ENHANCEMENT OF MECHANICAL PROPERTIES OF POLYMERIC BLENDS FOR BONE TISSUE REGENERATION

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HPMC: Hydroxy propyl methyl cellulose

SEM: Scanning electron microscope

XRD: X-Ray Diffraction

CHAPTER 1

INTRODUCTION

Numerous Scaffolds are made in order to replace, support or heal damaged tissues. Scaffolds provide a support or platform on which new cells can be grown into tissues. Scaffolds are mostly bio-mimetic and closely relate to the Extra Cellular Matrix structure which holds the cells. Scaffolds generally need to be bio-compatible so that when the tissue gains sufficient strength it degrades with passage of time naturally. Scaffolds for bone tissues must allow a 3-Dimensional structure to support attachment and proliferation of osteoblasts.

Tissue engineering is the use of a combination of cells, engineering, and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological tissues. Tissue engineering involves the use of a tissue scaffold for the formation of new viable tissue for a medical purpose. While it was once categorized as a sub-field of biomaterials, having grown in scope and importance it can be considered as a field in its own. [1]

Bone diseases are a major problem these days especially in older patients. Bone acts as a scaffold for whole body it takes most of the load and provides support and protection to the body. Currently, musculoskeletal maladies that result in tissue degeneration and inflammation are the main reasons for the disability and associated diseases around the globe. In 2013, as reported in the Global Burden of Disease Study 2013 (GBD 2013) led by the Institute for Health Metrics and Evaluation (IHME), the burden caused by musculoskeletal maladies around the globe was 149 435 700 disability-adjusted life-years (DALYs), that mainly included rheumatoid arthritis, osteoarthritis, gout, low back and neck pain and other musculoskeletal disorders. Bone structure depicts remarkable regenerative abilities. But in case of more serious problem the bodies healing process slows down or does not function, so some more additional treatments are required to provide a platform for healing process. Slow

immune response occurs due to decrease in vascular supply in the effected region. [3-5]

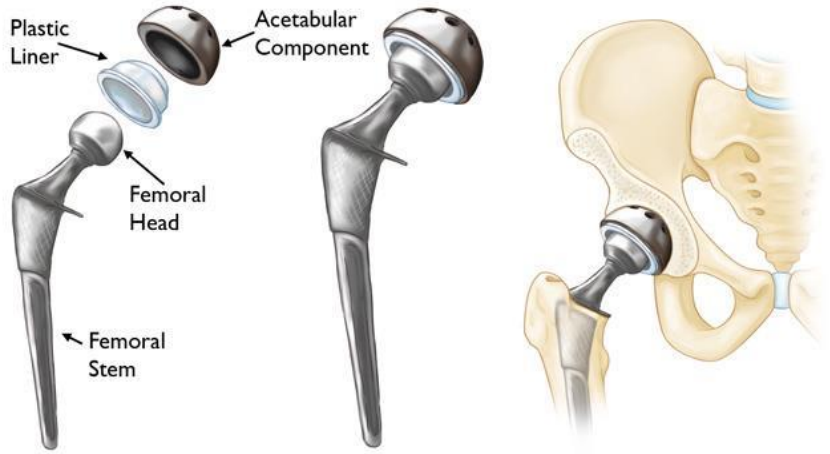

Figure 1: Illustration of Hip Implant

Scaffolds with ideal properties are very difficult to process. Mostly nowadays ideal properties are achieved by a combination of polymers and bio-ceramics. When designing scaffolds for tissue engineering constructs, it is considered particularly appropriate to use a resorb able or biodegradable polymer as the scaffold so that 100% pure and viable biological tissue can be obtained for implantation without the risk of a chronic inflammatory response. Once placed in the body, the composite coatings are expected to have specific properties depending on the location and the function required from the implant. In this context, three types of composite coatings have been defined as anti-wear, biocompatible, and antibacterial coatings. [2-3]

The main aim of our project is the development of natural scaffolds for bone tissues. The scaffolds must cost efficient as well easily degradable. We would be focusing majorly on surface modification of the scaffold to enhance the regeneration of tissue. And in order to achieve our primary goal we would synthesize and characterizing a combination of polymeric blend with a ceramic filler to form a composite having properties close to that of an ideal scaffold. [3]

CHAPTER 2

LITERATURE REVIEW

A bone is a high strength complex tissue structure which forms the whole skeletal structure of body. Bones support and protect various parts of the body. Bone is not a solid material instead it is a matrix and the bone tissues are mostly made up of composite materials containing inorganic mineral calcium phosphate. This bone mineral provides rigidity to the bone and collagen, an elastic protein, provides fracture resistance. Defects and functional bone disorders have become a global health problem and bone repair has become a major clinical and socio – economical need with the increasing aging population and social development.

Current treatment for bone injuries largely focuses on replacement of defect bones that are limited by many aspects such as amount of donor tissue available, complications at the donor site. The basic idea of tissue regeneration is to take the advantage of the natural healing potentials of the patients and holds great promise for the future treatment of large bone defects. The main three ingredients of tissue engineering and tissue regeneration process are signals, stem cells and scaffolding technique. Tissue Engineering has evolved out of the need to repair organs and damaged tissues due to any kind of disease or injury. The concept of tissue engineering embodies the creation of scaffold structure, which has an appropriate physical, chemical and mechanical property in order to enable the cell penetration and tissue formation in three dimensions. [4]

2.1 Bone Regeneration Techniques

Bone is one of the most frequently transplanted body tissues despite being used for a long time clinically bone grafts exhibit some disadvantages that limit their application. Different techniques for bone regeneration are: [5]

- **Allografts:** Allografts is the process of bone regeneration by taking some part of bone or tissue from a living process. But it is a very difficult process; it is

hard to find donors and can generate abnormal immune responses in the acceptor.

- **Auto-grafts:** Auto grafting is the process of taking cells or tissues from patients own body. It can be highly efficient as no donor complications are involved. But auto-grafts generate another potential site for infection in the body.

- **Xeno-grafts:** Xeno-grafts involves taking cells from animals, it can be very harmful as grafting from animals increases potential risk of harmful viruses and pathogens entering the body.

- **Synthetic Implants (Tissue Engineering):** In BTE, a biomaterial can be defined as a temporary matrix that provides a specific environment and architecture for bone growth and development. A scaffold can be described as an artificial structure used to support three Dimensional (3D) tissue formations. Scaffolds can be used as cellular systems or as vehicles for cells and/or drugs. Once implanted into the injured site, a cellular material should allow proper host cell colonization for regeneration purposes. Alternatively, scaffolds can be combined with different types of cells able to promote bone formation in vivo. [6]

2.2 Scaffold Features for Bone Tissue Regeneration:

An ideal scaffold should have following properties:

- Should improve cell viability
- Enhance cell attachment and Proliferation
- Should not generate abnormal inflammatory response
- Should not cause infection
- Bio-Compatible
- Bio-Degradable

- Bio-Mimetic
- Should promote Osseo induction
- Load bearing ability where necessary
- Easy to handle [8-9]
- Could be sterilized on industrial scale
- Cost Efficient

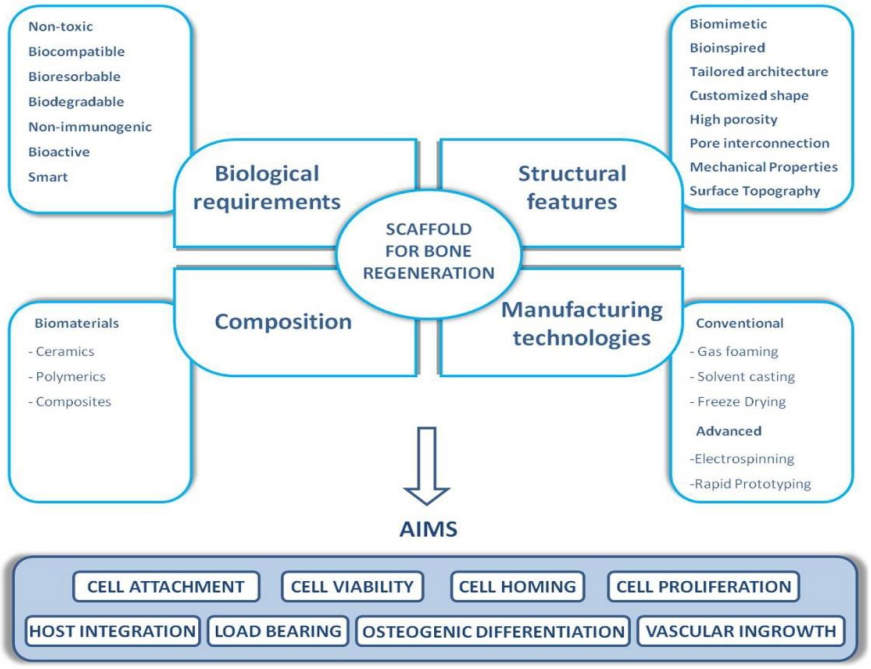

Figure 2: Flowchart illustrating features of an ideal scaffold for bone regeneration [7]

2.3 Materials for Bone Regeneration Scaffolds

Number of has been used for making scaffolds. Scaffolds with ideal properties are mostly made by a combination of polymer and bio-ceramics. The degradation rate of scaffold depends upon the mechanical strength of scaffold. Scaffolds with comparatively lower mechanical strength degrade more easily to be replaced by tissues than with higher mechanical strength. [8]

2.4 Polymeric Materials

Generally, polymeric materials provide more controllability on physiochemical characteristics of scaffolds such as pore size, porosity, solubility, biocompatibility, enzymatic reactions, and allergic response. Polymers can be natural or synthetic.

