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Bioactive Nanocomposites with Applications in Biomedicine

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Abstract

Nowadays one of the big challenges is to obtain materials that can find applications in biomedicine. In order to develop tailor-made biomaterials different aspects need to be considered such as selection of materials in terms of purity, toxicity and biocompatibility, manufacturing process and final application of the new material. For such materials to be used in biomedicine, certain mechanical properties are typically desired (i.e., low friction coefficient, wear resistance, thermal stability). Nanocomposites materials have been tested for biomedical use and especially those based on layered silicates have shown great properties. Due to special properties that layered silicates possess, and by dispersing them into different polymer matrices, bioactive materials can be obtained which can find applications as: drug delivery systems and targeting into sites of inflammation or tumours, wound healing patches, covers for implants biointegration, tissue engineering, bone repair, a.s.o.

Keywords: Nanocomposites, biomaterials, bioactive, biocompatibility, layered silicates, collagen, scaffolds, drug delivery systems.

1.1 Introduction

Research in biomaterials has increased in the last years, due to promising results that have been observed and due to the versatility within these materials.

Mayuri Prasad and Paolo Di Nardo (Eds.), Innovative Strategies in Tissue Engineering, 1–24.

Materials developed in this sense are important for drug delivery systems and tissue engineering approaches and can play a key role in developing of artificial organs or on growing organs.

Many types of polymers can be used as carrier systems due to their ability to provide delivery of active substances to specific sites. Moreover, such biomaterials can deliver cells to the surrounding tissue, which makes them excellent candidates for controlling the attachment, growth and differentiation of the cells [1].

Bioactive ceramics can form a mechanically strong interfacial bond with bone depending on the conditions. Thus, bioactive composites present excellent biochemical compatibility, but less optimal biomechanical compatibility. To be an ideal bone replacement material, composite biomaterials have to combine bioactivity with biomechanical properties [2].

Biomaterials can find applications in biomedicine as soft or hard implants and can be used as: joint replacements, bone cements, artificial ligaments, dental implants, blood vessels, heart valves, skin repair, contact lenses, and cochlear replacement [3].

Considering the various types of implants that can be engineered and their functions, they need to be designed with appropriate geometry, size and weight for a given patient. To obtain tailored made prosthesis, many researchers have incorporated different features to promote tissue ingrowth [4], several described rapid prototyping [5, 6] to create artificial tissue by means of computer numerical controlled machining and others used electron beam melting [7] to fabricate complex shape implants. In the future years it is overseen a dramatically increase in use of biomaterials and development of advanced materials in medical-device industry due to materials complex properties and shapes [8].

At the beginning, the only requirement for materials when they were used for the first time in biomedical applications was to be "inert" so as to reduce the inflammatory response [9]. These type of materials were classified as "first – generation" and had to possess proper combination of physical properties as for the replaced tissue with a low toxic response of the body.

The second-generation of biomaterials appeared between 1980 and 2000. They were completely opposite to the first generation in terms of interaction to the human body. These materials had the ability to interact with the biological environment, to improve their biointegration and some were bioabsorbable; they undergo degradation while the new tissue was regenerated.

The third-generation of biomaterials combines bioactivity and biodegradability with the ability to stimulate cellular response [10]. Moreover,

three-dimensional porous structures are being developed that can stimulate cell proliferation or can act as drug delivery systems [11–13]. Tissue engineering and the third-generation of biomaterials appeared approximately at the same time as a potential solution to tissue transplantation and grafting [14–16]. Regenerative medicine is a recent research area, which explores ways to repair and regenerate organs and tissues using combination of stem cells, growth factors and peptide sequences with synthetic scaffolds [17, 18].

1.2 Factors that Influence the Quality of a Biomaterial

Polymer matrices used in therapeutic applications are often resorbed or degraded in the body. In this case, two key challenges can be identified. First, the degradable polymers used in tissue engineering were selected from materials used for other surgical uses and thus such materials may have deficiencies in terms of mechanical and degradation properties [19]. To overcome this, new classes of polymers and biopolymers are being developed. The second major challenge concerns tailoring these polymers into scaffolds with defined and complex porous shapes, which can undergo cell attachment and proliferation [20, 21].

Biodegradable three-dimensional scaffolds play an important role in maintaining the cell functions. The cells adhere to the porous scaffold in all three directions, proliferate and replace the temporary scaffold. Moreover, the scaffold should be biodegradable, biocompatible, and highly porous with a large surface area, with a specific mechanical strength and shape to permit cell attachment, proliferation and maintaining of differentiated cell functions [22].

When obtaining a biomaterial several factors need to be considered starting with selection of raw materials in terms of purity, toxicity and biodegradability, the obtaining process and last but not least the mechanical and structural properties of the final material.

Polymer purity as well as purity of other additives is important for biocompatibility of medical devices and drug delivery systems. Residual monomers, catalyst residue or impurities strongly affect cell viability; a special consideration must be given also to the biodegradability compounds. Therefore, quality assurance of productions processes must be taken into account especially for biocompatibility of newly obtained biomaterials [23].

Many natural polymers have been extensively used in biomaterials in tissue repair and regeneration, for example collagen, gelatine and

chitosan [24–27]. The use of natural polymers in tissue engineering as scaffolds or biomaterials is limited by poor mechanical properties and by the loss of biological properties during processing [28]. Thus, natural polymers in general are chemically treated or mixed with other materials to improve their mechanical properties and maintain their biological activity. However, some problems were observed in the chemical treatment of some biopolymers i.e. when collagen was modified with glutaraldehyde, which promoted calcification of heart valve [29, 30].

Implants in general are used for several years, thus they must perform adequately, must not cause locally abnormal reactions and should not produce toxic or carcinogenic effects inside the body. Similar, biodegradable scaffolds should not release toxic products or affect the healing process anyhow, while serving their intended function [31].

New approaches are needed for processing the polymers to make implants with complex architectures and macroscopic shapes, allowing the composition to change to accommodate changes in tissue development. A great challenge arises in tissue engineering from materials issues, that the biological processes are not yet well understood to set the design parameters specifically. The development of materials and understanding of biological processes take place simultaneously. The development of new materials gives indications about the complexity of biological processes, which consequently improves the design of scaffolds. From tissue engineering great challenges and opportunities arise for material science in terms of materials design and processing. Scaffolds must be designed in three-dimensional configuration and direct the cells proliferation to form the desired tissue structure, especially in a way that can be reproducible and on a large scale. From molecular point of view it is important that the new materials interact with cells controlling adhesion and proliferation phenomena [32].

Usually, implanted devices are subjected to high stresses and cycle loading; consequently, the materials used are exposed to environmental aggressive conditions inside the body, which often leads to failure. During erosion of an implanted device, fragments can be detached which can lead to an acute host-tissue reaction by producing highly corrosive enzymes and chemicals which in return will affect the biomaterial. Thus, it is necessary to develop methods for fatigue evaluation for biomaterials to understand the host-tissue reaction to fragments formation and simulate accurately *in vivo* stress-strain behaviour and environmental conditions. The development of such biomaterials with high resistance to fatigue and wear conditions is still in its early stage [33].

