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# Passive and Bioactive Films on Implant Materials and their Efficiency in Regenerative Medicine

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# Passive and Bioactive Films on Implant Materials and their Efficiency in Regenerative Medicine

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*The article presents various behavior aspects of passive and bioactive films on Ti and CoCr alloy and their efficiency in regenerative medicine, taking into account that metallic materials are typically inert, but various surface treatments are devoted to build films which change them into active materials. The studied passive films were oxides and bioactive films with collagen and phosphate coatings. All films were characterized from the point of view of their electrochemical stability. Their composition was evaluated using infrared spectroscopy and electronic microscopy type SEM with and EDAX module The cytotoxicity test for fibroblast growth using direct contact method was the in vitro biocompatibility evaluation for all studied films.*

**Keywords:** bioactivation; CoCr alloys; passive films; titanium



## INTRODUCTION

There are many classifications of biomaterials taking into account various points of view [1]. The article is related to a classification governed by the tissue response. This one divides biomedical materials roughly into three main categories; bioinert materials which no or minimal tissue response, bioactive materials which encourage bonding to surrounding tissue, and resorbable materials which are incorporated into the surrounding tissue. Metals are typically inert, but various surface treatments [2–4] are devoted to build films which change them into bioactive materials. Our results on titanium [5] and on CoCr [6,7] biomaterials reveal interesting aspects regarding

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stability and biocompatibility of passive and bioactive films. Depending on surface preparation, passive films are: (1) Natural passivated being non continuous, and having weak protection; (2) Passive non natural film (electrochemical, chemical, physical and chemical vapor deposition, laser deposition, etc.) which are continuous and represent a good protection. In the physiological environment, passive films could be changed into active films through adsorption phenomenon.

Bioactivation process could be performed chemically, physically, electrochemically, and biochemically and during last decade many studies were devoted to surface bioactivation and functionalization [8–10].

The goal of this article is to compare chemical and electrochemical bioactive films obtained on Ti and CoCr alloys with the passive films on the same materials in the same conditions. The behavior to be compared includes electrochemical stability in various saliva, morphological features and composition of passive and bioactive films.



## EXPERIMENTAL PART

The composition of the metallic samples is the subject of Table 1.

Regarding the surface preparation in order to obtain passive and bioactive films on Ti and CoCr alloys, investigations were performed using surface treated physically, chemically (chemically polished in 3% $HF + 20\%HNO_3$) or biochemically with collagen gel, taking into account the correlation between surface quality and film stability.

The biocompatibility was evaluated in an ethical manner using in vitro culture of cell and a cytotoxicity test for fibroblast growth with direct contact method [9]. In this method, secondary cultures of human skin fibroblasts (HSF) were obtained, grown, and subcultured at 37°C in a humidified incubator equilibrated with 5% $CO_2$. The cells were seeded on the films at a density of $1 \times 10^5$ cells/mL and cultured for up to 7 days.

**TABLE 1** Chemical Composition of Metallic Biomaterials

| | % Wt. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sample | Al | Fe | Mo | Cr | C | O | N | Ti | Co |
| Ti | 0.005 | 0.095 | | – | 0.04 | 0.056 | 0.045 | 98.04 | – |
| CoCr | – | 0.54 | 6.44 | 28.56 | – | – | – | – | 64.43 |

Evaluation of film stability and morphological features behavior were done using electrochemical and complementary methods of stability determinations as follows:

- monitoring of the variance of electrode potential in open circuit (using a METRIX system) in short, medium and in long term, simultaneously with pH determination;
- cyclic voltametry experiments with a VOLTALAB 40 equipment;
- Infrared (FT-IR) spectra for structure determination of the passive film modification after immersion in various saliva, performed with a JASCO model 620;
- Determining the morphology and the composition of films before and after immersion in bioliquids by means of an Environmental Scanning Electron Microscope FEI/Phillips XL30 ESEM equipped with an EDAX (X ray energy dispersive) module.



