Multifunctional carrier based on Halloysite/Laponite hybrid hydrogel for kartogenin delivery

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ABSTRACT: A novel carrier system based on halloysite nanotubes (HNT), for the potential intraarticular delivery of kartogenin (KGN) by means laponite (Lap) hydrogel (HNT/KGN/Lap), is developed. The drug was firstly loaded into HNT and the hybrid composite obtained was used as filler for laponite hydrogel. Both the filler and the hydrogel were thoroughly investigated by several techniques and the hydrogel morphology was imaged by transmission electron microscopy. Furthermore, the gelating ability of laponite in the presence of the filler and the rheological properties of the hybrid hydrogel were also investigated. The kinetic release of kartogenin from HNT and HNT/Lap hybrid hydrogel was studied both in physiological conditions and in *ex-vivo* synovial fluid. In the last case, the kinetic results highlighted that HNT carrier can effectively release KGN in a sustained manner for at least 38 days. Finally, a preliminary biological assays showed that the HNT/KGN/Lap hybrid hydrogel did not exhibit any cytotoxic effect.

Kartogenin (KGN) is a CBFβ-RUNX1 pathway activator and has recently emerged as a potential osteoarthritis disease therapeutic.[1]

Osteoarthritis (OA) is a type of joint disease that results from breakdown of joint cartilage degradation, which is often associated with pain and functional limitations.

KGN promotes the selective differentiation of multipotent mesenchymal stem cells into chondrocytes thus stimulating the repair of damaged cartilage. Unfortunately, like most of organic molecules with biological properties, kartogenin is hydrophobic and possesses a short-term stability in an aqueous medium; therefore, its use for pharmacological applications is limited. Due to the localized nature of the disease, intraarticular (IA) drug injection[2-3] is an attractive therapeutic treatment approach. Therefore, an efficient treatment for osteoarthritis can benefit from the IA local release of a high KGN therapeutic dose over an extended period of time. Such a treatment will avoid the inconvenience of frequent injections and drastically reduce systemic side effects.[4-5] Several slow release KGN systems[6-8] are currently under investigation in order to develop a potential new local therapeutic approach to improve life quality of OA patients.

Halloysite nanotubes (HNTs) may represent an excellent slow release drug delivery platform for IA KGN injection. HNTs are an aluminosilicate clay with a predominantly hollow tubular structure and chemically similar to the platy kaolinite ($Al_2Si_2O_5(OH)_4$). Generally, the inner and outer diameters of the tubes are in the ranges of 10–30 nm and 40–70 nm, respectively, while their length is in the range of 0.2–1.5 μm. HNTs, naturally occurring in considerable amounts at low cost, show good bio-[9] and eco-compatibility.[10] Halloysite possesses different charged surfaces: positive in the inner lumen, where mostly of aluminum hydroxide are present; negative in the external one, which consists in silicon dioxide. Due to the different chemical composition, halloysite nanotubes can be selectively functionalized at the inner and/or outer surfaces leading to the synthesis of several interesting nanomaterials.[11-12] HNTs are widely used as drug carrier[13-15] and delivery,[16] pollutant removal,[17-19] catalyst[20] and so on.

In the last years, hydrogel systems have gathered interest, since they can act both as drug carrier and they can self-organize *in situ* to allow minimally invasive delivery of stem-cells and/or growth factors, overcoming the clinical drawbacks of cell based engineered tissue such as invasive surgery, inflammation, and subsequent infection. Among the different gel systems,[21-22] the clay ones are particularly attracting since they possess appealing properties.[23] Laponite (Lap) is a synthetic clay mineral, from smectite group, which possesses the peculiarity to form, in an aqueous regime, thixotropic hydrogels that can be injected by 21G needles and therefore, they could be

useful for the delivery and subsequent application of kartogenin in the tissue regeneration.[23]

Herein, we report a carrier system based on halloysite nanotubes for the potential intraarticular delivery of KGN by means laponite hydrogel (HNT/KGN/Lap).

In particular, we realized an alternative approach for KGN synthesis,[24] by means of C-C cross coupling reaction mediated by Pd nanoparticles supported on *ad hoc* modified HNT, and its delivery by HNT/Lap hybrid hydrogels. Therefore, in this context, we studied for the first time, both the gelating ability of laponite in the presence of halloysite in different aqueous solutions (H_2O, PBS (1x) and phosphate buffer at pH 7.4) and the physico-chemical properties of the HNT/Lap hybrid hydrogels. Specifically, the mechanical properties of the hydrogels were examined by rheology and their self-repair abilities were analyzed by subjecting them to ultrasound and magnetic stirring. The morphology of the hydrogels was investigated by transmission electron microscopy (TEM). Furthermore, the changes in the hydrodynamic radii and in the colloidal stability were investigated in terms of translational diffusion from Dynamic Light Scattering (DLS) and ζ-potential measurements.