Natural Polymers used in scaffolds:

- collagen
- gelatin
- fibrinogen
- elastin
- keratin
- Silk
- Polysaccharides (glycosaminoglycan, cellulose, amylose, dextran, chitin,)
- Polynucleotides (DNA, RNA)
- Chitosan [9]

Synthetic Polymers used in Scaffolds are:

- Poly-Lactic Acid (PLA)
- Poly-Glycolic Acid (PGA)
- Poly-Caprolactone (PCL)
- TOPAS
- PMMA
- PE
- HPMC
- Polyurethane [10-11]

2.5 Different Polymers used in Bone Scaffolds

Gelatin: Gelatin is a mixture of peptides and proteins produced by hydrolysis of collagen extracted from the skin, bones, and connective tissues of animals. Gelatin exhibits enzymatic bio-degradability and is highly bio-compatible as it is derived from

collagen of living animals, water solubility, low immunogenicity, plasticity, adhesiveness, promotion of cell adhesion, growth, and cost economy, as well as the ability to form transparent gels under specific conditions. [10]

Chitosan: Chitosan is an amino polysaccharide, produced from the de-acetylation of chitin obtained mostly insects. It is widely used in medical fields because of its properties such as it behaves an anti-oxidant, strongly antibacterial and biocompatible. It has hemostasis ability which enables it to be used in wound healing applications.

Hydroxy propyl methylcellulose (HPMC): HPMC is linear chained polysaccharides. HPMC is used as a thickening agent, binder, film former, and hydrophilic matrix material. It also forms a thermo-reversible gel like gelatin and is bio-compatible. It is mostly used in capsules for drug delivery. [11]

2.6 Some Ceramics used in Bone scaffolds

Hydroxyapatite: HA coatings mostly as a filler material are used for bone tissues because its composition is similar to bone and teeth. They are mostly used as a composite with synthetic polymers such as PLA or PGA to reduce down the stress shielding effect. The native dissolution rate of hydroxyapatite in-vivo, around 10wt% per year, is significantly lower than the growth rate of newly formed bone tissue. [13-16]

- **Alumina:** Porous Alumina like other ceramics greatly resembles the structure of bone. Alumina shows high tensile strength in range of 290-310MPa this causes stress shielding effect and slows down durability. Alumina (Al2O3; Aluminum oxide) as the first clinical bio ceramics is highly consumed in orthopedic and dental implants due to its chemical inertness, resistance to oxidation, corrosion and biocompatibility.

2.7 Gelatin/HPMC BLENDS:

We would be using blends of gelatin and HPMC of varying compositions in order to form a bio-compatible and degradable scaffold. We would be carrying out variety

of characterizations such as XRD, contact angle measurement, SEM imaging, Tensile testing etc. to validate our results. [12]

2.7.1 Gelatin in Bio-medical

History

In 1682, a Frenchman named Denis Papin (1647-1712) recorded his research experiments on the subject. His experiments resulted in a method of removing the glutinous material from animal bones by boiling. It has no taste, no odor, and when combined with liquid, no color, but it is pure protein. After 1940's and during World War-2 Gelatin was excessively used in medical field. [13]

Types of Gelatin:

Gelatin is commonly used for pharmaceutical and medical applications because of its enzymatic biodegradability and biocompatibility in physiological environments. Of the two types, acidic and alkaline gelatin, the former has an isoelectric point similar to collagen. The isoelectric point depends on its extraction procedure from collagen, and variations in it allow gelatin to bind with either positively or negatively charged therapeutic agents.

Composition:

Gelatin composition depends upon amino acid content. On hydrolysis gelatin contains about 19 amino acids.

Table 1

Amino Acid	Percentage
Proline or Hydroxy proline	25%
Glycine	20%
Glumatic acid	11%
Arginine	8%
Alanine	8%
Other non-essential amino acids	12%
Other essential amino acids	16%

2.7.2 Properties of Gelatin

- Gelatin readily dissolves in hot water and forms a thermo reversible gel on cooling.
- Gelatin hydrogels closely resemble some essential properties of native extracellular matrix (ECM) due to the presence of cell-attaching and matrix metalloproteinase responsive peptide motifs, which allow cells to proliferate and spread in Gelatin-based scaffolds.
- It crosslinks on irradiation with light to form hydrogels.
- A hybrid network can be formed by combining it with nano-particles such as CNT's , Silica, Alumina , SiC etc
- Gelatin is highly bio-compatible.
- Can be broken down easily by enzymes in body. [14]

2.7.3 Production

Most gelatins are derived from pork skins, pork and cattle bones, or split cattle hides. Gelatin made from fish by-products avoids some of the religious objections to gelatin consumption.

The manufacturing processes of gelatin consist of several main stages:

- Pretreatments to make the raw materials ready for the main extraction step and to remove impurities that may have negative effects on physicochemical properties of the final gelatin product.
- Hydrolysis of collagen into gelatin.
- Extraction of gelatin from the hydrolysis mixture, which usually is done with hot water or dilutes acid solutions as a multistage process.
- The refining and recovering treatments including filtration, clarification, evaporation, sterilization, drying, rutting, grinding, and sifting to remove the water from the gelatin solution, to blend the gelatin extracted, and to obtain dried, blended, ground final product. [9-13]

2.8. Hydroxyl propyl methylcellulose (HPMC)

Hypromellose (INN), short for hydroxyl propyl methylcellulose (HPMC), is a semisynthetic, inert, viscoelastic polymer used as eye drops, as well as an excipient and controlled-delivery component in oral medicaments, found in a variety of commercial products.

Properties

- Superior film-forming capability
- Good biocompatibility
- Biodegradability
- It is usually used in the pharmaceutical industry as a drug delivery matrix (film or gel) and in the food industry as a film former, emulsifier, stabilizer, or thickening agent.

HPMC is a nonionic and water-soluble polymer, this enables it to be used for drug delivery specially capsules. [11-15]

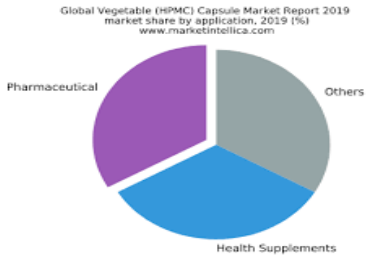

Figure 3: Illustrating wide range of HPMC application in Pharmaceutical Industry [15]

2.9 Blend Formation

Gelatin and HPMC are two of most bio-compatible polymers that are formed from natural products. They both exhibit lower strength individually but this disadvantage is overcome by making a composite of different combinations of compositions. The main of this composite blend is to provide a platform where cells can attach and form tissues. As this scaffold is used for bone tissue regeneration so we take gelatin as one component because of its ability to attach cells more efficiently and degrade at room

temperature. While HPMC is used because it is hydrophilic in nature allows good adhesion and it has the ability to carry drug up to 98% for 24 weeks in accelerated conditions. This property is very essential as when the implant is placed an infection might occur so drug encapsulation is necessary. [13-14]

The filler material in the composite film is nanoparticles of alumina. Because it slows down the degradation rate of blend so that the scaffold does not degrade more quickly before the formation enough tissue which can itself provide a support to healing bone.

The procedure for film formation has been explained in detail in the next section.

