Título: DEVELOPING PHYSICAL PROPERTIES OF HIERARCHICAL NANOSTRUCTURES FOR BIOMEDICAL APPLICATIONS

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Resumen: Understanding the physicochemical basis and the different models of nanosystems is, currently, of an essential importance on a significant number of scientific areas and industrial processes. In the present dissertation, specific focus has been put on nanosystems created by self-assembly. The formation, organization and behaviour of single units at these scales are a challenging topic in light of the inherently small dimensions involved, the susceptivity and vulnerability to minimal fluctuations, and the intrinsic problems that appear when trying to scale up these structures for extensive use and general implementation.

Theoretical concepts and the development of computational models to predict different properties of self-aggregation processes of mixed molecular systems, are remarkably convenient and useful for a better knowledge of this topic and for reaching more effective approaches. For that end, in the first part of this report (Chapter 3), a combination of computational and experimental studies was performed to analyse protein aggregation, binding and interactions. Bovine serum albumin (BSA) and fibrinogen (Fib), two widespread used proteins because of their wide range of physiological and biological functions, were chosen as the biomolecule

models. Their behaviour in presence of surfactants and drugs of different nature was assessed by means of molecular dynamic simulations (MD) and experimental procedures. Small angle X-ray scattering (SAXS) was used to validate those models and isothermal titration calorimetry (ITC), zeta potential, UV-vis, fluorescence, synchrotron radiation circular dichroism (SRCD), differential scanning calorimetry (DSC) and Raman spectroscopy were applied to further corroborate the proposed theoretical mechanisms, the interactions involved and the conformational changes suggested. The combined results might be of a key importance to pave the way to better understand the early steps of numerous neurodegenerative diseases and shall open new avenues towards the application of complex supra-molecular information in rational drugs-design with biomedical applications.

While bionanotechnology led to an increasing interest in the study of the interactions of nanostructures with biological entities, in parallel, important investigations in the field of biomedical research have demonstrated the usefulness of nanoparticles as the centerpiece of drug/gene delivery, visualization, or tissue engineering. Bioceramics are considered suitable materials for bone repair because of their high osteoconductive capacity. Among them, hydroxyapatite (HA) is the most common choice to develop synthetic substitutes due to its chemical composition that is similar to the mineral phase in the bone. It is thus a mineral or bioceramic that can be produced by the living beings. In vivo, hydroxyapatite nucleation and mineral growth occur within the extracellular matrix of cells and has the general formula Ca10(OH)2(PO4)6.

Particularly, nanoparticles have many potential applications within the biomedical device industry. However, these applications demand a precise control of their sizes, shapes and morphology which play a main role in most properties. In this regard, a new route for the synthesis of hydroxyapatite nanoparticles using a microfluidic device was successfully established (Chapter 4). The process is carried out by continuous laminar flow through the device. The obtained nanoparticles have shown same properties of composition, length, orientation and roughness than those produced by conventional methods. Besides, this type of microfluidic systems allows to fine tune the structure via simple engineering and produce nanoparticles of different size by only varying the flow velocities and ratios.

The excellent features that these bioceramic nanoparticles exhibit, enable them to function as ideal material for hard tissue engineering. On the other hand, it should not be overlooked the fact that their biological effect depends on the ability to reach the target organs or cells inside the body. Achieving these goals involves routes of administration such as intravenous, where the nanoparticles interact with blood components, namely plasma proteins, thus determining their hemocompatibility. Hence, the evaluation of hemocompatibility requires several approaches, including particularly, the study of these interactions. One of the concepts that best defines this type of relationships is the ¿protein corona¿, that is, the protein layer absorbed on the nanoparticle. To characterize it, the biological interaction of bovine serum albumin and hydroxyapatite nanoparticles was presented by using a battery of techniques: ITC, zeta potential, UV-vis, fluorescence and CD (Chapter 5). Experimental data was analysed to determine important parameters such as rates, affinities, and stoichiometries of protein associated with the nanoparticles. Besides, the important role the protein flexibility plays on the mentioned biomedical purposes has been also evaluated.

Having defined and characterized the self-aggregation processes of mixed molecular systems, and having introduced the importance of the protein corona, the further step is to design and create complex hybrid

materials with enhanced biological attributes. The following Chapters of the current doctoral thesis describe the synthesis, physicochemical and biological characterization of diverse materials with the potential use in guided bone regeneration (GBR) and implants or fixation devices as well.

Synthesized structures are presented from the more subtle and delicate samples: bilayer membranes (Chapter 6); to the more intricate and dense ones: bioinspired composite hydrogels and mixed hydrogel microparticles (Chapters 9 and 10, respectively). Likewise, ultrathin films (Chapter 7), and biomimetic scaffolds (Chapter 8) were also studied in terms of their bioactivity, i. e. mineralization capacities; and their cellular response, proving their biocompatibility and osteoconductive qualities. Special focus has been put on the viscoelastic properties, mechanical stiffness and strength. While allowing the natural growth of bone tissue, designed samples must also withstand properly the mechanical efforts, and therefore, a complete rheological analysis of different hydrogels has been carried out (Chapter 9). The attributes inferred from the data showed the notable impact the addition of bioceramic nanorods to the soft matrix has in the final mechanical performance alongside the predicted enhancement of the biological functions.

Equally important, it should also be highlighted the application of innovative experimental approaches to substitute traditional ones, by the utilization of microfluidics systems for purposes that, generally, were only addressed with bulk processes. In addition to the aforementioned HA synthesis, such techniques have been used to automate the creation of complex hybrid materials as well. For its part, computational flow simulations were probed to be an exceptional tool to predict comportment of the liquid phases, to model the droplet formation and to study the dependence on the physical properties and the rates of the respective flows. This subject, due to its relevance and topicality, remains the centre of study of present researches and future plans.

Conclusively, the importance and novelty of this proposal lies in the study and optimization of self-aggregate systems that promote the triggering of sequences leading to the deposition, nucleation and growth of hydroxyapatite crystals with the proper geometry and orientation. New strategies and novel synthetic routes were effectively developed, resulting in economically viable materials, that mimic the structure and composition of the extracellular matrix, making them eligible and suitable for numerous applications in tissue regeneration and biomedical engineering.