The kinetic *in vitro* release of KGN, from both HNT/KGN composite and HNT/KGN/Lap hybrid hydrogels, was studied in phosphate buffer solution at pH 7.4 as well as in *ex-vivo* synovial fluid.

Finally, a preliminary *in vitro* cytocompatibility and dose-response of the HNT/Lap and HNT/KGN/Lap hybrid hydrogels was studied by MTS assay choosing a model cell lines to evaluate the toxicological effects of the new hybrid hydrogel.

Results and Discussion. The kartogenin was synthetized according to the synthetic route shown in Scheme S.1. Firstly, the 1,1'–biphenyl-4-amine (**1**) was synthetized by a Suzuki cross-coupling mediated by palladium nanoparticles supported on HNT, under microwave irradiation. The advantage to use Pd supported on HNT relies in the possibility to work in heterogeneous conditions, with an irradiation time of only 10 min compared with 12 h of the traditional synthesis, and noticeably without loss in the catalytic activity even after 10 cycles.[20] In this way, compound **1** was obtained in quantitative manner and, more importantly, without metal contamination, as estimated by ICP-OES measurements, according to government regulations for parenteral drug administration.[20] Compound **1** was reacted with phthalic anhydride to finally obtain the kartogenin molecule.[24]

The loading of KGN into halloysite was carried out by mixing halloysite with a highly concentrated kartogenin solution. Then, the obtained suspension was stirred and maintained under vacuum for 3 to 5 min, resulting in light fizzling, which indicated that air was being removed from the tubes.[25]

Once the vacuum was removed, the solution entered the lumen and the loaded compound condensed within the tubes. This procedure was repeated 2 to 3 times to improve the loading efficiency. After loading, the HNT/KGN complex was washed with water in order to remove free KGN.

The drug loading of HNT/KGN was estimated by UV-*vis* spectroscopy. The amount of KGN loaded in the HNT nanomaterial, expressed as the percent amount of drug in the final composite, was ca. 6 wt% (60 mg g^{-1}) with an entrapment efficiency of 99.4%.

The HNT/KGN composite was further characterized by means of FT-IR spectroscopy (see Figure S.1) and thermogravimetric analysis.

Figure 1 presents the thermogravimetic (TG) curves of HNT/KGN composite and kartogenin.

We observed that the composite shows three mass losses in the investigated temperature interval: i) the mass loss at 25-150 °C (ML_{25-150}) due to the physically adsorbed water; ii) the mass loss at 200-330 °C ($ML_{200-300}$) that reflects the kartogenin degradation; iii) the mass loss at 400-600 °C ($ML_{400-600}$) as a consequence of the expulsion of the two water molecules in the HNT interlayer. Table 1 collects the values of the mass losses for the investigated materials.

According to literature,[26] we estimated the amount of KGN adsorbed onto HNT by the mathematical combination of ML_{25-150} values with the residual masses at 600 °C (MR_{600}) through the rule of mixtures. Based on the data in Table S.1., we calculated a KGN loading of 7.2 wt%.

Furthermore, we observed that the interactions with the HNT surfaces influence the thermal stability of kartogenin. Within this, we determined the onset temperature (T_{ons}) of the weight change in the interval between 200 and 330 °C. As reported in Table 2, the HNT/KGN composite shows a lower T_{ons} compared to that of pristine kartogenin. In addition, the effect of the adsorption onto HNT on the KGN thermal stability was evidenced by the differential thermogravimetric (DTG) curves. In particular, we observed a consistent shift of the DTG peak temperature (T_{peak}) in the range 200-330 °C (Table S.2). Being that T_{peak} represents the maximum rate of the KGN degradation, this result confirms that loading into HNT favors the thermal decomposition of kartogenin.

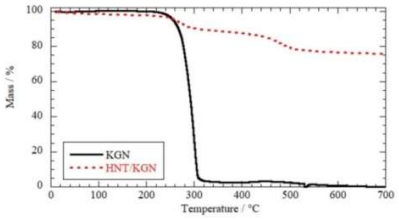


Figure 1. Thermogravimetric curves for KGN and HNT/KGN composite.

