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Surface functionalization of a silica-based bioactive glass with compounds from *Rosa canina* bud extracts.

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Abstract

Bud extracts are a new category of vegetal products which are used in gemmotherapy. They are liquid preparations sources of bioactive molecules (phytochemicals) and this explain their medicinal use as health-promoting agents. *Rosa canina* is a medicinal plant belonging to the family *Rosaceae*. The *Rosa canina* bud extracts, in particular, possess anti-inflammatory and antioxidant activities due to the presence of flavonoids and other phenolic compounds. The combination of *Rosa canina* bud extracts with biomaterials can be promising for the obtainment of functional materials carrying both inorganic and biological properties.

A silica-based bioactive glass (CEL2) was used as substrate for the grafting of various bud extracts of *Rosa canina*.

The Folin&Ciocalteu method was used to determine the amount and redox activity of total polyphenols in the extracts and on functionalized solid samples. XPS and Fluorescence microscopy were employed to investigate the presence of phenol substances on the material surface. Bioactivity (in terms of ability of inducing hydroxyapatite precipitation) has been investigated by soaking the samples, with or without functionalization, in simulated body fluid (SBF).

Keywords

Surface functionalization, Bioactive Glasses, Polyphenols

1. Introduction

In the last years, the use of herbal medicinal products increased strongly due to their potential health benefits and low toxicity. According to the World Health Organization (WHO), about 80% of world populations are using products based on medicinal herbs, especially in the developing countries [1, 2].

One type of phytoderivates products are bud extracts, which are defined as exclusively obtained from fresh buds, sprouts, young leaves and other meristematic tissue, which are macerated in a mixture of water, ethanol (henceforth: alcohol) and glycerol, the result consisting in concentrated solutions of bioactive phyto-ingredients. Buds are rich in bioactive compounds as vitamins, enzymes, proteins, amino acids, nucleic acids, growth factors, micropolypeptides, plant hormones and cytokines. In addition, gemmo-derivatives contain beneficial substances that can no longer be

found in the adult plant, such as gibberellin, auxin, or cytokinins [3, 4, 5]. The use of buds makes it possible to obtain a more active medication than remedies that are prepared from the whole plant.

The official procedure for bud-preparation is detailed in the monograph "Homeopathic preparations" published in the 8th edition of the French Pharmacopoeia and subsequent edition [6].

Commercial liquid preparations derived from *Rosa canina L.* (Dog Rose) buds or young sprouts are one of the most used in traditional folk medicine for its high phenolic contents. Almost all of the studies available in literature have been focused to evaluate rosehip and seeds extracts, while are minimal, or completely absent, to date scientific papers on the bud extracts.

Several compounds from rose hip extracts have been reported to display in vitro anti-inflammatory and antioxidant activities [7-9]. In particular, rose hip extracts inhibited the chemotaxis and chemiluminescence of peripheral blood polymorph nuclear leucocytes in vitro [9]. Moreover, it has been evidenced that *Rosa canina* extract inhibits the carrageenan-induced rat paw edema following a time-course similar to that of indomethacin administration [7].

Orodan et al. reported that the proanthocyanidins and flavonoids contained in *Rosa canina* fruits, possess radical scavenging properties. The rose hip extract activities were higher than other reference antioxidants (such as 2-mercaptoethane sulphonate (mesna) and N-acetylcysteine) against HClO and H_2O_2 [10]. Chrubasik et al. reported a beneficial effect of rose hip powder in the treatment of osteoarthritis [11]. Schwager et al. demonstrated that rose hip powder has enhanced in vitro anti-inflammatory and chondroprotective properties in human peripheral blood leukocytes and primary chondrocytes [12]. Rose hip extracts are nowadays used as diuretic, laxative, for kidney and lower urinary tract disorders, arthritis, gout, fever, colds and for vitamin C deficiency [11]. Rose hips are known to have a very high vitamin C content, far exceed the one found in citrus fruits [13-16]. In addition, rose hips contain other vitamins and mineral components, carotenoids, tocopherols, flavonoids, fruit hydroxy acids, tannins, pectin, sugars, amino acids and essential oils rich in volatile substances [16]. Recent studies revealed that the *Rosa canina L.* extracts were effective for the inhibition of growth and biofilm formation of methicillin-resistant *Staphylococcus aureus* (MRSA) [17, 18].

Surface functionalization is a useful and versatile procedure to realize multifunctional materials, combining the properties of both substrates and grafted molecules. It is currently possible to modify biomaterial surfaces for implants with chemical and biological functionalization following three methods: 1) realization of drug delivery systems which consists of a controlled release of bioactive molecules, 2) grafting molecules with a covalent bonding, 3) simple adsorption [19, 20].

Bioactive glasses are a particular class of biomaterials of interest for bone contact applications due to their ability to form chemical bonds with bone and stimulate its growth and regeneration. One of the main applications of bioactive glasses are bone implants, it is therefore necessary to control the physical, chemical and biochemical properties of implants surfaces in order to improve tissue integration. Some studies have been developed in the last few years, concerning the opportunity to bind natural molecules to bioactive glasses in order to couple the properties of inorganic materials with those of phytochemicals [21-23]. Gallic acid, a natural molecule present in many plants, has been combined with a bioactive glasses as model molecule for polyphenols and in order to take advantages of its antioxidant, anti-allergic, antibacterial, anti-carcinogenic and anti-mutagenic properties [22, 23]. Polyphenols extracted from grape skins and green tea leaves have been grafted to the surface of a bioactive glass without the use of any synthetic spacer [21, 23, 24].

