Innovative immobilization matrices

GS Alvarez[1,2], MI Alvarez Echazú[2], JA Bertinatto[2], PN Catalano[2], GJ Copello[1,2], ML Foglia[1,2], JA Gonzalez[2], SA Giorgieri[2], SL Iglesias[2], AM Mebert[1,2], PL Santo Orihuela[2], MV Tuttolomondo[1,2], ME Villanueva[1,2], MF Desimone[1,2]*

[1]IQUIMEFA-CONICET.

[2]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Química Analítica Instrumental. (1113) Junin 956 Piso 3. Ciudad de Buenos Aires. Argentina. Tel/Fax: +54-11-49648254.

*Corresponding author: desimone@ffyb.uba.ar

Running title: **Survey in memory of Professor Luis E. Díaz**

Abstract

We present a brief survey of some of the recent work of Professor Luis E. Díaz, performed together with his students and collaborators at the University of Buenos Aires. Dr Luis E. Díaz has been involved in research on biochemical and pharmaceutical sciences solving scientific and industry problems for over 40 years until he passed away. Prof. Díaz scientific interests included various topics from NMR spectroscopy to biomedicine but fundamentally he focused in various aspects of chemistry (analytical, organic, inorganic and environmental). This is not a complete survey but a sampling of prominent projects related to sol-gel chemistry with a focus on some of his recent publications.

Keywords: antimicrobial surface; biomaterials ; biosorbents; immobilization; nanomaterials; sol-gel.

1. Introduction

The sol-gel process has several well-known advantages such as the numerous possibilities offered by the choice within a vast assortment of high purity precursors which leads to an increased homogeneity of the obtained materials and specially to the possibility to tune the mechanical and chemical properties in order to obtain new hybrid and composite materials [1-3]. As a result, sol gel chemistry has attracted the interest from researchers across different disciplines [4]. Indeed, through multidisciplinary research interesting advances concerning pharmaceutical and biotechnological applications have been made, improving the quantity and quality of several commercialized products [5-7]. Additionally, these materials could be potentially employed in the development of new eco-friendly processes compared to conventional chemical ones [8-9]. It is noteworthy to mention that there is also an increased interest in the development of biohybrid and biomimetic materials exhibiting improved structural and functional properties, which may prove valuable for applications involving disciplines such as engineering, biotechnology, medicine, pharmacy, agriculture and nanotechnology among others [10]. Nowadays, the sol-gel process provides a robust and versatile technology for the immobilization of biological materials. Indeed, a wide range of inorganic, composites and hybrid materials can be prepared to encapsulate drugs, proteins, nucleic acids, polymers, prokaryotic and eukaryotic cells into bulk gels, particles and films [11]. Different immobilization alternatives have been studied by Dr. Díaz group. These were specially focused on the sol-gel immobilization approach mainly through the use of tetraethylorthosilicate (TEOS) or sodium silicate as silica gel precursors. Gel stability, cell viability and performance were also studied in depth. The reason sol-gel silica matrices were chosen

was that they offer a number of advantages including improved mechanical strength, chemical stability, porosity control and negligible swelling [12].

This review highlights, as mentioned above, the more recent successful applications of sol-gel materials within the biotechnological and pharmaceutical fields obtained by Dr. Díaz´s group. The diversity of materials that can be achieved depending on the desired function is presented with emphasis in the broad variety of applications of sol-gel chemistry.

2. Immobilization of biologicals

Due to the high cost of protein and enzyme purification, there is great interest in developing immobilization techniques for biomolecules and cells. Several researchers devoted their research to these techniques, which include the use of polyacrilamide gels [13], agar [14] and cellulose triacetate polymers [15] among many others [16-17]. There were approaches where adsorbed microorganisms to a solid surface were then immobilized by crosslinking with different agents or even direct linkages of these microorganisms with zirconium hydroxide [18]. The applications of these immobilized cells are growing steadily and across several fields such as biosensing, affinity chromatography and bioreactors.

Sol–gel immobilization can occur by simple dispersion of the cells or enzymes during the gelling of the sol under mild conditions or through alternative routes such as the Biosil method where an aqueous TEOS solution is sprayed over living cells [19]. Over the past years Dr. Díaz research group has been working in the immobilization of cells for different purposes mainly within silica matrices . As a first attempt, *Saccharomyces cerevisiae* was entrapped in alkoxide derived matrices and it was found that their tolerance to ethanol increased, showing that the specific death velocity decreased from

2×10^5 c.f.u. min^{-1} for free cells to 2×10^4 c.f.u. min^{-1} for immobilized cells probably due to an enhanced stability of the hydration layer around cells within the pores of the silica network [20]. Additionally, immobilized *S. cerevisiae* was protected from the effects of five organic solvents obtaining the highest protection against the most hydrophobic solvent [21]. In a second instance, the best conditions for bacteria immobilization were studied. In this case, it was observed that the use of citric acid in the polymerization of TEOS instead of hydrochloric acid improved bacteria viability and long-term preservation. When gels were stored at 4°C, *E. coli* viability was approximately two orders of magnitude higher in citric acid gels than in those gels where hydrochloric acid was used. When gels were stored at 20°C, the difference between acids was lower but still significantly different. Furthermore, no differences were observed in bacteria viability for silica matrices obtained either from sodium silicate or SiO_2 aqueous precursors [22]. In an additional study, the effect of the addition of different molecules (trehalose, glycerol and mannitol) during the preservation of immobilized bacteria was investigated. It was observed that exogenous trehaloses as well as glycerol and mannitol induced bacterial death after a 550-day storage period, while bacteria entrapped in gels with no additives remained viable. In contrast to these results, osmotic-induced bacteria, which accumulated internal trehalose in high levels before their entrapment, were viable after 550 days [23].

