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Biological and mechanical characterization of carbon fiber frameworks for dental implant applications

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ABSTRACT

Objectives: The aim of the present study was to investigate the biocompatibility and mechanical characteristics of dental implant frameworks made of carbon fiber composite.
Methods: The biocompatibility of intact samples and fragments was evaluated by cell count and MTT test according to EN-ISO 10993-5:2009 directions.
Destructive and non-destructive mechanical tests were performed in order to evaluate: porosity, static and dynamic elastic modulus of carbon fiber samples. These tests were conducted on different batches of samples manufactured by different dental technicians. The samples were evaluated by optical microscope and by SEM.
A compression test was performed to compare complete implant-supported fixed dentures, provided with a metal or carbon fiber framework.
Results: Carbon fiber intact and fragmented samples showed optimal biocompatibility. Manufacture technique strongly influenced the mechanical characteristics of fiber-reinforced composite materials.
The implant-supported full-arch fixed denture provided with a carbon fiber framework, showed a yield strength comparable to the implant-supported full-arch fixed denture, provided with a metal framework.
Significance: Carbon fiber-reinforced composites demonstrated optimal biocompatibility and mechanical characteristics. They appear suitable for the fabrication of frameworks for implant-supported full-arch dentures. Great attention must be paid to manufacture technique as it strongly affects the material mechanical characteristics.

1. Introduction

In implant prosthodontics metal frameworks are used to improve the prosthesis rigidity and stiffness, reducing possible complications such as prosthesis fractures while rigidly splinting the implants together [1]. Stiff prosthesis materials are supposed to distribute the stress more evenly to the abutments and implants [2].

In particular, accuracy and rigidity of prosthodontic frameworks have been reported as fundamental prerequisites for the predictable osseointegration of dental implants that will be immediately loaded [2–3]. Splinting implants with rigid prostheses immediately after implant placement seems to protect them from overloads and micromotions [2,4–6].

Metal alloys allow fabrication of a sufficiently rigid and stiff prosthesis even if the prosthodontic space is limited. The clinical result is a thin prosthesis showing a more natural appearance without pink soft tissue reconstruction and avoiding aggressive bone remodeling. The latter is necessary in order to accommodate a sufficiently rigid and thick full-acrylic prosthesis.

However, metal frameworks supporting fixed prostheses are expensive and time-consuming to fabricate and for this reason possible alternatives are emerging. Recent improvements in composite materials have made it possible to fabricate metal-free fixed partial dentures by using fiber-reinforced frameworks [7–8]. Fiber-reinforced acrylic resin prostheses offer a cheaper alternative for the patient and additional advantages for the clinicians (avoidance of casting) [9–10]. Fiber reinforcements may carry the loads, providing stiffness, strength and thermal stability. The polymeric matrix binds the fibers together transferring the load among them in the direction perpendicular to the fiber axis and guarantees the fibers protection against chemical attack and mechanical damage [11].

In dental literature glass fibers have been mainly used as reinforcement of resinous prostheses, due to their esthetic characteristics [7–10]. However their mechanical behavior appeared unsatisfactory compared to metal alloys [9]. Carbon fibers may guarantee better mechanical properties when compared to glass fibers (greater stiffness and strength). Carbon fibers are filaments made of 99.9% chemically pure carbon with a 5–10 μm diameter. They provide high stiffness, light

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Fig. 1. Extraction flasks containing fragmented samples (on the left) and intact samples (on the right).

weight, low density, low coefficient of thermal expansion, low abrasion, good electrical conductivity and vibration damping, biological compatibility, chemical inertness (except in strongly oxidizing environments or when in contact with certain molten metals), elasticity to failure at normal temperature, high fracture strength, high fatigue and creep resistance [12–14].

These characteristics make Carbon Fiber Reinforced Composites (CFRC) appear excellent for fabrication of frameworks in fixed implant-supported prostheses. However, their application in this field has not been investigated yet.

The first aim of the present study was to investigate the biocompatibility and the mechanical characteristics of CFRC samples realized by different dental technicians.

The second aim was to compare implant-supported full-arch fixed dentures provided with a CFRC framework or a gold alloy framework via a compression test.

2. Materials and methods

2.1. Biocompatibility analysis

The biocompatibility of CFRC was evaluated in vitro following the EN ISO 10993-5:2009 directions. Both intact and fragmented (residues of manufacturing) samples were evaluated.

L929 mouse fibroblasts (BS CL 56) were exposed to extracts of the samples. 7 g of samples were put in 20 ml of culture medium Minimum Essential Medium + glutaMAX (MEM-glutaMAX) supplemented with 10% fetal bovine serum (FCS), penicillin and streptomycin into T25 sterile flasks (Falcon, Becton & Dickinson Labware, Lincoln Park, NJ) (Fig. 1).

The flasks were then incubated at 37 °C in a humidified 5% carbon dioxide incubator (relative humidity at 98%) for 72 h. The following day (day 2), a suspension of $2.14 \pm 0.08 \times 10^5$ fibroblasts/ml in 2.0 ml of Minimum Essential Medium + glutaMAX (MEM-glutaMAX) supplemented with 10% fetal bovine serum (FCS), L-glutamine, penicillin and streptomycin was put into 12-well cell culture sterile polystyrene plates (Cellstar, Greiner Bio-One GmbH, Kremsmünster, Austria). On the third day, the growth medium of the multi-wells was removed and substituted with 2 ml of extraction medium for each well.

Control cells were cultured in parallel in fresh culture medium and in contact with polyethylene (negative controls) and in fresh culture medium supplemented with 1% dioctylphthalate (positive control) (Fig. 2).

The 12 multi-wells containing the extracts and the controls were incubated at 37 °C in a humidified 5% carbon dioxide incubator (relative humidity at 98%) for 48 h. After 72 h of growth, the cells were observed using an inverted light microscope (Leica, Wetzlar, Germany) and photographed.

4 wells were then used to evaluate cell mitochondrial activity by using the 3-[4,5-dimethylthiazol-2yl]-2,5 diphenyltetrazolium bromide (MTT test, Sigma-Aldrich Srl, Milan, Italy). MTT (1 mg/ml) added to each well. After 2 h of incubation at 37 °C, the MTT precipitate was solubilized. The optical density was read at 570 nm using a spectrophotometer (GEnios, Tecan srl, Männedorf, Swiss).

The cells of 4 other wells were used for cell count. The extraction medium was removed and cells rinsed with phosphate buffered saline DPBS (Life Technologies, San Giuliano Milanese (MI), Italy) and immersed with 500 μl of 0.5 trypsin/EDTA 10× (Life Technologies). After 5 min the trypsin action was stopped by adding 100 μl of FCS. Then 10 μl of the cell suspension were added at 10 μl of 0.4% trypan blue solution. After 5 min, 10 μl of suspension were put into TC10 counting slides (Biorad, Hercules, CA, USA) and cell counting was performed 8 times for each sample (intact and fragmented samples) using TC10 Automated Cell Counter (Biorad).