Despite of the increasing interest in the application of buds extract in homeopathic treatments, in the scientific literature there are no studies that combine bud extracts with biomaterials. In this work, a protocol of functionalization have been studied to apply the active principles of the buds and their properties *in situ*, by linking them to a bioactive glass in a stable and reproducible way.

Bioactive glasses functionalized with natural buds extracts can be promising materials for bone contact applications in critical situations, such as bone loss due to cancer resection or infections. Particularly, the use of *Rosa canina* buds extract can be of interest for bone contact application due to the above cited properties.

Moreover, in the perspective of a circular economy, the utilization of natural sources offers the opportunity to exploit the by-products of buds extracts (buds post-maceration) still rich in active ingredients [25], thus transforming a waste into a resource.

The aim of the present work was to study the possibility to graft different bud extracts of *Rosa canina* to a bioactive glass in different grafting conditions. The glass surface analysis after functionalization was performed in order to assess the effectiveness of the grafting procedure and its eventual influence on the glass bioactivity (in terms of ability to induce hydroxyapatite precipitation).

2. Materials and methods

2.1 Sample preparation

In the present research work, a silica based bioactive glass (CEL2) developed and characterized in previous works [26-28], was used as substrate (in bulk and powder form), for the grafting of various buds extracts,. The glass was produced by the traditional melt and quenching route and its molar composition is: 45% SiO_2, 3%P_2O_5, 26% CaO, 7% MgO, 15% Na_2O, 4% K_2O. After melting of the precursors (SiO_2, $Ca_3(PO_4)_2$, $CaCO_3$, $C_4H_2Mg_5O_{14}{\cdot}5H_2O$, Na_2CO_3 and K_2CO_3, >99%, Sigma Aldrich) in a platinum crucible at 1500°C for 1 hour, the melted glass was poured in water to obtain a frit, or poured on a brass plate to obtain bars. The glass bars were annealed at 500°C for 13 hours in order to release residual stresses [21, 29], cut in slices 2 mm thick (Struers Accutom 5) and polished with SiC abrasive papers (120-4000 grit). Glass slices with homogeneous surfaces and total area of 124.12±12.16 mm^2 were obtained.

The frit, instead, was milled and sieved up to grain size lower than 20 µm. Each powder sample used for the tests was composed by 100 mg of CEL2 powder.

2.2 Phenol compounds handling

The biomolecule used for the functionalization of the bioactive glass were bud extracts of *Rosa canina* (Table 1).

Table 1: Acronyms of samples/solutions and description of the functionalization procedures with bud extracts of *Rosa canina.*

Sample acronym	Sample description
MG ROSA	**Glyceric macerate of Rosa canina**
MG ROSA WEG	**Glyceric macerate of Rosa canina diluted 1/10 in water/ethanol/glycerol**
MG ROSA W	**Glyceric macerate of Rosa canina diluted 1/10 in water**
BUDS ROSA	**Rosa canina fresh bud extract**
BY-PRODUCT ROSA	**Rosa canina glyceric macerate by-product extract**
CEL2	**CEL2 washed (acetone an water)**
CEL2+MG ROSA	**CEL2 functionalized with glyceric macerate of Rosa canina**

CEL2+MG ROSA WEG	**CEL2 functionalized with glyceric macerate of Rosa canina diluted 1/10 in water/ethanol/glycerol**
CEL2+MG ROSA W	**CEL2 functionalized with glyceric macerate of Rosa canina diluted 1/10 in water**
CEL2+BUDS ROSA	**CEL2 functionalized with Rosa canina fresh bud extract**
CEL2+BY-PRODUCT ROSA	**CEL2 functionalized with Rosa canina glyceric macerate by-product extract**

The glyceric macerate of *Rosa canina* (MG ROSA) were provided by GEALPHARMA (Bricherasio, Torino, Italy), a small company manufacturing glyceric macerates and mother tinctures.

Glyceric macerate (hencefort, MG) were prepared according to the European Pharmacopea 8th edition, following the procedure deriving f the French Pharmacopea [6] with some changes. Briefly, buds were left to macerate in solvents 50/20/30 w/w/w water/ethanol/glycerol with the solid:liquid ratio 1:15. After 3 months of maceration, the suspension was filtered, and the residue was pressed. The percolate was added to the filtrate, and the obtained solution was stored in stainless steel containers and then transferred in glass vessels (MG ROSA) or diluted as explained in Table 1 and in section 2.3 (MG ROSA WEG and MG ROSA W).

Rosa canina fresh bud extract was obtained from buds collected in the north west of Italy (Prali, Piedmont). Conventional solvent extraction was performed in a water: ethanol mixture (20:80 volume ratio) with a solid : liquid ratio 1:20. The extraction was made in a thermostatic bath, 60 °C for 60 min under shaking (120 rpm). The extraction solution was separated from the buds using a filter and, then, put into an incubator at 37°C until the total ethanol evaporation. In the end, the extracts was picked and suspended in double distilled water and freeze dried (BUDS ROSA).

Another type of extraction was made by using the buds post-maceration that was used for made a *Rosa canina* glyceric macerate by-product extract. The extraction was the same followed by the fresh buds but, probably due to residual glycerol contained in the buds after the first maceration, it was not possible freeze dry it.

2.3 Glass surface activation

In order to functionalize a surface, it is essential the presence of reactive functional groups on it, such as free hydroxyl groups [22, 23]. The method of exposition of the -OH groups has already been optimized in previous works [27, 28] and, briefly, consists in a first washing step in acetone in an ultrasonic bath for 5 min, to remove the surface contaminants, and then 3 further washing steps for 5 min in double-distilled water in order to expose -OH groups. The samples with the surface activated will be called glass-washed from now on.

