Elastomer-Based Bio-Nanocomposites

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Abstract This chapter encompasses an overview of recent advances in the field of natural, synthetic and semi-synthetic elastomer based bio-nanocomposites. Several naturally occurring polymers are considerably elastomeric and biocompatible. Elastin and its various derivatives are essential component of mammalian bio-systems. Various soft to semi-soft tissues of animals containing collagens, elastin proteins or other extra-cellular materials are classic examples of natural elastomeric bionanocomposites. There have been needs for developing synthetic or semi-synthetic elastomeric bio-nanocomposites for replacement or regeneration of such soft or semi-soft tissues or organs. Most of the elastomeric biopolymers lack in mechanical properties. Various modified cellulose components derived from plants like, cellulose whiskers, micro-fibrillated cellulose in their nano-scale size have proven promising in improving thermal, mechanical and moisture absorption properties of the elastomeric biopolymers. Similarly, biocompatible and bio-inert particulate nano filler systems including hydroxyapatite, bio-glass, silicates or other minerals can be potential reinforcing units for synthetic or semi-synthetic bionanocomposites. Non-toxicity of these precursors is utmost important for their actual bio-medical applications. Several synthetic methodologies have been adopted for the preparation of other novel and or bio-mimetic elastomeric bio-nanocomposites. Biodegradable polyurethanes and polycaprolactones, when modified with chitin or chitosan based chain extenders form novel nanocomposites, having wide applications in the area of biomaterials. Various precise and sophisticated characterization techniques namely, NMR, TEM, SEM, CD-spectroscopy have been routinely employed to evaluate the structure-property correlation of these novel bio-nanocomposites. Research is progress throughout

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P. M. Visakh et al. (eds.), *Advances in Elastomers II*,
Advanced Structured Materials 12, DOI: 10.1007/978-3-642-20928-4_7,

the globe for further improvement in properties of such materials and subsequently to figure out novel practical applications of these elastomeric bio-nanocomposites.

1 Introduction

Bio-nanocomposites have attracted a focal research interest in the recent past. The assembly of molecular or polymeric species of biological origin and other components through interactions, on the nano-metric scale constitutes the basis for preparation of bio-nanohybrid materials. This is entirely an interdisciplinary area encompassing biological sciences, material sciences and nanotechnology. Elastomer based bionanocomposites, which is a sub class of bio-nanohybrid materials, have several advantages of long range elasticity and viscoelasticity in addition to their inherent eco-friendly, biodegradable and renewable characteristics [1]. This special class includes composites of polysaccharides such as starch, cellulose, proteins such as collagen, elastin and synthetic bio-polymers like poly-lactic acid reinforced with particulated solids. They are also well known as green-nanocomposites [2, 3].

The modern definition of bio-elastomers can be given as: it is a material or composite having favorable bio-compatibility with adjacent tissues, with a low glass transition temperature than that of body temperature, exhibiting hyper-elasticity.

This chapter elucidates the basic idea for natural, synthetic as well as semi-synthetic varieties of elastomeric bio-nanocomposites. Traditional elastomers mainly consist of hydrocarbon chains flexible enough to allow C–C bond rotation within a specified temperature to execute long range elasticity. To make elastomeric bionanocomposites one major question is: how can we incorporate the whole range of attributes of elastomers as well as those of biomaterials in elastomeric bio-nanocomposites? If one has to do this, several parameters have to be taken into consideration. Intermolecular interaction especially inter-chains interaction and entropic elasticity are the utmost controlling factor for a material to behave as an elastomer. In true sense, most of the classical elastomeric molecules where intermolecular force is negligible are neither biological in origin nor are they biocompatible. These molecules also lack bio-relevant characteristics including biocompatibility, bio-functionality, and bio-degradability like in cellulose fibers, chitin, elastin etc.

Bio-mimetic (soft tissue) preparation of elastomeric bio-nanocomposites mainly starts with collagen or elastin based matrix mimicking cartilage type soft tissues. Amongst the biodegradable synthetic polymers, few block copolymers behave as thermoplastic elastomers with limited range of elasticity. Thus to coin the biological characteristics with long range elasticity one has a very limited choice. Thus initial developments in elastomeric bio-nanocomposites have been initiated with above mentioned class of bio-polymers following bio-mimetic preparation route.

Several synthetic methodologies have been developed in the recent past in pursuit of more versatile materials of this class. These materials exhibit excellent structural properties owing to special arrangements at the nanometric level of their assembled components. Recently, elastomers based bionanocomposites are prepared, mimicking the natural materials. Most of the works aims to develop new bio-hybrid materials having both biocompatibility and functionality. It is interesting that in the cell wall of prokaryotes, nano-structured silica nano-spheres could be assembled by the participation of cationic polypeptides [4].

As per a statistical data, the fascinating growth of research in the area of 'biomaterials including in bionanocomposites can be realized for the recent past as shown in Fig. 1 and it can be easily realized from the bar chart that the future of research in the allied area is very bright [2, 5].

Elastomers assembled with biological species are of diverse nature having different compositions, structures and textures [6]. They ultimately determine the properties of such bio-nanohybrid materials. The affinity among the components determines the stability of the morphology of resulting bio-nanocomposites guided by intermolecular interaction [7]. The coating of micro or nano-particulate solids with biopolymers occurs through hydrogen bonding or via formation of metal complexes. Magnetic ferrite nanoparticles embedded in biopolymers have extensive applications in MRI, hyperthermia and drug delivery systems [8]. Development of novel bio-hybrid materials e.g. hydro-gels displays a homogeneous dispersion resulting in multiple functionality [9]. Bio-composites are vividly useful in biomedical or eco-friendly applications. They are based on the biogenic components with well known biocompatibility, such as silica, nanoclay, iron-oxide, and hydroxyapatite to name a few.

Recent studies have shown that the great interest of interfacing a biological entity with non-biogenic metal oxides has promising futures. Such assemblies have been made with the aim of improvement of biosystems. Among protein based

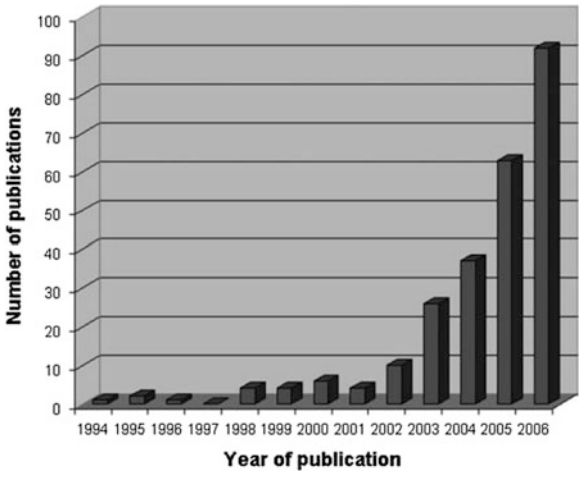

Fig. 1 Progressive development in bio-material/bio-medical related research publications in recent past. [Web of Science Databases-ISI web of knowledge, 2, 5]

polymers, gelatin is widely used in pharmaceuticals. Combination of bioactive and bio-resorbable properties are utmost important. The successful combination of bio-polymer with suitable bio-active or bio-inert hard fillers constitutes the basis of design of such materials. Elastomeric bio-nanocomposites exhibits substantial viscoelastic properties and can be easily fabricated into complex structures [10]. These materials are important for soft to semi soft-tissue replacements and making scaffolds.

2 Classification on Elastomeric Bio-Nanocomposites

Several classifications of the biomedical relevant polymers are possible. For example, some authors have distinguished between synthetic polymers like polylactic acid (PLA) and poly lactic-co-glycolic acid (PLGA) or their copolymers with polycaprolactone (PCL), and polymers of biological origin like polysaccharides (starch, alginate, chitin/chitosan, gelatin, cellulose, hyaluronic acid derivatives), proteins (say, collagen, elastin, fibrin, silk), and a variety of bio-fibers, such as ligno-cellulose containing elastomeric natural fibers [10].