2.2. Mechanical characterization of CFRC samples

2.2.1. CFRC samples

CFRC are not isotropic materials. This means that the mechanical, electrical and thermal properties of this particular material are extremely variable when measured in different directions. This happens either on the microscale, i.e. at the fiber level, and on the macroscale, i.e. at the final produced device. As a consequence, the final characteristics of the object produced with CFRC will be extremely influenced by the total fiber percentage, the fiber orientation and the geometrical lay-up of the various layers adopted to create the sample.

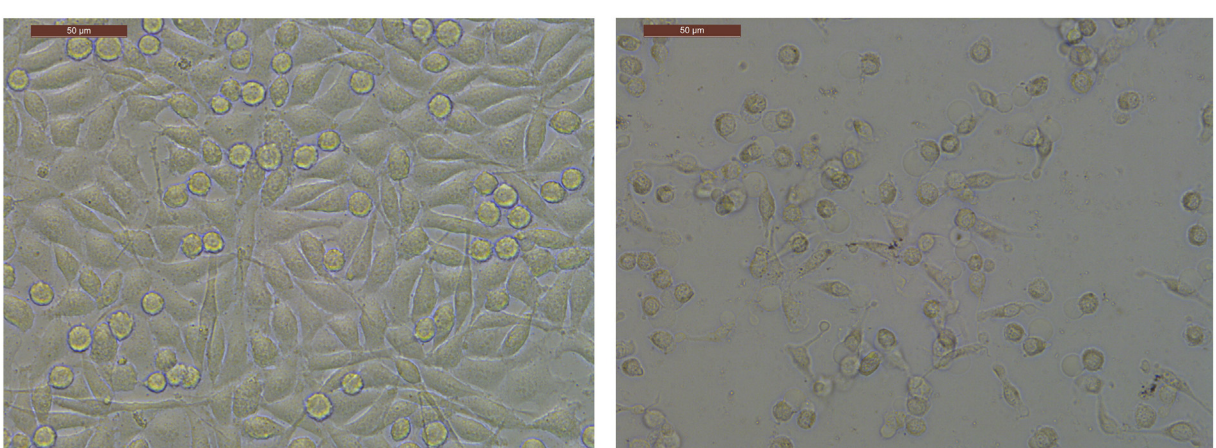

Fig. 2. Negative control (on the left) and positive control (on the right).

To explore the importance of the material, the resin and the different technological paths followed to create and apply CFRC in dental practice, in the present study; 5 dental technicians employed in 2 different dental laboratories who were already experienced in fiber-reinforced composite material manufacturing, were recruited. Four of them (employed in dental lab A) produced samples Aa, Ab, Ac, Ad. A fifth technician (employed in dental lab B) produced samples B.

Overall 34 samples of CFRC were fabricated by the 5 dental technician working in the 2 different dental laboratories.

Dental technicians were requested to fabricate beam samples with the following standard dimensions: 70 mm long, 5 mm wide, and 3 mm thick. Each sample had to be fabricated superimposing 15 plain fabrics of isotropic carbon fibers with 0°-90° orientation (Dream Frame) impregnated with a vegetal-origin epoxy resin (Bio Resin, DEIitalia).

All the dental technicians were told to follow the specific protocol suggested by the carbon fiber supplier (Dream Frame, DEIitalia, Mercallo (VA), Italy) for manufacturing CFRC prostheses. However, only technician B (of lab B) followed a 1-day training course on the manufacturing of CFRC prostheses following the above-mentioned protocol.

Samples manufacturing protocol:

1. Arrangement of 15 carbon fiber sheets (Dream Frame, DEIitalia, Mercallo (VA), Italy) impregnated with resin matrix components (with a 3:1 relationship between base and catalyst);
2. The resin must be accurately spread out with a brush on each carbon fiber sheet in order to impregnate the entire fabric before superimposing the subsequent sheet; resin excess is removed using a rubber spatula;
3. Compression of layers with a roller to remove exciding resin;
4. The samples are inserted in an aluminum muffle;
5. Firing of composite at 75 °C for 2 h and 15 min in a specially design oven (Dream Frame Black Oven, DEIitalia).

2.2.2. Microscopic observation of the samples

A microscopic analysis of one sample A and one sample B was performed before and after the sample fracture, using Nikon® Eclipse LV100 microscope (Nikon Instruments Europe BV, Amsterdam, Netherlands).

The magnifications used were 50×, 100×, 200× and 500×. The transversal sections of the samples were analyzed using two digital microscopes perpendicularly positioned (one oriented along the vertical axis of the samples, while the other one along their horizontal axis). Pictures were elaborated using Dinocapture software (Dino-lite, New Taipei City, Taiwan).

2.2.3. Measure of porosity

To measure the total volume of the pores in the samples a device constituted by a 25 cm diameter aluminum basin was used, which was hermetically enclosed by a glass bell, below which a tube linked with a vacuum pump and one with a water tank pass. Samples were placed in the basin and after having positioned the bell above this in order to prevent air from entering, the vacuum pump was activated for 30 min to eliminate all air from the samples' pores.

After that, the second tube was introduced in the water tank and it sucked into the basin enough water to completely cover all samples, maintained in immersion for 30 min. At the end, the samples were dabbed with a damp cloth to eliminate the excess water: through a precision balance measure of the difference between the weight of the dry and that of the wet samples, the quantity of water filling the samples pores was calculated and, considered that water's density is equal to 1 g/cm^3, the total volume of pores for each sample was deduced.

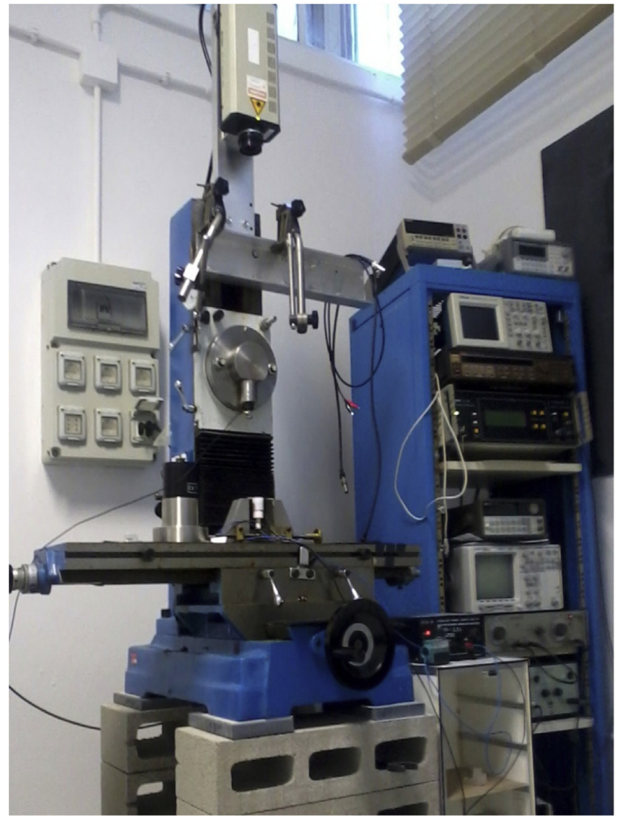

Fig. 3. Laser complex modulus measuring device.