The improved immobilization technique was also applied to the development of practical solutions in many and diverse areas such as bioremediation, agriculture, or in the production of recombinant proteins [24-25]. Bioremediation examples include the reduction of high levels of chromate from aqueous environments and contaminated soils by *Burkholderia sp*. immobilized in inorganic silicate matrices obtained through the sol–gel process. It was possible to recycle the silicate pearls with immobilized bacteria

for consecutive reduction processes without losing their activity as well as gaining an extra advantage derived from the characteristics of silica matrices which favor the adsorption of Cr(III) to their surfaces after bacteria reduction, thus helping to diminish the total concentration of chromium ions in the media (**figure 1**) [26]. Another example of remediation developed by Dr Díaz group is the sol–gel immobilization of *Pseudomonas sp*. able to enzymatically reduce azo groups which were used for the decoloration of water containing azo dyes observing that compared to free bacteria the immobilized ones produced an amount of extracellular enzymes involved in the biodegradation of azo dyes more than seven times higher. Same as in the case mentioned before, the reusability of the immobilized bacteria was successfully evaluated therefore presenting a cost-effective and efficient treatment to remove dyes from effluents [27].

In the agriculture field the potential of silica gels as an alternative support for the long term storage of rhizobia at room temperature was successfully evaluated for its possible application as a novel inoculant formulation. The study was mainly focused on the immobilization of *Mesorhizobium spp*. cells, which could keep their ability to develop nitrogen fixing nodules in narrow leaf birdsfoot trefoil (*Lotus tenuis*) roots after being immobilized in silica matrices, which also offer the entrapped microorganisms a better protection against adverse environmental stresses such as acid soils [28].

Finally, the biotechnological applications of immobilized cells were explored by Dr. Díaz group studying the functionality and quantitative level of recombinant protein produced from bacteria immobilized and preserved in sol–gel matrices [29]. In this context, matrices were prepared from two precursors, silicon dioxide and TEOS for the inclusion of *Escherichia coli* BL21 transformants containing different DNA coding for a T-cell receptor beta chain, which are expressed as inclusion bodies in the cytoplasm

and the other two encoding bacterial superantigens *Staphylococcal Enterotoxin* G and *Streptococcal Superantigen*, which are expressed as soluble proteins in the periplasm. It was concluded that bacteria immobilized in SiO_2-derived matrices remain genetically stable, preserve their antibiotic resistance and recombinant protein production capability when stored at 4 and 20 °C for two months. However, when alkoxide derived matrices were employed; no viable cells were detected at either 4 or 20°C after 42 days due to the deleterious effect of remaining ethanol after precursor hydrolysis [30].

The immobilization of enzymes in silica matrices is also a promising area [31] and was explored by Dr. Díaz´s group as well. In this sense, the stability of urease was examined after immobilization in sol–gel silica matrices and in two types of nanocomposites obtained by the addition of either trehalose or glycerol. These samples were aged at 20ºC under different relative humidity (RH) for 16 days and the influence of formulation composition and ambient relative humidity on the preservation of the enzyme activity was evaluated. It was seen that immobilized urease was more stable when the formulations were equilibrated at RH 80% and that the highest urease activity was retained in the presence of glycerol probably due to changes in pore volumes, surface area, and state transitions of water and solid components in the presence of polyols [32].

Moreover, monodispersed magnetic silica nanoparticles obtained using a water-in-oil reverse microemulsion system were functionalized and employed to covalently bind urease. The immobilized enzyme presented long-term stability and the enzyme-linked particles were stable over repeated uses and storage retaining more than 75 % activity after four months with the advantage of easy recovery due to their magnetic properties [33].

2. Silicon containing biomaterials

Among the various definitions for biomaterials available, the most accepted one is the one employed by the American National Institute of Health that describes a biomaterial as ‘‘any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual’’[34]. Therefore, for their design the concept of biocompatibility is closely linked to the material’s functionality and the elicited host innate and acquired immune response of the organism in contact with it [35]. Alternatively, drug delivery systems associated with biomaterials have been developed over the recent decades to stimulate wound healing without any side effects [36-37]. Indeed, Protein, gene, and small molecule therapies hold great potential for facilitating comprehensive tissue repair and regeneration [38].

Silicon is one of the most common elements in the earth crust often found in combination with oxygen in the form of silicates. Soluble silicates are frequently taken up by living organisms, some of which produce siliceous structures as internal or external skeletons, scales or secondary walls [39]. Among these, diatoms, a major component of phytoplankton, are one of the largest groups of silicifying organisms, and most species have an obligate requirement for silicon (Si) for cell wall formation [40]. However, in more complex organisms, silicon is found in trace amounts, though it performs important functions in the development of connective tissue, such as bone and cartilage [41].

