

## Biodegradable polymer matrix nanocomposites for tissue engineering: A review

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### ABSTRACT

Nanocomposites have emerged in the last two decades as an efficient strategy to upgrade the structural and functional properties of synthetic polymers. Aliphatic polyesters as polylactide (PLA), poly(glycolides) (PGA), poly( $\epsilon$ -caprolactone) (PCL) have attracted wide attention for their biodegradability and biocompatibility in the human body. A logic consequence has been the introduction of organic and inorganic nanofillers into biodegradable polymers to produce nanocomposites based on hydroxyapatite, metal nanoparticles or carbon nanostructures, in order to prepare new biomaterials with enhanced properties. Consequently, the improvement of interfacial adhesion between the polymer and the nanostructures has become the key technique in the nanocomposite process. In this review, different results on the fabrication of nanocomposites based on biodegradable polymers for specific field of tissue engineering are presented. The combination of bioresorbable polymers and nanostructures open new perspectives in the self-assembly of nanomaterials for biomedical applications with tuneable mechanical, thermal and electrical properties.

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## 1. Introduction

Tissue engineering (TE) is a multidisciplinary field focused on the development and application of knowledge in chemistry, physics, engineering, life and clinical sciences to the solution of critical medical problems, as tissue loss and organ failure [1]. It involves the fundamental understanding of structure–function relationships in normal and pathological tissues and the development of biological substitutes that restore, maintain or improve tissue function [2]. For in-vitro engineering of living tissues, cultured cells are grown on bioactive degradable substrates (scaffolds) that provide the physical and chemical cues to guide their differentiation and assembly into three-dimensional structures. One of the most critical issue in TE is the realization of scaffolds with specific physical, mechanical and biological properties. Scaffolds act as substrate for cellular growth, proliferation, and support for new tissue formation. Biomaterials and fabrication technologies play a key role in TE.

Materials used for tissue engineering applications must be designed to stimulate specific cell response at molecular level. They should elicit specific interactions with cell and thereby direct cell attachment, proliferation, differentiation, and extracellular matrix production and organization. The selection of biomaterials

constitutes a key point for the success of tissue engineering practice [3]. The fundamental requirements of the biomaterials used in the tissue regeneration are biocompatible surfaces and favourable mechanical properties. Conventional single-component polymer materials cannot satisfy these requirements. In fact, although various polymeric materials are available and have been investigated for tissue engineering, no single biodegradable polymer can meet all the requirements for biomedical scaffolds. Therefore, the design and preparation of multi-component polymer systems represent a viable strategy in order to develop innovative multi-functional biomaterials. In particular, this review deals with the introduction of nanostructures in biodegradable polymer matrices to obtain nanocomposites with specific properties able to be used in tissue engineering.

The basic functional subunits of cells and tissues are defined at the nanoscale, hence understanding nanobiology and application of nanotechnology represents a new frontier in TE research [4]. Nanotechnology enables the development of new systems that mimic the complex, hierarchical structure of the native tissue. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine [5]. Nanotechnology involves materials which possess at least one physical dimension in the nanometer range, to construct structures, devices, and systems with novel properties. Many biological components, such as DNA, involve nano-dimensionality, hence it has logically given rise to the interest in using nanomaterials for tissue engineering. There are already several

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scientific reports on the impact of nanomaterials in TE. For example, iron oxide super-paramagnetic nanoparticles and quantum dots have been used to track the biodistribution of cells [6]. Interestingly, nanomaterials can also be multifunctional systems capable of both targeting and imaging [7]. Carbon nanomaterials, in particular, have the potential for multiple uses in tissue engineering [8].

Generally, polymer nanocomposites are the result of the combination of polymers and inorganic/organic fillers at the nanometer scale [9,10]. The interaction between nanostructures and polymer matrix is the basis for enhanced **mechanical** and functional properties of the nanocomposites as compared to conventional microcomposites. In the last two decades there has been a continuous increase of research for the improvement of material properties employing nanometric engineered structures taking advantage of the inherent high surface area–volume ratio of nanomaterials [11]. Nanocomposite materials often show an excellent balance between strength and toughness and usually improved characteristics compared to their individual components [12]. As a matter of fact, natural bone matrix is an organic/inorganic composite material of collagen and apatites. From this point of view, composite materials are excellent choices as bone tissue engineering scaffolds [13]. Indeed, current opportunities for polymer nanocomposites in the biomedical field arise from the multitude of applications and the vastly different functional requirements [14].

The **mechanical** properties of available polymeric porous scaffolds revealed insufficient stiffness and compressive strength compared to human bone, so the possibility to use inorganic/organic nanostructures to include in biodegradable polymers could be an important possibility to increase and modulate **mechanical**, electrical and degradation properties. The interface adhesion between nanoparticles and polymer matrix is the major factor affecting the nanocomposite properties. In order to increase the interfacial strength between the two phases, various methods have been tried in the past [15–19]. Therefore, the **mechanical** properties of nanocomposites are controlled by several microstructural parameters such as the properties of the matrix, properties and distribution of the fillers as well as interfacial bonding, and by the synthesis or processing methods. The interfaces may affect the effectiveness of load transfer from the polymer matrix to nanostructures. Thus surface modification of nanostructures is needed to promote better dispersion of fillers and to enhance the interfacial adhesion between the matrix and the nanophase [18–20]. Recently, a variety of nanocomposites based on polyester and carbon nanostructures have been explored for potential use as scaffold materials in our laboratory [21–23].

The aim of this paper is to put in evidence the evolution and potentiality of emergent nanocomposite approaches in tissue engineering applications. So, this paper reviews current research trends on relevant nanocomposite materials for tissue engineering: biodegradable polymers, organic/inorganic nanostructures, matrix–nanostructure interaction, including strategies for fabrication of nanocomposite scaffolds with inter-connected pores. Dense nanocomposite films and 3D porous scaffolds are reviewed, as well as the effects of the sterilization process and the surface modification of the nanocomposites. Moreover, the in-vitro degradation behaviour of polymer nanocomposites for TE and **stem cell–bionanocomposite interactions** are discussed.

## 2. Current polymer matrices for bionanocomposites

Polymers are the primary materials for scaffold fabrication in tissue engineering applications and many types of biodegradable polymeric materials have been already used in this field. They can be classified as: (1) natural-based materials, including

polysaccharides (starch, alginate, chitin/chitosan, hyaluronic acid derivatives) or proteins (soy, collagen, fibrin gels, silk); (2) synthetic polymers, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly( $\epsilon$ -caprolactone) (PCL), poly(hydroxyl butyrate) (PHB) [24–26].

Many advantages and disadvantages characterize these two different classes of biomaterials. Synthetic polymers have relatively good **mechanical** strength and their shape and degradation rate can be easily modified, but their surfaces are hydrophobic and lack of cell-recognition signals. Naturally derived polymers have the potential advantage of biological recognition that may positively support cell adhesion and function, but they have poor **mechanical** properties. Many of them are also limited in supply and can therefore be costly. This review will focus on synthetic biodegradable polymers, that can be produced in large-scale under controlled conditions and with predictable and reproducible **mechanical** properties, degradation rate and microstructure.

PGA, PLA, and their copolymers, poly(lactic acid-co-glycolic acid) (PLGA) are a family of linear aliphatic polyesters, which are most frequently used in tissue engineering [27–30]. They have been demonstrated to be biocompatible and degrade into non-toxic components with a controllable degradation rate in-vivo and have a long history of use as degradable surgical sutures, having gained FDA (US Food and Drug Administration) approval for clinical use. These polymers degrade through hydrolysis of the ester bonds [31], with degradation products eventually eliminated from the body in the form of carbon dioxide and water; their degradation rates can be tailored to satisfy the requirements from several weeks to several years by altering chemical composition, crystallinity, molecular-weight value and distribution.

PGA is widely used as polymer for scaffold [32], due to its relatively hydrophilic nature, it degrades rapidly in aqueous solutions or in-vivo, and loses **mechanical integrity** between two and four weeks [32,33]. PGA has been processed into non-woven fibrous fabrics as one of the most widely used scaffolds in tissue engineering. The extra methyl group in the PLA repeating unit (compared with PGA) makes it more hydrophobic, reduces the molecular affinity to water, and leads to a slower hydrolysis rate. PLA is degraded by hydrolytic de-esterification into lactic acid. The morphology and crystallinity strongly influence PLA rate of biodegradation and **mechanical** properties [34–36], therefore PLA scaffold degrades slowly in-vitro and in-vivo, maintaining **mechanical** integrity until several months [37,38]. To achieve intermediate degradation rates between PGA and PLA, various lactic and glycolic acid ratios are used to synthesize PLGA [39–42]. PLGA copolymers, with different PGA/PLA ratio (50:50, 65:35, 75:25, 85:15, 90:10) are currently applied in skin tissue regeneration and generally for suture applications [43]. These polymers (PLA, PGA, and PLGA) are among the few synthetic polymers approved by the FDA for certain human clinical applications.

There are other linear aliphatic polyesters, such as poly( $\epsilon$ -caprolactone) (PCL) [44,45] and poly(hydroxyl butyrate) (PHB) [46], which are also used in tissue engineering research. PCL degrades at a significantly slower rate than PLA, PGA, and PLGA [47]. The slow degradation makes PCL less attractive for biomedical applications, but more attractive for long-term implants and controlled release applications. PCL has recently been synthesized to improve degradation properties [48] and it has been used as a suture material and as a long-term drug delivery system. PCL has appeared as a candidate polymer for bone tissue engineering; in fact, it showed sufficient **mechanical** properties to serve as scaffold in applications, such as bone substitution, where physical properties have to be maintained for at least 6 months [49–55]. Scaffolds are involved in a bone regeneration process, and this could be enhanced by the addition of a carbonated apatite component, i.e.

the main constituent of the inorganic phase of bone [3,56,57]. Commonly used biodegradable polymers, along with their selected physical and chemical characteristics, are listed in Table 1.

### 3. Current nanostructures for bionanocomposites

#### 3.1. Hydroxyapatite

Hydroxyapatite (HA) has been widely used as a biocompatible ceramic material in many areas of medicine, but mainly for contact with bone tissue, due to its resemblance to mineral bone [58]. Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) is the major mineral component (69% wt.) of human hard tissues, it could be natural or synthetic, and it possesses excellent biocompatibility with bones, teeth, skin and muscles, both in-vitro and in-vivo. HA promotes bone in-growth, biocompatible and harden in situ and it has Ca/P ratio within the range known to promote bone regeneration (1.50–1.67). HA is biocompatible and osteoinductive and it is widely employed for hard tissue repair in orthopaedic surgery and dentistry [59,60].

Inorganic–organic composites aiming to mimic the composite nature of real bone combine the toughness of the polymer phase with the compressive strength of an inorganic one to generate bioactive materials with improved **mechanical** properties and degradation profiles. For such composites, the alkalinity of the inorganic particle as hydroxyapatite neutralizes acidic autocatalytic degradation of polymers such as PLA, exploiting a bioactive function [61].

