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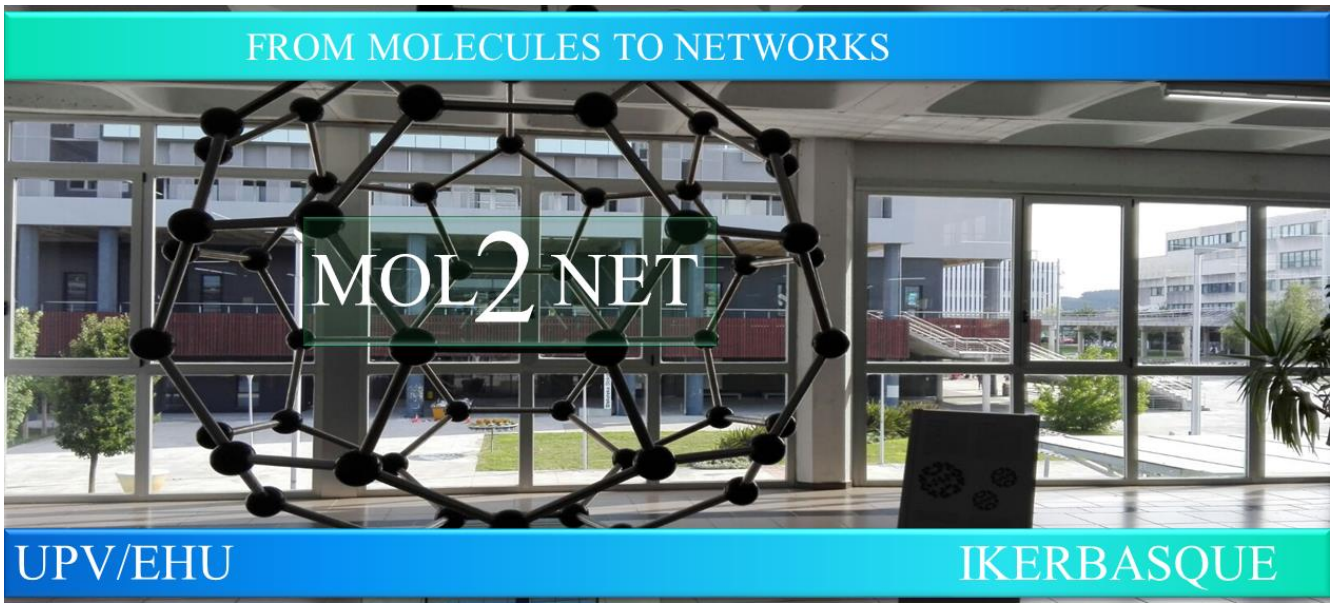

Liquid crystal gel based on sensitive pH and temperature composites

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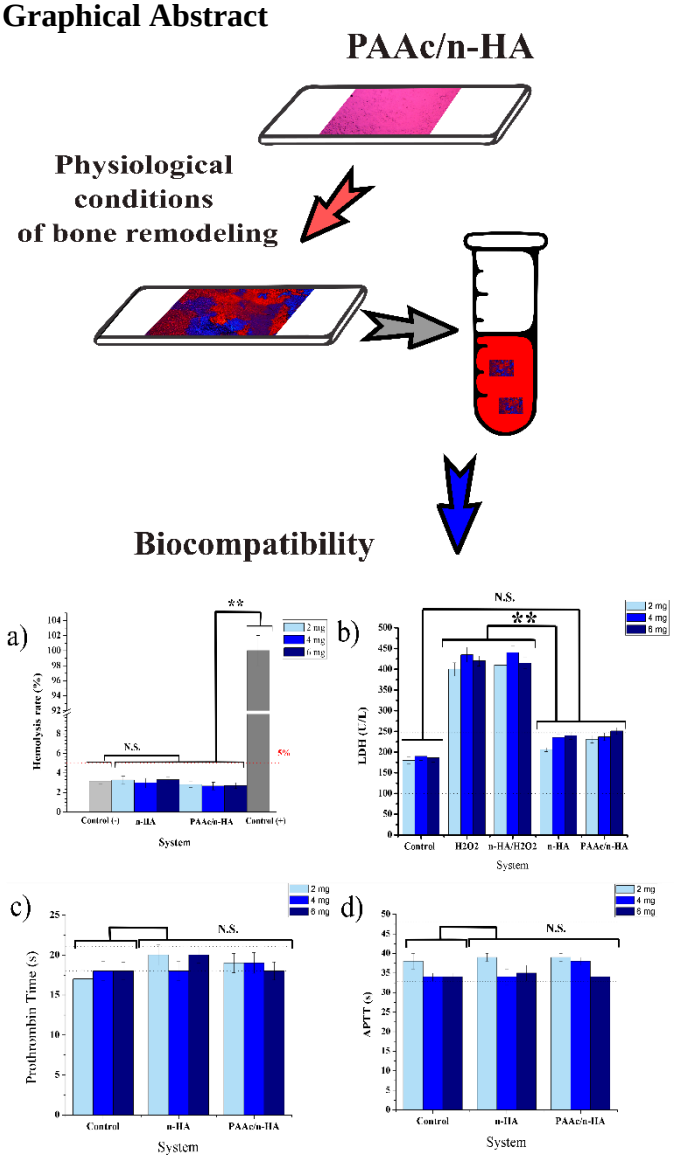
Graphical Abstract