Gelating ability and study of gel properties. Pristine halloysite nanotubes were used as fillers for the laponite hydrogels. In general (Table S.3), it was observed that Lap dispersion gradually lost mobility and formed transparent gel in water, which was due to formation of "house of card" structure. The addition of the same amounts of laponite to a phosphate buffer pH 7.4 or a PBS solution does not form gels even after 7 days, suggesting a combination of a weakly screened Coulomb repulsion between the platelet faces that stabilized the cluster and an attractive interaction that acted as the driving force for clustering.[27] When both buffers were diluted 1:10, both mobility and transparency changed over time, and the dispersion turned to an opaque hydrogel. The introduction of halloysite filler, in each case investigated, helps the gel formation, resulting in opaque, strong hydrogels (Table S.3).

Aqueous dynamic behaviour and surface charge. The translational diffusion dynamics of laponite and HNT/laponite mixtures was investigated by Dynamic Light Scattering (DLS). Based on the Stokes-Einstein equation, we estimated an apparent hydrodynamic radius of the equivalent hard sphere (R_h) of 18 ± 2 nm for pure laponite. This result is in agreement with the literature.[28] DLS data of the HNT/Lap mixtures showed the simultaneous presence of nanoparticles with different diffusion coefficient in water. In particular, we determined R_h val-

ues of ca. 20 and 220 nm, which reflect the dynamics of single laponite nanodisks and single halloysite nanotubes, respectively. Accordingly, formation of large clusters can be ruled out in HNT/Lap dispersions. Moreover, we performed DLS experiments at variable HNT/Lap ratio. As reported in Table 1, the composition of the mixture slightly affects the hydrodynamic radii.

As concerns the ζ–potential data (Table 1), we determined a negative value (-34.4 ± 0.7 mV) for pure laponite, which is in agreement with the peculiar surface properties of the clay nanodisks. The presence of HNT generated an increase of the ζ–potential that cannot be attributed to a simple additive effect being the lower ζ–potential of halloysite (-19.4 mV).[28] The enhancement of net negative charge in the composite mixtures could be related to the electrostatic interactions between the negative HNT outer surface and the laponite edge, which possesses a positive charge. Namely, the increase of the negative charge for HNT/Lap composite could be due to the partial neutralization of the positively charged edge of the nanodisks.

Table 1. Hydrodynamic radius and ζ–potential for pure Lap and HNT/Lap mixtures in water.

Dispersion	Hydrodynamic radius (nm)	ζ–potential (mV)
Lap 0.1 wt%	18 ± 2	-34.4 ± 0.7
Lap 0.1 wt% + HNT 0.002 wt%	20 ± 4; 235 ± 20	-43.4 ± 1.9
Lap 0.1 wt% + HNT 0.005 wt%	21 ± 5; 219 ± 13	-47.4 ± 1.4

Rheological properties. The rheological properties of Lap and HNT/Lap hybrid hydrogels were investigated at room temperature, for hydrogels in PBS solution.

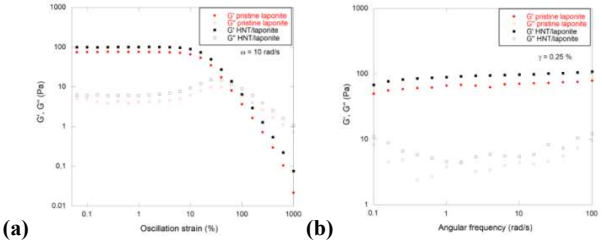
The evolution of the storage, G′, and the loss modulus, G″, as a function of the percentage strain (γ) and angular frequency (ω) have been studied within the linear viscoelastic region (LVR) of the gels. Soft materials here reported presented the viscoelastic behaviour typical of gel phases. In particular, in strain sweep measurements at low values of γ, a solid like behaviour was observed (G′ > G″). However, at higher γ values moduli trends showed a liquid-like behaviour (G′ < G″) (Figure 2a). In addition, the gel-like behaviour of the soft materials was also supported by frequency sweep measurements, where the storage modulus was always larger than the elastic one independently from the angular frequency applied (Figure 2b). A measure of the gel strength can be also obtained from the crossover points of yield strain (γ at which G′= G), indicating the level of stress needed to detect the flow of the material and from the loss tangents (tan δ = G″/G′), that can be defined as a measure of gel's stiffness.