To date, calcium phosphate biomaterials have been widely used clinically in the form of powders, granules, dense, porous blocks and various composites. Calcium phosphate materials form the main mineral part of calcified tissues. HA has already been widely used in clinic due to its similarity to bone mineral in structure and composition. Hydroxyapatite promotes faster bone regeneration, and direct bonding to regenerated bone without intermediate connective tissue. It has been developed as bone graft substitute and it is currently used in clinical applications [62–65]. Recent research suggested that better osteoconductivity would be achieved if synthetic HA could resemble bone minerals in composition, size and morphology [66]. In addition, nano-sized HA may have other special properties due to its small size and huge specific surface area. Webster et al. have shown significant increase in protein adsorption and osteoblast adhesion on the nano-sized ceramic materials compared to traditional micro-sized ceramic materials [67]. Thus, there is a growing recognition that a nano-sized inorganic component is likely to be more bioactive than a micro-sized one [68]. In the case of nano-hydroxyapatite (n-HA), studies have shown that due to nanometer surface topography, n-HA particles influenced the conformation of adsorbed vitronectin (a linear protein 15 nm in length that mediates osteoblast adhesion), underlying **mechanisms** of enhanced osteoblast functions have been elucidated [69]. Moreover, it has been reported in the literature that increased initial calcium adsorption to nanoceramic surfaces enhanced binding of vitronectin that subsequently promoted osteoblast adhesion [70].

In this review we focused on synthetic n-HA, prepared by precipitation method [71]. In Fig. 1 a transmission electron microscopy (TEM) image of n-HA is reported. Image shows the as-precipitate powder that consisted of needle-like particles, 10–30 nm width and 50–100 nm length. Nanocomposites based on HA particles and biodegradable polymers have attracted much attention for their good osteoconductivity, osteoinductivity, biodegradability and high **mechanical** strengths. PCL/n-HA nanocomposites were processed and they combine the osteoconductivity and biocompatibility exhibited by HA ceramic with PCL properties [23,37,59,72]. HA materials are very advantageous to

be used in hard-tissue replacement composites. However, due to the brittleness of the HA and to the lack of interaction with polymer, the ceramic nanoparticles may present deleterious effects on the **mechanical** properties, when added at high loadings. Coupling agents are generally used to overpass the lack of interaction with polymer and n-HA aggregation.

Therefore, the incorporation of hydroxyapatite in a polymeric matrix has to overcome processing and dispersion challenges, since it is of great interest to the biomedical community. Consequently, a desirable material in clinical orthopaedics should be a biodegradable structure that induces and promotes new bone formation at the required site. To date, primarily polysaccharide and polypeptidic matrices have been used with hydroxyapatite nanoparticles in hybrid composites [73]. Nanocomposites produced from gelatine and hydroxyapatite nanocrystals are conducive to the attachment, growth, and proliferation of human osteoblast cells. Collagen-based, polypeptidic gelatin has a high number of functional groups and is currently being used in wound dressings and pharmaceutical adhesives in clinics [74]. The flexibility and cost-effectiveness of gelatin can be combined with the bioactivity and osteoconductivity of hydroxyapatite to generate potential engineering biomaterials. The traditional problem of hydroxyapatite aggregation can be overcome by precipitation of the apatite crystals within the polymer solution. The porous scaffold generated by this method exhibited well-developed structural features and pore configuration to induce blood circulation and cell in-growth.

#### 3.2. Metal nanoparticles

Nanoparticles of noble metals have been studied with growing interest, since they exhibit significantly distinct physical, chemical and biological properties from their bulk counterparts. Discoveries in the past decade have demonstrated that the electromagnetic, optical and catalytic properties of noble-metal nanoparticles such as gold, silver and platinum, are strongly influenced by shape and size. The size-dependant properties of small metal particles are known to yield particular optical [75], electrochemical [76] and electronic [77] properties. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size.

Biomedical applications of metal nanoparticles have been dominated by the use of nanobioconjugates that started in 1971 after the discovery of immunogold labeling by Faulk and Taylor [78]. Currently metal-based nanoconjugates are used in various biomedical applications such as probes for electron microscopy to visualize cellular components, drug delivery (vehicle for delivering drugs, proteins, peptides, plasmids, DNAs, etc), detection, diagnosis and therapy (targeted and non-targeted). However biological properties of metal nanoparticles have remained largely unexplored. Therefore, in this review we discuss the novel biological properties and applications of gold and silver nanoparticles in the nanocomposite development.

Currently, there is a very strong interest for the use of metal and semiconductor clusters as advanced additives for plastics and considerable research activities are being done in this novel field of composite science [79,80]. The goal is to obtain small particle sizes, narrow size distributions and well-stabilized metal particles. Because of surface effects and the dramatic changes in properties occurring when the critical length, which governs some physical phenomenon (magnetic, structural, etc.) becomes comparable with size, metal clusters have unique properties (e.g. plasmon absorption, near-IR photoluminescence, superparamagnetism, etc.). The embedding of nanoscopic metal structures into polymeric matrices represents the most simple way to protect clusters and take advantage of their physical characteristics. Polymer-embedded

**Table 1**  
Physical properties of biodegradable polymers used as scaffolds.

Polymers	Thermal & Mechanical Properties			Degradation Properties		Processing and Applications		Polymer repeat unit structure	Ref.
	Melting Temperature (°C)	Glass Transition Temperature (°C)	Tensile Modulus (GPa)	Time (Months)	Products	Solvent	Applications		
Poly(lactic acid) PLA	173–178	60–65	1.5–2.7	12–18	L-lactic acid	Choloroform Dioxane Dichlorometane Etylacetate Acetone Tetrahydrofuran hexafluoroisopropanol	Fracture fixation, interference screws, suture anchors, meniscus repair		[24,26,39,44]
Polyglycolic acid PGA	225–230	35–40	5–7	3–4	Glycolic acid	Hexafluoroisopropanol Acetone Dicholoromthane Choloroform	Suture anchors, meniscus repair, medical devices, drug delivery		[24–26]
Poly(ε-caprolactone) PCL	58–63	–60	0.4–0.6	>24	Caproic acid	Choloroform Hexafluoroisopropanol Dichlorometane Toluene	Suture coating, dental orthopaedic implants		[45–48]
Poly- latic-co-glycolic PLGA (50/50)	Amorphous	50–55	1.4–2.8	3–6	D,L-lactic acid and glycolic acid	Choloroform Dichlorometane Etylacetate Acetone Tetrahydrofuran hexafluoroisopropanol	Suture, drug delivery		[24–26,44]
Poly- latic-co-glycolic PLGA (85/15)	Amorphous	50–55	1.4–2.8	3–6	D,L-lactic acid and glycolic acid	Choloroform Dichlorometane Etylacetate Acetone Tetrahydrofuran hexafluoroisopropanol	Interference screws, suture anchors, ACL reconstruction		[24,44]
Poly- latic-co-glycolic PLGA (90/10)	Amorphous	50–55	–	< 3	D,L-lactic acid and glycolic acid	Choloroform Dichlorometane Etylacetate Acetone Tetrahydrofuran	Artificial skin, wound healing, suture		[24,44]
Poly(Propylene Fumarate) PPF	30–50	–60	2–3	Depends on the formulation and composition several months >24	Fumaric acid, propylene glycol and poly(acrylic acid-cofumaric acid)	Tetrahydrofuran, Acetone, Ethanol	Orthopaedic implants, detal, foam coatings, drug delivery		[174,175]

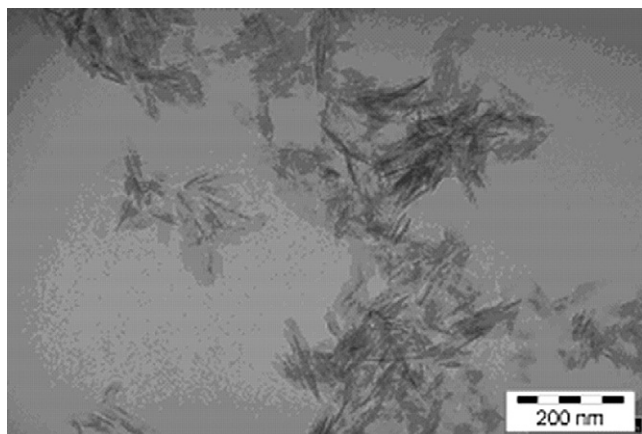


Fig. 1. TEM micrograph of synthetic n-HA, prepared by precipitation method. Reproduced with permission by Bianco et al. [23].

gold nanoparticles have been frequently investigated [80]. The unique physical characteristics, gold/polymer nanocomposites are potentially useful for a number of advanced functional application, especially in the optical and photonic fields [81–86].

Silver (Ag) has been known to have a disinfecting effect and has found applications in traditional medicines. Several salts of silver and their derivatives are commercially employed as antimicrobial agents. Thus, Ag nanoparticles have aptly been investigated for their antibacterial property [87–89]. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size-dependent interaction of silver nanoparticles with bacteria [88]. Silver nanoparticles have drawn considerable interest for their capability to release silver ions in a controlled manner which in turn leads to a powerful antibacterial activity against a large number of bacteria [90,91]. It has been shown that the use of nanostructured silver materials enhances the inhibitory capacity likely because nanostructured materials have a high surface area to contact [90–92]. However, their use has been limited by difficulties associated with handling and processing nanoparticles. In fact, they are easily aggregated because of their high surface free energy, and they can be oxidized or contaminated in air. Embedding of nano-sized metals into biodegradable polymer matrices represents a valid solution to these stabilization problems and permits a controlled antibacterial effect [93]. Moreover, low concentrations of silver nanoparticles are able to induce surface morphological changes in the polymer matrix and affect surface nanocomposite wettability and roughness, all of these aspects can influence the bacterial adhesion process on the nanocomposite surface [94,95]. Fig. 2 shows field emission scanning electron microscopy (FESEM) image of commercial silver nanoparticles, supplied by Cima NanoTech (Corporate Headquarters Saint Paul, MN USA) deposited on indium thin oxide substrate. The particle size distribution is ranging from 20 nm to 80 nm.

### 3.3. Carbon nanostructures

Carbon nanostructures (CNS) are the most celebrated products of nanotechnology to date [96], since the discovery of fullerenes, carbon nanotubes (CNTs), carbon nanofibres (CNFs), graphene and a wide variety of carbon related forms [97].

Carbon nanotubes are tubes made of a single sheet of graphene (SingleWallCarbonNanoTubes, SWCNTs) or more sheets (Multi-WallCarbonNanoTubes, MWCNTs). The regular geometry gives CNT excellent mechanical and electrical properties, which makes them attractive for the development of innovative devices in several

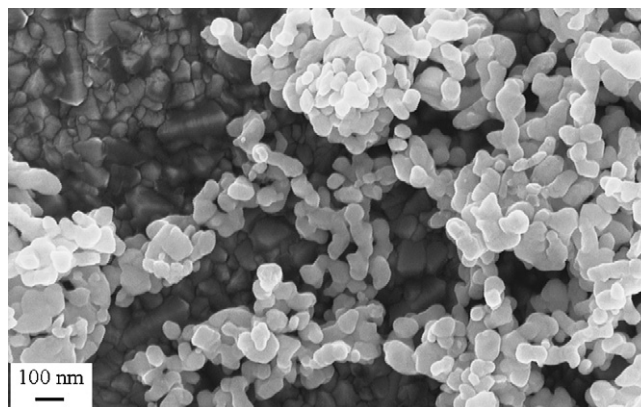


Fig. 2. FESEM images of silver nanoparticles on ITO substrate.

applied fields, including composites, sensors and nanoscale electronic devices [98–100].

Carbon nanofibres (CNFs) are cylindrical or conical structures with diameters varying from few to hundreds nanometers and lengths ranging from less than a micron to few millimeters. The internal structure of carbon nanofibres is comprised of different arrangements of modified graphene sheets ordered [97].

