In vitro Cytocompatibility Assessment of Ti-modified Silicon-oxycarbide Based Polymer-derived Ceramic Implantable Electrodes under Pacing Conditions

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This document is the accepted manuscript version of the following article:
Vallachira Warriam Sasikumar, P., Müller, E., Clement, P., Jang, J., Kakkava, E., Panusa, G., ... Blugan, G. (2020). In vitro cytocompatibility assessment of Ti-modified silicon-oxycarbide based polymer-derived ceramic implantable electrodes under pacing conditions. ACS Applied Materials and Interfaces. <https://doi.org/10.1021/acsami.0c01465>

Abstract

Polymer derived ceramics (PDC) have recently gained increased interest in the field of bioceramics. Among PDC's, carbon rich silicon oxycarbide ceramics (SiOC) possess good combined electrical and mechanical properties. Their durability in aggressive environments and proposed cytocompatibility makes them an attractive material for fabrication of bio-MEMS devices such as pacemaker electrodes. The aim of the present study is to demonstrate the remarkable mechanical and electrical properties, biological response of PDCs modified with titanium (Ti) and their potential for application as pacemaker electrodes. Therefore, a new type of SiOC modified with Ti fillers was synthesized via PDC route using a Pt-catalyzed hydrosilylation reaction. Preceramic green bodies were pyrolyzed at 1000 °C under argon atmosphere to achieve amorphous ceramics. Electrical and mechanical characterization of $\text{SiC}_x\text{O}_{2(1-x)}/\text{TiO}_x\text{C}_y$ ceramics revealed a maximum electrical conductivity of 10^1 Scm^{-1} and a flexural strength of maximal 1 GPa, which is acceptable for pacemaker applications. Ti incorporation is found to be beneficial for enhancing the electrical conductivity of SiOC ceramics and the conductivity values were increased with Ti doping and reached a maximum for the composition with 30 wt% Ti precursor. Cytocompatibility was demonstrated for the PDC SiOC ceramics as well as SiOC ceramics modified with Ti fillers. Cytocompatibility was also demonstrated for SiTiOC20 electrodes under pacing conditions by monitoring of cells in an in vitro 3D environment. Collectively, these data demonstrate the great potential of polymer derived SiOC ceramics to be used as pacemaker electrodes.

Keywords: Polymer derived ceramics, Bioceramics, Implants, Cytocompatibility, Pacemaker electrodes

1. Introduction

Polymer derived ceramics have become a promising material for various industrial applications, including sensors ¹⁻², anodes in Li-ion batteries ³⁻⁴, thermal insulators ⁵⁻⁶, coatings ⁷⁻⁸ or microparts for microelectro-mechanical system (MEMS) applications ⁹⁻¹⁰. When developing biomedical implants, the tissue-biomaterial interaction is crucial for the performance of the implant. The properties of implant materials strongly influence the intensity of the body's defense mechanism and the response of the tissue to the electrode determines the efficiency and performance of an implant system¹¹. Electrodes based on titanium or platinum have been the material of choice for pacemaker applications owing to their high conductivity and ease of fabrication. However, metal implants during long term implantation were reported to release metallic ions into surrounding tissue which can lead to cytotoxicity¹²⁻¹³. Ceramic electrodes are one of the best alternatives to metal electrodes offering excellent biocompatibility and mechanical properties. The combination of stability in harsh environments and the excellent electrical and mechanical properties make polymer derived silicon oxycarbide (SiOC) ceramic materials also a promising candidate for application in the field of bioceramics for medical applications ¹⁴⁻¹⁸.

In vitro biocompatibility of SiOC systems has been investigated more recently, demonstrating their potential use as pacemaker electrodes or as antimicrobial coatings ^{16-17, 19-23}. Investigated compositions included SiOC ceramic materials with different free carbon ratios and metal doping (Ca, Mg, Ag), and were mostly synthesized by the PDC method. Analysis of cell attachment, survival, and proliferation on these SiOC materials revealed good cytocompatibility, making them promising candidates for use as electrodes for pacemaker applications. Biocompatibility of PDC electrodes were already demonstrated within our previous work and the tested ceramics demonstrated biocompatible properties similar to those of currently used standard implantable materials²²⁻²³. However, the application of SiOC materials in pacemaker electrodes is still under

investigation and a more detailed understanding of biological responses to these materials under pacing conditions is required.