2.2.4. Dynamic elastic modulus

Young Modulus, defined as the slope of the elastic part of a stress-strain diagram, is mainly used to evaluate the materials stiffness. Carbon fibers show a very high rigidity, much higher than metals, such as strength and overall mechanical properties. Indeed these properties are related to the fiber itself not to the final CFRC devices that are affected by the fiber pattern, the polymer matrix and the possible presence of defects. Consequently it is not unusual to see CFRC devices with a Young Modulus that is equal or minor than Inox or Titanium made devices. Young Modulus, usually measured by a stress-strain test of a sample prolonged till the evidence of plastic deformation, could be also supported by Complex Modulus evaluation. This measure, normally recorded for viscous and plastic materials like polymers, also provide information related to the energy dissipation of the material, or imaginary part. The stiffer the material, the smaller the energy dissipation.

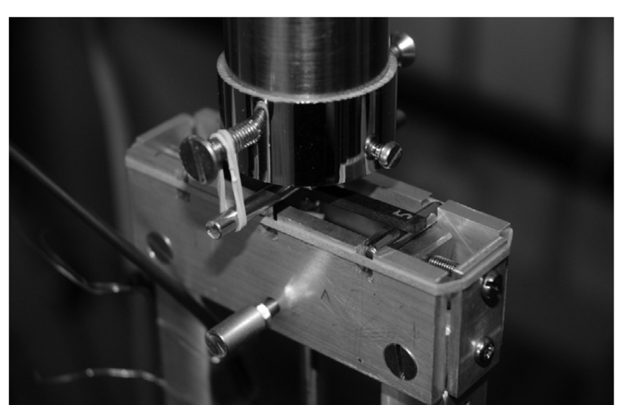

Fig. 4. Detail of the sample support.

Fig. 5. Carbon fiber composite prosthesis and corresponding cast.

For stiff materials complex Modulus evaluation is nearly meaningless, while CFRC, composed of stiff fibers annealed in viscous polymer matrix, can be fruitfully tested with Complex Modulus analysis.

To calculate the complex modulus, an experimental Complex Modulus apparatus (Fig. 3) was used, which was built by the Authors (FB, AL) in their Lab at the Department of Civil, Chemical and Environmental Engineering of the University of Genoa, Genoa, Italy. This system is composed of five different components: 1) vertical optical head, which directs a perpendicularly oriented laser beam over the sample surface and captures its reflection due to the vibration of the sample itself (POLYTEC OFV 302, Germany), 2) vibrometer controller, which sets the capture frequency of the beam reflection at 5 mm/s for each Volt of tension with which the sample is hit, 3) band-pass filter ($Lf = 2$ kHz and $Hf = 6$ kHz) applied to the frequencies of vibrations captured, 4) electro-magnetic force generator providing a 15 Volt tension to push a little plastic sphere against the inferior surface of the samples and generate the vibration, 5) trapezoidal symmetrical sample's support.

Labview data acquisition board version 8.5 (National Instruments Italy srl, Assago, Italy) was used to drive a homemade routine, developed by some of the Authors, tested and validated in many projects and experiments.

This system calculates the sample fundamental frequency of vibration f, the velocity and the internal friction of the elastic weaves Q^{-1}, max bending stress MBS and the dynamic elastic modulus.

The complex Modulus test is a dynamic way to evaluate materials. An energetic input is provided to the sample and the feedback recorded by a sensor, without stressing or breaking the sample itself. Therefore the same sample may subsequently undergo classic destructive stress-

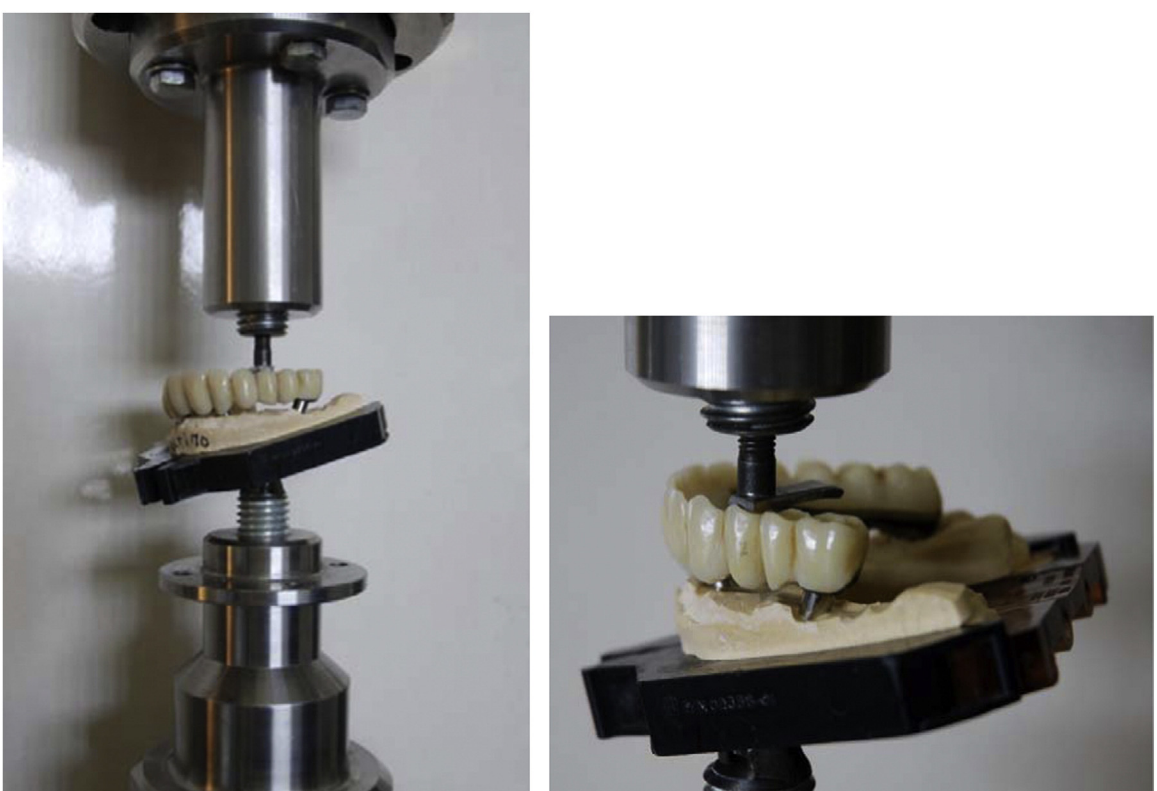

Fig. 6–7. Detail of compression test 1 with interposition of a lead laminate between the punch and the denture.

Table 1
MTT test and cell count results.