Graphene is a single layer two-dimensional material composed of carbon atoms forming six membered rings and it presents long and reactive edges [101–104]. Graphene became available in 2004, by the “simple” expedient of cleaving a single atomic layer from a sample of graphite using a piece of sticky tape. This discovery stimulated a whirlwind of activity and graphene sheets are novel nanofillers for composites with many unique properties [105–107].

Fig. 3,a, shows individually separated carbon nanofibres, characterized by rough surface sidewalls and the diameters ranging between 100 and 200 nm. Moreover, some hollow CNFs were also detected within the sample. Fig. 3,b shows pristine SWCNT bundles of about 10 nm in diameter, showing uniform diameter distribution [21,22].

Carbon nanostructures can mismatch with the interface layer in composite systems. Polymers that incorporate carbon nanostructures have been investigated for a variety of biomedical applications [8,20–22,108]. Carbon nanotubes have the potential in providing the needed structural reinforcement for biomedical scaffold. By dispersing a small fraction of carbon nanotubes into a polymer, significant improvements in the composite **mechanical** strength have been observed. CNTs, in fact, are one of the most promising candidates for the design of novel polymer composites [109,110]. Considerable efforts have been made to fabricate different carbon based molecular structures and to explore new applications in different fields including nanocomposites. The physical properties and performance of polymer matrix in nanocomposites can be in fact significantly improved by the addition of small percentages of carbon nanotubes less than 1% wt. [111]. The main objective in the development of nanocomposites is to transfer the unique properties of SWCNTs to matrix increasing their added value and creating a good interface between the nanotubes and the polymer. The role of the interface between the nanotubes and polymer matrix is essential in transferring the load from the matrix to the tubes, thereby enhancing the **mechanical** and electrical properties of the composite. In our research, different techniques were explored to improve the SWCNT dispersion in different biopolymer matrix and improve the bioactivity of the composite [21,22,110]. Both covalent and non-covalent functionalization of the nanotube surface were considered in order to control the interactions between polymer and carbon nanostructures. The advantage

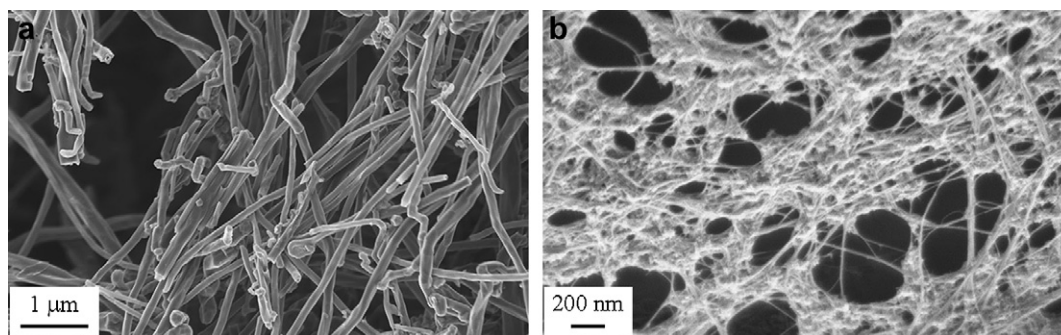


Fig. 3. FESEM images of CNFs (a) and SWCNTs (b).

of a non-covalent attachment is that the perfect structure of the SWCNTs is not damaged and their properties remain intact. The disadvantage is that the forces between the polymer and the SWCNTs are very weak, which means that the load may not be transferred efficiently from the polymer matrix to the nanotube. Covalent functionalization could include fluorine, radicals, amine groups, etc., but the group that is most frequently attached to the CNT sidewall are the carboxylic acid groups [112–114]. The nature of the functional group at the CNT surface seems to play a determinant role in the **mechanism** of interaction with cells.

In order to transfer their outstanding properties from nano to micro-scale, one essential step involves CNS assembling and processing with polymers, which is hindered by their intrinsic poor solubility and processability. To improve their dispersion in polymer matrix and their compatibility in biological fluids, sidewall carboxylic functionalization has been investigated [108]. In a previous work, we have shown that SWCNTs influenced the mineralization process that was also affected by the surface SWCNT functionalization. Nanotubes sustained osteoblast matrix deposition and allowed mineralization, cell differentiation and bone-like tissue forming functions which indicates that SWCNTs provide an effective nucleation surface to induce the formation of a biomimetic apatite coating [115].

However, wide attention has been dedicated to analyze the eventual interactions of carbon nanotubes with living entities [116–118] and any biomedical application should also consider these aspects. Furthermore, there has been a tremendous interest in using the properties of CNTs to promising biological applications [119]. There have been several recent investigations concerning the use of carbon nanotubes for biological purposes and their introduction in biological systems taking advantage of the fact that all living entities are carbon based and nanotubes are solely made of carbon with a similar scale size of DNA [118]. CNTs could be ideal in designing new tissue-engineered products in biological applications and promising possibilities can be expected by introducing them to reinforce scaffolds for tissue engineering. On this point there are different in-vitro investigations and very limited toxicology information. The different results are due to different cells investigated, the difference CNT morphology and aggregation. CNTs could be nanometric powders, but also they can be aggregated in two and in three-dimensional structures (buckypaper), so the way to interact with cells could be very different. However, the toxicity and biocompatibility of carbon nanotube nanocomposites need to be thoroughly investigated [108,120,121]. Although a large number of investigations have been conducted on carbon nanotubes in recent years, at different concentration, purification and functionalization, and in the form of nanocomposites, using a range of cell types, the results reported offer a quite disparate range of conclusions, underlining in many cases the positive effect of the SWCNT

functionalization that induces an adequate solubility and individually dispersion in the biological environment [119]. The first application of CNT technology to neuroscience research methods were developed for growing embryonic rat-brain neurons on MWCNTs. Considering the unmodified nanotubes, neurones extend only one or two neurites, in contrast neurons grown on nanotube coated with bioactive molecule elaborate multiple neurites, which exhibited extensive branching. These findings establish the feasibility of using nanotubes as substrates for nerve cell growth and as probes of neuronal function at the nanometer scale [122]. In-vitro experiments have shown that several different cell types have been successfully grown on carbon nanotubes or CNT based nanocomposites. Carbon nanotubes are similar in shape and size to nerve cells, hence they could help to structurally and functionally reconnect injured neurons. Hippocampal neurons grown on nanotubes display a six-fold increase in the frequency of spontaneous post-synaptic currents, evidence of functional synapse formation [123]. The data give information on the performance of carbon nanotubes as support devices for bridging and integrating functional neuronal networks in-vitro. The researchers foresee an impact of carbon nanotubes on novel chronic neural implants. Investigating nanomaterial interactions with nervous tissue will also favour the design of acceptably small electrodes to provide spinal microstimulation without causing significant neural damage [123].

Honeycomb-like matrices of MWCNTs were fabricated as potential scaffolds for tissue engineering [124]. Vertically aligned carbon nanotubes on a silicon substrate were treated with an acid solution that generates carboxylic acid groups at defects and the ends of the nanotubes. Mouse fibroblast cells were cultured on the nanotube networks. After seven days of growth, the fibroblasts form a confluent layer and no cytotoxicity effects were observed. These carbon networks can be used as biocompatible mesh for restoring, maintaining, or reinforcing damaged tissues [117].

Recent studies have focused on the development of composite materials incorporating carbon nanotubes to enhance the electrical and **mechanical** properties of synthetic polymers commonly used in biomedical applications [125–128]. The electrical conductivity of CNS based nanocomposites is a useful tool in order to direct cell growth, since they can conduct electricity stimulus into the tissue healing process. For example when an alternating current is applied to the substrate, nanocomposites of poly(lactic acid) and MWCNTs have been shown to increase osteoblast proliferation and calcium production [129]. Despite an explosion of research into potential biomedical applications of carbon materials, it is only recently that information on toxicity and biocompatibility has become available [130]. If the unique clinical potential of carbon nanotubes is to be exploited, toxicological studies and pharmacological development must continue in parallel, before eventually converging to provide a clear acceptable framework to regulatory authorities and the

public with toxicological and pharmacological studies that may suggest guidelines for the safe use of carbon nanotubes in medicine [131].

#### 4. Current process in bionanocomposite technology

Tissues, in the body, are organized into three-dimensional (3D) structures as functional organs and organ systems. To engineer functional tissues and organs successfully, the scaffolds have to be designed in order to facilitate cell distribution and guide tissue regeneration in three dimensions [28,57–59,132]. Scaffolds with designed microstructures provide structural support and adequate mass transport to guide the tissue regeneration [133]. In TE the scaffold also serves as a 3D template for cell adhesion, proliferation, differentiation, extracellular matrix (ECM) formation and provides an appropriate environment for the newly formed tissue. Generally, the ideal scaffold for tissue regeneration should possess good biocompatibility, biodegradability with controllable degradation kinetics, easy fabrication and sufficient **mechanical** properties.

One critical issue is the realization of artificial supports, with specific physical, **mechanical** and biological properties [1,2,59]. In this part of the review different techniques to assembly the nanocomposites for tissue engineering applications will be shown. Firstly dense 2D films will be analyzed, in order to investigate the effect of composition on the final properties, then 3D architectures will be considered, in order to show how the morphology affects the nanocomposite properties.

##### 4.1. Nanocomposite films

The approach in developing dense films as a prequel to 3D scaffold development is a useful strategy, since it facilitates the introduction of single variables with the purpose of observing their impact on cell growth. Before a scaffold can be considered for use as a substrate for cell culture, its properties must first be properly characterized and optimised.

Researchers have tried a variety of processing techniques to make dense polymer nanocomposite films. The incorporation of nanostructures into polymer can generally be done in different ways as follows:

- (a) Solution method: it involves dissolution of polymers in adequate solvent with nanoscale particles and evaporation of solvent or precipitation.
- (b) Melt mixing: the polymer is directly melt-mixed with nanoparticle.
- (c) In situ polymerization: the nanoparticles are first dispersed in liquid monomer or monomer solution. Polymerization is performed in presence of nanoscale particles.
- (d) Template synthesis: using polymers as template, the nanoscale particles are synthesized from precursor solution.

The first mentioned method, solvent casting, represents a flexible, low-cost and short-term process widely used for the fabrication of polymeric nanocomposite films, by using a solvent in which the polymer is soluble. The effects of different solvents used represent a key point in the film realization that needs to be elucidated. The choice of solvent influences film properties, heterogeneity of the surface structure, reorientation or mobility of the surface crystal segment, swelling, and deformation [134–136]. The polymer solubility appeared to be the dominant factor, as this correlated with the surface structure. In nanocomposite development by solvent casting process the effects of solvents used for the realization of films need to be elucidated. Specific properties of solvent (i.e., electron-pair donicity, solvochromic parameter,

hydrogen bond donation parameter and dielectric constant) can support an effective dispersion of nanostructures in the solvent and consequently in the polymer matrix.