2.4 Surface functionalization

Five solutions of bud extracts were prepared for glass functionalization: 1.0 mg/ml of buds rosa lyophilized in double-distilled water (BUDS ROSA), glyceric macerate of *Rosa canina* (MG ROSA), MG ROSA diluted 1/10 in water/ethanol/glycerol 50/20/30 by weight (MG ROSA WEG), MG ROSA diluted 1/10 in water (MG ROSA A) and 10 mg/ml of buds post-maceration (BY-PRODUCT ROSA).

The glass slices was put into a holder coated with aluminum foil to prevent the UV light degradation of phenol, covered with 5 ml of one of the five solutions previously prepared and incubated for 3 h at 37 °C following a protocol developed from previous works [22, 23]. After that time, the slices were washed twice in double distilled water and dried at room temperature. Three samples functionalized with each solutions were prepared for each test.

The samples grafted with buds extract will be named CEL2+MG ROSA, CEL2+MG ROSA WEG, CEL2+MG ROSA A, CEL2+BUDS ROSA and CEL2+ BY-PRODUCT ROSA (Table 1).

2.5 Photometric analysis

The total phenolic content and redox activity of the bud extracts was measured using the Folin&Ciocalteu method [30]. 2 ml of the solution were mixed with 6 ml of double-distilled water and with 0.5 ml of Folin&Ciocalteu reagent (Folin&Ciocalteu phenol reagent, Sigma Aldrich). After 3 min, 1.5 ml of 20% (p/V) Na_2CO_3 solution were added and after 2 h of reaction the photometric reading is done. The absorbance was measured at λ = 760 nm, using a Beckman DU 64 UV-VIS spectrophotometer. A standard curve of calibration was prepared by using different concentrations of gallic acid (0.0025, 0.005, 0.01, 0.02, 0.03 and 0.04 mg/ml) as described in [1] as reference. The total phenolic content was expressed as mg gallic acid/ml functionalization solution (GA equivalent, GA = gallic acid).

To quantify the phenol grafted on the surface, a modified version of the Folin&Ciocalteu test was performed: the glass slices functionalized was put into a holder covered with 8 ml of water, 0.5 ml of Folin&Ciocalteu reagent and 1.5 ml of 20% (p/V) Na_2CO_3 solution [31].

All determinations were performed in triplicate.

2.6 Fluorescence microscopy observations

In order to verify the presence and the distribution of the biomolecules grafted on the surface and their distribution, functionalized glasses were observed in different areas by fluorescence microscopy (Leica DM5500 B, Leica Microsysems, IL, USA) exploiting the natural autofluorescence of polyphenols [32].

2.7 XPS analysis

To evaluate the presence of the polyphenols on the surface a X-ray Photoelectron Spectroscopy analysis (XPS, PHI 5000 VERSAPROBE, PHYSICAL ELECTRONICS) of bulk samples was made. Both functionalized and not functionalized samples were analyzed.

Survey spectra were acquired in order to determine the chemical composition of the surfaces while the high resolution spectra of the most significant elements (C and O) were recorded in order to investigate the chemical state of elements and determine the presence of chemical groups characteristic of the polyphenols from buds extract.

2.8 Apatite-forming ability tests

To investigate the bioactivity of the new biomaterial in term of apatite-forming ability of glass before and after functionalization, the glass bulk samples were soaked in simulate body fluid (SBF) [33, 34]. The powder samples, one for each type, were put in a bottle coated with aluminum foil, to avoid polyphenols photodegradation, and covered with 25 ml of SBF according with previous work [22, 23] (Ion concentrations RE reported in Table 2) [33, 34].

Table 1: Ion concentration of the SBF (mM)

Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5

All the samples were incubated at 37 °C up to 14 days, the SBF were refreshed every 3 day and the pH measured in order to evaluate the variation due to the ionic release from the glass. After the soaking in SBF, the samples were dried at room temperature. The samples were analyzed after 3, 7, 14 day by means of FTIR (Nicolet iS50 FTIR Spectrometer) on pellets of the samples with 198 mg of kBr and 2 mg of glass powder.

3. Result and discussion

3.1 Macroscopic observations and pH measurements

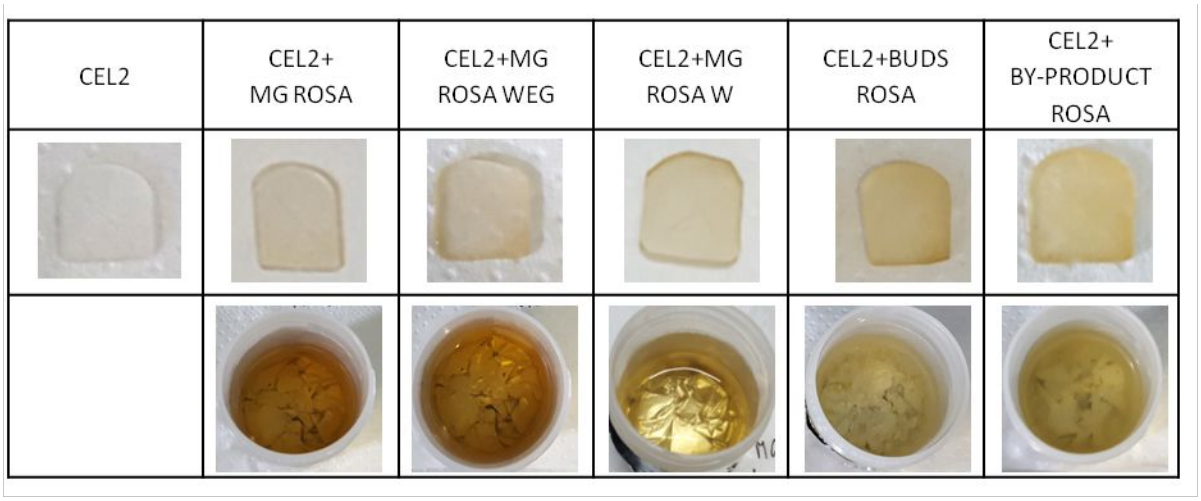

Figure 1. Glasses after functionalization and solution before samples soaking

Figure 1 shows the CEL2 bulk samples functionalized and solutions before the functionalization process. It is clearly visible that the surface of CEL2 change the color from colorless to yellow-orange after functionalization.