The matrices obtained by Dr. Díaz´s group through the sol-gel process possess some interesting characteristics such as optical transparency, chemical inertness, outstanding mechanical properties and customizable surface moieties. Enzymes and cells usually

perform their functions in crowded environments and a more rigid silica containing matrix would be able to provide adequate support as well as protection from harsh external conditions.

The use of water-soluble alkoxide incorporating non cytotoxic alcohols was more recently explored in an attempt to boost the production of monoclonal antibodies secreted by hybridoma cells. Sol-gel silica matrices synthesized from tetraethoxysilane (TEOS) and tetrakis(2-hydroxyethyl)orthosilicate (THEOS) were tested for their abilities to maintain cell viability and enable antibody diffusion from the hybridoma cells to the surrounding media. THEOS matrices showed a higher performance when compared to TEOS, a phenomenon attributed to the alcohol released during the alkoxide hydrolysis. Alternatively, THEOS as a precursor presents some interesting characteristics as it can be directly dissolved in water at pH 7 in the presence of the cells, forming a gel within a few minutes. Overall, it was shown that hybridoma cells immobilized in THEOS matrices were able to produce monoclonal antibodies to the same extent as free cells, thus introducing the possibility to use them in the design of bioreactors for large-scale production [42].

Similarly, rat mature ovarian follicles were encapsulated in sol–gel silica matrices obtained through hydrolysis and condensation of THEOS and TEOS for the evaluation of in vitro steroid hormones production. Although cell viability and proliferation were not favored when cultured in these matrices, estradiol secretion was maintained for up to 9 days in culture. Additionally, significant differences were observed between TEOS and THEOS. When the latter was used, follicle viability was conserved to a higher extent when compared to silica matrices derived from TEOS. Furthermore, an efficient interaction between theca and granulosa cells within the sol–gel silica matrix was

demonstrated. This is novel, as no previous reports have described such a collaborative cellular interaction between two different cell types encapsulated in silica matrices [43].

Even though silica pure matrices have been used successfully for the immobilization of non-adherent cells, the majority of cells derived from solid tissues grow as adherent monolayers, thus requiring a proper surface to attach and spread before proliferation can occur. Indeed, apoptosis may be triggered when adhesion to the presented surface is impaired, resulting in cell death. The use of hydrogels and additives in the design may render the hosting biomaterial suitable for the adhesion of the selected cells [44]. In particular, the use of collagen, a structural protein commonly present in the extracellular matrix of connective tissues, provides them a natural anchoring moiety for attachment and survival. Bearing this in mind, silica–collagen scaffolds were obtained by covalent binding of an aminosilane to glutaraldehyde fixed collagen hydrogels, resulting in a three dimensional network of silicon coated collagen fibrils. The obtained matrices were able to support cell attachment and growth for over three weeks, presenting additionally a 60 fold increase in the rheological properties and an improved enzymatic digestion profile (**figure 2**) [45]. Alternatively, dye-modified hydrogels with enhanced thermal and mechanical stability may also find applications in the biomedical field [46].

Additionally, the sol-gel process can be easily manipulated in order to obtain non bulk materials such as fibers and particles. Among these, silica nanoparticles have recently attracted interest for their biodegradability and potential use as vehicles, offering a number of advantages over organic polymers, given silica´s natural stability and reactivity, for which they could be easily dispersed in aqueous media and be equally suitable for a variety of water insoluble therapeutic agents [47]. When the material is intended to be in contact with a living organism, biocompatibility should be taken into consideration. Nanoparticulated systems have raised concern regarding the possibility

of cell toxicity, as cell entry may be facilitated through different pathways [48]. However, it is possible to tune the nanoparticles characteristics in order to meet the requirements of each specific cell and desired application. Therefore, through the optimization of size, morphology and concentration, among others, nanoparticles could be successfully introduced for the delivery of chemicals and biologicals, such as antibiotics and growth factors [49-50]. As an example, the antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* of silica-collagen nanocomposites bearing 100, 300 and 500 nm plain silica nanoparticles (SiNPs) loaded with gentamicin or rifamycin was evaluated. Results showed that the loading capacity was affected by particle size and drug type. Gentamicin incorporation was favored over rifamycin´s, leading to higher concentrations which rose with the increase in particle size. As gentamicin loaded SiNPs were incorporated into collagen hydrogels, their fibrillar structure was conserved and at the same time an increase in the proteolytic stability as well as an unparalleled prolonged antibacterial activity was observed. The composites thus obtained demonstrated no cytotoxicity towards fibroblastic cells *in vitro* and the additional sustained antibacterial activity could prove beneficial for the aid of persistent dermal chronic infections (figure 3) [51].

Sol-gel chemistry covers different techniques which lead to the synthesis of materials with differential characteristics. The biomaterials reviewed so far introduce the use of different forms of silica for the generation of scaffolds designed to be in contact with living organisms, from pure inorganic silica matrices to organic-inorganic composites.