Sample	Cell count (n. cell/ml)		Absorbance (OD570)		Cell vitality (%)	Reduction of vitality (%)
	Mean	Std dev	Mean	Std dev		
Negative control	10.250	0.9284	0.2685	0.0056	100	–
Negative control (polyethylene)	10.079	1.1960	0.2650	0.0054	98.69	1.31
Positive control	0.190	0.1144	0.0402	0.0013	14.95	85.05
CFRC – intact samples	9.013	0.2927	0.2562	0.0028	95.43	4.67
CFRC – fragmented	9.048	0.3061	0.2454	0.0068	91.40	8.60

strain tests, thus providing the Young Modulus information by two different techniques on the same sample, avoiding any possible misleading data related to differently produced samples.

2.2.5. Static elastic modulus: flexion test

Classic destructive stress-strain tests were performed using an Instron machine (Instron 8501, ITW Test and Measurement Italia S.r.l. Torino, Italy).

All samples were subjected to a three points flexion test realized with a steel support specifically created for the present study (Fig. 4). The sample leaned on a system of two hinged cylinders, equidistant from its extremities and completely adjustable by articulated housings, screwed on the lower piston, while a third cylinder was fixed on the load cell by another semi-cylindrical slot, centered to coincide with the center of the sample's upper surface. The load, imposed to 300 N for all samples, was directly registered by the Instron machine, while the downward shift of the sample's lower surface under the normal force was measured by an extensimeter with a maximum range of 2.5 mm, placed in a dedicated cavity of the support. The piston's velocity was set at: 8 mm/min for samples A batch (a) and batch (c), 6 mm/min for samples A batch (b), 12 mm/min for samples A batch (d) and 4 mm/min for samples B. Different piston velocity were set in order to keep constant the deformation velocity of the different samples.

The fractured surface of one of samples B was analyzed by scanning electron microscope (SEM).

2.3. Mechanical characterization of CFRC vs. golden alloy prostheses

2.3.1. Carbon fiber composite and golden alloy prostheses

Two identical full-arch implant-supported prostheses, derived from the impression taken from a real patient, were realized on the base of a maxillary master cast made by type IV plaster. Four conical angled abutment analogs, with a 4 mm diameter (with 30° inclination in the distal sites, and 17° in the anterior sites, Biomet 3i, Palm Beach Gardens, FL) were inserted at the level of the two lateral incisors and the two first molars according to the bone availability. The master cast had been realized on the base of a patient who had been rehabilitated at the Implant Prosthodontics Department of Genoa University following the Columbus Bridge Protocol described by Tealdo et al. [4,14]. The prostheses were screw-retained on the analogs and retaining screws were torqued to 10 N cm with a torque instrument (Contra-Angle Torque Driver, Biomet 3i). The only difference between the two prostheses was the material of which the framework was made: one prosthesis was provided with a CFRC framework (Dream Frame, DEitalia), the other one with a gold alloy framework (Ney-Oro CB, Dentsply Int, York, PA) (Fig. 5). The occlusal material was acrylic resin (SR Ivocron, Ivoclar Vivadent, Schaan, Liechtenstein).

2.3.2. Compression test of the prostheses

CFRC and golden alloy dentures were screwed on to the cast and subjected to two compression tests using the Instron machine (Figs. 6–7) to measure the downward shift of the lower surface of the denture due to the vertical load and the flexion of the denture portion enclosed between the first molar and the lateral incisor, and between the two lateral incisors. The measured spans were: 27 mm between the right first molar and the right lateral incisor, 28 mm between the left first molar and the left lateral incisor, and 20 mm between the two lateral incisors. In the first test, the load and the maximum flexing moment were applied on the first premolar, while in the second test they were applied at the level of the interincisive line.

A spherical housing was excavated in the lower surface of the master cast along the loading line in order to contain a 10 mm diameter steel sphere screwed on the Instron piston. A 6 mm diameter punch was inserted on the load cell and a thin lead laminate was interposed between the punch and the denture to guarantee load distribution: it was a 4 mm^2 surface lead laminate in the first test, while in the second test aluminum and copper were used on the golden alloy and the carbon denture respectively.

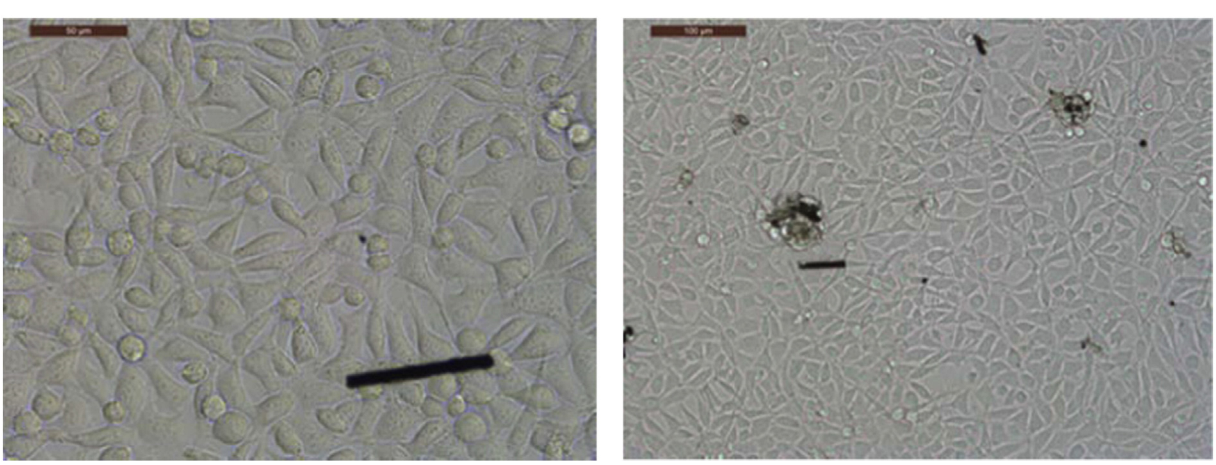

Fig. 8. Mouse fibroblastic cells L929 with extracts of fragmented samples (left) and with extracts of intact samples (right). Black objects represent Carbon fibers and fragments of CFRC left in the extracts.

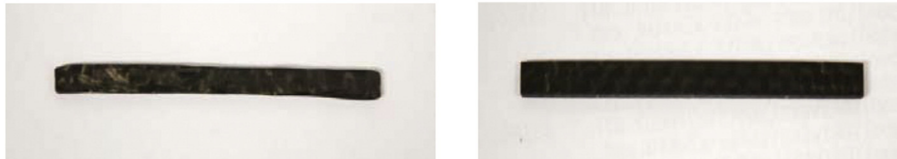

Fig. 9. One of the samples A (left) and one of the samples B (right) CFRC samples.

The maximum load was gradually increased from 100 N to 300 N, with a corresponding piston velocity of 8 and 16 mm/min, and the downward translation of the denture lower surface was measured.

3. Results

3.1. Biocompatibility analysis

Extracts of CFRC caused no signs of cytotoxicity to L929 mouse fibroblasts and MTT assay (Table 1) showed a significant difference between the tested samples and the positive control (dioctyl-phthalate).

Cell count and cell vitality presented high value both for CFRC intact samples and the fragmented ones. Cell count was 9.013 cell/ml for the intact samples and 9.048 for the fragmented ones, cell vitality was 95.43% for the intact samples and 91.40 for the fragmented ones.

