FABRICATION OF SILK-BASED COMPOSITE SCAFFOLD FOR BONE-LIGAMENT-BONE GRAFT USING AQUEOUS POLYMERIC DISPERSION TECHNIQUE

A THESIS SUBMITTED FOR PARTIAL FULFILLMENT OF
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BACHELOR OF TECHNOLOGY
IN
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By

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CERTIFICATE

This is to certify that the project titled, "**Fabrication of Biodegradable silk-based composite scaffold for Bone-Ligament-Bone graft using Aqueous Polymeric Dispersion (APD) technique**" submitted by **Suraj Prakash Behera** is an authentic work carried out by him under my supervision and guidance for the partial fulfilment of the requirements for the award of **Bachelor of Technology (B. Tech) Degree in Biotechnology** at **National Institute of Technology, Rourkela.**

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

Date: 12/05/2014
Place: Rourkela

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"*Knowledge Is Incomplete Without Motivation & Direction***"**

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ABSTRACT

Tissue engineering is a promising technology for treating tissue defects or replacing non-functional tissues/organs. It relies upon a temporary scaffold that is basically an artificial structure which provides the support for 3D tissue formation or organogenesis. Ideally, scaffolds should be able to accommodate human cells, orchestrate their growth and differentiation leading to tissue regeneration and ultimately makes it feasible for implantation. Major sports injuries involve the damage of cartilages, ligaments, tendons and the enthesis (also known as an 'insertion site', or an 'osteotendinous' or 'osteoligamentous' junction). Thus, enormous effort is being given to musculoskeletal tissue engineering by the researchers globally. Since ligament injury is most common and ligament-alone grafts are not so successful to replace the injured ligaments, the researchers are experimenting with the construction of a composite scaffold which can guide the stem cells to differentiate into fibrocartilage that bridges of Bone-Ligament interface i.e. enthesis. In the current project, a composite silk-based scaffold was fabricated by incorporating multiple compartments for B-L-B graft. The core scaffold was prepared by knitting the silk fibers (from *Bombyx mori*) to provide required mechanical strength. The individual compartments over the knitted scaffold were coated with specific biocompatible components (i.e. hydroxyapatite for bone, Polyethylene oxide and & Polylethylene glycol for ligament and cartilage) blended with gelatin using Aqueous Polymer Dispersion (APD) Technique. The morphology of fabricated scaffolds was studied under optical microscope and SEM (Scanning Electron Microscope) while the mechanical properties were analysed through the Texture Analyzer. The particle sizes were found to be between 10-1000 nm. It was concluded that silk based multi-compartmental scaffolds fabricated from APD technique are suitable for enthesis tissue engineering due to their porosity and matching mechanical properties. However, the scaffolds need to be confirmed for their bioactivity by culturing live cells on respective compartments.

Keywords: Enthesis, Tissue Engineering, Aqueous Polymer Dispersion, Silk-based Scaffold, Gelatin

CHAPTER 1

1. INTRODUCTION:

1.1 IMPORTANCE OF ENTHESIS:

Entheses are also known as the osteoligamentous junctions, osteotendinous junctions or insertional sites as they are the connective tissues joining the tendon or ligament with the bone and act as the sites of stress concentration. Because of acting as stress concentration at the hard-soft tissue interface, entheses are very much sensitive to acute injuries during sports. In orthopedic reconstruction surgery, a significant challenge arises in constructing an extended functional integration of soft tissue grafts with subchondral bone. Entheses are of great clinical significance while constructing a graft for a damaged ACL (Anterior Cruciate Ligament) where there is a big challenge of reattaching the ligament or tendon to the bone for achieving musculoskeletal motion. According to the type of tissues present at the attachment site, there are two types of entheses i.e. fibrous entheses and fibrocartilaginous entheses. As the name suggests in case of fibrocartilaginous entheses there will be differentiation of cartilage tissue which is absent in case of fibrous entheses.

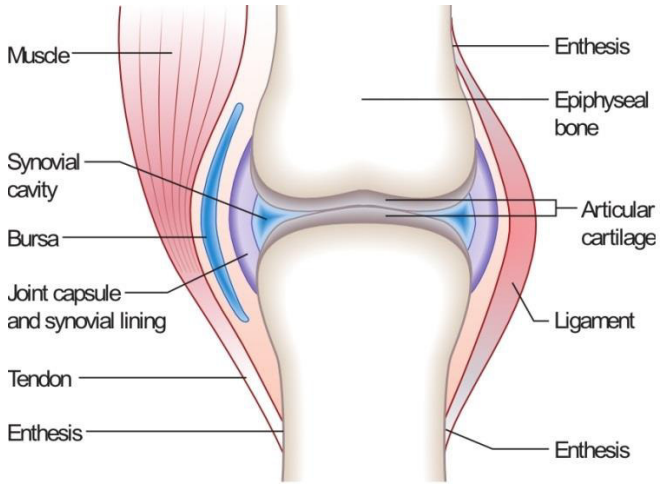

Figure 1.1 Synovial Joint

1.2 TISSUE ENGINEERING:

All over the world, there is great demand of donors for organ transplantation which is very rare to find. Because the transplanted organs are also not very biocompatible, there is a need to get organs from the individual by regenerating cells which is

achievable by tissue engineering. Tissue engineering or Regenerative medicine is the most promising technique involving medicine, biology and engineering used for the treatment of the damaged tissues by repairing or regenerating the tissues with increased maintenance and function. With the effective therapeutic applications, tissue engineering has a great role in diagnostic applications where tissues are cultured in vitro and utilized for testing drug uptake & metabolism, their toxicity level and pathogenicity level.

