

## Fabrication of Cellulose Acetate/Cellulose-HA Composite Films for Bone Fixation

FATIMA Nisar<sup>a\*</sup>, USMAN bin Khalid<sup>b</sup>, MUHAMMAD Aftab Akram<sup>c</sup>,  
SOFIA Javed<sup>d</sup> and MOHAMMAD Mujahid<sup>e</sup>

School of Chemicals and Materials Engineering, NUST, Islamabad, Pakistan

<sup>a</sup>fatima\_me06@scme.nust.edu.pk, <sup>b</sup>usmanbinkhalid992@gmail.com,

<sup>c</sup>aftabakram@scme.nust.edu.pk, <sup>d</sup>sofia.javed@scme.nust.edu.pk, <sup>e</sup>m.mujahid@scme.nust.edu.pk

**Keywords:** Micro Cellulose, Cellulose Acetate, Membranes, Bone, Hydroxyapatite, Biomedical Material, Bone, X-Ray Diffraction, Scanning Electron Microscopy, Simulated Body Fluid, FTIR.

**Abstract.** Bone is a rigid and constantly remodeling organ, a type of tissue which provides support and protects organs in the body, and together they form the skeleton. Sometimes due to trauma or injury, the damage requires orthopedic surgery. Materials generally used for implants bear tissue rejection and produce toxins on degradation. The objective is to synthesize a biocompatible film which mimics the properties of natural bone that can be used for bone replacements. Hydroxyapatite (HA) and Cellulose are used as a reinforcement and Cellulose Acetate as a matrix. The experimental procedure is divided into two major steps; extraction of cellulose microfibrils (CMF) from raw cotton followed by dispersion of cellulose and HA in cellulose acetate then casting membranes of the composite. The composite film is successfully fabricated through biometric route. The porous and flexible films obtained allow osteoconductivity and Hydroxyapatite growth in simulated body fluid. X-Ray diffraction; scanning electron microscopy and Fourier transform infrared spectroscopy are used to characterize the films.

### Introduction

Bones, being an integral part of the human body, perform numerous functions and are responsible for the structural framework of our body [1]. They start developing at a very early stage by the bone apposition process and with the passage of time this process slows down which leads to weakening of bones [2]. Damage may also result due to injuries caused by accidents. Synthetic materials are required to compensate for this deterioration of bones which are used as implants but most of these are composed of metals and other materials which are not readily accepted by the body and result in tissue rejection. Therefore, an alternative biocompatible material is required with similar structural properties.

A material is required which is flexible and can be made into a form which can replace the bone as an implant. The process selection and the synthesis of that material is the main focus of this project. First the components of bone are analyzed and then material selection is done based on the composition of the actual bone. These are hydroxyapatite, cellulose and cellulose acetate [3, 4]. Hydroxyapatite is a calcium phosphate which is already found in the bone and makes up most of the bone [5, 6]. It is a ceramic with hexagonal crystal structure. It is a bioactive and biocompatible material [7-9]. Cellulose from plant is the other material because it is also biocompatible and abundant. It is used to mimic collagen which is present in the bones as cellulose is the structural material in plants just as collagen is the structural material in bones of humans and animals [5, 10]. First phase of the project was the extraction of cellulose from cotton fibers as cotton has lignin and hemicellulose in it. This was done by the acid hydrolysis method and the concentration of acid was varied to obtain microfibrils of varying lengths [10, 11].

In the next phase two methods were selected for the formation of the composite of hydroxyapatite which was used as obtained. The first method was to deposit hydroxyapatite on the cellulose fibers in a 1.5 r-sbf using the method similar to that which happens in body [12, 13]. The second method was a simple solution casting method in which cellulose acetate [14, 15] was used

as a matrix in which hydroxyapatite and cellulose were dispersed as reinforcements and then the membrane developed was analyzed [16].

The former process follows a biometric route and allows bone regeneration due to osteoconductivity through porous membranes. This being a unique approach would open the doors for further research on self-healing bones. In future the membranes can be stacked together to enhance their properties or even rolled to obtain flexible rods with good mechanical properties.

