Novel Clay Intercalation for Design of Clay-Polymer Nanocomposites for Bone Tissue Engineering

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Statement of Purpose: Montmorillonite (MMT) based smectite clays have recently been extensively used for biomedical applications. These layered silicate-polymer nanocomposites have been used in biomedical applications with synthetic as well as biopolymers. In addition, extensive studies exist on use of clays as reinforcements for design of polymer clay nanocomposites. Also, addition of nanoclay to polymer has shown significant improvement in mechanical and thermal properties Therefore, polymer/layered silicate nanocomposites are widely investigated for structural applications. The clays used in polymer clay nanocomposites require the use of non-biocompatible small molecule organic modifiers (OMMT) to enhance the miscibility of polymer and clay. These modifiers inhibit the use of the clays for biomedical applications and not using the modifiers inhibits the use of clays with the most benefit in biomedical nanocomposites. Here we describe the use of unnatural amino acids as potential new modifiers appropriate for biomedical application. The choice of these modifiers is based on our extensive previous studies on effect of chain length, functionality etc of the modifiers through experiments and molecular modeling in polymer clay nanocomposites.

Methods: Na-montmorillonite (Swy-2) which has a cationic exchange capacity of about 76.4 mequiv/100g was obtained from the clay minerals repository at the University of Missouri, Columbia, MO. The chemical formula of this clay is $NaSi_{16}$ (Al_6FeMg) O_{20} $(OH)_4$. An unnatural amino acid , aminopimelic acid with 97% purity was obtained from Sigma Aldrich, St. Louis, MO. In this work, OMMT containing 38% by mass ±2-aminopimelic acid was prepared.

Nicolet 850 FTIR spectrometer with KBr beam splitter was utilized in the range of 4000-400 cm^{-1} with a spectral resolution of 4 cm^{-1}.

XRD patterns were collected using X-ray diffractometer, model Philips X'pert, Almelo, Netherlands, equipped with secondary monochromator and Cu-tube using CuKα radiation of wavelength 1.54056 Å.

For bioactivity and cell proliferation and growth study, human osteoblast cells (obtained from ATCC) were grown over samples of composites. The cells were allowed to grow in presence of cell culture medium. (90% HyQ DMEM – 12 (1:1), 10% FBS and 0.6% G418 solution (antibiotic)). All the samples were placed in an incubator at a temperature of 35^oC and the growth of cells over each sample was investigated by taking images at a magnification of 200x, after 1 day, 3 days and 7 days of feeding the cells on the sample using a inverted microscope.

Results: Interactions between MMT and amino acid are studied experimentally using infrared spectroscopy. MMT containing 38% by mass ±2-aminopimelic acid was prepared. Transmission FTIR spectra of pure MMT, OMMT and the H-O-H bending region of these two spectra are shown in Figure 1.

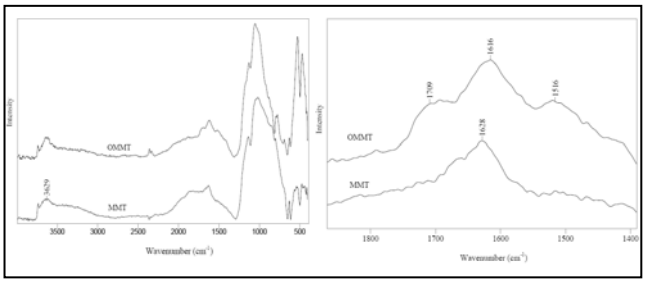

Figure 1. a. Transmission spectra of MMT and OMMT, b. H-O-H bonding region of transmission FTIR spectra of MMT and OMMT.

Bands at 1628, 1616 cm^{-1} are attributed to H-O-H bending vibration of interlayer water. The band at 1709 cm^{-1} is attributed to the disassociated C=O stretching where as band at 1516 cm^{-1} is attributed to N-H stretching of amino acid. The H-O-H bending band of interlayer water in OMMT shifts to lower energy by about 12 cm^{-1}. This is due to the formation of new hydrogen bonds between water molecules and the amino acid molecules that enter into the interlayer. Amino acid molecules that are hydrogen bonded to the interlayer water molecules weaken the bending vibration of the interlayer water molecules. In addition we also observe a new band in OMMT. We attribute the 1709 cm^{-1} band to disassociated C=O stretching of carboxylic group. Normally, in amino acids this band occurs at around 1735 cm^{-1}. Due to the disassociation of carboxylic group, this band shifts to lower energy.

XRD results indicate that d (001) spacing of MMT has increased by 2.29 A^0 after MMT is modified by aminopimelic acid confirming the intercalation of aminopimelic acid in the clay structure. In addition cell culture experiments indicate that the modified clay is biocompatible and leads to excellent cell proliferation and growth.

Conclusions: Here we have successfully modified nanoclays with biocompatible unnatural amino acid enabling a potential use of clays in tissue engineering scaffold application s that will take advantage of excellent capability of nanoclays in enhancing properties with applications of scaffolds. FTIR spectroscopic studies indicate non bonded interactions at amino acid-clay interfaces.

References:

Katti KS, et al. Polymer 2006; 47:403-414.
Sikdar D, Katti KS, Katti DR. J. Nanosci. Nanotech. 2008; 8:1638-1657.
Sikdar D, Katti KS,Katti DR. J. Appl. Pol. Sci. 2008;107:3137-3148.