1.3 Layered Silicate Nanocomposites for Biomaterials

Nanocomposites are materials with unique properties which have found applications in wide areas of activity: aircraft industry, automotive, packaging, construction, electronics, and especially in medicine and in the pharmaceutical industry. Materials with 2 to 7% inorganic nanofillers (i.e. a layered silicate) exhibit improved properties like composites with 20 to 40% inorganic filler dispersed at macro or microscopic scale. Among these, several polymer nanocomposites have potential to be used as biomaterials in the biomedical field.

1.3.1 Layered Silicate Properties

Clay minerals used in nanocomposites belong to the general family of layered silicates whose main representative in terms of "utilization degree" is the montmorillonite. The crystal structure of montmorillonite consists in layers of oxyanions (with thickness of 0.95 nm), formed by fusion of two sheets of $[SiO_4]^{4-}$ silica tetrahedral arranged in hexagonal rings with a sheet of $[AlO_4(OH)_2]$ alumina or magnesia octahedral, sandwiched between two opposing tetrahedral sheets (Figure 1.1.).

The ratio between the octahedral and tetrahedral sheets is 2:1. A feature of the montmorillonite is that it contains molecular water that is fixed by adsorption between layers. Between the layers of montmorillonite, water molecules associate in according with hexagonal symmetry. Therefore, montmorillonite formula is $Al_2O_3 \cdot 4SiO_2 \cdot H_2O + xH_2O$ [35].

Studies of the composition of the natural montmorillonite (bentonite) showed that in the sheet of tetrahedrons, Si^{4+} ion might be partially replaced by Al^{3+} ion, and in the sheet of octahedral, Al^{3+} ion might be replaced by Mg^{2+}, Fe^{2+}, Zn^{2+}, etc. ions, such as, by the replacement of Si^{4+} and Al^{3+} ions with lower valence ions, resulted an excess of negative charges. To obtain the electric neutrality between layers hydrated cations Na^+, K^+, Ca^{2+}, etc. are adsorbed [36].

By arranging of oxyanions layers, spaces (galleries) are formed, connected by weak Van der Waals links [37]. These galleries are occupied by mobile hydrated cations that compensate the charge deficit generated by isomorphic substitution between the layers. The water from the space between the layers tends to be associated with mobile cations and forms around them "hydrated coatings", whose number depends on the relative humidity and of cations nature between the layers [38].

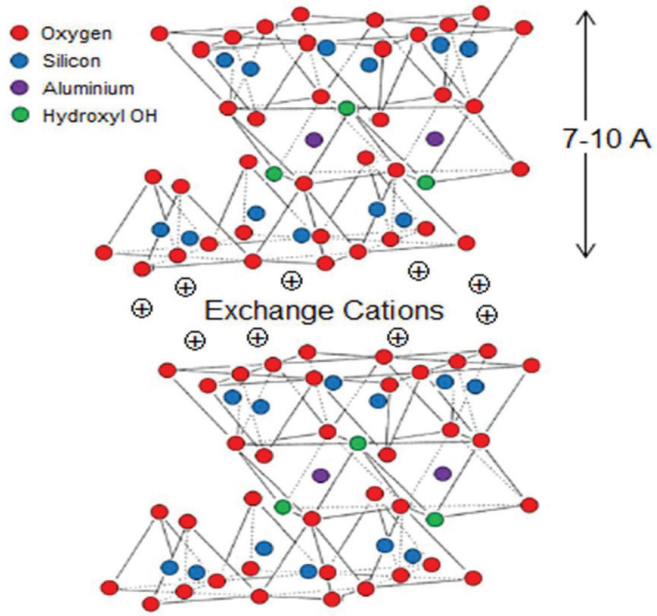

Figure 1.1 Montmorillonite structure (Modified form [34])

Montmorillonite can absorb water increasing the volume of 20–30 times. Each layered sheet is smaller than 1 nm thick, with surface of about 1 μm (1000 nm). For an aspect ratio (length to thickness ratio of silicate layers) of about 1000, clays specific surface area is about 750 m^2/g, resulting in high reinforcement efficiency at lower clay concentrations (2–5%) [39]. However, natural layered silicates are not suitable for obtaining nanocomposites because they are too hydrophilic and layers are compacted too tightly by inorganic cations to interact with the hydrophobic molecules of polymer and to be able to disperse among them. To ensure compatibility between the clay and the polymer matrix, modification of the layered silicate surface (organophilisation) is necessary. This technology implies two steps:

- layered silicate purification to a sufficiently high level requested by the field of application;
- modification of the layered silicate surface (organophilisation).

The large specific surface area, layer charge, swelling capacity and adsorption properties of different organic/ inorganic substances make clay minerals (hydrated layered silicates) to be useful as materials for pollution control [40],

carriers of pesticides [41], liners in waste disposal [42], barriers in nuclear waste management [43] and last but not least as materials beneficial to human health.

The intelligent properties of clays are known from antiquity in all part of the world: antiseptic, bactericides, scar action, antitoxic properties, sans microbial germs. Since prehistoric times, a large variety of clays and clay minerals (i.e. bentonite, kaolinite, montmorillonite, smectite) have been used for therapeutic purposes, including the treatment of wounds, inhibition of haemorrhages and as a preservative in mummification (in ancient Egypt), used as antiseptic cataplasms to cure skin diseases, as scars, or as an anti-inflammatory agent for snake bites [44]. Based on the capacity to adsorb and retain harmful and toxic substances, the mineral clays have beneficial effects in the treatment of gastrointestinal disorders. Clays can adsorb a variety of toxic substances, such as toxins, pesticides, viruses, bacteria, and other digestive irritant [45]. However, clay minerals can be harmful to human health when they are inhaled over a very long period. The toxicity of these minerals is generally related to the presence of very fine-grained quartz and cristobalite in the range from 0 to 24% [46], which inhaled in the lung, can cause cancer. For this reason, the purification process of clay minerals is very important especially for uses in biomedicine.

1.3.2 Layered Silicate Purification

Usually, the natural layered silicates are mixtures or associations of minerals and/or amorphous materials. In order to enrich one clay mineral and/or to remove other unwanted clay mineral, a purification step is necessary. Purification is also required for identification and for characterization of clay minerals. Very important is the chemical composition of layered silicates when they are used as reinforcing agent for preparing nanocomposites. The final properties of nanocomposites depend on stable compositions. The montmorillonite (MMT) is the most important layered silicate used for preparation of nanocomposites. Different minerals such as quartz, zeolite, calcite, feldspar and pyrite have a great effect on the composition and influence the purification technology [47].

The purification procedure involves two main steps: one of them consists in removal of carbonates, hydroxides, and organic materials and the other one consists in fractionation by sedimentation. However, not all the purification steps are always necessary. The purification of layered silicates from identified

geological deposits consists in replacing the exchangeable cations with Na^+ followed by washing with water.

The natural layered silicates (bentonites) may contain different percentages (5–40%) of quartz and other impurities, which act as a sterile, hindering the surface modification process, in order to ensure the compatibility between the silicate and a polymer matrix [48]. Purification is made by dispersing the layered silicate in distilled water at 60–90 °C. Quartz and other hydrophobic impurities are separated by decantation [49]. Often is required additional purification to ensure a high degree of purity, imposed in the biomedical field. By additional purification, the concentration of MMT increases with 7–10% [49].