Composites based on HA particles and biodegradable polymers have been used clinically in various forms due to the good osteoconductivity and osteoinductivity of HA and biodegradability of polymer matrix in the composites. In an ordinary PLA/HA blending system, only physical adsorption is achieved between HA particles and PLA matrix, consequently, its **mechanical** properties are low and its load-bearing applications are limited. The interface adhesion of HA particles and polymer matrix plays a very important role and it represents the major factors affecting the properties of the PLA/HA composites. In order to increase the PLA/HA interfacial strength, various methods have been tried in the past [16–19]. In order to improve the bonding between hydroxyapatite particles and poly(L-lactide) (PLLA), and hence to increase the **mechanical** properties of the PLLA/HA composite, the HA nanoparticles were surface-grafted (g-HA) with the polymer and further blended with PLLA [15]. Uniform nanocomposites were successfully prepared and exhibited improved tensile strength, bending strength, bending modulus and impact energy at the particle content of 4% wt. compared to corresponding PLLA/HA composites. However, the properties decreased with further increasing filler content for both PLLA/g-HA and PLLA/HA. The tensile modulus and the bending modulus increased with increasing filler content for both PLLA/g-HA and PLLA/HA. The g-HA particles had both reinforcing and toughening effects in the composites, in the filler content range examined, from 2% wt. to 20% wt. These improvements could be ascribed firstly to the grafted-PLLA molecules, which played a role of the molecules between the fillers and the PLLA matrix, and secondly to the g-HA particles which were uniformly distributed in the composites and played the role of the heterogeneous nucleating agents in the crystallization of the PLLA matrix. The PLLA/g-HA composites also demonstrated improved cell compatibility due to the good biocompatibility of the HA nanoparticles and a more uniform distribution of the g-HA nanoparticles on the film surface [15–19].

The **mechanical** improvement was also observed in PCL-POE-PCL block copolymer with HA introduction. In this case the effect could be explained on the basis of a close bonding between polymeric matrix and HA grains, not only of physical nature, but also chemical [137]. The interaction takes place with molecules of  $\epsilon$ -caprolactone or PCL thanks to the presence of -OH groups at the surface of HA grains which act both as chain-forming promoters and as their traps in forming a bond.

Nanocomposite films based on carbon nanostructures and biodegradable polymers show enhanced **mechanical**, thermal, and electrical properties. In particular, nanocomposite based on PLLA and SWCNTs and carboxylated SWCNTs at 1% wt. were investigated in our laboratory. Thermal investigation (DSC) demonstrated different PLLA crystallites were formed and a fraction interface-polymer was organized around the nanotube sidewalls, as confirmed by the presence of a shoulder during melting scans and by decrease in melting temperatures [138,139]. DSC measurements revealed that SWCNTs and their COOH groups created heterogeneous nucleation on the carbon nanotube sidewalls. At the carboxylated nanotube-polymer interface chemical affinity modulated and enhanced the crystal order [139]. This good interfacial adhesion as well as good homogeneous dispersion in the polymer system is a major player in transferring SWCNT properties to the polymer matrix and in achieving the full SWCNT reinforcing potential [109,140,141].

A homogeneous dispersion of CNFs was also revealed within the PCL matrix and a good affinity between the polymer and nanofibre sidewalls was also obtained. The enhanced crystal nucleation, due

to the CNF presence, reduced the polymer chain bulk ability to be fully incorporated into growing crystalline lamella [142] leading to the formation of less ordered polymer crystals characterized by more defected crystalline lamella. As a result of this bulk effect, nanocomposite films showed lower crystallinity values (or at least comparable) than neat PCL [22].

Dynamomechanical analysis (DMA) showed SWCNTs modified the relaxation mechanism induced by polymer–nanostructure interaction. PLLA and SWCNTs showed a good interface affinity, inducing an increase in DMA storage modulus which was caused by a reduction in the polymer chain molecular mobility at the PLLA/SWCNTs interface [143]. The PLLA/SWCNTs-COOH nanocomposite exhibited a better interaction with the polymer matrix, than SWCNT nanocomposites, as indicated by the highest storage modulus ( $G'$ ) and by the greatest shift in the glass transition temperature  $T_g$  attributed to the partial decrease in PLLA chain mobility due to the presence of SWCNTs and COOH groups [144]. An increase in the mechanical properties was evaluated also in the PLGA polymer, by using DMA. Nanocomposite based on 1% wt. carboxylic nanotubes (PLGA/SWCNTs-COOH) showed the higher storage modulus that indicates stress transfers from the matrix to the functionalized CNTs [21].

The addition of few CNF weight percentage, in PCL polymer matrix, resulted in a strong reinforcing effect, raising up the tensile modulus and inhibiting polymer drawing [22]. The increase of the nanocomposite tensile modulus proceeded linearly with the CNF content, from 1% wt. to 7% wt. Nanocomposite **mechanical** properties depend on the strength of interface that relays on the interaction between the polymeric matrix and the nanostructure. In CNF reinforced films, carbon nanofibres inhibit the macromolecular sliding of chains. A remarkable reinforcement effect was observed in nanocomposites, since tensile strength increased 14% with respect to the tensile strength of the neat matrix but was increased by 150% for the same level of deformation with only 7% wt. of CNFs. Moreover, the tensile modulus increased one order of magnitude respect to neat PCL film, resulting 1.4 GPa [22]. **Mechanical** properties revealed that incorporation of high aspect ratio CNFs into the PCL matrix significantly enhanced the polymer stiffness [145].

Novel ultra-high strength polymer composites demand a uniform dispersion of the nanofillers in the polymer matrix and, consequently a strong interaction between CNS and polymer is needed [9–12,146]. Numerous efforts worldwide are addressing all aspects of the rapidly developing nanohybrid field, including synthesis, CNS dispersion, characterization and integration within commercial products, such as those capitalizing the exceptional enhancement in electrical conductivity resulting from nanostructure addition (<10% wt.) [138]. By now, at least 200 scientific publications report on the electrical percolation threshold of carbon nanotubes in different polymer systems [138,147,148]. Experimental results showed that 3% wt. of SWCNTs in PCL, after 1.5 h sonication treatment, increased the polymer conductivity to  $10^{-3} \text{ S}\cdot\text{m}^{-1}$  [149]. It has been observed that SWCNTs can act as a nucleating agent for PCL crystals, and the composite structure has **shown a significant mechanical reinforcement of the polymer** [150]. Generally, several papers have been focused on composites based on PCL and functionalized CNFs [151,152].

Composites with low CNF content (<3% wt.) showed a purely insulating behaviour, as indicated by the frequency dependent increase in conductivity with a slope of unity on the log–log plot of specific conductivity [153]. A different behaviour is obtained increasing CNF content, with 7% wt. of CNFs, conductivity remain constant at a given frequency range [154]. Dielectric measurements highlight the importance of carbon nanostructure clustering for the formation of a conductive network. The change in AC conductivity

with frequency provides information about the overall connectivity of the conducting network. The addition of carbon nanostructures decreased the electrical resistivity ( $\rho$ ) values of biodegradable polymer films. Among the carbon structures, SWCNTs were the most effective fillers to reduce the  $\rho$  of biodegradable polymer films. The addition of SWCNTs lowered the  $\rho$  of PLLA film from  $1.6 \times 10^5 \Omega\text{cm}$  to values lower than  $1 \times 10^2 \Omega\text{cm}$  (filler concentration of 10% wt.). Furthermore, even the addition of 1% wt. SWCNT caused dramatic decrease in  $\rho$  from  $1.6 \times 10^5$  to  $3\text{--}4 \times 10^3 \Omega\text{cm}$ . Such high effects of SWCNT can be ascribed to its needle-like structure, which obliges SWCNT to effectively contact with each other [155].

Nanocomposite films containing silver nanoparticles have been also extensively used to prevent attack of a broad spectrum of microorganisms and to reduce infections, although there is a debate to explain the inhibitory effect of silver on bacteria [92]. Hence, the dispersion of silver nanoparticles in biodegradable polymers would allow to obtain plastics able to release silver species in a controlled manner thus preserving antibacterial action for extended times.

Few reports on the development and use of silver nanoparticles in PLGA matrix nanocomposites can be found in the scientific literature [156,157]. Results demonstrated a prolonged antibacterial effect of electrospun biodegradable fibres containing finely dispersed silver nanoparticles, with antibacterial efficacy duration longer than 20 days [158]. The metal nanoparticles enhance the thermal conductivity of the nanocomposites that can speed up the degradation process of the polymeric matrix [93]. Moreover, low concentrations of silver nanoparticles are able to induce surface morphological changes in the polymer matrix and affect the surface nanocomposite wettability and roughness; all of these aspects can influence the bacterial adhesion process on the nanocomposite surface [94,95,159]. Contact angle increases with silver content above 5% wt. Highly hydrophobic behaviour is associated to surface roughness increase induced by the presence of silver nanoparticles. Contact angle, in fact, depends on several factors as surface preparation, roughness, chemical and physical configuration that can influence biomaterial bacteria adherence [160,161].

#### 4.2. Porous scaffolds

Scaffolds might be defined as an artificial structure capable of supporting three-dimensional tissue formation, that allows cell attachment and migration, deliver and retain cells and biochemical factors enable diffusion of vital cell nutrients and expressed products. To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. High porosity and pore interconnectivity are key requisites to increase the specific surface area available for cell attachment and tissue ingrowth, so facilitating the uniform distribution of cells and the adequate transport of nutrients and cellular waste products. Taking into account the intimate correlation between specific cells and pore sizes for optimal cell attachment and growth, it is crucial to develop polymeric scaffolds with a high degree of porosity but, simultaneously, with good control over the pore size and morphology [57–59,162]. The development of novel biomaterials with different fabrication techniques is critical for the success of tissue engineering. Nanocomposite 3D scaffolds based on biodegradable polymers have been developed by using different nanostructures and processing methods. These techniques mainly include solvent casting and particulate leaching, gas foaming, emulsion freeze-drying, electrospinning, rapid prototyping, and thermally induced phase separation [28,33,37,57–59,74].



Solvent casting particulate leaching is an easy technique that has been widely used to fabricate biocomposite scaffolds [163,164]; it involves the dissolution of the polymer in an organic solvent, mixing with porogen particles, and casting the solution into a pre-defined 3D mould. The solvent is subsequently allowed to evaporate. Porogen particles are removed by leaching following the main processing step. Fig. 4 shows FESEM image of a PLGA scaffold prepared in our laboratory by solvent casting-particulate leaching technique. PLGA scaffold surfaces show a continuous microstructure of well inter-connected pores, 100–200  $\mu\text{m}$  in diameter and spherical shape.

To avoid toxicity effect of organic solvent, gas foaming process can be used to fabricate highly porous polymer foams without the use of organic solvents [165,166]. In this approach, carbon dioxide ( $\text{CO}_2$ ) is usually used as an agent for the formation of polymer foam.

To combine the osteoconductivity of calcium phosphates and good processability of polyesters, polymer/ceramic composite scaffolds have been developed for bone tissue engineering. Ceramic nanoparticles have been used in the scaffold, in order to **increase mechanical properties of the polymer matrix and increase osteoconductive properties** [167,168]. They present good bioactivity, manipulation and control over both macro and microstructure in shaping to fit bone defects. However, most results reveal that, while the incorporation of a ceramic phase improved the bioactivity of the polymeric scaffold, this advantage is not usually combined with a **commensurate enhancement of the mechanical properties of the composite** [169]. Authors have described the limited reinforcement offered by HA micrometric particles within a PCL matrix, indicated by particle overexposure on the pore surfaces, combined with a tendency to form clusters [170].

Recently, ceramic/polymer nanocomposites, particularly nano-hydroxyapatite (n-HA) reinforcement and polymer matrix, have gained much recognition as bone scaffolds not only due to their composition and structural similarity with natural bone but also because of their unique functional properties such as larger surface area and superior **mechanical** strength than those of their single phase constituents. Nanocomposite scaffold based also on rod shaped nano-sized HA was developed in order to mimic natural bone apatite morphology [168]. The incorporation of synthesized n-HA instead of micro-sized hydroxyapatite (MHA) reinforcement enabled the composite scaffolds to possess higher **mechanical** strength, and more regular microarchitecture due to its more interfacial area, surface reactivity and ultra-fine structure. It can be suggested that the newly developed PLLA/n-HA composite scaffold fulfil most of the requirements as a suitable bone substitute for bone tissue engineering applications [168].