The experiments were performed in simulated artificial saliva solutions with phosphate content and in buffer environment with the following compositions:

Afnor (Af): 0.7 g/L NaCl, 1.2 g/L KCl, 0.26 g/L $Na_2HPO_4$, 1.5 g/L $NaHCO_3$, 0.33 g/L KSCN, 1.3 g/L uree;

Carter-Brugirard (CB): 0.7 g/L NaCl, 1.2 g/L KCl, 0.26 g/L $Na_2HPO_4$, 1.5 g/L $NaHCO_3$, 0.33 g/L KSCN, 0.2 g/L $K_2HPO_4$, 0.13 g/L uree;

Fusayama, this is the only studied saliva with calcium content (Fus): 0.4 g/L NaCl, 0.9 g/L KCl, 0.795 g/L $CaCl_2 \cdot 2H_2O$, 0.69 g/L $NaH_2PO_4$, 1 g/L uree;

Tani–Zucchi (TZ): 1.5 g/L KCl, 1.5 g/L $NaHCO_3$, 0.5 g/L $NaH_2PO_4$, 0.5 g/L KSCN, 0.9 g/L lactic acid;

Duffo-Quezada: NaCl0.600 g/l, KCl 0.720 g/L, $CaCl_2 \cdot 6H_2O$ 0.330 g/L, $KH_2PO_4$ 0.680 g/L, $Na_2HPO_4 \cdot 12H2O$ 0.856 g/L, KSCN 0.060 g/L, $KHCO_3$ 1.500 g/L;

Buffer solution: (NaCl, 8.74 g/L; $NaHCO_3$, 0.35 g/L; $Na_2HPO_4 \cdot 12H_2O$, 0.06 g/L; $NaH_2PO_4$, 0.06 g/L; pH = 6.5.

The measurements were done at 25°C, the usual temperature of the environment.

## RESULTS AND DISCUSSIONS

Titanium and CoCr implants show an outstanding corrosion resistance due to the spontaneous formation of a thin protective oxide film (the $TiO_2$, and respectively $Cr_2O_3$, CoO, passive film).

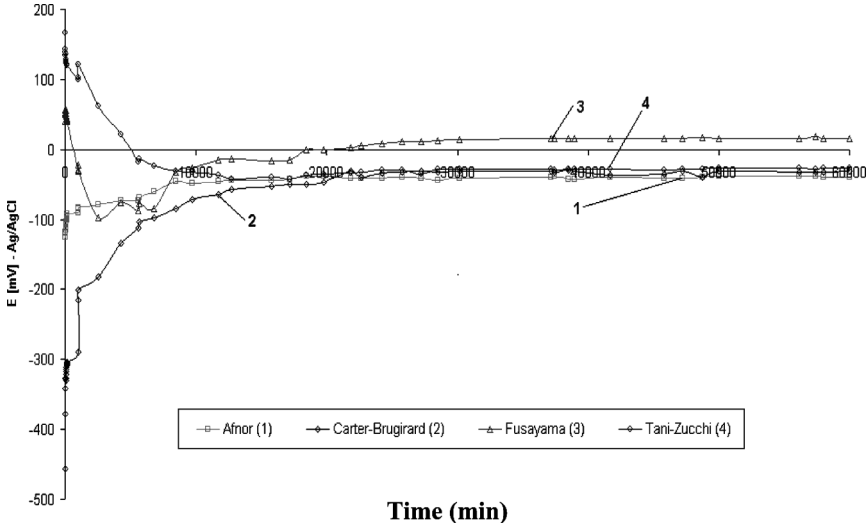


**FIGURE 1** Variation of Ti potential in open circuit in various saliva.

Long-term stability of our implant materials was studied from the monitoring of the open circuit potentials in time, simultaneously with pH determinations. As a result of pH changes, potential gradients can appear. Infections can cause variations in the pH from 4 to 9 and local acidification can arise from the corrosion product reaction. Taking into account that in the oral cavity the pH changes are expected frequently this subject was treated in another paper [6].