In SiOC ceramics, silicon is bonded simultaneously to both oxygen and carbon atoms resulting in a stoichiometry of $\text{SiC}_x\text{O}_{2(1-x)} + \text{C}_{\text{free}}$, where 'C_{free}' refers to a disordered free carbon network present in the system²⁴⁻²⁷. A SiOC mixed tetrahedral network is formed by the incorporation of tetravalent carbon atoms in silicate glasses by replacing some of the bridging oxygen atoms between SiO_2 units. A better understanding of the microstructure and composition of the final ceramics plays a key role in explaining their associated structural, electronic, thermal and other functional properties. Electrical conductivity and mechanical strength of SiOC ceramics can be influenced by changing the pyrolysis temperature, as reported previously^{6, 28}. However, while SiOC ceramics pyrolyzed at 1400 °C exhibited a maximum conductivity of 10^1 Scm^{-1} , the material showed a reduced flexural strength at these temperatures²⁹. Modification of SiOC microstructure by incorporating active nanofillers including but not limited to CNTs, Ti, and Al in preceramic polymers was reported to have a significant influence on their physical properties such as thermal stability, electrical conductivity or their mechanical properties³⁰⁻³³. Among these nanofillers, Ti based materials demonstrated cytocompatibility in a variety of applications and in general also offer acceptable electrical and mechanical properties³⁴⁻³⁵. In previous studies, pyrolysis of polysiloxane precursors modified with Ti fillers at 1000 °C was shown to create amorphous ceramics having a combination of SiOC and Ti oxycarbide phases ($\text{SiOC-TiC}_x\text{O}_y$)³⁶.

Currently, many different methods are used to fabricate monolithic SiOC ceramics. The most common method proceeds via a powder processing route. Thereby, starting precursors in powder form are converted into monolithic pieces of desired shapes by applying high pressure, followed by pyrolysis at temperatures above 800 °C to obtain the final ceramics³⁷⁻³⁸. In comparison to

powder processing, liquid polymer precursors have many advantages when it comes to the fabrication of ceramic material parts with complex shape. Preceramic green bodies of a large variety of shapes down to micrometer scale precision can be fabricated by different ways such as casting in a specific mold^{23, 29, 39}, photo crosslinking⁴⁰⁻⁴¹ and stereolithography⁴⁰⁻⁴³.

Herein, SiOC and SiTiOC ceramics with variable Ti contents were synthesized from tailor made liquid preceramic precursors. After a thorough investigation of their structural, electrical and mechanical properties, cytocompatibility of the fabricated surfaces was investigated with and without pacing conditions to assess their potential for application as pacemaker electrodes.

2. Materials and Methods

2.1. Synthesis of SiOC ceramics

Polyhydromethyl siloxane (PHMS) (Sigma Aldrich, Switzerland, MW ~ 1900), divinyl benzene (DVB), titanium ethoxide and a platinum Karstedt's catalyst (Platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex solution in xylene, Pt ~2 %) were purchased from Sigma Aldrich and used as received. The synthesis procedure for SiOC ceramics was the same as reported previously²⁹. In brief, DVB and PHMS were crosslinked in the presence of a Pt catalyst and casted in Teflon molds for fabrication of preceramic green bodies. Gelation was observed after 30 minutes and the gels were left overnight at ambient conditions. Thereafter, preceramic polymer films were removed from the Teflon molds and tempered at 80 °C for 48 h to complete crosslinking of the gels. For the synthesis of SiTiOC ceramics, titanium(IV)ethoxide (TiOET) was used along with the precursors described above. For this, TiOET solution was mixed with DVB and PHMS (2:1 weight ratio of DVB:PHMS) prior to addition of Pt catalyst for crosslinking. Ti content was varied by changing the concentration of TiOET precursor and an amount corresponding to 10, 20, 30 and

40 wt% of TiOET in DVB-PHMS precursor solution was used for fabrication of SiTiOC preceramic green bodies.

Pyrolysis of preceramic gels was carried out in an alumina tubular furnace under controlled argon atmosphere (Carbolite STF 16/450, Germany). Samples were pyrolyzed at 1000 °C with heating and cooling rates of 50 °C/h and a holding time of 1 h at the maximum temperature. Samples were named accordingly as SiOC, SiTiOC10, SiTiOC20, SiTiOC30 and SiTiOC40 respectively according to the starting Ti content.

2.2. Structural characterization

FTIR spectra of cross-linked preceramic green bodies were recorded in a Tensor 27, Bruker (Massachusetts, USA) using a Golden Gate ATR. Raman spectra of SiOC and SiTiOC samples after pyrolysis were recorded using a confocal Raman spectrometer (Renishaw, UK) with a laser beam (488 nm). Thermogravimetric analyses (TGA) were carried out using a Netzsch STA449 F3 Jupiter instrument under argon atmosphere. The surface microstructure after pyrolysis was examined via SEM imaging (VEGA Plus 5136 MM, Tescan instruments, Brno – Kohoutovice, Czech Republic).