Even if, both systems, behave as gelatinous materials, the introduction of a small amount of HNT reinforces the rheological properties of the gel. Indeed, while the stiffness of the gel is almost unchanged, a higher G′ value can be recognized for hybrid gel, especially the crossover point occurs at larger percentage of strain, indicating that the hybrid gel needed a higher strain to flow (Table S.4). This trend is in agreement on what previously observed comparing hybrid gels with pure ones,[29] and in particular hybrid halloysite hydrogels and pure hydrogels.[14]

In general, crossover points observed for our laponite gels are much more larger than the ones reported in literature for similar laponite gels, where the gel break down occurred at γ < 10 %.[30]

The ability of gel phases to self-repair after an event of disruption caused by external stimuli, such as magnetic stirring (thixotropy) and ultrasound irradiation (sonotropy) can be extremely useful considering the pharmaceutical application of these gels as tissue regeneration systems. In general, these properties are mainly analysed for gels hold together by supramolecular interactions, which reversibility warrants, in proper conditions, the regeneration of the gelatinous matrix.

Bearing in mind that laponite in aqueous medium self-organizes *via* face-edge aggregation forming an open, macroporous and reversible (thixotropic) gel network, the ability to self-repair of gels here reported has been also investigated. It is known that laponite in aqueous medium self-organize *via* face-edge aggregation forming an open, macroporous and reversible (thixotropic) gel network. The results obtained showed that the hybrid gels retained the properties of the pure gels. All the hydrogels, indeed, gave good responses to the thixotropic tests, demonstrating self-repair after disruption, while most of the hydrogels were stable to ultrasound irradiation (Table S.5).



(a) (b)

Figure 2. (a) Strain sweeps of pristine laponite and HNT/Lap hydrogels; (b) frequency sweeps of pristine laponite and HNT/Lap hydrogels.

Morphological studies. The hydrogel phases were further characterized by TEM measurements (Figure 3). In particular, we chose to analyze the morphologies of both the pure and hybrid gels in PBS. The formation of aggregates with 'house of cards' structure in the pure laponite hydrogel is confirmed by the presence of black lumps in the TEM images (Figure 3a). When 5.0 wt% of HNT was added, the phenomenon of aggregation was avoided and the region became brighter (Figure 3c). Furthermore, the halloysite tubes organize themselves in the hydrogel matrix as single tube.

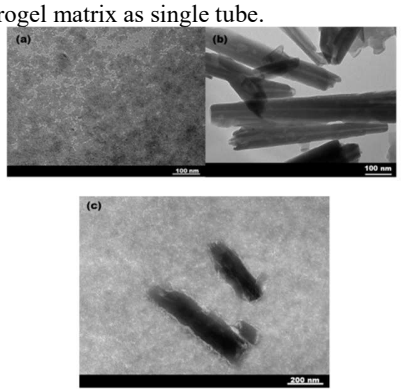


Figure 3. TEM image of (a) pure laponite (b) pristine halloysite and (c) HNT/Lap gel in PBS.

Kinetic release. The main goal of this study was to control/study/analyze the kartogenin release kinetic in various

conditions from the Lap hydrogels, which might be beneficial for cartilage regeneration.

The KGN release behavior of the HNT/KGN composite and from HNT/KGN/Lap hybrid hydrogel was investigated in phosphate buffer solution at pH 7.4 mimicking the conditions presented at normal physiological conditions.

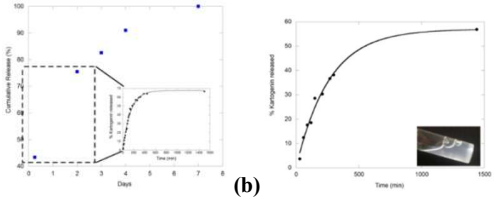
The KGN release-time profile from HNT/KGN is reported in Figure 4a. The system exhibited sustained release of KGN with an initial rapid-release phase, followed by a gradually slower release pattern. The cumulative release of KGN was around 70% in the first 600 min after which the molecule is still slow released for 7 days. The experimental data were analyzed according to the first order kinetic and double exponential model. It was found that the release of KGN from the HNT/KGN composite follows the first order kinetic (M_∞ = 67.5 ± 1.1 wt%, k = 0.0073 ± 0.0002 min^{-1}).

Because OA pathology is restricted to the affected joint, IA administration of biomolecules targeting chondrocyte activity or synovial inflammation is a promising strategy for therapeutic intervention. Therefore, to better simulate the joint environment the kinetic release of kartogenin from halloysite was also performed in ex-vivo inflamed synovial fluid.