In order to investigate the effect of pH, pH value of *Rosa canina* solution was measured before and after soaking CEL2 for 3 hours (the term "uptake" concerns the functionalization solutions after soaking) and the results were showed in figure 2.

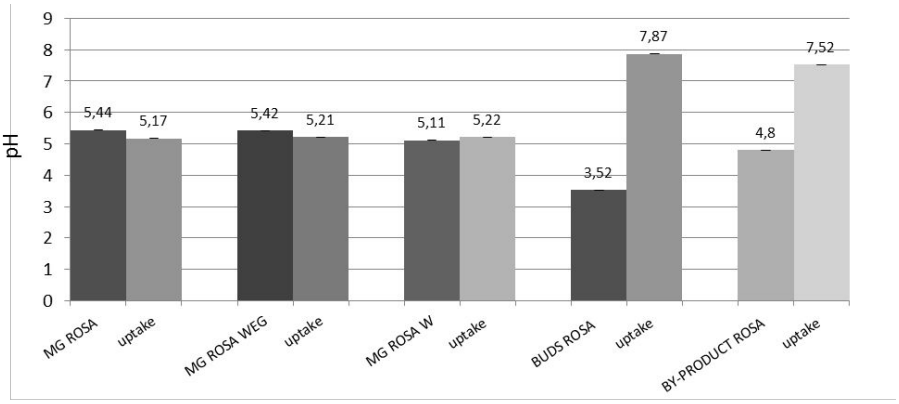

Figure 2: pH value of Rosa canina solutions before and after 3 h soaking of different glass bulks.

All five utilized solutions were characterized by an acid pH, but pH changes after functionalization depending on the presence or not of glycerol. The ion release of bioactive glasses in the solution medium BUDS ROSA and BY-PRODUCT ROSA (solutions without glycerol) causes an increase of pH up to a basic value; the initial pH values of the functionalization solutions are 3,52 and 4,80 respectively, while they become 7,87 and 7,52 after the soaking (3h, functionalization time).

It must be underlined, as already performed for the gallic acid, tea and grape polyphenols [21-23] that, in the present setup, the glasses were soaked in unbuffered solutions. MG ROSA, MG ROSA WEG and MG ROSA W contain glycerol and it is in these three solutions that no particular pH changes were recorded, probably due to a barrier effect opposed by the glycerol adsorbed in the liquid phase on the glass surface that hinders ion exchange.

3.2 Folin&Ciocalteu test

The Folin&Ciocalteu test was performed on the samples functionalized and on the solutions before and after the procedure of functionalization, in order to measure the quantity of polyphenols and their redox activity.

This test is not only a quantitative measurement of polyphenols in the solutions or on the surfaces after grafting, but it also reveals whether the molecules are still active (redox reactivity) after coupling with bioactive glasses.

Figure 3 (a) Concentrations in GA equivalent of polyphenols in the solutions used for grafting. It can be observed that the phenol concentration in MG ROSA results significantly higher (1,634 mg/ml GA equivalent) than all the other solutions. Diluting MG ROSA with a mixture of ethanol/glycerol/water (20/30/50 by weight) and pure water, the concentration of polyphenols is significantly lowered, as expected.

To check interferences, a measurement was made on samples treated in ethanol/glycerol/water, which gave zero as a response: it can be concluded that no interference from glycerol is expected in this measurement.

Functionalized bulk samples were investigated after functionalization with *Rosa canina* extracts (Figure 3 (b)). It can be noted that the amount of bud extracts grafted on the glass strongly depends on the medium used. Dilution of MG in water increases the concentration of polyphenols on the surface probably due to the lower presence of glycerol. Glycerol acting like a barrier seems to reduce the reactivity of glass, as observed with the pH measurement reported in Figure 2, and it can also act as a physical barrier that isolates the surface of the samples from grafting and inhibits ion exchange between the glass surface and solution.

