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COLLAGEN/HYDROXYAPATITE COMPOSITE MATERIALS: A REGENERATIVE PLATFORM VERSUS DRUG DELIVERY SYSTEM

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Abstract. The current review is intending to highlight the main advances in the field of collagen/hydroxyapatite (COLL/HA) composite materials as regenerative supports in bone tissue engineering but also as a platform for the delivery of different biological active agents with curative application. Basically, the review will highlight the most important synthesis routes as well as parameters able to tailor the characteristics of the COLL/HA composite materials as well as the most important achievements in the field of drug delivery systems based on COLL/HA using various classes of biologically active agents with different activity.

Keywords: COLL/HA composite grafts; drug delivery systems; material processing and design; bone cancer.

MATERIALE COMPOZITE COLAGEN / HIDROXIAPATITĂ: O PLATFORMĂ REGENERATIVĂ VERSUS SISTEM DE LIVRARE A MEDICAMENTELOR

Rezumat. Lucrarea de faţă evidenţiază principalele progrese în domeniul materialelor compozite pe bază de colagen si hidroxiapatită (COLL / HA) ca suporturi regenerative în ingineria ţesuturilor osoase, dar şi ca platformă pentru livrarea diferiţilor agenţi biologic activi cu aplicare curativă. Practic, lucrarea evidenţiază cele mai importante rute de sinteză, precum şi parametrii capabili să inducă caracteristicile materialelor compozite COLL / HA, precum şi cele mai importante realizări în domeniul sistemelor de administrare a medicamentelor bazate pe COLL / HA utilizând diverse clase de agenţi biologic activi cu activitate diferită.

Cuvinte cheie: materiale compozite de tipul COLL/HA; sisteme cu eliberare controlata; proiectarea si procesarea materialelor; cancer osos

1. Introduction

Collagen/Hydroxyapatite composite materials are extensively studied in the literature because of the high similarity with the bone, this being an remarkable composite materials based on hydroxyapatite reinforced with mineralised collagen fibers [1]. Starting from the compositional similitude, COLL/HA composite materials became of high interest in pure regeneration but also in drug delivery [2-5]. From regenerative point of view there are a lot of manuscripts highlighting the influence of different ternary components, including ions, biomacromolecules (PVA, Alginate, Chitosan, hyaluronic acid, etc.), vitamins but even cells or bone morphogenic factors, etc. Certainly, an important advance in bone grafting is assured by the use of modern, additive manufacturing methods such as 3D printing.

COLL/HA composite materials in tissue regeneration

According to the literature, the development of the bone grafting materials involves 4 ages, as presented in Fig 1. It is worth to mention that, step by step, some properties and performances are improved, the most important improved properties being the biological properties. Based on the paper published by Murugan and Ramakrishna [6] four generations of materials for bone grafting were identified, the compositional changes being less, and less important over the time. It can see that the first generation of materials are exclusively not bioresorbable and nor bioactive (the representatives of this generation being metals and alloys: Stainless Steel, Titanium Alloys, Co-Cr Alloys, etc.) which, even can lead to inflammation because of their corrosion. The second generation are bioactive or bioresorbable being represented by ceramics (Calcium Phosphates, Bioglasses, etc.) or Polymers (collagen, hyaluronic acid, chitosan, …) which, even better tolerated by the body, unfortunately have some lower mechanical properties comparing to metals and this is why, in the clinic, alloys are still using even if revisions are required in less than 10-15 years. The third generation of materials for bone grafting are both bioactive and resorbable and are trying to combine the previous materials and to keep their advantages and limit their shortcomings. In fact, we are talking about (nano)composite materials such as collagen/hydroxyapatite – COLL/HA, polylactic acid/hydroxyapatite - PLA/HA; chitosan/hydroxyapatite - CS/HA, etc. the fourth generation of bone grafting materials are also based on nanocomposites but, these are loaded with biological active molecules or even cells and these materials can be considered biomimetic materials with higher biological properties (osteoinduction, osteoconduction as well as osteointegration, etc.).

Starting from pure COLL/HA composite materials, it can observe that the processing conditions are important and can drastically change the morphology of the composite materials. One of the most important factors affecting final characteristics is related to the initial precursors [7-9].