Other authors have differentiated between resorbable or biodegradable polymers e.g. poly (α-hydroxyesters) polysaccharides and proteins and non-resorbable (e.g. cellulose) polymers. As synthetic polymers can be produced under the controlled conditions, they in general exhibit predictable and reproducible mechanical and physical properties such as strength, modulus, elongation at break and rate of degradation. Control of impurities is a further advantage of synthetic polymers. Essentially, the central idea has been to mimic elastic biological tissues by using a suitable polymeric matrix. The mechanical reinforcement and bioactive character of the composite are utmost important.

Based on physiological activities, classifications of bio-degradable elastomers are as follows [11]:

1. The elastomers suitable for long term physiological contact.
2. Bio-degradable elastomer for a determined time of contact.

Many tissues in the body have elastomeric properties like long range elasticity and dynamic properties [12]. Repair of such tissues projects to the development of elastomeric scaffolds that can sustain and recover in deformed state without disturbing the surrounding tissues [13].

Based on the source of precursor material, bio-elastomers can be further classified into three categories as:

1. Naturally extracted or bio-synthetic bio-elastomers; majority of protein and peptide based elastomers are included under this head.
2. Bio-synthetic degradable polyesters and polyhydroxyalkanoates.
3. Chemically synthesized bio-degradable elastomers.

Both linear and branched polyethylene (PE) can be used as a matrix and the resultant semi-flexible bio-composites have been found to give rise to a higher modulus [14]. Generally, if a reactive filler system is embedded, the polymeric matrix might be affected by the filler through reduction of molecular weight during composite processing. Formation of a shell of polymer around the particles (trans-crystallization, surface induced crystallization, or epitaxial growth) and changes in conformation of the polymer due to particle surfaces and variation in inter-particle spacing can markedly influence the characteristics of the matrix [15].

Creating nano-fibrous scaffolds with material dimensions at the nanometer scale allows for the efficient replication of the physical structure of natural bio-nano-composites. Biomaterials fabricated as nano-fibers can positively influence the physical and mechanical performance of the biomaterial scaffolds. Scaffolds fabricated from nano-fibers have been considered for use in the engineering of cartilage and wound healing applications. Some advantages of employing nano-fibrous biomaterials in tissue engineering, includes formation of polymeric nano-fibrous matrix for cellular adhesion, formation of neo-tissue and growth into a mature tissue in a bioreactor [16]. Tissue engineering process employs a nano-structured polymeric scaffold material. Advantages of nano- and bio-composites include physical mimicking of natural polymers, ease of surface functionalization, and improving mechanical properties of scaffolds of such materials can serve as a medium through which diffusion of metabolites can occur freely. Synthetic bio-polymers and their composites have been playing a pivotal role for making synthetic and semi-synthetic bio-nanocomposites. The advantages they offer includes that they can be tailored to provide a wide range of properties e.g., reduced immunogenicity, simplicity of processing and ease of sterilization. Classical example is PLGA possessing thermoplastic elastomeric characteristics and it can also easily be degraded by simple hydrolysis. PLA is an α-polyester having two enantiomeric form: D-lactide and L-lactide. PLA has certain advantages including tensile strength up to 60 MPa which is very useful for reinforcement. The high mechanical integrity possessed by these classes of polymers is often used as such for elastomeric body components and cartilage tissue engineering. The degradation and resorption kinetics need to be designed and controlled in such a way that the nano-structured scaffold retains its structural and functional integrity. Composite scaffolds consist of two or more materials. These materials together produce scaffolds that ideally draw from the properties of the individual properties of components [17]. Most of the natural polymers like chitosan and collagen display significantly lower values of tensile strength and elastic modulus, even upon cross-linking, as compared to the synthetic class of biopolymers. This in turn unveils scopes for integrating properties of synthetic and natural elastomeric composites. Their combination can overcome several drawbacks of individual components.

Chitosan and collagen based bio-nanocomposites alone could not provide higher tensile strength and enhanced mechanical properties. Nano-structured three-dimensional scaffold materials are promising options which can mimic natural soft and semi-soft tissues and facilitate growth and vasculature resulting in new tissue

regeneration. For example, hydroxylapatite (HA)/collagen composites are usually prepared by self-assembly processes that can promote such tissue regeneration. Spinning of chitosan nano-fibers is a challenging task as chitosan is extensively intermolecular hydrogen bonded. As the polymer concentration is enhanced, the number of direct interacting chains increases. Thus chitosan forms a three dimensional crosslinked network and viscous gel. Addition of PEO can reduce the viscosity of chitosan and makes it spinable. Chitosan has extensively been used in wound dressing systems and tissue engineering applications. Another natural biopolymers namely, gelatin, and a synthetic biodegradable polymer, PCL, can form a favorable composition. The gelatin provides hydrophilic and cellular affinity enabling continuous release of the protein from the scaffold to create a favorable condition for cell attachment and proliferation. It gives superior mechanical properties and permits cell penetration.

Polyether and polycaprolactones are very useful precursors for preparation of elastomeric matrix of synthetic bio-nanocomposites.

3 Advancement on Elastin Based Elastomeric Bio-Nanocomposites

Elastin is an extra-cellular matrix protein which provides elasticity of tissues and organs such as lungs, muscle, and blood vessel. Elastin is mainly derived from tissues like endothelial cells, fibroblasts, chondrocytes etc. The precursor of elastin is known as tropoelastin which can self-assemble depending on physiological condition. The well known phenomenon associated with elastin is coacervation, a second order phase transition which gives proper alignment of elastin tissues [10]. The intermolecular crosslinked structure in elastin can be typically encoded by single copy gene. Coacervation process can be affected by various parameters like temperature, protein concentration, salt concentration, pH etc. Different living beings have similarities in the structure of their respective elastin proteins.

There exist elastin-laminin receptors in various types of biological cells. The receptors contain two subunits of molecular weight 61 and 55 kDa, respectively. The subunit of 67 kDa is a binding site for elastin and laminin [18]. The binding for both tropoelastin and the bound membrane at 55 kDa receptor site is significantly lower helping in release of tropoelastin. The structural features of elastin can be represented as shown in Fig. 2.

Elastin contains uncommon amino acid sequence, making it highly insoluble and resistant to fracture and rupture. Glycine and proline residues have major contribution in the sequence. Elastin has two natural verities, named as α-Elastin and κ-Elastin. The entire protein consists of a repeating polypeptide chains, used as a model of protein structures. Elasticity, glass transition temperature and coacervation are the most important parameters those determine their properties. For ideal elastin chain, energy is consumed by the backbone and recovered by the

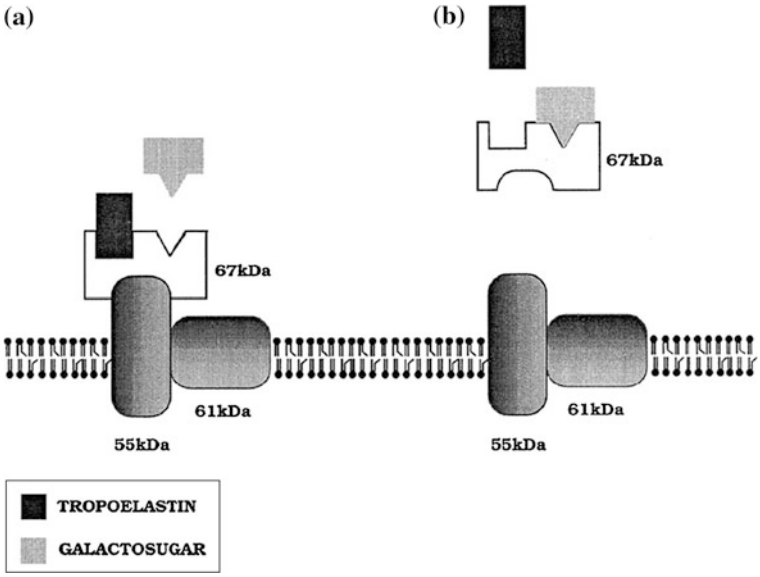

Fig. 2 Pictorial depiction of a model for the elastin-laminin receptor consisting of three subunits. Tropoelastin usually binds to the complex via elastin binding protein [19]. [Reproduced from B. Vrhovski, A.S. Weiss, Biochemistry of tropoelastin, Eur. J. Biochem, 1998, 258, 1–18]