3. Silicon oxide immobilized biosorbents

In the search for the effective application and technology transfer of his projects, Dr. Díaz spotted a growing field that combines two areas that were vaguely studied together

at that time. His experience in the immobilization of biomolecules and microorganisms in silicon oxide matrices led him to propose several systems for the immobilization of biosorbents. The basic concept of this idea was to make use of low-cost biosorbents that have proved their efficiency on their own original form and immobilize them in a supporting matrix by means of the chemical and mechanical stability provided by the silicon oxide network. Thus supported, the biosorbents can be applied in heterogeneous phase systems, working in batch or continuous flow. The main objective of this project was to develop low-cost alternatives for the remediation and/or concentration of pollutants or high cost biomolecules. With this aim two groups of biosorbents were studied, the first involved metal chelating polyphenols, and the second studied two polysaccharides consisting on chitin and chitosan.

In the past decades there has been an increased interest in finding biosorption properties from untreated waste from industries or domestic residues. In literature it can be found a vast quantity of works dealing with these sorbents, such as discarded coffee beans, sugar beet pulp, grainless stalk of corn, etc. Also, there are some studies using different types of teas (green, black, red). In these plants, it has been pointed that polyphenols, such as tannins, are responsible for their metal chelating properties. Yerba mate (*Ilex paraguariensis*) is the most popular tea-like beverage of South America. As a consequence, a huge amount of residues of exhausted yerba mate are generated every year. In order to evaluate if they contain polyphenols that could be extracted and supported in a silicon oxide matrix, the capability of exhausted yerba mate leaves in the removal of metals was evaluated in a study [52]. The yerba mate leaves showed adsorption capacity for Pb(II), Cr(III) and Cr(VI) mainly at pH below 5. As a continuation of this work, the polyphenols from the milling residual dust of yerba mate leaves were extracted and then immobilized within a SiO_2 matrix [53]. In order to avoid

polyphenol leakage from the matrix they were crosslinked with glutaraldehyde. By means of FT-IR spectroscopy it was confirmed that the crosslinking occurred among carbonyl groups of glutaraldehyde and phenols from the A-ring of the polyphenols, while the chelating capacity was maintained by the ortho-hydroxyl groups in the B-ring. This hybrid material proved to have Pb(II), Cr(III) and Cr(VI) adsorption capacity. The pH dependence of the adsorption was similar to the observed for the yerba mate leaves, making this hybrid an ideal sorbent for acid media. Also, Electron Spin Resonance (ESR) spectroscopy confirmed that Cr(VI) adsorption occurred via an adsorption-coupled reaction, favored in acid media, and the adsorbed specie was Cr(V). Finally, the hybrid matrix proved its adsorption capacity of Cr(III) in a non-treated tannery wastewater.

A completely different approach arises from the success of the polysaccharide chitosan in the adsorption of different pollutants, such as heavy metals and organic dyes, and also different biomolecules. The literature studying chitosan's adsorption capacity is extensive and also arise the interest in the developing of applicable materials for heterogeneous phase processes. On the other hand, chitin also presents an interesting field since it is chitosan's precursor and, therefore, has a lower cost. Chitin drawback is evident in its difficult manipulation which has hindered the number of studies related to this polysaccharide. Chitin consists predominantly of unbranched chains of β-(1→4)-2-acetoamido-2-deoxy-D- glucose. It can be extracted from shrimp, crab shell, fungi and other crustaceans [54]. Chitosan (β-(1→4)- 2-amino-2-deoxy-D-glucose) is a hydrophilic and cationic polymer product of chitin deacetylation. The presence of amino groups in chitosan increases its adsorption capacity compared to that of chitin. Thus, the choosing of chitosan over chitin as a sorbent, or vice-versa, implies a compromise between costs and sorption capacities.

In a study that advocates for high sorption capacities, chitosan was immobilized in a surface by the generation of layer-by-layer silicate–chitosan composite biosorbent [55]. The relevance of using silicate as the immobilizing agent relies in the capability of the monomers of forming an independent network from chitosan chains. Silicate monomers will condensate in a silicon oxide matrix without interacting with chitosan's metal adsorption sites generating an interpenetrated polymer that do not diminish the polysaccharide original sorption capacity. The films were evaluated on its stability regarding the polymer leakage in different pH media and how this media influences its capability of Cd(II), Cr(III) and Cr(VI) removal from an aqueous solution. Silicate–chitosan films with a final layer of silicate demonstrated to retain chitosan and had better sorption capacities than those without it. For metal species, such as Cd(II) and Cr(III), the greatest adsorption was obtained when the pH of the solution was 7. When Cr(VI) was evaluated, pH 4 was the optimal for its adsorption. An acid incubation allowed a recovery of 80% of the adsorbed metal. This non-covalent immobilization allowed chitosan to maintain an equal sorption capacity to the free chitosan powder.