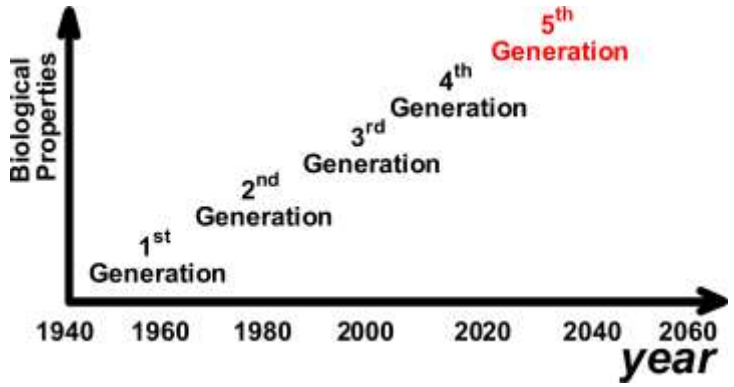

Figure 1. Evolution of the bone graft materials

For instance, Figure 2 is highlighting the morphology of some COLL/HA composite materials obtained by starting form collagen gels, matrices or fibres.

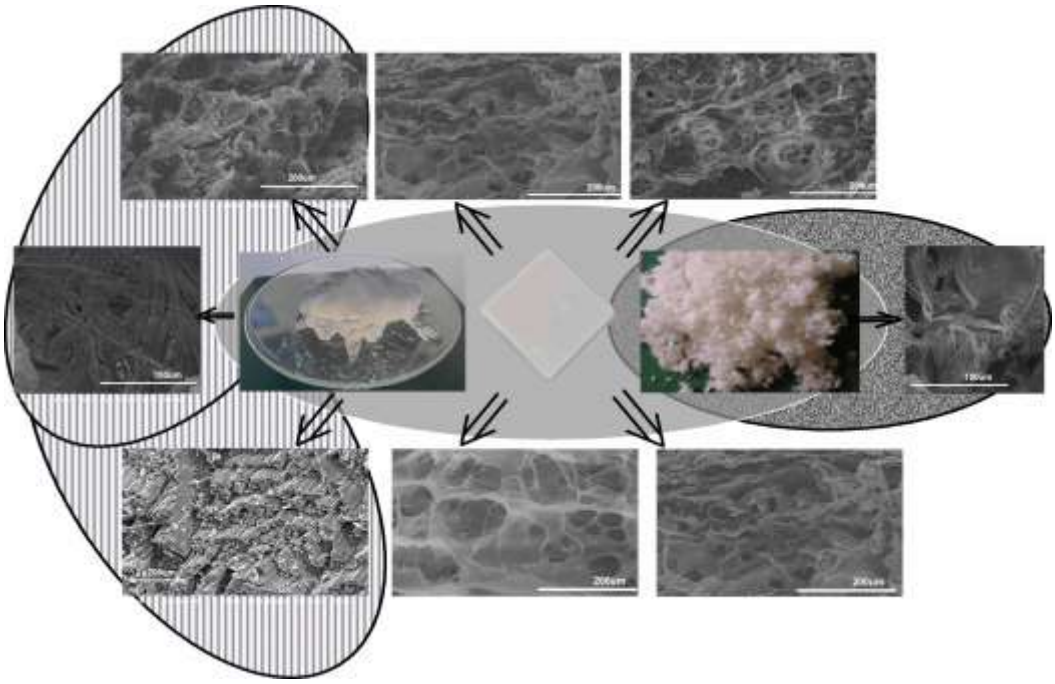

Figure 2. The morphology of the COLL/HA composite materials

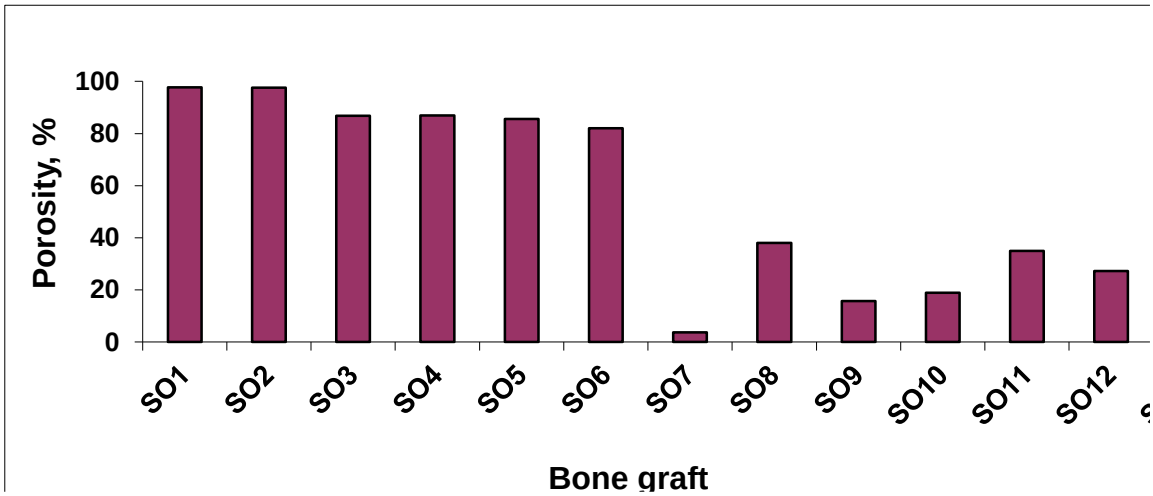

Figure 3. Porosity of the COLL/HA composite materials

It can conclude that dense or porous materials can be obtained, while the fibers can be in an oriented or un-oriented morphology. The porosity of the samples obtained from