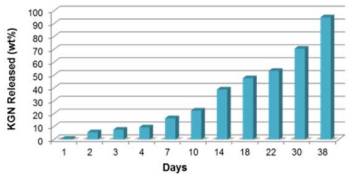
In Figure 4c is reported the amount of kartogenin released as function of time. As it is possible to note, in these conditions, a different behavior respect to the buffer solution was observed: a stronger prolonged release from 7 up to 38 days was achieved. In these conditions, the kinetic release follows the zero-order model (k = 0.1031 ± 0.002 h^{-1}; R^2 = 0.9943).

The slower release in synovial fluid respect to that in phosphate buffer solution, could be due to the existence of supramolecular interactions between halloysite and the components of synovial fluid. Synovial fluid has a pH of 7.54; in these conditions, hyaluronic acid, one of its major components, exists as hyaluronate and therefore it could partially interact with the halloysite positively charged inner surface, creating a kind of stopper which slow down the KGN release from the HNT lumen as already reported for similar molecules.[31]

Finally, the release of KGN from the HNT/KGN/Lap gel was studied to verify if incorporation of HNTs into the gel matrix could induce a time-controlled drug release process. The trends of cumulative KGN release from the hybrid gel as a function of time are displayed in Figure 4b. As it is possible to note the system can effectively release in a sustained manner KGN for at least 24 h; after this time, we observed the dissolution of gel matrix in a physiological medium (Figure 4b) and therefore the KGN kinetic release from Lap gel became very similar to that from pristine halloysite. Similar results were obtained the release of KGN from the HNT/KGN/Lap gel in synovial fluid. These findings show that Lap can act as inert carrier to the efficient delivery of the HNT/KGN composite directly in the affected joints by means of intraarticular injections. Therefore, these results substantiated that the KGN release from halloysite loaded with KGN hybrids underwent a sustained releasing manner, which offered promise for the long-term administration using KGN *in vivo* for cartilage regeneration in the treatment of osteoarthritis.



(a) (b)



(c)

Figure 4. Kinetic release of KGN from (a) HNT/KGN carrier system in phosphate buffer solution at pH 7.4; (b) HNT/KGN/Lap hydrogel in phosphate buffer solution at pH 7.4; (c) HNT/KGN carrier system in synovial fluid.

Cytocompatibility. The cytotoxicity of the hybrid hydrogels was evaluated in human liver HepG2 cells by means of the MTS assay (Figure 5). They were chosen since liver is the mayor xenobiotic metabolizing organ in the human body and therefore, cultured liver cells are widely used for xenobiotic metabolism studies.[32] As human hepatocytes produce various drug metabolizing enzymes at comparable levels to those found in vivo, they are used for modelling toxicology in humans.[33]

The survival rates of the cells, incubated with HNT/Lap and HNT/KGN/Lap hybrid hydrogels in PBS for 72 h with a KGN concentration ranging from 0.1 to 10 μM, were found to be in the range of 98% to 85% for HepG2 (Figure 5). Since both KGN and Lap hydrogel did not have any cytotoxicity in the concentration range investigated,[6, 23] the slight decrease in the cellular viability could be due to halloysite. Indeed, as reported in literature, the uptake and toxicity of halloysite nanotubes depend on the cell culture and it was demonstrated that HNTs significant reduce MCF-7 cell viability at concentrations up to 75 μg mL^{-1}; whereas they show an IC_{50} of 300 μg per 10^5 cells towards A549 cells.[9]

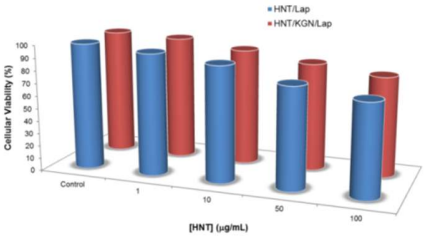


Figure 5. MTS test for cell viability of HepG2 cells cultured for 72 h in presence of HNT/Lap and HNT/KGN/Lap hybrid systems. The data are mean values obtained from at least three estimations (see Figure S.2).

Conclusions

An attractive therapeutic treatment of osteoarthritis requires intraarticular injections which can release the drug in an extended period of time. Kartogenin is an emerging small molecule that promotes the selective differentiation of multipotent mesenchymal stem cells into chondrocytes thus stimulating the repair of damaged cartilage. Unfortunately, kartogenin is hydrophobic and possesses a short-term stability in an aqueous medium.

In this context, we have developed, for the first time, a novel carrier system based on halloysite nanotubes and laponite hydrogel, for the potential intraarticular delivery of KGN. The introduction of halloysite filler, in laponite hydrogel, helps the gel formation with an improvement of the rheological properties as a consequence of electrostatic interactions between the negative HNT outer surface and the laponite edge, which possesses a positive charge.