Tissue engineering basically involves four areas as follows:

a) Collection of Stem cells

b) Construction of biocompatible and biodegradable scaffold

c) Seeding of stem cells over the scaffold and supply of required nutrients for their proliferation and differentiation inside a bioreactor.

d) Preservation of implants for future use.

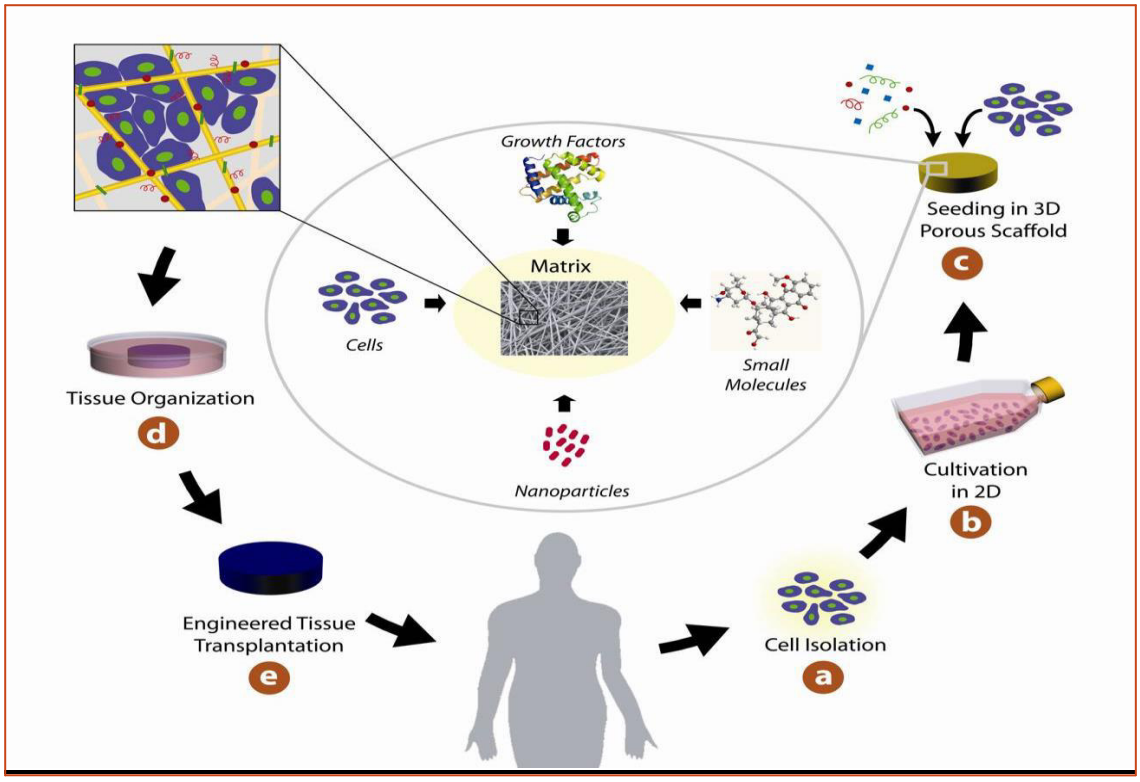

Figure 1.2 Concept of Tissue Engineering

1.3 SCAFFOLDS:

For constructing a tissue engineering scaffold, we need to consider some factors during choosing a material such as: biocompatibility, biodegradability, mechanical strength, chemical and biological properties etc. Ideally, scaffolds should be able to

accommodate human cells, guide their growth and help in tissue regeneration in three-dimension. Scaffolds with different architecture and properties can be produced depending on the fabrication process and type of material used. Potential materials having these characteristics are: natural polymers, synthetic polymers, ceramics, metals, and combination of these materials. The microspheres were uniformly distributed throughout the Nanoporous/Nano-fibrous scaffold and their incorporation did not interfere the macro-, micro-, and nanostructures of the scaffold. The surface area/volume ratio is large in case of nanofibres because of which its attachment with the host cell is more i.e. in multiple focal points.

Figure1.3 Knitted silk scaffold

Figure 1.4 Scaffold microstructure

1.4 CELL CULTURE:

Cell culture is the complex process which is generally takes place under a controlled environment (bioreactors usually) providing healthy proliferation and differentiation of cells which can be used for tissue engineering applications. In case of animal cell culture, the cells are usually derived from stem cell sources which have self-renewal property and ability to differentiate into other type of cells. When the cells occupy all the substrate in the culture media, these should be subcultured by transferring them into a new vessel containing fresh culture media.

1.5 BIODEGRADABLE POLYMERS:

The polymers which are going to be used in Tissue engineering should be Bio-degradable means the rate of natural tissue regeneration should be equal to the rate of scaffold degradation.

1.5.1 GELATIN:

Gelatin is a soluble protein which can be obtained by hydrolysis of collagen which is available naturally. Gelatin is found as a suitable material for tissue engineering

because of presence of a number of attributes in it. They are also lower in cost, biocompatible, biodegradable, and having lower immunogenicity, increased cell adhesion, migration, proliferation & differentiation. Gelatin promotes cell adhesion, migration and forms a polyelectrolyte complex because of containing an Arg-Gly-Asp (RGD)-like sequence. Gelatin has free carboxyl groups in its backbone which enables it to blend with cationic polymers to form a network by hydrogen bonding.