Microscope observation revealed high density of cells grown in contact with the extracts (all the available space has been colonized), cells showed a polygonal morphology and firmly adhered to the bottom of the wells. Very rare multinucleate giant cells were observed. These observations were analogous to the negative controls. Fibroblastic cells also grew in direct contact with some residues of CFRC left in the extracts (Fig. 8).

3.2. Mechanical characterization of CFRC samples

3.2.1. Carbon fiber composite samples

Samples B showed a mean variance of measures equal to 0.1 mm for length and thickness and equal to 0.04 mm for width, while the mean variances for samples A were equal to 0.46 mm for length, 0.53 mm for width, and 0.40 mm for thickness (Fig. 9).

The mean weight was equal to 1.498 g for samples B and 1.503 g for samples A. Mean density was 1.287 g/cm^3 for samples A and 1.474 g/cm^3 for samples B.

3.2.1.1. Surfaces and transversal sections analysis by optical microscope. Fig. 10 shows samples images taken at 10× and 50× magnifications. Samples A showed irregular shapes with irregular edges and corners, air bubbles compromising the superficial state of conservation and an un- balanced fibers/resin ratio. Samples B had squared and regular edges and corners, a lower percentage of superficial pores and a more homogeneous fibers distribution. Transversal section sample A was not homogeneous and showed a higher percentage of defects, lack of fibers, air bubbles in resin matrix and irregular section shapes, whereas samples B showed a balanced distribution of fiber layers and resin matrix and a squared section shape with parallel and compact fiber layers.

3.2.1.2. Optical and SEM microscope analysis of the fractured surface. After static elastic modulus destructive tests, fractured samples were observed by optical microscope (Fig. 11). Optical microscope Nikon observation (Fig. 11) revealed that the fracture line proceeded more easily in samples A than in samples B. Moreover, the fracture line resulted much more linear and clean-cut in samples B, without frayed fibers or irregular borders. In samples A the fracture line followed an area composed by almost only resin matrix. Samples B were able to maintain their transversal section shape even in spite of the fracture, whereas samples A showed the detachment of entire portions of fiber layers.

Fig. 12 shows SEM images of one of samples B after fracture. The picture revealed a regular alternation of carbon fiber layers, which guarantee a better resistance to load. The fractured surface presents a net cut which demonstrates how the fibers interaction and their ability to carry the load allow the sample to have a homogeneous response to stress, while torsion and lengthening of the fibers are reduced to a minimum percentage.

3.2.2. Porosity

Porosity values are reported in Table 2. Two batches of samples (Aa and B) showed a minimum volume of pores, whereas the other batches exhibited not homogeneous values with pores volumes up to ten times higher than Aa and B samples.

3.2.3. Dynamic elastic modulus

Values of dynamic elastic modulus are reported in Table 2. Samples B showed the greatest values of dynamic elastic modulus, the highest vibration fundamental frequency and the best internal friction. Samples A showed the worst results with a lack of homogeneity in the values recorded.

3.2.4. Static elastic modulus

Results for static elastic modulus are shown in Table 2. Samples A were marked by a considerable lack of homogeneity in terms of values of static elastic modulus and of yield strain. On the contrary Samples B presented the greatest values of static elastic modulus and the greatest resistance to fracture, with a greatest homogeneity in the results. Mean yield strain for samples B was equal to 582 MPa.

Fig. 13 presents the stress and strain graphs. A lower flexion (deformation or strain), a greater resistance to fracture, and a greater homogeneity among the curves was found for samples B.

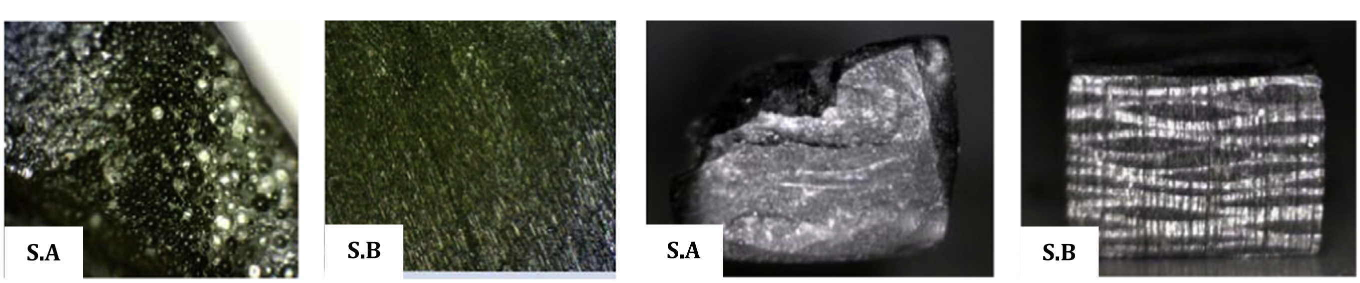

Fig. 10. Sample A (a, c) and sample B (b, d) surface analysis by optical microscope Nikon at 50× magnification (a, b) and by Dinocapture at 10× magnification (c, d).

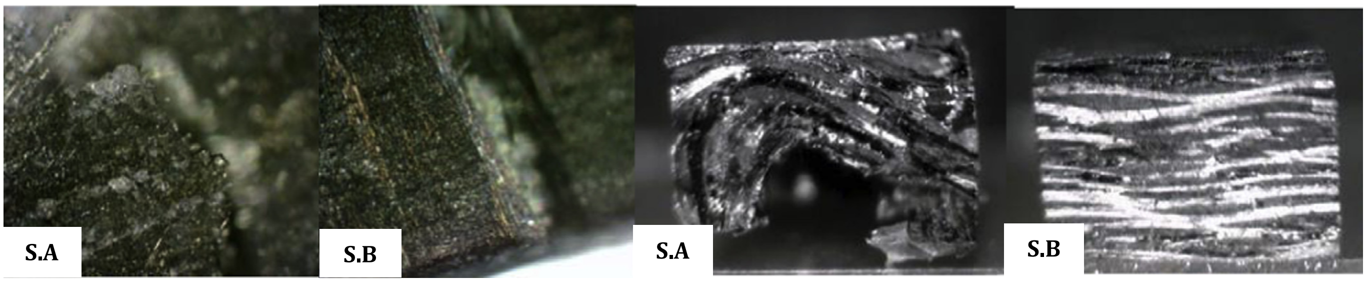

Fig. 11. Comparison of the transversal sections of samples A (a, c) and samples B (b, d) observed by optical microscope Nikon at 50× magnification (a, b) and by Dinocapture at 10× magnification (c, d).

3.3. Carbon fiber composite and gold alloy denture: compression test

The prosthesis made of carbon fiber framework was lighter (10.06 g) than the golden alloy prosthesis (28.52 g).

The results for CFRC and gold alloy denture are collected in Fig. 14. The denture with a gold alloy framework showed a more plastic behavior and a smaller deformation than the carbon fiber composite one.

