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***Abstract*—Actually, the main focus of the research in the biomaterials field is represented by multifunctional surfaces able to promote tissue integration, to control inflammation, to reduce bacterial adhesion and finally to modulate host response for physiological healing. In this context, the present research is aimed at coupling bioactive surfaces (chemically treated Ti6Al4V alloy and bioactive glasses) with natural polyphenols.**

***Keywords*—polyphenols, bioactive glasses, Ti6Al4V, host response, bioactivity.**

I. Introduction

The requirements for biomaterials intended for bone contact applications evolved, together with the scientific research in the field, including several levels: from mechanical resistance (in order to sustain and transmit load) and biocompatibility (no cytotoxic reactions) to bioactivity (ability to chemically bond to bone) and fast bone integration, to modulation of inflammation and reduction of bacterial contamination. In order to satisfy this complex set of needs, innovative multifunctional materials are required.

Titanium and its alloys are widely employed in orthopaedic and dental applications due to their good mechanical properties and biocompatibility, however, they are not bioactive and several surface treatments have been developed in order to improve their bone bonding ability [1]. On the other hand, bioactive glasses have an excellent bioactive behaviour, and are often used as small bone substitutes or coatings, in order to overcome poor mechanical properties as bulk materials [2].

Polyphenols are widely investigated for their numerous interesting properties such as antioxidant, anticancer, anti-inflammatory, antibacterial, bone stimulating and vasculoprotective effects [3].

Despite of a wide research on natural molecules (and expecially polyphenols), few attempts of combination with inorganic bioactive materials have been reported.

The aim of this research work is coupling of natural polyphenols (extracted from green tea leaves and grape skins or pomaces) to bioactive surfaces (chemically treated Ti6Al4V and bioactive glasses) in order to obtain multifunctional materials able to promote bone integration and to modulate host response.

II. Materials and Methods

A. Samples preparation

A bioactive glass (CEL2) with molar composition 45% SiO_2, 3% P_2O_5, 26% CaO, 7% MgO, 15% Na_2O, 4% K_2O, has been prepared by melt and quenching technique in the form of bars which have then been cut and polished [4].

Ti6Al4V has been chemically treated (CT) according to a patented process which includes a first acid etching for the removal of the native oxide layer and a subsequent controlled oxidation in hydrogen peroxide that allows the development of a new surface oxide layer with nanotextured morphology and high density of hydroxyls groups [5].

B. Biomolecules preparation

Polyphenols were extracted from green tea leaves (TPH), red grape skins (GPH) and red grapes pomaces (PPH) by conventional solvent extraction in water:ethanol mixture at 60°C, evaporated, suspended in water and freeze-dried [4].

Polyphenol solutions were prepared dissolving freeze dried extract in a proper solvent (water or simulated body fluid) at define d amount.

Gallic acid (GA) was used as model molecules for process optimization.

C. Surface functionalization

Surface grafting of biomolecules was performed by soaking of the activated substrates in the prepared solution for 3h at 37°C. Surface activation was obtained by acetone/water ultrasonic washing for the bioactive glass [4] and by UV exposure for Ti6Al4V-CT.

D. Characterizations

The presence and activity of grafted polyphenols was verified by means of the Folin&Ciocalteu test adapted to solid surfaces [6]. The chemical composition and the presence of specific chemical groups was determined by means of XPS analyses. The presence and distribution of polyphenols on the biomaterials was observed by means of fluorescence microscopy. In vitro bioactivity was investigated by samples soaking in Simulated Body Fluid. The selective antitumor activity of polyphenols upon grafting was investigated on safe (hFOB) and tumor (U2OS) osteoblast cells by means of direct and indirect cellular tests.

III. RESULTS AND DISCUSSION

The Folin&Ciocalteu tests performed on solid samples demonstrated the effective grafting of polyphenols on both bioactive glass and Ti6Al4V-CT with maintenance of molecule redox activity. A certain dependence of grafting ability on surface reactivity was evidenced. XPS analyses noticed the presence of specific functional groups of polyphenols on all the tested surfaces after grafting (OH and CO signals in the high resolution spectra of oxygen and carbon). Polyphenols autofluorescence was employed for biomolecules visualization on the samples surface (Figure 1). This technique demonstrated a complete surface coverage for both glass and metal with some more dense zones.

