rupp, et al., Wetting behavior of dental implants, Int. J. Oral Maxillofac. Implants 26 (6) (2011) 1256–1266.
[10] D. Buser, et al., Enhanced bone apposition to a chemically modified SLA titanium surface, J. Dent. Res. 83 (7) (2004) 529–533.
[11] F. Schwarz, et al., Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs, J. Periodontol. 78 (11) (2007) 2171–2184.
[12] F. Schwarz, et al., Bone regeneration in dehiscence-type defects at non-submerged and submerged chemically modified (SLActive) and conventional SLA titanium implants: an immunohistochemical study in dogs, J. Clin. Periodontol. 35 (1) (2008) 64–75.
[13] B. Ghezzi, et al., Osteoblast adhesion and response mediated by terminal -SH group charge surface of SiOxCy nanowires, J Mater Sci Mater Med 30 (4) (2019) 43.
[14] A. Toffoli, et al., Thermal treatment to increase titanium wettability induces selective proteins adsorption from blood serum thus affecting osteoblasts adhesion, Mater. Sci. Eng. C Mater. Biol. Appl. 107 (2020) 110250.
[15] S.M. Hamlet, et al., Hydrophilic titanium surface-induced macrophage modulation promotes pro-osteogenic signalling, Clin. Oral Implants Res. 30 (11) (2019) 1085–1096.
[16] K.M. Hotchkiss, et al., Titanium surface characteristics, including topography and wettability, alter macrophage activation, Acta Biomater. 31 (2016) 425–434.
[17] T.J. Webster, R.W. Siegel, R. Bizios, Osteoblast adhesion on nanophase ceramics, Biomaterials 20 (13) (1999) 1221–1227.
[18] T.J. Webster, et al., Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics, J. Biomed. Mater. Res. 51 (3) (2000) 475–483.
[19] T.J. Webster, et al., Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin, Tissue Eng. 7 (3) (2001) 291–301.
[20] E. S.S., Silicon Carbide Biotechnology - Silicon Carbide Materials for Biomedical Applications, ed. E. Inc., 2012.
[21] A. Cacchioli, et al., Cytocompatibility and cellular internalization mechanisms of SiC/SiO2 nanowires, Nano Lett. 14 (8) (2014) 4368–4375.
[22] M. Negri, et al., Tuning the radial structure of core–shell silicon carbide nanowires, CrystEngComm 17 (6) (2015) 1258–1263.
[23] F. Finetti, et al., Synthesis mechanism of SiC–SiO2 core/shell nanowires grown by chemical vapor deposition, Nano Express 1 (2) (2020), 020038.
[24] F.R.F. Fabbri, P. Lagonegro, M. Negri, J.S. Ponraj, M. Bosi, G. Attolini, G. Salviati, 3C–SiC nanowires luminescent enhancement by coating with a conformal oxides layer, J. Phys. D. Appl. Phys. 47 (2014) 394006.
[25] M. Ting, et al., Classification and effects of implant surface modification on the bone: human cell–based in vitro studies, Journal of Oral Implantology 43 (1) (2017) 58–83.
[26] R. Smeets, et al., Impact of dental implant surface modifications on Osseointegration, Biomed. Res. Int. 2016 (2016) 6285620.
[27] K.A. Kilian, et al., Geometric cues for directing the differentiation of mesenchymal stem cells, Proc. Natl. Acad. Sci. 107 (11) (2010) 4872.
[28] C. Yin, et al., Effects of the micro–nano surface topography of titanium alloy on the biological responses of osteoblast, J. Biomed. Mater. Res. A 105 (3) (2017) 757–769.
[29] Ghezzi, B., et al., Sub-Micropillar Spacing Modulates the Spatial Arrangement of Mouse MC3T3-E1 Osteoblastic Cells. Nanomaterials (Basel, Switzerland), 2019. vol 9(12): p. 1701.
[30] P. Lagonegro, et al., Titanium dioxide nanowires grown on titanium disks create a nanostructured surface with improved in vitro osteogenic potential, J. Nanosci. Nanotechnol. 19 (8) (2019) 4665–4670.
[31] F. Rupp, et al., Enhancing surface free energy and hydrophilicity through chemical modification of microstructured titanium implant surfaces, J. Biomed. Mater. Res. A 76A (2) (2006) 323–334.
[32] M.M. Bornstein, et al., Bone apposition around two different sandblasted and acid-etched titanium implant surfaces: a histomorphometric study in canine mandibles, Clin. Oral Implants Res. 19 (3) (2008) 233–241.
[33] R.A. Gittens, et al., The effects of combined micron−/submicron-scale surface roughness and nanoscale features on cell proliferation and differentiation, Biomaterials 32 (13) (2011) 3395–3403.
[34] E. Calciolari, et al., Protein expression during early stages of bone regeneration under hydrophobic and hydrophilic titanium domes. A pilot study, J. Periodontal Res. 53 (2) (2018) 174–187.
[35] C.J. Wilson, et al., Mediation of biomaterial-cell interactions by adsorbed proteins: a review, Tissue Eng. 11 (1–2) (2005) 1–18.
[36] Y. Xie, et al., Improvement of surface bioactivity on titanium by water and hydrogen plasma immersion ion implantation, Biomaterials 26 (31) (2005) 6129–6135.
[37] L. Parisi, et al., Titanium dental implants hydrophilicity promotes preferential serum fibronectin over albumin competitive adsorption modulating early cell response, Mater. Sci. Eng. C 117 (2020) 111307.
[38] E. Farrell, et al., A collagen-glycosaminoglycan scaffold supports adult rat mesenchymal stem cell differentiation along osteogenic and chondrogenic routes, Tissue Eng. 12 (3) (2006) 459–468.
[39] E. Farrell, et al., A comparison of the osteogenic potential of adult rat mesenchymal stem cells cultured in 2-D and on 3-D collagen glycosaminoglycan scaffolds, Technol. Health Care 15 (1) (2007) 19–31.
[40] I.V. Yannas, et al., Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin, Proc. Natl. Acad. Sci. U. S. A. 86 (3) (1989) 933–937.
[41] C. Galli, et al., Actin cytoskeleton controls activation of Wnt/β-catenin signaling in mesenchymal cells on implant surfaces with different topographies, Acta Biomater. 8 (8) (2012) 2963–2968.
[42] Y. Hayashi, et al., Integrins regulate mouse embryonic stem cell self-renewal, Stem Cells 25 (12) (2007) 3005–3015.
[43] M. Arnold, et al., Activation of integrin function by nanopatterned adhesive interfaces, Chemphyschem 5 (3) (2004) 383–388.
[44] E.A. Cavalcanti-Adam, et al., Lateral spacing of integrin ligands influences cell spreading and focal adhesion assembly, Eur. J. Cell Biol. 85 (3–4) (2006) 219–224.
[45] E.A. Cavalcanti-Adam, et al., Cell spreading and focal adhesion dynamics are regulated by spacing of integrin ligands, Biophys. J. 92 (8) (2007) 2964–2974.
[46] E.A. Cavalcanti-Adam, et al., Cell adhesion and response to synthetic nanopatterned environments by steering receptor clustering and spatial location, Hfsp j 2 (5) (2008) 276–285.