CHAPTER 3

EXPERIMENTAL METHODOLOGY AND CHARACTERZATIONS

3.1 Materials

- DIEJUNG granular Gelatin
- HPMC Powder

3.2 Experimental Methodology

All the components were available so we just perform the experiment by selecting a different ratio of Gelatin to HPMC. Pure films and then blends were made to check the optimum value of tensile strength.

3.2.1 Procedure

For pure films:

The following procedure was followed:

1. Take 2gram powder of Gelatin and pour it in 20 ml of distilled water and stir it for around 20 mints at room temperature.
2. Similarly, take 2gram of HPMC and pour it in 20 ml of distilled water and stir it for around 25 mints.
3. Firstly, heat the distilled water at around 70'C and after that pour the powder of HPMC in it so that it will completely dissolve in it and don't make suspension.
4. After that, cast the viscous liquid in separate petri dishes and allow it to solidify (It will take one day to solidify).
5. After the films gets solidify, peel it off carefully from the petri dishes.

For making blends of different compositions in total of 2 grams:

1. Heat the distilled water at around 100'C in media bottle and pour 70% G and 30% HPMC in a total of 2 gram in 20ml of water.
2. Stir it around 25 mints while maintaining the temperature.
3. Cast it in petri dish and allow it to solidify.

After that

4. We take 50% of G and 50% of HPMC in a total of 2 gram in 20 ml of water and follow the previous procedure. [16]

We tried different combination of compositions:

1. 70 G + 30 HPMC
2. 30 G + 70 HPMC
3. 50 G + 50 HPMC
4. 90 G + 10 HPMC
5. 95 G + 5 HPMC
6. 97 G + 3 HPMC
7. 99 G + 1 HPMC
8. 99.9 G + 0.1 HPMC

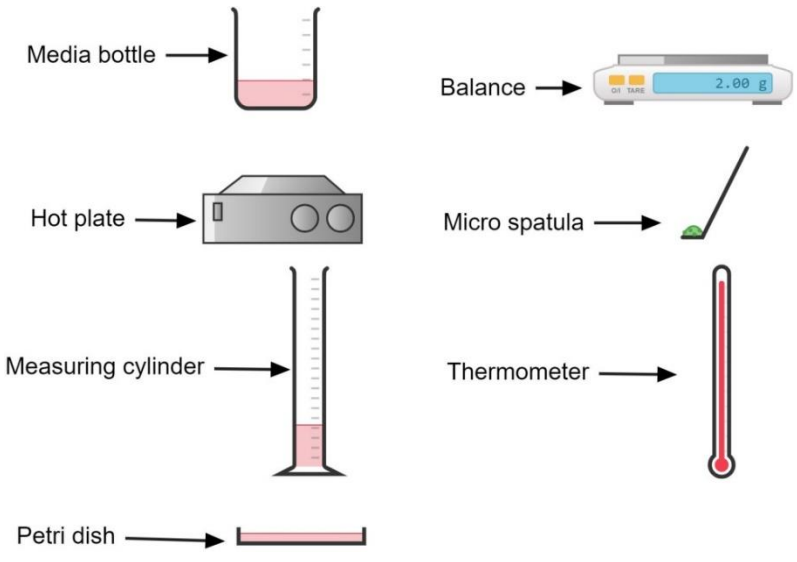

Figure 4: Illustrating the apparatus (Glassware) used in the experiment. [17]

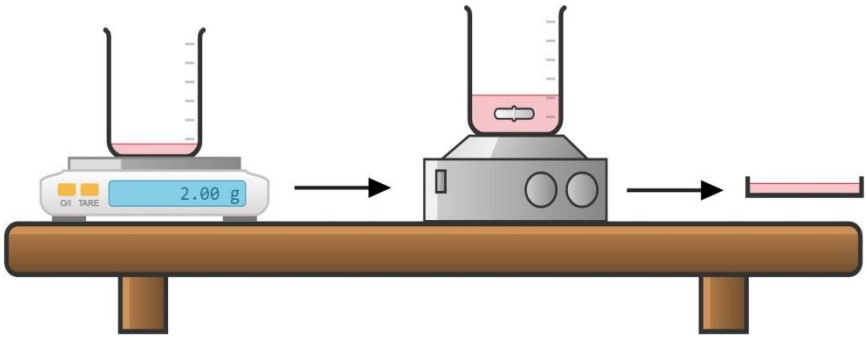

Figure 5: Schematic of blends preparation [18]

Table 2: Summary

Sr. no	Gelatin	HPMC	Temp	Stirring time	Drying	Total solution
1	30	70	100C	20min	Air dry	2gm in 20 ml water
2	50	50	100C	20min	Air dry	2gm in 20 ml water
3	70	30	100C	20min	Air dry	2gm in 20 ml water
4	90	10	100C	25min	Under fan	2gm in 20 ml water
5	95	5	100C	25min	Under fan	2gm in 20 ml water
6	97	3	100C	25min	Under fan	2gm in 20 ml water
7	99	1	100C	25min	Under fan	2gm in 20 ml water
8	99.9	0.1	100C	25min	Under fan	2gm in 20 ml water

3.3 Characterizations

3.3.1 Mechanical Properties

The mechanical properties of pure films and blends were determined according ASTM standard D882. The testing machine was equipped with 50kg load cell. Testing was done to determine the tensile strength of blends because when dealing with soft tissues some strength is required to retain the load.

Procedure:

- Sample of both blends and pure films prepared with dimensions 27mm×7mm. With thickness of 1mm.
- Three samples for each composition were prepared.
- Use a paper cutter or scissor to cut out the sample.
- Samples of blends free from physical defects were attached to tensile grips.
- The test speed was set at 50mm/min.
- Data was recorded and graph was plotted on ORIGIN software to determine tensile strength and elongation.

3.3.2 Surface Roughness

Surface roughness of blends was determined using an Optical Profilometer. Optical Profilometer uses the properties of wavelength of light to determine the path difference of waves on test surface. Optical profilometry was used because it is non-contact type and prevents the blends from damage as compared to stylus type. Surface roughness is important because as roughness increases cell attachment to an appreciable value. If roughness is very high proteins on the surface tend to coagulate and cell attachment reduces. So, we require a moderate value of roughness for our application.

Procedure:

- Cut out small samples of blends roughly of rectangular shape.
- Use a double side tape to stick the sample on glass slide.
- Place it under the platform of Profilometer.
- Start the software.
- Record the height and intensity profiles of samples.
- Determine the average roughness of each sample.

3.3.3 Contact Angle Measurement

The contact angle of polymeric blends and pure films was measured was measured using an optical tensiometer. In order to enhance cell attachment surface should be hydrophilic. In literature contact angle less than 90º is considered hydrophilic. So, contact angle measurement was important to determine whether our blends have enough hydrophilicity to support cell attachment or not.

Procedure:

- Cut out small samples of blends roughly of rectangular shape.
- Use a double side tape to stick the sample on glass slide.
- Place it under the platform of Tensiometer.
- An ultrapure water droplet of 5µL is dripped onto the surface of blend by a precision microsyringe.
- Record the image of drop deposited on surface by Attension Theta analysis software.

3.3.4 Scanning Electron Microscopy

Surface morphology of blends was observed using a TM300 microscope. This was done to observe the general morphology of films for comparison.

Procedure:

- Cut out small samples of blends roughly of rectangular shape.
- Use a double side tape to stick the sample on glass slide.
- Images were captured at 250X,500X,1000X,2500X,5000X,7500X,10000X and 15000X respectively.
- Acceleration voltage was set at 10kV for the analysis of surface.
- Analyze and compare the images.

CHAPTER 4

Results and Discussion

4.1 Tensile Testing Results

Results of samples were obtained with respective composition. The data was then plotted on Origin to get a graphical representation. Tensile testing was determined to evaluate the mechanical properties of blends. Our research's main focus is on enhancement of mechanical properties. Good mechanical properties are required to bear load in case of soft tissues and also a comparable value in case of hard bone tissues. Simultaneously, we would be selecting a composition have tensile strength comparable to bone to avoid stress shielding as well.

4.1.1 Pure Gelatin

According to the previously mentioned procedure mechanical testing of Pure Gelatin film of 2g by weight was carried out. Maximum strength of pure Gelatin film was around 18 MPa which is relatively very low.