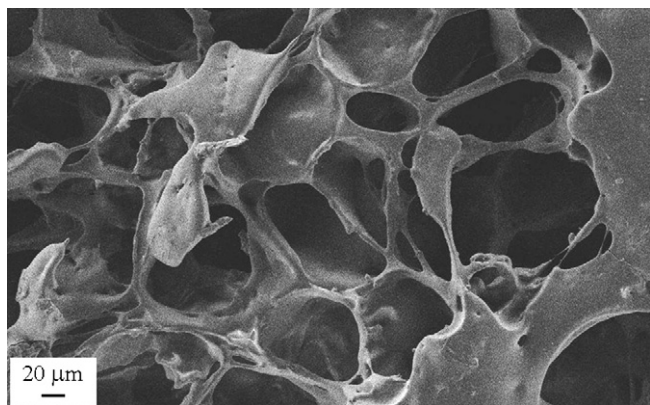


Fig. 4. FESEM image of PLGA scaffold prepared by solvent casting particulate leaching technique.

The Ma group has developed a variety of scaffolds using thermally induced phase separation (TIPS) [37,171,172]. The controlled TIPS process was first used for the preparation of porous polymer membranes. This technique was recently utilized to fabricate biodegradable 3D polymer scaffolds [27]. Pore structure and pore wall morphology can be controlled by phase separation parameters. They have demonstrated that the addition of MHA increases the adsorption of proteins and extracellular matrix (ECM) components [173]. Different solvent systems were used to obtain scaffolds with different microarchitectures and properties. When dioxane was used alone, the porous structure resulted from a solid–liquid phase separation of the polymer solution. During quenching, the solvent crystallized and the polymer were expelled from the solvent crystallization front. Solvent crystals became pores after subsequent sublimation. To better mimic the mineral component and the microstructure of natural bone, novel nano-hydroxyapatite composite scaffolds with high porosity and well-controlled pore architectures were prepared using TIPS techniques. The high porosity (90% and above) was easily achieved and the pore size was adjusted by varying phase separation parameters. The introduction of HA particles into the polymer solution perturbed the solvent crystallization to some extent and thereby made the pore structure more irregular and isotropic. The perturbation by n-HA particles, however, was small even in high proportion up to 50% due to their nanometer size scale and uniform distribution. Microscopy images showed that the n-HA particles were dispersed in the pore walls of the scaffolds and bound to the polymer very well. n-HA/polymer scaffolds prepared using pure solvent system had a regular anisotropic but open 3D pore structure similar to plain polymer scaffolds while MHA/PLLA scaffolds had an isotropic and a random irregular pore structure. The introduction of HA greatly increased the **mechanical** properties and improved the protein adsorption capacity. The results suggest that the newly developed n-HA/polymer composite scaffolds may serve as an excellent 3D substrate for cell attachment and migration in bone tissue engineering. n-HA/PLLA composite scaffolds maintained the main characteristic pore architecture of solid–liquid phase separation which was anisotropic and regular. In contrast to n-HA/PLLA, the regular anisotropic pore structure was obtained only when the HA content was very low in MHA/PLLA scaffolds. In this case, low content of HA did not affect the solvent crystallization significantly enough to alter the pore structure [37]. These results suggest that the newly developed n-HA/polymer composite scaffolds may be a superior choice for bone tissue engineering.

Novel composite scaffold was proposed by Ambrosio et al. which combines the use of two reinforcement systems in different forms, particles and long fibres, **to optimize the final mechanical response of the scaffold**. 3D porous PCL-based composite scaffolds, tubular in shape, were prepared by the combination of the filament winding technique and a phase inversion/salt leaching process. The synergistic contribution between ceramic phase and a highly organized, **continuous fibre network influenced the mechanical response of a scaffold oriented to mimic bone functional behaviour**. The integration of a solid porogen (i.e. sodium chloride crystals) within a 3D polymer matrix enables creation of an inter-connected pore network with well-defined pore sizes and shapes [162].

Recently, a variety of nanocomposite materials made of poly(propylene fumarate) (PPF) and single-walled carbon nanotubes have been explored for potential use as scaffold materials [105,174,175]. These nanocomposites are injectable, thermally crosslinkable, and cytocompatible in-vitro, making them promising biomaterials for bone tissue engineering. SWCNTs, especially ultra short SWCNTs, significantly reinforced PPF polymer, whose inferior **mechanical** properties often limit its use as a highly porous scaffold for load-bearing applications. Chemical functionalization of

SWCNTs can improve their dispersion into PPF, augmenting their reinforcing effects [105]. Therefore, functionalized ultra short SWCNTs were introduced in the polymer to investigate their effects on scaffolds for bone tissue engineering. They demonstrated that up to 90 vol% scaffolds of nanocomposites can be reproducibly created via thermal-crosslinking and salt porogen leaching. They found that there was no significant difference in porosity, pore size, and pore interconnectivity among scaffolds made of the three different materials [124]. There was also a general trend of enhancement in compressive **mechanical** properties of nanocomposite scaffold based on functionalized nanotubes. It has been established that scaffold porosity plays a major role in determining the compressive **mechanical** moduli and yield strengths in accordance with power-law relationships [176]. These power-law declines in **mechanical** properties with higher porosity set a tradeoff for the benefit of increasing porosity of scaffolds to improve pore interconnectivity for better tissue in-growth.

Smart scaffolds were also developed by Misra et al. [177]. They have, for the first time, incorporated multiwalled carbon nanotubes in a novel bioresorbable/bioactive composite, and they have developed a ternary nanocomposite scaffold involving three different materials. The addition of MWCNTs to the bioactive composites material makes new highly conducting material, since it produces a three-dimensional electrical conducting network. The MWCNT composites obey Ohm's law and exhibit classic ohmic conduction. The results showed that combining two different nanostructures it is possible to develop multifunctional biomaterials with tailored bioactivity, structural and **mechanical** integrity as well as electrical conductivity of porous scaffolds. The production of a smart system, having the ability to perform all the required tasks in tissue engineering, is the main task in scaffold development.

#### 4.3. Nanohybrid membranes

Electrospinning is a straightforward technique to produce non-woven micro- or nanofibrous mats, based on the application of high voltage to a polymeric solution, in order to create an electrically charged jet randomly collected onto a grounded target [178]. Electrospinning technology is a simple and versatile method to prepare ultra thin fibres from polymer solutions or melts. The obtained fibres usually have a diameter from several nanometers to a few micrometers, and mostly in hundreds of nanometers. Electrospun polymer nanofibres possess many extraordinary properties including small diameters, the concomitant large specific surface areas, a high degree of structural perfection and the resultant superior **mechanical** properties. Additionally, the non-woven polymer fabrics offer a unique capability to control the pore sizes among nanofibres [179]. In the last decade, electrospinning technique has attracted a great interest since it allows to produce fibrous non-woven micro/nano fabrics for tissue engineering, mainly due to the structural similarity to the tissue extracellular matrix. Several studies have reported the performance of nanofibrous materials in guiding cells to initially adhere and spread over the material, as well as further triggering them to secrete the appropriate ECM molecules targeted to skin, blood vessel, cartilage, muscle, adipose, nerve and bone. The intriguing features of a fibrous morphology with diameters ranging from tens of nanometers to a few micrometers have attracted considerable attention focused on exploiting the properties as well as structural tuning to the tissue of concern for the applications as a tissue engineering scaffold [22,180–182].

Electrospun nanocomposite scaffolds based on bioresorbable polymers and hydroxyapatite particles allow osteoblast proliferation and differentiation, and are thus considered very promising tissue engineering [23,183–185]. Nanocomposite mats based on

PCL and n-HA show different properties respect to the polymer matrix. Crystallization temperature of nanocomposites occurred at higher temperature with respect to the neat sample, clearly evidencing that n-HA nanoparticles promote the crystallization of the PCL matrix, acting as heterogeneous nucleating agents. Thermal analysis (DSC) also evidenced that the presence of low n-HA contents (e.g. up to 6.4% wt.) did not significantly affect the crystallinity degree ( $X_c$ ) value (50%), the effect of the fibre-forming process being predominant. The **mechanical** behaviour of fibre-based polymeric structures [186–188] and their nanocomposites have been extensively investigated [183]. As a general trend, we found that **mechanical** properties of nanohybrids were not strongly affected by the incorporation of n-HA up to 6.4% wt. According to [189], blending PCL with nanoparticles is an effective approach to afford dramatic improvement in elongation at break of the resulting nanocomposites.

It is known that the critical material parameters and the main challenges for manufacturing nanocomposite are the homogeneous dispersion of the nanoparticles in polymer solutions and the interactions between the particles and the polymer chains [15–19]. Therefore, HA nanoparticles were grafted with PLA, in order to easily disperse in a PLA matrix to form a PLA-g-HA/PLA composite. The composite was electrospun into porous fibre mats. Uniform PLA-g-HA/PLA composite nanofibre mats were successfully prepared by electrospinning and they exhibited improved **mechanical** properties compared to corresponding HA/PLA fibre mats and the pristine PLA fibre mats. Especially at PLA-g-HA content of 4% wt. the composite fibres showed highest tensile strength and tensile modulus due to the uniform distribution of PLA-g-HA in the composite fibres and the relative good interaction and adhesion between the fillers and PLA matrix. The content and the distribution of PLA-g-HA nanoparticles in the composite fibres also affected the degradation rate of the composite fibre mats [190]. Aligned nanocomposite fibres of PLGA/HA were fabricated by using a rotating collector by electrospinning. At low concentrations the fibres had no agglomerates and good dispersion was achieved. However, higher concentrations of HA resulted in increased diameter and broken fibres due to agglomeration. The glass transition temperature ( $T_g$ ) of the polymer was markedly reduced by the fast processing technique of electrospinning. This reduction brought the  $T_g$  down to be equal to or less than physiological temperature. In addition, the low  $T_g$  resulted in oriented amorphous chains that folded, resulting in significant shrinkage. However the presence of well-dispersed nanoscopic HA particles reduced the chain mobility and hence helped to prevent shrinkage to some degree. The glass transition was affected by the incorporation of n-HA into the polymer matrix which hinders chain motion. This hindering resulted in a slight increase in the  $T_g$  as the n-HA concentration increased from 0% to 10%, and thereafter a plateau was reached [191].