Abstract.
In this work, a liquid crystal system as a potential bone-repairing booster has been induced from a polyacrylic acid (PAAc) gel loaded with synthetic hydroxyapatite nanoparticles (nHA) under physiological conditions of pH and temperature that resemble the early stages of bone formation. Dynamic processes at the microscale in biological systems require a material capable of behaving following a specific order and fluency. Besides, they must fulfill conditions such as biocompatibility. The occurrence of mesophases at different environments of pH and temperature has been shown by an optical microscope with crossed polaroids and retardation plate. The biocompatibility has been demonstrated by hemolysis analysis and coagulation assays. The results have shown a biocompatible system with excellent adaptation properties under physiological in vitro conditions.

Introduction

The self-assembly and auto-organization of structures suitable to replicate and replace those belonging to living systems in case of disease or miss function tissue states are still a subject of study[1]. Based on this prompt, composed materials formed by a biocompatible polymer, PAAc, and a homemade biocompatible inorganic material, n-HA[2], have been designed. These materials are configured as a tridimensional arrangement (liquid crystal) as a compatible structure with bone under physiological conditions of bone remodeling (pH and temperature)[3]. The formation of this mesophase has been shown by optical microscopy with crossed polaroids (OM). Their biocompatibility in vitro, with elements of entire blood, was demonstrated by hemolysis percentage assay (%HR) and lactate dehydrogenase (LDH) assay. Their influence on coagulation time was studied by activated partial thromboplastin time (APTT) and prothrombin time (PT) tests. The knowledge of the affectation of these properties is essential in the first application stage of materials for bone remodeling because coagulation influences bone healing[4]. Accordingly, under specific conditions, it is possible to obtain lyotropic liquid crystals that provide nucleation points for the growth of calcinated tissues behaving as

environmental-sensitive bone-repairing boosters, and they could be used as fillers or membranes for guided bone tissue regeneration.

Results and Discussion

1. Optical microscopy with crossed polaroids. Lyotropic liquid crystals formation

In **figures 1a** and **1b**, no birefringence is shown. In both cases, systems are formed only by the PAAc without n-HA at 37°C controlled by the hot plate. In **figure 1b**, the acetate buffer (ACE)(pH=4.2) was added to simulate the bone remodeling environment. In **figure 1c** no refringence is shown for the PAAc/n-HA system at 37°C. Birefringence is shown starting from **figure 1d** (23°C) to **1i** (37°C) for the PAAc/n-HA system with ACE added. The mesophase appears when the temperature is increased. For that reason, for the appearance of these arrangements, the system must be composed of PAAc/n-HA/water (ratio 3/6/10-5) under bone remodeling conditions (pH=4.2 and 37°C temperature). This assay demonstrates the potential behavior of the PAAc/n-HA system as bone-filling material because these tridimensional arrangements simulate those of the bone tissue that can generate calcium deposits from the negative nucleation points of the carboxylic groups[5].