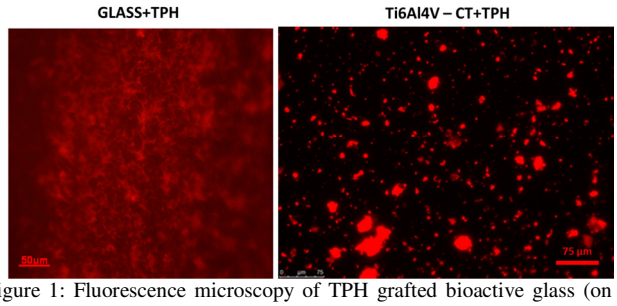

Figure 1: Fluorescence microscopy of TPH grafted bioactive glass (on the left) and Ti6Al4V-CT (on the right).

In vitro bioactivity tests showed the ability of both polyphenols grafted bioactive glasses and Ti6Al4V-CT to induce apatite precipitation after SBF soaking Figure 2). This result indicate that polyphenols do not inhibit the inorganic bioactivity of the tested surfaces. This point is particularly important for the realization of multifunctional surfaces.

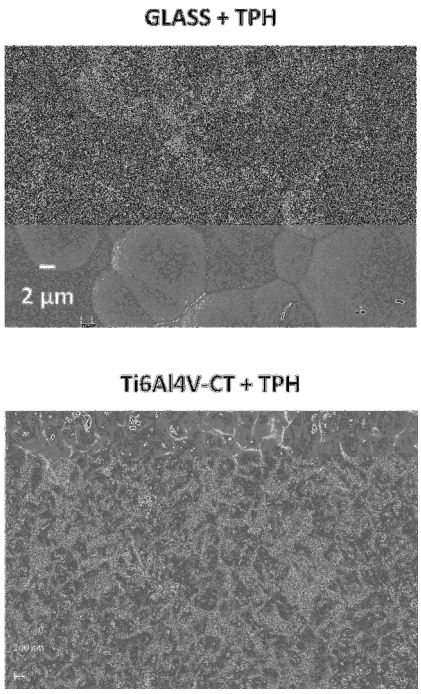

Figure 2: hydroxyapatite on the surface of TPH grafted bioactive glass (upper panel, after 14 days in SBF) and Ti6Al4V-CT (lower panel, after 28 days in SBF).

A selective cytotoxic activity for tumor cells (U2OS) of polyphenols-grafted glasses was evidenced, with particular significance in direct tests. This result suggest a direct action of polyphenols from the surface of biomaterials without release, in accordance with preliminary results of biomolecular leaching in solution. Moreover RONS production and permanent DNA damage were noticed on U2SO cells.. On the contrary, an anti-inflammatory action was evidenced for the safe cells (hFOB) [7].

IV. CONCLUSION

The present research demonstrated the possibility to graft natural polyphenols to bioactive surfaces (bioactive glass and chemically treated Ti6Al4V alloy) maintaining molecular activity and materials inorganic bioactivity.

The modified surfaces were widely characterized from the physico-chemical point of view in order to investigate biomolecular presence.

Preliminary biological characterization evidenced the ability of polyphenol grafted bioactive glasses to inhibit the growth of tumoral osteoblasts (U2OS) preserving healthy ones (hFOB).

The produced materials appear promising for bone contact applications in critical situations.

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REFERENCES

[1] S. Spriano, S. Ferraris, “Metallic Surfaces for Osteointegration” in Surface Tailoring of Inorganic Materials for Biomedical Applications, , L. Rimondini, CL Bianchi, E Vernè Eds,Bentham Science Publishers, 2012, pp 279-296

[2] M N. Rahaman et al. Bioactive glass in tissue engineering, Acta Biomaterialia 7 (2011) 2355–2373

[3] S. Quideau, D. Deffieux, C. Douat-Casassus, L. Pouységu, Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis, Angew. Chem. Int. Ed. 50 (2011) 586- 621.

[4] M. Cazzola, I. Corazzari, E. Prenesti, E. Bertone, E. Vernè, S. Ferraris, Bioactive glass coupling with natural polyphenols: Surface modification, bioactivity and anti-oxidant ability, Appl. Surf. Sci. 367 (2016) 237-248.

[5] S. Ferraris, S. Spriano, G. Pan, A. Venturello, C. L. Bianchi, R. Chiesa, M. G. Faga, G. Maina, E. Vernè, Surface modification of Ti–6Al–4V alloy for biomineralization and specific biological response: Part I, inorganic modification, J Mater Sci: Mater Med 22 (2011) 533 545.

[6] S. Ferraris, X. Zhang, E. Prenesti, I. Corazzari, F. Turci, M. Tomatis, E. Vernè, Gallic acid grafting to a ferrimagnetic bioactive glass-ceramic, J. Non-Cryst. Solids 432 (2016) 167-175..

[7] M. Cazzola et al.Bioactive glasses functionalized with polyphenols: in vitro interactions with healthy and cancerous osteoblast cells, J Mater Sci (2017) 52:9211–9223