Final Publishable Summary Report for Project No. 236739

Bone repair and regeneration is considered an important area of research concerning human health. This project targets at fabrication of novel biomimetic hydroxyapatite (HAP)-fibrous clay-biopolymer hybrid membranes by layer-by-layer assembly (LBL) for potential uses in this field. To achieve the project goal, we have carried out a series of experiments and obtained the following main results.

First, we chose natural fibrous nanoclay, namely sepiolite, as the template for synthesis of hydroxyapatite (HAP) nanocrystals using a chemical co-precipitation method, due to its porous structure and negative surface charge. The dimensions and morphology of HAP nanocrystals grown on the fibre surfaces can be tailored by adjusting the surface chemistry of sepiolite and synthesis conditions such as the pH value of the solution, temperature and aging time. It was found that carbonated HAP nanorods were successfully grown on the sepiolite surface with a preferred orientation to the *c*-axis. Strong acid-activation increased the specific surface area of the sepiolite by 205% and also transformed the sepiolite to silica fibers with an elastic modulus being 395% of the original value. The novel HAP/acid-activated sepiolite biocomposite has a specific surface area of 182 m^2/g and an elastic modulus of over 20 GPa, considerably higher than those of the HAP synthesized without sepiolite. Such hierarchically assembled HAP/sepiolite biocomposites with the controlled size, improved modulus and similar biological functions to HAP are promising in tissue engineering and biological load-bearing devices. The research results are published in ***Nanoscale*** **(2011, 3, 693-700**) (Objective 1).

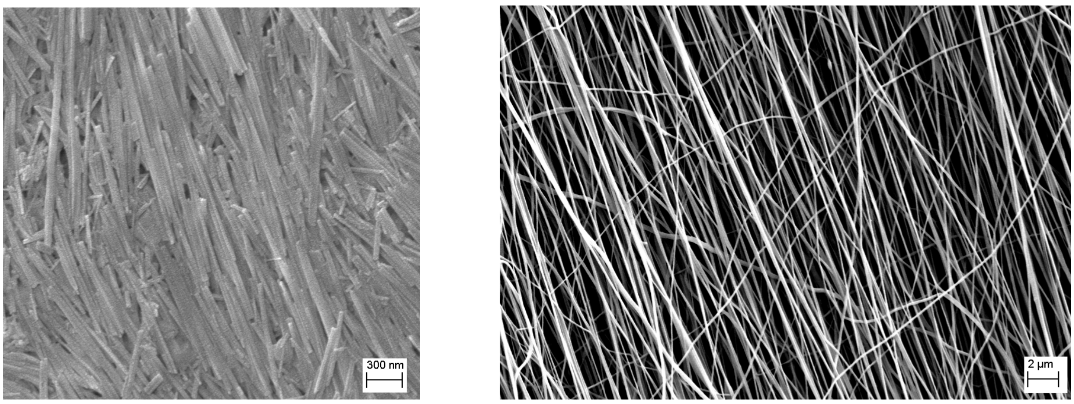

Figure 1. Scanning electron microscopy images of well aligned nanofibres: (a) fibrous sepiolite/ chitosan hybrid prepared by layer-by-layer assembly; and (b) PCL nanofibres fabricated by electrospinning.

Next, we fabricated sepiolite-chitosan hybrid membranes by a layer-by-layer assembly method. Due to the negative charged surface of sepiolite and positive charged properties of chitosan, the sepiolite nanofibers and chitosan macromolecular chains acting as polyelectrolytes were alternatively adsorbed onto a glass substrate layer by layer during a dip-coating process. As shown in Figure 1a, the sepiolite nanofibers were aligned to one direction and a novel hybrid membrane was formed on the glass slide. But, such membrane was too thin and fragile to be peeled off for property assessment, which limits its further application as tissue scaffolds. As a feasible and versatile technique, electrospinning can produce two or three dimensional nanofibrous scaffolds, demonstrating its high potential in tissue engineering. To solve the problem associated with LBL, we set up an electrospinning facility to fabricate polymer or nanofiller-reinforced polymer nanofibers, and obtained nanofibrous membranes with randomly dispersed or unidirectionally oriented fibres (Figure 1b), depending on the design and type of collectors, the electrospinning conditions and the materials in use (Objective 2).

In order to improve the mechanical properties of the polymer nanofibers, we have compared the reinforcement effects of sepiolite and graphene oxide (GO) on a biopolymer, i.e. gelatin. Under the same processing and testing conditions, sepiolite exhibited lower reinforcing efficiency than GO due to its lower modulus (ca. 10 GPa versus ~217 GPa), aspect ratio (ca.20 versus ~1000) and specific surface area (148 m^2/g versus ~460 m^2/g) as well as less oxygen-containing functional groups on the surface. For example, the Young's modulus of gelatin was improved by 10% with the addition of 1 wt% sepiolite, while it was increased by 65% in the presence of the same amount of GO. The research results of the reinforcement effects of fibrous sepiolite on cellular and non-cellular gelatin, and of GO on gelatin films have been published in ***J Mater Chem*** **(2011, 21, 9103-9111)** and ***Soft Matter*** (**2011, 7, 6159-6166**), respectively (Objectives 2 and 3).

To further elucidate the reinforcing effects of GO on polymers, a series of polymer/GO nanocomposites, including bio-nanocomposites, with different chemical structures were prepared and their reinforcement mechanisms were investigated by applying classical composite theories and considering the interphase regions. The research results have been accepted for publication in ***J Mater Chem*** (**DOI: 10.1039/C2JM15062J**). A research paper on GO-reinforced biodegradable poly(butylene succinate) has been submitted for journal publication. These research outcomes can be used to guide the design and application of polymer/GO nanocomposites in biomedical engineering and beyond (Objectives 2 and 3).

Besides the above advantages of GO over sepiolite, GO has also been proven to be nontoxic in biomedical applications such as drug delivery and biosensing by other researchers. Our work showed that GO promoted the formation of calcium phosphates on both biopolymer (gelatin) films and electrospun nanofibrous biopolymer (poly(ε-caprolactone), PCL) membranes by inducing more and finer HAP nanocrystals, and that the nanocomposite films or fibrous membranes exhibited good bioactivity. Therefore, GO acted as both an effective reinforcement filler and biological activator in biopolymers such as gelatin and PCL, offering biopolymer/GO nanocomposites great potential to be further developed as bone scaffolds. The bioactivity of sepiolite/gelatin was also assessed and the results showed sepiolite nucleated the formation of HAP crystals to a less extent compared to GO. The work on PCL/GO biocomposties and nanofibrous membranes has been published in ***Biomed Mater*** (**2011, 6, 055010 (8pp)**) (Objective 3).

In conclusion, we have prepared a range of biopolymer/fibrous clay/HAP and biopolymer/GO/HAP hybrids, assessed their potential application in bone repair and regeneration, and performed fundamental investigation for understanding of these promising materials. Our research results not only present a number of promising biopolymer nanocomposites which are of technical interests in biomedical fields, but offer a good understanding of this relatively new class of materials. They will have positive impact on the research into Biomimetics, Nanotechnology, Hybrid Materials, Biomaterials and Bone Tissue Engineering, and on the development of novel medical devices and materials in the relevant industry sectors.