2.3. Electrical conductivity

Room temperature DC electrical conductivities of the pyrolyzed ceramics were measured using a four point collinear probe (Microworld, Grenoble, France) connected to a Keithley 2450 source meter (Tektronix UK Ltd., Berkshire, England). A current of 1 μ A was applied through the outer probes and the corresponding voltage between the inner probes was used for measurement of the resistance values.

2.4. Flexural strength

To measure the flexural strength of the fabricated ceramic discs, a ball-on-three-balls (B3B) test was applied. Results were analyzed using the following equation:

$$\sigma = f(\alpha, \beta, \nu) * F/t^2 \quad \text{eq. (1)}$$

Where σ is the flexural strength [MPa], F the applied force [N], and t the thickness of the specimen [mm]. “ F ” is a dimensionless factor depending on the ratio of thickness and radius of the disc ($\alpha=t/R$), the ratio of the support radius of the 3 balls to the discs radius ($\beta=Ra/R$) and the Poisson's ratio of the material which was in this case defined as 0.22. The radii of the balls were 1.9 mm and the crosshead displacement was set to 5 mm/min.

2.5. Cytocompatibility of SiOC and SiTiOC ceramics

Cytocompatibility of the fabricated SiOC and SiTiOC surfaces was analyzed using primary human dermal fibroblasts (NHDF) (PromoCell, cat. C12302, Lot 410Z037.5). NHDF were chosen because fibroblasts represent an important component of cardiac tissues and because of their common use in cytocompatibility tests. Disc-shaped samples with a diameter of 5 mm and a thickness of 0.5 mm were placed into a 96 well plate and sterilized with UV-light. Afterwards, 3350 cells suspended in 100 μ L cell culture medium (Dulbecco's Modified Eagle's Medium, DMEM) supplemented with 10% Fetal Calf Serum (FCS), 1% Penicillin-Streptomycin-Neomycin (PSN), and 1% L-Glutamine (all Sigma) were seeded on top of the surfaces and cultivated for 5d at 37°C and 5% CO₂. Tissue culture plastic (TCP) was used as reference substrate. After this cultivation period, the metabolic activity of the cells was measured with an MTS assay kit (Promega). The supernatant was removed and 120 μ L of the MTS working solution (16% MTS reagent from Roswell Park Memorial Institute (RPMI) 1640 medium without phenol red (Gibco)) added. After 1 h of incubation, 100 μ L of the supernatant were transferred into a new 96-well plate and the absorbance was measured at 490 nm (Mithras2 plate reader, Berthold Technologies).

To analyze cell attachment on top of the surfaces, samples were washed three times with phosphate buffered saline (PBS) (Sigma) and fixed with 4% paraformaldehyde (PFA) (Sigma) dissolved in PBS for 1h. The cells were then lysed in 0.1% Triton X-100 (Sigma) for 10 min, washed three times in PBS and blocked with 5% goat serum (Sigma) and 1% FCS in PBS for 1 h. To stain the actin cytoskeleton and nuclei of the cells, 100 μ L of a solution with 1% FCS/PBS, Alexa Fluor 488-labelled phalloidin (1:200; Thermo Fisher Scientific) and DAPI (4',6-diamidino-2-phenylindole, (1:1000, Sigma)) were added to each sample for 1h. Samples were then washed three times with PBS and analyzed with a confocal laser scanning microscope (LSM780, Carl Zeiss AG).

2.5.1. Electrochemical characterization of the PDC electrodes under pacing conditions

To assess the electrochemical characterization and cytocompatibility of the PDC electrodes, a custom platform was fabricated in order to provide continuous and parallel monitoring of cells in an *in vitro* 3D environment. The platform consists of various components designed to allow good electrical contact between the electrodes and the PCB (printed circuit board) as well as sealing of the electrodes from the buffer solution, as shown in **Figure 1**.

Figure 1: Schematic illustration of the custom pacing set up; a) exploded view showing PCB with PDC sample and Pt reference electrodes (bottom), PDMS wells filled with buffer (middle) and top cover, and b) cross sectional view of each layer showing electrode assembly on PCB, PDMS block and top cover (drawing not to scale).