1.3.3 Layered Silicate in Drug Release Systems

Layered silicates are widely used ingredients in pharmaceutical products as both excipients and active substances. Based on their adsorption capacities, swelling and colloidal properties, clay minerals can be used in drug delivery systems to achieve technological (taste masking), chemical (increasing stability), biopharmaceutical (decreasing or increasing dissolution rate, delaying and/or targeting drug release) and pharmacological (prevention or reduction of side effects) benefits [50].

Layered silicates, especially montmorillonite and saponite, because of their high cation exchange capacity, were used and studied in controlled-release drug delivery systems. The interaction between clay minerals and active substances depends on the type of mineral involved and on the functional groups and the properties of the organic compounds.

There are some mechanisms of interaction or complexation between montmorillonite and drug [51–67]:

- Cation exchange with cationic drugs. This produces a strong interaction bonds between montmorillonite and basic molecules;
- Anion exchange of anionic drugs at slightly positive-charged platelet edges. This produces weak interaction bonds with anionic drugs;
- Hydrogen bonding at platelet faces;
- Intercalation of non-ionic drugs via ion-dipole interactions;
- Adsorption by solvent deposition onto the high surface area of the clay to increase the dissolution rate of poorly soluble drugs.

Depending on the degree of interaction between montmorillonite and drug, nanostructured systems with intercalated, partial exfoliated or exfoliated

lamellar structures, able to release faster or slowly the bioactive substance could be obtained [68].

1.3.4 Biopolymers Properties

Biopolymers are materials produced from renewable resources. In recent years, the worldwide interest in biopolymers increased due to their positive environmental impact such as reduced carbon dioxide emissions. Many biopolymers are biodegradable, being degraded and gradually absorbed and/or eliminated by the body. This property is of high interest for biomedical applications (tissue engineering, bone replacement/repair, dental applications and controlled drug delivery).

Many biomedical applications require biomaterials with high performance and mechanical properties. For such materials to be used in biomedicine is not enough to be biocompatible and biodegradable, certain mechanical properties are imposed (i.e., low friction coefficient, wear resistance, thermal stability, modulus, strength and toughness). Not all these properties can be achieved by using the biopolymer alone [69–71]. By dispersion of inorganic/organic fillers at the nanometer scale into a biopolymer matrix, new class of bionanocomposites, with enhanced mechanical properties as compared to conventional microcomposites, was developed.

One of the most researched and used biomaterial in various fields of medicine is collagen, due to its biocompatibility, biodegradability, and weak antigenicity [72]. It is well known the use of collagen as biomaterial, biocompatible and bioresorbable for connective tissue prosthesis in which collagen is the basic protein. To use collagen as a scaffold in bone reconstruction, modifications are necessary in the structure and composition of the matrix to achieve the osteoconductive and osteoinductive effect. This was achieved by preparation of biocomposites with SiO_2, TiO_2, clays, hydroxyapatite, etc. [73, 74]. The collagen fibrils have high elasticity while the mechanical properties are relatively limited. Substantial improvement of its properties can be achieved by the nanoscale dispersion of the layered silicate in a collagen matrix. Depending on the collagen morphologies, nanocomposites with intercalated or exfoliated lamellar structures and improved thermal stability were obtained [75].

In order to use a material in biomedicine, both its bulk and surface properties are important to be known, especially interfacial behaviour with aqueous environment. The wettability capacity, the swelling behaviour, the presence of surface roughness, liquid and vapour water absorption are only

a few of the properties required for biomaterials with biomedical purposes. Valuable informations about these properties were achieved by water contact angle and determination of water absorbency [68].

1.4 Routes for Obtaining Bio-Nanocomposites

In literature, there have been described three main processes to prepare polymer layered silicate nanocomposites [76]:

The first process is known as intercalation from solution. The layered silicate is swelled in the same solvent in which the polymer is soluble. Using an adequate solvent and due to the weak forces that are between silicate platelets, the sheets of layered silicate can be dispersed and form a stable suspension. Furthermore, the solvated polymer penetrates into the silicate galleries. In this way the sheets are delaminated and an ordered multi-layered structure is formed when the solvent is evaporated [77, 78].

The second technique also takes place in solution and is called in situ intercalative polymerization. As compared to the previous one, this time the silicate is swelled into liquid monomer or prepolymer followed by polymerization reaction. This reaction takes place between the silicate platelets if the initiator is introduced in the swelling step or in special cases if the sample is subjected to heat or radiation [77, 78].

Melt intercalation technique is the third process in which the already modified clay is mixed with melted polymer [77]. To obtain nanocomposites in which the polymer melt must be intercalated between modified silicates, a temperature with 10–12 °C higher than polymer softening temperature is required when using static or shear stress thermal treatment [79]. In this case, no solvent is involved and thus if the compatibility between polymer and modified silicate is good enough, intercalated or exfoliated nanocomposites can be formed [78].

It has been demonstrated that melt intercalation technique is more effective in terms of dispersion rather than intercalation of a monomer followed by polymerization or by intercalation of a polymer from solution [80]. However, when obtaining nanocomposite biomaterials, other aspects need to be considered, hence all these techniques have some limitations. There are biopolymers like collagen, which cannot be extruded or melted due to low polymer degradation temperature.

Intercalative polymerization technique can be used to obtain nanocomposite biomaterials if the solvent in which the polymer is soluble should not produce toxic or carcinogenic effects, since traces of solvent may still be present in the final material.

Among all techniques, in situ intercalative polymerization presents most limitations, since traces of monomer, initiator and other impurities, which can produce abnormal reactions inside the body, may still be present in the scaffold.

Figure 1.2 synthesise the main steps for obtaining a nanocomposite, furthermore one must take into consideration all the implications discussed above and the factors that influence the quality of a biomaterial.

Complex polymer bio-nanocomposite materials are being developed as scaffolds, tissue regenerating patches and control drug release systems for the hope of better and faster treatment of diseases. Implants in general must have superior performance and mechanical properties as well as biological function. Thus, facing these challenges, implants properties and design can be tailored so to optimize the functionality and performance [81].

Taking into consideration the final application of the scaffold and the necessity to create biomaterials with complex structures, porous matrices or transparent membranes can be obtained (Figure 1.3.)

