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The effect of nanoclay and crosslink density on the mechanical and tribological properties of a hydrogel composite for cartilage replacement

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ABSTRACT

Millions of individuals suffer from the bone joint disease arthritis, every year, worldwide. Total joint replacements, the standard for current treatment has certain drawbacks such as wear of the surface, a negative immune response to wear particles, non-matching of the mechanical and tribological properties with natural bone joint tissue. In order to overcome the drawbacks of current materials used in total joint replacements, and mimic natural cartilage, hydrogel composite materials were investigated. Inter penetrating networks of Poly Hydroxyethyl Methacrylate (P-HEMA) and Poly Acrylamide (PAAM) were synthesized with nano clay particles as reinforcement. Tribological and compression tests were carried out. There was a significant change in stiffness and failure load with the addition of nanoclay. Four lubricants-simulated body fluid (SBF), and SBF with 1, 2, and 3mg/ml of Hyaluronic acid (HA) were incorporated in the tribological tests. The counter material was a stainless steel pin. It was found that the nanoclay particles significantly improved the strength of the composite. Increasing the HA concentration led to an increase in viscosity of the lubricant but had no significant effect on the coefficient of friction. An increase in crosslink density also led to an increase in the coefficient of friction. The addition of nano clay led to a decrease in the coefficient of friction.

KEYWORDS: Biotribology, arthritis, biomaterial, cartilage, hydrogel.

INTRODUCTION

The debilitating disease arthritis caused due to cartilage failure, has hampered lives worldwide. Cartilage, being avascular and aneural, has limited self-healing capacity. In the United States alone, approximately 26% of all adults (approximately 78 million people), are projected to suffer from the disease [1]. It is the leading cause of work disability, causing annual costs for medical care and lost earnings of $303. bn.

Complications due to arthritis do not show up until it is really severe. The gold standard for its treatment is still Total Joint Replacement/Arthroplasty (TJR/TJA), developed by Sir John Charnley, in the 1960's [2]. A report has indicated that by the year 2040, the Total Hip Arthroplasties (THA) annually in the US itself would be 1429,000 while the number of Total Knee Arthroplasties (TKA) would be 3416,000 [3].

Total joint replacements have a life span of approximately 10-15 years and need to be replaced thereafter [4, 2, 5, 6, 7]. With more joint replacements being performed in individuals below the age of 50, this can be a serious concern. The primary reasons of failure are surface wear, asceptic loosening, stress shielding and osteolysis. There are also reports of toxicity of the metal ions from the implants [8,9,10,11,12,13].

It is necessary for the replacement biomaterials to have mechanical and physical properties as close to natural surrounding cartilage and bone tissue, as possible [14]. Therefore, there is a great necessity to provide a joint repair technique that is less invasive, having less surgery, and providing recovery to a more natural condition than a total joint replacement. Other advantages would be reduced time in hospital with lowered cost.

Several investigators have attempted to develop synthetic materials that can possibly act as a replacement for cartilage tissue [15,16,17]. Hydrogels are ideal candidates for cartilage replacement, as they possess several cartilage-like properties [18, 19]. They are hydrophilic, deformable and porous. They are also known to provide excellent lubrication [20, 21].

Awasthi et al. have investigated a composite of Poly Acrylamide Hydrogel with carbon nanotube and Titanium di oxide nanoparticles [22]. The investigations showed some promise. Liu et al [23] have investigated hydrogels that could potentially be injectable for cartilage and bone tissue engineering. Bao et al [24] investigated the performance of natural hydrogels for cartilage tissue engineering. Wu et al [25] worked on designing injectable hydrogels for cartilage repair. Swieszkowski et al [26] studied a soft cryogel material, which has shock absorbing properties similar to that of cartilage tissue. Covert et al [27] studied the friction characteristics of PVA cryogel. Oka et al [28] studied a polyvinyl alcohol hydrogel material having a titanium mesh interface with bone. Some of these materials have been found not to have adequate mechanical strength and some have not been tested clinically. It is necessary to identify and synthesize a hydrogel material with nanoparticle fillers such that the composite would ideally mimic natural cartilage. The nanofillers would provide strength and hydrophilicity similar to collagen fibres and proteoglycan molecules respectively in natural cartilage. The biocompatibility of several hydrogels has already been established and this group of materials have already found applications in the medical industry [29, 30, 31, 32].

The use of the hydrogel poly(2-hydrozyethyl methacrylate) [p(HEMA)] has been studied as a biomaterial [33]. It has been as a potential cartilage replacement material by several authors [34, 35, 36]. It is already being used as a biomaterial in contact lenses [37, 38]

Poly Acrylamide (PAAM) has been studied as a potential cartilage replacement material [39, 40]. Yang et al [41] have reported that Alginate/Polyacrylamide hydrogels can be strengthened using various multivalent cations. Risbud et al studied the in-vitro biocompatibility and sustained antibiotic release of polyacrylamide-chitosan hydrogels and have reported success. Gong et al. [42] have reported on the high toughness of double network hydrogels.