Flexion values of the dentures are collected in Table 3.

4. Discussion

In the present in vitro investigation, CFRC possible application as a framework in fixed prostheses supported by dental implants was evaluated. The dental technicians involved in the study were asked to provide simple bars of CFRC. This was done in order to separate the properties of the material itself from the features provided by the denture shape and manufacturing. Subsequently, full-arch fixed prostheses were tested to simulate a clinical setting. The aim was to evaluate if CFRC and gold alloy determined any difference when the characteristics of a fixed denture (shape, occlusal material, titanium cylinders etc.) were simulated. In particular, the prosthesis was realized following the Columbus Bridge Protocol (CBP) described by Tealdo et al. [4,15]. The master cast was poured on the base of an impression of a patient rehabilitated according to the CBP. In this protocol, to avoid bone regeneration in the atrophic maxilla the implants are inserted in a tilted position. Tilting may be in a mesio-distal direction or also in a lingual-vestibular direction and both mesial and distal implants may be tilted depending on bone availability. Angled abutments are used to correct the implants inclination in order to produce a correct screw-retained dental prosthesis. For this reason, angled abutments analogs were used in the present study both at the level of distal and mesial implants.

The test set-up was simplified compared to the clinical situation. This is one of the main shortcomings of in vitro studies. However, the advantage of such simplification within in vitro studies is the possibility to reduce variables possibly affecting the results.

CFRC devices can be produced following many different techniques mainly developed in the aerospace and automotive environments. The final properties of the items created adopting CFRC may show surprising differences due to the various technical procedures followed to realize them.

Moreover, in contrast with metal alloy, which is an isotropic, homogeneous material, CFRC is an anisotropic and non-homogeneous material. It is therefore extremely important to consider during manufacturing that mechanical, electrical and thermal properties are different, along the various directions of the material. In CFRC best properties are provided along the fibers axis direction (thanks to covalent bonds between Carbon atoms), while they are usually very low in a direction perpendicular to them. In fact, the polymer matrix provides very low mechanical properties but vibration damping and fiber coating. The mechanical properties of the final devices are dependent upon the fiber direction axis.

CFRC devices are usually created superimposing several carbon fiber layers, normally 0.1 mm thick, until the final desired dimension is reached. Using monodirectional layers, known as “prepregs”, because resin is already present in the layer, extremely high mechanical performance devices can be realized. Indeed, being highly directional materials, the stacking sequence of the superimposed layers should be aligned along the directions of the forces acting on the material. This task can be easily achieved when dealing with a regularly shaped device, but it could be a very challenging goal with a non-regular, rounded, non-symmetric shape like a dental arch. Moreover, it has to be underlined that the devices created with this technique show an X-Y range of fiber disposal direction, while no fibers are placed along the Z axis. This means that any curvature and deformation may provide stresses unmanageable by the structure itself, leading to delamination.

To skip these problems different layer typologies, called “fabric”, are adopted when it is not necessary to obtain the top of the mechanical properties. Fabric CFRC layers are created with perpendicularly crossed fibers following the same patterns adopted in tissue industries. In this way fibers are no longer aligned in one single direction but are crossed in their disposal. As a consequence, the mechanical behavior of the

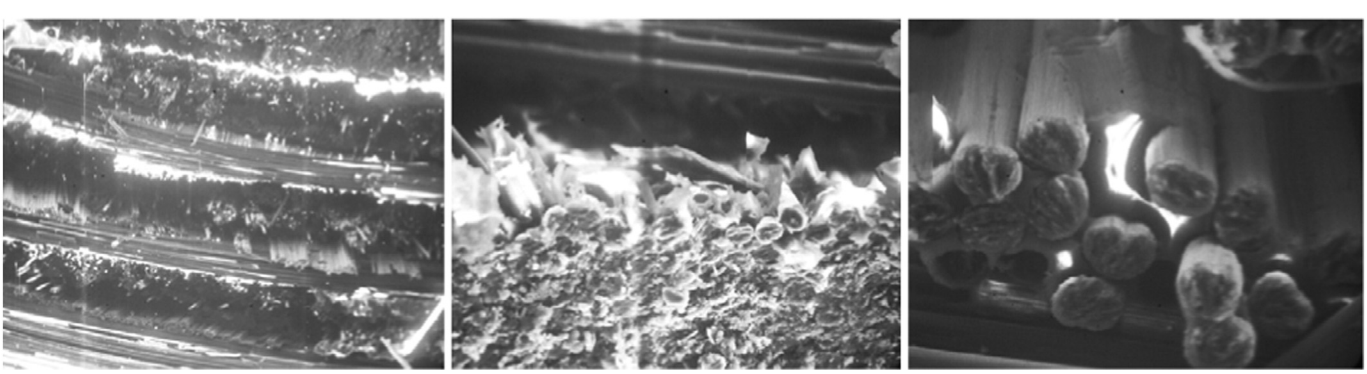

Fig. 12. SEM images of one of samples B with evidence on fiber layers alternation and fiber heads at 100×, 1000× and 2500× magnification.

Table 2
Porosity, dynamic and static elastic modulus of samples.

Sample	Pores' volume [g/cm^3]	Dynamic E [MPa]	Static E [MPa]
Aa1	$5\cdot10^{-3}$	41,845	38,030
Aa2	$4\cdot10^{-3}$	36,114	33,950
Aa3	$4\cdot10^{-3}$	39,663	36,630
Aa4	$4\cdot10^{-3}$	40,126	37,290
Aa5	$3\cdot10^{-3}$	53,903	49,290
Aa6	$3\cdot10^{-3}$	45,314	36,730
Aa7	$4\cdot10^{-3}$	43,291	35,180
Aa8	$5\cdot10^{-3}$	36,026	32,730
Mean Aa	$\mathbf{4\cdot10^{-3}}$	**42,035**	**37,479**
Ab1	$15\cdot10^{-3}$	60,364	52,810
Ab2	$9\cdot10^{-3}$	62,972	50,850
Ab3	$6\cdot10^{-3}$	61,012	50,810
Ab4	$11\cdot10^{-3}$	56,125	50,380
Ab5	$9\cdot10^{-3}$	66,041	58,160
Ab6	$23\cdot10^{-3}$	72,142	65,780
Ab7	$13\cdot10^{-3}$	33,283	29,150
Ab8	$8\cdot10^{-3}$	80,821	70,020
Mean Ab	$\mathbf{11\cdot10^{-3}}$	**61,595**	**51,134**
Ac1	$67\cdot10^{-3}$	37,342	35,260
Ac2	$19\cdot10^{-3}$	40,022	34,570
Ac3	$11\cdot10^{-3}$	68,683	62,280
Ac4	$54\cdot10^{-3}$	42,554	37,276
Ac5	$7\cdot10^{-3}$	44,109	40,000
Ac6	$19\cdot10^{-3}$	41,882	48,380
Ac7	$15\cdot10^{-3}$	31,883	28,260
Ac8	$19\cdot10^{-3}$	31,964	27,490
Mean Ac	$\mathbf{26\cdot10^{-3}}$	**42,305**	**39,190**
Ad1	$77\cdot10^{-3}$	27,173	16,290
Ad2	$73\cdot10^{-3}$	35,531	33,540
Ad3	$46\cdot10^{-3}$	19,028	15,240
Ad4	$33\cdot10^{-3}$	27,432	24,530
Mean Ad	$\mathbf{57\cdot10^{-3}}$	**27,291**	**22,400**
B1	$5\cdot10^{-3}$	98,288	89,010
B2	$5\cdot10^{-3}$	95,706	84,180
B3	$3\cdot10^{-3}$	92,066	86,460
B4	$5\cdot10^{-3}$	88,060	82,370
B5	$5\cdot10^{-3}$	90,781	83,200
B6	$2\cdot10^{-3}$	88,216	81,640
Mean B	$\mathbf{4\cdot10^{-3}}$	**92,186**	**84,477**

layers is no longer defined by a single direction but the material is “more isotropic” in its performances. This also allows non-specialist, non-aerospace technicians to successfully apply CFRC in their manufacturing.