The efficacy of HNT/Lap hydrogel as carrier for KGN was proved by in vitro release experiments performed at pH 7.4 and in ex-vivo synovial fluid at 37 °C, in order to simulate both the physiological conditions and the joint environment, respectively. The two components of the hybrid hydrogel system HNT/Lap, act in a complementary way: the Lap acts as inert carrier since the gel matrix is dissolved in a physiological medium after 24 h and thus does not affect the KGN release; on the contrary, a sustained release of the drug was observed from HNT. It was found that KGN is slower release in synovial fluid than phosphate buffer pH 7.4. We have hypothesized that the different behaviour could be due to the existence of supramolecular interactions between halloysite and the components of synovial fluid.

Finally, the cytotoxicity of the hybrid hydrogels was evaluated in human liver HepG2 cells by means of the MTS assay. The collected results have shown that the hybrid hydrogel system did not possess any cytotoxicity against the cell lines investigated. We also observed a slight decrease in cell viability at higher HNT concentrations according to literature data.

Future work will be devoted to assessing the feasibility of the hybrid systems for their use in the regenerative medicine using animals as model.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Experimental details, synthesis of kartogenin, FT-IR spectra, TGA data, gelation tests, rheological parameters, MTS test (PDF)

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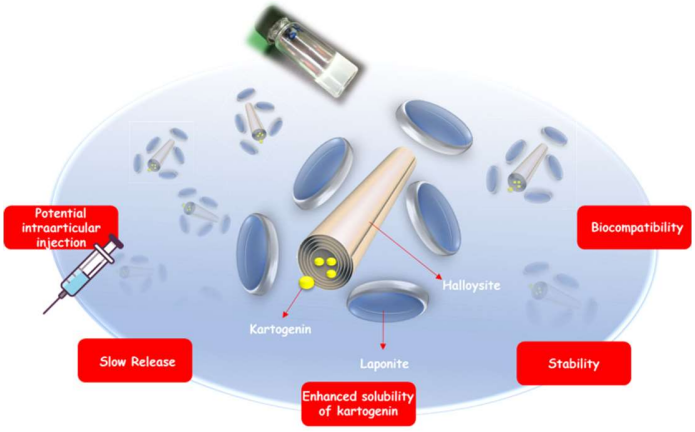
REFERENCES

