3D PRINTING FOR DRUG DELIVERY: POTENTIALITIES OF ALGINATE HYDROGELS

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Thanks to the manufacturing flexibility, 3D printing technologies could allow to fulfil the growing request of therapeutic personalization, complex drug delivery systems production and on-demand manufacturing. However, to unleash the potential of 3D printing, there are still several technological issues that must be overcome, especially in the case of semi solid extrusion (SSE), where the 3D model is built up through the well-defined extrusion of polymeric ink. Focusing on polymers, even if the hydrogels are widely used in pharmaceutical compound, their physico-chemical properties should be deeply investigated to evaluate the 3D printability. This study aimed to produce tablets easy to swallow exploiting as 3D printing-ink a crosslinked alginate hydrogel added of sorbitol speculating that plasticising action of sorbitol should allow to improve tablets swallowability. Three different amounts of 70% sorbitol solution (5, 15, 25 % v/v) were evaluated to identify the best composition in terms of both printing and technological properties. The data obtained from the chemical, rheological and mechanical evaluation of each ink was related to the printing performance to reach the best manufacturing reproducibility (mass average 384.1±19.2 mg). Moving to DDS properties, the increase of residual mass was related to the sorbitol amount loaded, as confirmed by linear regression fitting study (r2=0.9970). The highest sorbitol concentration induced changes in the network entanglement, leading to a spreading of the forms, after printing. In accordance with the information about the swallowability requirement, all the dried batches were subjected to mechanical evaluation highlighting differences in the soft-ability between platform with 5 and 15% of sorbitol, this latter showed matrix elasticity comparable to conventional soft and chewable tablet. Differently, 25% v/v of sorbitol used in the ink did not affect the elasticity of the matrix, but it led to an increase in the adhesivity that could negatively impact on the swallow process.

CONVENTIONAL AND HA-COATED LIPOSOMES FOR OCULAR DELIVERY OF THYMOQUINONE

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Thymoquinone (TQ) is the main constituent of the essential oil of Nigella sativa L. It has a protective effect against oxidative stress induced by H2O2 in human retinal pigment epithelium cells; furthermore, in vivo evidence demonstrated a positive effect in decreasing corneal neovascularization and reducing the inflammation in a mouse dry eye model [1]. However, the poor bioavailability, solubility and permeability of TQ limit its therapeutic application. In this study, two liposomal formulations have been developed to improve its biopharmaceutical performance and enhance its delivery and the activity at the ocular level [2]. Liposomes consist of phosphatidylcholine and Plurol Oleique, and one formulation has been coated with hyaluronic acid (HA). Their dimensions are less than 200 nm with an encapsulation efficiency of 70%. Both liposomes increase the TQ solubility and HA-coated liposomes are stable over 2 months at +4 °C. Both liposomes exhibit a gradual and sustained release. Two cell lines, human corneal epithelial cells (HCE-2) and human conjunctival epithelial cells (HConEC) have been used to test the safety of the formulations. Uptake studies have been also performed using fluorescent liposomes. Both liposomes and in particular HA-coated liposomes reduce the TQ toxicity observed at high doses in both HCE-2 and HConEC cells, and both formulations increase the absorption at the cellular level and in particular at the nuclear level, with a most marked effect for HA-coated liposomes. Furthermore, TQ and liposomal formulations have anti-inflammatory effect on HCE-2 cells in an in vitro model of dry eye obtained by hyperosmolarity (450 mOsm) [3]. The anti-inflammatory effect of TQ and liposomal formulations quantified by RT-qPCR was achieved through a significant reduction of IL-1β, IL-6 and TNFα. Our data suggest that these formulations have potential as a therapeutic agent in dry eye disease, which should be further investigated in in vivo model.