relaxation process. It is surprising that its glass transition temperature (T_g) depends on the elastin content. In dehydrated state, its T_g is 200 °C and at 30 % dehydrated state, T_g is approximately 30 °C. Upon heat treatment, elastin becomes insoluble. Polypeptides in elastin chains lacks in hydrophobic domain. In its structure it contains type II ß-turns. By using centrifugal action, phase separation can be observed. During the turn, segmental motion increases the entropy of the whole system, which is responsible for the elasticity of the elastin itself [20]. During stretching, the damping of this system is also significant. The decrease in entropy can be compensated by release of water, which gives self-assembled structure to align tropoelastin molecule. An elastic ligament is majorly made by elastin having rod-like structure. Molecular dynamics predicts that ß- spiral structure are highly unstable. From both NMR data and MD (molecular dynamics) simulations it has been proven that the β-turns are characterized by an extensive sliding; that is, they are inter-converting to each other along the chain. This phenomenon is proposed as one of the possible sources of entropy in the relaxed state of elastin [21].

C. Hillery and S. Greenwald have carried out dynamic experiments on purified elastin samples. The elastin tissues were cycled at 20 Hz, under a load which was equivalent to arterial pressures. The fracture of elastin tissues occurred after $5–10 \times 10^6$ cycles. It is evident that at 1 Hz, a significant tolerance of dynamic strain has been observed as the number of cycles of application of dynamic strain is increased [22].

Elastin fibers are composed of amorphous elastin and micro-fibrils; those adopt honey-comb like structure. Tropoelastin is mainly synthesized in endoplasmic reticulum with certain modifications. This component mainly acts as a scaffold material, assisting fiber formation. Crosslinked structure has been observed in mature elastin, which is insoluble and extremely stable. Elastin and its derivatives mainly perform several biological functions in chemotaxis, astrocytes, glioma cells, arterial cell, protease activity etc. Incorporation of elastin into bio-systems generates significant effect. The major drawback of elastin is calcinations phenomena. Neucleation can also occur at elastin and collagen based sites. Elastin may exist in different forms depending on the nature of the bio-composites. Elastin has good compatibility with de-cellularized tissues, but these systems lacks in higher degree of purity. De-cellularization is mainly done by extraction methods. The hydrophobic domains are responsible for the elasticity of the protein. The hydrophobic domains are rich in glycine residues which are localized in the N-terminal and C-terminal regions of the protein [10]. Elastin is known as a fractal protein even with a comparatively short sequence showing molecular and supramolecular features resembling the whole protein.

Far-ultraviolet (UV) CD spectroscopy is a handy technique for the conformational studies of bio-molecules like peptides and proteins. This technique is based on the observation that different secondary structures show different characteristic CD spectra. Conformational analyses of α-helices as well as β-sheets are easily identified by CD spectroscopy. The spectral characteristics of conformation of elastin structures are solely dependent on solvent and temperature. The CD spectra are assumed to be a linear combination of all the different secondary structures of elastin. NMR spectroscopy is also extensively used, but due to the timescale of NMR experimentation, it is unable to predict the individual contribution of each conformers of elastin. Thus, the NMR spectroscopy provides the average preferred secondary structure of the polypeptide sequence of elastin. The polypeptide chains oscillate between extended conformations is the key player for the elasticity of elastin. A rapid flipping is suggested between extended and folded conformations [23]. The temperature induced phase transition i.e. coacervation process of elastin is utmost important. This process is completely reversible, leads to formation of two phases: one is rich in protein content, another is rich in aqueous solvent. Soluble peptides released from elastin via proteolytic decomposition, leading to slow aggregation due to the mutated microenvironment. The human tropoelastin gene controls the supramolecular structure of the entire protein which can be reproduced by the single domain of elastin.

E. Wang, S. H. Lee, S.W. Lee synthesized biomimetic matrices using elastin like polypeptides (ELP) and hydroxyapatite (HA) based bionanocomposites. Enhancements in properties of such nanocomposites with improved injectibility and mechanical properties have been observed. ELP exhibits specific interaction to bind and disperse HAP nano-particles. When these bio-composites are incorporated into calcium phosphate cements improvement in adhesion properties has been observed [24].

Mimicking of elastin based materials is an interesting topic for soft tissue engineering [25]. Various modification of elastin structure is underway of research interest. In near future, elastin based nanocomposites will play a pivotal role in the field of semi-synthetic elastomeric bio-nanocomposites.

4 Advancement on Cellulose Based Elastomeric Bio-Nanocomposites

Cellulose is the most abundant natural polymer of this planet. Cellulose and its different derivatives are extensively used during the development of Science and Technology. Chemically cellulose is a carbohydrate, which is obtained by the hydrolysis of mono-saccharides. In its structure, two glucose units are joined by a ß-1, 4 glycosidic linkages. Extensive hydrogen bonding makes a network structure of polymeric cellulose highly stable. This hierarchical structure is built up by smaller and mechanically stronger entities consisting of native cellulose fibrils. These fibrils interact strongly and aggregate to form the natural or native cellulose fibers. The lateral dimension of these fibrils depends on the source of the cellulose but it is typically of the order of a few nanometres. The chemical structure of cellulose is represented as shown in Fig. 3.

Structure of cellulose and its own crystalline organization determine the mechanical properties. Researchers have already proposed that wood contains around 10000 glucopyranose units and cotton contains 15000 repeating units [26]. The basic structural component of cellulose is cellulose microfibril that is formed during the biosynthesis. Actually, the chains of β-1,4-D-glucosyl residues aggregate to form a fibril, which is a long thread-like bundle of molecules laterally stabilized by intermolecular hydrogen bonds. The repeating units have 20 nm lengths. FTIR and XRD studies clearly reveal that the main portion of cellulose is constituted by crystallites interspersed with amorphous regions having low degree of order with parallel chains. When the cellulose is precipitated out of alkaline

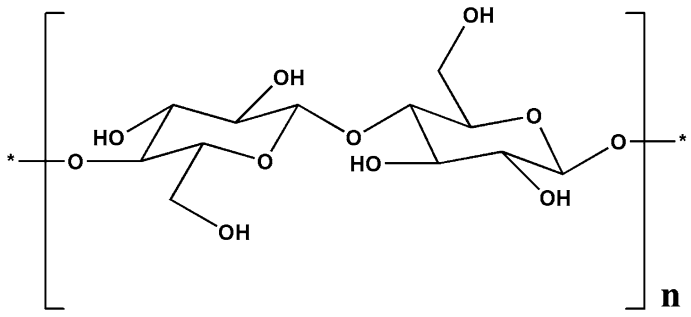

Fig. 3 Chemical structure of cellulose molecule

Table 1 Mechanical properties various cellulose based nano-fibers [28, 29]

Fiber type	Young's modulus (GPa)	Specific Young's modulus ($GPa \cdot Mg^{-1}m^3$)	Breaking strength (GPa)	Breaking strain (%)
Flax	27.0	18.0	0.81	3.0
Jute	25.8	17.2	0.47	1.8
Hemp	32.6	21.7	0.71	2.2
Ramie	21.9	14.6	0.89	3.7

[Reproduced from **a** current international research into cellulose nanofibres and nanocomposites-S.J. Eichorn, A. Dufresne, M. Aranguren et al., J. Mater. Sci. 45 (2010) 1–33, and **b** W.E Morton, J.W.S Harle, Physical properties of textile fibers, William Heinnmann Limited, London, 1993]

solution it is known as regenerated cellulose. Inter-conversion between two forms of cellulose is performed by strong alkaline hydrolysis [27]. The material used for cellulose nano-filler contains mainly native cellulose (Cellulose I) which is extracted by traditional bleaching treatment of lignocellulosic fibers. Cellulose I is responsible for mechanical properties due to its inherent high modulus and crystalline nature. Table 1 displays the mechanical properties of various cellulose based nano-fibers. Incorporation of such nano-fibers into suitable bio-elastomeric matrices gives promising improvement of mechanical properties of the resultant nanocomposites.