Variations of open circuit potentials of Ti in saliva (Fig. 1) reveal that these potentials are active at the beginning and denote slow dissolution and re-passivation processes on the surface of Ti and its alloys. The tendency for a constant level, denoting passive, protective films is the characteristic of Ti behavior in various environment for the longer exposure hours [11]. According Figure 1, a scale of stability (SC) in the stationary range is:

*SC Fusayama* > *SC Tani-Zucchi* > *SC Carter-Brugirard* > *SC Afnor*

The dependence of potential in time in saliva for CoCr alloy has the same trend having a tendency to reach a constant level [6].

The data from potentiodynamic polarization curves of Ti and CoCr in artificial saliva before bioactivation are shown in Table 2.

The corrosion parameters, including corrosion potential ($E_{cor}$,) density current ($I_{cor}$) passivity current ($I_{pas}$) obtained from the potentiodynamic polarization test are listed and the corrosion rate as penetration index $I_p$ [mm/year] and gravimetric index $K_g$ were

**TABLE 2** Electrochemical Parameters for Ti and CoCr Alloy in Various Saliva

| Electrode | Saliva | Passive domain [mV] | $I_{pas}$ [μA/cm] | $I_{cor}$ [μA/cm$^2$] | $E_{cor}$/SC [mV] | $I_p.10^3$ [mm/year] |
|---|---|---|---|---|---|---|
| Ti | Afnor | 2430 | 20.08 | 2.96 | −475.9 | 51,5 |
| | Carter-Brugirard | 2497 | 17.62 | 2.14 | −511,4 | 37,2 |
| | Fusayama | 2949 | 22.76 | 1.99 | −426,4 | 34,6 |
| | Tani-Zucchi | 2408 | 25.02 | 0.94 | −450,5 | 16,4 |
| CoCr | Duffo-Quezada | 1079 | 24.05 | 1.3 | −160 | 18.4 |



computed, taking into account the relation between them, as following $I_p = K_g \times 9760/1000$x density. In this equation 9760 is the number of hours in a year. For CoCr alloys the biomaterial density was computed as a function of each component percentage.

Our infrared spectral data are arguments that the almost immediate event that occurs upon implantation at the interface Ti, $TiO_2$/bioelectrolyte is adsorption of various molecules and ions. Such data support adsorption models existing in literature [12–14]. The composition modification of passive film after immersion in a saliva without calcium or with calcium (Fusayama saliva) indicates in both cases characteristic phosphate band (570 $cm^{-1}$ and 1077 $cm^{-1}$ for bonded phosphate), and in the same time a decrease of 3400 $cm^{-1}$ absorption band related to the OH of hydrogen bonding. The corresponding reaction with complex formation is supposed to take place in both cases according first of all to $TiO_2$ hydrolisis and formation of hydroxyl rich surface: $TiO_2 + 2H_2O \rightarrow TiO(OH)_2$

$$TiO(OH)_2 + 2H2PO_4^- \rightarrow TiO(H_2PO_4)_2 + 2OH$$

$$TiO(OH)_2 + HPO_4^{2-} \rightarrow TiO(HPO_4) + 2OH$$

In the Fusayama environment the process is more complex calcium being involved according to the reaction: $TiO\ (H_2PO_4)_2 + Ca^{+2} + 2OH^- \rightarrow CaTiO\ (H_2PO_4)_2(OH)_2$. Calcium consumption was put in evidence with atomic adsorption in a previous work [15].

ESEM analysis is presented in Figure 2 and a slight corrosion attack can been seen at inter granule level in all saliva, but more evident in Afnor saliva. Such data support electrochemical measurement in open circuit and cyclic polarization which indicated this environment as the most aggressive studied saliva.

The EDAX results presented in Table 3 are arguments for adsorption process, the ions from saliva being present in the new surface composition.

