CHAPTER 1

INTRODUCTION

From time immemorial, the highly developed species on earth i.e humans have constantly evolved from one stratum to another. Innovations and discoveries were the key words for betterment, not only of the three basic needs of food shelter and clothing but of each and every finer aspects of life that can be dreamt of. The game which started with natural and raw innovations slowly shifting to artificial and synthetic innovations has now gradually shifted to more pronounced natural and environment friendly means.

Taking onto account the third basic need i.e clothing, needless to say that over the centuries the advancement in clothing material is as significant as the other needs. Clothes or fabrics are basically composed of fibers which are either natural or synthetic. Natural fibers include cellulosic fibers (cotton, jute, bamboo etc.) protein fibers (silk, hair, wool) and mineral fibers. Synthetic fibers are based on natural polymers (cellulose acetate) or synthetic polymers (polyolefines, polyacrylonitrile, polyamide or polyester fibres).

Silks are natural protein polymers prevalent in human civilizations from a long time primarily as a prestigious textile material. Silks symbolize pride and dignity to the wearer and signify a cultural realm of a civilization. Silks are polymers of protein which are spanned into elongated fibers by different lepidopteran larvae. Notable among them are silkworms, spiders, flies scorpions and mites (Kaplan et al., 1992; 1994; 1998). Silkworms produce silk in the form of cocoons which signifies the success of nature in
the synthesis of materials, of which the production is very smooth and natural but the mechanical properties are not compromised, making them useful in many ways (Kaplan et al., 1991). Silkworm silk is the most extensively studied and manifested silk although in recent time’s spider silk is also gaining prominence due to its strong mechanical properties with the limitation of low production and predatory nature of spiders.

Around 400-500 species of silkworm are producers of silk. The majority of the silk producing insects belong to the Order Lepidoptera, and Families Bombycidae or Saturniidae. Nearly 95% of commercial insect silk comes from the mulberry silkworm B. mori, known as mulberry silk, rest from all other sources collectively called Nonmulberry silk and it includes Muga (Antheraea assamensis), Eri (Samia ricini) and Tropical tasar (Antheraea mylitta) etc. The B. mori silkworm is monophagous, feeding only on mulberry leaves (Morus sp.). On the contrary, non mulberry silkworms are polyphagus having more than one host plants. The most extensively studied and manifested silks are those produced by silkworm B. mori. This accounts for nearly 90% of silk production in the world. The whole process from 1st instar larvae to the cocoons and to the moths results in a highly refined, flawless and mesmerizing structure i.e. silk, which has captivated human conscience since centuries and will proudly continue to do so in the future. As the Northeastern part of India is considered a hotspot of biodiversity and microbiology, it can also be considered a silkworm hotspot as this region produces three major commercially important silks namely Eri, Tasar and Muga along with B. mori.
Amongst the highly sophisticated natural engineering processes, silk production is surely a chart buster. Silk proteins are stored within a pair of silk glands after synthesis in epithelial cells of the silkworm. Before fiber production the proteins remain in liquid state, which is spanned by the larvae to form fibers (Altman et al., 2003). When the larvae matures its starts secreting silk through its spinneret in a circular motion due to which a cocoon is formed. The liquid silk after coming in contact with air becomes hard and forms the fiber. The formation of cocoon during morphogenesis of the larvae is considered to protect them from microbial degradation and predators (Zhao et al., 2005).

The composition of silk cocoons is fibroin and sericin. The core is made up of fibrous protein fibroin (75–80%) and surrounded by sericin (20–25%) which acts as a glue. The amount of fibroin and sericin vary from season to season, place to place and also with the races of silkworms. Percentage of sericin and fibroin reflect the quality of the silk fiber (Basavaraja et al., 2000). Silk having more sericin percentage are not considered economical as these result in the lower yield of fabric (Choudhury, 1981). The silk gland is divided into three parts, posterior silk gland, which secretes fibroin, middle silk gland, where sericin is synthesized of the mature silkworm larvae, anterior silk gland, from where the fibroin comes out after coating with sericin during spinning. (Lucas et al., 1958). Sericin holds the fibroin fiber together forming sticky layers which helps in formation of the cocoon. The fibroin is the highly crystalline core component of silk fibre (Horan et al., 2005).