Several methodologies regarding preparation of cellulose fibers have been adopted. The fibers are first milled and are made to undergo alkali treatment using NaOH followed by bleaching using sodium hypochlorite ($NaClO_2$)/acetate buffer solution. The cellulose moieties remain intact. The bleached fibers are lead to acid hydrolysis and disintegrated by mechanical shearing. Then the cellulosic nano-whiskers as highly pure single-crystal are extracted. These nano-fibers don't show surface regularity, as it contains both amorphous and crystalline domains. The surface morphology of typical Baggase whiskers has been evaluated by TEM as represented in Fig. 4. The amorphous regions are found to be randomly oriented compared to the crystalline regions.

The equatorial positions of the glucopyranose residues play the crucial role as it stabilizes the structure of cellulose. Extensive intra and intermolecular hydrogen bonding also causes insolubility in water and accordingly increase its mechanical rigidity. The mechanism of cellulose nano-crystal production has been proposed by De Souza Lima and Borsali [30]. The hydroxonium ion penetrates into the cellulose and the amorphous region promotes the cleavage of the glycosidic linkage giving individual crystallites. Reaction time is assumed to be an important parameter during acid hydrolysis of the wood-pulp. It has also been observed that sulfuric acid prepared nano-crystals present a negatively charged surface, due to the esterification of surface hydroxyl groups leading to charged sulfate groups [31]. Nano-whiskers promote reinforcement in all bio-nanocomposites. Its size, shape and dimensions solely depend on the source of cellulose. It is customary to write that cellulose nano-whiskers are devoid of chain folding, so they contain a few crystal defects.

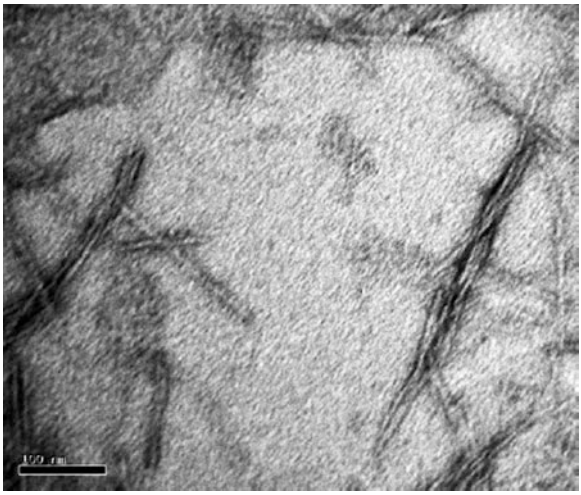

Fig. 4 TEM photomicrograph of Baggase cellulose whiskers [33], [J. Bras, M.L. Hassan, C. Bruzesse, E.A. Hassan, N.A. El-Wakil, A. Dufrense, Industrial Crops and Products, 32(2010) 627–633]

Micro-fibrillated cellulose (MFC) belongs to a special classes of cellulose derivative, obtained from wood cell by means of mechanical degradation. MFC belongs to the class of long and flexible nanoparticles which is composed of individual cellulose micro fibrils. It exhibits a typical web-like structure [32]. Homogenization treatment also gives a diluted dispersion of MFC having a sol–gel structure. Micro fluidizer is also used to destroy the fibril structure of cellulose pulps producing MFC. During production it consumes a large amount of energy, and it has been an obvious drawback of this process. An optimization process for micro-crystalline cellulose was already established depending on various parameters like concentration, temperature etc.

Another facile route for the preparation of MFC is enzymatic hydrolysis of the cellulose and its derivatives, e.g. cellulose can undergo hydrolysis by pure C-type endoglucanase. Cellulose based bio-nanocomposites has immense application as high performance materials. Improvement of mechanical properties of a model elastomeric bio-nanocomposite by the incorporation of MFC matrix can be statistically proven from literature [Table 2].

A large volume of interfacial materials are associated with nanocomposites in their micro-structure. Thus bulk properties differ significantly from the bulk elastomeric materials. It is worthy to note that prediction of the properties of cellulose nano-fiber based nanocomposites has certain limitations also. However cellulose nanoparticles like whiskers and MFC imparts significant reinforcement in composites. They have lots of advantages like—low weight, biodegradability, minimum energy consumption, lower abrasion along with some drawbacks including moisture absorption and incompatibility [35]. Preparation of cellulose based nanocomposites follows two methods namely solvent casting and mechanical extrusion. During nanocomposite preparation two methods are generally employed to modify surface of cellulose e.g., coating of the surface using

Table 2 Table of tensile modulus (E_i, MPa) and shrinkage (r_i, %) calculated for cellulose nanoparticles/NR nanocomposites during successive tensile tests [34]

Sample	E1	E2	E3	E4	E5	E6	E7
NR	0.64	0.58	0.36	0.27	0.20	0.17	0.16
NR-W1	1.58	0.75	0.38	0.27	–	–	–
NR-MF1	1.50	0.79	0.32	0.22	–	–	–
Sample	r_1	r_2	r_3	r_4	r_5	r_6	
NR	64	133	194	252	303	–	–
NR-W1	50	105	156	–	–	–	–
NR-MF1	35	84	117	–	–	–	–

[Reproduced from A. Bendahou, H. Kaddami, A. Dufresne, European Polymer Journal, 46(2010) 609-620]

specific surfactants or grafting of the hydrophobic chains onto the fibrils. Use of cellulose nanoparticles at the dry state is a challenging work as extensive hydrogen bonding forms agglomerated structures. Formation of rigid whiskers is also possible by solution cast method. Cellulose nanocomposite films are prepared in aqueous solution due to its inherent stability in water soluble polymers. Polymers in the form of latex can also be used for emulsion systems which also include natural rubber (NR), Poly-vinyl chloride (PVC) and Poly-vinyl acetate (PVAc). In non-aqueous media mainly suspension systems are used. Tunicin based whiskers are mainly used in this methods. Acid hydrolysis of MCC produces whiskers with controlled porosity. Typical nanocomposites have been prepared using cellulose tunicin whiskers in DMF solution exhibiting better thermal and mechanical properties [36]. Another method for preparation of tunicin whiskers is acid hydrolysis using HCl.

Whiskers behave differently when modified in protic solvents as protic solvents disrupt hydrogen bonding and it promotes the dispersion as mentioned earlier. Surface chemical modification is another promising technique for stabilization and dispersion of the nano-crystals. Grafting agents also improves the compatibility of cellulose fibers in bio-polymers. MFC can be chemically surface modified using silanes and isocyanates showing improved dispersion in acetone and THF. Silane coupling agents like 3-trimethoxy aminosilane used for the surface modification of cellulose nano-crystals causes a change in the hydration behavior of nano-crystals [37]. Polysaccharide nano-crystals can be transforms into a co-continuous material, improving bondability between the filler and polymer. Polycaprolactone is also a promising material with a huge scope for grafting purpose. “Grafting from” method is also a versatile one for surface modification. This results in change of crystalline features. It is evident that aspect ratio plays a vital role for reinforcement which varies significantly depending on the sources of materials [38]. Dynamic mechanical analysis (DMA) reveals important clues on complicated relaxation processes involved in such materials. But still, origin of the mechanical reinforcing effect is poorly understood for such systems. Characterization of the prepared nanocomposites in a precise and repeated manner is very essential for

structure- property correlation. Transmission Election Microscopy is an essential tool for analysis of the morphology of such nanocomposites.

Cellulose with tendon produces bio-based elastomeric nanocomposites. These bio-composites give both biocompatibility and biodegradability including excellent mechanical properties.

Highly deacetylated chitosan and wood cellulose whiskers have been used to make elastomeric bio-nanocomposites. They show improved viscoelastic and mechanical properties. These materials are extensively used in biomedical and packaging applications [39].