Since chitin and chitosan contain a similar chemical structure and both polysaccharides are highly and lowly acetylated respectively, both can eventually adsorb the same type of sorbates. Of course, each one presents a major tendency towards the sorption of a particular type of sorbate than the other. With the purpose of comparing the capability of the two polysaccharides in the sorption of pollutant dyes they were immobilized in the form of hydrogels that were interpenetrated by a silicon oxide matrix. The advantage of immobilizing the polysaccharides in the hydrogel form relies in the swelled state of the polymeric chains that allows the maximum exposure of the sorption sites. Thus, chitosan hydrogel/SiO_2 and chitin hydrogel/SiO_2 hybrid mesoporous materials were tested in their adsorption capabilities against four dyes (Remazol Black B, Erythrosine

B, Neutral Red and Gentian Violet) in order to evaluate chitin as a plausible replacement for chitosan considering its efficiency and lower cost. In this work it was concluded that chitosan containing hybrids were more efficient in removing highly charged dyes [56]. Nevertheless, when these dyes were exposed to the sorbent in a slightly charged stated or they were non-polar, the chitin containing matrix have similar or higher adsorption capacity than the chitosan one. Therefore, the chitin hydrogel/SiO_2 hybrid was presented as a potential replacement for the chitosan containing one.

In a different approach the chitin hydrogel/SiO_2 hybrid was evaluated for its use as a downstream processing chromatographic matrix for lysozyme recovery from egg white [57]. Most lysozyme purification processes take advantage of the high isoelectric point of the protein by using ion exchange chromatography. Other processes make use of the affinity interaction between lysozyme and N-acetyl-D-glucosamine monomers of chitin or its affinity for dyes. However, in almost all of these processes the dilution of the egg white is required in order to obtain high adsorption capacity, thus preventing its further utilization as food additive. The chitin hydrogel/SiO_2 hybrid make use of the affinity of lysozyme for chitin and can recover the enzyme even from undiluted egg white. An 87% of the lysozyme was removed from the egg white and the matrix was easily recovered by a simple filtration through a strainer. The overall yield of the process was 64% with a purification factor of 20.

The experience from the above mentioned works showed that the silicon oxide matrices, although very useful in supporting the polysaccharides, endow the materials with unspecific adsorption when the SiO_2 is in high proportion [58]. This experience encouraged the development of chitosan/SiO_2 hybrids in which the inorganic moieties were in lower proportion than the organic ones. The decrease in SiO_2 content was carried down to 1 mmol TEOS per gram of chitosan. In order to evaluate the

performance of the SiO_2 matrix as a crosslinker for chitosan beads, the hybrids were compared with the two most widely studied chitosan crosslinkers at the same crosslinker/chitosan ratio: glutaraldehyde and epichlorohydrin. The performance of the anionic dye Remazol Black (RB) and the cationic Cd(II) adsorptions was assessed in order to characterize the sorbate–sorbent interaction. For both sorbates the TEOS cross-linked beads showed the higher maximum adsorption capacity, followed by epichlorohydrin and glutaraldehyde cross-linked beads, confirming the innocuousness of the SiO_2 for chitosan's adsorption sites.

4. Antimicrobial surfaces via sol-gel

In a surface most bacteria live in a biofilm state to enhance their survival and propagation. Biofilms are microbial communities of surface-attached cells embedded in a self-produced extracellular polymeric matrix which are adherent to an inert or living surface [59].

The process of bacteria attachment to an available surface and the subsequent development of a biofilm are dictated by a number of variables, including the bacteria species, the surface composition, and environmental factors [60-63]. Bacterial adhesion can be divided into two stages: the primary adhesion stage and the secondary or locking phase [59]. The initial microbial adhesion plays a key role in microbial biofouling on abiotic surfaces, which can be explained in terms of microbial and substrate surfaces properties. Primary adhesion constitutes the first meeting between a conditioned surface and a plantonic microorganism. This stage is reversible and it is generally mediated by nonspecific interactions (i.e., hydrophobic, electrostatic, and Van der Waals forces, steric hindrance, temperature, and hydrodynamic forces) [59, 62-64].

The second stage of adhesion is the locking phase and employs molecularly mediated

binding between specific adhesins and the surface. At this point, loosely bound organisms consolidate the adhesion process by producing exopolysaccharides. At the conclusion of the second stage, adhesion becomes irreversible in the absence of physical or chemical intervention, and the organism is attached firmly to the surface. Once bacteria have irreversibly attached to a surface, the process of biofilm maturation begins. The overall density and complexity of the biofilm increase as surface-bound organisms begin to actively replicate and extracellular components generated by attached bacteria interact with organic and inorganic molecules in the immediate environment to create the glycocalyx [64-66].

Biofilm formation can cause significant problems in many areas, such as in medical and industrial environments [66-69]. According to the previously described process of biofilm formation, possible antibiofilm strategies are based on physicochemical modification of the material surface (anti- adhesive surface) or the use of biocides agents either bound to the surface or to be released to the surroundings. Sol-gel technology has been widely used in these developments [70-74].

Since primary adhesion is influenced by the surface´s physicochemical characteristics, many developments varying surface free energy (hydrophobicity/ hydrophilicity), electrostatic charge or roughness in order to minimize bacteria – surface affinity can be found in literature [75-78]. Surface charge, surface free energy and surface wettability are related and often have joint influence on bacterial adhesion. Besides, they all depend on the surface chemical composition whose effect on bacterial adhesion is typically studied through surface wettability, free energy and charge of the material.