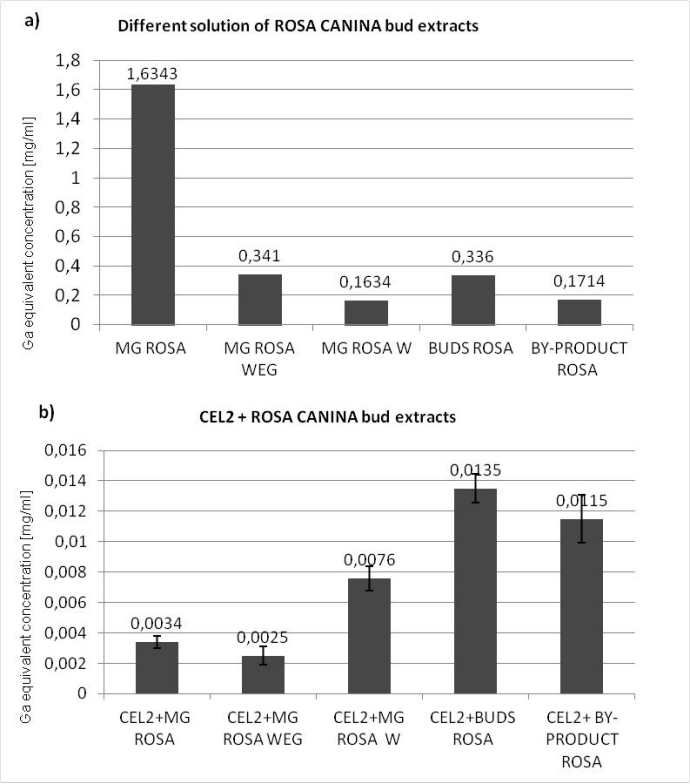

Figure 3: natural polyphenols amount expressed in GA equivalent for the uptake solutions (a) and for the sample (b)

3.3 Fluorescence microscope observations

Representative Fluorescence images of control (CEL2) and functionalized samples (CEL2+ CEL2+MG ROSA, CEL2+MG ROSA W, CEL2+BUDS ROSA and CEL2+BY-PRODUCT ROSA) are reported in Fig. 4. The control CEL2 does not show any signal as expected, instead of the other glass functionalized, which show a marked fluorescent signal due to the

grafted biomolecules onto the surface. These images highlighted the success of the procedure of functionalization and the presence of a homogeneous layer of polyphenols on the surface of the glass with some brighter spots on the samples CEL2+BUDS ROSE and CEL2+BY-PRODUCT ROSA due to a local higher presence of polyphenols.

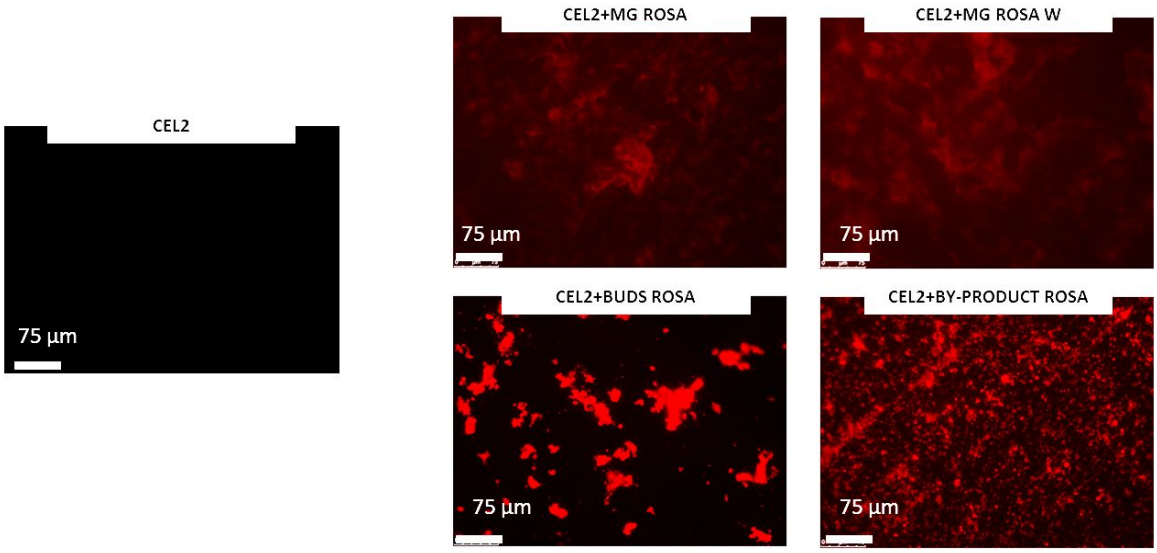

Figure 4: Fluorescence images of CEL2, CEL2+MG ROSA, CEL2+MG ROSA W, CEL2+BUDS ROSA, CEL2+BY-PRODUCT ROSA.

3.4 XPS analysis

XPS analysis was employed to characterize the chemical composition and bonds on the surface of CEL2 bulk samples bare and functionalized with *Rosa canina*.

Table 3 reports the atomic percentages of the elements detected on the surface of bioactive glasses before (CEL2) and after polyphenols grafting. It can be observed that a certain amount of carbon contaminants are observable on CEL2 surface, as reported in literature for reactive surfaces [28, 35-37].

Table 2: Atomic percentages of elements from XPS survey analyses detected on samples.

	CEL2	CEL2+MG ROSA	CEL2+MG ROSA W	CEL2+BUDS ROSA	CEL2+ BY-PRODUCT ROSA
C	30,5	57,2	53,7	53,4	55,9
O	43,3	34,2	36,4	36,9	36,1
Si	13,5				
Ca	1,8	2,8	2,6	2,7	2,3
Na	2,1	1,3	3,1	2,6	3,1
Mg	0,9	0,5	0,6	1,3	
P		1,6	0,5	0,6	0,4
S		1,5	1,2	1,9	1,5
N		0,9			0,7
Al	1,4				
Zn					
F				0,6	

The absence of Si on all the functionalized samples suggests the presence of layer of natural molecules (thicker than XPS penetration depth, at about 4-5 nm) that covers the glass. A significant increase in the carbon content after functionalization suggests the presence of organic molecules on the surface.

In order to identify the chemical groups exposed on the surfaces, the detailed analyses of carbon and oxygen regions have been performed and reported in Figure 5 and 6.

Figure 5 shows the high resolution XPS spectra of carbon region of CEL2 bulk samples.