Acetylated microfibrillated cellulose and PLA forms useful elastomeric bio-nanocomposites. A dramatic improvement of compatibility of fibre matrix interface has been observed. Accordingly, thermal, mechanical and viscoelastic properties of such nanocomposites are found to be significantly enhanced [40].

In near future, these forms of cellulose reinforced elastomeric bio-nanocomposites will occupy a pivotal role for bio-medical applications.

5 Advancement on Chitin Based Elastomeric Bio-Nanocomposites

Chitin and its derivatives are now proven to be a promising representative in the area of bio-polymers [41]. Recently, a successful method of interest is to develop chitin and chitosan based biomaterials. Hydroxy and acetamide derivatives of chitin are interesting candidates for bio-degradable elastomers. Chitin with pyranose ring has rigid cellulosic structure. Chitin is chemically composed of 1, 4-2–acetimido-2-deoxy-β-D-glucose [42]. It occurs in the skeletal materials of insects and in bacterial cell wall. Chemically, it is an aminopolysaccharide having acetamido group at C-2 position. Nanocomposites of chitin have several advantages being non-toxic, bio-degradable and anti-bacterial. Chitin as a material has vast unexplored commercial applications. Novel modification techniques of PU structure by incorporating starch, polycaprolactone and chitin as a chain extender is also a thrust area of research [43]. The hydroxyl and acetamido functionalities of chitin based bio-polymers allow chemical reaction with conventional diisocyanates or urethanes. Actually, it is evident that the –OH group at C-6 position of chitin molecule react with the pre-polymer giving final hybrid-PU. The chemical structure of chitin is represented in Fig. 5.

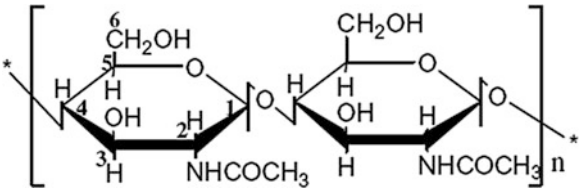

Fig. 5 Chemical structure of chitin bio-molecule [44]

It is evident that, hybrid-PU shows optimum hydrophobicity than virgin chitin based nanocomposites. Improvement in bio-compatibility of these systems has been clearly indicated in literature [44]. Structure—property relationship for elastomers based nano-structured chitin solely depends on hydrogen bonding, hydrophobicity and chitin content in the elastomeric matrix [45]. Chitin and polyurethanes gives a favorable combination to form composite structures of PU having micro-phase separated structure leading to good compatibility. Successful methods of increasing bio-degradability of such materials are developing and there have been an increasing trend of using chitin with other elastomeric nano-composites. Chitin in combination with polysaccharides is bio-degradable in bio-active environments, where bacterial population is higher. The major drawback of chitin is its inferior solubility, which mimics its application to replace the existing members of elastomeric bio-nanocomposites [46]. Chitin with pyranose ring has rigid cellulosic structure. Overall chitin based nanocomposites have sufficient scope as a potential elastomeric bio-nanohybrid material.

6 Advancement on Starch Based Elastomeric Bio-Nanocomposites

Starch is one of the most important and abundant bio-molecules since the genesis of this universe. Various types and classes of starch molecules are found in the animal and plant kingdom. Starch molecule can be modified by esterification reaction, leading to numerous applications [47]. Modified starch can also be blended with natural rubber latex, producing a typical elastomer based bio-composites. Starch-NR composites are known to have superior thermal and mechanical properties along with unique morphological characteristics [48]. The crystal structure of starch disintegrates and uniformly dispersed in the NR matrix. It can be assumed that, improved inter-phase interaction between NR and starch gives better physical properties [49]. Modification of starch reduces its size with improved dispersion in polymer matrix. Cornstarch-NR bio-composites are an effective alternative for tyre-tread applications [50]. Various factors control the mechanical properties. Starch molecules having higher sizes are bonded through adjacent hydroxyl groups. The tear resistance and elasticity usually degrades when starch is incorporated without surface modification [51]. Another approach is the use of methacrylate grafted copolymer of natural rubber and methyl methacrylate [52]. Polyvinyl acetate (PVA)/starch, poly-ethylene octane copolymer (POE)/starch composites, poly-caprolactone/starch, polyester/starch, poly-lactic acid (PLA)/starch nano-composites are well established as elastomeric bio-nanocomposites [53]. Starch-elastomeric bio-nanocomposites has immense in application in medicine, agricultural and industrial aspects.

7 Advancement on Synthetic and Semi-Synthetic Elastomer Based Bio-Nanocomposites

To overcome several drawbacks of the available natural bio-elastomers, scientists have been engaged towards the synthesis of bio-elastomers with well optimized properties. Synthetic bio-elastomers can be associated with the certain advantages including high purity, good processibility, and optimized physical, chemical, mechanical properties. For a tissue engineered scaffold, the rate of degradation must match with the rate of tissue regeneration. Scaffolds should degrade chemically following hydrolytic or enzymatic degradation mechanism [11]. Several external factors influence the degradation pathway including types of chemical bond, crystallinity, hydrophillicity, pH and temperature. Degradation can also be assisted through the change of mechanical properties like, weight loss, surface porosity, residual monomer etc. Polyurethanes and polyphosphazenes have provoked a new era in the field of synthetic bio-elastomers. Apart from these, a handful of bio-polymers (block copolymers) have been found to exhibit thermoplastic elastomeric properties making them suitable matrices of choice for the preparation of elastomeric bio-nanocomposites.

7.1 Biodegradable Polyurethanes

Segmented polyurethanes (SPU) belong to a distinct class of synthetic biomaterial well known over the last decade. SPU provides biodegradability via hydrolysis reaction with tailored physical properties as the properties can be varied from biostable to rapidly bio-degradable elastomer [54]. It is customary that bio-degradable polyurethanes (PUs) are designed to undergo bio-degradation via *in vivo* mechanistic pathways [55]. SPU comprises of two segments namely, hard and soft segments. The hard segment is composed of diisocyante and the chain extender, on the contrary, the soft segment contains an amorphous macro-diol. SPU provides uniform elastomeric properties. These materials possess tensile strength (TS) ranging from 4–60 MPa with an elongation at break of 100–950 %. Average degradation time is also significantly lower for SPU [56]. The main degradation products are α-hydroxyl acids, urethanes, urea fragments, lysine etc. Biodegradable SPU contains polyether or polyester macrodiol components. The polyol are mainly made of PEO, PPO, PCL, Polylactide and polyhydroxy butyrate. It has been found that PEO based PU are weak and amorphous in nature [57]. PEO can accelerate the degradation rate but PCL enhances the crystallinity of the matrix [58]. Commercially biodegradable SPU was launched as DegraPol (Trade name) which uses mainly lysine based macrodiol. The diisocyanate component has several limitations as biomaterial because it produces toxic products during degradation leading to inflammatory response in the body. Newly invented lysine

methyl ester diisocyanate (LDI) and 1, 4-diisocyanatobutane is extensively used for bio-medical application [11, 59].

Recently, degradable chain extenders have been invented which are mainly diammine based. Phosphate ester linkage containing lactic acid based chain extenders are also bio-degradable [51]. Bio-degradable SPU is designed to undergo enzymolysis/hydrolysis [49]. The degradation is controlled mainly by the degree of cross linking. Chemical links present in soft segment hydrolyze more quickly than that of the hard segment. Polyurethans can be surface modified using poly (N-vinyl pyrollidine) i.e. PolyNVP which provides well known bio-compatibility. These biomaterials have high water solubility also. These precursors have applications in coated catheters [60].

The mechanical properties and resistance to urinary encrustation of IPN network formed by polyurethane (PU) and PMMA have been studied by Jones et al [61]. Maximum elongation at break has been observed for pure PU and it decreases with increasing PMMA content. These biomaterials provide high degree of resistance to compression of the ureter in comparison with native PU [61].