Bacterial retention can be controlled by means of electrostatic interactions. When a negatively charged bacteria approaches a negatively charged surface, no attraction takes place due to electrostatic repulsion. Thus, anti-adhesive surfaces can be obtained, for

example, by modifying the surface point of zero charge (PZC). There is a pH at which the number of positively charged (basic) groups equals the number of negatively charged (acidic) ones in the absence of other adsorbed ions; this is called the PZC of the oxide. For a typical hydroxylated surface, the charge of the oxide changes from positive at pH < PZC to negative at higher values of pH

Recently, sol-gel coated aluminum plates were prepared to analyze bacterial retention on these surfaces, especially considering electrostatic interactions. [79]. In order to achieve these objectives, aluminum plates were functionalized with sulfonic, amine and hydroxyl groups with different silane reagents (3-mercaptopropyltrimetoxysilane with oxidation of thiol groups to obtain sulfonic funcionalized surfaces; 3-aminopropyltrimethoxysilane to obtain amine derivatized surfaces and tetraethoxylsilane for hidroxy derivatized surfaces). PZC of *Pseudonomonas aeruginosa* cell surfaces and coated aluminum materials were evaluated with the obtention of different pH values. Bacterial surface PZC was 4.5 and coated surfaces were divided in two groups: those with high PZC, amine funcionalized (PZC = 7.1) and nude aluminum (PZC = 6.8); and those with low PZC, sulfonic funcionalized (PZC = 4.7) and hidroxy funcionalized (PZC= 5.0). The coated plates were characterized by Scanning Electron Microscopy (SEM) and images did not show major topographical variations between the coatings. In order to assess the coating homogeneity, Energy Dispersive X-Ray Spectroscopy (EDX) elemental analysis and mapping were performed. All the coated plates revealed the presence of Al, C, Si and O, coming from aluminum alloy and the silicon oxide coating. For EDX mapping, Al and Si were chosen as representative elements. The distributions of the elements indicated that the coatings' elemental composition was homogeneous. Infrared Spectroscopy (FT-IR) was also performed. The observed bands confirmed that the coatings were performed successfully. The

corrosion potential (E_{corr}) and the corrosion density current (I_{corr}) were also evaluated by potentiodynamic polarization curves and determined by Tafel polarization technique. These electrochemical assays revealed a decrease of the I_{corr} and an increase in the E_{corr}, showing that the presence of the coatings improved the corrosion resistance of the plates. The coating acted as a barrier to prevent electrolyte from reaching the metal surface. *P. aeruginosa* bacterial retention at two different interaction times (24 and 48 h) on these surfaces has also been analyzed. Low PZC surfaces showed low bacterial retention due to electrostatic repulsion. In high PZC surfaces, bacterial retention raised along with pH, due to the image charge generated in these metallic surfaces. According to these results, when bacterial attachment was undesired the use of low PZC surfaces showed to be the best choice as they presented low attachment efficiency in bacterial retention in the range of pH 4–8. Therefore, the PZC of the coatings should need to be similar to the bacterial membrane PZC in order to minimize electrostatic interaction.

Sol-gel chemistry has been also used to give antimicrobial properties to the materials by including antimicrobial drugs. Indeed, the antimicrobial compound dodecyl-di(aminoethyl)-glycine was immobilized in a silicon oxide xerogel matrix and used for glass surface coating [80-81]. These coated glasses were tested for surface antimicrobial activity. The utilization of TEOS as a silicon oxide polymer precursor, using the dip-coating process, allowed for the generation of transparent thin films over glass surfaces. Different concentrations of the antimicrobial compound were used to generate the coatings. The presence of dodecyl-di(aminoethyl)-glycine on coated and uncoated slides was analyzed by FTIR spectra. The antimicrobial efficacy test was performed by exposing coated slides to suspensions of common food-borne pathogens: *Escherichia coli*, *Staphyloccocus aureus*, *E. coli* O157:H7, *Salmonella typhi*, *Salmonella cholerasuiss*, *Listeria innocua* and *Listeria monocytogenes*. The coating activity was not

only bacteriostatic but also bactericidal. The percent reduction of viable microorganism exposure over 24 h to the coated surface ranged between 99.5%, for the more resistant gram-positive bacteria, and over 99.999%, for most gram-negative bacteria. The silicon matrix itself did not account for any reduction of viable microbial, even more an increase was observed.

In order to get antimicrobial surfaces, nanomaterials can be used as antimicrobial drugs carriers. Recently, a process for antimicrobial finishing of cotton textiles with laundry durability was reported [82]. The antimicrobial treatment was performed by treating a cotton textile with chitin nanowhiskers (CNW) loaded with the well-known preservative methylparaben. CNW were fixed in a silicon oxide matrix in order to achieve a methylparaben controlled release considering hydrophobic characteristics for both CNW and the preservative. SEM analysis and tensile strength tests showed that the coating did not introduce major changes in morphologic characteristics and in mechanical properties. The antimicrobial activity was assessed against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Acinetobacter baumanii* and *Salmonella choleraesuis.* Treated textile has shown antimicrobial activity with laundering durability up to 20 washing cycles. Methylparaben leaching from the textile has been assessed by liquid chromatography showing a Methylparaben controlled release which could be responsible for the obtained antimicrobial laundry durability (**figure 4**).