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SEMI-SYNTHESIS OF GLYCOSAMINOGLYCAN-MIMETICS FROM BACTERIAL SOURCES FOR BIOMEDICAL APPLICATIONS

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Polysaccharides are the most abundant biomacromolecules on our planet, possessing enormous structural diversity and functional versatility. They are currently employed for several purposes, both in their natural and structurally modified forms. However, several polysaccharides used in pharmaceutical field are obtained from animal sources (e.g. glycosaminoglycans, GAGs) and this limits their use both for ethical and ecological reasons and for problems related to the possible contamination of the batches (e.g. heparin crisis in 2007). A solution could be the development of new strategies for the modification of polysaccharides from sustainable sources (bacteria, algae and fungi). Therefore, our work is focused on the development of appropriate semi-synthetic strategies for the regioselective modification of polysaccharides, in order to obtain new polysaccharide-based products, which can be proposed as substitutes for drugs already existing but obtained from less eco-sustainable sources. The regioselective derivatization that is carried out is focused on the insertion of negatively charged functionalities (sulfate, phosphate), in order to mimic the structural characteristics of natural sulfated GAGs. The starting materials are two polysaccharides extracted from bacterial sources such as chondroitin produced by fed-batch fermentation of Escherichia coli O5:K4:H4, and an exopolysaccharide (EPS) from the marine bacterium Vibrio diabolicus HE800 strain, firstly isolated from the polychaete annelid Alvinella pompejana. They structurally resemble GAGs and in their modified form they could mimic GAGs activities. Our final aim is to obtain new regioselectively modified polysaccharides with unprecedent sulfation or phosphorylation patterns with potential employment in the formulation of novel drugs.

GLYCO-GOLD NANOPARTICLES REPOLARIZE MACROPHAGES TOWARDS A RESTORATIVE PHENOTYPE IN PRIMARY BILIARY CHOLANGITIS

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Background: The role of glycans is of increasing interest in nanomedicine. Binding molecules such as mannose (Man) and sialic acid (Sia) to nanocarriers could enhance their immune-mediated effect with relapses in autoimmune disorders. The link with the nanocarriers could have a double role: to increase their stability and increase their affinity towards the target. A third potential benefit could come from the tropism of the nanocarriers to the liver. To combine these three factors we decided to link selected glycans to gold nanoparticles (GNPs) to deliver them to a mouse model of biliary autoimmune disease (primary biliary cholangitis) after systemic administration.
Methods: This study is integrated, at 4 and 24 hours after treatment with naked GNPs or functionalized with the two glycans of interest, a histological analysis was performed and then, flow cytometry analysis was carried out separating the various liver populations and measuring the gene expression of pro and anti-inflammatory cytokines by rt-PCR.
Results: First, the comparison of the immunophenotype between the liver of healthy and pathologic untreated mice revealed important differences. Subsequently, it was shown that single administration of naked GNPs does not significantly modify the pathological condition. In contrast, both glyco-GNPs were capable of modifying the immunophenotype by reducing the production of TNF-α, IL-1β, Arg1 and IL-6. Finally, it is important to underline that Man-GNPs are able to re-polarize macrophages towards a restorative phenotype.
Discussion: Our study is a proof-of-concept that Man and Sia can be effective against inflammatory disorders. However, this is a single intravenous administration, chronic treatments to perpetuate the restorative phenotype in the cells must be assessed, to discard possible toxic effects over time.
Conclusion: Man-NPs are an effective targeting strategy that might be applied as therapeutic for selective targeting of autoimmune cells.