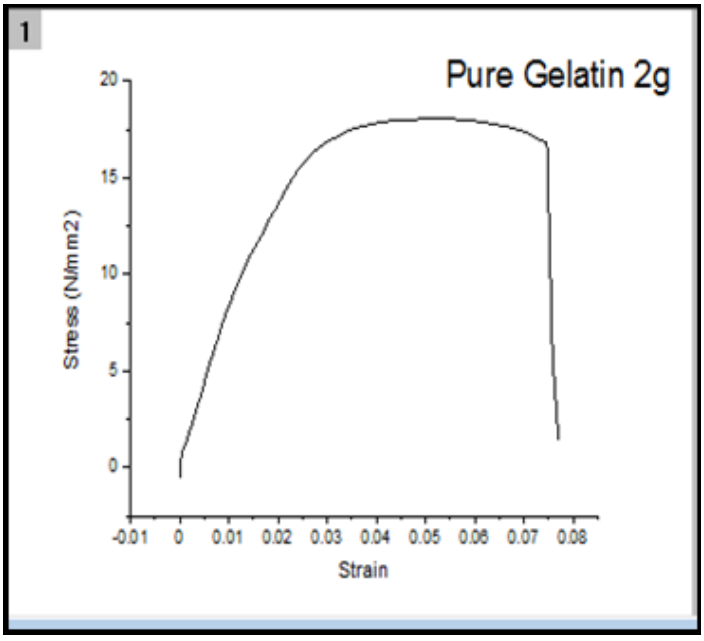

Figure 6: Tensile Strength of Pure Gelatin

4.1.2 Combined Graph (70%HPMC , 50%HPMC ,30%HPMC,10%HPMC)

When the HPMC content is high strength of the blend decreases considerably because HPMC is has naturally low density and the hydroxyl group has very weak hydrogen bonding between adjacent layers.

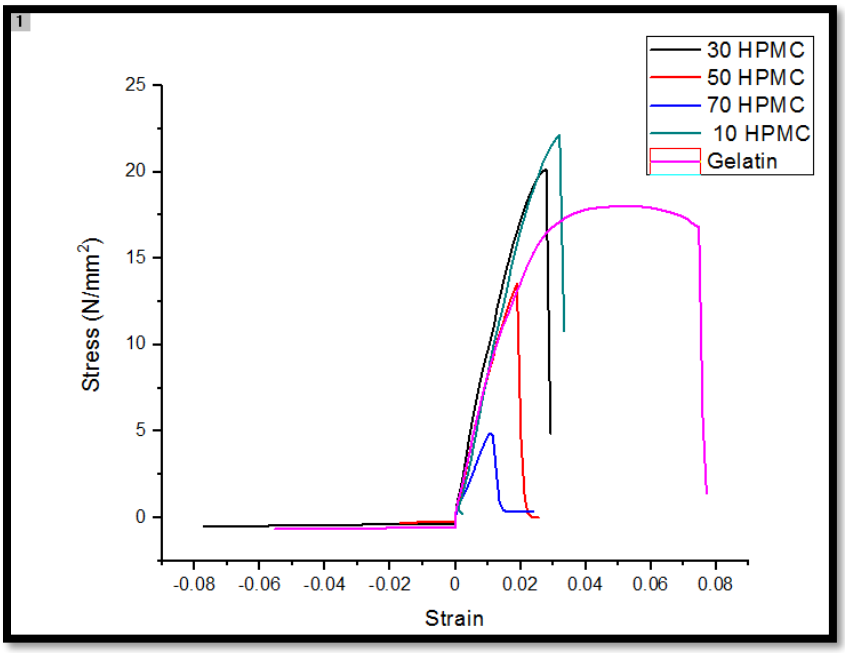

Figure 7: Effect of very high concentration of HPMC on strength

4.1.3 99% Gelatin and 1%HPMC

In the second phase of our experiments we tested 1% ,3% and %5 HPMC content. The strengths were much higher as compared to previous ones. 1% HPMC shows a TS of 44 MPa.

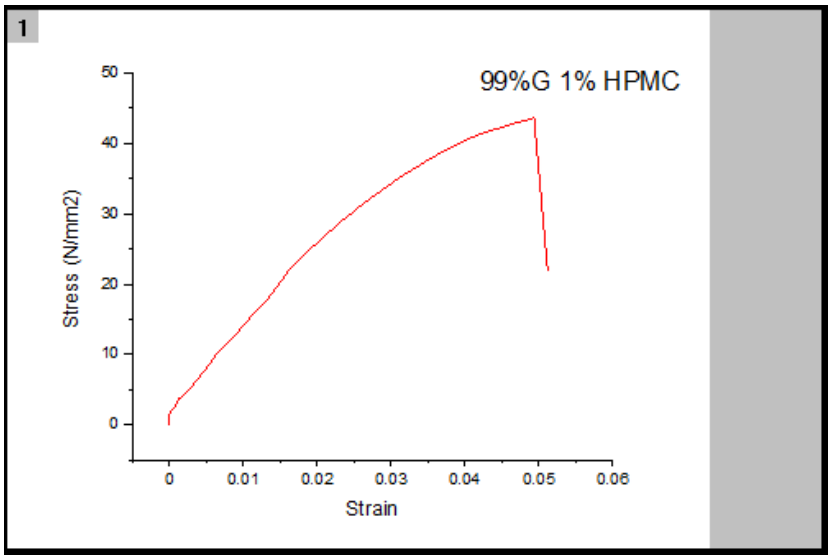

Figure 8: Tensile Strength of 99% Gelatin and 1% HPMC blend

4.1.4 97% Gelatin and 3% HPMC

When the concentration of HPMC was increased to 3% the tensile strength increased was increased to 48MPa.

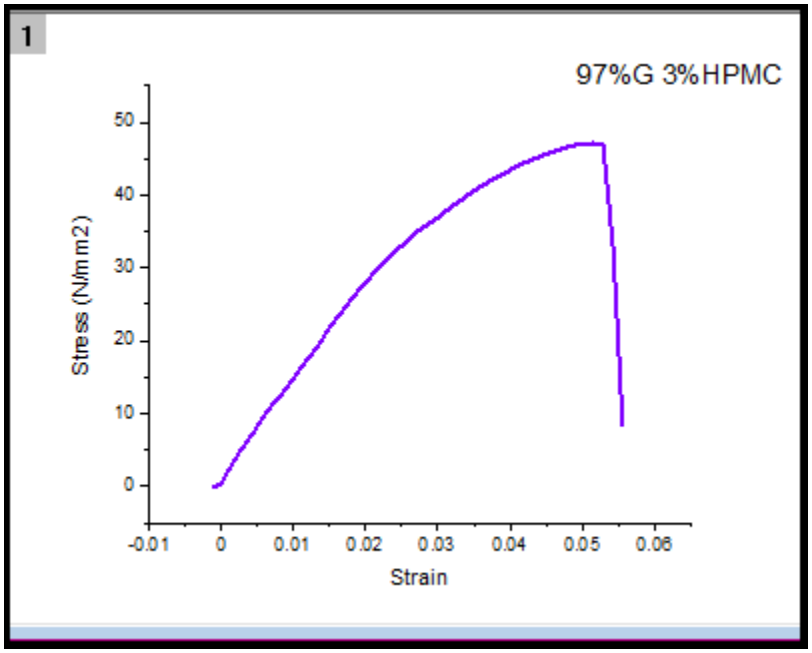

Figure 9: Tensile Strength of 97% Gelatin and 3% HPMC

4.1.5 95% Gelatin and 5% HPMC

When the HPMC content was increased to 5% the blend showed highest strength of about 52 MPa which considerable good strength. Strength should be enough to withstand mechanical damage during production, handling and application.