1.5.2 SILK:

Bombyx mori silk fibres are usually 10-20 micron in thickness and each fibre consists of its silk coating (sericin protein) & an inner core (fibroin protein). From the fibroin, we can get nanofibres which are present as parallel fibrils. The sericin protein is removed because of its toxic nature. The unique amino acid composition of silk which is translated into a specific primary structure makes the properties of silk quite unique.

1.5.3 PEO (Polyethylene Oxide):

PEO-based scaffolds have a great advantage in tissue engineering applications because of its ability to control the mechanical properties of the gel which are biocompatible and biodegradable simultaneously.

1.5.4 PEG (Polyethylene Glycol):

Synthetic hydrogels like PEG-based hydrogels have advantages over natural hydrogels, such as the ability for photo polymerization, adjustable mechanical properties, and easy control of scaffold architecture and chemical compositions. (Zhu J, Biomaterials; 2010; PMID: 20303169)

1.5.5 HA(Hydroxyapatite):

HA is one type of calcium phosphate bioceramics whose biocompatibility property in bone tissue engineering is thought to be due to its chemical and structural similarity to the mineral phase of native bone.

1.6 FABRICATION TECHNIQUES:

For constructing functional tissues and organs which should be organized into 3D architecture inside body, tissue engineering scaffolds have to be fabricated using different methodology for facilitating uniform cell distribution and guiding their proliferation & differentiation.

1.6.1 SOLVENT CASTING:

This method of scaffold preparation is very simple and cost effective. Here, the polymer is added over the mould and sufficient time is allotted for the evaporation of solvent that forms a polymeric membrane layer adhered to the mould. The main disadvantage of this method is that the solvent can denature protein because of toxicity. This problem can be overcome by vacuum process but it is very much time consuming.

1.6.2 PARTICULATE LEACHING:

It is a very popular technique where porogens like wax, salt or sugar can be used to create pores inside the mould. Here, the size of pores can be modified by changing the porogen size. A polymer solution is casted into the porogen filled mould and after evaporation of the solvent, the porogen crystals will be leached out using water forming desired pores in the scaffold.

1.6.3 GAS FOAMING:

This technique does not involve high temperature and organic solvents. It uses CO_2 gas with high pressure for fabricating the scaffold with high porosity which depends upon the amount of gas dissolved in the polymer. This is also dependent upon the porogen size for constructing 3D porous scaffold after completion of foaming process. Until the completion of saturation with CO_2, the mixture of porogen and polymer are exposed to high pressure.

1.6.4 PHASE SEPARATION:

This technique is for designing scaffold which involves temperature change for separating the polymeric solution into two phases with different polymer concentration. Biologically active molecules are dispersed over the polymer dissolved in naphthalene or phenol. It can give better result combined with particulate leaching and rapid prototyping.

1.6.5 ELECTROSPINNING:

It is the method for producing nanofibres from the polymeric solutions. This process involves high voltage supply (10kv to 50kv) between two electrodes having opposite charge polarity. The thickness of fibre produced depends on the concentration of polymer solution, voltage supply and distance between the collector plate and the solution container.

1.6.6 FIBRE MESH:

This technique for fabricating scaffold consists of fibre which may be woven or interweave into 3D pattern of various porosity. This can be achieved by the deposition of polymer solution over a nonwoven mesh of different polymer followed by evaporation which provides large surface area for cellular attachment and quick diffusion of nutrients. But it lacks structural stability.

1.6.7 COLLOIDAL DISPERSION:

It is the process in which one substance (dispersed phase) is dispersed in another substance known as dispersion medium which are very fine particles within nano range. These particles can be visible in an optical microscope easily but for better visualization, SEM is required. In a solution like salt solution, the particles exist as atoms whereas while dispersion the atoms aggregate to form nanoparticles on the dispersion medium.

Emulsion droplets are formed in the aqueous phase when the polymeric solution is added over it. After the droplet formation, acetone quickly diffuses out from each emulsion droplet by reducing its size drastically to become nanoparticles.

1.7 OBJECTIVE:

- To fabricate a multicompartment silk-based scaffold system for B-L-B graft.
- To add mechanical strength by incorporating the core scaffold from knitted silk fibroin.
- To compartmentalize the scaffold by installing tissue specific material (HA for bone, PEO with gelatin for cartilage and PEG with gelatin for ligament) for tissue specific induction.
- To use aqueous polymer dispersion (APD) technique for embedding the tissue specific material of core silk scaffold.
- To characterize the scaffold for mechanical strength and morphology.

CHAPTER 2

2. LITERATURE REVIEW

2.1 ENTHESIS INJURY IN ACL (Anterior Cruciate Ligament):

While sports, there is always a probability of over injury or acute injury of connective tissues like tendons, ligaments, cartilages and the immediate junctions between these tissues and the bone which are known as entheses. This transition (enthesis) facilitates force transmission between the two different tissues by reducing potentially damaging interfacial stress concentrations. If it is damaged due to some injury, its regeneration by surgical repair or natural recovery is quite difficult because of less self-repair capacity. Biomechanically, articular cartilage is often represented as a multiphasic fiber-reinforced permeable composite material having material properties like in homogeneousity, anisotropic, nonlinearity, and viscoelasticity.[1] These unique features provide healthy joints the ability to bear applied loads continuously, dissipate energy, and provide lubrication to the articulating joint surfaces over a lifetime. So there is a need to construct a composite Bone-Ligament-Bone scaffold for treating the common injury occurred in ACL (Anterior Cruciate Ligament).

There are some problems associated with the tissue engineering solution for Bone-Ligament-Bone injury. From the perspective of the mechanical properties associated with the function of the ligament-to-bone insertion site, few challenges emerge. Firstly, the ligament-to-bone insertion site (enthesis) must be stabilized mechanically during regeneration. Secondly, the architecture present during healing must guide reconstruction of a natural tissue. Thirdly, there must be maintenance of the seeded cells over the composite scaffold.