Bioactive glasses are a promising reinforcing material used in the formation of several types of bio-nanocomposites. Ciobanu et al. [62] has investigated the hydroxyapatite formation on the porous surface of polyurethanes. Using solvent casting method, they have developed three dimensional porous scaffold materials. Then HA was coated on the scaffold has been precisely characterized using XRD, SEM. These elastomeric bio-nanocomposites containing uniformly coated HA can be applied to obtain uniformly coated scaffold material [61].

Zuber et al. [63] have synthesized chitin based polyurethane/clay nanocomposites. It has been observed that interaction among the clay and the polymer chain improves their dispersion in the PU matrix. Optical microscopy clearly reveals that the MMT clays have been well dispersed and intercalated layers of clays have been formed [63].

Wu et al. [64] have synthesized novel high strength elastomeric bio-nanocomposites using microcrystalline cellulose and PU. Significant improvement in tensile strength, stiffness and strain to failure properties has been observed. Due to extensive hydrogen bonding as well as covalent bonding between PU and cellulose network such improvement in properties has been observed [64].

Wang et al. [65] have embedded starch nano-crystals and cellulose whiskers in waterborne polyurethanes. It is evident that the Young's modulus and tensile strength of such elastomeric bio-nanocomposites along with thermal properties have been significantly improved than that of pristine PU. Actually, polysaccharide molecules and whiskers forms a hydrogen bonded network, giving synergistic effect. They belong to the class of eco-friendly elastomeric bio-nanocomposites [65].

Chen et al. [66] have prepared elastomeric bio-nanocomposites using starch nano-crystals and PU. Significant enhancement in tensile strength, elongation at break and Young's modulus has been observed up to an optimum loading of starch nano-crystals [66].

7.2 Bio-Degradable Polyphosphazenes

Polyphosphazenes belong to a special class of inorganic polymer used in biomedical applications. The inorganic backbone consists of alternating phosphorous and nitrogen atoms with adjacent groups. The properties of this polymer are entirely governed by the adjacent substituents [11]. During degradation, its mainly convert itself into ammonia, phosphates and alkyl moieties. The general structural representation is given as:

$-[N = PR_2]-_n$

It is evident that the structure of polyphosphazenes has a high degree of freedom of bond rotation and low glass transition temperature. Most PN derivatives show elastomeric properties. Small group containing derivatives renders lower T_g values. The movement of chains can also be restricted by inter-atomic interaction. Amine substituted polymers generally exhibits higher T_g values. The flexibility of PN backbone arises as it undergoes structural changes in the solid state [67]. Biodegradable PNs comprise hydrophilic substituents in a maintained ratio. This active group can be grafted or trapped in the polymeric matrix. Imidazole, glucose and polyether groups are incorporated to favour hydrolysis. PN also degrades via erosion mechanism depending on several factors such as lability of bonds, water permeability etc. Polyphosphazenes are classified into two categories e.g., PN substituted with amines and substituted with alcoholic moieties.

Aminated PNs are extensively used in biomedical applications [68]. Alkoxy substituted PNs, such as glyceryl substituted ones are bio-compatible in both in vitro and in vivo conditions.

Glycine containing polyphosphazenes derivatives have also been synthesized which are fully biocompatiable. They support cell adhesion and growth [69, 70]. Degradable polyphosphazenes/poly (α-hydroxy ester) blends have also been studied. The major degradation products associated with this material consist of phosphate, glucose, ammonia etc.

Liyan Qiu [71] has synthesized chitosan coated polyphosphazenes-Ca^{2+}-hydrogel i.e. a novel class of elastomeric bio-nanocomposites. Polyphosphazenes have been dropped into $CaCl_2$/chitosan gelling solution. The interaction among the polymeric components have revealed by using turbidimetric titration method. It has been observed that drug loading efficiency of such elastomeric bio-nanocomposites is significantly high [70].

Nukavarapu et al. [72] have synthesized polyphosphazene/nano-hydroxyapatite composite microsphere scaffolds. These composite have been fabricated into a three dimensional microporous scaffolds. These elastomeric bio-nanocomposites provide compressive modulus of 46–81 MPa with mean pore diameter 86–145 μm. These scaffolds have certain advantages including good cell adhesion inducing proliferation of cells [72].

Allcock et al. [68] have also studied radiation crosslinking of hydrogel based on (methoxyethoxyethoxy) polyphosphazenes. It has been observed that increasing

radiation dosages enhances the degree of crosslinking. Superior mechanical properties of such elastomeric bio-nanocomposites have been observed [68].

Andrianov [73] have synthesized ionically crosslinked polyphosphazene hydrogel microsphere. The synthesized microdroplets are highly stabilized when crosslinking with Ca^{2+} ions. This microsphere can be extensively used as vaccine delivery vehicles [73].

It is already established that glycerol and sebacic acid monomers produce a novel elastomeric material, poly (glycerol sebacate) PGS a useful elastomeric material for soft tissue engineering [74]. The degradation products of PGS are non-toxic. PGS has certain advantage e.g. simple synthesis procedure, tailored mechanical and biodegradation characteristics. Extensive research on preparation and modification of novel PGS are still underway.

Elastomeric bio-nanocomposites of poly-vinyl pyrollidine, chitosan and PVA belong to a novel class of elastomeric bio-nanocomposites. The thermal and mechanical properties of such novel bio-nanocomposite are considerably high [75].

Citric acid is a non-toxic metabolic product of the body, and it can form network structure. Several advantages of poly (diol citrate) elastomers include non-toxicity, availability and lower cost. Biodegradable crosslink's can introduce elasticity in these systems. Various diols provide flexibility in the elastomer. Intermolecular hydrogen bonding can enhance mechanical properties of the elastomer. It's synthesis can be conducted under very mild condition. Citric acid reacts with various diols without using any catalyst. Improvement in tensile properties has been observed e.g., T.S increases up to 11.5 MPa with significant improvement in percentage elongation [76].

Chung et al. [77] have precisely investigated the synthesis, characterization and biological response of citric acid based elastomeric bio-nanocomposites. They have prepared a novel elastomeric bio-nanocomposites using poly (1, 8-octanediol-co-citrate) and hydroxyapetite nanoparticles. It has been observed that mechanical properties of such elastomeric bio-nanocomposites including strength and stiffness is remarkably improved compared to the virgin polymer. The tissue response has been found to be dependent on the content of HA [77].

Webb et al. [78] have fabricated novel elastomeric bio-nanocomposites using poly (L-lactic acid) and poly-diol citrate. A significant improvement in tensile strength, elongation at break and modulus has also been reported. This system has potential to serve as novel elastomeric bio-nanocomposites for soft tissue engineering in near future [78].

Poly ether esters (PEE) belong to the group of novel elastomeric material, consisting of soft segmented polyethers and hard crystalline segment of polyesters. The ratio of ether to ester dictates wide range of mechanical properties and biodegradability. It's synthesis procedure have been widely reported and it is synthesized using PEG 1000, 1, 4 butane-diol and dimethyl terepthlate as the precursor materials [79]. The families of silk-elastin like polymer hydrogels are newly invented, consisting alternating blocks of silk-like and elastin-like blocks [80]. These types of polymers can be categorized under hydrogels as well as under

bio-nanocomposites. These typical elastomeric bio-nanocomposites provide solubility, higher mechanical strength, immunogenicity and *in vivo* degradation by alternating the amino acid sequence. The properties of these typical materials can be altered by several factors including ionic strength, polymer concentration, cure time etc. It is already established that macroporus scaffolds can be fabricated from a biocompatible and bio-degradable elastomers [81]. Porous collagen matrix can be cross linked using elastomeric hyaluronic acid and fibro-nectin results improved cell attachments [82]. Elastomeric proteins have certain novel properties also e.g., long range elasticity, reversible deformation and high resilience. Synthetic polypeptides have recently applied to prepare multi-block protein based copolymers. Nanoclay based elastomeric polypeptide composites are found to be relevant in the field of bio-nanocomposites. It gives the advantages like bio-degradability and gas and water permeability. These have potential applications in living bio-systems. Immunogenic response of peptide sequences limits the choice of polypeptides for biomedical applications.