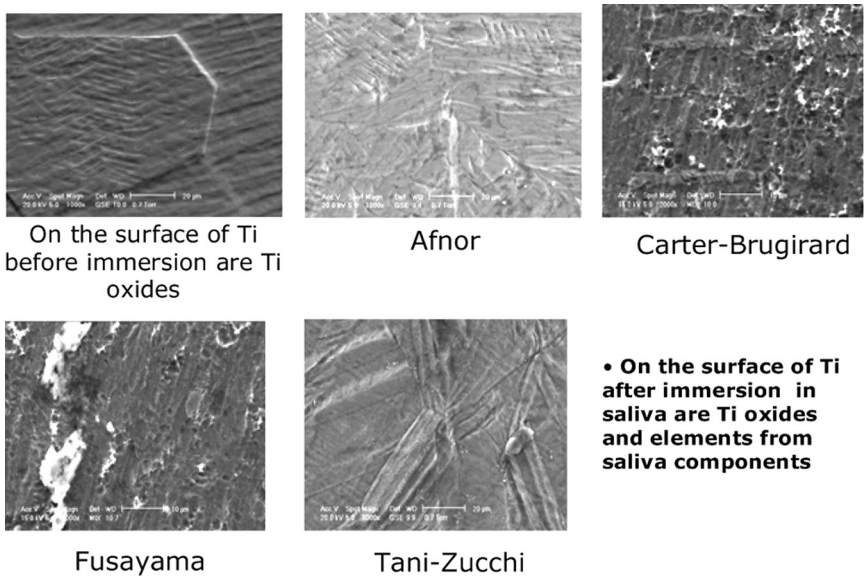


**FIGURE 2 ESEM** analysis on Ti surface before and after 5 month immersion in saliva Afnor, Carter-Brugirard, Fusayama and Tani-Zucchi.

**TABLE 3** Surface Composition of Ti Before and After Immersion in Saliva

| Element | Ti–Before immersion [% weight] | Ti–immersed 5 month in saliva Afnor [% weight] | Ti–immersed 5 month in saliva Carter-Brugirard [% weight] | Ti immersed 5 month in saliva Fusayama [% weight] | Ti immerssed 5 month in saliva Tani-Zucchi [% weight] |
|---|---|---|---|---|---|
| Ti | 100,00 | 95,79 | 93,61 | 95,56 | 90,70 |
| O | 0,00 | 1,30 | 2,97 | 3,46 | 5,15 |
| Ca | 0,00 | 0,00 | 0,00 | 0,31 | 0,00 |
| Na | 0,00 | 0,26 | 0,24 | 0,23 | 0,65 |
| Cl | 0,00 | 0,26 | 0,13 | 0,38 | 0,36 |
| P | 0,00 | 0,25 | 0,15 | 0,06 | 0,22 |
| C | 0,00 | 2,14 | 2,90 | 0,00 | 2,92 |
| $TiO_2$ | 0,00 | 95,14 | 93,85 | 99,18 | 92,66 |
| CaO | 0,00 | 0,00 | 0,00 | 0,28 | 0,00 |
| $Na_2O$ | 0,00 | 0,19 | 0,18 | 0,17 | 0,48 |
| $Cl_2O$ | 0,00 | 0,19 | 0,09 | 0,29 | 0,26 |
| $P_2O_5$ | 0,00 | 0,33 | 0,21 | 0,08 | 0,28 |
| $CO_2$ | 0,00 | 4,16 | 5,68 | 0,00 | 6,32 |