DISACCHARIDES APPLICATION FOR LIPOSOMES AND EXTRACELLULAR VESICLES PHARMACEUTICAL FREEZE-DRYING SOLUTIONS

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In recent years extracellular vesicles (EVs) have become an increasingly used tool for developing pharmaceutical nanomedicines. Although these vesicles are always better characterized and engineered, their storage is still challenging since it affects the possibility of further functionalizations or direct administration for safe care and treatment. The preservation of the EVs' morphofunctional integrity is a demanding goal due to their peculiar structure. These vesicles consist of an external phospholipid bilayer containing native proteins or engineered targeting and/or signaling moieties such as homing peptides and antibodies. The lipid bilayers' temperature-dependent fluidity can strongly be affected by physical and chemical stresses such as crystals formation, pressure variation, and drying stresses. In addition, since native EVs carry cytosolic aqueous compounds, proteins, and nucleic acids, the vesicles' inner core can be affected by ice crystal formation and, eventually, osmotic freezing changes.
In this study, we evaluated the possibility of preserving lymphocyte-derived EVs using freeze-drying, where disaccharides acted as excipients to protect the EVs' morphology and their intrinsic characteristics. We screened the ability of selected disaccharides to preserve liposomes, and freeze-thawing and freeze-drying experiments showed that lactose, sucrose, and trehalose successfully preserve membrane integrity from the stresses that occurred during the process. Then, we tested the same disaccharides with EVs, alone at different concentrations or combined with dextran, a collapse temperature modifier, and with glycine, a bulking agent. Results demonstrated that both the concentration and the dimensions of EVs were maintained after freeze-drying in the presence of excipients. We also evaluated the EVs' biological activity in an in vitro system, made of a healthy cell line, B-lymphocytes, and a cancerous one, Daudi. Cytotoxicity remained similar to the one of the untreated EVs, while the uptake was even improved, probably thanks to the presence of disaccharides that made EVs more attractive for cells.

HYALURONIC ACID DECORATED-LIPOSOME LOADED WITH IMATINIB: AN IN VITRO STUDY AS TARGETED DRUG DELIVERY SYSTEM FOR LUNG FIBROSIS

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Interstitial Lung diseases is a rare fibrotic disorder with scarcity of adequate therapeutic options. Nanomedicine introduces the possibility to administer drugs locally, increasing drug accumulation in alveola and reducing side effects. In order to study the interactions of liposomes with lung fibroblasts (LFs) derived from CTD-ILD and BOS, we analyzed liposomes internalization by flow cytometry and we observed higher interaction of Hyaluronic acid decorated-liposome (LIP-HA) with A549 cells, BOS- and CTD-ILD-LFs rather than liposomes without HA. Moreover, we detected a significant increase in LIP-HA uptake proportionally with HA molecular weight. Data confirmed by confocal microscopy, further demonstrating that most of the internalized liposomes, in all cell types, co-localize with cellular endosomes after 24 h of incubation.
The encapsulation of Imatinib (Im) inside LIP and LIP-HA reduce the vitality of LFs by 30%, while Im alone reduced 80%. In the case of A549 cells, after 72 h of treatment, a reduction in cell viability by LIP-HA-Im is observed (30%) compared to LIP-Im (0%). Furthermore, in this cell line LIP-HA-Im exert the same effect as Im alone (30%). Knowing that Im has cAbl as a specific target, we decided to evaluate the effectiveness of LIP-HA-Im to inhibit the activity of cAbl in BOS and CTD-ILD LFs. The level of phosphorylation of the protein is evaluated by western blot analysis and we found a 50% reduction in the activity of cAbl with both LIP-Im and LIP-HA-Im in BOS LFs after 24 h of treatment. Analyzing the LFs derived from CTD-ILD, LIP-Im reduces the activity of cAbl by 60%, while LIP-HA-Im reduces it by 70%.
In conclusion, the encapsulation of Im inside targeted liposomes could be a promising option to vehicle locally those drugs, such as Im or other Tyrosine Inhibitors, that might be difficult to use in chronic lung disorders due to their systemic toxicity.