2.2 ENTHESIS TISSUE ENGINEERING:

Currently, tissue engineering is the most promising technology for regeneration of diseased or injured tissues. In this technique, we are combining the bioactive elements with the cells which are then seeded over the scaffold prepared from various biomaterials. Biomaterial scaffolds are also helpful in determining the functional characteristics of engineered tissues such as anisotropy, nonlinearity, inhomogeneity, or viscoelasticity. It is very much easier to construct scaffolds for homogeneous tissues

using one of the suitable biomaterials but significant challenges arise in developing scaffolds that have the capability to provide the required functional properties of load-bearing tissues such as ACL. So, for fabricating complex scaffolds that can induce the regeneration of engineered tissues having all functional properties, there has been development of composite scaffolds consisting of two or more distinct materials.

2.3 METHODS OF COMPOSITE FABRICATION:

In this literature, there has been mentioned a method to fabricate composite scaffolds for cartilage tissue engineering is to embed textile materials, either as fibres dispersed randomly or as higher-order structures, within hydrogels or bulk polymers. The central advantage of this method is that the constituent fibers provide reinforcement to the scaffolds and improve their mechanical characteristics.

It has been seen that bone-to-bone interfaces integrate quite better and faster than cartilage-to-bone interfaces. Several attempts have been done to develop bilayered constructs of cartilage-bone by the combination of two different scaffold materials. (Caplan, Elyaderani et al. 1997; Goldberg and 18 Caplan 1999; Schaefer, Martin et al. 2000; Schaefer, Martin et al. 2002)

Fiber-reinforced composites are very much useful for tissue engineering applications where high strength and light weight are important. In these two-phase materials, matrix bound with load-bearing fibers maintains their relative location and orientations, while transferring loads between them. Traditional composites reinforced with textile materials are typically made with 2-dimensionally assembled fabrics, including weaves, knits, and nonwovens. But to construct composite parts which require specific thickness or complex shapes, multiple layers of these fabrics must be cut, stacked, and laminated together to achieve the desired geometry.[3] Multi-layered fabrics and fabrics with some fiber oriented in the thickness direction can be constructed with slight modification to standard weaving machinery.

In order to fabricate 3D scaffold, there has been utilization of some techniques for processing biomaterials. As the tendons and ligaments are fibrous in nature, textile techniques can be used to fabricate scaffolds for tendon and ligament tissue engineering. But the single fiber filament does not match with the mechanical properties of natural tendon/ligament tissue. So, to improve mechanical properties of the fibres, fiber bonding techniques were developed to build strong biocompatible scaffold with

interconnecting fiber network (Hutmacher 2001). 3D braiding is one of the common methods to construct a scaffold with controlled pore size which are resistance to physical or chemical damage, and have high mechanical properties. Researchers (Cooper et al. 2005; Laurencin et al. 2005) used 3D braiding technique to fabricate a three-region (femoral tunnel attachment; ligament region; tibial tunnel attachment) PLGA graft for ACL tissue engineering. Three regions had different pore size and fiber orientation to trigger different tissue formation thus formed to biomimetic bone-ligament-bone graft.

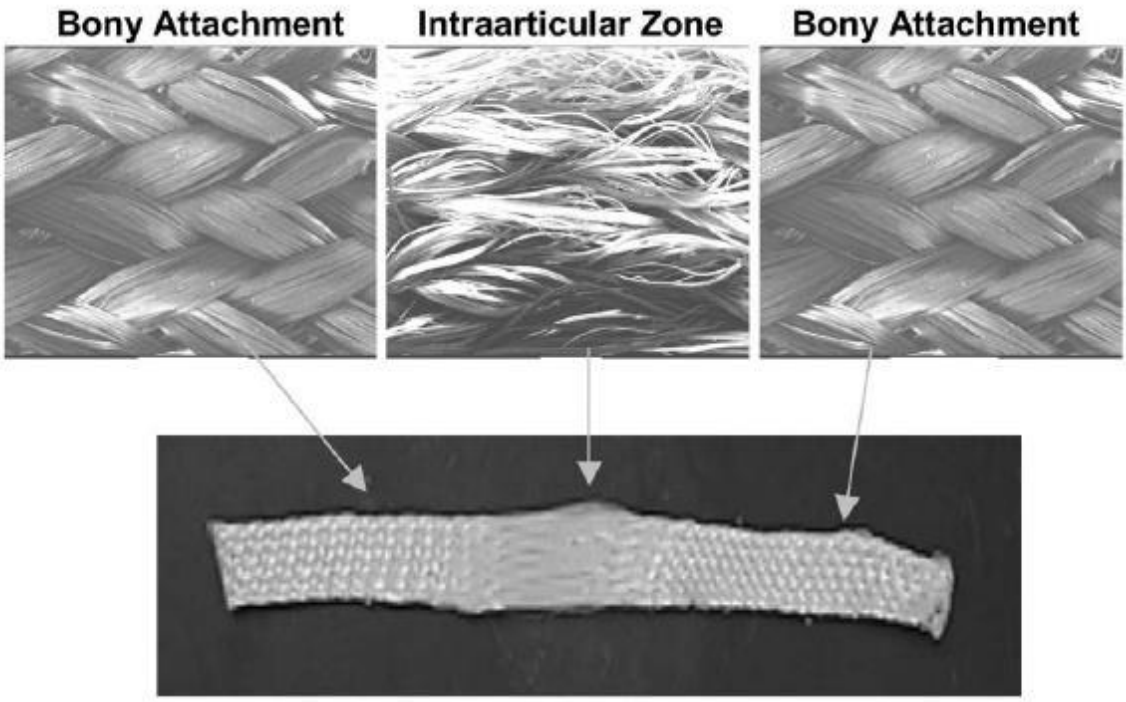

Figure 2.1 Schematic of three-region braided scaffold (Cooper et al. 2005)