A master mold was created by 3D printing (Multi Jet Modelling) of Vero clear polymer using a Connex 500 printer and the mold was subsequently coated with 5 μm of parylene (Comelec C-30-S deposition system). Polydimethylsiloxane (PDMS, 1:10 w/w ratio, Sylgard 184, Dow Europe) was mixed uniformly and degassed in a desiccator for 20 min. The mixture was then poured into the 3D master mold and degassed a second time for 1h. Finally, the PDMS substrate was cured overnight at 50°C in air. The bottom of the wells were manually punched to create holes of 1 mm in diameter and the electrodes were attached with a medical grade glue (EPOXY technology, kit part A: kit part B, 1:1) to the back side of the PDMS substrate and let dry at room temperature overnight. The platform cover was fabricated by a similar above-mentioned 3D printing method and attached on top of the PDMS substrate. A potentiostat (Ref. 600+, Gamry Instruments, USA) was used to characterize electrochemical properties of the PDC samples by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The EIS was performed in the frequency range between 1 Hz and 100 kHz. The CV was carried out in the range of -1.2 to 1.2 V with a 25 mV s^{-1} sweep rate (see Figure 6).

2.5.2. Cytocompatibility of the PDC electrodes under pacing conditions

Cytocompatibility of the SiTiOC20 electrodes under pacing conditions was assessed with NHDFs encapsulated in collagen hydrogels. For cell encapsulation, NHDFs (PromoCell, cat. C12302, Lot 410Z037.5) were harvested and a cell suspension with 6'000'000 cells per mL cell culture medium

was prepared. Collagen hydrogels at a ratio of collagen (10mg/mL, FibriCOL, Advanced BioMatrix) : 10X DMEM (Sigma) : 0.5M NaOH (Sigma) + cell-media suspension (8:1:1) were prepared to reach a final concentration of 100'000 cells per 250 μ L of hydrogel. The collagen-cell suspension was mixed gently and the pH of the hydrogel precursor was measured and adjusted to physiological pH ($\sim$ 7) with 0.5M NaOH. After UV-irradiation of the platform (for 1h), 250 μ L of the hydrogel precursor were placed into each channel between the electrodes (SiTiOC20 working electrode, Pt counter electrode) of the PDMS pacing device (Fig. S1d). Gelation was allowed for 1 h at 37°C. Afterwards, 400 μ L of cell culture medium (DMEM supplemented with 10% Fetal Calf Serum (FCS), 1% Penicillin-Streptomycin-Neomycin (PSN), and 1% L-Glutamine (all Sigma)) were added into each well. Air bubbles above the electrodes were removed with a pipette and the experiment was performed for 48 h at 37 °C and 5% CO₂ in an incubator. Half of the samples were paced for 30min with bipolar electrical pulses (10 μ A, 1ms, 1Hz) after 6h, 18h, 30h, and 42h, respectively. The other half was kept without electrical pacing under identical conditions. Finally, all hydrogel samples were transferred into the wells of a new 48 well plate. The metabolic activity of the cells within the hydrogels was assessed by adding 250 μ L of 10% presto blue (PB) (Thermo Fisher Scientific) dissolved in cell culture medium to each sample with subsequent incubation for 3 h at 37 °C and 5% CO₂ in an incubator. Fluorescence intensities were measured at λ_{Ex} 547nm/ λ_{Em} 582nm (Mithras2 Plate reader, Berthold Technologies). Afterwards samples were fixed for 1h with 4% PFA (Sigma), cells were permeabilized with 0.1% Triton-X100 (Sigma) solution for 20 min and blocked with 5% goat serum (Sigma) and 1% FCS (Sigma) dissolved in PBS for 30min. Samples were then stained with Alexa Fluor 546-labelled phalloidin (1:200; Thermo Fisher Scientific) and DAPI (1:1000, Sigma) dissolved in 5% FCS/PBS for 2h. After each step, samples were washed three times with PBS (Sigma). Images were taken with a confocal laser scanning microscope (LSM780, Carl Zeiss AG).

3. Results and Discussion

3.1. Ti incorporation into SiOC ceramics

In order to ensure a homogenous dispersion of Ti fillers in SiOC ceramics several titanium precursors were analyzed and a good miscibility of titanium(IV)ethoxide (TiOET), DVB and PHMS solutions was found (data not shown). The platinum catalyzed hydrosilylation reaction of the mixture resulted in stable preceramic green bodies. The proposed reaction scheme and preceramic polymer mixtures along with a schematic illustration of the teflon mold used for casting as well as preceramic green bodies are presented in **Figure 2**. After pyrolysis, fabricated ceramics had a shiny black appearance due to the presence of excess free carbon in their amorphous structure. Notably, sintering of SiTiOC with 10, 20 and 30 wt% of Ti at 1000 °C lead to ceramics without significant cracks or ruptures. On the contrary, samples with 40 wt% of Ti were very brittle, greatly limiting the possibility of subsequent characterization and analysis.