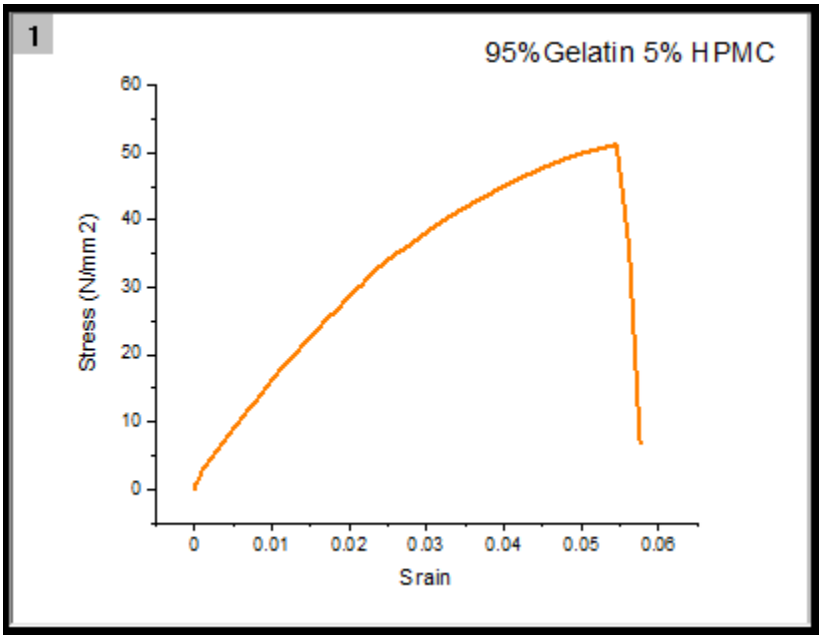

Figure 10: Tensile Strength of 95% Gelatin and 5% HPMC

4.1.6 Combined Graph (1% HPMC,3%HPMC and 5%HPMC)

When the graph of these three compositions and pure gelatin was combined it was observed that a trend was being followed TS increased from 1% HPMC content and reached a maximum value at 5% HPMC content. After this strength started to decrease. This increase occurs due to increase in adjacent polymer interactions. This results in formation of numerous protein-protein bonds which results in high cohesion and low flexibility. Furthermore, the flexibility of Gelatin is much lower than HPMC.

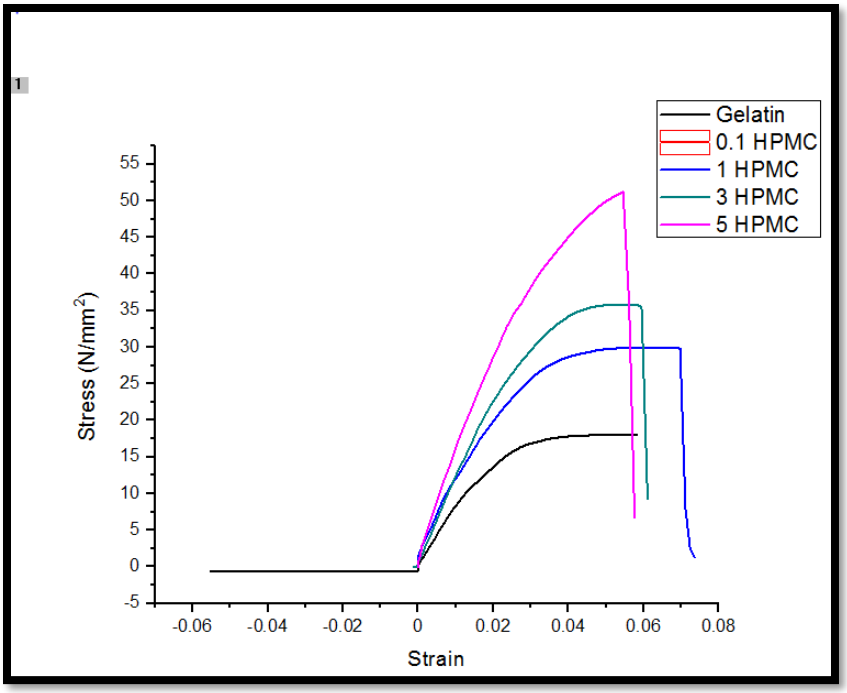

Figure 11: Trend for increasing HPMC content to 5%

Due to this fact we selected our optimum composition of 95% Gelatin and 5% HPMC.

Optimum Composition Selected= 95% Gelatin and 5%HPMC

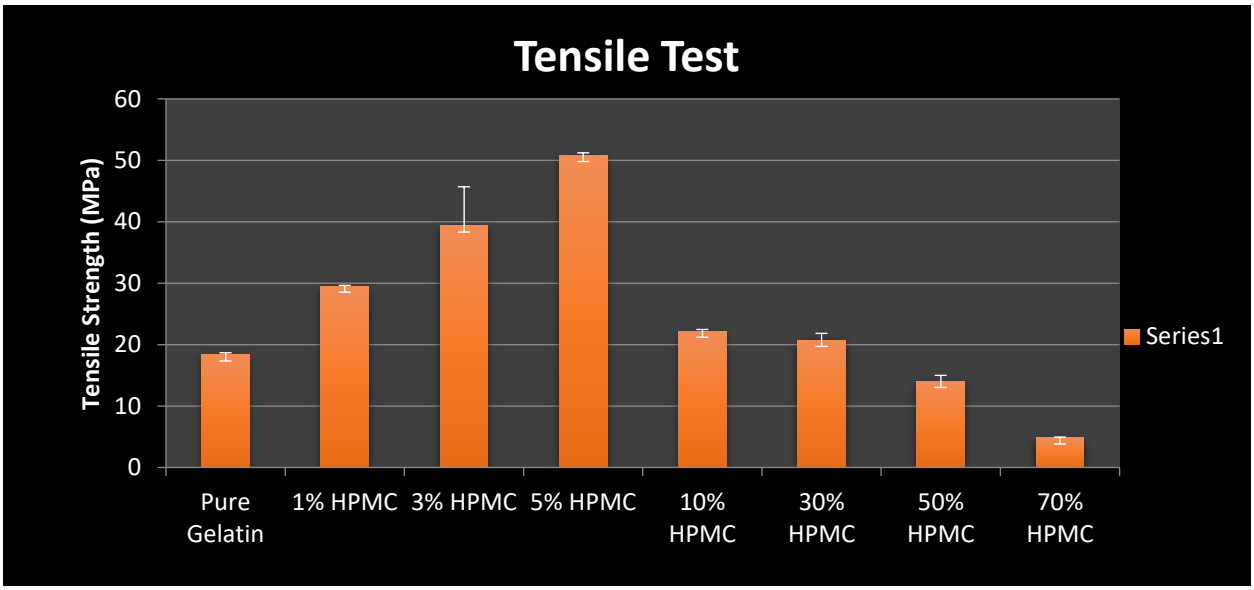

Figure 12: Bar chart showing trends for increasing HPMC content

4.2 Surface Roughness Results

Surface roughness is a very important feature when dealing with biomedical applications. Surface roughness greatly enhances cell attachment. It provides a site where cells can attach and proliferate to form tissues. According to the previously mentioned procedure we evaluated the surface roughness. Our major focus would be on our optimum composition of 95% Gelatin and 5% HPMC.

4.2.1 Pure Gelatin Surface Roughness

The average surface roughness of pure gelatin is 5.22µm which is lower than an average cell size in body is larger so it would be difficult for cell to incorporate in such surface features.