Bentonite clay is known to absorb high quantities of water [43]. It is known to be biocompatible externally as well as if ingested orally. Bentonite clays are found to possess therapeutic and healing properties [44]. An extensive review by Erezuma et al. [45] have reported that nanoclays exhibit no toxicity and assist in healing of bone and cartilage tissue. Sakr et al [46] reported success in the development of bentonite-gelatin nanocomposite hybrid hydrogels for tissue engnineering.

However, the above attempts have not yet succeeded in finding a material that can be commercialized as a widespread cartilage repair material. In this study, hydrogels poly hydroxyethyl methacrylate and poly acrylamide have been synthesized together to form double notwork hydrogels. Some of the samples were also combined with bentonite nanoclay to investigate the difference in properties. A simulated synovial fluid was synthesized with varying concentration of hyaluronic acid. Tribological properties were investigated using this fluid as lubricant. It was found that the Equivalent Water Content (EWC) increased with the addition of clay and increase in cross-linking. The modulus increased with the addition of clay but failure took place at a lower strain. The coefficient of friction decreased with the addition of clay and was minimum at a certain concentration of HA in the lubricant.

Double network hydrogels have been found to have enhanced toughness [43]. They have also been found to mimic the modulus, strength and lubricity of cartilage [47]

In this study, double network hydrogels of pHEMA and PAAM have been synthesized. Some samples were combined with nanoclay. Compression tests showed an enhanced strength due to the presence of nanoclay. The coefficient of friction when articulated against a stainlesssteel 316L stainless steel pin was found to decrease. The effect of increasing the concentration of Hyaluronic acid in the lubricant had a marginal effect on the coefficient of friction. The samples with clay and increased crosslinking were found to absorb more water.

MATERIALS

The following table shows the two polymer systems

Table 1. The two polymer systems of the inter-penetrating network.

S.No.	Monomer	Cross-linker	Initiator
1	Hydroxyethyl methacrylate (HEMA)	Ehthylene Glycol Diethyl Methacrylate (EGDMA)	Benzoyl Peroxide (BPO)
2	Acrylamide	NN' Bis Acrylamide	Ammonium Persulphate (APS) + Catalyst Tetramethylenediamine (TEMED)

Monomer HEMA, BPO, Monomer Acrylamide, NN' Bis Acrylamide, APS and TEMED were obtained from Loba Chemie Pvt. Ltd.

EGDMA was obtained from TCI chemicals

Bentonite nano-clay powder was obtained from NICE chemicals

The following were the ratios of the chemicals.

Monomer:Cross-linker- 20%, 30%, 40% by molecule

Initiator: Monomer- 2% by molecule

For poly Acrylamide, monomer:catalyst was also 2% by molecule

HEMA:Acrylamide = 2:1 by wt.

One set of samples were selected with nanoclay and the others without.

Samples with nanoclay had monomer (HEMA):nanoclay = 1:1 by wt.

The calculated and measured ratios of monomer:crosslikner and monomer:initiator were mixed in a beaker with the help of a magnetic stirrer, until the solute was completely dissolved. For Poly Acrylamide, the momomer:catalyst weight ratio was also calculated and measured and added initially. After this, each solution was purged separately, by passing nitrogen gas through it for five minutes, to displace the oxygen.

The two solutions were then mixed using a magnetic stirrer.

Part of the combined solution was separated and nanoclay was added to it and stirring continued until the entire nanoclay was mixed evenly.

The solutions with nanoclay and without nanoclay were poured into separate containers and allowed to polymerize in an oven at 60ºC. The solution with nanoclay was stirred every five minutes until it started solidifying

The pin in the pin-on disk tests were made of Stainless Steel 316L (used as a biomaterial) [48] rod of 6mm diameter. The end which came in contact with the disk specimen was machined to a hemispherical shape of 6mm diameter.

Simulated body fluid and simulated synovial fluid:

A standard solution of simulated body fluid (SBF) was prepared with the following ionic concentrations shown in table 2, according to a reference [49].

Table 2. The ionic concentrations in Simulated Body Fluid [50].

Ion	**Concentration mM**
Na^+	142.0
K^+	5.0
Mg^{2+}	1.5
Ca^{2+}	2.5
Cl^-	147.8
HCO_3^-	4.2
HPO_4^-	1.0
SO_4^{2-}	0.5

To this solution Hyaluronic acid (HA) concentrations of 1, 2 and 3 mg/ml were added to prepare three different simulated synovial fluid lubricants. Plain SBF was also used as a lubricant.