## Experimental Procedure

**Extraction of Cellulose.** The cotton is first washed then sourced and bleached in 20 wt. % sodium hydroxide NaOH for 2 hours at 70°C. This swells the cotton and dissolves impurities such as lignin, hemicellulose and see, etc. After soaking and stirring in NaOH cotton is washed by distilled water to neutralize the pH. Thereafter the washed cotton is torn into short fiber and stirred for further 2 hours at 70°C in sulfuric acid H<sub>2</sub>SO<sub>4</sub>. This step is basically to reduce the chain length and degree of polymerization of cellulose as acids interacts with the amorphous region in cellulose and breaks intermolecular bonds [5, 10]. The acidic suspension of cellulose microfibers is then left to settle and acid is decanted, distilled water is used to wash and neutralize the acidic microfibers. The fibers are obtained by filtration and dried in oven at 60°C for an hour. The dried product is then treated mechanically, that is pestle and mortar is used to crush fibers to reduce their size further and also to de-agglomerate the fibers [11, 17]. The long cellulose molecules are now converted to short micro fibers which can be used to enhance the strength as well as provide flexibility.

**Simulated Body Fluid.** It can be used for not only measurement of bioactivity of artificial materials *in vitro*, but also for apatite coatings on various materials through biomimetic route in controlled environment. In our case simulated body fluid is used for precipitation of calcium phosphate in the form of hydroxyapatite (HA) on pre-treated micro cellulose [18].

Standard steps to prepare revised simulated body fluid (R-SBF) were followed. Firstly, apparatus was washed with HCL (to sterilize) and then by deionized or double distilled water, thereafter while stirring, each chemical was added to 1000ml deionized water at 36.5°C in the quantity as mentioned in the table 1 till order#8 and reagent 9 added slowly in quantity less than 1g to avoid increase in pH locally. After adjusting pH to 7.25 at a temperature of 36.5°C the fluid was left for 1—2 days to be stable [19].

Table 1 Reagents used to prepare simulated body fluid

Order	Reagent	SBF in 1000ml	1.5 SBF in 1000ml
1	Ultrapure water		
2	NaCl	7.996 g	11.994 g
3	NaHCO <sub>3</sub>	0.350 g	0.525 g
4	KCl	0.224 g	0.336 g
5	K <sub>2</sub> HPO <sub>4</sub> .3H <sub>2</sub> O	0.228 g	0.342 g
6	MgCl <sub>2</sub> .6H <sub>2</sub> O	0.305 g	0.458 g
7	1 kmol/m <sup>3</sup> HCl	40 cm <sup>3</sup>	60 cm <sup>3</sup>
8	CaCl <sub>2</sub>	0.278 g	0.417 g
9	Na <sub>2</sub> SO <sub>4</sub>	0.071 g	0.107 g
10	(CH <sub>2</sub> OH) <sub>3</sub> CNH <sub>2</sub>	6.057 g	9.86 g

**Composite Formation.** 50ml of SBF prepared as mentioned previously is used with a pH adjusted around 7.5 with Tris(hydroxymethyl) aminomethane, at a temperature of 36°C. CNC suspension is made (~0.34 mmol/g) and placed in ultrasonic bath for 15min with 35 kHz [20]. The mixture is then placed in water bath at a temperature of 37°C for 1 hour. The process of ultra-sonication and maintaining temperature in the water bath is repeated 3 times. Precipitated products were collected and washed with ethanol several times. The filtrate is dried at 60°C for 1 hour [21].

**Film Formation.** The matrix used for film formation is cellulose acetate with cellulose microfibrils and HA dispersed in it using CTAB as the surfactant [22-24]. Acetone is used as a solvent which evaporates when poured into the glass molds leaving behind thin flexible porous membranes. The ratios of the three can be varied according to the required flexibility and thickness of the membranes [5, 25].