Porous scaffolds play an important role in tissue engineering and they were used to construct cartilage, bone, skin, ligaments a.s.o [82–85].Three

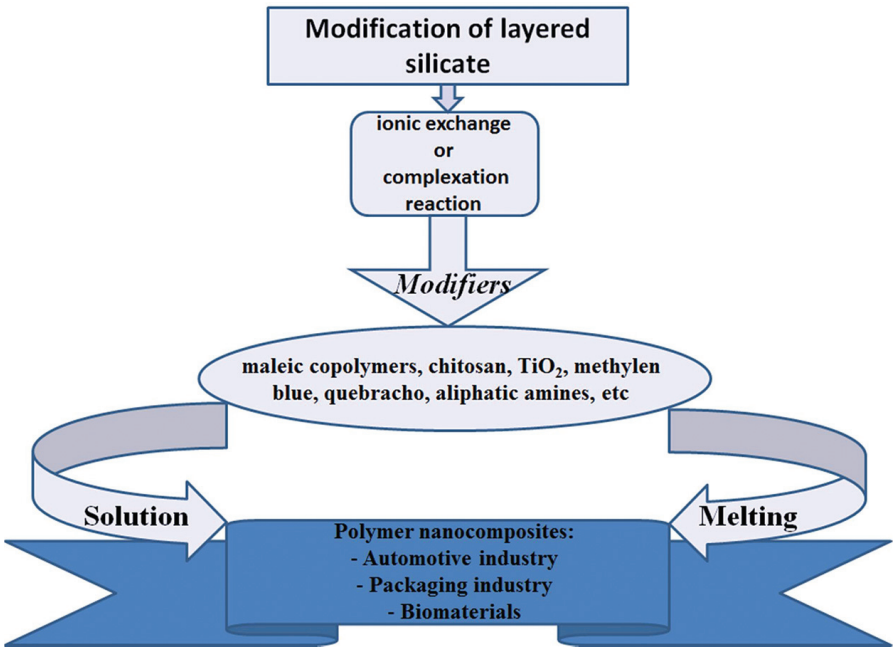

Figure 1.2 Scheme flow for obtaining layered silicate nanocomposites

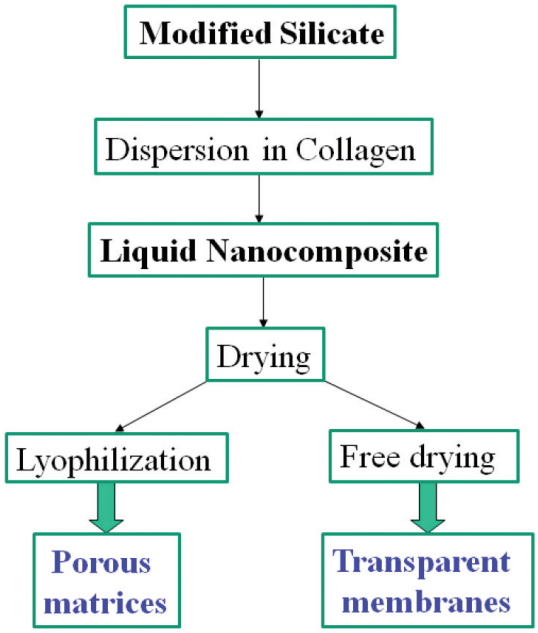

Figure 1.3 Scheme flow for obtaining collagen based nanocomposites

dimensional porous scaffolds can be obtained from synthetic polymers such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymer poly(DL- lactic-co-glycolic acid) (PLGA), and from naturally derived polymers such as collagen [86–88].

To obtain hybrid collagen microsponges, gaseous glutaraldehyde was used as cross-linking agent to form their pores and to stabilize the collagen in water [22, 89, 90].

1.5 Biomaterials Development

The quality of biomaterials is strongly influenced by composition, architecture and three-dimensional design, biocompatibility, but also by mechanical strength of the scaffold that mimics the mechanical strength of the tissue intended to repair or replace. Pore size distribution as well as pore types influences the attachment of specific cells and interaction of biomaterials with the body. Furthermore, it is important also to identify and isolate the appropriate cells from the primary source, when selecting the cells for the engineered scaffold [91].

In the recent years, more and more encouraging results are being obtained. Biomaterials that may find applications in bone tissue engineering were developed which showed impressive results. Such hybrids were synthesised by dispersing of layered silicate modified with maleic anhydride methyl methacrylate copolymer into collagen gel denoted CG/PB/MA-MMA. Nanocomposites in form of microporous matrix (scaffold) having a spongy structure, which contains macro, and micro interconnected nanopores were obtained at different pH's using freeze-drying technique.

The spongeous matrix was *in vitro* tested on osteoblast cell cultures and followed after 24 and 72 hours from hatching. Figure 1.4. presented that cells proliferated on the ternary nanocomposite and no cytotoxic response was observed. The cells presented normal phenotype and moreover, the viability was reported to be 97% [49].

Layered silicate/collagen membranes with different amounts of methylene blue and quebracho were also obtained and their biocompatibility was tested

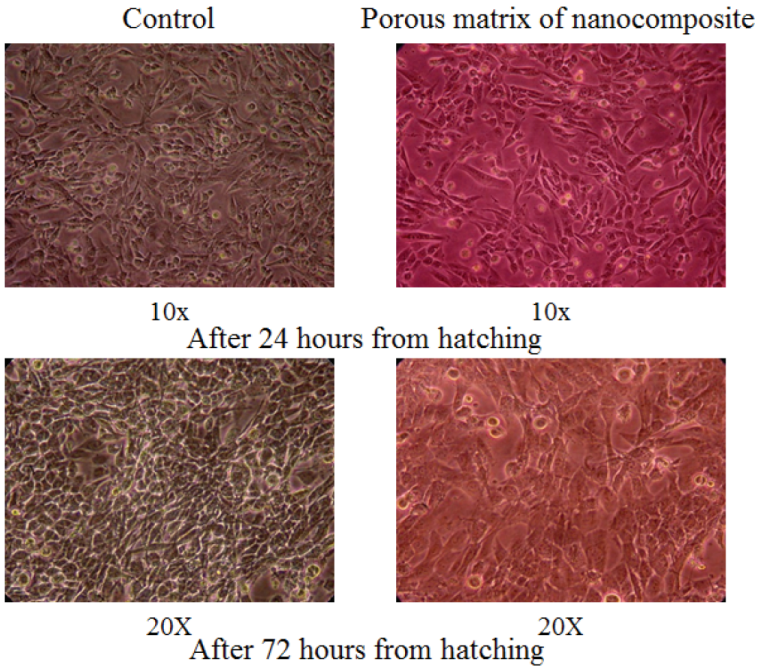

Figure 1.4 Microscopic analysis of osteoblast culture samples sowed on CG/ PB/ MA-MMA ternary nanocomposite (Originally published in [49] under CC BY 3.0 license. Available from: http://dx.doi.org/10.5772/25947).

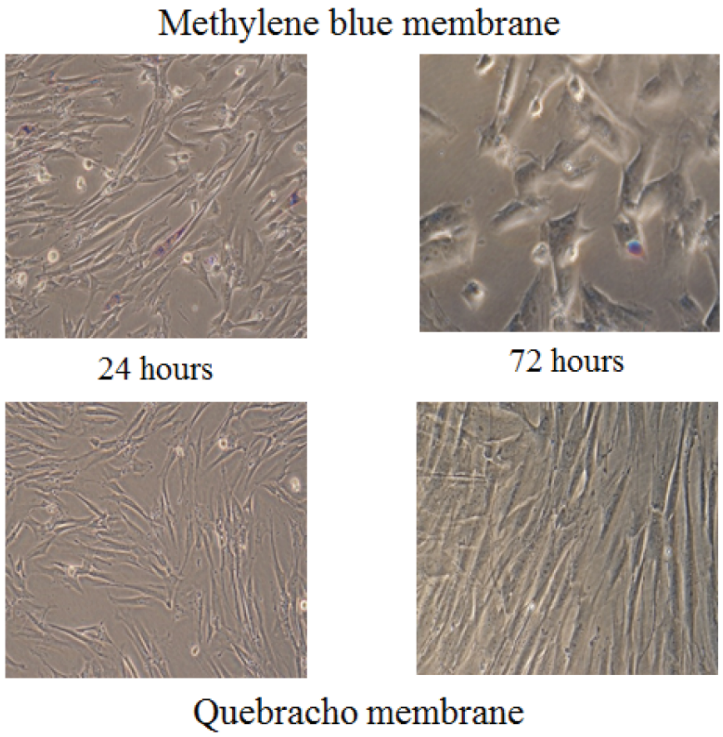

Figure 1.5 Microscopic analysis of human dermal fibroblast culture samples sowed on collagen/clay/bioactive substance membranes (Reproduced with permission from [92])

on human dermal fibroblast (HDF) cells. Figure 1.5. presents *in vitro* results after 24 and 72 hours, respectively. The cells were uniformly distributed and presented a normal phenotype after 24 h. However, quebracho membrane showed a 99% cell viability as compared to methylene blue membrane which after 72 h presented only 30% viable cells [92].