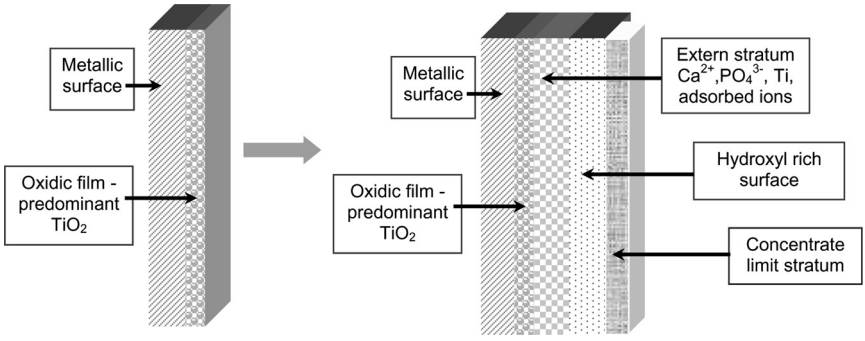


**FIGURE 3** Modification of passive film composition due to adsorption.

In Figure 3 an empirical model for passive and modified film at interface was proposed based on literature data and the above results.

It is a double layer model with the presentation of biointerface adsorption after immersion in saliva environment, where release ions as titanium are together with ions from saliva.

As an example of bioactive coating on titanium and CoCr alloy the behavior of collagen film and phosphate coatings were studied. The effect in increasing corrosion resistance after bioactivation is presented in Table 4 and 5 respectively.

We can see in Table 4 that the corrosion rate after collagen treatment is less than in the titanium covered with a passive film, but the effect on biocompatibility is an unexpected one.

In Table 5 are presented the electrochemical parameters for CoCr alloy treated with collagen at 2 different pH. The collagen treatment increases the corrosion rate. Regarding the bioactivation with phosphate, the quality of phosphate coating on CoCr alloy and conditions of deposition were described previously [7]. The data from polarization studies show that both CoCr alloys, the naturally passivated and the bioactivated one, present good stability in artificial saliva. The corrosion rate for passive sample is $5.9 \cdot 10^{-3}\,g/m^2 \cdot h$ and corrosion

**TABLE 4** Increasing Corrosion Resistance Using Collagen Coating on Ti Surface Immersed in Buffer Solution and Cell Viability for Passive and Bioactive Surface

| Sample | $E_{cor}$ (mV) | $I_{pas}$ ($\mu A/cm^2$) | $I_{cor}$ (A) | $I_p$ (mm/year) | Viability (%) | Spreading (%) |
|---|---|---|---|---|---|---|
| Ti | −131 | 26 | $4.81 \cdot 10^{-6}$ | $4.17 \cdot 10^{-4}$ | 74.3 | 8.5 |
| Ti-col | 617 | 25 | $3.17 \cdot 10^{-6}$ | $2.75 \cdot 10^{-4}$ | 70.0 | 11.7 |

**TABLE 5** Electrochemical Parameters of CoCr Alloy Untreated (UN) and Treated with Collagen Gel (TC) in Artificial Saliva Duffo-Quezada

| | $E_{cor}$ (mV/SCE) | | $I_{cor}$ ($\mu A/cm^2$) | | $I_p$ (mm/year) | | Passive domain (mV) | |
|---|---|---|---|---|---|---|---|---|
| pH | UN | TC | UN | TC | UN | TC | UN | TC |
| 7.4 | −110 | −370 | 0.6 | 0.98 | 0.0061 | 0.0099 | 1210 | 1340 |
| 2.2 | −160 | −630 | 1.3 | 7.33 | 0.0131 | 0.0744 | 1079 | 1288 |



rate for bioactivated surface is a round a half of it being $2.5 \cdot 10^{-3}$ $g/m^2 \cdot h$. All data correspond to stable range.

The data from Table 4 and 5 clearly indicate a better stability after bioactivation process, despite some other results obtained with hydroxiapatite coating [5].

The fact that stability of films means less ions release in the environment and could be related to biocompatibility [16], to the fibroblasts culture and to cell viability, on surface of titanium and CoCr alloy.

Figure 4 As can be seen in Figure 4 fibroblast morphology did not vary significantly during the culture period and the cells showed a normal behaviour, and the same phenotype for anodized (A) and bioactivated with phosphate samples (B).