Silicone rubber-hydrogel bio-nanocomposites have been extensively investigated by P. Lopour et al. A matrix has been developed composed of polysiloxane and hydrogels. The hydrogel phase consists of crosslinked poly (2-hydroxy ethyl methacrylate). High water permeation rates have been obtained with retention of mechanical properties [83]. Elastomer based bio-nanocomposites containing silicone rubber matrix and particulated hydrogels is an emerging field of biomaterial research interest. Several factors including size, shape and aggregation of hydrogels those affect tensile properties and tear strength of such composites. It has also been observed that chemical composition of such bio-nanocomposites affect its mechanical properties [9]. In near future, silicone rubber based biomaterials are likely to serve to the entire mankind.

7.3 Bio-Inspired Preparation of Elastomeric Bio-Nanocomposites

Recent patents in the field of bio-inspired, bio-mimicked and bio-hybrid materials are an emerging facet of research. Several international patents have already been filed based on the development of such novel materials [84–88]. Biological preforms may begin to replace some classes of light duty composite materials. Collagen tissues are abundant in bio-systems which are capable of supporting tensile loads. Biomimicry is a well known method to exploit existing biologically derived materials with a nano-porous structure. Biopreform have been made from several plants like coconut, date palm etc. [89, 90]. These plants are dried under pyrolytic condition. Silicon and silicon carbide based ceramics have been made using this procedure.

Syntheses of peptide/peptide like molecule for in vitro and in vivo applications have already been patented [91]. Here, targeted protein expression can be tracked

and properly monitored. Polymeric micelle for drug delivery has already been patented; a drug loaded sample contains triblock amino acids have been developed [92]. The blocks consist of one hydrophilic block, a crosslinked poly amino acid block, an uncrosslinked poly amino acid block. Surface functionalization method may be adopted for the attachment of the drug using PEG, HMPA as the novel monomer. Fluorescence resonance energy transfer method is used for their characterization. Use of amphiphilic multiblock copolymers has already been patented. A liposome is a bilayered sphere of amphiphilic molecule. Amphiphilic block copolymer contains both hydrophobic and hydrophilic block. Click chemistry is a new promising technique regarding the development of such kind of smart materials.

8 Conclusions

Elastomeric bio-nanocomposites have emerged as unique advanced material with potential biological applications. It is a challenging task for the researchers and scientists to unify characteristic properties of traditional elastomers, nano-scale reinforcements and biomaterial attributes within a single composite. A lot of these materials of this class have been invented by bio-inspired and bio-mimetic research in the allied areas. The naturally occurring bio-elastomeric matrices have been mainly protein and carbohydrate based materials having comparatively lower intermolecular force and glass transition temperature. The deficiencies of mechanical properties of the matrix are reinforced with various bio-based nano scale fillers preserving their bio-compatibility. These materials have wide range of applications including in soft tissue engineering, wound dressing, drug delivery, packaging etc. Because of the several draw backs of properties of purely bio based elastomeric bio-nanocomposites, synthetic and semi-synthetic elastomeric bio-nanocomposites are emerging on fast track. Elastomeric bio-nanocomposites have a promising future for the next decade as novel advanced materials.

References

1. http://en.wikipedia.org/wiki/composite material
2. Darder, M., Aranda, P., Hitzky, E.R.: Adv. Mater. **19**(10), 1309–1319 (2007)
3. Darder, M., Colilla, M., Hitzky, E.R.: Chem. Mater. **15**, 3774 (2003)
4. Glawe, D.D., Rodriguez, F., Stone, M.O., Naik, R.R.: Langmuir **21**, 717 (2005)
5. http://images.isiknowledge.com
6. Evans, S.L., Gregson, P.J.: Biomaterials **19**, 234 (1998)
7. de Groot, K., Geesink, R.G.T., Klein, C., Serekian, P.: J. Biomed. Mater. Res. **21**, 1375–1381 (1987)
8. Lagally, M.G.: Jpn. J. Appl. Phys. **32**, 1493 (1993)
9. Lapour, P., Plichta, Z., Volfova, Z., Hron, P., Vondracek, P.: Biomaterials, **14**(14), 1051–1055 (1993)

10. Lau, A.K.T., Hussain, F., Lafdi, K. (eds.): Nano and Biomaterials 1st edn. CRC press, Taylor and Francis group, USA (2010)
11. Shi, R., Chen, D., Li, Q., Wu, Y., Xu, X., Zhang, L., Tian, W.: Int. J. Mol. Sci. **10**, 4223 (2009)
12. Planeix, J.M., Jaunky, W., Duhoo, T., Czernuszka, J.T., Hosseini, M.W., Bres, E.F.: J. Mater. Chem. **13**, 252 (2003)
13. Suchanek, W., Yoshimura, M.: J. Mater. Res. **13**, 94 (1998)
14. Unwin, A.P., Ward, I.M., Ukleja, P., Weng, J.: J. Mater. Sci. **36**, 3165 (2001)
15. Rea, S.M., Bonfield, W.: J. Aust. Ceram. Soc. **40**, 43 (2004)
16. Zhang, Y.Z., Lim, C.T., Ramakrishna, S., Huang, Z.M.: J. Mater. Sci. **16**, 933 (2005)
17. Du, C., Cui, F.Z., Zhu, X.D., de Groot, K. : J. Biomed. Mater. Res. **44**(4) 407–415 (1999)
18. Daamen, W.F., Veerkamp, J.H.: J.C.M van Hest, T.H van Kuppevelt. Biomaterials **28**, 4378 (2007)
19. Vrhovski, B., Weiss, A.S.: Eur. J. Biochem. **258**, 1 (1998)
20. Venkatachalam, C.M., Urry, D.W.: Macromolecules **14**, 1225 (1981)
21. Debelle, L., Tamburro, A.M.: Int. J. Biochem. Cell. Biol., **31**(2), 261–272 (1999)
22. Rachev, A., Greenwald, S.E.: J. Biomechanics **36**, 661 (2003)
23. Woody, R.W.: Adv. Biophys. Chem. **2**, 37 (1992)
24. Wang, E., Lee, S.H., Lee, S.W.: Biomacromolecules **12**, 672 (2011)
25. Silva, S.S., Mano, J.F., Reis, R.L.: Crit. Rev. Biotechnol. **30**(3), 200–221 (2010)
26. Vrhovski, B., Jensen, S., Weiss, A.S.: Eur. J. Biochem. **250**(1), 92–98 (1997)
27. Anderson, M., Johansson, L.S., Tanem, B.S., Stenius, P.: Cellulose **13**, 665 (2006)
28. Eichhorn, S.J., Dufresne, A., Aranguren, M., Macrovich, N.E., Capadona, J.R., Rowan, S.J., Weder, C., Thielemans, W., Roman, M., Renneckar, S., Gindl, W., Veigel, S., Keckes, J., Yano, H., Abe, K., Nogi, M., Nakagaito, A.N., Mangalam, A., Simonsen, J., Benight, A.S., Bismarck, A., Berglund, L.A., Peijs, T.: J. Mater. Sci. **45**, 1 (2010)
29. Morton, W.E., Harle., J.W.S.: Physical Properties of Textile Fibers 3rd edn. William Heinemann Limited, London (1993)
30. de Souza, M.L., Borsali, R.: Macromole. Rapid Comm. **24**, 4168 (1991)
31. Gray, D., Raman, M., Candanedo, S.B.: Biomacromolecules **11**, 551 (2009)
32. Rowell, R.M., Han, J.S., Rowell, J.S.: Cellulose **13**, 665 (2006)
33. Bras, J., Hassan, M.L., Bruzesse, C., Hassan, E.A., El-Wakil, N.A., Dufresne, A.: Ind. Crops Prod. **32**, 6273 (2010)
34. Bendahou, A., Kaddami, H., Dufresne, A.: Europ. Polym. J. **46**, 609 (2010)
35. Henrikson, M., Henrikson, G., Berglund, L.A., Lind Storm, T.: Europ. Polym. J. **43**, 3434 (2007)
36. Azizi Samir, M.A.S., Alloin, F., Durfence, A.: Biomacromolecules **6**, 612 (2005)
37. Lu, J., Askland, P., Drazel, L.T.: Polymer **49**, 1285 (2008)
38. Favier, V., Chazy, H., Cavaille, J.Y.: Macromolecules **289**, 6365 (1995)
39. de Mesquita, J.P., Donnici, C.L., Pereira, F.V.: Biomacromolecules **11**, 473 (2010)
40. Tingaut, P., Zimmermann, T., Suevos, F.L.: Biomacromolecules **11**, 454 (2010)
41. Chandra, R., Rustgi, R., Prog, J.: Polym. Sci. **23**, 1273 (1998)
42. Muzzarelli, R.A.A. (ed.) : Chitin 1st edn. Pergamon press, USA (1977)
43. Barikani, M., Hepburn, C.: Cell. Polym. **6**, 47 (1987)
44. Zia, K.M., Barikani, M., Zuber, M., Bhatti, I.A., Seikh, M.A.: Carbohydr. Polym. **74**, 149 (2008)
45. Alfani, R., Lannancee, S., Nicholas, S.: J. App. Polym. Sci. **68**, 738 (1998)
46. Jang, M.K., Kong, B.G., Jeong, Y.I., Lee, C.H., Nah, J.W.: J. App. Polym. Sci. **42**, 3423 (2004)
47. Wang, Z.F., Peng, Z., Li, S.D., Lin, H., Zhang, K.X., She, X.D., Fu, X.: Compos. Sci. Technol. **69**, 1797 (2009)
48. Rouilly, A., Rigal, L., Gilbert, R.G.: Polymer **45**(23)7813–7820 (2004)
49. Shang, X., Fu, X., Yang, L.: J. Reinfo. Plast. Compos. **28**(3), 279–288 (2009)
50. Qi, Q., Wu, Y.P., Tian, M., Liang, G.H., Zhang, L.Q., Ma, J.: Polymer, **47**(11), 3896–3903 (2006)