The main commercial use of silkworm silk has been in textile production from long times. Chinese were the first to develop textile materials from silk and exporting to
the rest of the world. In the 3rd Century BC sericulture was developed in other parts of Asia and gradually spread to other parts of the planet. Through these centuries silk has attained a reputation of opulence and exquisiteness unmatched by other textile fibers. The next major use of silk was discovered few decades ago as biomedical sutures. Due to its biocompatibility and biodegradability, the effectiveness of silk fibers reflects in many clinical applications. Meanwhile, some allergic reactions and responses due to the protein have marked for more in depth research of the same. The main barrier in manipulating the biological responses of the silk fibers is the lack of proper information. There is no detailed Characterization of the fibers used and to what extent sericin has been extracted, the coatings of wax-like substances may also contribute towards the reactions, and many correlated factors during processing of the fibers (Altman et al., 2003).

Recently, the scientific community is trying to utilize silk as different types of biomaterials in related fields with remarkable achievements. A biomaterial is defined as quantified matrices, surfaces, or engineered constructs that interacts with living systems. They are either from natural resources or sometimes synthesized in the laboratory using variety of chemical approaches. The main application of biomaterials is in the medical field, and thus comprises strata of a living structure which performs, augments, or replaces a natural function. E.g- heart valve, artificial ligaments etc.

Recently, silk protein fibroin extracted from *B. mori* has been exploited as a versatile biomaterial for the fabrication of films, microparticles and 3D scaffolds for numerous clinical applications. Films are 2D structures which act as a substrate for cell
growth. Similarly scaffolds are 3D structures which fit into the bodies and helps in cell
growth. Microparticles and nanoparticles are also made of silk protein which aids in
drug delivery and cell growth. The success of this regenerated structures are mainly due
to its biocompatibility, meaning they are compatible to the human body without eliciting
any immunogenic reactions. Secondly, slow degradability of the materials gives ample
time to augment the function it was meant to do and lastly robust mechanical properties
like crystallinity, thermal stability, insolubility, slow enzymatic degradation etc (Vepari
et al., 2007). Fibroin from B. mori has been highly explored and reported to be utilized
for a wide range of tissue engineering applications including osteoblast, fibroblast,
hepatocyte and keratinocyte adherence and growth in vitro (Gotoh et al., 2004). All this
types of cell lines are made possible to grow and divide in a substrate made of fibroin.

Silks when compared with globular proteins are found to be highly
environmentally stable. The main factor is because of the extensive hydrogen bonding,
the surface of the protein is hydrophobic and they are naturally crystalline or it can be
induced. Solubility of silks are limited, they are almost insoluble in normal solvents, like
water, dilute acid and alkali (Kaplan et al., 1994). But to design biomaterials silk fibroin
needs to get dissolved so that it can be regenerated in a suitable form. Silk dissolution
was possible in few solutions namely lithium thiocyanate, lithium bromide etc.

During degumming, some amount of sericin is left in the fiber, which causes
adverse problems with biocompatibility and allergic reactions to silks because sericin is
hydrophilic and amorphous in nature which attract water molecules and thus microbial
infections and immunogenic reactions. If sericin is completely eliminated the biological
responses of the tissues to the fibroin fibers is like other commonly used biomaterials. The process of removal of sericin is termed ‘degumming’ and is carried out using number of chemicals. Different types of chemicals including alkali and acid and other natural materials are used as degumming agents for silk (Choudhury, 1981). These include sodium carbonate, ‘kolakhar’, ‘ritha’, cowdung, papain etc. There is also reported that silk being a protein is a candidate for proteolytic degradation inside the body and along the timeline it gets slowly absorbed (Altman et al., 2003). So the design of the biomaterial is done in such a way so that the function is performed completely before it gets degraded by the proteolytic enzymes.

The secondary structure of the biomaterial is the most important aspect of it being used as a biomaterial. Silk protein contains both amorphous and crystalline structures and it is of utmost importance for biomaterials to be in a crystallized form for application as biomaterials. For cell culturing and similar purposes the structure of the substrate needs to be in ordered crystalline form. For this purpose alcohol treatment is given to the biomaterials. Treating with alcohol changes the amorphous structures to ordered crystalline forms.