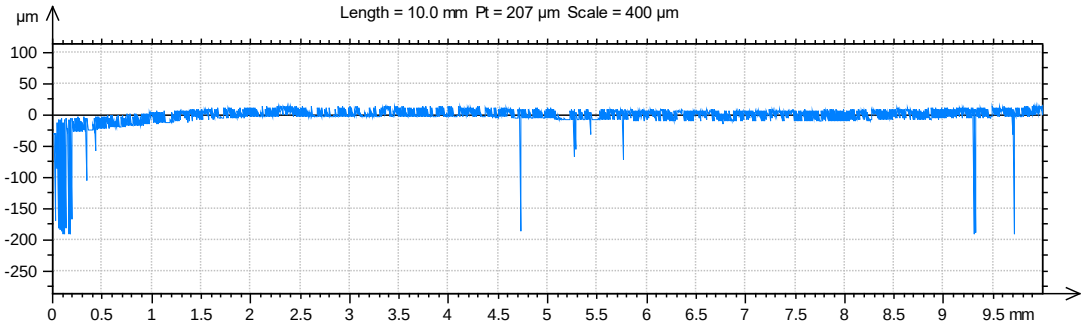

Figure 13: Height Profile of Pure Gelatin Film

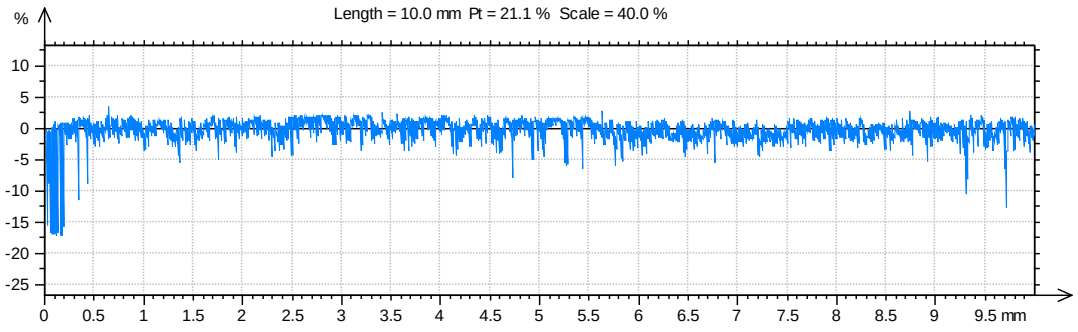

Figure 14: Intensity Profile of Pure Gelatin Film

Table 3: Roughness Profile of Pure Gelatin

ISO 4287			
Amplitude Parameters- Roughness profile			
Rp	11.5	µm	Gaussian Filter, 0.8mm
Rv	34.2	µm	Gaussian Filter, 0.8mm
Rz	45.7	µm	Gaussian Filter, 0.8mm
Rc	15	µm	Gaussian Filter, 0.8mm
Rt	206	µm	Gaussian Filter, 0.8mm
Ra	5.22	µm	Gaussian Filter, 0.8mm
Rq	6.39		Gaussian Filter, 0.8mm
Rsk	-1.27		Gaussian Filter, 0.8mm
Rku	21.3		Gaussian Filter, 0.8mm
Material Ratio parameters- Roughness profile			
Rmr	0.0738	%	c= 1 µm under the highest peak, Gaussian Filter, 0.8mm
Rdc	11.3	µm	p= 20%, q= 80%, Gaussian Filter, 0.8mm

4.2.2 Pure HPMC Surface Roughness

While in case of pure HPMC average roughness is about 18.4µm which is much higher than size of average cell. When roughness is very high proteins responsible for cell attachment tend to coagulate and cell adhesion is decreased.

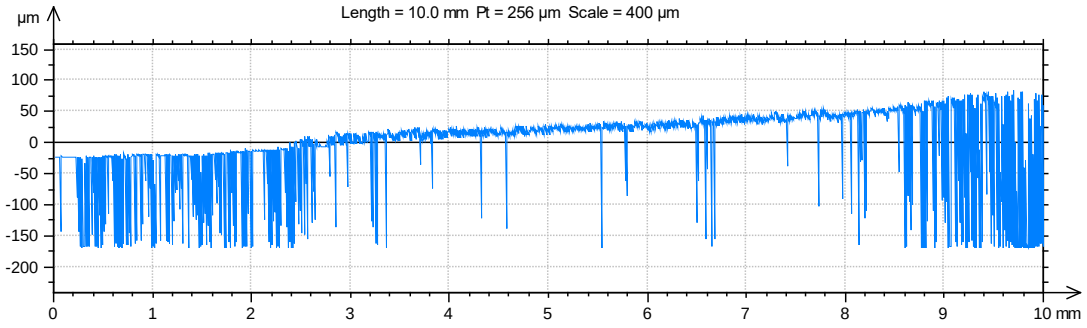

Figure 15: Height Profile of Pure HPMC Film

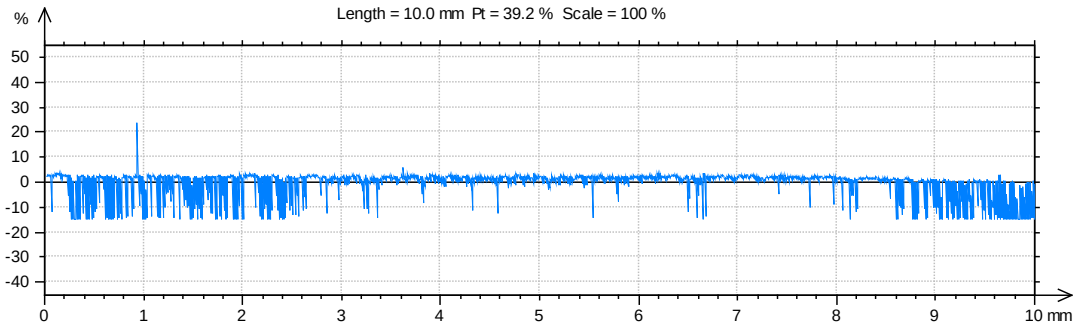

Figure 16: Intensity Profile of Pure HPMC Film

Table 4: Roughness Profile of Pure HPMC Film

ISO 4287			
Amplitude Parameters- Roughness profile			
Rp	26.5	µm	Gaussian Filter, 0.8mm
Rv	147	µm	Gaussian Filter, 0.8mm
Rz	174	µm	Gaussian Filter, 0.8mm
Rc	80.4	µm	Gaussian Filter, 0.8mm
Rt	310	µm	Gaussian Filter, 0.8mm
Ra	18.4	µm	Gaussian Filter, 0.8mm
Rq	28.3		Gaussian Filter, 0.8mm
Rsk	-4.8		Gaussian Filter, 0.8mm
Rku	44.9		Gaussian Filter, 0.8mm
Material Ratio parameters- Roughness profile			
Rmr	0.0109	%	c= 1 µm under the highest peak, Gaussian Filter, 0.8mm
Rdc	27.1	µm	p= 20%, q= 80%, Gaussian Filter, 0.8mm

4.2.3 95% Gelatin 5% HPMC Surface Roughness

When we take a look at surface roughness of our optimum composition it has an average surface roughness of around 12.1µm. While, in literature the average bone cell size is about 8µm. Thus, cells are smaller and can be incorporated in the roughness sites. This increases cell attachment on the film. This validates the fact that the optimum composition selected also shows reasonably good surface properties.

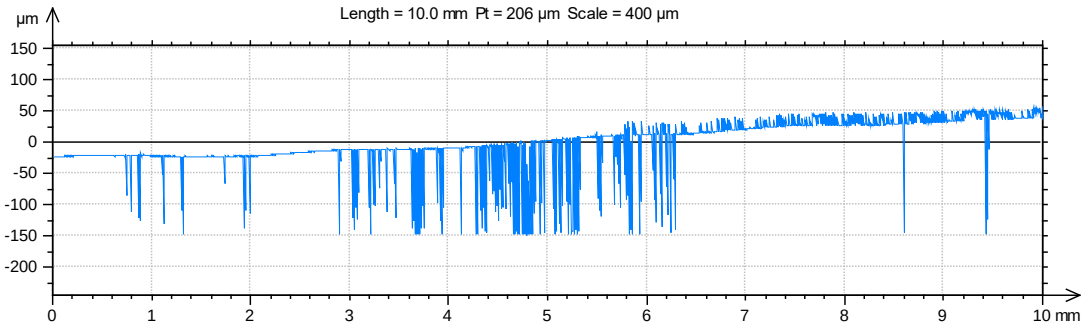

Figure 17: Height Profile of 95% Gelatin 5% HPMC

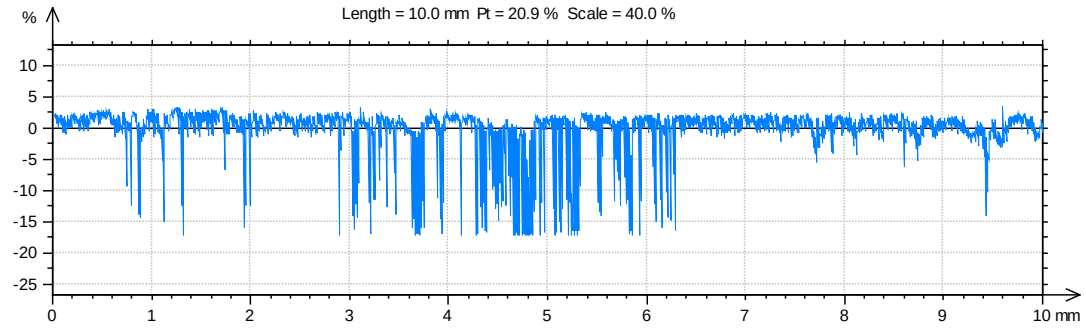

Figure 18: Intensity Profile of 95%Gelatin 5%

Table 5: Roughness Profile of 95%Gelatin 5%HPMC

ISO 4287			
Amplitude Parameters- Roughness profile			
Rp	19.5	µm	Gaussian Filter, 0.8mm
Rv	97.8	µm	Gaussian Filter, 0.8mm
Rz	117	µm	Gaussian Filter, 0.8mm
Rc	68.7	µm	Gaussian Filter, 0.8mm
Rt	240	µm	Gaussian Filter, 0.8mm
Ra	12.1	µm	Gaussian Filter, 0.8mm
Rq	19.2		Gaussian Filter, 0.8mm
Rsk	-2.88		Gaussian Filter, 0.8mm
Rku	26.8		Gaussian Filter, 0.8mm
Material Ratio parameters- Roughness profile			
Rmr	0.0363	%	c= 1 µm under the highest peak, Gaussian Filter, 0.8mm
Rdc	13.6	µm	p= 20%, q= 80%, Gaussian Filter, 0.8mm