Water vapour adsorption was also tested for the same type of materials and compared to collagen membrane. Figure 1.6. shows the adsorption curves of the membranes for 48 hours. It was observed that the collagen membrane presented a continuous increasing variation as compared to nanocomposite membranes. The hybrids continuously adsorbed water vapours in the first 24 hours from exposure, then reached a plateau and the adsorption remained constant [92].

These findings showed that such type of materials especially the membrane with quebracho, which presented a biostimulating effect on the growth

and development of fibroblast cells, could be used as antiseptic and good regenerating patches [92].

Other collagen/layered silicate nanocomposites which contained gentamicine as active substance were obtained. *In vitro* biocompatibility test was performed on human dermal fibroblast cells and the results were compared to a collagen membrane as it can be seen in Figure 1.7. The layered silicate increased the biocompatibility of the materials; the cells presented a normal phenotype and proliferated. As compared to the collagen

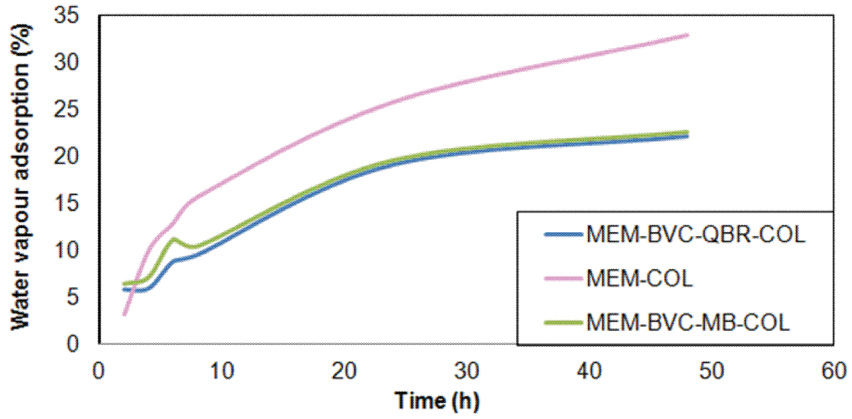

Figure 1.6 Water vapour adsorption for nanocomposite membranes with quebracho and methylene blue (Reproduced with permission from [92])

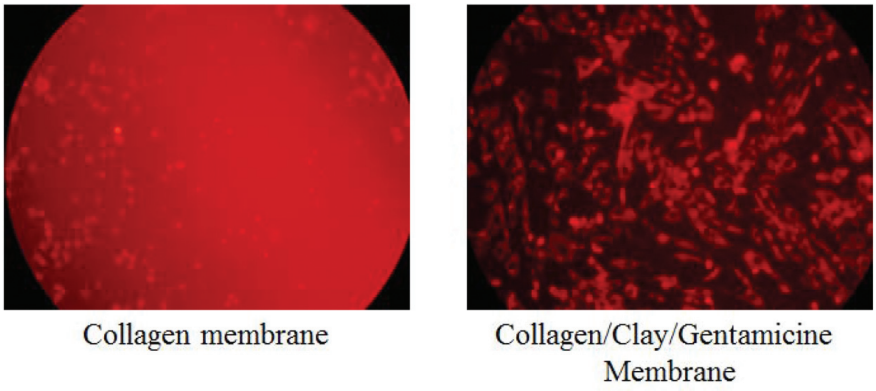

Figure 1.7 Microscopic analysis of human dermal fibroblast culture samples sowed on collagen/clay/gentamicine membrane (Reproduced with permission from [74])

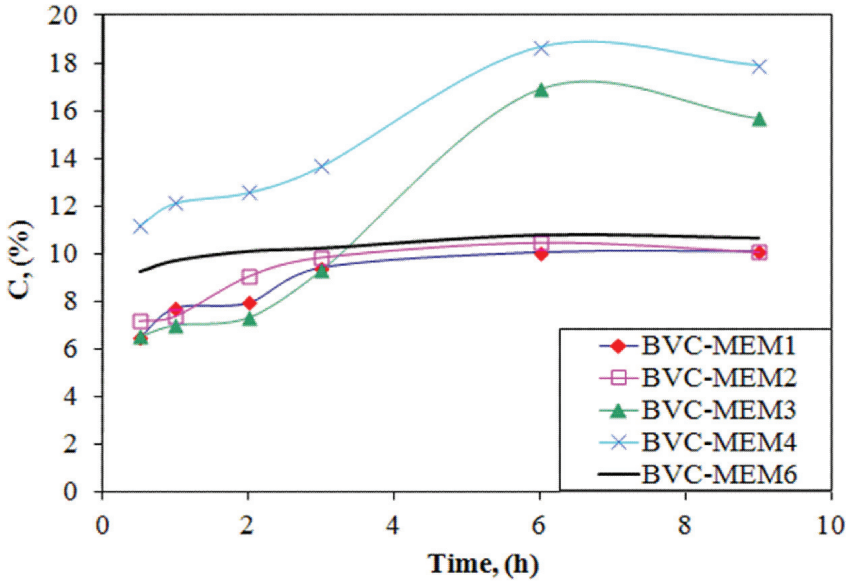

Figure 1.8 Gentamicin release concentration in time from collagen/layered silicate membranes (Reproduced with permission from [74])

membrane, the viability of the cells increased which showed that gentamicine had a positive effect on cells viability and diminished the cytotoxic effect.

In Figure 1.8. it is presented the drug release capacity of gentamicine from such membranes. This is evidence that these materials can act like drug delivery systems [75], moreover it shows that the drug release can be tailored by changing the order of introducing the components when mixing the materials [74].

1.6 Conclusions

Today, advances in tissue engineering are extending the possibility to treat and heal diseases that have put medicine in difficulty. To achieve a successful development in this filed, researchers from various disciplines like biology, medicine, material science, mechanical engineering a.s.o. have to cooperate. It has been showed that there is the possibility to tailor biomaterials so to obtain scaffolds with specific properties. Moreover, nanotechnology is accelerating the development of biomaterial-based systems with effective

individual approaches. However, from material science point of view to ensure a successful development of biomaterials, certain aspects need to be considered to realize their maximum potential:

- The raw materials must be selected from natural based resources so they exhibit good compatibility with the human body and must be as pure as possible to avoid inflammatory response.
- While processing the material, contamination with impurities, residual initiator or catalyst, which can affect the biocompatibility and biointegration of implant and additionally cytotoxic and carcinogenic effects, must be avoided.
- Materials must be selected such that cell adhesion and/or proliferation occur so the scaffolds perform as intended.
- Performance of scaffolds must be considered from mechanical point of view, so that the implant should resist to fatigue and cyclic loading as long as possible.
- Last but not least and probably most important, the final application of the scaffold needs to be considered starting from materials selection.