Extensive work on silk biomaterials has led to new innovations, like blending silk protein with other components to increase the efficiency and improved properties. Silk based composites are also gaining importance. Silk fibroin/polyacrylamide semi-interpenetrating network hydrogels are fabricated to use in controlled drug release. Hydrogels are polymers fabricated in a 3D polymeric fashion, with water playing a major role, made from polymers stabilized through physical or chemical cross linking.
Silk with its unique properties is designed into many forms like fibroin scaffolds, silk microspheres, silk fibroin films and woven mats, and its potentiality lies in the fact that the release and growth kinetics can be controlled. As a carrier and coating of drugs, silk biomaterials are reported to be excellent candidates. The properties of films, scaffolds and hydrogels are further enhanced by blending silk with various other polymeric materials as well as natural molecules like gelatine, collagen, elastin etc (Mandal and Kundu, 2009).

Sericin is the adhesive protein which binds the fibroins together. Considered a waste product of the textile industries, as it comes out as a byproduct after the degumming is completed, but sericin is now being utilized in fascinating new applications (Freddi et al., 2003). Sericin is hydrophilic in nature with polar side chains which enables cross-linking, copolymerization and blending with other polymers to achieve materials with improved properties (Cho et al., 2003). Applications of sericin are numerous ranging from applications in food industries, drug delivery, cosmetics, medical and clinical sectors, mostly in powdered forms.

To use the silk proteins as biomaterials it is important to regenerate the material in a form that can be easily molded into different forms, it is usually done in a solution form and called regenerated silk solutions. With the ease of fabrication in the liquid form, various substances can be made out of it, some common items are regenerated fiber, powder or particle, membrane or film, gel or porous matrix as per the need and their applications (Zhang et al., 2007). Powdered silk has been extensively utilized in surface improving materials, health foods, cosmetics and industrial materials. Fibroin
and sericin nanoparticles have been prepared and used in applications like drug delivery
due to their biocompatibility and biodegradability (Mathur and Gupta, 2010).

Bio-composites fabricated from natural polymers are adapted to environmental
problems as they are environment friendly and contribute to sustainable development,
the main advantage being they are renewable and degradability is high, either by
enzymes or by composting process without any harmful byproducts (Bleddzki et al.,
1999). Biocomposite materials have gained importance in recent times due to non-
availability of cost-effective, readily available materials. Amongst the ingredients,
biological polymeric materials, viz., carbohydrates, protein and lipids are the most
attractive because of their peculiar molecular properties and behavior and most
importantly the natural degradability (Fanga et al., 2005). The most advantageous feature
of composite materials is that they can be custom made and designed keeping in mind
the specific requirements (Mohanty et al., 2000). The applicability of a polymer
composite mainly depends on the selection of parent components, the interaction
between them and the bonding between them. It is necessary to modify the matrix In
order to meet the specific needs, along with the reinforcement.

Most of the recent innovations of silk as biomaterials involve B. mori silk. It has
all the requisite of becoming or rather performing as a successful biomaterial. But the
thought was that if there are some superior silks in terms of properties than those of B.
mori silk, will it not be feasible to augment those as more reliable and successful
biomaterials.
The extent to which \textit{B. mori} silk has been exploited in terms of biomaterials is simply remarkable. But to the same extent the non mulberry silks remain unexplored mainly due to its geographical demarcations and low availability. However, non mulberry silks are found to be different from that of \textit{B. mori} silk in respect of strength, porosity, robustness, amino acid composition, UV absorption capacity and thermal properties. Silk belonging to Saturniidae family have higher components of amino acids which are polar, bulky, basic, and have hydrophilic side chains as compared to the domestic mulberry silk \textit{B. mori} (Tashiro et al., 1970). Bioactivity of Nonmulberry silk is more than \textit{B. mori} as found out by studies. Previous studies on fibroin from tasar silk of \textit{Antheraea mylitta} and \textit{Antheraea pernyi} in comparison with \textit{B. mori} silk showed that they have better growth and cell attachment. At this hour, it becomes immensely important to further study different non-mulberry silk species to explore their biomedical applications and better understandings of their kind.