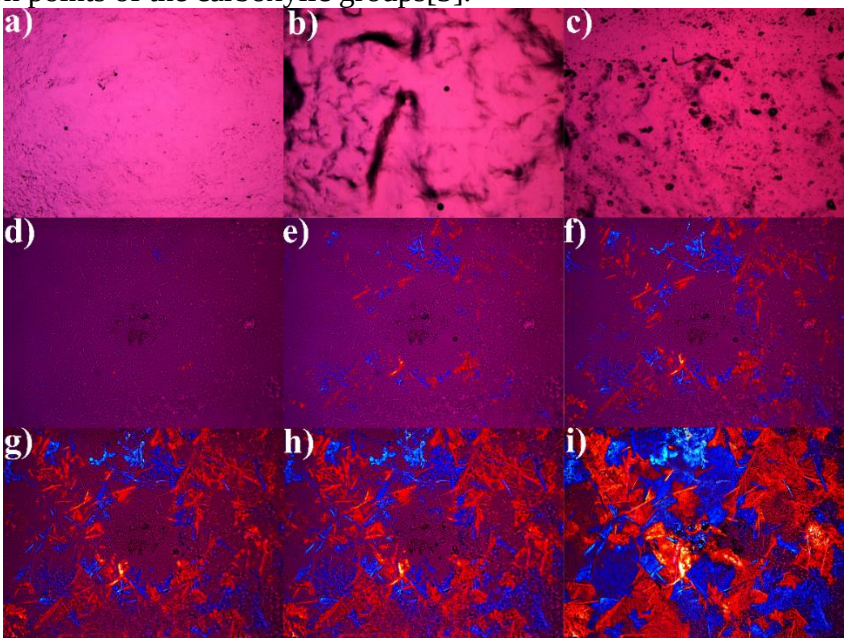


Figure 1: Liquid crystals formation by variation pH and temperature conditions. Optical microscopy with crossed polaroids micropictures **1a.** PAAc at 37°C without n-HA and **1b.** PAAc at 37°C without n-HA with ACE. **1c.** Composite material PAAc/n-HA at 37°C without ACE. Form **1d.** to **1i.** Composite material PAAc/n-HA with ACE. **1d.** at 23.4 °C. The temperature is increased to 37°C in figure **1i**.

2- Biocompatibility of composite materials by interaction with entire blood

The biocompatibility has been studied by the %HR, quantification of released LDH enzyme from red blood cells, and coagulation from PT and APTT tests.

Absorbance peaks at 414, 541, and 576 nm indicate free hemoglobin, with the highest peak at 414 nm[6]. Measures were obtained by a UV–vis-NIR scanning spectrophotometer. The higher the absorbance in plasma samples, the higher the degree of the hemolysis. Less than 5% of release is considered as safe level for hemostatic materials. **Figure 2a** shows the release of hemoglobin after incubating n-HA and composite materials with entire blood (%HR). Negative control (-) is incubated with no materials inside and (+) with H_2O_2. The obtained %HR is similar among samples and control (-) with non-significative differences (N.S.) but with $p < 0.05$ compared with control (+). This assay

confirms that materials did not increase the rupture of the red blood cells. This is comparable with other works applying PAAc as tissue regeneration[7].

The integrity of red blood cell membranes was corroborated by the LDH test[8]. The typical value range of LDH using this kit is 100-240 U/L. First, the optimal incubation conditions were established. F**igure 2b** represents the determination of LDH after 24 h incubation time. The **figure** shows that the presence of materials (n-HA or composites, PAAc/n-HA) slightly increases the release of the LDH enzyme but inside the range of the validation. For this reason, it is essential to compare the results with positive and negative controls. Increased concentration of materials increases the release of LDH among the normal range.

The PT test is the most relevant clinical screening test for disorders evaluation of the extrinsic pathway of coagulation. Its sensitivity to qualitative and quantitative alterations of extrinsic and common pathway factors allows it to be used for detecting simple or combined deficiencies of coagulation factors. It is used in pre-surgical studies, for the specific determination of the activity of factors: II, V, VII, and X and for monitoring therapy with oral anticoagulants, due to their sensitivity to vitamin K-dependent factors (II, VII, and X). This assay demonstrates that neither nanoparticles nor materials in all experiments show influences on PT. Reference typical values for this test ranged from 17 to 21 s[9]. The results of this test are shown in **figure 2c.**