1. Johnson, K.; Zhu, S.; Tremblay, M. S.; Payette, J. N.; Wang, J.; Bouchez, L. C.; Meeusen, S.; Althage, A.; Cho, C. Y.; Wu, X.; Schultz, P. G. A Stem Cell–Based Approach to Cartilage Repair. *Science* **2012,** *336* (6082), 717-721.
2. Gerwin, N.; Hops, C.; Lucke, A. Intraarticular Drug Delivery in Osteoarthritis. *Adv. Drug Del. Rev.* **2006,** *58* (2), 226-242.
3. Iannitti, T.; Lodi, D.; Palmieri, B. Intra-Articular Injections for the Treatment of Osteoarthritis: Focus on the Clinical Use of Hyaluronic Acid. *Drugs in R&D* **2011,** *11* (1), 13-27.
4. Im, G. I. Application of Kartogenin for Musculoskeletal Regeneration. *J. Biomed. Mater. Res. A* **2018,** *106* (4), 1141-1148.
5. Maudens, P.; Seemayer, C. A.; Thauvin, C.; Gabay, C.; Jordan, O.; Allémann, E. Nanocrystal–Polymer Particles: Extended Delivery Carriers for Osteoarthritis Treatment. *Small* **2018,** *14* (8), 1703108.
6. Hu, Q.; Ding, B.; Yan, X.; Peng, L.; Duan, J.; Yang, S.; Cheng, L.; Chen, D. Polyethylene Glycol Modified Pamam Dendrimer Delivery of Kartogenin to Induce Chondrogenic Differentiation of Mesenchymal Stem Cells. *Nanomed. Nanotechnol. Biol. Med.* **2017,** *13* (7), 2189-2198.
7. Zhu, Y.; Tan, J.; Zhu, H.; Lin, G.; Yin, F.; Wang, L.; Song, K.; Wang, Y.; Zhou, G.; Yi, W. Development of Kartogenin-Conjugated Chitosan-Hyaluronic Acid Hydrogel for Nucleus Pulposus Regeneration. *Biomater. Sci.* **2017,** *5* (4), 784-791.
8. Kang, M. L.; Ko, J.-Y.; Kim, J. E.; Im, G.-I. Intra-Articular Delivery of Kartogenin-Conjugated Chitosan Nano/Microparticles for Cartilage Regeneration. *Biomaterials* **2014,** *35* (37), 9984-9994.
9. Kamalieva, R. F.; Ishmukhametov, I. R.; Batasheva, S. N.; Rozhina, E. V.; Fakhrullin, R. F. Uptake of Halloysite Clay Nanotubes by Human Cells: Colourimetric Viability Tests and Microscopy Study. *Nano-Structures & Nano-Objects* **2018,** *15*, 54-60.
10. Bellani, L.; Giorgetti, L.; Riela, S.; Lazzara, G.; Scialabba, A.; Massaro, M. Ecotoxicity of Halloysite Nanotube–Supported Palladium Nanoparticles in Raphanus Sativus L. *Environ. Toxicol. Chem.* **2016,** *35* (10), 2503-2510.
11. Massaro, M.; Colletti, C. G.; Guernelli, S.; Lazzara, G.; Liu, M.; Nicotra, G.; Noto, R.; Parisi, F.; Pibiri, I.; Spinella, C.; Riela, S. Photoluminescent Hybrid Nanomaterials from Modified Halloysite Nanotubes. *Journal of Materials Chemistry C* **2018,** *6* (27), 7377-7384.
12. Massaro, M.; Cavallaro, G.; Colletti, C. G.; Lazzara, G.; Milioto, S.; Noto, R.; Riela, S. Chemical Modification of Halloysite Nanotubes for Controlled Loading and Release. *J. Mater. Chem. B* **2018,** *6* (21), 3415-3433.
13. Liu, M.; Chang, Y.; Yang, J.; You, Y.; He, R.; Chen, T.; Zhou, C. Functionalized Halloysite Nanotube by Chitosan Grafting for Drug Delivery of Curcumin to Achieve Enhanced Anticancer Efficacy. *J. Mater. Chem. B* **2016,** *4* (13), 2253-2263.
14. Rizzo, C.; Arrigo, R.; D'Anna, F.; Di Blasi, F.; Dintcheva, N. T.; Lazzara, G.; Parisi, F.; Riela, S.; Spinelli, G.; Massaro, M. Hybrid Supramolecular Gels of Fmoc-F/Halloysite Nanotubes: Systems for Sustained Release of Camptothecin. *J. Mater. Chem. B* **2017,** *5* (17), 3217-3229.
15. Massaro, M.; Riela, S.; Guernelli, S.; Parisi, F.; Lazzara, G.; Baschieri, A.; Valgimigli, L.; Amorati, R. A Synergic Nanoantioxidant Based on Covalently Modified Halloysite-Trolox Nanotubes with Intra-Lumen Loaded Quercetin. *J. Mater. Chem. B* **2016,** *4* (13), 2229-2241.
16. Massaro, M.; Riela, S.; Baiamonte, C.; Blanco, J. L. J.; Giordano, C.; Lo Meo, P.; Milioto, S.; Noto, R.; Parisi, F.; Pizzolanti, G.; Lazzara, G. Dual Drug-Loaded Halloysite Hybrid-Based Glycocluster for Sustained Release of Hydrophobic Molecules. *RSC Adv.* **2016,** *6* (91), 87935-87944.
17. Peng, Q.; Liu, M.; Zheng, J.; Zhou, C. Adsorption of Dyes in Aqueous Solutions by Chitosan-Halloysite Nanotubes Composite Hydrogel Beads. *Micropor. Mesopor. Mater.* **2015,** *201* (C), 190-201.
18. Cataldo, S.; Lazzara, G.; Massaro, M.; Muratore, N.; Pettignano, A.; Riela, S. Functionalized Halloysite Nanotubes for Enhanced Removal of Lead(Ii) Ions from Aqueous Solutions. *Appl. Clay Sci.* **2018,** *156*, 87-95.
19. Massaro, M.; Riela, S.; Cavallaro, G.; Colletti, C. G.; Milioto, S.; Noto, R.; Lazzara, G. Ecocompatible Halloysite/Cucurbit[8]Uril Hybrid as Efficient Nanosponge for Pollutants Removal. *ChemistrySelect* **2016,** *1* (8), 1773-1779.
20. Massaro, M.; Colletti, C. G.; Buscemi, G.; Cataldo, S.; Guernelli, S.; Lazzara, G.; Liotta, L. F.; Parisi, F.; Pettignano, A.; Riela, S. Palladium Nanoparticles Immobilized on Halloysite Nanotubes Covered by a Multilayer Network for Catalytic Applications. *New J. Chem.* **2018,** *42* (16), 13938-13947.
21. Mahinroosta, M.; Jomeh Farsangi, Z.; Allahverdi, A.; Shakoori, Z. Hydrogels as Intelligent Materials: A Brief Review of Synthesis, Properties and Applications. *Mater. Today Chem.* **2018,** *8*, 42-55.
22. Rizzo, C.; Arrigo, R.; Dintcheva, N. T.; Gallo, G.; Giannici, F.; Noto, R.; Sutera, A.; Vitale, P.; D'Anna, F. Supramolecular Hydro- and Ionogels: A Study of Their Properties and Antibacterial Activity. *Chemistry – A European Journal* **2017,** *23* (64), 16297-16311.
23. Boyer, C.; Figueiredo, L.; Pace, R.; Lesoeur, J.; Rouillon, T.; Visage, C. L.; Tassin, J. F.; Weiss, P.; Guicheux, J.; Rethore, G. Laponite Nanoparticle-Associated Silated Hydroxypropylmethyl Cellulose as an Injectable Reinforced Interpenetrating Network Hydrogel for Cartilage Tissue Engineering. *Acta Biomater.* **2018,** *65*, 112-122.