Benedetta Ghezzi graduated in Medical, Veterinarian and Pharmaceutical Biotechnologies at the University of Parma in 2016. After her graduation she joined a PhD course in Molecular Medicine at the TBLab of the Dental School of the University of Parma and she defended her thesis on the use of nanostructured materials for tissue regeneration in March 2020. She is currently involved in the research division of the Dental School and she is focusing on the development of innovative surfaces and biomaterials for bone regeneration.

Paola Lagonegro is a postdoctoral researcher at Institute of Chemical Sciences and Technologies Giulio Natta-National Research Council. Currently, her research is focused on synthesis and characterization of biomaterials and nanomaterials for biomedical applications. She is graduated in Biomedical engineer at Polytechnique of Milan in 2011 and in 2016 she received her Ph.D. in “Innovative material science and technology” from University of Parma with a dissemination on the interaction between Si (O, C) nanowires and the biological system.

Giovanni Attolini was born in 1951. He studied at the University of Parma, where he took a degree in Physics. From 1971 he worked at the IMEM-CNR in Parma. Dr. Attolini has been working in vapour growth of bulk crystals and epitaxial deposition (by CVD and MOCVD) of several binary and ternary compounds. He is presently involved in CVD growth of SiC nanowires on silicon. He is co-authors of 220 papers in national, international specialized journals book chapters and more than 300 contributions to conferences. Finally, he published two books on crystal growth and epitaxy from vapour phase.