The successful development in this field lies in the complexity and understanding of how to rationally design biomaterials. Even though there is no ideal biomaterial that satisfies all the requirements needed for implants, the possibility to regenerate tissues and organs and even more to grow organs from stem cells should not be neglected. The possibility to modify surfaces and obtain tailored materials constitutes one of the major breakthroughs because it opens new horizons in this young field of study.

References

[1] G. Orive, E. Anitua, J. L. Pedraz, D. F. Emerich, 'Biomaterials for promoting brain protection, repair and regeneration', Nature Reviews, pp. 682–692, 10, 2009.

[2] W. Cao, L. L. Hench, 'Bioactive Materials', Ceramics International, pp. 493–507, 22, 1996.

[3] A. Tathe, M. Ghodke, A. P. Nikalje, 'A brief review: biomaterials and their apllication', International Journal of Pharmacy and Pharmaceutical Sciences, pp. 19–23, 2, 2010.

[4] A. Ovsianikov, B. Chichkov, O. Adunka, H. Pillsbury, A. Doraiswamy, R. J. Narayan, 'Rapid prototyping of ossicular replacement prostheses', Appl. Surf. Sci., pp. 6603–6607, 253, 2007.

[5] Y. He, M. Ye, C. Wang, 'A method in the design and fabrication of exact-fit customized implant based on sectional medical images and rapid prototyping technology', Int. J. Adv. Manuf. Technol., pp. 504–508, 28, 2006.
[6] T. Burg, C. A. P. Cass, R. Groff, M. Pepper, K. J. L. Burg, 'Building off-the-shelf tissue engineered composites', Phil. Trans. R. Soc. A, pp. 1839–1862, 368, 2010.
[7] L. E. Murr, S. M. Gaytan, F. Medina, H. Lopez, E. Martinez, B. I. Machado, D. H. Hernandez, L. Martinez, M. I. Lopez, R. B. Wicker, J. Bracke, 'Next-generation biomedical implants using additive manufacturing of complex, cellular and functional mesh arrays', Phil. Trans. R. Soc. A, pp. 1999–2032, 368, 2010.
[8] R. J. Narayan, 'The next generation of biomaterial development', Phil. Trans. R. Soc. A, pp. 1831–1837, 368, 2010.
[9] L. L. Hench, 'Biomaterials', Science, pp. 826–831, 208, 1980.
[10] L. L. Hench, J. Polak, 'Third generation biomedical materials', Science, pp. 1014–1017, 295, 2002.
[11] D. Hutmacher, M. B. Hurzeler, H. Schliephake, 'A review of material properties of biodegradable and bioresorbable polymer for GTR and GBR', J. Oral Maxillofac. Implants, pp. 667–678, 11, 2000.
[12] J. S. Temenoff, A. G. Mikos, 'Tissue engineering for regeneration of articular cartilage', Biomaterials, pp. 431–440, 21, 2000.
[13] C. M. Agrawal, R. B. Ray, 'Biodegradable polymeric scaffolds for musculoskeletal tissue engineering', J. Biomed. Mater. Res., pp. 141–150, 55, 2001.
[14] J. C. Fernyhough, J. J. Schimandle, M. C. Weigel, C. C. Edwards, A. M. Levine, 'Chronic donor site pain complicating bone graft harvest from the posterior iliac crest for spinal fusion', Spine, pp. 1474–1480, 17, 1992.
[15] J. C. Banwart, M. A. Asher, R. S. Hassanein, 'Iliac crest bone graft harvest donor site morbidity. A statistical evaluation', Spine, pp. 1055–1060, 20, 1995.
[16] J. A. Goulet, L. E. Senunas, G. L. DeSilva, M. L. V. H. Greengield, 'Autogenous iliac crest bone graft. Complications and functional assessment', Clin. Orthop., pp. 76–81, 339, 1997.
[17] P. Hardouin, K. Anselme, B. Flautre, F. Bianchi, G. Bascoulenguet, Bouxin, B. 'Tissue engineering and skeletal diseases', Joint Bone Spine, pp. 419–424, 67, 2000.

[18] M. Navarro, A. Michiardi, O. Castano, J. A. Planell, 'Biomaterials in orthopaedics', J. R. Soc. Interface, pp. 1137–1158, 5, 2008.
[19] L. G. Griffith, 'Emerging design principles in biomaterials and scaffolds for tissue engineering', Ann. NY Acad. Sci., pp. 83–95, 961, 2002.
[20] L. G. Griffith, M. A. Schwartz, 'Capturing complex 3D tissue physiology *in vitro*', Nat. Rev. Mol. Cell. Biol., pp. 211–224, 7, 2006.
[21] A. D. Metcalfe, M. W. J. Ferguson, Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration', J. R. Soc. Interface, pp. 413–437, 4, 2007.
[22] G. Chen, T. Ushida, T. Tateishi, 'Development of biodegradable porous scaffolds for tissue engineering', Materials Science and Engineering C, pp. 63–69, 17, 2001.
[23] R. Zange, Y. Li, T. Kissel, 'Biocompatibility testing of ABA triblock copolymers consisting of poly(L-lactic-co-glycolic acid) A blocks attached to a central poly(ethylene oxide) B block under *in vitro* conditions using different L929 mouse fibroblasts cell culture models', Journal of Controlled Release, pp. 249–258, 56, 1998.
[24] J. J. Grzesiak, M. D. Pierschbacher, M. F. Amodeo, T. I. Malaney, J. R. Glass, 'Enhancement of cell interactions with collagen/glycosaminoglycan matrices by RGD derivatization', Biomaterials, pp. 1625–1632, 18, 1997.
[25] S. Sofia, B. M. McCarthy, G. Gronowicz, D. L. Kaplan, 'Functionalized silk-based biomaterials for bone formation', J Biomed Mat Res, pp. 139–148, 54, 2001.
[26] P. J. VandeVord, H. W. T. Matthew, S. P. DeSilva, L. Mayton, B. Wu, P. H. Wooley, 'Evaluation of the biocompatibility of a chitosan scaffold in mice', J Biomed Mater Res, pp. 585–590, 59, 2002.
[27] A. G. A. Coombes, E. Verderio, B. Shaw, X. Li, M. Griffin, S. Downes, 'Biocomposites of non-crosslinked natural and synthetic polymers', Biomaterials, pp. 2113–2118, 23, 2002.
[28] J. A. Hubbell, 'Biomaterials in tissue engineering', Biotechnology, 565–576, 13, 1995.
[29] F. J. Schoen, R. J. Levy, 'Bioprosthetic heart valve failure: pathology and pathogenesis', Cardiol Clin, pp. 717–739, 2, 1984.
[30] J. Dong, Q. Sun, J.-Y. Wang, 'Basic study of corn protein, zein, as a biomaterial in tissue engineering, surface morphology and biocompatibility', Biomaterials, pp. 4691–4697, 25, 2004.