An attractive feature of electrospinning technique is the chance to align conductive nanoparticles with high aspect ratio within the polymeric fibres. CNFs can orientate along the axis of electrospun fibres due to the sink flow and the high extension of the electrospun jet [192]. The carbon nanofibre alignment however, depends upon the CNF dispersion in the polymer solution [193]. The idea of dispersing and aligning carbon nanostructures in polymer matrix to form highly ordered structures and composite materials has significant technological implications [21,194]. Fig. 5 shows a SEM micrographs of a neat PCL electrospun mat (a) and PCL/CNFs electrospun mats loaded with 1% wt. CNFs [22]. All electrospun fabrics showed a well-defined non-woven fibrous architecture, and PCL and PCL nanocomposite samples were comprised of homogeneous and uniform fibres. However, there is a clear difference between the diameter of composite fibres with respect to the pure matrix,

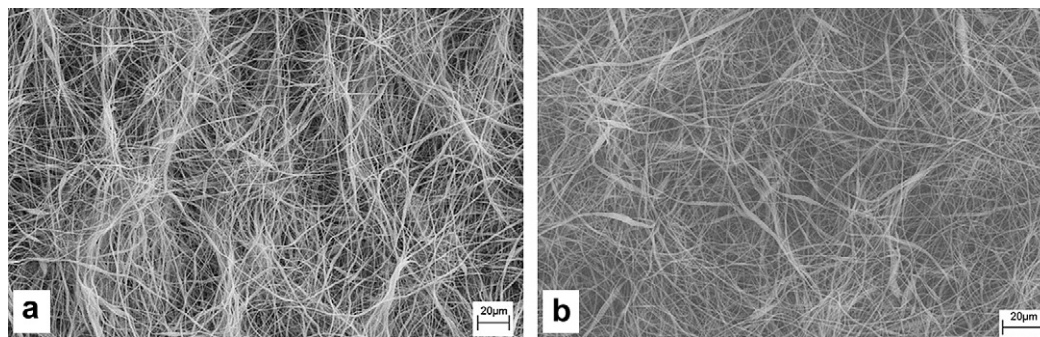


Fig. 5. SEM micrographs of a neat PCL electrospun mat (a) and PCL/CNFs electrospun mats loaded with 1% wt CNFs. Reproduced with permission by Armentano et al. [22].

probably ascribed to the increased electrical conductivity of the suspension to be spun.

Electrospun nanofibrous scaffolds aimed to mimic the architecture and biological functions of ECM, are considered as very promising substrates for tissue engineering [195,196].

Nanocomposite membranes based on multiwalled carbon nanotubes and PCL were prepared by in situ polymerization, whereby functionalized MWCNTs and unfunctionalized MWCNTs were used as reinforcing materials. The functionalized MWCNTs were chemically bonded with the PCL chains, as indicated by the appearance of amide II group in FT-IR spectrum. The functionalized nanocomposite showed better dispersion and thermal stability compared to pristine tubes. The MWCNTs/PCL nanofibers were electrospun from the solutions with different concentrations. The nanofibre morphology is strictly connected with the process parameters and composition. The beads formation decreased by increasing the concentration of the PCL and the number of beads in the MWCNTs/PCL composite nanofibers increased by increasing the amounts of MWCNTs. The MWCNTs were embedded within nanofibres and they were well oriented along the axes of the nanofibres during electrospinning [194].

Significant effort has been devoted to fabricate various biomaterials to satisfy specific clinical requirements. Recently researchers have employed the electrospinning technique in the incorporation of multiwalled carbon nanotubes/hydroxyapatite (MWCNTs/HA) nanoparticles into PLLA and the fabrication of a composite membrane to satisfy the specific requirements of guided tissue regeneration. This work represents the first trial on the fabrication of a biomedical membrane which possesses dual biological functions [197]. This new type of membrane shows excellent dual biological functions and satisfied the requirement of the guided tissue regeneration (GTR) technique successfully in spite of a monolayer structure.

## 5. Sterilization of nanostructured bionanocomposites

Nanocomposite scaffold materials must be easily and accurately sterilizable to prevent infection. The method of sterilization, however, must not interfere with the bioactivity of the material or alter its chemical composition which could, in turn, affect its biocompatibility or degradation properties. The selection of an appropriate sterilization method is an important step in the use of polymer and nanocomposite films and scaffolds for biomedical purposes. A lot of work has been reported on the effects of sterilization methods on the properties of several polymers. In fact, each method has its own advantages and disadvantages. The method that may finally be used is dependent on many factors including the

material to be sterilized and its resistance to the sterilization procedure [198,199]. Although sterilization undoubtedly has effects on the properties of biodegradable polymers and scaffolds, these can be limited by adopting the less destructive sterilization technique.

Sterilization can be done by a variety of procedures including steam sterilization, ethylene oxide sterilization,  $\gamma$ -irradiation, e-beam sterilization, UV exposure, and dry heat sterilization. Because of the high temperature range, autoclave or steam sterilization can melt the polymer or alter its morphological structure. Energy methods such as gamma and e-beam irradiation are instantaneous, penetrating and non-toxic but may be associated with changes in the molecular structure [200]. Gamma sterilization is perhaps the most popular procedure for the terminal sterilization of heat-sensitive medical devices. Sterilization by ionizing radiation typically uses gamma rays ( $\gamma$ ), that are photons of electromagnetic radiation with energies in the range of 1 keV–10 MeV.  $\gamma$ -irradiation, causes substantial degradation of polyester chains with increasing dosages of radiation. For example, at the standard 2.5 Mrad sterilization dose, considerable damage was observed on PGA sutures [201]. By using  $\gamma$ -irradiation a polynomial correlation between dose and molecular weight was observed in the PLLA polymer, in which the molecular weight decreased with increasing dose of  $\gamma$ -irradiation. Clearly, irradiation of PLLA leads to significant molecular damage affecting the entire spectrum of material properties [202].

Biomedical devices prepared from biodegradable polyesters are usually sterilized by ethylene oxide (ETO). Ethylene oxide gas sterilization is almost exclusively used for bioabsorbable medical devices, as it is generally regarded as having few destructive effect on properties, with many workers reporting limited or zero effects [203]. In comparison, gamma irradiation can cause chain scission and crosslinking at doses of 2.5 Mrad [202]. Other sterilization procedures, such as heat, steam or acid, cause extensive deformation of the devices and accelerated polymer degradation [204]. ETO sterilization has its limitations as well it includes accelerated degradation of the polymer, and residual ethylene oxide gas within the bulk of the sterilized device.

Isopropanol washing may be an alternative for polymer sterilization, as well as ethanol treatment. However, appropriate sterilization may not be achieved [205]. Disinfection in 70% ethanol (EtOH) for 30 min is often used in-vitro and it is shown to produce no morphological and/or chemical damages to polyester scaffolds. However, while gram-positive, gram-negative, acid-fast bacteria and lipophilic viruses show high susceptibility to concentrations of EtOH in water ranging from 60 to 80%, hydrophilic viruses and bacterial spores are resistant to the microbial effects of ethanol

[206]. Therefore, EtOH is considered as a chemical disinfectant instead of a sterilizing media and cannot be used for in-vivo applications of biomedical devices. Data obtained by Hooper et al. confirm that, as a general rule, PLLA can be exposed to ethylene oxide without detrimental changes in molecular weight, polydispersity, **mechanical** properties, surface chemical composition, and the degradation rate after sterilization. There were no obvious trends related to the backbone or pendent chain structure of the polymers [202].

Recently, a low-temperature radiofrequency glow discharge (RFGD) plasma treatment was introduced as a sterilizing method for polyester devices. While the RFGD plasma was shown to induce surface crosslinking or branching of the polymer, it did not affect polymer crystallinity, **mechanical** properties, or overall melting temperature [207]. The sterilization efficiency of plasma gas was recently demonstrated by a  $10^5$  reduction of bacteria, bacterial endospores, yeast and bacterial viruses within 90 s of exposure to an atmospheric uniform glow discharge plasma [208], indicative of a similar sterilization efficiency to that of ETO and  $\gamma$  [205].

## 6. In-vitro biodegradation study of bionanocomposites

Degradation properties are of crucial importance in biomaterial selection and design in tissue engineering [209–212]. Thus, a polymer nanocomposite scaffold must meet certain design and functional criteria, including biocompatibility, specific biodegradability profiles, **mechanical** properties, and, in some cases, aesthetic demands. The underlying solution to the use of polymer nanocomposites in vastly differing applications is the correct choice of matrix polymer chemistry, filler type, and matrix–filler interaction for which the degradation process can be tailored [213].

The biomaterial should not only stimulate and support tissue growth, but it may also degrade with the same rate at which new tissue forms, and importantly, it has to possess the additional ability to withstand the loading conditions experienced in situ. The **mechanical** support is continuously needed as the material degrades, until the new tissue can take up the load [28,33,38,57,130]. Since the tissue engineering aims at the regeneration of new tissues, hence biomaterials are expected to be degradable and absorbable with a proper rate to match the speed of new tissue formation. The degradation behaviour has a crucial impact on the long-term performance of a tissue-engineered cell/polymer construct. The degradation kinetics may affect a range of processes such as cell growth, tissue regeneration, and host response. The **mechanism** of aliphatic polyester biodegradation is the bio-erosion of the material mainly determined by the surface hydrolysis of the polymer. Extensive literature on biodegradation of polymer materials reveals the complexity of the hydrolysis **mechanism**, in which it is important to understand not only the time the material employs to bio-erode itself but also in what conditions it will happen, in relation to the chemical composition of the samples, the pH of the medium, temperature, surface treatments, sample size and shape, reinforcing particles and particle functionalization [25,212,214]. Fig. 6 shows scheme of the biodegradation process; the factors affecting the degradation are underlined and correlated to its importance in biomedical application. When the water molecules attack the ester bonds in the polymer chains, the average length of the degraded chains becomes smaller. The process results in short fragments of chains having carboxylic end groups that render the polymer soluble in water. Very often, the molecular weights of some fragments are still relatively large such that the corresponding diffusion rates are slow. As a result, the remaining oligomers will lower the local pH value, catalyze the hydrolysis of other ester bonds and speed up the degradation process. This **mechanism** is termed autocatalysis, which is frequently observed in

thick biodegradable materials [215–217]. The degradation in semi-crystalline polyesters undergoes preferentially within the amorphous regions because of a higher rate of water uptake in the free volume than in the crystalline regions. The degraded segments could then diffuse and give rise to re-crystallization; this increase of crystallinity during hydrolytic degradation can be detected from the whitening of the specimens and from the change in properties [218].

Addition of nanostructures to bioresorbable polymers can alter the polymer degradation behaviour, by allowing rapid exchange of protons in water for alkali in the glass or ceramic. Inclusion of bioactive glasses has been shown to modify surface and bulk properties of composite scaffolds by increasing the hydrophilicity and water absorption of the hydrophobic polymer matrix, thus altering the scaffold degradation kinetics. Composite materials based on inorganic nanoparticles, showed a strongly enhanced polymer degradation rate if compared to the pure polymer. Tricalcium phosphate filled polymers showed deposition of small, 10 nm sized hydroxyapatite crystals on the surface of the composite, while for pure PLGA no hydroxyapatite formation was observed during degradation. This indicates improved osteoconductive properties of PLGA nanocomposites. The fast degradation and the superior bioactivity make these nanocomposites a promising material for application in orthopaedic medicine [38,214,219]. The differences between the composites and pure polymers in decomposition were due to both biodegradation **mechanism** of the polymers and dissolution of nanoparticles.