5. Conclusions / Final comments.

As final comments, we can say that Professor Dr. Luis E. Díaz has made outstanding contributions to the scientific world in the areas of research summarized in this manuscript. As mentioned above, the topics of immobilization of biologicals,

biomaterials containing silicon, biosorbents and antimicrobial surfaces obtained by sol-gel chemistry represent the highlighted topics developed at the end of his scientific career. As a mentor of human resources and director of this group, Dr. Díaz has always been of exceptional quality in the human and scientific aspects and he provided everyone the opportunity to learn and develop scientific knowledge with absolute freedom and unconditional guideness. Indeed, he was a very generous man. We will greatly miss all these aspects and specially the person behind the scientist.

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Legend to figures

Figure 1: A) Cr(III) (●) and Cr(VI) (▲) adsorption isotherms for silicate matrices without immobilized bacteria. Langmuir (dashed lines) and Freunlich (complete lines) plots are presented ([26]-Reproduced by permission of The Royal Society of Chemistry). B) The Cr(VI) reduction capacity as a function of the initial Cr(VI) concentration for immobilized Burkholderia sp. ([26]-Reproduced by permission of The Royal Society of Chemistry).

Figure 2: Overview of the stepwise reaction involved in the obtention of silica-coated collagen hydrogels. ([45]-Reproduced by permission of The Royal Society of Chemistry).

Figure 3: Quantification of the gentamicin release from nanocomposites with various silica contents: (A) cumulative released dose and (B) cumulative released percentage of initial loading calculated for each nanocomposite composition. The dashed line on (B) shows gentamicin release from collagen hydrogels. Results are expressed as mean $\pm$ SD from triplicate experiments. ([51]-Reproduced by permission of The Royal Society of Chemistry).

Figure 4: Scheme of the synthesis of an antimicrobial textile finishing with the SEM images of untreated and treated textiles (FMWT). ([82]- Reproduced by permission of The Royal Society of Chemistry).

Figure 1

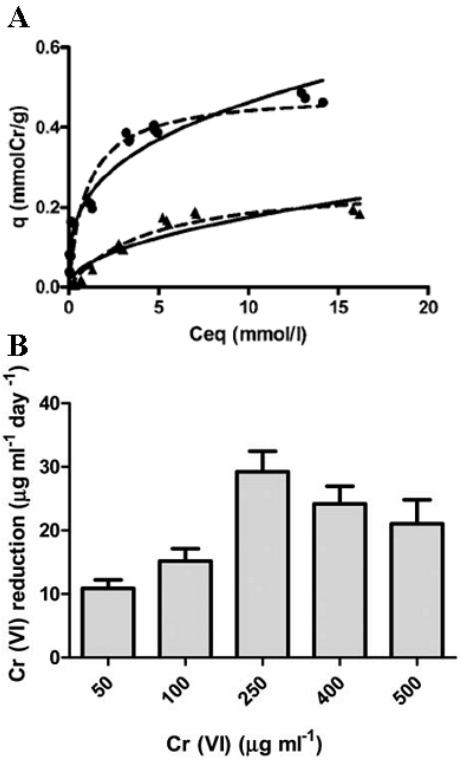

A
0.6
0.4
0.2
0.0
q (mmolCr/g)
0
5
10
15
20
Ceq (mmol/l)
B
40
30
20
10
0
Cr (VI) reduction (µg ml-1 day -1)
50
100
250
400
500
Cr (VI) (µg ml-1)