EXPERIMENTAL PROCEDURES

A. Viscosity

The viscosity of plain SBF and that mixed with various concentrations of HA were measured using an Ostwald's vicometer. The temperature of the fluid was maintained between 36 and 40ºC by heating in a magnetic stirrer batch, prior to testing, as normal body temperature is 37ºC. The time of flow between standard markings on the viscometer was measured using a stopwatch, for distilled water and the other solutions.

The equation used for calculating the viscosities is

$$V(liq) = \frac{D(liq)\times t(liq)\times V(water)}{D(water)\times t(water)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where, V refers to viscosity, D to density, and t to time of flow

B. Equivalent water content

Post polymerization, the samples were weighed in dry condition. They were re-weighed after soaking in distilled water. The equivalent water content was calculated using the following equation.

$$EWC = \frac{(Wet\ weight\ of\ hydrogel - Dry\ weight\ of\ hydrogel)}{Wet\ weight\ of\ hydrogel}\ X\ 100 \ldots\ldots\ldots..(2)$$

C. Compression tests

Samples for the compression tests were not hydrated and were kept in dry condition to determine their behaviour in this condition before testing in the hydrated condition. This was done to determine the behaviour of the materials without superimposition of the effects of water exudation. The effects of hydration would be studied in a future investigation. The samples were of cylindrical shape having diameter 30mm and height 20mm. The compression tests were conducted using a 3T Universal Testing Machine (Asian Instruments). The sample was compressed between two flat plates. The upper plate was kept stationary, while the lower plate had a speed of 10mm/min. Force-displacement values were measured and displayed.

D. Friction tests

The friction tests were conducted in pin-on-disk configuration. The normal load was 5N and speed of rotation was 60rpm. The various lubricants prepared were added to the interface prior to starting the tests and additional lubricant was sprayed using a Pasteur Pipet. Tests were conducted for 10minutes each, to determine the friction behaviour. The sample surface was a bit rough and therefore, with each rotation, the friction values went through a cycle.

RESULTS AND ANALYSIS

Table 3 shows that the viscosity increases with increase in concentration of HA. The measured viscosities are still much lower than the viscosity of healthy synovial fluid [50]. This indicates that other constituents (not present in these formulations but present in synovial fluid) have a great effect on the viscosity of synovial fluid. The viscosities of the lubricants synthesized are in the range found for synovial fluid of arthritic joints. The viscosity increases with increase in concentration of HA. From plain SBF to the sample with 1mg/ml HA, the viscosity increases by approximately 26%. From 1mg/ml to 2mg/ml HA, the viscosity increases by approximately 11%. From 2mg/ml to 3mg/ml, the viscosity increases by approximately 23%. Therefore, the increase in viscosity with increase in HA concentration is not linear.

Table 3. Viscosity of Simulated Synovial Fluid, with varying concentrations of Hyaluronic acid.

Lubricant	**Viscosity (m Poise)**
Plain SBF	7.03
SBF with 1mg/ml HA	8.84
SBF with 2mg/ml HA	9.8
SBF with 3mg/ml HA	12.1

Table 4 shows the change in EWC with change in cross-linking concentration and with addition of nanoclay. It is seen that EWC increases with increase in cross-linking concentration. This is probably caused by the hydrophilic groups present on the cross-linking molecules. It also increases with increase in clay content confirming the details of the report [44]. The equivalent water content for all samples is much lower than that found in natural cartilage, which is in the range 60 to 80% by weight [51, 52, 53] but the trend of increase with increase in nanoclay content is encouraging.

Table 4. Equivalent water content with varying cross-linking, with and without clay.

Percentage cross-linking	EWC% (without clay)	EWC% (with clay)
20% cross-linker	17.62	22.69
30% cross-linker	19.90	25.89
40% cross-linker	21.03	26.11