2.4 Comparative Analysis of Biodegradable Polymers used in Tissue Engineering for Bone, Cartilage & Ligament:

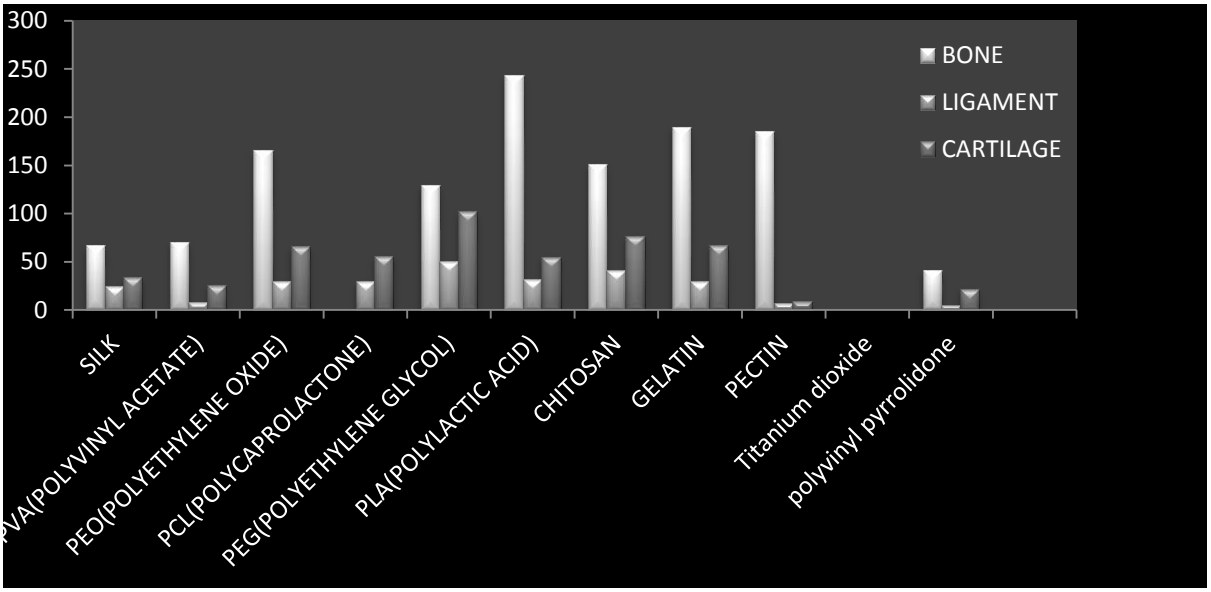

Figure 2.2 Comparative Analysis of Biodegradable Polymers used in Tissue Engineering for Bone, Cartilage & Ligament

After going through this analysis, we have found that gelatin which is derived from a natural source i.e. collagen can be used for better bone-ligament-bone tissue engineering.

2.5 POLYMER DISPERSION:

Polymer dispersion technique can give rise to stabilized water-borne emulsion polymers containing colloidal particles in water within the range of 50-500 nm and with 70 % solid content. But for the polymers which are not responsive to emulsion polymerization, dispersion should be prepared by dispersing the water insoluble polymer into an aqueous phase like acetone. Film formation happens by the aggregation of the individual particles which are held apart by stabilizing forces. The particles are packed densely as water evaporates which makes the coated layer even stronger and by deformation fills the empty cavities in the knitted scaffold.

Polymers like gelatin or its blend with other biodegradable polymers containing drugs with variable solubility can be released as nanoparticles by using colloidal polymer dispersion technique in a completely aqueous environment.

Emulsion droplets are formed in the aqueous phase when the polymeric solution is added over it. After the droplet formation, acetone quickly diffuses out from each emulsion droplet by reducing its size drastically to become nanoparticles.

Figure 2.3 Polymer Dispersion Test

CHAPTER 3

3. MATERIALS & METHODS:

3.1 Collection of degummed silk:

Degummed Bombyx mori silk fibres were collected from the State Silk Board, Dhenkikote, Keonjhar district, Odisha, India.

Figure 3.1 Degummed silk (B. mori)

3.2 Knitting of silk fibre:

At first, silk fibres were wound into yarns of various turns (3, 5, 8, 12). Then, scaffolds were woven from all respective turns of silk. Following the instructions mentioned in the manual, the machine was adjusted with a tension between 0-4 to carry out knitting with the desired porosity. The following steps were undertaken for arranging the fibre in the knitting machine. The dimension of scaffolds was adjusted to (5cm*2cm). Hence, there is a requirement of 9 needles to be used. Before starting the knitting procedure, the silk fibre bundle was kept perpendicular under K-Carriage arm. The pressure was maintained throughout knitting to avoid any knot in scaffolds or loosening during fabrication. Required tension was given to the scaffold using clip to keep it elongated during knitting. Then, the knitted scaffolds were arranged inside metal strips for keeping them stretched.

Figure 3.2 Knitting Machine

Figure 3.3 Knitted Scaffold

3.3 Mechanical strength of Knitted Scaffold:

The tensile strength of the knitted scaffold was determined by utilizing the TA-HD Plus Texture Analyser.