According to our results presented in Table 4 for titanium the viability for bioactive sample is a bit less, despite that collagen, being bone component, has a very good biocompatibility [17]. The spreading coefficient which represent the ratio between area occupied with cell and total area is higher and that can be an argument for a better

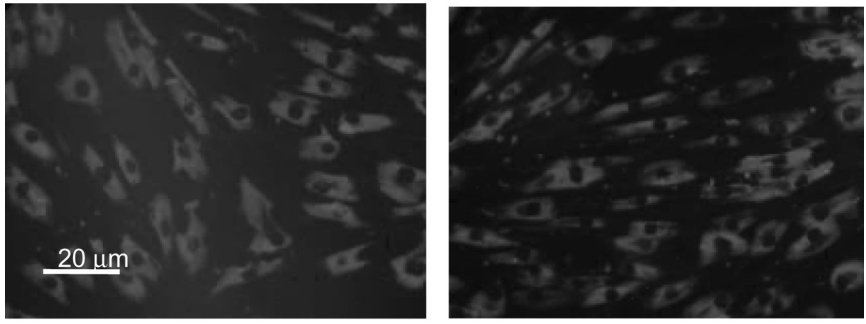


**FIGURE 4** Cell growth on passive (A) and on phosphate bioactive coating on CoCr.

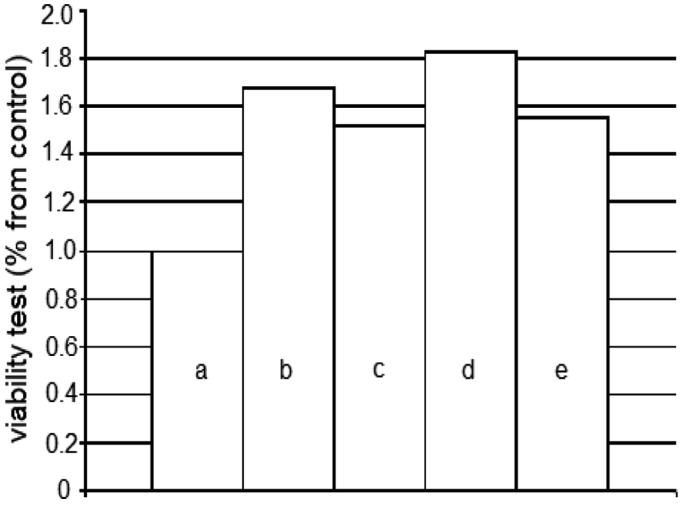


**FIGURE 5** (a) Cell viability on passive and bioactive films on CoCr alloy a control; (b) passive spontaneous; (c) bioactive phosphate (3,5 V; 60 min, 25°C); (d) bioactive phosphate (3,5 V; 20 min, 60°C); (e) electrochemical passivation.

integration in the case of bioactive sample. For CoCr the cell viability is presented in Figure 5 as a dependence of surface film preparation.

The stability of passive film obtained in an anodic process is higher than the stability of a natural oxide film, but the biocompatibility [18] in terms of cells viability is a function of more parameters. As can be seen in Figure 5 the phosphate coating obtained at higher temperature has a higher cell viability.

## CONCLUSIONS

1. Electrochemical, surface analysis, dissolution and in vitro tests were used to estimate the stability of the films in artificial saliva for different passive and bioactive films, and its behavior in the presence of fibroblast cells. The results prove a good electrochemical stability of the chosen samples, combined with positive results regarding its biocompatibility.
2. The morphology and the composition of films before and after immersion in illiquid determined with an Environmental Scanning Electron Microscope FEI/Phillips XL30 ESEM confirm electrochemical data and adsorption phenomenon at biointerface. The ions adsorption at interface was put in evidence also from infrared spectra.

3. An empirical model for passive and active film at interface was proposed based on literature data and paper results.

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