Compression results

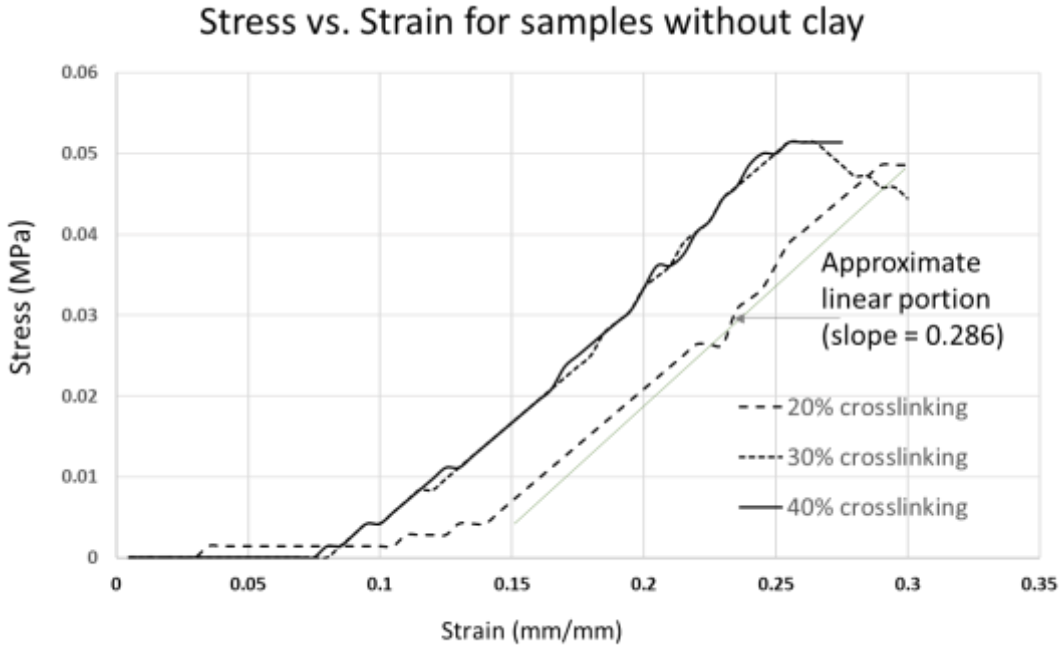

Figure 1. Stress vs. strain curves for the samples with clay.

Fig. 1 shows that the samples without clay are able to resist deformation upto a stress of approximately 0.052MPa force. The strain at failure is approximately 0.27 for samples with 30% and 40% cross-linking. The sample with 20% cross-linking deforms relatively more easily as compared to the other two samples. However, the failure stress is slightly below the other samples at approximately 0.48MPa. The slope of the approximately linear portion represents the modulus and is equal to 0.286MPa.

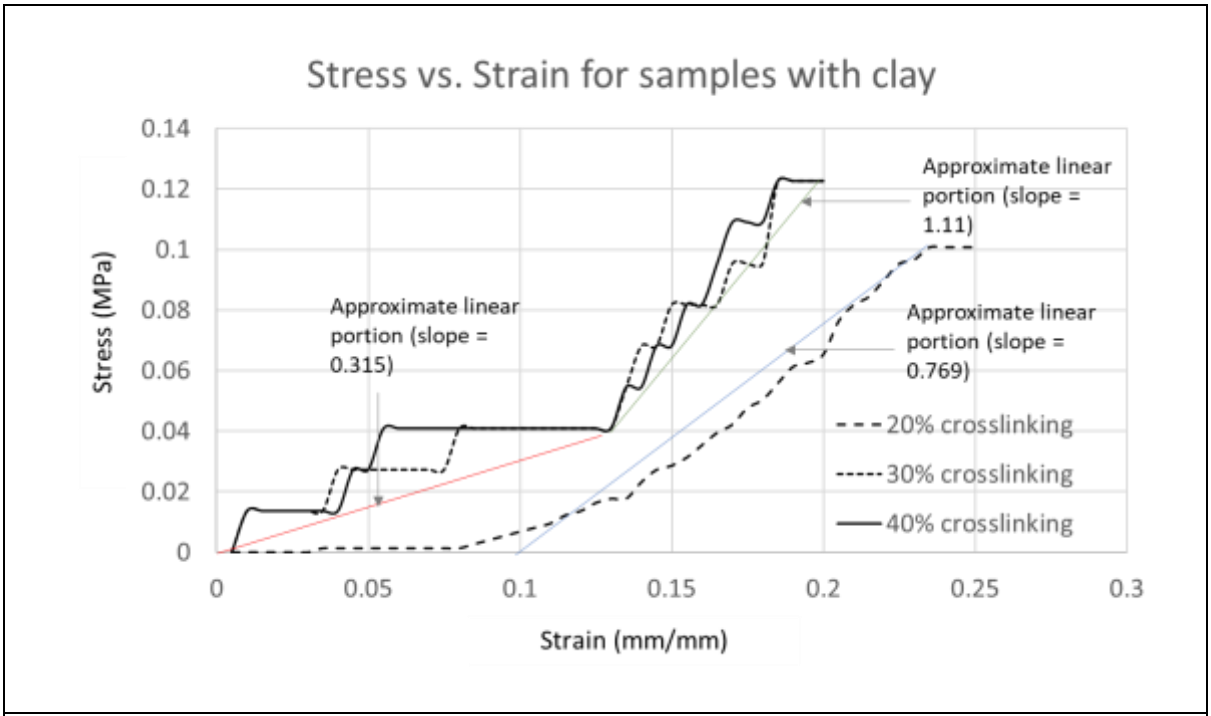

Figure 2. Force vs. displacement curves for the samples with clay.