Figure 3.4 TA-HD Plus Texture Analyser

3.4 BIODEGRADABILITY TEST:

Preparation of 1X Phosphate Buffered Saline (PBS Buffer):

8g of NaCl, 0.2g of KCl, 1.44g of Na_2HPO_4 & 0.24g of KH_2PO_4 were dissolved in 800ml of distilled water. $_PH$ was adjusted to 7.4 with addition of HCl. Then, the volume was adjusted to 1L with additional distilled water. PBS was autoclaved to become sterilized. Then, the prepared scaffolds with a dimension of 5cm*2cm were soaked in PBS for (3, 7, 14, 21 days). During the period of soaking, the $_PH$ was checked in every alternative day, and in every 2 days, the PBS was replaced. After the stipulated time period was completed, the tensile strength of all scaffolds was tested using the texture analyzer.

After this analysis, the mechanical stability of the degraded scaffold is done by the TA-HD Plus Texture Analyzer.

3.5 SCAFFOLD FABRICATION:

3.5.1 Preparation of Gelatin Solution:

Gelatin crystals were mixed with distilled water (H_2O) and then gently stirred at 40 ^{0}C for 1 hour to obtain 50ml of 6-wt% gelatin solution.

3.5.2 Preparation of Polymer blends:

The prepared gelatin solution was blended separately with PEO, PEG & HA-PEG blend (6-wt%) by mixing 25% of these polymers with 75% of gelatin solution using the magnetic stirrer at 40 ^{0}C. Separate solutions were prepared according to make scaffold on the basis of bone, ligament and cartilage on respective manner.

3.5.3 Aqueous Polymer Dispersion:

Two knitted silk scaffold were taken and these are spread tightly over two glass slides. Then each scaffold is divided into 5 compartments using aluminium foil accordingly so that we can differentiate between the compartments while dispersing the respective polymer solution over it as shown below in the figure. Then the polymer solution was put inside a syringe and the solution was put drop wise over acetone (58.08% from NICE Chemicals Pvt. Lmt.) for the formation of colloidal nanoparticles which are sedimented over the glass slide dipped inside the acetone. Then the particle size was analysed by optical microscope (OPTIKA) and image was taken through fluorescence microscope (OPTIKA) at 10X & 40 X resolutions. After confirming the particle formed over the slide, the knitted silk micro-scaffold was dipped under acetone and the gelatin solution was put drop wise through syringe. Similarly, the

gelatin blended polymer solutions were put drop wise over different sides of knitted scaffold respectively as shown below the figure for different type of cell regeneration. Then, both the scaffolds were kept for drying at room temperature.

BONE	CARTILAGE	LIGAMENT	CARTILAGE	BONE
GELATIN, HYDROXY APATITE & PEG	GELATIN AND PEO	GELATIN AND PEG	GELATIN AND PEO	GELATIN, HYDROXY APATITE & PEG

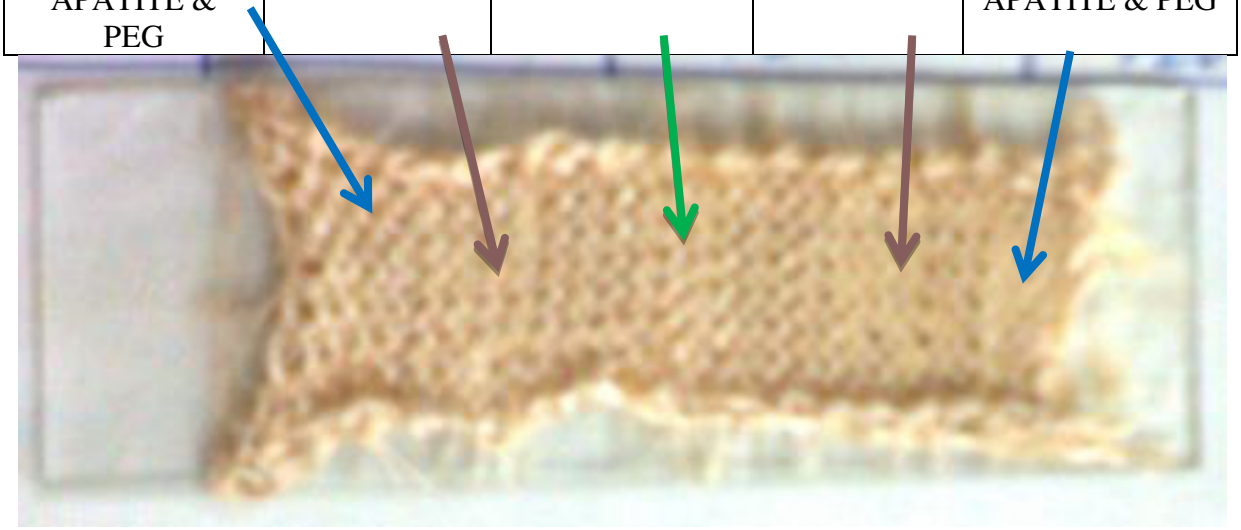

Figure 3.5 Composite scaffolds for Bone-Cartilage-Ligament-Cartilage- Bone Tissue Engineering

3.6 ANALYSIS OF FABRICATED SCAFFOLD:

After the drying of knitted silk scaffolds dispersed with different polymer solutions, microstructure of the scaffolds were viewed through optical microscope and picture of these were taken through fluorescence microscope at 10 X and 40 X resolutions.

CHAPTER 4

4. RESULTS & DISCUSSIONS:

Following the above mentioned procedure, we have fabricated the silk-based scaffolds for Bone-Ligament-Bone tissue engineering. These are characterised by different methods as described above whose results are given below.