24. Shi, D.; Xu, X.; Ye, Y.; Song, K.; Cheng, Y.; Di, J.; Hu, Q.; Li, J.; Ju, H.; Jiang, Q.; Gu, Z. Photo-Cross-Linked Scaffold with Kartogenin-Encapsulated Nanoparticles for Cartilage Regeneration. *ACS Nano* **2016,** *10* (1), 1292-1299.
25. Massaro, M.; Campofelice, A.; Colletti, C. G.; Lazzara, G.; Noto, R.; Riela, S. Functionalized Halloysite Nanotubes: Efficient Carrier Systems for Antifungine Drugs. *Appl. Clay Sci.* **2018,** *160*, 186-192.
26. Massaro, M.; Cavallaro, G.; Colletti, C. G.; D'Azzo, G.; Guernelli, S.; Lazzara, G.; Pieraccini, S.; Riela, S. Halloysite Nanotubes for Efficient Loading, Stabilization and Controlled Release of Insulin. *J. Coll. Interf. Sci.* **2018,** *524*, 156-164.
27. Li, N.; Du, J.; Tan, Y.; Ling, J.; Yang, X.; Ma, J.; Wu, R.; Xu, S.; Zhang, Y. Dispersion and Rheological Behaviors of Laponite in 2-Acrylamido-2-Methylpropanesulfonic Acid Solution. *Appl. Clay Sci.* **2017,** *137*, 94-100.
28. Cavallaro, G.; Lazzara, G.; Milioto, S. Exploiting the Colloidal Stability and Solubilization Ability of Clay Nanotubes/Ionic Surfactant Hybrid Nanomaterials. *J. Phys. Chem. C* **2012,** *116* (41), 21932-21938.
29. Rizzo, C.; Arcudi, F.; □or□evi□, L.; Dintcheva, N. T.; Noto, R.; D'Anna, F.; Prato, M. Nitrogen-Doped Carbon Nanodots-Ionogels: Preparation, Characterization, and Radical Scavenging Activity. *ACS Nano* **2018,** *12* (2), 1296-1305.
30. Thrithamara Ranganathan, V.; Bandyopadhyay, R. Effects of Aging on the Yielding Behaviour of Acid and Salt Induced Laponite Gels. *Coll. Surf. A* **2017,** *522*, 304-309.
31. Dzamukova, M. R.; Naumenko, E. A.; Lvov, Y. M.; Fakhrullin, R. F. Enzyme-Activated Intracellular Drug Delivery with Tubule Clay Nanoformulation. *Sci. Rep.* **2015,** *5*, 10560.
32. Turpeinen, M.; Ghiciuc, C.; Opritoui, M.; Tursas, L.; Pelkonen, O.; Pasanen, M. Predictive Value of Animal Models for Human Cytochrome P450 (Cyp)-Mediated Metabolism: A Comparative Study in Vitro. *Xenobiotica* **2007,** *37* (12), 1367-1377.
33. Tyson, C. A.; Mitoma, C.; Kalivoda, J. Evaluation of Hepatocytes Isolated by a Nonperfusion Technique in a Prescreen for Cytotoxicity. *J. Toxicol. Environ. Health* **1980,** *6* (1), 197-205.

SYNOPSIS


Potential intraarticular injection
Biocompatibility
Halloysite
Kartogenin
Slow Release
Laponite
Stability
Enhanced solubility of kartogenin