\textit{Antheraea assamensis} Helfer (Muga silkworm) and \textit{Samia ricini} Donovan (Eri silkworm) are non diapausing silkworm species prevalent in the Northeastern states of India, comprising of Assam, Meghalaya, Manipur and Mizoram. Muga silk is produced by the moth \textit{Antheraea assamensis} Helfer (Lepidoptera: Saturniidae), which is available particularly in Assam, India (Helfer, 1837 and Choudhury, 1981). Muga is an assamese word from which the silkworm derives its name, meaning golden brown colour of cocoon. Being polyphagous in nature, it feeds on leaves trees like som, \textit{Machilus bombycina} King, \textit{L. citrata} Roxy, sualu, \textit{Litsaea polyantha} A. Juss, \textit{L. salicifolia} Roxy and several other plants belonging to the family Lauraceae. It is multivoltine in nature.
having 5-6 generations in a year. The fibers produced by this silkworm have an inherent
golden-yellow colour which can withstand sunlight, cannot be bleached easily and no
reaction of detergents. Tensile strength of Muga fibre is higher in comparison with *B. mori*
and other non-mulberry silk fibers and many more characters make it unique from
the *B. mori* silk fiber (Devi *et al*., 2011). The durability of Muga is very high which
raises its demand internationally, along with the local markets. Considering these
properties, the effectiveness of Muga silk in the field of biomaterials was tried to be
explored in this study.

The name ‘Eri’ is derived from the Assamese word ‘era’ which denotes the
castor-oil plant (*Ricinus communis*) the main food plant of this silkworm. Eri silkworm,
*Samia ricini* (Lepidoptera: Saturniidae) is multivoltine, polyphagous and feed on a wide
range of host plants. It is found widely in various parts of NE India. The domesticated
nature of Eri is an advantage to the rearers. Eri silk fiber is also unique like the Muga
fiber in many ways; it has good thermal insulation properties. Production rate of Eri is
high and fiber is of low cost which is another advantage for exploiting this fiber as
biomaterial.

In spite of a large number of artificial fibers overwhelming the textile arena, the
natural silk still occupies a respectful position as its poignant and prominent aura is
unrivaled. In Assam, the ‘Muga’ silk has aesthetic value and is traditionally used in
occasions and festivals, like ‘Bihu’, ‘puja’, marriage etc. Silk industries in this region are
home-based and are livelihood for many people. Apart from ‘Eri’ , ‘Muga’ and *B. mori*
silk, other wild silks of tasar (*A. mylitta, A. proylei* and *A. frithi*), kotkari Muga (*A.
atlas), amphutukoni Muga (C. trifenestrata) are found in the forest in wild form throughout the NE region. Exploitation in commercial scale could be a support to the local farmers to boost their livelihood.

It was not until recently that scientific community realized the tremendous potential of non-mulberry or wild silks. Sporadic study has been done regarding the physical and mechanical properties of these silks and only a few reports are available till date (Jolly et al., 1979; Iizuka et al., 1985; 1994; Sonwalker et al., 1989; Baruah, 1991; Freddi et al., 1994; Das, 1996; Rajkhowa, 1998 and Das et al., 2005). However the wild silks A. proylei, A. frithi, A. atlas and C. trifenestrata of NE India have not been studied systematically.

The ratio of fibroin to sericin is 4:1, therefore fibroin, is the major part of the silk protein obtained from cocoon. It has been reported that the fibroin of non-mulberry silkworm P. ricini is unique both in biochemical and immunological aspect from B. mori silk fibroin. A comparative study of the sericin obtained from the cocoon and peduncle of A. mylitta was done with B. mori and characterized to understand the relationship between them by Dash et al., (2006). Three major fractions of sericin of which lower fraction is around 70 kDa, middle fraction is approximately 200 kDa and other one is more than 200 kDa was reported in the study. A lower molecular weight sericin of 66 kDa is found in the cocoons of Muga and Eri (Ahmad, 2004). Serine and glycine dominates the amino acid composition along with glutamate which is acidic in nature. Sericin is a water-soluble protein because most of the residues present in the protein are either hydrophilic or does not have hydrophobic side groups. This dissimilarity in amino
acid composition of cocoon and peduncle is the result of the difference between the
tensile strength and interaction of fibroin-sericin. The peduncle of tasar silkworm
contains a major fraction of approximately 200 kDa sericin. Both high and lower
molecular weight sericin are present in the cocoon of the same species that protects the
pupa from various environmental stresses. Probably the higher molecular weight sericin
increases the strength of silk while the lower molecular weight sericin protects the pupa
from various environmental stresses. Based on the amino acid compositions, the
difference between A. mylitta peduncle sericin and B. mori cocoon (B. mori) sericin
were found out. This type of findings has attracted more researchers to study and
understand the non mulberry silks in comparison with the B. mori silk.