4.1 BIODEGRADABILITY TEST OF KNITTED SILK SCAFFOLD:

The knitted silk scaffolds are tested for their biodegradability property which is very much essential to be used as implants in the body.

Table 1: Mechanical strength of Scaffolds soaked in PBS

SL. NO.	NUMBER OF DAYS	TENSILE STRENGTH (M Pa)
1.	0^{th} day	48.14±0.17
2.	3^{rd} day	46.3±0.63
3.	7^{th} day	34.5±0.25
4.	14^{th} day	14.2±0.26
5.	21^{st} day	6.74±0.34

By analysing the above results for mechanical strength, the tensile strength of the knitted scaffold in PBS medium was found decreasing with increase in the number of days. So, it has been confirmed that the silk degradation is more for the scaffolds which are put in PBS media for more number of days.

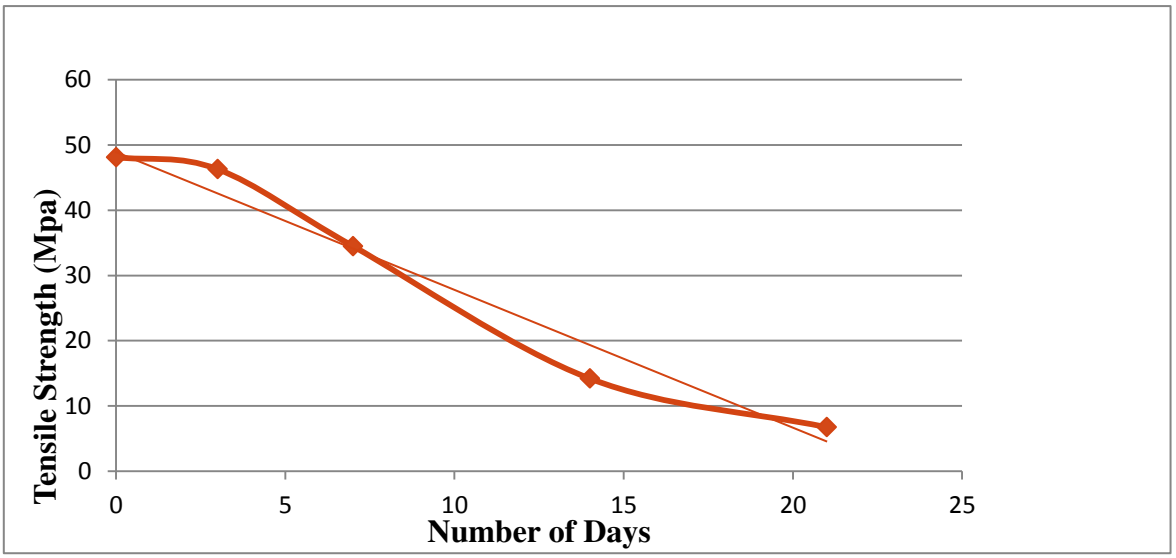

Figure 4.1 Data analysis for degradation of knitted silk scaffold in PBS medium

4.2 IMAGES TAKEN BY OPTICAL MICROSCOPE:

These are the images of the dispersed nanoparticles spreaded over glass slide, which are taken by optical microscope.

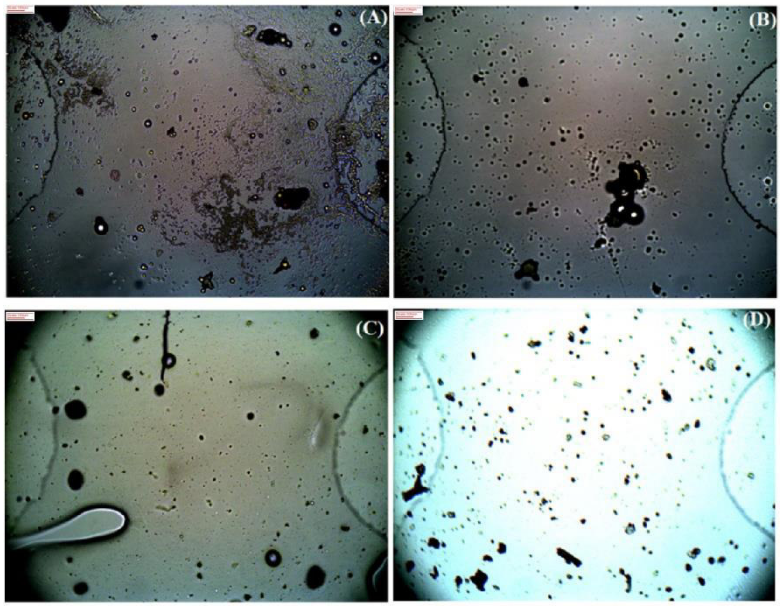

Figure 4.2 – Optical image of spreading of nanoparticle over glass slide after dispersion of polymer through acetone (a) Gelatin-PEO (b) Gelatin (c) Gelatin-PEG (d) Gelatin-Hydroxyapatite

After viewing the above images, it is confirmed that there are formation of nanoparticles when the polymer solutions blended with gelatin are dispersed into an aqueous media like acetone. Gelatin is easily soluble in distilled water but it is not soluble acetone. So, when gelatin solution is put drop wise through syringe into acetone, the perturbation of the interface creates a larger interfacial area spontaneously, which leads to nano ranged droplets of polymer solution. These particles are spread uniformly over the glass slide whose size is found to be in the range of 1 to 100 µm.