Figure 2. Schematic illustration of the entire synthesis and fabrication process, a) polymer precursor solutions without and with titanium ethoxide, teflon mold for polymer casting, preceramic green bodies and PDC electrode after pyrolysis b) proposed hydrosililation reaction leading to formation of final ceramics.

After pyrolysis, a similar shrinkage of ~ 25 wt% was observed for all compositions, i.e. 10-40%wt Ti. The TGA analysis revealed a mass loss in the range of 30-42 wt% leading to a ceramic yield of 58-70 %. TGA mass loss patterns are presented in **Figure 3a** and the corresponding ceramic yield and shrinkage values of the SiOC and SiTiOC ceramics after pyrolysis are presented in **Table 1**. SiOC ceramics had a total mass loss in the range of ~ 42 wt%. SiTiOC compositions demonstrated a similar weight loss as SiOC ceramics but with a higher ceramic yield (63-70 %) compared to SiOC. The major mass loss observed between 200 - 600 °C is attributed to the polymer to ceramic conversion with release of volatile organic groups and hydrogen. After 700 °C no further mass loss was observed up to 1350 °C^{38, 44-45}. The process of polymer to ceramic conversion did not lead to observable pore formation and the density of studied compositions were all in the order of 2.2 g cm⁻³, which is comparable to the previously reported values for ceramics of similar composition⁴⁶. Above 1400 °C, a second weight loss was observed, presumably due to the onset of carbothermal reduction with consumption of SiO₂ by C to form SiC and release of CO^{29, 47}. In the case of SiTiOC, the same carbothermal reduction reaction lead to formation of TiC at temperatures above 1350 °C and a weight loss was observed at this temperature for Ti incorporated samples.

The FTIR spectra of the preceramic green bodies are presented in **Figure 3c**. The majority of the observed bands represent Si-O-Si and other functional groups such as Si-C, C-H, and C=C bands, which are common in SiOC preceramic precursors⁴⁸. With the addition of Ti fillers, formation of a band at 1403 cm⁻¹ representing the formation of Ti-O-Ti bonding was observed and the intensity of

this band increased with Ti content, being largest for the 40 wt% Ti composition. However, bands representing Ti-O-Si (at 900 cm^{-1}) overlap with the one of Si-O-Si. Therefore, a broad pattern is visible in the FTIR spectra. The more pronounced existence of C=C (1603 cm^{-1}) bands in the Ti modified precursors and the presence of C=C bands compared to the SiOC samples could be attributed to the presence of more unreacted DVB precursor due to the reduced hydrosilylation reaction between Si-H and C=C bonds. FTIR spectra of samples after pyrolysis at $1000\text{ }^{\circ}\text{C}$ did not provide further information as the spectra only had broad peaks representing Si-O, Si-C bands, common for SiOC ceramics. In order to understand the polymer to ceramic conversion after pyrolysis, Raman spectra were recorded on pyrolyzed samples (**Figure 3d**).

Figure 3. Characterisation of preceramic greenbodies and final ceramics a) TG curve of SiOC and Ti incorporated samples b) XRD spectra of SiTiOC samples pyrolyzed at 1000 °C, c) FTIR spectra of preceramic green bodies and d) Raman spectra of samples pyrolyzed at 1000 °C

Raman spectra of both SiOC and SiTiOC compositions comprise mainly D and G bands corresponding to the free carbon network. The spectra of SiOC to SiTiOC samples with 40 wt% Ti showed typical D and G bands at ~ 1350 and 1600 cm^{-1} , characteristic for a disordered free carbon network. The D peak present at 1350 cm^{-1} corresponds to the A_{1g} breathing mode of vibration and G peak represents E_{2g} mode of vibration representing in-plane bond stretching motion of pairs of sp² carbon atoms. Two additional bands 2D (2700 cm^{-1}) and D+G (2900 cm^{-1}) are also present corresponding to an overtone of the D band and a combination of D+G bands⁴⁹. Intensity ratios of D (I_D) and G (I_G) bands represents the level of order in free carbon phase present in the PDC ceramics. As proposed by Ferrari and Robertson, the lattice parameter La is directly proportional to the intensity ratios of D (I_D) and G (I_G) bands⁵⁰. D and G peak positions are presented in Table 1 and no significant changes in peak positions have been observed. The highest I_D/I_G ratio of 1.45 was observed for SiTiOC30 composition representing the highest order of free carbon present in these ceramics. In case of SiTiOC40 the intensity ratios dropped to 1.2, most likely due to the reduced free carbon content. Starting preceramic polymer for this composition had lesser DVB content, which was the major carbon source and the ceramics after pyrolysis is expected to have lower content of disordered free carbon network. The obtained data suggests that the Ti content has minor influence on the domain size of free carbon clusters.