HYALURONIC ACID DECORATED-LIPOSOME AS TARGETED DRUG DELIVERY SYSTEM FOR FIBROTIC LUNG DISORDERS: EX VIVO ASSESSMENT ON HUMAN FIBROTIC

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Nanomedicine has proven to be a great opportunity to administer drugs by local route, to increase drug efficiency and to reduce side effects. In particular, the chance to administer drug-loaded nanoparticles directly into the lungs through inhalation has gained great attention. Based on our previous in vitro results obtained with liposomes conjugated with hyaluronic acid (HA), we aimed to assess the activity imatinib (Im) loaded liposomes [LIP-HA(Im)] coated with 40-60 kDa HA on Precision Cut Lung Slices (PCLSs) obtained from patients with idiopathic lung fibrosis.
By real-time PCR we evaluated time course of collagen type 1a1 (COL1a1) and collagen type3a1 (COL3a1), two key extracellular proteins, produced by pulmonary myofibroblasts during fibrogenesis. We assessed variations over 24-96 h of treatment with LIP-HA(Im), or LIP(Im) and imatinib alone. At 24h after treatment reduction in both COL1a1 and COL3a1 from baseline levels was detectable with all experimental conditions (60% and 30% for LIP-HA(Im), 70% and 50% for LIP(Im), 95% and 90% for imatinib alone). The expression levels of α-SMA, known mesenchymal marker, was also significantly reduced at 24 h in all conditions (60% for LIP-HA(Im) and LIP(Im) or 80% for imatinib alone) but maintained low levels up to 96 h only with LIP-HA(Im).
Knowing that Im has cAbl as a specific target, we decided to evaluate the effectiveness of LIP-HA(Im) to inhibit its activity. To study the activity of cAbl, the level of phosphorylation of the protein was evaluated: western blot analyses showed that LIP-HA(Im) reduced the phosphorylation level of cABL in PCLSs, analogously to what obtained with the free drug up to 96 h of treatment.
In conclusion, in ex vivo model of human lung fibrosis we confirm the therapeutic activity of HA coated Im liposomes as biocompatible nanovehicles for the inhalatory treatment of these disorders.

GLYPICAN-3 TARGETED CHITOSAN-SHELLED NANOBUBBLES FOR HEPATOCELLULAR CARCINOMA TREATMENT

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Background: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death. The high levels of Glypican-3 (GPC3) detected in HCC and the absence or very low levels in normal and non-malignant liver make GPC3 a promising target for cancer treatment strategies [1]. Polysaccharide-shelled nanobubbles have been proposed for anticancer drug delivery [2]. This work aimed at the development of anti-GPC3 conjugated chitosan (CS)-shelled and decafluopentane-cored NBs loaded with idarubicin (IDA) to treat HCC.
Methods: CS-shelled NBs were formulated according to a purposely-tuned protocol. Targeted NBs were prepared by the conjugation of the anti-GPC3 antibody 4A1, obtained as humanized miniantibody starting from the sequence of the GC33 antibody. Drug loaded NBs were obtained encapsulating IDA in the decafluoropentane core. Moreover, fluorescent-labelled NBs were produced binding cyanine 5.5 (Cy5.5) to the chitosan shell of targeted or untargeted NBs. The NB formulations were in vitro characterized determining their physico-chemical parameters. The capability of IDA-CS-NBs to kill HUH7 cells was evaluated by MTT assay. The in vivo evaluation was carried out using a HCC xenograft murine model. The biodistribution of targeted CS-4A1-Cy5.5-NBs compared to the untargeted ones was evaluated by IVIS Optical Imaging after their i.v. injection.
Results: NBs with sizes of about 400 nm and positive surface charge were obtained. The NBs were able to load IDA with a good encapsulation efficiency and release it with a prolonged in vitro release kinetics. CS-NBs appeared biocompatible and IDA-CS-NBs confirmed cytotoxic activity in vitro. At 48 hours after treatment a significantly higher accumulation was showed in the HUH7 tumor mass of CS-4A1-Cy5.5-NBs treated group compared with that in the CS-Cy5.5-NBs group. IDA-CS-NBs and IDA-CS-4A1-NBs treatments slowed tumor growth and statistically increased mice survival.
Discussion: Stable targeted and untargeted CS NBs were produced as biocompatible idarubicin delivery system with promising results for HCC treatment.