In fig. 2 it is seen that the samples with clay are able to resist failure upto higher stresses as compared to the samples without clay. The sample with 20% crosslinking deforms relatively more easily compared to the other samples and fails at a stress of approximately 0.1MPa. For a specimen diameter of 30mm, the failure stress was 0.097N/mm^2. The stress-strain curves for the samples with 30% and 40% crosslinking follow each other quite closely. For these two samples (30% and 40% crosslinked), it is seen that at certain stresses, there is a sudden increase in displacement. This took place due to sudden passage of nanoclay particles out through the pores of the hydrogel, until there was a temporary equilibrium with the load, as physically observed during the test. This phenomenon was not observed for the sample with 20% crosslinking. At higher crosslinking densities of 30% and 40%, the nanoclay particles needed to be compressed to a certain load, to overcome the resistance within the voids in the specimen and of the pores on the surface. The failure stresses for the samples with 30% and 40% crosslinkingwas approximately 0.125MPa. If we consider approximately linear portion of the sample with 20% crosslinking as the modulus, its value is 0.769MPa. The plots for the samples with 30% and 40% crosslinking can be split into two approximately linear portions (as shown). At lower stresses, the samples are more easily deformed (as indicated), having a modulus of approximately 0.315MPa (upto a stress of 0.04MPa and a strain of approximately 0.13). Beyond this, the slope (modulus) changed to approximately 1.11MPa. Therefore, as the sample deformed and clay exited the sample, the modulus increased indicating a higher stress increase required to deform the crosslinked network having less clay.

Table…..Summary of moduli for samples with and without clay

Crosslinking	Modulus (MPa)		Failure stress (MPa)		Failure strain (mm/mm)	
	Without clay	With clay	Without clay	With clay	Without clay	With clay
20%	0.286	0.769	0.048	0.1	0.3	0.25
30%	0.286	Initial: 0.315	0.052	0.122	0.253	0.2
40%	0.286	Later: 1.11	0.052	0.122	0.255	0.2

It is known that hydrogels, although having certain properties that mimic those of natural cartilage, lack the fracture strength and modulus required for load bearing at bone joints[16]. The review cited various references [54, 55, 56, 57, 58, 59, 60, 61, 62], which indicated that, for various hydrogels and their combinations, the tensile strength ranged from 0.02 to 5MPa and the Modulus ranged from 0.005 to 10MPa. An investigation by Awasthi et al [23] reported that investigated hydrogels exhibited a compressive strength in the range 0.148 to 0.43MPa. There was a lower modulus at low strains (0.011MPa to 0.027MPa) and at higher strains, the slope of the stress strain curve was higher, giving a higher modulus (0.091 to 2.34MPa). The strength and modulus were higher for hydrogels strengthened with nanoparticles. Compressive moduli of investigated hydrogels in the report by Arjmandi et al [41] indicated moduli ranging approximately from 0.5 to 5.8MPa and depended on the strain rate. The results from this investigation lie within these ranges. An investigation by Little et al [63]reported that the unconfined compressive modulus of native cartilage ranged

from 0.24 to 0.85 MPa. Kerin et al [64] reported that the failure stress of bovine cartilage under compression was 35.7MPa whereas its failure strain was 0.3.

An investigation by Kaukinen et al [65] reported the mean compressive modulus of bovine cartilage at 3.5%/s strain rate as 1.39MPa. The mean yield stress was 4.58MPa. The mean yield strain was 50.2%

Friction results

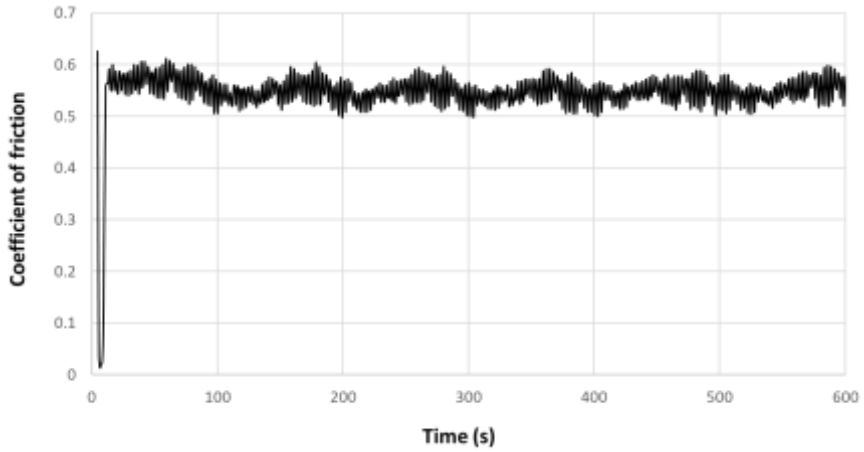

Figure 3. Coefficient of friction vs time with plain SBF lubricant for the sample with 40% crosslinking, without clay