4.3 Contact Angle Measurement

In literature, the contact angles of HPMC and Gelatin blends lie between 54.3º to 104.4º. Our optimum composition of 95% Gelatin and 5%HPMC has a contact angle of about 86º± 3º. As the angle is less than 90º this makes the blend hydrophilic thus, due higher wetting cell attachment onto the surface gets increased. Contact angle also allows us to control cell behavior via controlling protein adsorption onto the surface. HPMC shows more affinity for water than Gelatin. Both polymers contain polar groups but HPMC shows more prominent hydroxyl groups thus increasing its wettability. Hydrophilicity in blends increased with the addition of HPMC content but strength was decreased significantly. So, our optimum composition just comprises of 5% HPMC by weight. In short, contributing to both hydrophilicity and strength of blend. Contact angle also depends on the surface roughness, it increases as surface roughness increases. Thus, the heterogeneity in films also effects the contact angle. Likewise, in the blend with optimum composition showing average roughness of 12.1µm also enhances its wettability.

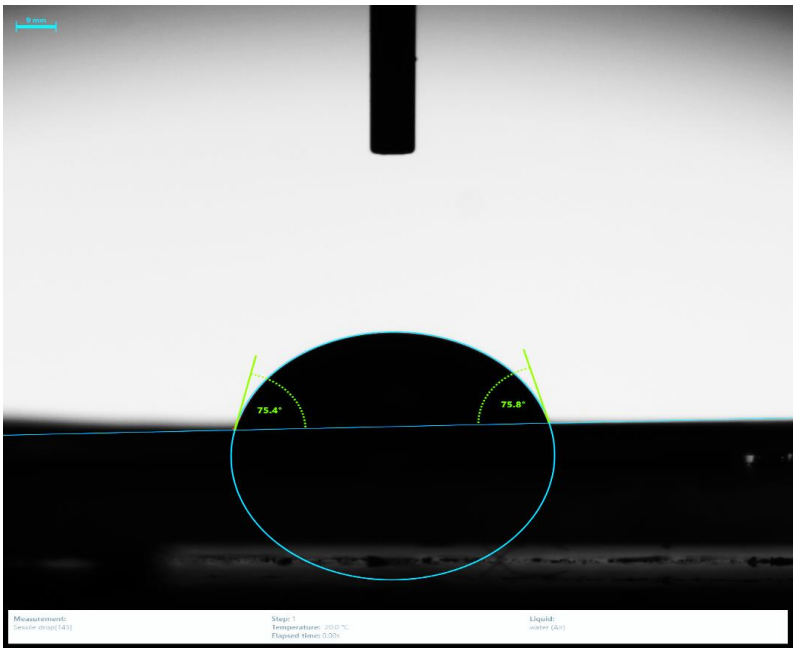

Figure 19: Contact Angle of Pure Gelatin

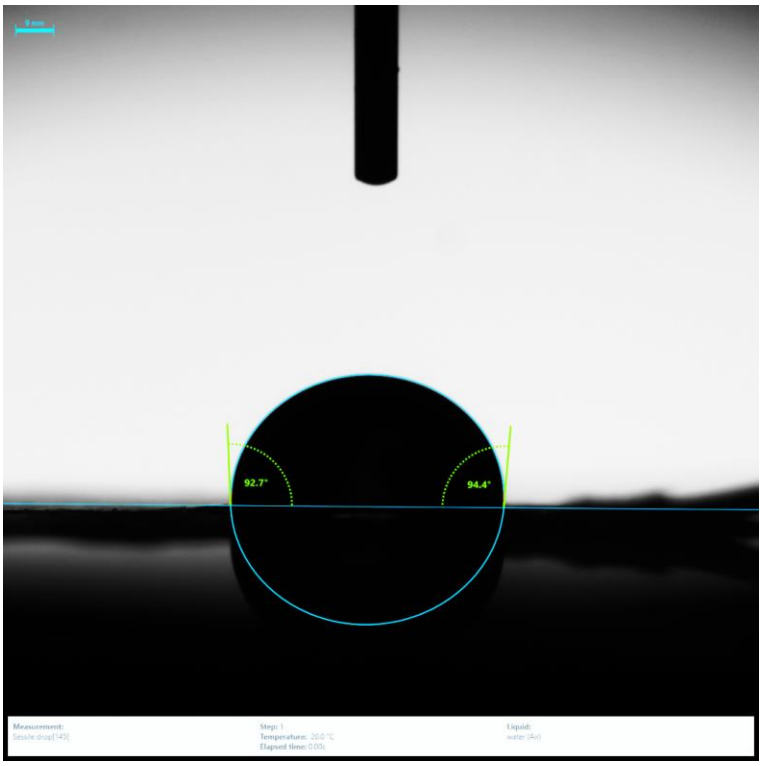

Figure 20: Contact Angle of Pure HPMC

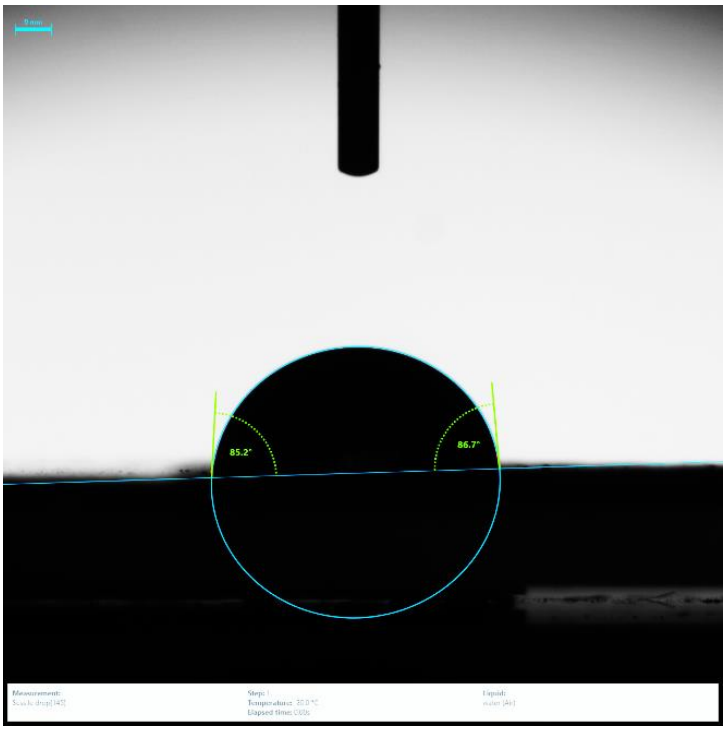

Figure 21: Contact Angle of 97%Gelatin and 3%HPMC

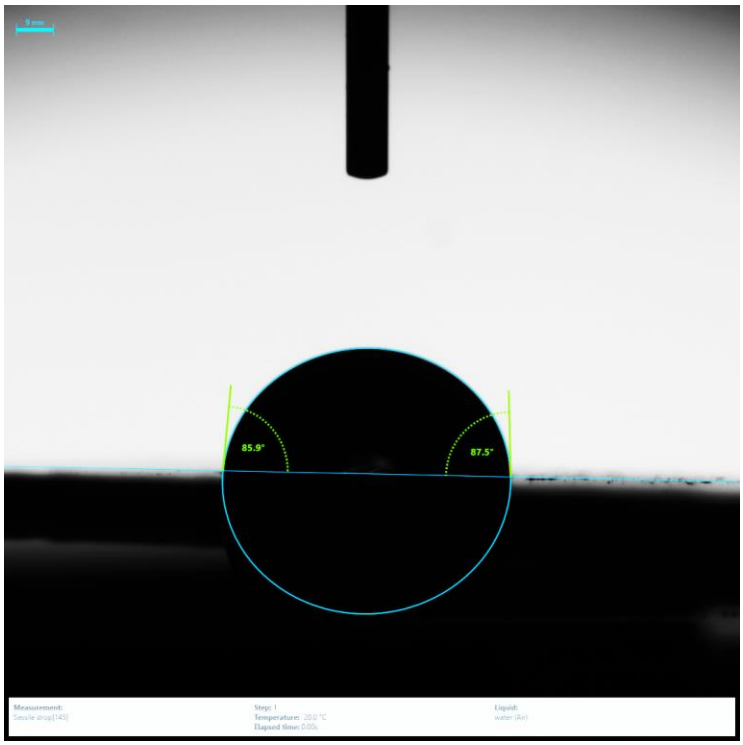

Figure 22: Contact Angle of 95%Gelatin and 5%HPMC

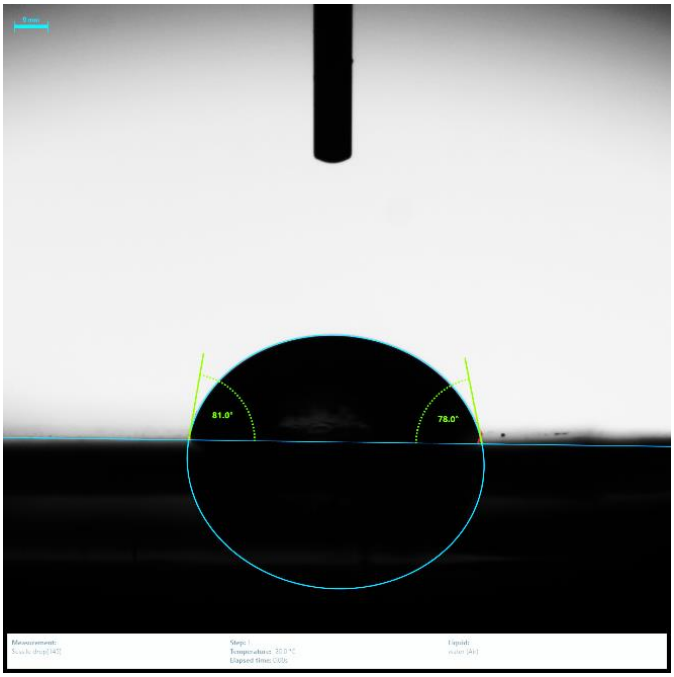

Figure 23: Contact Angle of 99%Gelatin and 1%HPMC

4.4 SEM Results

SEM analysis was carried out according to the above mentioned procedure to determine surface topography. In case of Pure Gelatin and HPMC films smooth surfaces were observed. However, in case of blends discontinuous areas were observed as HPMC content was increased due to increase in immiscibility of HPMC and Gelatin. Matrix density decreases with increase in discontinuous areas. At higher HPMC contents phase segregation occurs which reduces the strength of blends. So, we can also conclude that Gelatin shows good miscibility with HPMC at 5% of HPMC weight content.

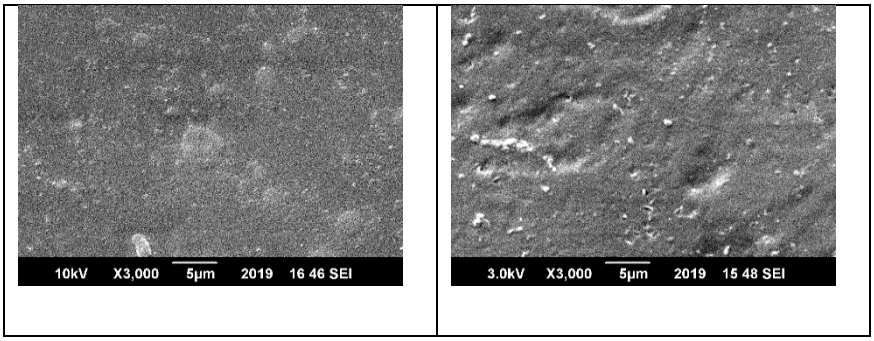

Figure 24: SEM result of Pure Gelatin and Pure HPMC films at 3000X

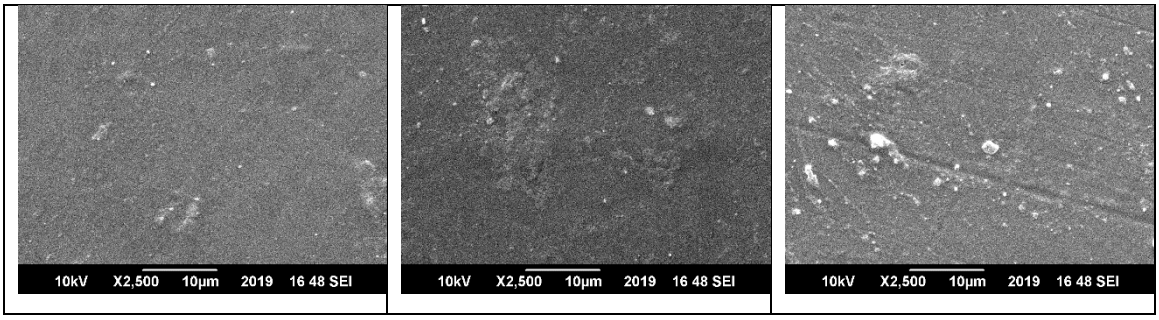

Figure 25: SEM results of 1%, 3% and 5% HPMC blends respectively at 2500X

CONCLUSION

The blends of HPMC and Gelatin were observed to be most suitable for biomedical based applications such as for bone tissue regeneration. Different composition of blends was made and then selects the most optimum value based on mechanical properties. Both HPMC and Gelatin are biocompatible and biodegradable as well, so they can easily mixed and form a blend. The maximum tensile strength of pure 2g gelatin is 18Mpa which is very low. So by adding a suitable amount of HPMC would impart greater strength for load bearing applications. When the HPMC content is high, strength of the blend decreases considerably because HPMC is has naturally low density and the hydroxyl group has very weak hydrogen bonding between adjacent layers.