[31] K. A. Athanasiou, G. G. Niederauer, C. M. Agrawal, 'Sterilization, toxicity, biocompatibility and clinical applications of polylactic acid/ polyglycolic acid copolymers', Biomaterials, pp. 93–102, 17, 1996.
[32] L. G. Griffith, 'Polymeric biomaterials', Acta mater., pp. 263–277, 48, 2000.
[33] S. H. Teoh, 'Fatigue of biomaterials: a review', International Journal of Fatigue, pp. 825–837, 22, 2000.
[34] K. Magniez, 'Development of novel melt-spun nanocomposite fibers', Society of Plastics Engineers, pp. 1–3, 10.1002/spepro.003802, 2011.
[35] O. Solacolu, 'Physical chemistry of technical silicates', Second Edition, Technical Publishing, Bucharest, 1968.
[36] J. C. Hutchison, R. Bissessur, D. F. Shriver, 'Enhancement of ion mobility in alumino-silicate-polyphosphazene nanocomposites', Mat. Res. Soc. Symp. Proc., pp. 489–494, 457, 1997.
[37] R. A. Vaia, E. P. Giannelis, 'Lattice model of polymer melt intercalation in organically-modified layered silicates', Macromolecules, pp. 7990–7999, 30, 1997.
[38] B. K. G. Theng, 'Formation and properties of clay - polymer complexes', Elsevier Scientific Publishing Co., Amsterdam, Oxford, New York, 1979.
[39] R. L. D'Aquino, 'A little clay goes a long way', Chem. Eng. Mag., pp. 1–2, 106, 1999.
[40] G. J. Churchman, W. P. Gates, B. K. G. Theng G. Yuan, 'Clays and clay minerals for pollution control', Handbook of Clay Science, pp. 625–675, Elsevier, Oxford, UK, 2006.
[41] S. Nir, Y. El Nahhal, T. Undabeytia, G. Rytwo, T. Polubesova, Y. Mishael, U. Rabinovitz, B. Rubin, 'Clays and pesticides', Handbook of Clay Science, pp. 677–691, Elsevier, Oxford, UK, 2006.
[42] K. Czurda, 'Clay liners and waste disposal', Handbook of Clay Science, pp. 693–701, Elsevier, Oxford, UK, 2006.
[43] R. Pusch, 'Clays and nuclear waste management', Handbook of Clay Science, pp. 703–716, Elsevier, Oxford, UK, 2006.
[44] M. I. Carretero, C. S. F. Gomes, F. Tateo, 'Clays and human health', Handbook of Clay Science, pp. 717–741, Elsevier, Oxford, UK, 2006.
[45] M. T. Droy-Lefaix, F. Tateo, 'Clays and clay minerals as drugs', Handbook of Clay Science, pp. 743–752, Elsevier, Oxford, UK, 2006.
[46] M. Ross, R. P. Nolan, A. M. Langer, W. C. Cooper, 'Health effects of mineral dusts other than asbestos', Health Effects of Mineral Dusts.

Reviews in Mineralogy, pp. 361–409, 28, Mineralogical Society of America, Washington, DC, 1993.

[47] Y. C. Ke, P. Stroeve 'Polymer-layered silicate and silica nanocomposites', Elsevier B. V., 2005.

[48] K. A. Carrado, 'Synthetic clay minerals and purification of natural clays', Handbook of Clay Science, pp. 115–139, Elsevier, Oxford, UK, 2006.

[49] Z. Vuluga, G. C. Potarniche, M. G. Albu, V. Trandafir, D. Iordachescu, E. Vasile, 'Collagen - modified layered silicate biomaterials for regenerative medicine of bone tissue', Materials Science and Technology, pp. 125–148, InTech, 2012.

[50] C. Aguzzi, P. Cerezo, C. Viseras, C. Caramella, 'Use of clays as drug delivery systems: Possibilities and limitations', Applied Clay Science, pp. 22–36, 36 (1–3), 2007.

[51] Y. Chen, A. Zhou, B. Liu, J. Liang, 'Tramadol hydrochloride/ montmorillonite composite: Preparation and controlled drug release', Appl. Clay Sci., pp. 108–112, 49 (3), 2010.

[52] M. S. Lakshmi, M. Sriranjani, H. B. Bakrudeen, A. S. Kannan, A. B. Mandal, B. S. R. Reddy, 'Carvedilol/montmorillonite: Processing, characterization and release studies', Appl. Clay Sci., pp. 589–593, 48 (4), 2010.

[53] G. V. Joshi, B. D. Kevadiyaa, H. A. Patel, H. C. Bajaj, R. V. Jasrab, 'Montmorillonite as a drug delivery system: Intercalation and *in vitro* release of timolol maleate', Int. J. Pharm., pp. 53–57, 374, 2009.

[54] N. Meng, N. Zhou, S. Zhang, J. Shen, 'Controlled release and antibacterial activity chlorhexidine acetate (CA) intercalated in montmorillonite', Int. J. Pharm., pp. 45–49, 382 (1–2), 2009.

[55] J. K. Park, Y. B. Choy, J.-M. Oh, J. Y. Kima, S.-J. Hwanga, J. H. Choy, 'Controlled release of donepezil intercalated in smectite clays', Int. J. Pharm., pp. 198–204, 359, 2008.

[56] J. P. Zheng, L. Luan, H. Y. Wang, L. F. Xi, K. D. Yao, 'Study on ibuprofen/montmorillonite intercalation composites as drug release system', Appl. Clay Sci., pp. 297–301, 36, 2007.

[57] T. Kollár, I. Palinko, Z. Konya, I. Kiricsi, 'Intercalating amino acid guests into montmorillonite host', J. Mol. Struct., pp. 335–340, 651–653, 2003.

[58] F. H. Lin, Y. H. Lee, C. H. Jian, 'A study of purified montmorillonite intercalated with 5-fluorouracile as drug carrier', Biomaterials, pp. 1981–1987 23 (9), 2002.