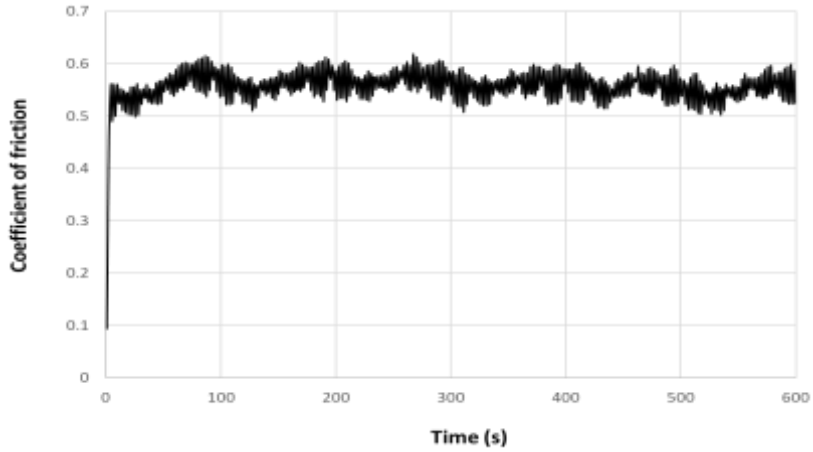

Figure 4. Coefficient of friction vs time with 1mg/ml HA lubricant for the sample with 40% crosslinking without clay

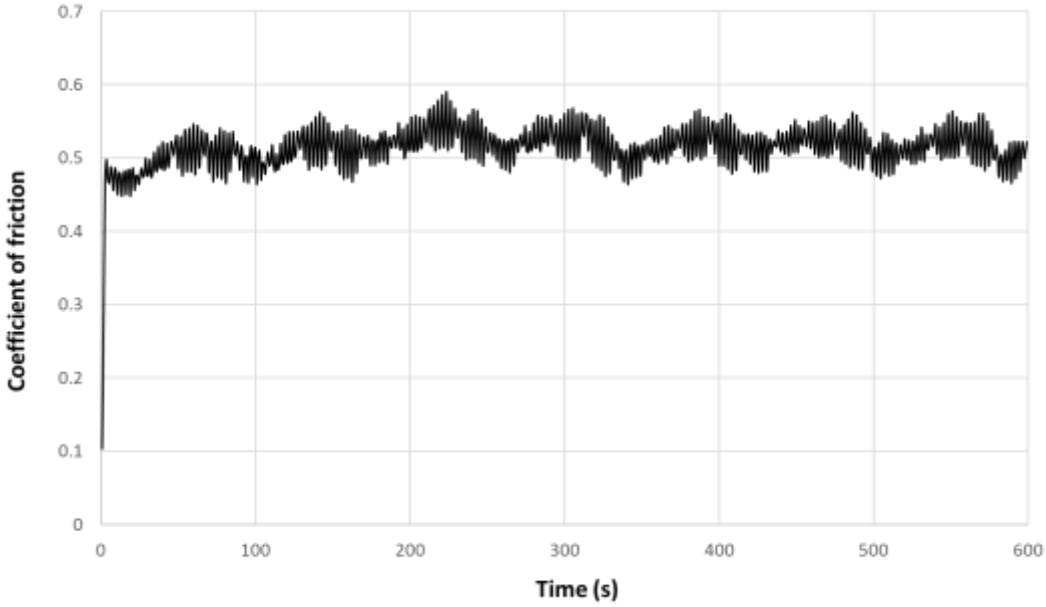

Figure 5. Coefficient of friction vs time with 2mg/ml HA lubricant for the sample without clay

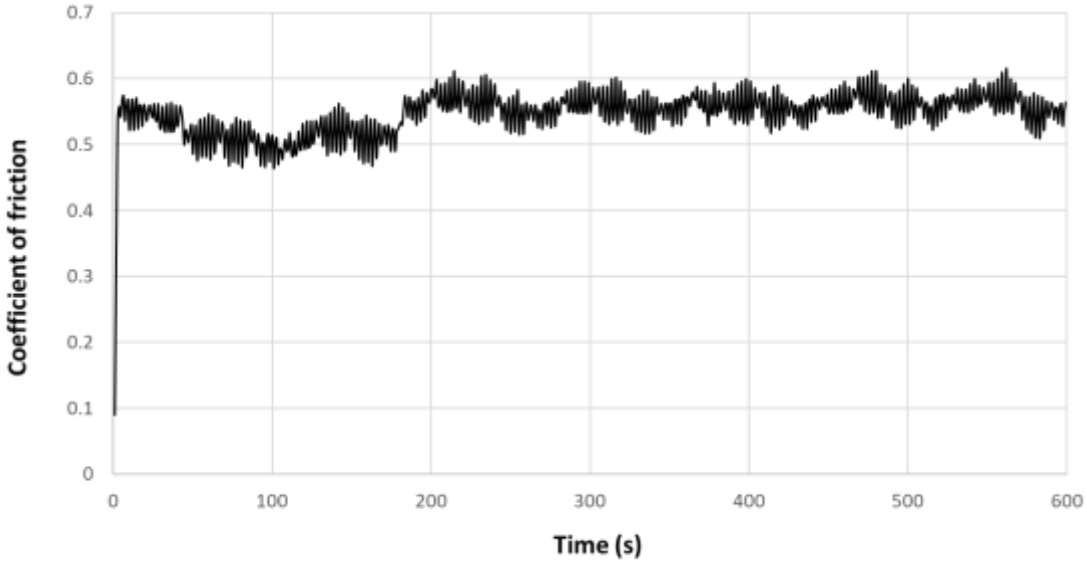

Figure 6. Coefficient of friction vs time with 3mg/ml HA lubricant for the sample without clay