In the second phase of our experiments we tested 1%, 3% and 5% HPMC content. The strengths were much higher as compared to previous ones. 1% HPMC shows TS of 44Mpa. When the concentration of HPMC was increased to 3% the tensile strength increased was increased to 48MPa. When the HPMC content was increased to 5% the blend showed highest strength of about 52MPa which considerable good strength. Strength should be enough to withstand mechanical damage during production, handling and application.

When comparing the overall results of different compositions of HPMC/Gelatin and pure 2g Gelatin, it is noticed that a trend was being followed TS increased from 1% HPMC content and reached a maximum value at 5% HPMC content. After this, strength started to decrease. This increase occurs due to increase in adjacent polymer interactions. This results in formation of numerous protein-protein bonds which results in high cohesion and low flexibility. Furthermore, the flexibility of Gelatin is much lower than HPMC. For this reason, we selected our optimum composition of 5% HPMC and 95% Gelatin having tensile strength corresponds to 52Mpa.

For biomedical applications, surface roughness is also an important factor. Surface roughness greatly enhances cell attachment. It provides a site where cells can

attach and proliferate to form tissues. According to the previously mentioned procedure we evaluated the surface roughness. Our major focus would be on our optimum composition of 95% Gelatin and 5% HPMC. The average surface roughness of pure gelatin is 5.22µm which is lower than an average cell size in body is larger so it would be difficult for cell to incorporate in such surface features. While in case of pure HPMC average roughness is about 18.4µm which is much higher than size of average cell size. When roughness is very high proteins responsible for cell attachment tend to coagulate and cell adhesion is decreased. When we take a look at surface roughness of our optimum composition it has an average surface roughness of around 12.1µm. While, in literature the average bone cell size is about 8µm. Thus, cells are smaller and can be incorporated in the roughness sites. This increases cell attachment on the film. This validates the fact that the optimum composition selected also shows reasonably good surface properties.

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SEM analysis was carried out according to the above mentioned procedure to determine surface topography. In case of Pure Gelatin and HPMC films smooth surfaces were observed. However, in case of blends discontinuous areas were observed as HPMC content was increased due to increase in immiscibility of HPMC and Gelatin. Matrix density decreases with increase in discontinuous areas. At higher HPMC contents phase segregation occurs which reduces the strength of blends. So,

we can also conclude that Gelatin shows good miscibility with HPMC at 5% of HPMC weight content.

By looking at all the above mentioned data and results, we conclude at the best possible optimum value of blend which is 95G/5HPMC for bone tissue regenerations.

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