collagen gel (SO7 – SO13) is ranging between 3.7 and 63% being a very good correspondence between the microscopic data and the porosity. Starting from matrices, usually the resulted composite materials are denser than the matrices (which are very porous) and thus their porosity is moderate to high and do not change the orientation of the fibres orientation. In Figure 3, samples SO3-6 correspond to the mineralised COLL/HA bone grafts derived from collagen matrices and have porosity of about 70 – 80% while the initial matrices had a porosity of ~96%.

Starting from these pure, COLL/HA composite materials, the performances of the materials can also be tailored by adding additional/third components as doping agents for HA or additional polymers making these ternary composite materials very attractive. In fact, we have to accept that the remarkable properties of the bone are also influenced by the minor components.

COLL/HA composite materials were also obtained by additive manufacturing, by depositing on the surfaces of the metallic implants (by using MAPLE deposition technique) [2] or by modelling the morphology by 3D printing [4].

COLL/HA composite materials in drug delivery

Drug delivery is exploited in many cases being very good solution to deliver the biological active agent directly to the desired area. Some of the most common diseases are: cancer, bone infections or osteoporosis and in all these diseases, the use of drug delivery systems can induce healing (cancer, infections) or amelioration (especially osteoporosis) of the effects of the diseases. So, Table 2 present some of the COLL/HA based systems suitable for bone cancer treatment. Some of the most relevant materials with potential applications in bone cancer treatment are presented in Table 2. Most of the compositions presented were already published in our group but still some compositions are of interest in order to develop new and improved platforms for cancer treatment, combining some different mechanisms of action: chemotherapeutic agents (such as any classic cytostatic); hyperthermia (induced by the presence of magnetite) and phototherapy (induced by the presence of silver nanoparticles). Pain management can be also very important and this is why the platforms containing analgesic agents should be very important.

Table 1. Evolution of the materials in bone grafting

Generation	Representatives	Characteristics	References
1st generation	**Metals**: titanium **Alloys**: stainless Steel; Titanium Alloy; Co-Cr Alloy;	These materials present very good short-term mechanical properties, can be easily implanted and the fixation is very good but they are not bioresorbable nor bioactive and in time, corrosion appear and can lead severe inflammation and even rejection. The revision should be made, usually within 1 year but for long-term implants, the time should not exceed 10-15 years.	[6, 10, 11]
2nd generation	**Ceramics**: Calcium Phosphates (hydroxyapatite, tricalcium phosphates, bioglasses, etc.); **Polymers**: collagen, gelatine, chitosan, alginate, polylactic acid, polyethylene glycol, polyethylene, etc.	These materials are bioactive or bioresorbable but usually cannot be used alone when the implants are exposed to high mechanical loadings and thus, even nowadays, metals and alloys are still used. Usually, these materials are used in filling defects and the results are very good and will be resorbed or coated by natural materials without the need of extraction. Ceramics are usually brittle while polymers have limited mechanical performances.	[6, 12-16]
3rd generation	**Composite** and **nanocomposite materials**: COLL/HA; Gelatine/HA; etc.	Composite materials are bioactive and bioresorbable. These materials are similar with the natural bone. The main advantage of the composite materials is related to the possibility of combining the advantages of the components and avoiding their shortcomings. COLL/HA composite materials, for instance, is a composite material, compositionally similar to the bone tissue. Even in this case, the composite materials are suitable for applications which are not requesting hard loading and, if necessary, metallic devices are used to immobilize them until the integration is good enough and the implant can take over the mechanical load.	[6, 17-20]
4th generation	**Tissue Engineered Materials = Biomimetic Materials**: nanocomposites loaded with biological active molecules such as bone morphogenetic proteins, interleukins, etc. but also cells.	From compositional point of with, the representatives of the 4th generation are similar with those of 3rd generation but, some biological components are loaded inside, this means that biomimetic materials are designed. So, the above mentioned materials are especially loaded with specific bone cells, but also bone morphogenetic proteins, interleukins, etc. can be exploited.	[6, 21, 22]
5th generation	**Grafts obtained by materials design**: all the above mentioned classes	Material design is extensively exploited in developing materials for medical applications because it was proved that the morphology can induce new and improved properties and performances. The roughness is well known to be responsible, for a wide range of properties, several times drastically changing the properties. One such example is the superhydrophobic surfaces of the wings of butterflies, the surfaces of several leaves (such as lotus leaves, for instance), etc. In bone grafting, the morphology can be exploited in modifying the surface and the bulk characteristics which allow a better attachment – faster osteointegration if only the surface characteristics are obtained by materials design while, if the entire material is developed by materials design, cells can penetrate deep inside the material and in this case, allow an in depth integration, resorption and new bone formation!	[4, 23-31]