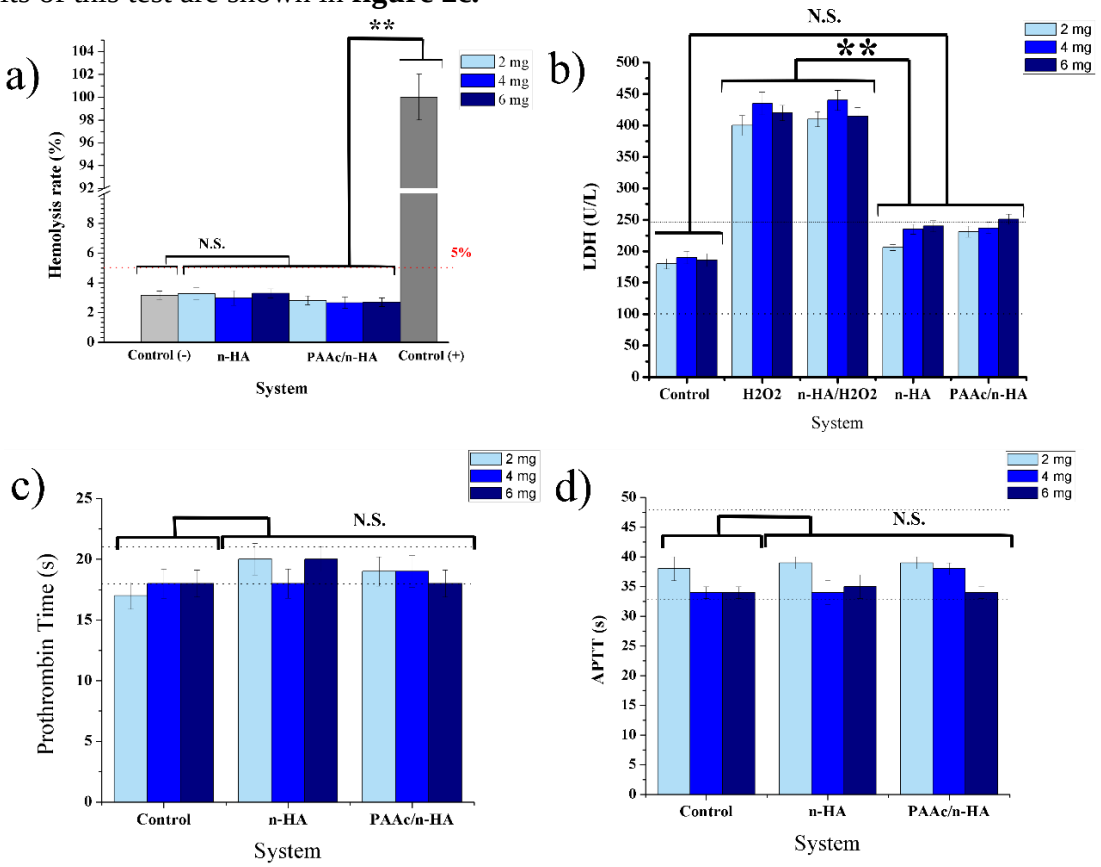


Figure 2: Biocompatibility by red blood cells interaction. **2a.** Hemolysis rate, 5% is shown as limit of acceptability. ** significant difference p< 0.05. **2b:** Determination of LDH concentration (cell membrane integrity assay). Horizontal dotted lines indicate the interval of reference for the determination of the concentration of this enzyme. N.S. non-significant difference p > 0.05. ** significant difference p < 0.05. **2c and 2d.** Coagulation assays. Prothrombin Time test and Activated Partial Thromboplastin Time test, respectively. Horizontal dotted lines indicate the interval of reference for determining these parameters.

The APTT is a sensitive test applied to study the deficiency of procoagulant factors in plasma, as well as the presence of specific coagulation inhibitors. This test is used for abnormalities detection in the intrinsic pathway of coagulation, such as the factors necessary for the prothrombin intrinsic activators

formation, that is, factors VIII, IX, XI, and XII. It also detects severe deficiencies of factors II, V, X, and fibrinogen, but this is not the case with platelet disorders, deficiencies of factors VII and XIII, or vascular problems. Its speed, simplicity, and reproducibility are very suitable for controlling heparin levels in anticoagulant therapy. The APTT allows the establishment of pre-surgical preventive treatments and avoids hemorrhagic problems in plasma[9,10]. This assay demonstrates that neither nanoparticles nor materials influence the coagulation time in all experiments. Such as the APTT and PT tests are carried out with serum, and it is not affected by particles or materials in all cases. The serum is not affected by particles or materials in all expanses. Up to this point, nanoparticles and systems based on them in three assayed concentrations have not generated hemorrhagic events; therefore, they could be applied as filling materials. The results of this test are shown in **figure 2d**.

Conclusions

We have developed composite materials for potential application as bone fillers based on synthetic hydroxyapatite, a biomimetic ceramic, and PAAc, a biocompatible polymer. These materials are organized by forming a three-dimensional arrangement compatible with a state resembling a previous structure of the bone formation, which is then mineralized. This structure is liquid crystal and is acquired when the material is subjected to variations in pH typical of the bone remodeling process and body temperature. Furthermore, these materials have demonstrated excellent hemocompatibility through tests of %hemolysis rate, LDH measurements, PT, and APTT. Further studies are necessary for their application; thus, we must perform in vivo trials to deepen our knowledge about these materials.

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