XRD spectra of samples pyrolyzed at 1000 °C, presented in **Figure 3b**, shows complete amorphous nature of the ceramic samples. SiOC, in general, is completely amorphous and even after addition of Ti in to the ceramic matrix no changes were observed in their amorphous nature. In previous

studies, pyrolysis of polysiloxane precursors modified with Ti fillers at 1000 °C was shown to create amorphous ceramics having a combination of SiOC and TiOC phases³⁶. However, with current data, we are not able to have a better understanding, and further work is needed to understand more about the presence and distribution of amorphous SiOC and TiOC units within the ceramics.

Table 1. Ceramic yield and shrinkage at different Ti concentrations (Data from Ti40 is not considered due to excessive warping and brittle nature) and Raman spectra details of samples pyrolyzed at 1000 °C.

Sample	Ceramic yield (%)	Radial shrinkage (%)	Raman peak position	
			D (cm ⁻¹)	G (cm ⁻¹)
SiOC	58 ± 0.83	25 ± 1.26	1357	1604
SiTiOC10	66 ± 1.01	26 ± 0.81	1348	1604
SiTiOC20	63 ± 1.50	25 ± 0.62	1360	1605
SiTiOC30	67 ± 2.03	28 ± 1.25	1353	1600
SiTiOC40	70 ± 4.72	-	1354	1605

Pyrolysis of preceramic polymer compositions containing Ti ethoxide resulted in formation of SiOC/SiTiOC ceramics. Final ceramic microstructure mainly contains SiOC and TiOC mixed units along with a network of disordered free carbon. Pyrolysis temperature plays a key role in the microstructure development of SiOC ceramics and this has a great influence on their properties. The microstructure of ceramics pyrolyzed at 1000 °C were completely amorphous in nature and a further increase in pyrolysis temperature will lead to the phase separation of SiC, SiO₂ and TiC phases. This phase separation is more active above 1300 °C pyrolysis temperature and generally results in reduced mechanical and electrical properties ²⁹

3.2. Mechanical and electrical properties

Mechanical characterization via the B3B method revealed a characteristic flexural strength from 600 to 1100 MPa for all ceramics. Variation of the strength was observed as a function of the Ti content. SiOC ceramics had a characteristic flexural strength of ~ 929 MPa and a similar flexural strength was observed for SiTiOC10 ceramics. Further increase of the Ti content resulted in a decrease of the flexural strength. The large variations observed for the flexural strength of the SiTiOC samples, especially that of SiTiOC30, might be attributed to inhomogeneities in the final ceramic composition. However, the strength was above 600 MPa in case of all SiTiOC ceramics.

Figure 4 a) Flexural strength of ceramics pyrolyzed at 1000°C with respect to Ti filler content (15 specimens from each composition). b) Electrical conductivity of final ceramics with respect to Ti filler content (5 specimens from each composition)

Electrical conductivity measured using a four point collinear probe showed, as expected, an increasing conductivity with increasing wt% of Ti-filler. Conductivity of SiOC ceramics is mainly attributed to the nature and amount of the free carbon content. A further increase in conductivity of SiOC ceramics was reported after pyrolysis at higher temperatures due to ordering of the free carbon phase ²⁹. Conductivity values reach up to 10^1 S cm⁻¹ for SiOC samples pyrolyzed at 1400

°C. However, those samples had poor mechanical properties and flexural strength less than 300 MPa. SiOC ceramics pyrolyzed at 1000 °C had a conductivity in the range of 10^{-2} S cm⁻¹. Upon incorporation of Ti into the SiOC ceramics, the conductivity values increased to 0.17 S cm⁻¹ (SiTiOC10), 0.46 S cm⁻¹ (SiTiOC20) and 6.09 S cm⁻¹ (SiTiOC30) respectively. Further increase of the Ti content (SiTiOC40) did not affect the conductivity and this is attributed to the reduction of the free carbon content in the obtained ceramics due to the lower proportion of divinylbenzene in the starting precursors. Moreover, a lower I_D/I_G ratio of SiTiOC40 compared to SiTiOC30, as seen from Raman spectra shows reduced ordering of the free carbon phase in these samples.