Table 2. COLL/HA based composite materials with potential applications in cancer treatment

Sample	Main characteristics
COLL/HA-Ag	Composite material based on collagen, hydroxyapatite and Ag-NPs with regenerative and antiseptic/ antitumoral role. Besides the native antitumoral activity of Ag-NPs, silver based materials are promising for cancer therapy due to the intrinsic but especially due to the antitumoral activity induced by phototherapy [32].
COLL/HA-CisPt	COLL/HA composite materials loaded with cisplatin are suitable for the cancer treatment and, after the cisplatin release, the composite material can assist in regeneration. After implantation, the cisplatin is released according to a profile dependent on several factors, depending on the COLL/HA characteristics but also die to the environmental conditions. The most important advantage of the system is related to the loco-regional delivery and thus lower systemic toxicity is observed because cisplatin is les in contact with healthy tissues/organs. Due to the "targeted delivery" of the biological active agent, the need of cisplatin is lower because a better administration (les cisplatin is lost in healthy tissues/organs) [33].
COLL/HA-Fe_3O_4	The ternary composite material based on collagen, hydroxyapatite and loaded with magnetite nanoparticles can assure both regenerative but also antitumoral activity. The antitumoral activity can be induced by exposing the ternary COLL/HA-Fe_3O_4 composites to alternative electromagnetic fields because of the hysteresis loop of the magnetite. Certainly, the produced hyperthermia is dependent on the content of magnetite and this is important in assuring personalized therapy [34].
COLL/HA-Fe_3O_4-Ag	The addition of the AgNPs is beneficial because of the additional, synergic antitumoral mechanism. Along with the intrinsic antitumoral and antimicrobial activity, AgNPs can be exploited also due to their photothermic behavior. This system combines the advantages of the above mentioned ternary systems, namely COLL/HA-CisPt and COLL/HA-Fe_3O_4 multifunctional systems.
COLL/HA-Fe_3O_4-CisPt (LbL)	The ternary composite COLL/HA-Fe_3O_4 can be loaded with cytostatics combining the antitumoral activity of magnetite (hyperthermia) and cytostatic activity of the chemotherapeutic drugs. It is also very important to mention that the overall antitumoral activity is more than cumulative because, once the hyperthermia is applied, the release rate is enhanced and thus, the antitumoral activity is enhanced too. Based on this synergy, the external control of the drug release and thus the external control of the antitumoral activity, these systems can be considered Smart Drug Delivery Systems. The overall antitumoral activity is given by the two components (chemotherapy and hyperthermia). The contribution of the chemotherapy is decreasing in time, because of the release of the cytostatic, but the hyperthermia can be activated for a longer period of time (certainly the used magnetite should be stabilized before) and, in this case, even at medium and long-term the antitumoral activity can be present and thus the remnant/residual tumoral cells are destroyed avoiding the recurrences [35].
COLL/HA-Fe_3O_4-Ag-CisPt	This complex system has regenerative, antiseptic and antitumoral activity. This system maintains all the properties of the COLL/HA-Fe_3O_4-CisPt multifunctional system and is even enhanced because of an additional antitumoral activity induced by the presence of AgNPs. The recurrences will be stronger limited because of the presence of silver nanoparticles.

COLL/HA-AT Ag-other components	AT Ag: antitumoral agent(s) – can be any antitumoral agent (nanoparticles for inducing hyperthermia by hysteresis loop or phototherapy); cytostatics, … Other components can be active components for pain management.

4. Conclusion

COLL/HA composite materials can be exploited in regeneration but also in the treatment of different bone-related diseases such as cancer, infections or osteoporosis. The characteristics of the COLL/HA composite materials are essential for the osteointegration and bone healing but also in the delivery of the biological active agents. According to the presented materials used in bone cancer treatment, new compositions were identified and could bring some important improvements in both the curative but also in the palliative part of the theranostics.

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