Indeed, the final device properties are greatly affected by the fabric layers superimposing technique.

Hand procedure is normally adopted when final shapes are complicated, rounded or unique in their creation and automated large-scale procedures are not recommended. Fabric layers are usually provided by the suppliers without any resin. This is essential in biomedical applications due to the necessity to adopt specific biocompatible resins, free of non-biocompatible solvents or chemical compounds normally found in aerospace applications. Fabrics are therefore superimposed by alternating resin deposition. In this phase it is extremely important to guarantee that resin penetrates among the layers and inside the patterns. Resin gaps can be assumed as mechanical weaknesses. In bioapplications this may also lead to potential bacteria-pockets, where infections or other medical related problems may arise.

It is therefore mandatory to create a very compact, robust and pore-free material. Many techniques are available to achieve this goal, mainly related to the specific skills of the technicians engaged in these creations. Clearly, as demonstrated in the present paper, the final results may be quite different even when the same fibers and resins are applied by different technicians.

Nowadays carbon fibers are successfully used in Dentistry to produce root posts, to increase the resistance of mobile prostheses and to create dental instruments. The findings of the present research suggest that carbon fibers may be also used as reinforcements of frameworks for fixed implant-supported restorations. In fact, the CFRC samples exhibited optimal biocompatibility and mechanical properties comparable to gold alloy.

Regarding biocompatibility, microscopic satisfying observations were confirmed by quantitative data (MMT test and cell count) (Table 1). In fact, cell vitality and cell count of L2929 fibroblasts presented high values both for CFRC intact samples and for the fragmented ones, comparable to the negative control, with a complete absence of cytotoxic effects. Fibroblasts and light microscopy were used in the present research according to EN ISO 10993-5:2009 directions for “biological evaluation of medical devices”. This regulation describes test methods to assess the in vitro cytotoxicity of medical devices. The aim in the present study was to evaluate CFRC biocompatibility, and not other possible biological characteristics of CFRC on adjacent tissues, which may require different methods of analysis and the use of different cells for testing.

Dealing with mechanical properties, the CFRC samples characteristics appeared strongly affected by manufacturing procedures. At microscope, analysis samples A did not exhibit homogeneity in resin and fibers distribution and showed air bubbles on their surfaces (Fig. 10). Those characteristics were probably due to incorrect application of the protocol (phases 2–3) with inadequate lamination process. Air bubbles may have resulted from improper resin mixing or a wrong firing treatment. On the contrary, samples B were more homogeneous in shape and showed a balanced ratio between resin matrix and reinforcing fibers. These differences in the surface state of conservation were due to differences in manufacturing, which in turn affected the properties of the samples. In fact, manufacturing defects jeopardized the mechanical characteristics causing the worst response to mechanical tests for samples A. In contrast, samples B showed the greatest values for both elastic and static modulus, the highest vibration fundamental frequency and

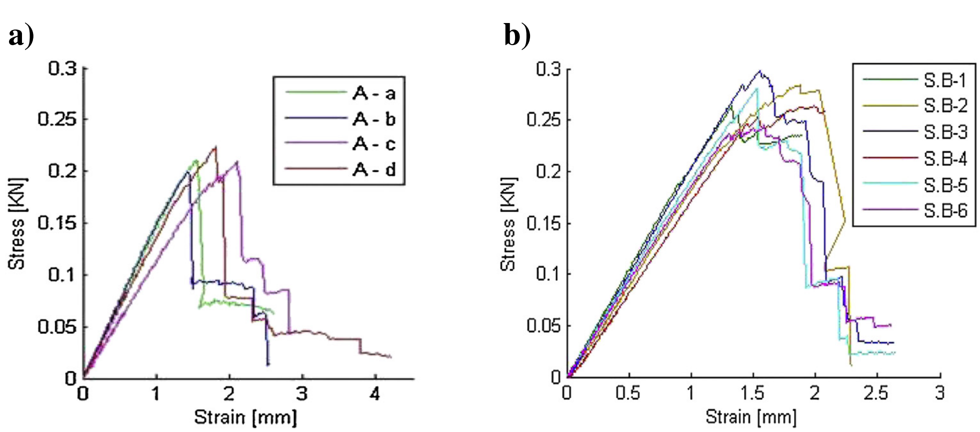

Fig. 13. Stress-strain graphs for samples A (left) and samples B (right).