3.3. Cytocompatibility of SiOC/SiTiOC ceramics

To assess the cytocompatibility of SiOC and SiTiOC samples with increasing Ti content, normal human dermal fibroblasts (NHDF) were cultivated for up to 5 days on the surface of the materials and were then evaluated for cell attachment, spreading and viability. While only a few cells attached on day 1 (data not shown), almost confluent cell monolayers with well spread cells could be observed after 5 days (Figure 5a). Furthermore, quantification of the metabolic activity after culture on SiOC and SiTiOC samples showed similar values compared to TCP controls (Figure 5b), demonstrating good cytocompatibility of the materials for NHDF. These results are in agreement with previous studies reporting cytocompatibility of SiOC ceramics, where SiOC was modified with Ca and Mg ions (SiMgCaOC), or where SiOC with different free carbon content and different structural morphologies was used^{16, 19-20, 22-23}.

Figure 5. Cytocompatibility of SiOC and SiTiOC samples. NHDF were cultivated on SiOC and SiTiOC samples for 5 days and a) stained for actin filaments (green) and cell nuclei (blue) and b) assessed for their metabolic activity via MTS assay (mean $\pm$ SD, n = 3).

While assessing the cytocompatibility of the fabricated SiOC ceramics we observed no harmful effects and the presence of Ti did not influence the cytocompatibility of SiOC ceramics. The present study therefore suggests that SiOC ceramics modified with Ti fillers can be considered as a potential material for fabrication of electrically conductive bio MEMS devices.

3.4 Electrochemical properties of SiTiOC ceramics

In view of the targeted pacing applications, the electrochemical properties of the PDC samples were characterized by measuring EIS and CV. A three-electrode setup is used for the electrochemical measurement. The working electrode (*i.e.*, SiTiOC20 or SiTiOC30) and Pt counter

electrode were loaded on the testing platform, and a Ag/AgCl reference electrode was immersed in a buffer of phosphate-buffered saline (PBS) solution (pH: 7.4) as shown in the Figure S(a).

Figure 6. Electrochemical characterization of SiTiOC20 and SiTiOC30 by a) electrochemical impedance spectroscopy ($n=4$) and b) cyclic voltammetry

Figure 6a shows the measured EIS curve with the average magnitude of impedance and phase. The EIS can be modeled as capacitance of the double layer effect (C_{dl}), charge transfer resistance (R_{ct}), and electrolyte solution resistance (R_s) as shown in the Figure S1b(Supporting information)²². Due to the capacitance properties of C_{dl} , the impedance decreases at the high frequency while the impedance is increased at the low frequency with a -90-degree phase shift. In addition, the C_{dl} is dominant for both PDC electrodes because the phase is dramatically changed at the high frequency region. Since the electrocardiogram (ECG) recording range is 0.5 – 40 Hz, a low frequency for stimulation is considered for *in vitro* studies²², so the average impedance of the SiTiOC20 and SiTiOC30 (electrodes) at 10 Hz was 86 ± 28 k Ω and 98 ± 21 k Ω , respectively. Figure 6b shows the measured CV curve of SiTiOC20 and SiTiOC30 electrodes. The CV curve of SiTiOC20 shows a wide stability window approximately from -0.9 to 0.9V, and there is no Faradaic reaction peak, which is similar to that of a titanium nitride (TiN) electrode as shown in Figure Sc. The cathodal

charge storage capacity (cCSC) of the SiTiOC20 and SiTiOC30 was calculated to be 200 and 425 $\mu\text{C}/\text{cm}^2$, respectively. Although the SiTiOC20 electrode could not show a cCSC value as large as for typical metal electrodes⁵¹⁻⁵², it has acceptable characteristics in terms of electrochemical stability and low standard deviation in mechanical properties as potential biomedical electrode application.

3.4. Cytocompatibility of SiTiOC ceramics under pacing conditions

To assess the cytocompatibility of the SiTiOC20 electrodes under pacing conditions, NHDFs encapsulated in collagen hydrogels were placed in the channel between the electrodes of the custom PDMS pacing platform (Figure 7a). Half of the samples were paced for 30min with symmetric bipolar electrical pulses (10 μA , 1ms, 1Hz). Within the 48h of culture, pacing was applied four times (total: 120 min pacing). Cell spreading within the collagen hydrogel and viability of the cells were assessed by measuring the cell metabolic activity via confocal microscopy (Figure 7b) or with a presto blue assay (Figure 7c) after culture for 48 hours, respectively.

Figure 7. Cytocompatibility of SiTiOC20 electrodes with and without pacing. a) Schematic representation of the platform with the hydrogel of cells that was seeded, b) representative CLSM images (maximum intensity projections of z-stacks) of well spread NHDF stained for actin (green) and nuclei (blue) within the collagen hydrogels after 48h of culture with and without pacing. c) NHDF cell viability assessed with a presto blue assay after 48h of culture and four times of 30 min pacing. Data are presented relative to the SiTiOC20 w/o pacing sample and as mean $\pm$ standard deviation of n=3 with 2-4 samples per condition and experiment

As shown in Fig.7b-c, no differences in metabolic activity were observed between paced and non-paced control samples. Furthermore, in both groups cells were well spread with long filopodia inside the hydrogels, demonstrating good attachment and viability. In conclusion, our results

demonstrate cytocompatibility of SiTiOC20 under pacing as no differences in metabolic activity of cells was observed without pacing.

