Gelatin/Clay Nanotubes Hybrid Nanocomposites for Cell Culture

Qi Xing, Yuri M. Lvov
Institute for Micromanufacturing, Louisiana Tech University, Ruston, Louisiana-71272

Introduction: Polymer nanocomposites containing nano-sized materials such as hydroxyapatite, carbon nanotubes attracted a great deal of attention in the application of biomedical research areas, due to their unique structure and properties. Recent studies showed that halloysite clay nanotubes are one of the promising materials to fabricate novel polymer nanocomposites. Halloysite is an alumino silicate clay which demonstrates a predominantly cylindrical geometry, the diameter of the hollow core varies from 16 to 50 nm and length 200nm to 1 micron. Compared with carbon nanotubes, halloysite is naturally available, much cheaper and non-toxic. Furthermore, some scientific studies have shown halloysite offers great potential for encasing and sustaining the release of bioactive molecules, which is very attractive for preparation of biomaterials and cell constructs for tissue scaffolds and drug evaluation. In this work, a novel biomimetic nanocomposite of gelatin- halloysite nanotubes was reported for the first time. The preparation of 2-dimensional (2D) and 3-dimensional (3D) gelatin-halloysite nanocomposites was presented. The morphology and mechanical properties of the nanocomposites were investigated.

Methods: Halloysite Clay was purchased from Atlas mining Corporation, Utah. Gelatin powder and other chemicals were bought from Sigma-Aldrich or Invitrogen and used without further purification. The 3D nanocomposite was prepared by solid-liquid phase separation and subsequent sublimation of the solvent. Briefly, the required amount of halloysite was mixed with deionized water and sonicated for 15 min. The resulting halloysite suspension was heated at 50° C. Gelatin powder was added to the halloysite suspension to make 2% (w/v) gelatin solution. A controlled amount of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were added to crosslink the gelatin. The final concentration of EDC and NHS was 5 mM at a molar ratio of 1:1. The mixture was put in ice bath to initiate gelation. After being kept in 4° C refrigerator overnight, the resulting gel was at -20° C to freeze. Then the frozen samples were lyophilized in a freeze-dryer for at least 24 h. For 2D nanocomposites, after gelation process the samples were put in air at room temperature to dry. Samples were cut into thin slices, and Scanning Electron Microscope was used to characterize the morphology of the scaffolds.

Results: From the TEM and SEM images (Figure 1)of halloysite, the cylindrical nature and empty lumen is clearly evident. For an ideal scaffold, high porosity is required to allow oxygen and nutrients diffusion into the matrix. The SEM images (Figure 2) showed the 3D gelatin/halloysite nanocomposites had porous and inter-connective structure as pure gelatin composites. The observation indicated that the halloysite nanotubes were integrated into the gelatin films. The pore size of 3D gelatin/hallosite nanocomposites ranges from 50 to 150 μm. Mechanical strength test showed that gelatin/ halloysite nanocomposite had comparable stiffness as pure gelatin composite, see Figure 3.

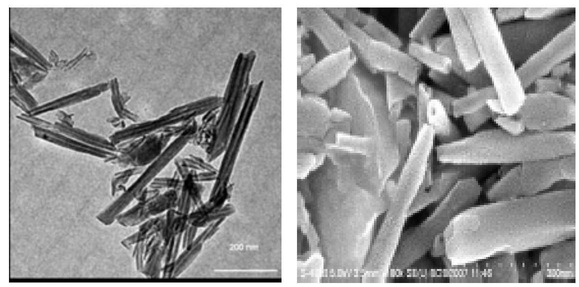

Figure 1. TEM and SEM images of halloysites.

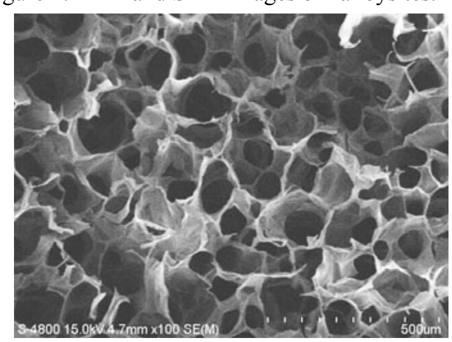

Figure 2. SEM images of 3D gelatin/halloysite nanocomposites

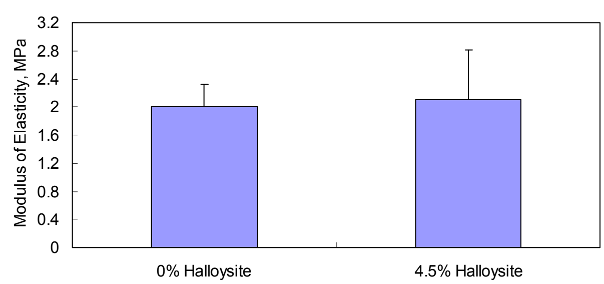

Figure 3. Mechanical strength of composites with and without Halloysite.

2D gelatin/halloysite film is transparent, flexible and strong.

Conclusions: Gelatin/halloysite hybrid nanocomposite was successfully fabricated into 2D membrane and 3D foam. The novel foam possesses suitable pore structure to be used as biomimetic materials for cell culture. Model bioactive reagents will be loaded into halloysites before nanocomposites fabrication. Release behavior of loaded reagents will be characterized.