Structural and conformational Characterization of the different silk and its
components have led to the applications of the said materials in different arenas and in
different ways. The natural biomaterials have superior properties compared to artificial
ones as they are more compatible with the surrounding matrix in the human system. The
properties that have made fibroin the most anticipated biomaterial are advanced
mechanical properties, permeability, and excellent biocompatibility (Vepari et al., 2007;
Reddy et al., 2011; Tao et al., 2012; Huang et al., 2014; Yucel et al., 2014; Partlow et
al., 2014). RGD motifs are said to be present in non-mulberry silk fibroin based matrices
which aids and acts as a substrate for stem cells differentiation and progenitor cells,
many of tissue engineering applications are dependent on stem cells and makes the
fibroin based matrices as a suitable biomaterial (Bhardwaj et al., 2015).
The most important aspect of using silk as biomaterials is the biocompatibility of the matrices and particles. Biocompatibility is the term used to describe the behaviour of the biomaterials in the live systems without eliciting any harsh effects. The use of B. mori silk fibers as has been started since the end of the 19th century, but some adverse biocompatibility issues were reported. The reason behind the immunogenic reactions was found out to be the glue protein sericin that holds the fibroin fibers together (Soong et al., 1984; Panilaitis et al., 2003).

Numerous in vitro studies have reported that, fibroin supports cell attachment and proliferation for a variety of cell types only after sericin is removed by degumming (Servoli et al., 2005; Roh et al., 2006; Gupta et al., 2007). RGD sequence is also said to be present in silk from the wild silkworm, Antheraea pernyi, due to which it supports cell attachment and growth better than B. mori silk. Usually alcohol treatment is used to induce crystallinity but the use of another method to induce β-sheet is through water vapour which is also reported to support better cell attachment and proliferation.

Inflammatory reactions to fibroin films in vivo are similar to that of collagen (Meinel et al., 2005). In case of B. mori silk braided into yarns a mild inflammatory response was elicited after seven days in vivo, whereas polyglycolide (PGA) and silk yarns which were sericin-coated caused an acute inflammatory response. Sericin has been identified as the main cause of the harsh inflammatory response to un-degummed silkworm silk, but it is also reported that only when combined with fibroin the activation of macrophages is elicited, sericin alone is not able to trigger the reactions (Fang et al., 2006). L929 murine fibroblasts and human skin fibroblasts grows and proliferates well
on sericin films. Sericin M, a particular component of sericin has been identified, that enhances cell attachment. Another component known as sericin S is also a potential supplement for serum-free culture medium, where cell proliferation and mitogenic activity of cells occur in normal conditions. Synthetic polymer fibers having sericin coatings have been found to have antibacterial and antifungal properties (Sarovart et al., 2003). Structural characteristics of silkworm silks are similar to amyloid (Chen et al., 2007) and fibroin in solution accelerates amyloid accumulation in mice. The presence of amyloids in the human body is linked with neurological diseases like Alzheimer’s and Parkinson’s.