If the dimension of biomaterials is small (the diffusion path is short), the hydrophilic oligomers can quickly escape from the surface [220,221]. This is exactly the case of the electrospun scaffolds, where the dimension of the nanofibres is small and the diffusion length of the degraded by-products (hydrophilic oligomers) is short. As a result, the possibility of autocatalysis in electrospun scaffolds is very limited [222]. Different aspects are involved in the case of carbon nanostructure composite materials. In a previous work, we investigated the in-vitro degradation of poly (DL-lactic-co-glycolic acid) (PLGA) nanocomposite films, and we analyzed the effects of the SWCNT incorporation and functionalization on the structural behaviour of the nanocomposite films produced [21]. Pristine (SWCNTs) and carboxylated (SWCNTs-COOH) carbon nanotubes were considered. The hydrolytic degradation of the PLGA matrix was clearly controlled by two mismatch **mechanisms**: chain-scission and crosslinking. The incorporation of SWCNTs increases the dimensional stability of the polymeric samples but they do not seem to significantly modify the kinetics of the hydrolytic erosion and the involved **mechanisms** with respect to the neat PLGA. PLGA/SWCNT film samples exhibited a similar weight loss behaviour than the neat PLGA with destruction after 24 days. Faster mass loss and different infrared spectra were revealed in SWCNT-COOH composites and this suggests higher interaction of the functionalized tubes with the polymer matrix and with water physiological solution leading to a more rapid erosion of the nanocomposite [21]. In the crosslinking/chain-scission mismatch, the second **mechanism** clearly dominated in PLGA/SWCNTs-COOH systems. In fact, the presence of carboxylic groups in functionalized SWCNTs-COOH accelerated the hydrolytic degradation of the PLGA matrix and the weight loss of the nanocomposites. This behaviour suggests the selective interaction of water at the interface between the nanotubes and the polymeric matrix similar to the behaviour reported at the fibre–matrix interface in conventional composites [223,224]. It is well known that this interaction is mainly controlled by the fibre treatment, functionalization and coatings of fibres. The interaction of the functionalized carboxylate nanotubes is high enough to promote higher hydrolytic degradation with respect to PLGA and PLGA/SWCNTs systems. Moreover, it should also be

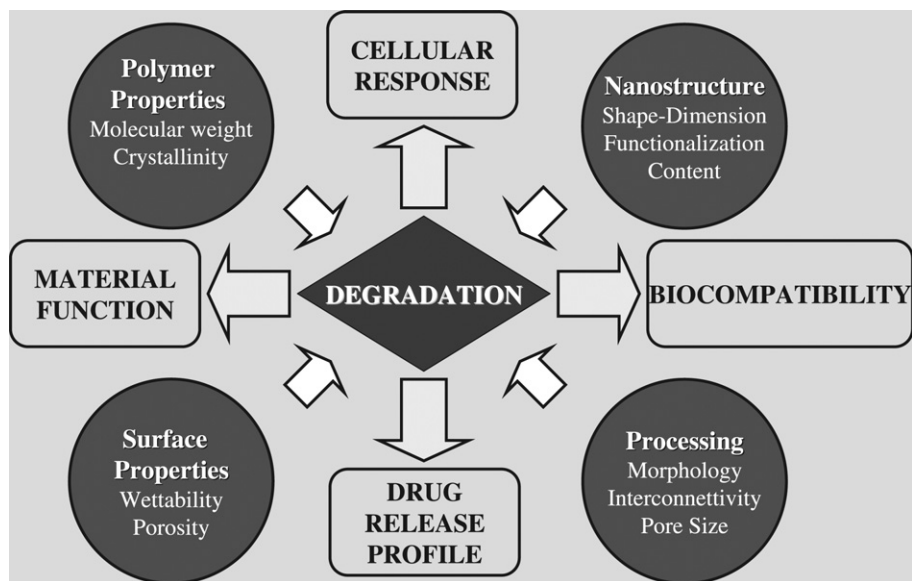


Fig. 6. Importance of biodegradability in biomedical application.

considered that the higher dispersion of the functionalized SWCNTs-COOH offers a better surface interaction with the biological milieu than in the case of SWCNTs that form bundles and offer a lower surface interaction with the polymer. Although the functionalization of carbon nanotubes offers better possibilities for their dispersion in the PLGA matrix, it is also clear that higher solubility of SWCNTs-COOH and the promotion of a higher PLGA-water interaction must be considered in the future development and possible standardization of biodegradable biomaterials.

The degradation rates of the MWCNTs composites in simulated body fluid (SBF) solution was reduced with the increase of nanotube percentage. The composites showed no cytotoxicity effects especially when the MWCNT loadings were above 1% wt. [125]. The existing reports on polymer–CNT nanocomposites have been mainly focusing on the CNT functionalization, composite preparation and property developments. Unfortunately the environmental durability of CNT nanocomposites has yet to be studied. CNT functionalization has opened in fact new horizons in the biocompatibility of carbon based materials. Pristine CNTs are insoluble in biological fluids, and properly functionalized CNTs seem to have high propensity to cross cell membranes. The chemistry of CNT offers the possibility to introduce more than one functionality on the same tube, so that targeting molecules, contrast agents, drugs, can be used at the same time. Though it is too early to establish CNTs for clinical use, these novel carriers are undoubtedly interesting and need further investigation [225].

The catalytic biodegradation of carbon nanotubes in-vitro by oxidative activity of horseradish peroxidase (HRP) and low concentrations of hydrogen peroxide was reported by Allen et al. [226]. Possible biotechnological and natural (plant  $H_2O_2$  peroxidases) ways for degradation of carbon nanotubes in the environment are presented. Results show that CNTs did not cause inactivation of the enzyme. Examination of the samples at 12 weeks, revealed that the bulk of nanotubes were no longer present, globular material had formed, contributing to the predominant species imaged. The evidence of the biodegradation of carbon nanotubes by HRP/ $H_2O_2$  over the period of several weeks was provided. These results marks a promising possibility for nanotubes to be degraded by HRP in environmentally relevant settings. It is tempting to speculate that other peroxidases in plants and animals

(e.g., myeloperoxidase) may be effective in oxidative degradation of carbon nanotubes and enhancement of these catalytic biodegradation pathways may be instrumental in avoiding their cytotoxicity in drug delivery, gene silencing, and tumor imaging. With further insight into this type of biodegradation process, it will be possible to engineer, more efficient drug delivery platforms, where the patient need not worry about the injection of materials that risk to accumulate causing cytotoxic effects. More studies are requested in order to ascertain the by-product of the biodegradation, as well as cellular studies for practical application. Further studies need to investigate the **mechanical** properties of materials during degradation as well as in-vivo degradation, cell response and toxicity studies.

## 7. Bionanocomposite surface functionalization

Surface properties of nanocomposite scaffolds are a key factor in governing the success of the engineered tissue, since the first interactions between the cells and the substrate are protein adsorption and then cell adhesion [227].

Multiple approaches have been developed to provide micrometer to nanometer scale alterations in surface architecture of nanocomposite films and scaffolds to enable improved protein and cell interactions, to guide cells to form tissue.

It is well-known that both chemical and topographical properties of material surfaces can influence cellular behaviour and can control cell shape, functions and motility. Recent studies have highlighted the **mechanisms of cell-surface recognition** and have provided solid data to obtain novel materials that are able to guide and activate specific cell behaviour on biomaterial [227–229]. Particularly the effects of micro-topography and more recently, the effect of nanotopography on cell biology also represents a critical issue [230–235]. Surface design generating biomaterial nanotopography for tissue engineering-based strategies has been demonstrated to enhance differentiation of progenitor cells into their programmed lineage pathway. To this aim, efforts have been made to tailor the surface of biomedical devices and biomaterials in general, to provide chemical and physical cues to become biocompatible to the surrounding tissues, or to guide cells to form tissue [236].

Chemical modification of nanocomposite scaffold surfaces is one of the upcoming approaches that enables enhanced biocompatibility while providing a delivery vehicle for proteins. Similarly, physical adsorption, radiation mediated modifications, grafting, and protein modifications are other methods that have successfully been employed for alterations of scaffold surface properties.

It is well-known that the aliphatic polyesters do not provide a desired environment for cell adhesion, due to the lack of biological recognition sites and its intrinsic hydrophobicity, compared to the natural extracellular matrix: they do not expose functional groups for the attachment of biologically active molecules [237–239].

In order to apply biodegradable polyester based nanocomposite in tissue engineering, their surfaces have been chemically and physically modified with bioactive molecules and cell recognizable ligands after the processing condition; this subsequently provides bio-modulating or biomimetic microenvironments to contacting cells and tissues. A variety of functionalization strategies of nanocomposite scaffolds with bioactive molecules including proteins, nucleic acids, and carbohydrates have been employed [240]. In this review, topographical and chemical immobilization methods of bioactive molecules on the surface of various polymeric scaffolds are described.

Therefore, many approaches to modify the surface of biodegradable polymer scaffolds have been undertaken in order to introduce useful surface characteristics to the polymer. Surface treatment techniques, such as plasma treatment, ion sputtering, oxidation and corona discharge, affect the chemical and physical properties of the surface layer without significantly changing the bulk material properties. Using plasma processes, it is possible to change the chemical composition and properties such as wettability, surface energy, metal adhesion, refractive index, hardness, chemical inertness and biocompatibility [241]. Plasma techniques can easily be used to induce the desired groups or chains on the surface of a material [242–244]. Plasma treatment of polymer substrates has been commonly employed to tailor surface adhesion and wetting properties by changing the surface chemical composition [244]. Appropriate selection of the plasma source enables the introduction of diverse functional groups on the target surface to improve biocompatibility or to allow subsequent covalent immobilization of various bioactive molecules. For example, typical plasma treatments with oxygen, ammonia, or air can generate carboxyl groups or amine groups on the surface [245–248]. Plasma treatment affects the chemistry of the biodegradable polymer surface, but at the same time it also introduces significant changes in topography [249,250]. A variety of extracellular matrix protein components such as gelatin, collagen, laminin, and fibronectin could be immobilized onto the plasma treated surface to enhance cellular adhesion and proliferation [251,252]. One of the surface modification methods for biopolymer substrate surfaces is attachment of extracellular matrix components or their derived synthetic peptides, such as Arg-Gly-Asp (RGD), that is the most effective and often employed peptide sequence for stimulating cell adhesion on synthetic material surfaces. This peptide sequence can interact with the integrin receptors at the focal adhesion points. Once the RGD sequence is recognized by and binds to integrins, it will initiate an integrin-mediated cell adhesion process and activate signal transduction between the cell and ECM, thus influencing cell behaviour on the substrate including proliferation, differentiation, apoptosis, survival and migration [253]. RGD peptide was immobilized on two-dimensional biodegradable polymer film surfaces [254] and 3D porous scaffolds [255].

Since three-dimensional scaffolds have larger surface area and highly inter-connected porous structure with suitable porosity and pore size, modification of scaffold surface to improve the

interaction between cell and scaffold surface has more potential in tissue engineering.

It was demonstrated that rat bone marrow stromal cell adhesion was significantly improved on the RGD-modified PCL films in a serum-free culture condition.

We previously reported that radiofrequency oxygen plasma treatment was effective in changing the surface properties of PLLA films and porous scaffolds. The treatment was shown to functionalize homogeneously the surface of the PLLA without affecting its bulk properties. The plasma treatment was found to be successful in achieving three-dimensional functionalization without any adverse effect on the chemical composition and structure of the scaffolds, thus preserving their properties for tissue engineering applications. The effects of the oxygen plasma treatments on the surface of the material have been shown to change wettability, roughness and to enable the selective interaction between the PLLA polymer surface and the protein, further improved **stem cell** attachment [256]. Fig. 7 shows contact angle images of pure PLLA (a) and oxygen treated PLLA, 10 Watt 5 min (b). In all the modified PLLA films, a decrease in the contact angle was registered, which means that the hydrophilicity increased greatly when oxygen plasma treatment was applied. When the sample was treated with a 10 W power supply for 5 min, the contact angles decreased from 90° to 50°, changing the original hydrophobic behaviour of PLLA surfaces to hydrophilic. Contact angles less than 10° were measured when applying a power supply of 20 W and a treatment time of 10, 5 or 2 min. The surface wettability of the modified PLLA films was obviously enhanced compared to the control film.

