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Sommario	In recent years, considerable attention has been given to chitosan (CS)-based biomaterials and their applications in the field of soft tissue engineering (TE). CS is a glycosaminoglycan derived from chitin, the primary structural polymer in crustacean exoskeletons. CS is biocompatible, biodegradable, easily formed into various structures (i.e. sponges, nanofibers and films) under mild processing conditions and can be chemically modified through graft copolymerization and crosslinking. However, the rapid degradation of CS and its low mechanical strength are concerns that may limit its use in clinical applications. In the first part of the thesis, different non cytotoxic crosslinkers were used aiming at improving the structural properties of CS. Genipin (GP), ?-glycidoxypropyltrimethoxysilane (GPTMS), dibasic sodium phosphate (DSP) were selected as biocompatible CS crosslinkers as reported in literature. After a preliminary physico-chemical and mechanical characterization, the proper crosslinking compounds were selected for the development of different typologies of CS scaffolds for both human and veterinary applications. CS- based scaffolds were developed as nerve guidance channels (NGCs) and internal fillers fabrication to promote peripheral nerve regeneration in humans. Two CS based hollow NGCs were prepared and tested in vitro and in vivo (coded as CS flat membrane and bi-layer CS membrane) and a CS based nanostructured internal filler was optimized and characterized in vitro. i. CS flat membranes

were prepared by solvent casting. According to the results obtained in the first part of the thesis, DSP alone (CS/DSP) or in association with the GPTMS (CS/GPTMS_DSP) were used as crosslinkers. CS crosslinked membranes showed permeation to nutrients and did not exert any cytotoxic effect on RT4-D6P2T. The higher mechanical stability of CS/GPTMS_DSP under wet state allowed to confirm the RT4-D6P2T attachment and proliferation as well as the neurite outgrowth of dorsal root ganglia (DRG) on CS substrates. Before in vivo implantation in rats, CS/GPTMS_DSP and CS/DSP membranes were easily rolled up to form a NGC. Then, membranes were used to bridge median nerve defects in rats. After 12 week post-operative CS/GPTMS_DSP tubes were found to be detached from the distal suturing site and functional recovery did not occurred. On the other hand, crushed nerve encircled with CS/DSP membranes, allowed nerve fibre regeneration and functional recovery, showing similar results to autografts. ii. Bi-layer CS membranes were developed using a two-step coating technique. CS/DSP and CS/GPTMS_DSP flat membranes were combined to produce scaffold structures with good biocompatibility in the inner layer (CS/DSP) and with the desired mechanical strength imparted by the outer (CS/GPTMS_DSP, GPTMS 25% wt./wt.). Gradual water uptake and permeation to small molecules was observed compared to single layers. From in vivo tests, median nerves treated with bi-layer tubes displayed regenerated and aligned fibres at the injury site. iii. CS crosslinked electrospun nanofibres were fabricated by electrospinning solutions containing CS, polyethylene oxide (PEO), and dimethylsulphoxide (DMSO). PEO and DMSO were introduced to allow the spinnability of CS solutions at high polymer concentration with controllable fiber size and increase fiber yields by relaxing CS chain entanglement. Optimization of the process and solution parameters allowed to obtain CS nanofibres with size of 128±17 nm. To increase CS stability in aqueous media, DSP was used as crosslinker After DSP crosslinking fibre size decreased to 109±17 nm while an increase in the mechanical strength (E, from 63±10 MPa to 113±8 MPa) was observed compared to uncrosslinked nanofibrous matrices. In the third part of the thesis, CS porous membranes with improved antimicrobial properties were prepared for veterinary application. The developed scaffolds were fabricated by freeze-drying to promote the wound healing process and to reduce the bacterial proliferation in chelonian shell injury site. Different ratios of silver nanoparticles (AgNPs, 5%, 10% and 15% wt. /wt.) and gentamicin sulphate (GS, 3.5 mg/ml) were loaded into the CS/GPTMS_DSP membranes to impart the proper antibacterial properties and to favor drug release avoiding the risk of systemic toxicity. After a preliminary in vitro characterization, CS/GPTMS_DSP loaded with AgNPs at a concentration of 10% wt./wt (CS/GPTMS_DSP_AgNP10) was selected as ideal candidate for this application field. GS release profile from CS/GPTMS_DSP_GS evidenced high burst release of the antibiotics in the first day (about 70%). Finally, GS and AgNPs (10 % wt./wt.) effect on bacterial inhibition was evaluated and confirmed against Gram+ and Gram-. The results reported in this thesis work demonstrate that CS is a promising candidate for applications in human and veterinary soft TE. Mechanical and physico-chemical properties of CS scaffolds can be tuned by using different crosslinking methods. By the in vitro characterization, GPTMS and DSP were selected as ideal compounds to the development of scaffolds for peripheral nerve regeneration (in human) and wound healing (in animals). Four different morphologies (3 for peripheral nerve regeneration and 1 for wound healing

	application) were obtained by varying the fabrication methods and the final composition. All membranes were found to satisfy the requirements for the application of interest. CS based membranes developed for peripheral nerve regeneration were found to be biocompatible, and successful functional recovery was observed in case of CS/DSP and bi-layer membranes. Porous membranes with improved antimicrobial properties were prepared to enhance wound healing in chelonians and were found to be effective against a broad spectrum of bacteria following the release of two different investigated antimicrobial agents (AgNPs and GS)
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