The above figures (3 to 6) show the coefficient of friction vs. time for the samples having 40% crosslinking and without clay, with varying concentration of hyaluronic acid in the lubricant. The coefficient of friction did not vary much during the tests and the average coefficient of friction remained almost the same with all lubricants (as shown in table 5). A similar result was obtained for samples with 20% and 30% crosslinking.

Table 5. Average coefficient of friction with different lubricants, for the sample with 40% crosslinking, without clay.

Lubricant	**Average coefficient of friction**
Plain SBF without HA	0.55
SSF with 0.1mg/ml HA	0.56
SSF with 0.2mg/ml HA	0.52
SSF with 0.3mg/ml HA	0.55

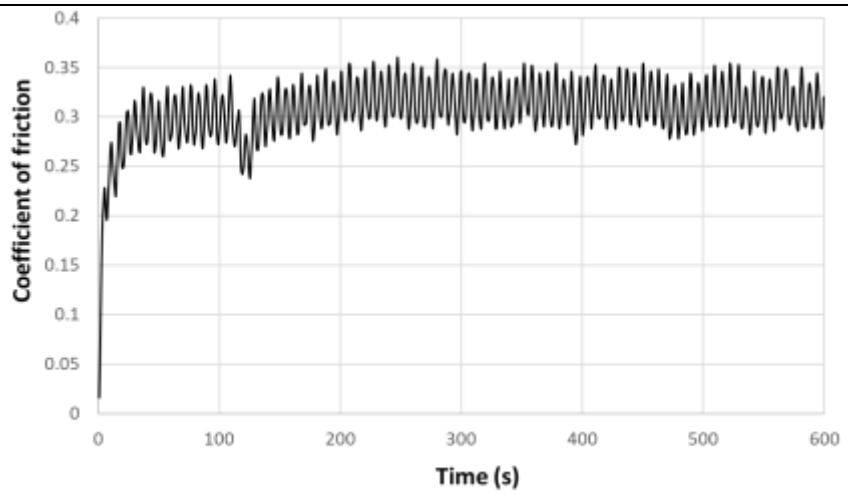

Figure 7. Coefficient of friction vs time with plain SBF (no HA) lubricant for the sample with clay.

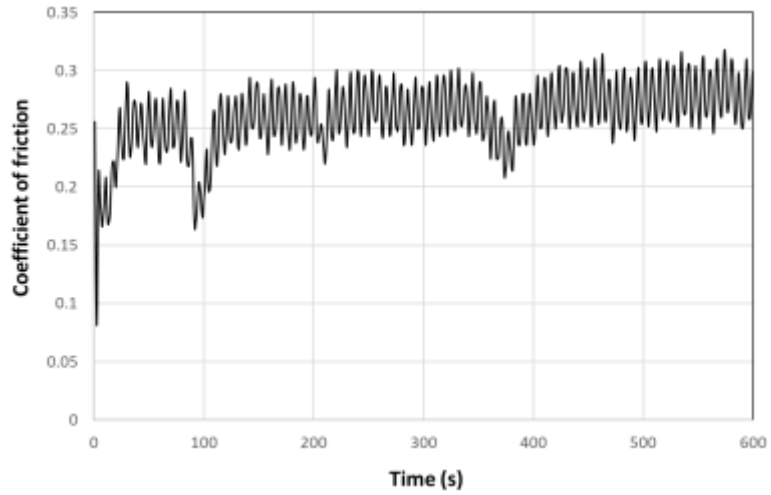

Figure 8. Coefficient of friction vs time with 1mg/ml HA lubricant for the sample with clay.