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MILD COATING OF HARD-GELATIN CAPSULES FOR COLON DELIVERY OF PROBIOTICS

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Hard-gelatin capsules represent a widely used dosage form for the oral administration of probiotics, avoiding application of possibly harmful compression forces. To achieve colon delivery of the conveyed formulation, coating with polymers preventing exposure of the gelatin shell to upper gastrointestinal fluids is required. Particularly, swellable/erodible coating layers based on hydroxypropyl methylcellulose (HPMC) were proposed for time-dependent colonic release. These were applied by aqueous spray-coating that, due to the water solubility of gelatin, involve technical issues and may be threatening to the bacteria. Recently, powder-layering, which would limit working temperatures, amounts of water employed and processing times, has successfully been explored as an alternative technique for HPMC coating of tablets. Hence, the aim of the present work was to investigate the feasibility of powder-layering in the application of low-viscosity HPMC to hard-gelatin capsules and, according to the time-based colon delivery strategy, of subsequent enteric-coating of the HPMC-coated units. For this purpose, size 0 hard-gelatin capsule shells were manually filled with freeze-dried Odoribacter splanchnicus culture (40%w/w, corresponding to 8.1x1010CFU/g), microcrystalline cellulose (Avicel®PH102, 51%w/w), sodium starch glycolate (Explotab®CLV, 4.5%w/w) and colloidal silica (Aerosil®200, 0.5%w/w). Paracetamol (4%w/w) was added to the mixture as a tracer drug. The filled capsules were checked for weight, dimensions and disintegration time, immediately coated in tangential-spray fluid bed (Glatt) with HPMC (Methocel®E50) powder, also used as the binder, and afterwards with an aqueous Eudragit®L (17.1%w/w) dispersion in top-spray fluid bed (Mini-glatt). The coated capsules were stored in vacuum sealed bags at -11ºC before being characterized for weight and dimensions and tested for release in an adapted disintegration apparatus (Sotax DT3, 250 ml 0.1N HCl for 2 h and phosphate buffer pH6,8, 37□0.5ºC, spectrophotometric assay at 248 nm, n=3). As desired, the HPMC-coated systems showed gastroresistance properties and reproducible lag phases followed by rapid release of the drug.

CHITOSAN DERIVATIVES-BASED FILMS AS pH-SENSITIVE DRUG DELIVERY SYSTEMS WITH ENHANCED FUNCTIONAL PROPERTIES

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Nowadays, attention has been focused on film preparation using eco-friendly "greener" methods, especially when using biomaterials extracted from renewable sources, including polysaccharides. Among the most studied biopolymers, chitosan, derived from chitin and composed of D-glucosamine and N-acetylglucosamine units, deserves special attention thanks to its biological and functional properties. However, its poor solubility in water limits its applications the biomedical field. Therefore, there is a growing interest regarding the application of low molecular weight (Mw) chitosan derivatives.

In this study, chitin derived from shrimp shells was deacetylated to chitosan (Ch) and varying Mw and acetylation degree (AD) chitosan depolymerization products (CDP) were prepared. The obtained chitosan and CDP were then applied to develop Ch/CDP-based films, to be applied as drug delivery systems. Blend films physicochemical properties in terms of color, light barrier behavior, microstructure, functional, mechanical and thermal behaviors. and their biological activities and in vitro drug release of ciprofloxacin (CFX), as a model, from these films, were also studied. As compared to Ch-film, most physicochemical and biological properties of Ch/CDP-based films were enhanced, depending on CDP-Mw and AD. SEM micrographs revealed homogenous and smooth surface. Further, to assess their in vitro release behavior, loaded-CFX Ch/CDP-based films were prepared and crosslinked with glutaraldehyde. Expect of elongation at break, crosslinked CFX-loaded films showed increased optical, water resistance, tensile strength and thermal properties, as compared to unloaded films. FTIR spectra of crosslinked films proved interactions by hydrogen bound between chitosan, CDP and CFX, and rough crosslinked films surfaces were observed in SEM micrographs. The CFX-release profiles were consistent with swelling studies showing that Ch/CDP-based films can release CFX for up to 54% in 6 and 24 h, at pH 1.2 and 7.4, respectively.