## Results and Discussion

**X-Ray Diffraction.** Curve Fig. 1(a) represents the XRD pattern of cellulose microfibrils the peaks shown at 2 theta of  $12^\circ$ ,  $20^\circ$  and  $22^\circ$  are broad presenting the polymeric structure of cellulose microfibrils that is it contains both amorphous and crystalline part [26]. The pattern is in accordance with the standard pattern found in reference library with an ICDD code of 00-003-0192. A little shift of peaks towards right that is, increased theta shows a decrease in d-spacing according to Bragg's law. The graph below in Fig. 1(b) represents the XRD pattern of treated and untreated raw cotton fibers. For comparison and to support our deduction it can be clearly seen that red graph representing treated fibers have sharper peaks with high intensity relative to that of untreated fibers. This suggests that after treatment the fibers become more crystalline and short therefore XRD peaks are sharper and intense with low full width half maxima [26].

**Scanning Electron Microscopy.** These images in Fig. 1 (c), (d), (e), (f) show the change in size and morphology of the raw cotton fibers after treatment in sulfuric acid. It can be seen from the images of scanning electron microscopy that the fiber length of cotton fibers is reduced by basic and acidic treatments which included sourcing, bleaching and acidic treatment [4, 11]. The raw cotton fibers are larger in length comparatively and the surface of fibers obtained is smooth except some of the depositions visible on the images which are mainly due to the presence of hemi cellulose and lignin type impurities. The average length of fibers after treatments is 85 micron with a thickness of around 11 microns. On the other hand raw cotton fiber length is more than 500 micron and a diameter of around 25 microns. This difference clearly represents a size reduction in fiber length and diameter due to treatments thus producing cellulose micro fibers.

**Fourier Transformation Infrared Spectroscopy.** Curve in Fig. 2 confirms the presence of labeled bonds by determining frequency of particular bond and comparing it with standard frequency of those bonds in literature thus confirming the cellulose molecule.

**SEM Images for Composite Membrane.** Images in Fig. 3 represent the surface morphology and texture of the composite membranes formed. Pure cellulose acetate membranes are smooth and have a plain texture although at higher magnification of 5000x porosity is identified of around 2-4 microns as shown in Fig. 3(a) and 3(b). While the other membranes in Fig. 3(c) and 3(d) contains CA as matrix with cellulose microfibrils and hydroxyapatite as reinforcement shows fibers dispersed in the matrix at a magnification of 500x and pore size in these membranes is around 1-2 microns and the thickness of membrane calculated using ImageJ software is in the range of 50-60 microns.