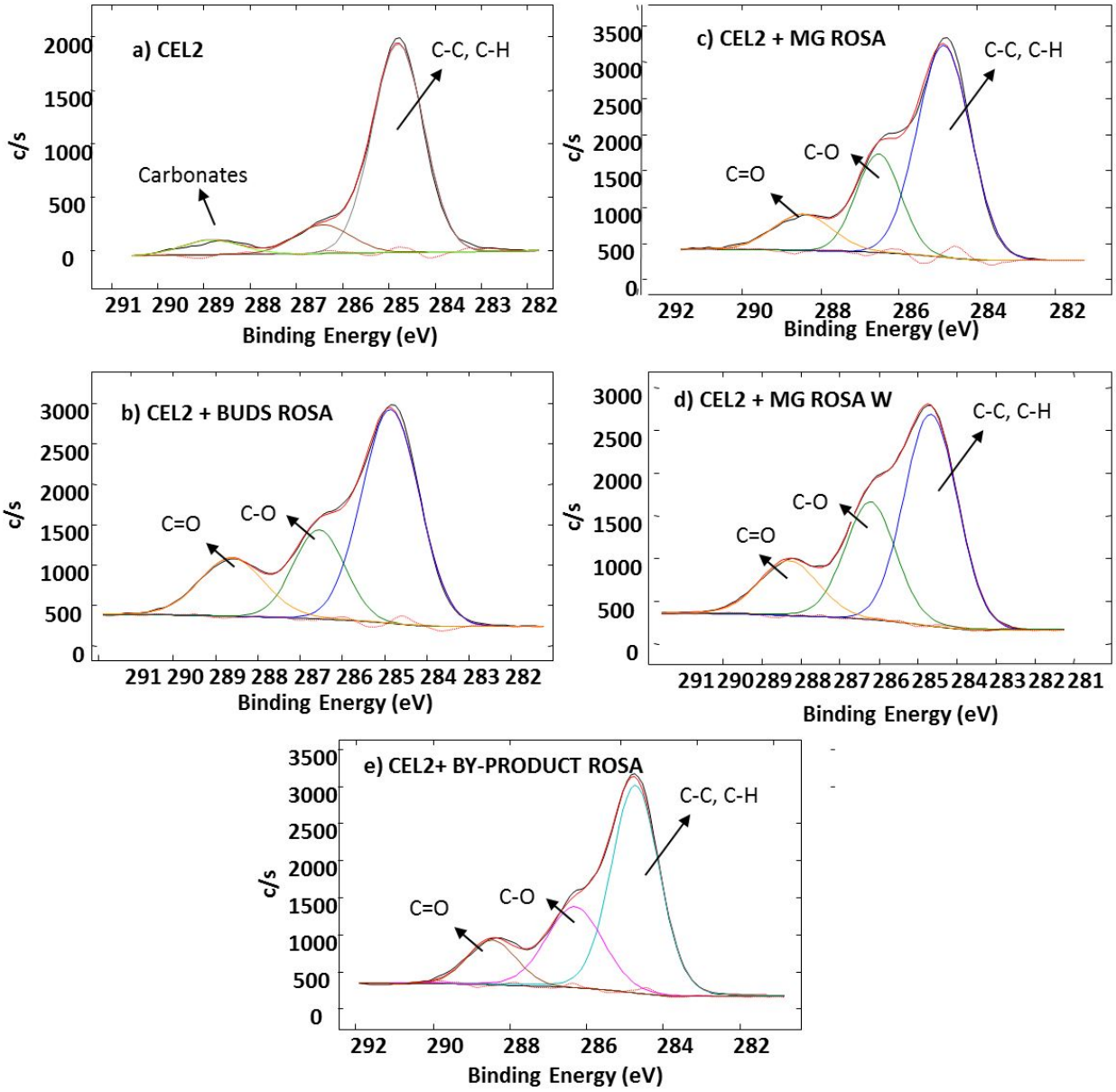

Figure 5: XPS high resolution spectra of the carbon region.

A notable signal at 284.79 eV was detected on surface of washed CEL2, which can be attributed to unavoidable hydrocarbon contaminations on reactive surfaces as mentioned in literature for XPS analysis of reactive materials [38-40]. The signal at about 289.25 eV can be assigned to carbonates, usually observed on the surface of bioactive glasses as contaminant [22, 23]. The signal attributed to carbonates disappears after Rosa canina functionalization, as previously observed by the authors after functionalization with gallic acid, grape and tea polyphenols [21-23]. Moreover, other two peaks at 286.54 eV and 288.60 eV were observed. This peaks can be attributed to C-O and C=O bonds according to literature [22, 23] and they are

characteristic of polyphenols, that confirms the presence of this molecules on the surface. On the contrary, the signal at 284.79 eV still persists and it can be attributed both to surface contamination and to C-C and C-H bonds in the polyphenols molecules. The increase on the surface of this last peaks can be correlated to the increase of the atomic percentage of carbon content on the surface functionalized with polyphenols. Figure 6 shows the high resolution spectra of oxygen region. The first spectrum is related to washed CEL2 and underlines the presence of the characteristic signal for silica at 530.80 eV and hydroxyls at 532.22 eV as reported in literature related for this glass after surface activation [38].