Interestingly, crosslinked Ch/CDP-based films could be applied as a promising pH-sensitive carrier for drug controlled-release for biomedical applications.

POLYSACCHARIDE-BASED MOLECULARLY IMPRINTED POLYMERS FOR THERAPEUTIC USE: A NEW APPROACH IN THE TREATMENT OF PHENYLKETONURIA

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Phenylketonuria (PKU) is an autosomal recessive disorder consisting of an inborn error of metabolism involving phenylalanine hydroxylase (PAH) gene mutations. This enzyme catalyzes the conversion of L- phenylalanine (Phe) into L-tyrosine (L-Tyr) in the presence of tetrahydrobiopterin (BH4). Defective PAH, in untreated PKU patients, results in a Phe accumulation in blood and tissues, which can lead to neurological damage and disorders including intellectual disability, microcephaly, seizures and psychiatric and behavioral problems. The main treatment for PKU is a low-protein diet that completely avoids high-protein foods and carefully measures many other foods. However, this rigorous diet can result complicated, especially for neonates born with PKU, expensive and can cause psychological and neurological issues to the patients. The inhibition of GI tract absorption of phenylalanine can represent a possible new approach. We designed a phenylalanine imprinted polymer able to selectively recognize and bind the aminoacid. The polymerization was carried out in water using pullulan and carrageenan as functional polymers and glutaraldehyde as crosslinker. A polymeric material made from natural polymers was successfully prepared by the casting method. The ability of the polymer to recognize and bind the template in aqueous media was evaluated. L-Tyrosine was used as functional analogue to test the specificity of the material. The imprinted polymer bound more Phenylalanine than the non-imprinted ones. Binding studies in the presence of Aspartame, an artificial non-saccharide obtained by condensation of aspartic acid and phenylalanine, were also carried out. The material was also characterized in terms of water regain ability, non-specific adsorption of proteins, in vitro bioavailability in simulated gastric and intestinal fluids and cytotoxicity, confirming the hydrophilic properties of the material and its biocompatibility. These polymers represent a potential green and water compatible polysaccharide-based system for recognition and separation of phenylalanine in PKU patients.

NOVEL ALGINATE HYDROGEL-BASED INKS WITH CURCUMIN LOADED-CELLULOSE ACETATE PARTICLES FOR THE 3D PRINTING OF DRUG RELEASING SYSTEMS

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In this work, new ink formulations were developed for 3D-printing of advanced 3D-constructs for drug release, based on an alginate (ALG) hydrogel (4% (w/V)) and curcumin-loaded spherical cellulose acetate particles (CApCUR). These particles act as modifiers of the rheological and mechanical properties of the inks and constructs, while simultaneously conferring the 3D-printed structures with drug-releasing capabilities. The particles showed diameters of 749 ± 232 nm and revealed no cytotoxic effect against HaCaT cells after 24h for concentrations of 1, 5 and 10% wt.%, with cell viabilities of 96.7 ± 8.4%, 88.9 ± 3.8% and 83.3 ± 3.4%, respectively. The presence of CApCUR in the inks improved their rheological features, with increased shear stress and shear viscosity, and the fully crosslinked hydrogels have a G' modulus higher than the G'', confirming their solid-like nature. Additionally, fully crosslinked hydrogels also have slightly improved mechanical performance, with higher compressive stress and Young's modulus with increasing CApCUR concentrations, with ALG showing 2.43 ± 0.84 MPa; and 2.82 ± 0.60 MPa, 2.99 ± 0.91 MPa and 3.28 ± 0.79 MPa for CApCUR concentrations of 1%, 5% and 10%, respectively. These hydrogels demonstrate no cytotoxic effect against HaCaT cells for 24h, 48h and 72h, with cell viabilities always above 80%.
The constructs obtained from the novel inks show higher resolution and definition of the grid-like structure, and the notorious yellow colour of curcumin. Moreover, the new hydrogels effectively release curcumin into the media, with nearly 50% of cumulative release after 24h at 37 ºC in PBS, confirming their drug-delivery potential. These constructs could be used for different dermal applications, for instance for wound healing.