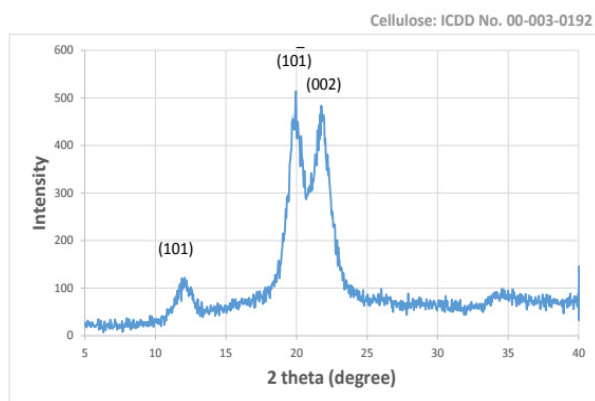


Fig. 1(a) XRD Curve for cellulose microfibrils

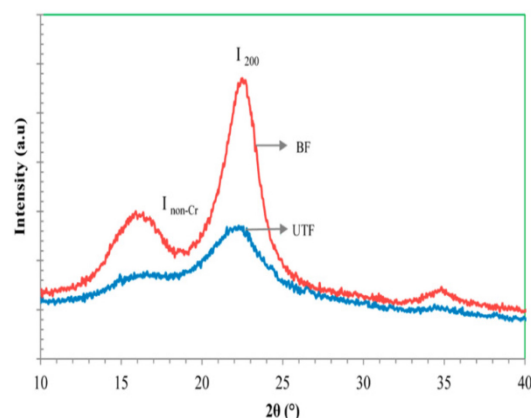


Fig. 1(b) XRD for CMF and cotton from literature

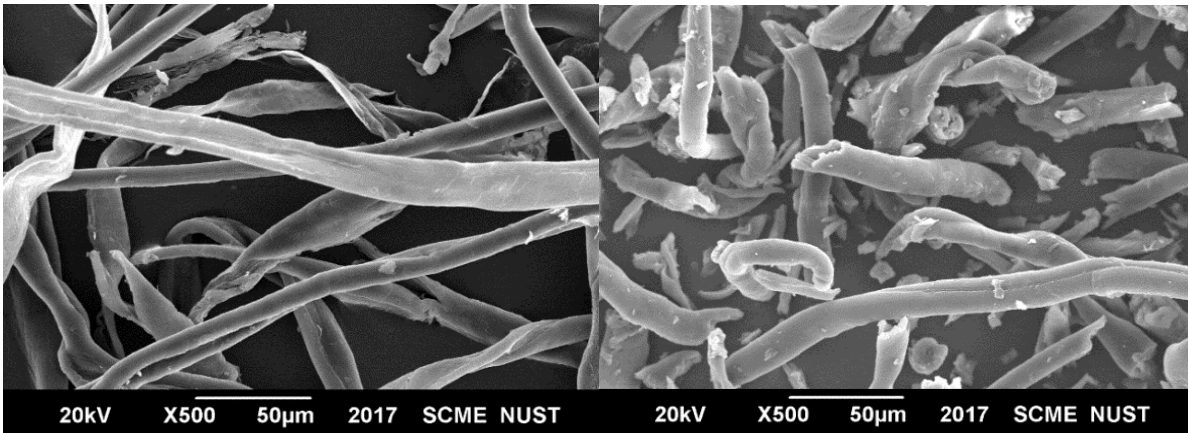


Fig. 1(c) SEM for raw cotton in fibrous form

Fig. 1(d) SEM for CMF at x500

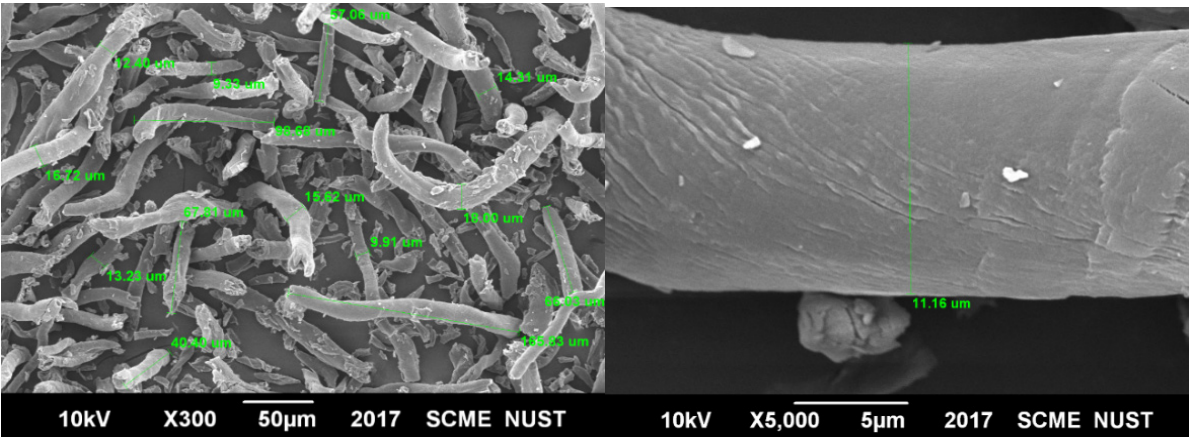


Fig. 1(e) SEM for CMF

Fig. 1(f) Surface of CMF

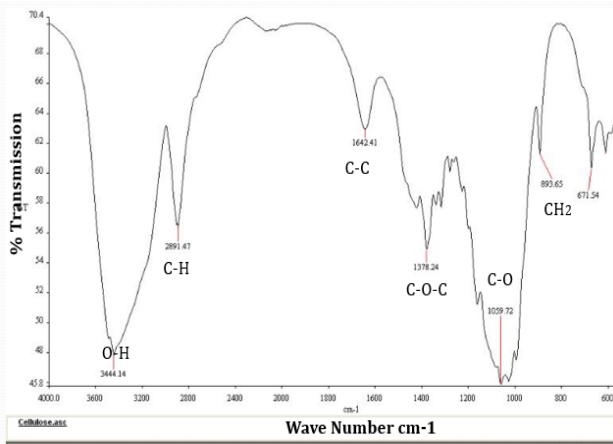


Fig. 2 FTIR of Cellulose Microfibers

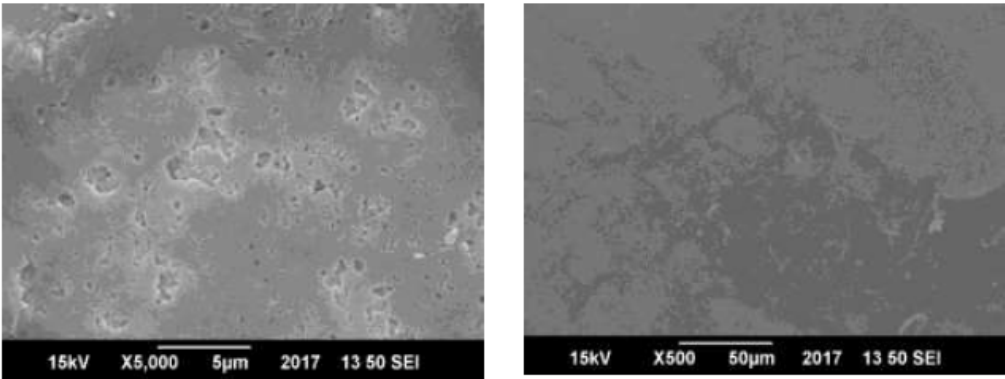


Fig. 3(a) Pure CA membrane surface

Fig. 3(b) Porous CA membrane surface

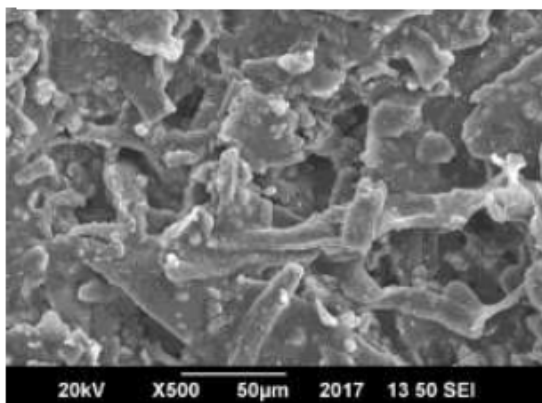


Fig. 3(c) Surface morphology

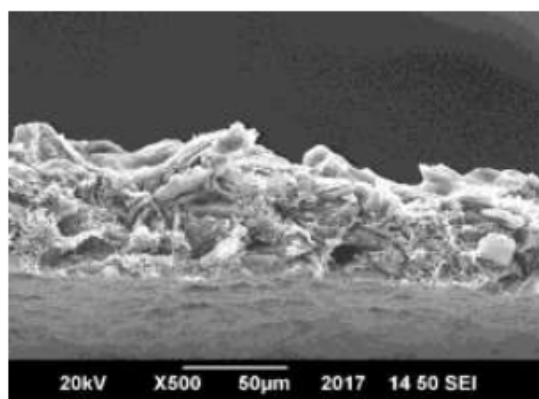


Fig. 3(d) Cross section of membrane

## Conclusions

The membranes prepared using the following protocol is flexible enough to get rolled and be utilized in biomedical applications due to the already approved biocompatibility of the materials being used. The porosity achieved would also help in osteo-conductivity and thus bone regeneration. The membranes can also be further tested for gas and liquid permeation. The flexible sheets can also be rolled and stacked to increase the strength and enhance anisotropic properties.

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