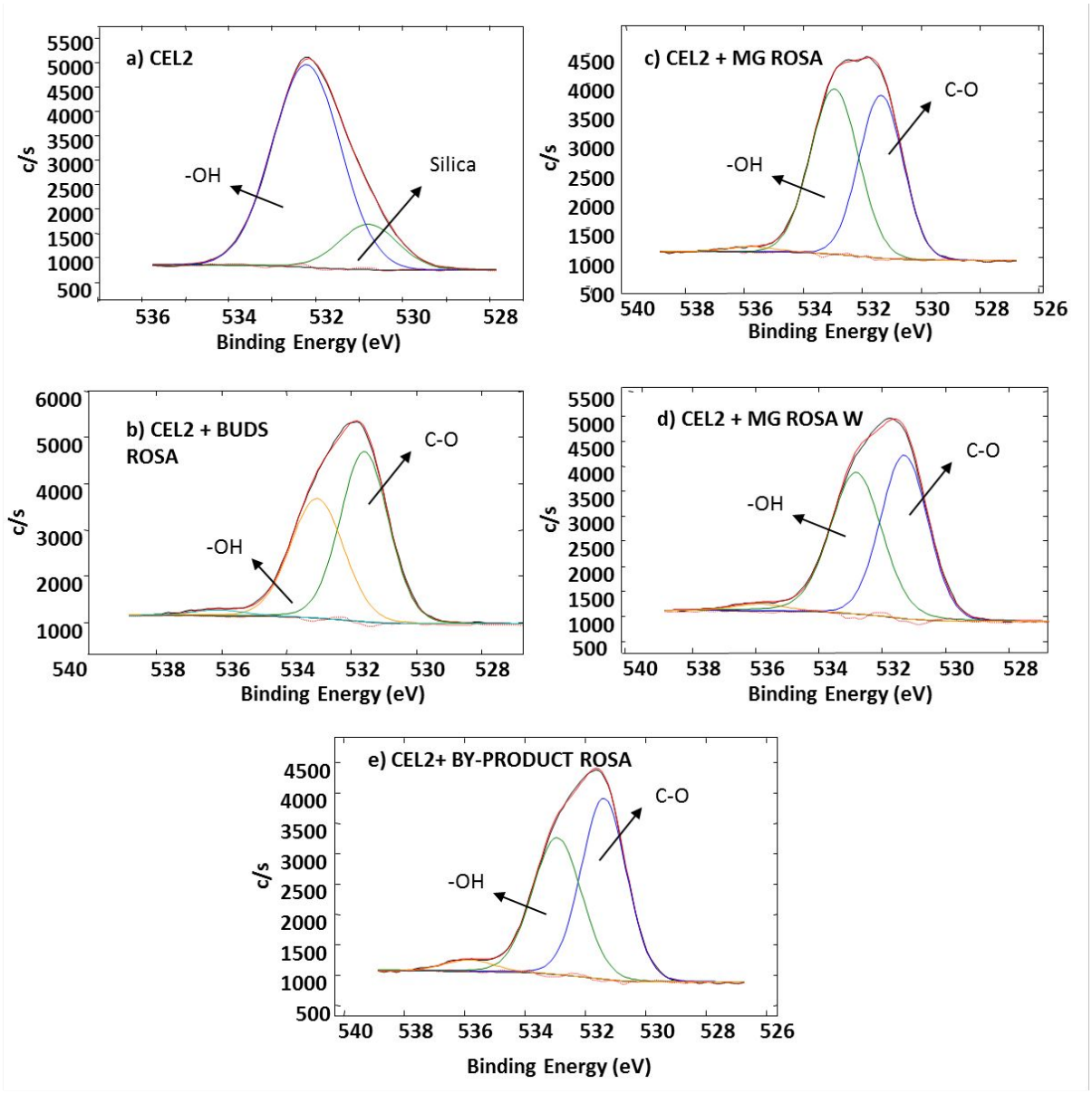

Figure 6: XPS high resolution spectra of the oxygen region.

The signal attributed to Si-O bonds disappears on functionalized samples, in accordance with the absence of the Si signal in the survey spectra. On the other hand, the signal of OH groups persist and results to be shifted to higher energies compared to the one of the washed glass. This shift can be associated to the presence of aromatic OH typical of phenols [38]. Moreover, a signal at about 531.6 eV appears on functionalized samples and can be attributed to C=O bonds, present in polyphenols, in accordance with the results obtained in carbon region. The functional groups of glycerol are mainly C-H, C-O and OH: as a consequence, it is not possible to discriminate them from the one of polyphenols and individuate eventual surface bonding of glycerol. Since the sample simply treated with ethanol,

glycerol and water mixture was not responsive to the Folin&Ciocalteu test, as reported in paragraph 3.2, the grafting of glycerol to the substrates can be considered negligible.

3.5 Apatite-forming ability tests

Powder glass samples were soaked in SBF up to 14 days to investigate bioactivity as hydroxyapatite precipitation. The pH was checked in order to evaluate the variation due to the ionic release from the glass and it ranged between 7.40 to 8.18.

Powder glass were analyzed after 3, 7 and 14 days by means of FTIR and the IR spectra are reported in Figure 7.