It is suggested that this approach can be used with various types of proteins and specific growth factors to modulate subsequent cell functions, such as proliferation, differentiation and migration on biomaterial surfaces.

## 8. Stem cell–bionanocomposite interactions

Engineered nanocomposite scaffolds made by biodegradable polymer matrices with organic/inorganic nanostructure phases, as reviewed here, will play a vital role in combination with **stem cell** seeding.

Bone marrow derived **human mesenchymal stem cells** (hMSCs) are an important cell source for cell therapy and tissue engineering applications. These **stem cells** have broad differentiation potential, being able to differentiate into a variety of anchorage-dependent cell types, including neurons, myoblasts and osteoblasts [257]. The interactions between stem cells and their environment are complex and not completely understood. In particular the relative importance of different physical parameters on the biocompatibility of biomaterials for tissue engineering is poorly understood. Previous studies showed that cells respond to the mechanical properties of the substrate on which they are growing [258]. Thus, polyester films with the lowest moduli provide the most favourable substrate for cell growth and also provide the most appropriate **mechanical** properties for the intended site of implantation. Rohman et al. demonstrated that PLGA and PCL films support the growth of both human bladder epithelial and smooth muscle cells [259]. Cell growth was affected by the mechanical properties of the films, with enhanced proliferation on films that had an elastic modulus closer to the native bladder tissue. This suggested an advantage in complementing the **mechanical** properties of a biomaterial to the intended site of implantation. The elastic modulus is a critical parameter, where it is relevant to the biology at the microscopic (cellular) level and may also have an impact at macroscopic (tissue/organ) scales. By developing new bionanocomposites it is possible to modulate **mechanical** properties for the specific sites, as reported in this review [260]. Many model systems and measurement tools

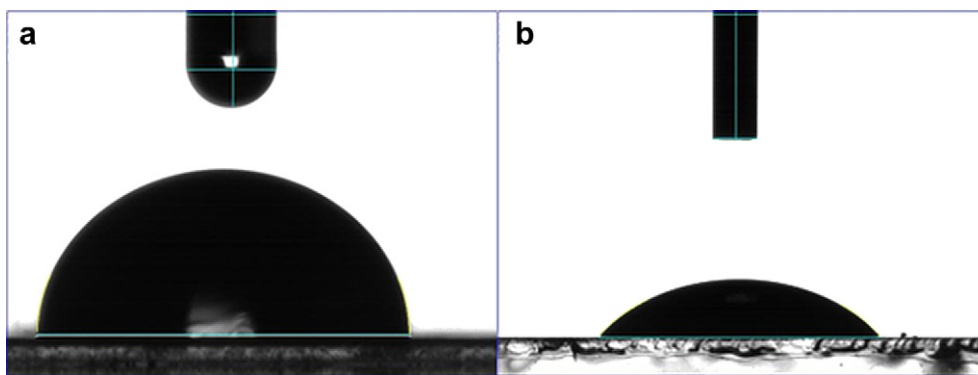


Fig. 7. Contact angle images of pure PLLA (a) and oxygen treated PLLA, 10 Watt 5 min (b). Reproduced with permission by Armentano et al. [256].

have been engineered for observing and [quantifying the effect of mechanics](#) on cellular response. Engineered synthetic polymeric nanocomposites can allow precise and systematic control over the [mechanical](#) properties of the cell substrate, and have provided quantitative information about the forces that are sensed and exerted by cells [261]. Recent studies have demonstrated the effect of matrix stiffness on the phenotype and differentiation pathway of [mesenchymal stem cells](#) (MSCs). They differentiated into neural, myogenic or osteogenic phenotypes depending on whether they were cultured on two-dimensional (2D) substrates of elastic moduli in the lower (0.1–1 kPa), intermediate (8–17 kPa) or higher ranges (34 kPa). Similar results were found for the three-dimensional (3D) culture [262].

The studies reported by Yeung et al. [263] have shown that [cellular response to matrix stiffness may be very different in different cell types and depends on the nature of the adhesion receptor by which the cell binds its substrate](#). There are also important differences between cells grown on two- and three-dimensional adhesive materials, but even when confined to adhesion on flat surfaces, the elastic constant of the surface can determine cell morphology and protein expression over a very wide range. Recent studies with aortic smooth muscle cells have shown that substrate stiffness is a more important determinant for cell shape than is the density of adhesive ligand to which the cell binds.

Fibroblasts and endothelial cells develop a spread morphology and actin stress fibres only when grown on surfaces with an elastic modulus greater than 2000 Pa, with a greater effect seen when bound to fibronectin compared to collagen. In contrast, neutrophils appear to be insensitive to stiffness changes over a very wide range. The stiffness-dependence of fibroblasts and endothelial cells is no longer evident when cells become

confluent, or in the case of fibroblasts even when two cells make contact suggesting either that [mechanosensing uses the cells internal stiffness](#) as a criterion or else that signaling from cadherins in cell–cell contacts overrides signals from the cell–matrix adhesion complexes.

Cell orientation and adhesion are known also to affect cell behaviours and functions in both natural and engineered tissues. Cell adherence to substrate plays a key role in morphogenesis and organogenesis [264]. Cells in biological tissues are typically orientated and spatially patterned. Oriented cells, for instance, could provide favourable adhesion due to higher density of focal contacts and rearrange the cytoskeletal structures (e.g., actin fibres) [265], and also determine the alignment of collagenous matrix in healing ligaments and tendons that are less organized after injury. Because regulation of cell adhesion and orientation is important for cell-based therapy and tissue engineering, several techniques, including surface topography modification and use of physical stimuli, have been developed to control cell orientation. For example, modifications in biomaterial surface properties such as the peak to valley height of the surface structures have been shown to induce cell orientation [265].

Electrical properties of the bionanocomposite substrates are important issue in cell interaction. Although induced cell adhesion, migration, and orientation in response to electrical stimulus have been well documented using 2D cultured cells, electrically induced cellular behaviours in the 3D scaffold remain unknown. Application of electrical stimulus could then offer a novel physical approach for controlling cell growth and differentiation in cell based therapy and engineered tissue constructs by regulating cell adhesion and orientation. Fibroblasts and rat MSCs seeded in the collagen scaffold respond differently to electrical stimulus. While fibroblasts are induced to reorient themselves perpendicularly to the axis of

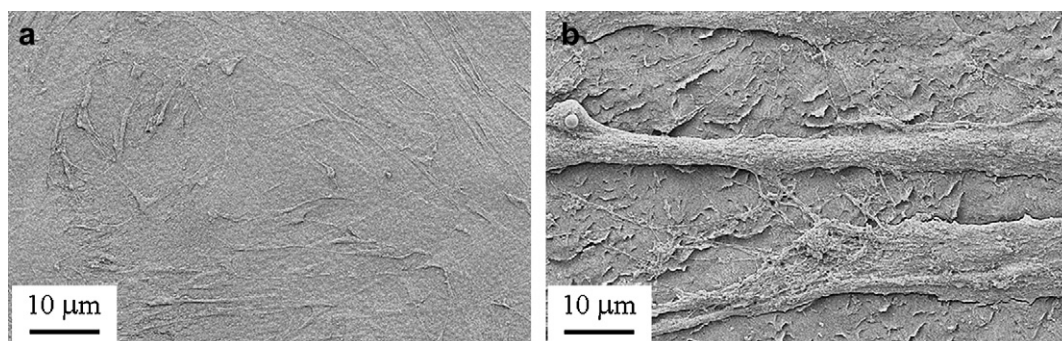


Fig. 8. Osteoblasts cells on PLLA/SWNTs-COOH films after 3h of cell attachment at different resolutions.

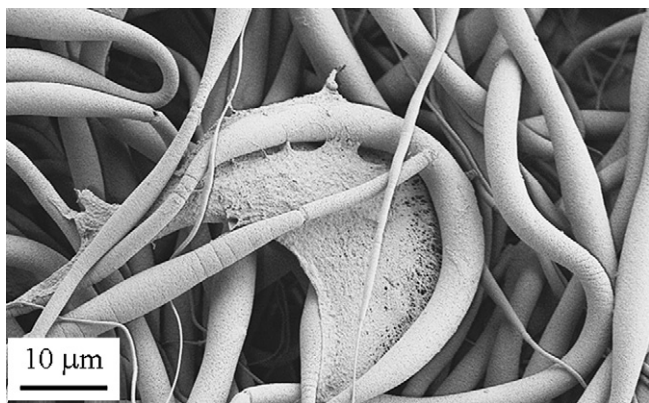


Fig. 9. Stem cells on PLLA/HA mats.

electrical stimulus, rat MSCs exhibit only a limited reorientation. At least two physical mechanisms could be postulated to mediate cell orientation in the 3D collagen scaffold. First, alignment of collagen fibres in response to an electrical stimulus could induce cell orientation. In summary, application of an electrical stimulus causes fibroblasts to change cell shape and reorient in the 3D collagen scaffold perpendicularly to the direction of electrical stimulus, accompanied by a preferential realignment of collagen fibres. The same electrical stimulus applied to MSCs induces much less significant reorientation or collagen fibre realignment. This may be attributed to differential cell adhesion mechanisms. Finally, optimal application of electrical stimulus could offer a novel engineering technique to regulate cell type-dependent cellular shape and orientation that are known to be involved in cell differentiation and growth. It also can be of potential use to selectively control a population of cells in coculture environment [126,129,264].

HA nanoparticles have been introduced in bionanocomposites to induce osteoconductivity. Bone regeneration was evaluated by using osteogenic cells derived from human embryonic stem cells (OC-hESCs) and an osteoactive PLGA/HA scaffolds, in-vivo [266]. They showed the first successful regeneration of bone tissues using osteogenic cells that had been differentiated from hESCs. In-vivo implantation of OC-hESCs and apatite-coated PLGA/HA scaffolds showed a significant amount of new bone formation in ectopic sites of implantation in immunodeficient mice.

Scaffold morphology, in term of interconnectivity, pore size, shape and morphology is a key point in stem cells interaction. Furthermore, there were some pores sized between 2 and 5 μm dispersed on the walls of the scaffolds, which would be helpful for fibrovascular colonization and nutrient transportation. Yuan et al. found that micropores on the macropore walls of the calcium phosphate ceramic were important in osteoinduction [267].

Therefore, small pores on the macropore surface of the scaffolds may be also helpful to improve the biological performance of the porous scaffolds and promote the favourable bioadsorption of the material. Fig. 8 shows osteoblasts cells seeded on PLLA/SWCNTs-COOH films after 3 h of cell attachment at different resolution. The osteoblast cells have attached and well flattened on the substrate surface, showing good affinity with the PLLA/SWCNTs-COOH polymer surface. Fig. 9 shows stem cells on PLLA/n-HA mats, where cell shape is entirely adapted to fibrous space in a peculiar “moon shaped” configuration.

## 9. Conclusions

Novel generation biomedical nanocomposites are expected to be hybrid, biofunctional, intelligent and containing active

component. Polymer matrix composites have the advantage of being very versatile, allowing for the tailoring of their final properties. Bionanocomposites can be designed and produced with specific requirements, using a wide range of polymeric matrices, reinforcements and processing routes.

As a result, much of the work is still ongoing and there is yet to be a definite conclusion on the effect of nano-sized inclusions on polymer systems. In this paper, a review has been presented on the materials, processing, experimental results, and possible interpretations of those results for polymer matrix nanocomposites.

The mentioned studies suggest that the combination of biodegradable polymer and nanostructures opens new perspective in the nanodevices for biomedical applications with tunable mechanical, thermal, morphological and electrical properties.

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