The second most important aspect of biomaterials is their degradability. The rate of degradation of biomaterials should match with the growth of new cells and tissues in that specific area so that the newly formed tissue can be integrated into the surrounding host tissue. Appropriate degradation rates also minimize any risk of stress building, which is most important in the case of engineering of tendons and ligaments. From studies it is demonstrated that in vitro degradation of silk fibroin biomaterials is slow, owing to its strong mechanical properties, with negligible loss of mass even after four weeks in culture (Meinel et al., 2004). Various degradation studies have demonstrated that silk degrades as a result of proteolysis, when treated with enzymes and it has been reported that protease enzymes have the greatest effect (Taddei et al., 2006). Smooth and porous silk films when exposed to enzymes are degraded to some extent even after one day. Studies involving silk yarns when exposed to protease enzymes for six week showed a loss of mass of more than 50% and also degradation of the mechanical
properties. The \textit{in vitro} degradation behaviour of silk fibroin depends to some extent on the method of silk processing including the degumming process. The highest degradation rates is shown by fibroin films and scaffolds which are aqueous derived than the chemically derived ones, also degradation is more compare to the fibers, the reason behind this may be due to the increased surface area. Treatment with methanol induces crystallinity which may also drastically reduce the degradation rate. The manipulation of the rate of degradation is significant for tissue engineering applications, the parameters for controlling the degradation are the specifically designed physical form and treatment after fabrication of a silk biomaterial. In situations, like wound healing, faster degradation rate is required. \textit{In vivo} studies involving silkworm silk biomaterials have resulted in slower degradation. Six weeks after implantation, the fibroin films did not degrade in a study done by Meinel \textit{et al}., (2005). The degradation of a fibroin based mesh, which was non woven, was found to be very low even after six months implantation (Dal \textit{et al}., 2005). In case of \textit{B. mori} silk bacterial degradation has been performed and reported that it is susceptible to degradation.

The vast aspects of tissue engineering have been miniaturely occupied by silk biomaterials as matrices, scaffolds and to some extent as nano/ microparticles. It is noteworthy that silk biomaterials have shown superiority to polymeric biomaterials in many aspects and hence they are noteworthy candidates considering the current scenario of environmental concerns and global warming. Some of the major applications of silk in tissue engineering is discussed below which primarily involves silk biomaterials derived from domesticated silkworm \textit{B. mori} with a few exceptions.
The first aspect considered is skin and wound healing. *In vitro* studies have shown that proliferation and spreading of dermal fibroblasts cells on fibroin films and scaffolds is significant and without eliciting immunogenic responses (Chiarini *et al.*, 2003). For wound healing applications oral keratinocytes are used which proliferate on fibroin biomaterials with meshes, a form that is likely to be used. Compared to clinically used materials fibroin films along with fibroin-alginate sponges have been found to enhance skin wound healing *in vivo*. A superior performance of fibroin-chitosan blend has been reported compared to materials normally used for repair of ventral hernias (Gobin *et al.*, 2006).

Considering the skeletal tissue engineering using silk based scaffolds the results are encouraging. The scaffolds have successfully resulted in chondrogenesis when used to culture human articular chondrocytes (Wang *et al.*, 2006) and mesenchymal stem cells *in vitro*. Cartilage has been synthesized on silk scaffolds in serum-free conditions which is highly successful for clinical applications. However, further investigation is going on to optimize the requirements as the mechanical and chemical properties of bioengineered cartilage are still inferior to those of native cartilage.

Silk-based biomaterials for, tissue engineering of bone are not lagging behind. Fibroin solutions for bone tissue engineering are used as films, electrospinning is used to form scaffolds or salt-leaching is used to process it into a 3-D porous scaffold. Additionally, RGD functionality or growth factors are also incorporated. When coupled
with RGD peptides, osteoblast cells easily grow on fibroin films, and produces mineralized matrix (Sofia et al., 2005). Osteogenesis has been achieved with stem cells grown in vitro on thin fibroin films, scaffolds electrospun from fibroin-poly (ethylene oxide) solution and porous fibroin scaffolds (Marolt et al., 2006). In vivo bone formation in non-load bearing and load-bearing sites has been done successfully. In vitro osteogenesis has been induced via Bone morphogenic protein-2 (BMP-2) grown on fibroin films and scaffolds successfully (Meinel et al., 2006) and initiates bone formation by pre-differentiated and undifferentiated MSCs seeded onto silk scaffolds and cell-free scaffolds in non-load bearing and load-bearing bone defects.

Ligament tissue engineering has been reported in B. mori silk after degumming, by designing into suitable matrices (Altman et al., 2002). These matrices have suitable mechanical properties for ACL reconstruction, and modification to the yarn design and surface construction can be manipulated which ultimately allows custom tailoring of the mechanical properties. Anterior cruciate ligament fibroblasts and MSCs adhered to and spread on silk yarns and expressed ligament specific markers, although cells did not infiltrate the centre of individual cords.

