Doctoral Dissertation
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Mesoporous bioactive glasses as smart platform to stimulate bone regeneration

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Summary

In the field of bone regeneration, considerable attention has been addressed towards the use of mesoporous bioactive glasses (MBGs), as multifunctional therapeutic platforms for advanced medical devices. Their extremely high exposed surface area and pore volume allow to store and release functional molecules (such as anti-inflammatory, antimicrobial agents, growth factors) and, additionally, their composition can be enriched through the incorporation of specific elements (*i.e.* Sr, Cu) with the aim to combine in a single biomaterial several therapeutic abilities, such as pro-osteogenic, pro-angiogenic and antibacterial properties.

The goal of this PhD research work was to design and develop a multifunctional platform based on Mesoporous Bioactive Glasses, to be used as therapeutic delivery system for biomedical application, in particular to treat compromised tissue healing and stimulate bone regeneration. To this purpose, two synthesis procedures were selected to produce the MBGs with a binary composition based on SiO_2 and CaO (ratio 85/15) enriched with different therapeutic ions, the base-catalysed sol-gel procedure and the aerosol assisted spray-drying method. These two different approaches allowed to produce nano-sized particles and micro-sized particles, respectively, with different peculiar features. Different Active Pharmaceutical Ingredients (APIs) were identified and selected to exert a specific therapeutic effect. Specifically, the amount and the type of therapeutic ions added during the synthesis were selected by following the idea to exert peculiar outcomes, in particular copper was added to provide an antibacterial and pro-angiogenic effect, while strontium ions were selected thanks to their well-known pro-osteogenic properties. In a second step, the therapeutic potential of the MBGs has been further enriched by loading ibuprofen and N-acetylcysteine, in order to develop a drug delivery system, able to co-release both the ions and the drugs at the pathological site. Ibuprofen was employed in combination with Cu-containing MBGs, aiming to design a multifunctional platform with anti-microbic/pro-angiogenic effects associated to the copper and anti-inflammatory potential exerted by ibuprofen to be used *in situ* for delayed bone healing applications. On the other hands, N-Acetylcysteine (NAC) was loaded into the Sr-containing MBGs to be used as osteogenesis-enhancing platform, able to deliver into the bony defect both strontium ions and NAC, thus improving bone regeneration and promote bone defect healing. Two different

loading procedures have been investigated, the adsorption and the incipient wetness methods, to identify the most suitable loading procedure in terms of yields and required time.

Furthermore, different surface modification approaches were investigated, with the aim to provide the final device with anti-adhesive properties and to obtain a prolonged and sustained release of the cargo. In details, the *zwitterionization* procedure has been selected to impart anti-adhesive properties to the MBGs, allowing to repel the protein adhesion and thus to prevent the biofilm formation. *Zwitterionic* surfaces are characterized by an equal number of both positive and negative charges in order to preserve the overall electrical neutrality and the related anti-adhesive properties are imparted by a strongly bonded water molecule layer which acts as a barrier against the adsorption of both proteins and bacteria. The resulting multifunctional device is supposed to target simultaneously all the causes, often mutually interlocked, of the pathologies related to compromised bone tissue healing. On the other hands, the Layer by Layer deposition has been chosen as promising strategy to modulate the cargo release rate. Two different routes were investigated: in the first strategy, chitosan, alginate and chitosan were assembled by forming the multi-layered surface and, successively, ibuprofen was loaded by incipient wetness impregnation. In the second one, alginate was replaced by the ibuprofen, by exploiting the ibuprofen negative charge (COO-), able to electrostatically interact with the chitosan positive charge.

Finally, to make MBGs suitable as long-term therapeutics delivery systems for bone healing, a hybrid formulation based on the conjugation of MBGs with an injectable thermosensitive hydrogel, acting as a vehicle phase, has been investigated in order to develop a multifunctional platform to be injected directly at the pathological site for the sustained delivery of therapeutic agent.