Pasquale Mario Rotonda studied engineering at the University of Parma and Bologna, then he started his own company in the textile sector, but never forgot his passion for science and created Lintes Research Laboratory in 2008, for which he built a Plasma Reactor in 2009, organized 3 Nanosimposia (in 2012, 2014 and 2017) reuniting professors and researchers from different Italian universities and research institutes, and assisted during the NGPT event at Rome Torvergata in 2016.

He was co-author of several posters and papers published by CNR and the University of Parma with which is pleased to collaborate.

Christine Cornelissen graduated in 1985 from Leuven University in Belgium as geologist. She was employed by Shell Petroleum Company in the Netherlands and the United Kingdom until 1991. In 1991 she moved to Italy, where she became the sole administrator of the company Lintes srl since 1998. Lintes srl has been working in the textile sector but since 2008 has also created its Research Laboratory, building a Plasma Reactor in 2009, organizing 3 Nanosimposia, reuniting professors and researchers from different Italian universities and research institutes, and assisting during the NGPT event at Rome Torvergata in 2016. She was co-author of several posters and papers published by CNR and the University of Parma.

Joice Sophia Ponraj is Director & Research Professor in Centre for Advanced Materials, Aaivalayam-DIRAC, India and Research Fellow at INL, Portugal. Joice is a recipient of DST INSPIRE Faculty Award and Marie-Curie CoFund. Her research interests include synthesis and studies of 2D materials and semiconducting nanostructures for bionanophotonics; 2D based cancer therapeutics, micro-nano fabrication; graphene-2D wearable electronics and optoelectronics. Joice received Ph.D from Anna University, India. She was awarded three times ICTP-TRIL to work in IMEM-CNR, Parma, Italy, "Italian-Indian Bilateral Program" hosted by University of Ferrara, Italy and AICTE-NDF, India during her PhD. She did post-doc in Suzhou University, China and served as Assistant Professor in UIST, North Macedonia and Bharathiar University, India.

Ludovica Parisi graduated in Biomaterials and Medical Biotechnologies at the University of Parma in 2014. Subsequently, she joined the Dental School of the University of Parma as a PhD student. During the PhD she further joined the University of Sao Paulo (2015) and the University of Pennsylvania (2017). Her work based on the control of selective protein adsorption on biomaterials for host-material interactions tailoring. She defended her doctoral thesis in March 2019. The same year she has been awarded with a one-year scholarship from the Osteology Foundation. She is currently post-doctoral research fellow at the Department of Orthodontics and Dentofacial Orthopedic of the University of Bern, Switzerland.

Giovanni Passeri graduated in Medicine and Surgery and became board specialist in Internal Medicine and Endocrinology. He got the PhD in Pathophysiology of Metabolic Bone Disease; he actually holds the chair of Internal Medicine at the University of Parma and is the Director of the Center for Metabolic Bone Diseases at the University Hospital of Parma. His main research interests are linked to the clinical aspects of bone and mineral metabolism, the interaction between bone and different pharmaceutical compounds, bone integration and pathophysiological aspects of the reaction between bone cells and biomaterials. He is author or co-author of more than 200 scientific papers published on both national and international medical journals.

Francesca Rossi graduated in Physics at the University of Parma in 2000 and got the PhD in Science and Technology of Innovative Materials from the same university in 2004. Since September 2009 she has a permanent position as Research Scientist at CNR-IMEM Institute. Her research activity deals with the application of electron microscopy techniques in materials science, to analyze the structural and morphological properties of advanced heterostructures, nanosystems and biomaterials. She has co-authored more than 130 papers in international journals and 9 book chapters.

Guido Maria Macaluso is the Chair of Periodontology and Implant Dentistry and the Director of the Center of Dentistry, University of Parma. He received his MD and DDS from the University of Parma and his MDS from the Catholic University of Leuven. Professor Macaluso leads the TBLab which specializes in the provision of translational research at all levels: in vitro, in vivo/preclinical and clinical. His research track record is mainly on biomaterials, regenerative technologies and reconstructive periodontics and implant dentistry, with an emphasis on biomaterial-cell interaction. The aim is to optimize biomaterials for oral and periodontal regenerative medicine, tissue engineering and personalized medicine.