Different approaches has led to use of B. mori fibers with gelatin coatings for ligament tissue engineering, as the gelatin coating fulfills the gaps in the mechanical properties that are reduced by degumming (Liu et al., 2007). Preliminary investigation on cytotoxicity has shown that these fibers are not toxic and does not cause any in vivo inflammatory response, but no specific ligament related work has been performed. Tendon tissue engineering using silk-based materials have also been investigated and
reported. Human tenocyte attachment and growth was increased on silk-RGD films compared to native silk films (Kardestuncer et al., 2006) and the highest levels of mRNA for decorin (the most abundant proteoglycan in tendon tissue) and type-I collagen were found on silk-RGD and more abundantly on silk films than on the control surface.

In the field of vascular tissues, sulphonated and heparinised silk fibroin films are used as suitable artificial blood vessels. Anticoagulant activity and platelet response of the films were satisfactory and supported endothelial cell spreading and proliferation. Collagen gel-silk filament composites for vascular tissue engineering have also been studied although the work is still in the preliminary stage (Couet et al., 2007). Grafting of water-soluble polymers such as 2- methacryloyloxyethyl phosphorylcholine (MPC) onto the surface of silk fibroin improves the blood compatibility and also by blending with blending with Scarboxymethyl kerateine. Silk fibroin nets and films are used to support endothelial cell attachment, maintenance of phenotype and the formation of microvessel-like structures when the fibroin is coated with fibronectin or collagen (Unger et al., 2004). Similarly, co-culture of endothelial and osteoblasts cells on silk fibroin biomaterials has been demonstrated to result in the formation of microvessel-like structures, even in the absence of pro-angiogenic factors and the fibronectin coating.

Formation of vascularised reticular connective tissue in non woven fibroin mesh and films was performed in a six month implantation study by Dal et al., (2005). The success of tissue engineering depends on vascularisation of the tissues occurs and the ability of silk fibroin and sericin related biomaterials to support this process, which is
considered most important factor for potential use of these materials for tissue engineering of bone and other tissues. Hepatocytes collagen-fibroin blends and lactose-fibroin conjugates have been reported to support and proliferation of hepatocytes. *B. mori* silk has also been demonstrated to support the growth and proliferation of Schwann cells and a dorsal root ganglion which has even broaden the applications of biomaterials in the tissue engineering of nerve grafts.

It can be concluded that silks can be processed into many suitable forms having applications for a variety of biomedical and tissue engineering aspects. They can be modified by chemical treatment or used in combination or blending with other materials in order to enhance mechanical properties and alter the surface chemistry. By these techniques, biomaterials appropriate to specific applications have been successfully produced. Biocompatibility of silk-based biomaterials is also satisfactory in terms of inflammatory response and ability to support cell proliferation compared to various materials currently in use. Although silk degrades slowly, the degradation behaviour can be manipulated, depending on the application, from a few days to many months. These materials are emerging as promising substrates for use in wound healing and as tissue engineering scaffolds, particularly for the development of skeletal tissue. Considerable research on other newer and unexplored applications, such as the use of silk for nerve regeneration, are going on and further application of the same in diverse fields is highly anticipated.
Attempt has been made to study the role of non mulberry silks of NE India as potential biomaterials with the following objectives:

1. To prepare 2-D film, micro/nano particles from silk proteins fibroin and sericin.

2. To characterize the above materials using SEM, FT-IR, XRD, DSC, TGA, contact angle etc.

3. To study the biocompatibility and biodegradability of the fabricated silk based materials in *in vitro* conditions.

This effort will provide insight into the multidimensional aspects of non mulberry silk i.e. characteristics of fibroin and sericin, mechanical properties, thermal behaviour, molecular conformation, crystallinity and the utility of the fabricated biomaterials. It is expected that this study will help to generate substantial information and applicability on the unexplored aspects of silks of NE India namely ‘Muga’ and ‘Eri’ which could be effectively used in the welfare of humans and allies. The artificial synthetic materials may be completely replaced by these natural materials which relates to the current global problems of environmental instability and pollution, and help to eradicate the problem completely.