[59] M. J. Sanchez, M. Sanchez-Camazano, M. T. Vicente, A. Dominguez-Gil, 'Physicochemical study of the adsorption of oxprenolol hydrochloride by montmorillonite', Drug Dev. Ind. Pharm., pp. 1019–1029, 9 (6), 1983.
[60] M. R. Harris, J. W. McGinity, 'Optimization of slowrelease tablet formulations containing montmorillonite. II. Factors affecting drug release', Drug Dev. Ind. Pharm., pp. 783–793, 8, 1982.
[61] M. R. Harris, J. W. McGinity, 'Optimization of slowrelease tablet formulations containing montmorillonite. III. Mechanism of release', Drug Dev. Ind. Pharm., pp. 795–809, 8, 1982.
[62] J. W. McGinity, J. A. Hill, 'Influence of monovalent and divalent electrolytes on sorption of neomycin sulfate to attapulgite and montmorillonite clays', J. Pharm. Sci., pp. 1566–1568, 64 (9), 1975.
[63] J. W. McGinity, J. L. Lach, 'Sustained-release applications of montmorillonite interaction with amphetamine sulphate', J. Pharm. Sci., pp. 63–66, 66 (1), 1977.
[64] J. W. McGinity, M. R. Harris, 'Optimization of slow release tablet formulations containing montmorillonite. I. Properties of tablets', Drug Dev. Ind. Pharm., pp. 399–410, 6 (4), 1980.
[65] M. Sánchez-Camazano, M. J. Sánchez Martin, M. T. Vicente, A. Dominguez-Gil, 'Adsorption of chlorpheniramine maleate by montmorillonite', Int. J. Pharm., pp. 243–251, 6, 1980.
[66] T. Takahashi, M. Yamaguchi, 'Host-guest interaction between swelling clay minerals and poorly water-soluble drugs: 1. Complex formation between a swelling clay mineral and griseofulvin', J. Incl. Phenom. Mol. Recognit. Chem., pp. 283–297, 10, 1991.
[67] T. Takahashi, M. Yamaguchi, 'Host-guest interaction between swelling clay minerals and poorly water-soluble drugs: II. Solubilization of griseofulvin by complex formation with a swelling clay mineral', J. Colloid Interface Sci., pp. 556–564, 146, 1991.
[68] M. Olteanu, D. Achimescu, Z. Vuluga and V. Trandafir, 'Measurement and interpretation of wetting properties of new collagen-silicate biomaterial', Revue Roumaine de Chimie, pp. 157–163, 53(2), 2008.
[69] A. Okada, A. Usuki, 'Twenty years of polymer-clay nanocomposites', Macromol. Mater. Eng., pp. 1449–1476, 291, 2006.
[70] R. A. Vaia, E. P. Gianellis, 'Polymer Nanocomposites: Status and Applications', MRS Bull., pp. 394–401, 62, 2001.
[71] H. D. Wagner, 'Nanocomposites-Paving the way to stronger materials', Nat. Nanotech., pp. 742–744, 2, 2007.

[72] M. G. Albu, I. Titorencu, M. V. Ghica, 'Collagen-based drug delivery systems for tissue engineering', Biomaterials Applications for Nanomedicine, pp. 125–148, InTech, 2011.
[73] Z. Vuluga, V. Danciu, V. Trandafir, C. Radovici, D. M. Vuluga, E. Vasile, S. Serban, C. G. Potarniche, 'Titania modified layered silicate for polymer/ inorganic nanocomposites', Mol. Cryst. Liq. Cryst., pp. 258–265, 483, 2008.
[74] J. deC. Christiansen, C.-G. Potarniche, Z. Vuluga, A. Drozdov, 'Nanomaterials in biomedical applications', 2nd International Conference on Wireless Comunications Vehicular Technology, Information Theory and Aerospace & Electronic Systems Technology". IEEE, Chennai, India, River Publishers, 2011.
[75] Zina Vuluga, Dan Donescu, Viorica Trandafir, Constantin Radovici et Sever Serban, 'L'influence de la Morphologie des Extraits de Collagene sur les Proprietes des Nanocomposites a Silicates Naturels', Revue Roumaine de Chimie, pp. 395–404, 52, 2007.
[76] C. Oriakhi, 'Nano sandwiches', Chem. Br., pp. 59–62, 34, 1998.
[77] Q. H. Zeng, A. B. Yu, G. Q. (Max) Lu, D. R. Paul, ''Clay-based polymer nanocomposites: research and commercial development', Journal of Nanoscience and Nanotechnology, pp. 1574–1592, 5, 2005.
[78] M. Alexandre, P. Dubois, 'Polymer-layered silicate nanocomposites: preparation, properties and uses of a new class of materials', Materials Science and Engineering, pp. 1–63, 28, 2000.
[79] Z. Vuluga, H. Paven, D. Donescu, 'Thermoplastic polymer layered silicate nanocomposites. I. Obtaining, properties and applications', Materiale Plastice, pp. 19–27, 39, 2002.
[80] R. A. Vaia, H. Ishii, E. P. Giannelis, 'Synthesis and properties of 2-dimensional nanostructures by direct intercalation of polymer melts in layered silicates', Chem. Mater., pp. 1694–1696, 5, 1993.
[81] C.-J. Wu, A. K. Gaharwar, P. J. Schexnailder, G. Schmidt, 'Development of Biomedical Polymer-Silicate Nanocomposites: A Materials Science Perspective', Materials, pp. 2986–3005, 3, 2010.
[82] L. E. Freed, J. C. Marquis, A. Nohria, J. Emmanual, A. C. Mikos, R. Langer, 'Neocartilage formation *in vitro* and *in vivo* using cells cultured on synthetic biodegradable polymers', J. Biomed. Mater. Res., pp. 11–23, 27, 1993.
[83] S. L. Ishaug, G. M. Crane, M. J. Miller, A. W. Yasko, M. J. Yaszemski, A. G. Mikos, 'Bone formation by three-dimensional stromal osteoblast

culture in biodegradable polymer scaffolds', J. Biomed. Mater. Res., pp. 17–28, 36, 1997.

[84] M. G. Dunn, J. B. Liesch, M. L. Tiku, J. P. Zawadsky, 'Development of fibroblast-seeded ligament analogs for ACL reconstruction', J. Biomed. Mater. Res., pp. 1363–1371, 29, 1995.

[85] G. Nechifor, S. I. Voicu, A. C. Nechifor, S. Garea, 'Nanostructure hybrid membrane polysulfone-carbon nanotubes for hemodyalisis', Desalination, pp. 342–348, 241, 2009.

[86] S. J. Peter, M. J. Miller, A. W. Yasko, M. J. Yaszemski, A. G. Mikos, 'Polymer concepts in tissue engineering', J. Biomed. Mater. Res., pp. 422–427, 43, 1998.

[87] L. E. Freed, G. Vunjak-Novakovic, R. J. Biron, D. B. Eagles, D. C. Lesnoy, S. K. Barlow, R. Langer, 'Biodegradable polymer scaffolds for tissue engineering', Bio/Technology, pp. 689–693, 12, 1994.

[88] B. S. Kim, D. J. Mooney, 'Development of biocompatible synthetic extracellular matrices for tissue engineering', TIBTECH, pp. 224–230, 16, 1998.

[89] L. H. H. Olde Damink, P. J. Dijkstra, M. J. A. van Luyn, P. B. van Wachem, P. Nieuwenhuis, J. Feijen, 'Glutaraldehyde as a crosslinking agent for collagen-based biomaterials', J. Mater. Sci., pp. 460–472, 6, 1995.

[90] N. Barbani, P. Giusti, L. Lazzeri, G. Polacco, G. Pizzirani, 'Bioartificial materials based on collagen: 1. Collagen cross-linking with gaseous glutaraldehyde', J. Biomater. Sci., pp. 461–469, 7, 1995.

[91] M. S. Chapekar, 'Tissue Engineering: Challenges and Opportunities', J Biomed Mater Res (Appl Biomater), pp. 617–620, 53, 2000.

[92] C.-G Potarniche, Z. Vuluga, C. Radovici, S. Serban, D. M. Vuluga, M. Ghiurea, V. Purcar, V. Trandafir, D. Iordachescu, M. G. Albu, 'Nanocomposites based on collagen and Na-montmorillonite modified with bioactive substances', Materiale Plastice, pp. 267–273, 47, 2010.