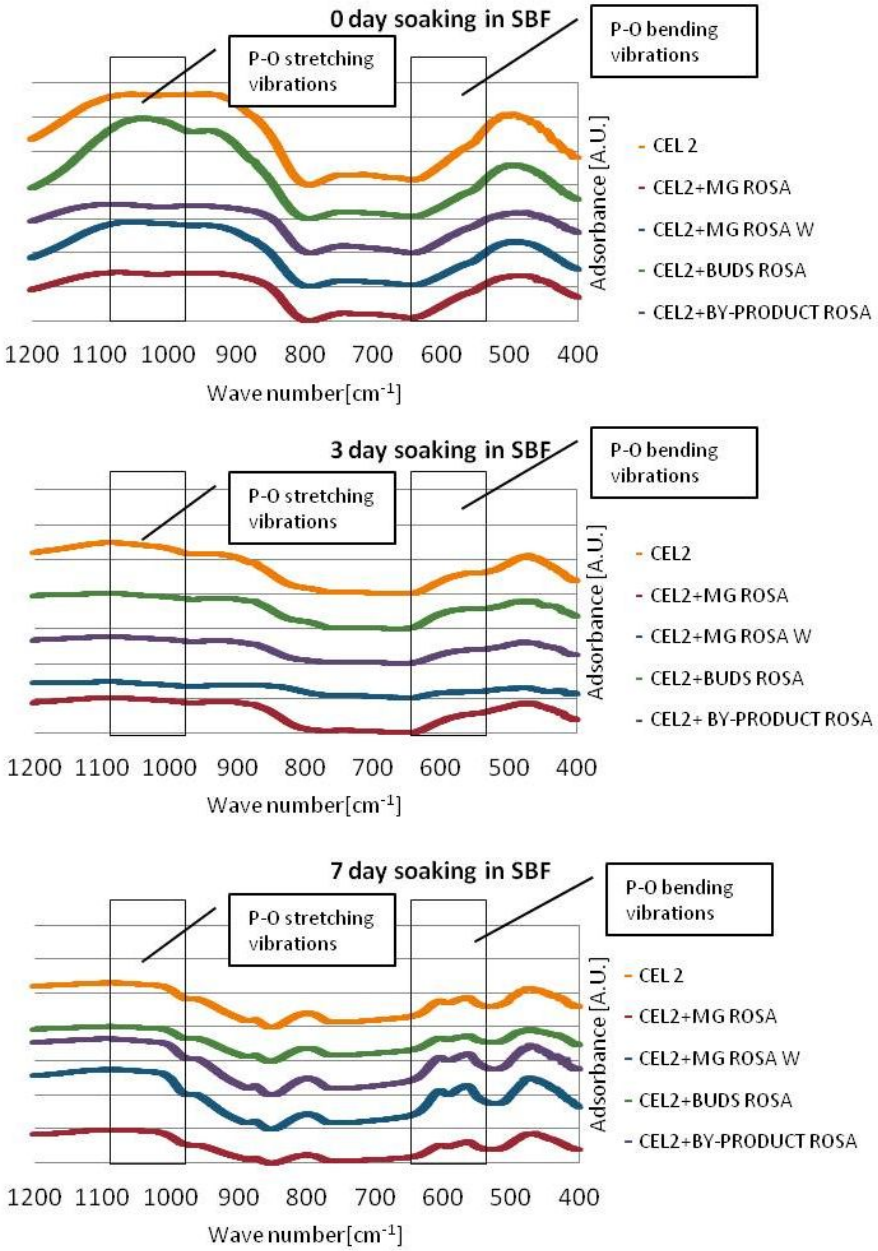

Figure 7: IR spectra of glass powder before and after functionalization with polyphenols after different times of soaking in SBF.

The presence of hydroxyapatite on pellets samples is shown by a double peak around 600 cm-1 and 560 cm-1. This double peak is correlated with the bending vibration of P-O bonds. It is evident that functionalization does not inhibit glass bioactivity of pure CEL2. These results are in accordance with the ones previously observed by the authors for surface functionalization of CEL2 with gallic acid and polyphenols from grapes and tea [21-23]. This point is extremely

important, because it confirms the possibility to couple the typical properties of the substrate (e.g. bioactivity for the bioactive glass) with the ones of the grafted molecules. Considering that glycerol seems to reduce the ion exchange of bioactive glasses (as reported in the investigation of pH variations in the functionalization media), the results of bioactivity tests support the hypothesis, previously reported in the XPS discussion, that glycerol does not remain grafted on the glass surface after functionalization, while it is adsorbed on the glass surface in the liquid phase.

4. Conclusions

In this work, a protocol of functionalization of bioactive glasses with phytoextract of *Rosa canina* buds was developed. A silica based bioactive glass named CEL2 was exploited as substrate and different extracts from buds were used for functionalization. The measurements of pH on the solutions of functionalization before and after soaking of the samples showed that the presence of glycerol avoids the basification of the solutions, suggesting a lower reactivity of the surface of the glass in this solutions. Glycerol reduces the ionic exchange of the glass with the solution, suggesting also a minor ability to bind polyphenols. However, it seems that this molecule does not remain grafted on the glass surface after functionalization.

This result was confirmed by both Folin&Ciocalteu and fluorescence microscope measurements, which highlighted an higher presence of polyphenols on the samples CEL2+BUDS ROSA and CEL2+BY-PRODUCT ROSA functionalized without the presence of glycerol in the solutions. Polyphenols result also present on the surface of the other samples, but in a lower amount. The XPS analysis, according to the fluorescence microscope images, showed the presence of an uniform layer of biomolecules on the surface of the samples, with much more agglomerates on CEL2+BUDS ROSA and CEL2+BY-PRODUCT ROSA.

In vitro bioactivity tests were performed in order to check whether the samples are still bioactive after the procedure of functionalization. From the FTIR analysis, it appears that the presence of the polyphenols from buds extracts, not only preserves bioactivity, but also enhances it promoting an abundant deposition of hydroxyapatite.

The solution which showed the greatest potential is the one obtained using the by-products of primary extraction in glycerol macerate. This solution has a great potential because it can promote the transformation of a byproducts (residuals from glycerol macerate production) in the source of high added value molecules (polyphenols) through a simple process (conventional solvent extraction). These molecules can effectively be used for the preparation of multifunctional materials with a green approach and a sustainable use of resources.

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Competing interests statement

The authors have no competing interests to declare.

CRediT statements

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