When the knitted silk scaffold was dipped inside the acetone medium and gelatin blended polymer solution was dispersed over it, then the droplets of polymer solution were dispersed into nanoparticles and spread uniformly over the scaffold which after drying makes the scaffold even stronger than previous and fills the micro compartments of knitted silk scaffold. Using this technique, we can insert drugs, growth factors etc. inside the scaffold by mixing these with the gelatin polymer solution.

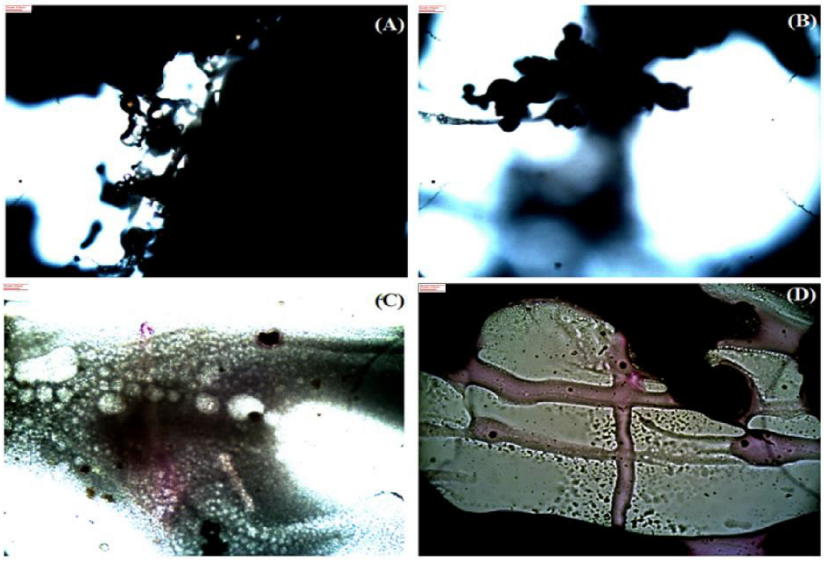

Figure 4.3 Optical image of deposition of particles on the surface of knitted silk scaffold.

4.3 SEM IMAGE:

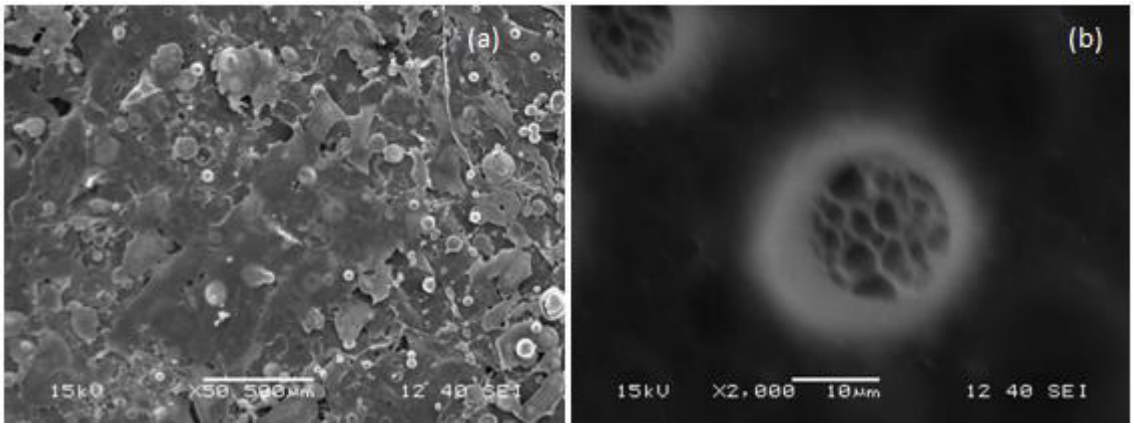

Figure 4.4 – (a) SEM image for gelatin PEG and Hydroxyapatite composite, (b) Presence of hydroxyapatite in the formulation

By viewing and analysing the SEM image of the composite scaffold for Bone-Ligament-Bone tissue engineering, we have found the presence of micro particles in the composite. Also, in the specific area where Hydroxyapatite was dispersed along with gelatin, its crystal type structure was visible through SEM imaging as shown in Figure 4.4 (b).

CHAPTER 5

5. CONCLUSION:

After going through various literatures and patents for analysing various biodegradable polymers which can be used for fabricating a composite scaffold for Bone-Ligament-Bone tissue engineering, gelatin was found to be suitable for this. Composite scaffold could be fabricated by dispersing various gelatin blended polymer solutions/minerals as nanoparticles over knitted silk scaffold through acetone aqueous medium. Mechanical strength of the scaffold was comparable with the mechanical strength of the bone and ligamentous tissues. The structural analysis of the silk-based composite scaffold was done through optical microscope imaging and SEM (Scanning Electron Microscope) imaging where the micro particles dispersed over the knitted scaffold are found to be adhered tightly to the silk fibre are sized in the range of 1-100 μm. The size distribution of suspended nanoparticles needs further confirmation through Transmission Electron Microscopy (TEM) and Differential Scanning Calorimetry (DSC).

5.1 FUTURE WORK:

In future, there will be seeding of fibroblast cells over the composite scaffold for the regeneration of cells for Bone-Ligament-Bone tissue engineering. This composite scaffold after being properly characterized can be implanted into the damaged synovial joints in animals for observing the regeneration potential of enthesis at the interface and ultimately the success of a ligament graft. The multi-compartmental scaffold with human cells can also be directly implanted in human synovial joints for replacing the damaged ligaments; however such a step needs proper validation of the scaffold-cell construct *in vitro*.

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