Figure 2

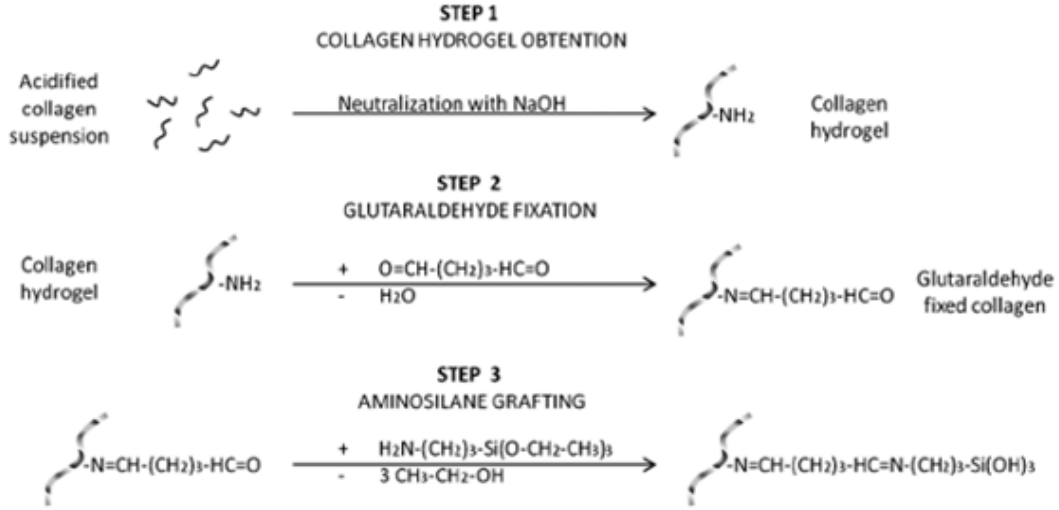

STEP 1
COLLAGEN HYDROGEL OBTENTION
Acidified collagen suspension
Neutralization with NaOH
-NH2
Collagen hydrogel
STEP 2
GLUTARALDEHYDE FIXATION
Collagen hydrogel
-NH2
+ O=CH-(CH2)3-HC=O
- H2O
-N=CH-(CH2)3-HC=O
Glutaraldehyde fixed collagen
STEP 3
AMINOSILANE GRAFTING
-N=CH-(CH2)3-HC=O
+ H2N-(CH2)3-Si(O-CH2-CH3)3
- 3 CH3-CH2-OH
-N=CH-(CH2)3-HC=N-(CH2)3-Si(OH)3

Figure 3

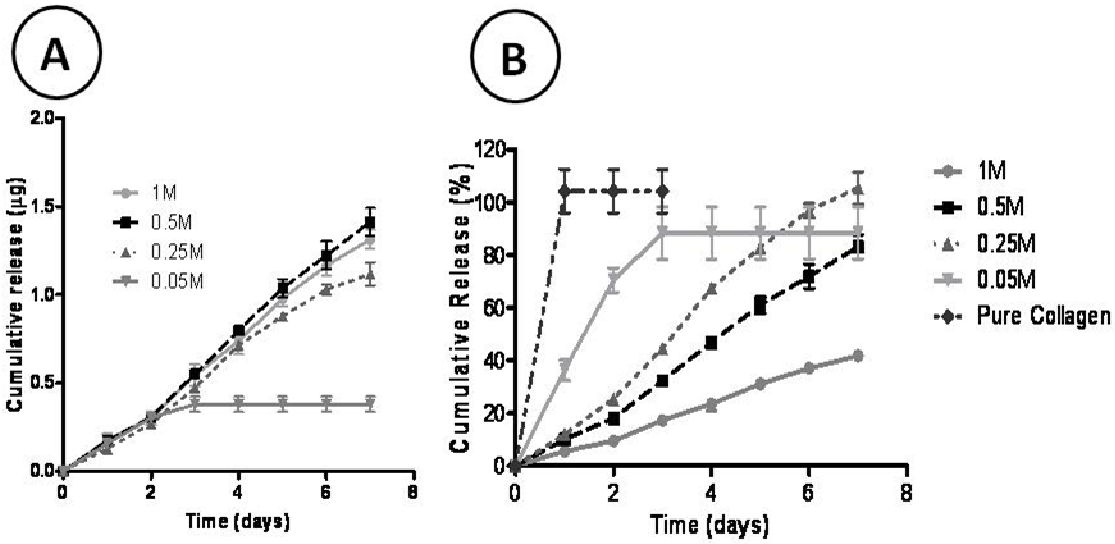
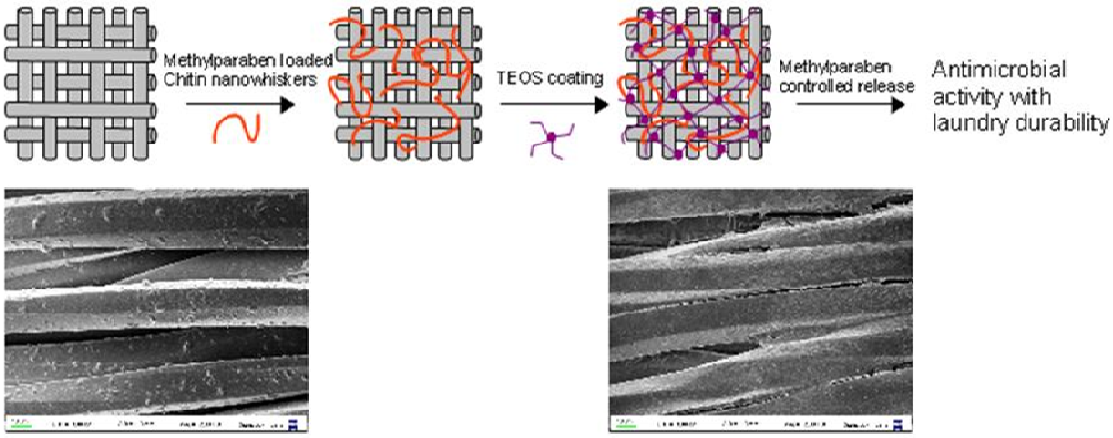

A
B
Cumulative release (µg)
Time (days)
1M
0.5M
0.25M
0.05M
Cumulative Release (%)
Pure Collagen

Figure 4


Methylparaben loaded
Chitin nanowhiskers
TEOS coating
Methylparaben
controlled release
Antimicrobial
activity with
laundry durability