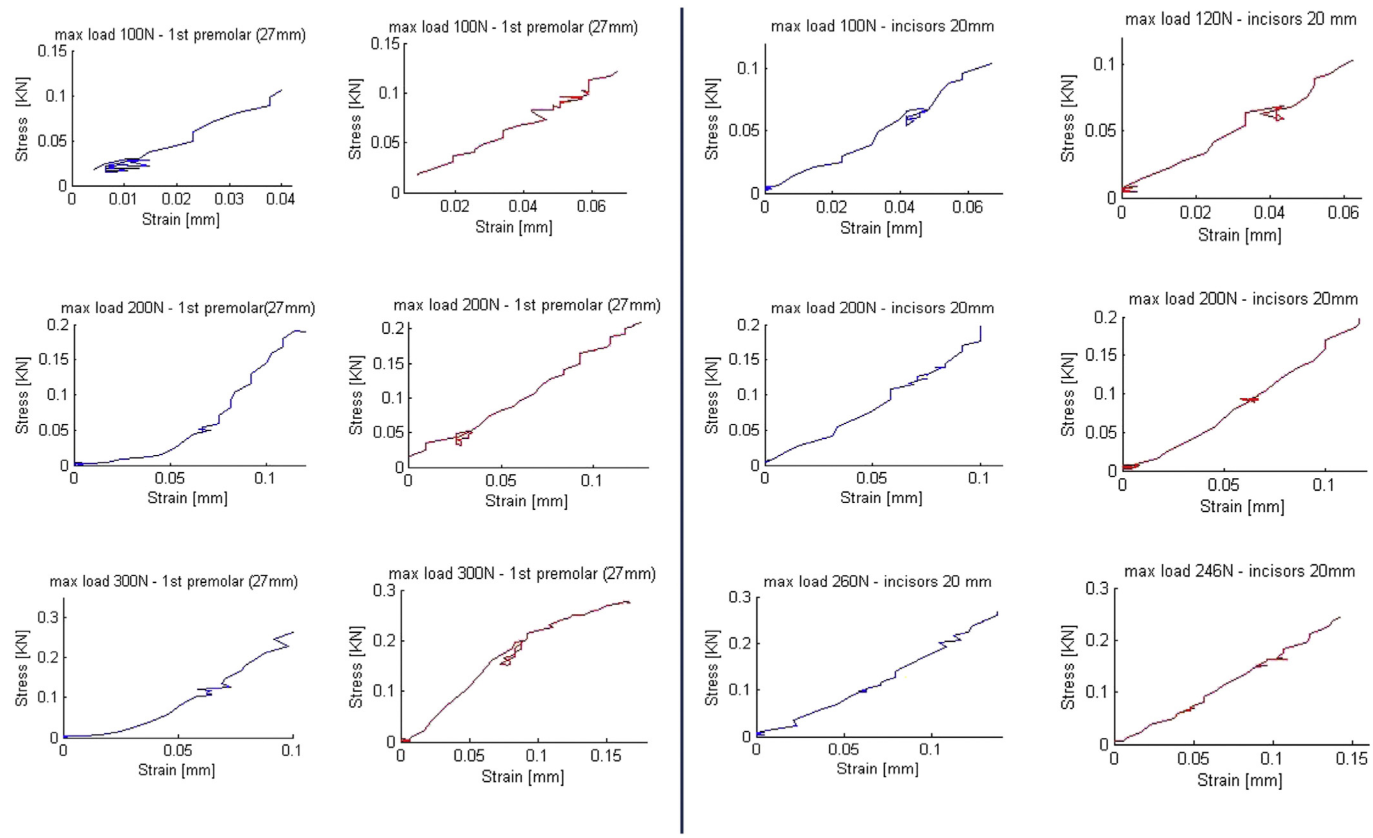

Fig. 14. Stress and strain graphs for dentures with a gold alloy (blue) or a CFRC (red) framework, submitted to a vertical load of 100 N (upper row), 200 N (middle row), and 300 N (lower row), on the right-hand first premolar (left) and on the incisors (right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the best internal friction, a parameter normally tested to dynamically evaluate the Young Modulus of stiff materials.

After fracture, samples B presented a net cut of the fibers (Fig. 11), which demonstrated how the fibers interacted and their ability to carry the load. These factors allowed samples B to have a homogeneous response to stress, and the torsion and the lengthening of the fibers were reduced compared to samples A.

The denture with a gold alloy framework showed a smaller deformation and a more plastic behavior than the denture with a CFRC framework. Given identical loads applied on the prosthesis, the downward shift of its lower surface was greater for the CFRC denture. However, once the load was removed, the prosthesis with the gold alloy framework did not completely restore its previous shape. These differences are related to the elastic properties of gold alloy and CFRC: metals reach plastic plateau, in order to deform and move from elastic to plastic regime while CFRC at room temperature show elastic behavior until they reach the fracture limit. [16].

The results were slightly different depending on the site of load application. In fact, the incisor region and the premolar region presented a different shape of the framework and a different ratio between framework and occlusal material. In particular, in the incisor region the framework was thinner and the quantity of acrylic resin was greater than in the premolar region. As a consequence, when load was applied on the incisors, CFRC and gold alloy frameworks provided similar flexion value.

Table 3
Flexion values for dentures with a gold alloy (right) or a CFRC (left) framework when load was applied at the incisors level or at the level of the first premolar.

Maximum load (N)	Carbon Fiber flexion (mm)		Golden alloy flexion (mm)	
	Incisors	Premolar	Incisors	Premolar
100	0,062	0,074	0,060	0,042
200	0,123	0,097	0,104	0,071
260	0,144	–	0,138	–
300	–	0,117	–	0,099

In immediate loading protocols the control of implant micromovements [17] is the main factor to obtain osseointegration. Low levels of compressive stresses and strains immediately after implant placement are preferred [18–19].

This can be achieved through high implant primary stability and load control. Primary stability depends on bone quality, implant macro-design and surgical technique [20,21]. Load control depends on patient's related factors and on prosthesis design. The presence of a rigid framework is one of the main prosthodontic characteristics to be respected in order to achieve a proper load distribution avoiding dangerous load concentration at the site of load application [1].

It is the authors' opinion that, in multiunit prostheses, a stiff substructure rigidly splinting the implants would be the best option to evenly distribute loads. The shock absorption capacity of more resilient restorative materials such as resin could be used at the level of occlusal surface in association with a stiff substructure. [22] Materials with relatively low Young modulus, produce a biomechanical improvement by transferring less tension to the supporting structures. In fact, elastic prosthodontic materials (such as acrylic resin) have been demonstrated to have a shock absorption capacity as opposed to stiffer materials (such as zirconia and dental ceramics) when simulating single crowns [22–25].

In a previous paper [1], Finite Element Analysis (FEA) suggested that a rigid framework is biomechanically advantageous compared to a full-acrylic prosthesis. In fact, testing prostheses provided with a metal framework, stresses transmitted to implants, prosthesis and peri-implant bone were more homogeneous compared to full-acrylic prostheses. The stress was partly distributed to contralateral implants reducing the maximum values of stress recorded next to the load application point. In that study [1], the carbon fiber framework induced a load distribution similar to the metal framework. This is due to the higher stiffness of metal and CFRC frameworks compared to acrylic resin. Using a CFRC framework a compression applied perpendicularly to the surface of the denture is also perpendicular to the axis of the fibers. Giving the continuity of the fibers, a tensile stress is expected to arise on the opposite part of the denture, especially on the external side. This aspect was not specifically investigated in the present paper. However, similar yield strengths were recorded for the denture provided with a metal framework and the one provided with a CFRC framework.

Compared to metal frameworks, CFRC frameworks present some advantages: they are cheaper, easy to produce (avoidance of casting), lighter, they allow chemical adhesion to the veneering acrylic resin, and no costly machineries or instruments are needed for their manufacturing. In particular, chemical adhesion to the resinous veneering material is expected to reduce the occurrence of chipping of the veneering material, which is a common technical complication in implant prosthodontics.

Laboratory techniques for fabrication of CFRC devices must be standardized in order to have a predictable behavior of carbon CFRC prostheses. In fact, the present study demonstrated that manufacture technique strongly affects the material mechanical characteristics. For this reason the development of a protocol for fabrication of these devices and a specific training for dental technicians is recommended. Further studies are needed in order to develop clinical guidelines for manufacturing CFRC prosthodontic devices.

5. Conclusion

Frameworks made of CFRC might be a viable alternative to traditional metal frameworks in implant prosthodontics, providing similar stiffness and rigidity and optimal biocompatibility. The development of a protocol for fabrication of these devices and a specific training for dental technicians is recommended to achieve satisfactory results.

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