4. Conclusions

Using the PDC method SiTiOC ceramics with Ti filler content up to 30wt% in the preceramic polymer composition were successfully fabricated. The Ti fillers were distributed homogenously in the samples. After pyrolysis at 1000 °C the obtained ceramics remained amorphous and the structure of the final ceramics were made up of mixed $\text{SiC}_x\text{O}_{2(1-x)}/\text{TiO}_x\text{C}_y$ phase along with the network of disordered free carbon. Ti incorporation in SiOC matrix improved electrical conductivity of the final ceramics. Notably, SiTiOC ceramics showed electrical conductivity of up to 10^1 S cm^{-1} and flexural strengths above 600 MPa. Newly developed PDCs, SiOC and SiTiOC ceramics were found to be cytocompatible for NHDF. Notably, electrodes from SiTiOC20 were applied in pacing studies using NHDF encapsulated in a 3D hydrogel model. Thereby cytocompatibility of the electrodes even under pacing conditions was demonstrated for the first time. While this makes them potentially interesting candidates for biomedical applications such as pacemaker electrodes, a more detailed understanding of the cell/tissue-material interaction is now required.

Acknowledgements

This work received financial support from the ETH domain, through the Strategic Focus Area (SFA) – Advanced Manufacturing programme under the project “Ceramic X.0 – High-precision micro-manufacturing of ceramics”.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding Sources

Pradeep Vallachira Warriam Sasikumar, Eike Müller, Pierrick Clement, Jongmoon Jang, Eirini Kakkava, Giulia Panusa, Demetri Psaltis, Katharina Maniura-Weber, Markus Rottmar, Juergen Brugger and Gurdial Blugan received financial support from the ETH domain, through the Strategic Focus Area (SFA) – Advanced Manufacturing programme under the project “Ceramic X.0 – High-precision micro-manufacturing of ceramics”

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Schematic illustration of the custom pacing set up and cytocompatibility of PDC electrode with and without pacing

Figure 1. Schematic illustration of the custom pacing set up; a) exploded view showing PCB with PDC sample and Pt reference electrodes (bottom), PDMS wells filled with buffer (middle) and top cover, and b) cross sectional view of each layer showing electrode assembly on PCB, PDMS block and top cover (drawing not to scale).

446x175mm (300 x 300 DPI)

Figure 2. Schematic illustration of the entire synthesis and fabrication process, a) polymer precursor solutions without and with titanium ethoxide, teflon mold for polymer casting, preceramic green bodies and PDC electrode after pyrolysis b) proposed hydrosilylation reaction leading to formation of final ceramics.

Figure 3. Characterisation of preceramic greenbodies and final ceramics a) TG curve of SiOC and Ti incorporated samples b) XRD spectra of SiTiOC samples pyrolyzed at 1000 °C, c) FTIR spectra of preceramic green bodies and d) Raman spectra of samples pyrolyzed at 1000 °C

Figure 4 a) Flexural strength of ceramics pyrolyzed at 1000°C with respect to Ti filler content (15 specimens from each composition). b) Electrical conductivity of final ceramics with respect to Ti filler content (5 specimens from each composition)

Figure 5. Cytocompatibility of SiOC and SiTiOC samples. NHDF were cultivated on SiOC and SiTiOC samples for 5 days and a) stained for actin filaments (green) and cell nuclei (blue) and b) assessed for their metabolic activity via MTS assay (mean $\pm$ SD, n = 3).

Figure 6. Electrochemical characterization of SiTiOC20 and SiTiOC30 by a) electrochemical impedance spectroscopy (n=4) and b) cyclic voltammetry

Figure 7. Cytocompatibility of SiTiOC20 electrodes with and without pacing. a) Schematic representation of the platform with the hydrogel of cells that was seeded, b) representative CLSM images (maximum intensity projections of z-stacks) of well spread NHDF stained for actin (green) and nuclei (blue) within the collagen hydrogels after 48h of culture with and without pacing. c) NHDF cell viability assessed with a presto blue assay after 48h of culture and four times of 30 min pacing. Data are presented relative to the SiTiOC20 w/o pacing sample and as mean $\pm$ standard deviation of n=3 with 2-4 samples per condition and experiment

288x224mm (300 x 300 DPI)