51. Rosa, D.S., Lopes, D.R., Calil, M.R.: Polym. Test. **24**, 756 (2005)
52. Nakason, C., Kaesman, A., Eardrod, K.: Mater. Lett. **59**(29), 4020 (2005)
53. Yew, G.H., Mohd. Yusuf, A.M., Mohd. Ishak, Z.A., Ishiaku, U.S.: Polym. Degrad. Stabil. **90**(3), 488–500 (2005)
54. Skarja, G.A., Woodhouse, K.A.: J. Biomater. Sci. Polym. Ed. **12**, 851 (2001)
55. Santerre, J.P., Woodhouse, K., Laroche, G., Labow, R.S.: Biomaterials **26**, 7457 (2005)
56. Wang, Y., Kim, Y.M., Langer, R.: J. Biomed. Mater. Res. A **66**, 192 (2003)
57. Skarja, G.A., Woodhouse, K.A.: J. Appl. Polym. Sci. **75**, 1522 (2000)
58. Guan, J., Fujimoto, K.L., Sacks, M.S., Wagner, W.R.: Biomaterials **26**, 3961 (2005)
59. Ganta, S.R., Piesco, N.P., Long, P., Gassener, R., Motta, L.F., Papworth, G.D., Stolz, D.B., Watkins, S.C., Agarwal, S.: J. Biomed. Mater. Res. **64** A, 238–242 (2003)
60. Wetzels, G.M.R., Koole, L.H.: Biomaterials **20**, 1879 (1999)
61. Jones, D.S., Bonner, M.C., Gorman, S.P., Akay, M., Keane, P.F.: J. Mater. Sci. **8**, 713 (1997)
62. Ciobanu, C., Ignat, D., Luca, C.: Chem Bull., Politehnica. **54**, **68** (1)57–60 (2009)
63. Zuber, M., Zia, K.M., Mahboob, S., Hassain, M.: Int. J. Biological. Macromol. **47**, 196 (2010)
64. Wu, W., Henrikson, M., Liu, X., Berglund, L.A.: Biomacromolecules, **8**(12), 3687–3692 (2007)
65. Wang, Y., Tian, H., Zhang, L.: Carbohydr. Polym. **80**, 665 (2010)
66. Chen, G., Wei, M., Chen, J., Huang, J., Dufresne, A., Chang, P.R.: Polymer **49**, 1860 (2008)
67. Potin, P., Jaeger, R.D.: Eur. Polym. J. **415**, 341 (1991)
68. Allcock, H.R.: Acc. Chem. Res. **12**, 351 (1979)
69. Allcock, H.R., Kwon, S., Riding, G.H., Fitzpatrick, R.J., Bennet, J.L.: Biomaterials **9**, 509 (1988)
70. El-Amin, S.F., Kwon, M.S., Starnes, T., Allcock, H. R., Laurencin, C.T.: J. Inorg. Org. Polym. Mat. **16**(4), 387–396 (2006)
71. Qiu, L.: J. Appl. Polym. Sci. **92**, 1993 (2004)
72. Nukavarapu, S.P., Kumbar, S.G., Brown, J.L., Krogman, N.R., Weikel, A.L., Hindenlang, M.D., Nair, L.S., Allcock, H.R., Laurencin, C.T.: Biomacromolecules **9**, 1818 (2008)
73. Andrianov, A.K., Chen, J., Payne, L.G.: Biomaterials **19**, 109 (1998)
74. Wang, Y., Ameer, G.A., Sheppard, B.J., Langer, R.: Nat. Biotechnol. **20**, 602 (2002)
75. Chrissafis, K., Paraskevopolous, K.M., Papageorgiou, G.Z., Bikiaris, D.N.: J. Appl. Polym. Sci. **110**, 1739 (2008)
76. Yang, J., Webb, A.R., Pickerill, S.J., Hageman, G., Ameer, G.A.: Biomaterials **27**, 1889 (2006)
77. Chung, E.J., Qiu, H., Kodali, P., Yang, S., Sprauge, S.M., Hwong, J., Koh, J., Ameer, G.A.: J. Biomed. Mater. Res. A, 96(1), 29–37 (2011)
78. Webb, A.R., Kumar, V.A., Ameer, G.A.: J. Mater. Chem. **17**, 900–906 (2007)
79. Fairkov, S., Gogeva, T.: Macromol. Chem. **191**, 603 (1990)
80. Megeed, Z., Cappello, J., Ghandehari, H.: Pharma. Res. 19(7), 954–959 (2002)
81. Gao, J., Capro, P.M., Wang, Y.: Tissue Eng. **12**(4), 917–925(2006)
82. Doillon, C.J., Silver, F.H., Berg, R.A.: Biomaterials **8**, 195 (1987)
83. Lopour, P., Vondracek, P., Janatova, V., Sulc, J., Vaclk, J.: Biomaterials **11**, 397 (1990)
84. Layton, B.: Recent Pat. Nanotechnol. **2**, 72 (2008)
85. Pancrazio, J.J., Jr.Bey, P.P., Cuttino, D. S. et al., Portable Cell based biosensor system for toxin detection. Sens. Actuators B. Chem. **53** 179–185 (1998)
86. Marquez, G., Renn, M.J.: US20070280866A1 (2007)
87. Patel, R.A., Legum, B., Gogostri, Y., Layton, B.E: 8th biennial asme conference on engineering system design and analysis ESDA. Turin, Italy (2006)
88. Layton, B.E., Sastry, A.M., Lastoskie, C.M. et al. : Biotechniques, **37**, 564–573 (2004)
89. Foran, D.J., Chen, W.: US2003000358935 (2003)
90. Darby, P.C.: US20040126561A1 (2004)
91. Mandell, A.J., Selz, K.A., Shlesinger, M.F.: US2004000777829 (2007)
92. Breitenkamp, K., Sill, K.N., Breitenkamp, R.: US2006000396872 (2006)