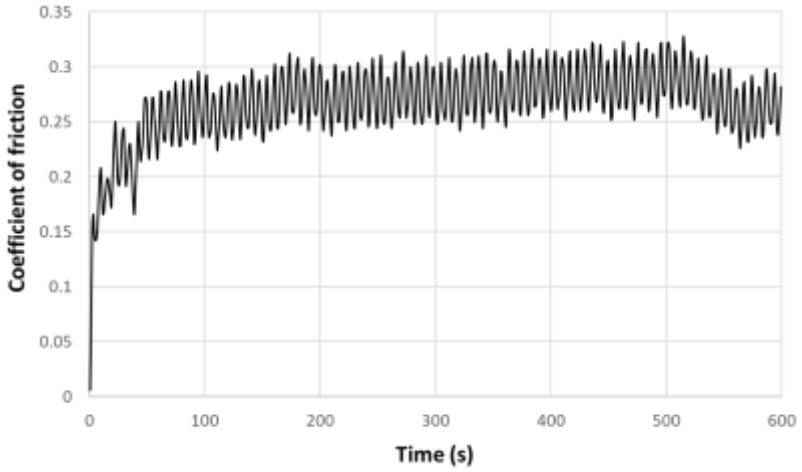

Figure 9. Coefficient of friction vs time with 2mg/ml HA lubricant for the sample with clay.

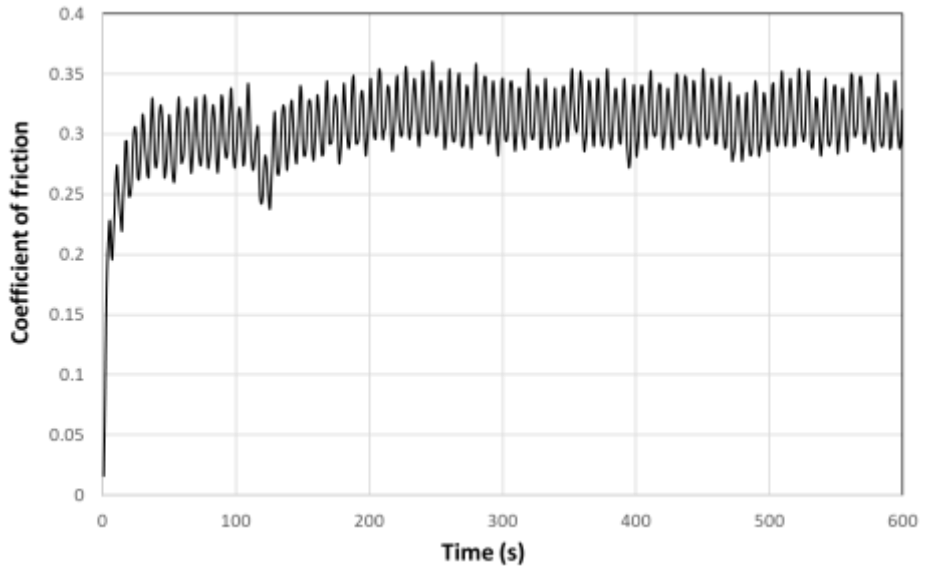

Figure 10. Coefficient of friction vs time with 3mg/ml HA lubricant for the sample with clay.

Figs. 7 to 10 show the coefficient of friction vs time for the samples with 40% crosslinking with clay, with varying concentration of hyaluronic acid concentration in the lubricant. The average coefficient of friction for the tests is shown in table 6 below. It is seen that for the plain SBF lubricant, the coefficient of friction is higher than for the lubricants with hyaluronic acid. The coefficient of friction for all these tests is markedly below that for the samples without clay. This indicates that the presence of nanoclay can decrease the coefficient of friction. The coefficient of friction remained higher that found in natural bone joints.

Table 6. Average coefficient of friction with different lubricants, for the sample with 40% crosslinking, without clay.

Lubricant	**Average coefficient of friction**
Plain SBF without HA	0.328
SSF with 0.1mg/ml HA	0.254
SSF with 0.2mg/ml HA	0.26
SSF with 0.3mg/ml HA	0.3

The reference by Mow et al [66] reported coefficients of friction in human and animal bone joints. The values ranged from 0.002 for bovine shoulder to 0.04 for the human hip. A study by Bavaresco et al [67] on pHEMA hydrogels reported friction coefficients below 0.1. A study by Yarimitsu et al [68] on different hydrogels, reported friction coefficients in the range 0.04 to 0.22. The study by Freeman et al [69] on different hydrogels, reported friction coefficients in the range 0.2 to 0.68. The coefficients of friction obtained in this study are therefore on the higher side as compared with natural bone joints and other studies on hydrogels. Using a lubricant with higher viscosity and a pin materials which is softer may bring down the coefficient of friction. Bringing the coefficient of friction down to levels found in natural bone joints is therefore complex and depends on the type of lubricant and nature of interacting materials.

CONCLUSIONS

Form this study, it can be concluded that:

The presence of nanoclay in the hydrogels led to an increase in equivalent water content, increase in strength and a decrease in the coefficient of friction when reciprocated against a stainless steel 316L pin. This information offers encouragement towards developing a suitable composite material for cartilage replacement.

The behavior of the composite materials depended on the presence of the nanoclay filler as well as the amount of crosslinking in the hydrogels. Therefore, future studies on variation of composition and test conditions are required in order to optimize the materials sufficiently.

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DECLARATION OF INTEREST

The authors confirm that there is no conflict of interest related to this work.

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