HYALURONIC ACID BASED NANOPARTICLES: CAN THE HYALURONIC ACID MOLECULAR WEIGTH AFFECT THE BIOLOGICAL BEHAVIOUR?

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Hyaluronic acid-based nanoparticles (HA NPs) can be used to transport drugs to cells overexpressing HA receptors (CD44) since they combine the low toxicity of the nano-vehicle, the retention of the payload integrity with the receptor-mediated internalization. HA properties play a crucial, but sometimes unclear, role in managing the formation and stability of the NPs, the cell interactions and ultimately the payload entrapment efficacy. In this work special attention was paid to the cellular response to HA NPs. HA NPs manufacturing was performed by a microfluidics platform starting from HA with different molecular weight (Mw 280, 540, 820 kDa) through polyelectrolyte complexation with chitosan (CS). HA/CS NPs with comparable physical features (size of around 200 nm and PDI < 0.2) were produced and only the effects of HA Mw on CD44-overexpressing cells (human mesenchymal stem cells, hMSCs) were investigated. This work provides evidence of the HA/CS NPs biocompatibility and their effect on cell proliferation. Endocytic mechanisms used to enter hMSCs was investigated. Results showed the notable role of CD44 and the evident effect of HA Mw in the NPs internalization. HA/CS NPs uptake occurs via different endocytic pathways simultaneously and most notably NPs with 280 kDa HA were internalized by clathrin-mediated endocytosis instead NPs with 820 kDa HA revealed a greater contribution of caveolae and cytoskeleton components. Lastly, effect of HA Mw on the encapsulation efficiency was evaluated by using Myoglobin as model macromolecule and encapsulation efficiency was always higher than 50%.

FREEZE DRYING VERSUS SPRAY DRYING TO OBTAIN GRAPEFRUIT INTEGROPECTIN POWDER

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Background: IntegroPectin is a new family of citrus pectins of broad biological activity obtained via hydrodynamic cavitation of citrus biowaste carried out in water only. These pectins have already been isolated by freeze drying, a time-consuming and expensive procedure difficult to be scaled up. Since recently the spray drying technique has been greatly appreciated for food and pharmaceutical applications, this work proposes a comparison between Grapefruit IntegroPectin powders isolated via spray drying (SD) and via freeze drying (FD).
Methods: A mini Spray Dryer B-290 with B-295 inert loop (Büchi) and a FreeZone 2.5 Liter freeze dry system (Labconco) were used. Dried powders comparison was carried out in terms of total phenolic content (Folin-Ciocalteu), amount of the most representative flavonoids (HPLC-DAD analysis), radical scavenging activity (DPPH assay), total protein content (Bradford assay) and pH on the re-dissolved powders.
Results and discussion: the optimized SD process led to a very high yield (>95%) referred to the fully quantitative yield of FD process and resulted extremely quick and thus advantageous (30-50 min/100 mL of water extract versus 3 days/30 mL, respectively). The two resulting IntegroPectin powders displayed analogous Naringin and Hesperidin content and similar pH in water solution (≈4-5). The total phenolic content and the antioxidant power of the SD IntegroPectin were slightly higher than those of the FD powder. Instead, in the SD sample the protein content resulted nearly 4 times lower than the FD one, probably due to denaturation which might occur at the relatively high temperature required for the SD process. This could be favourable to achieve long-term protection of the biophenols contained in the powder.
Conclusions: considering the broad-spectrum potentiality of these new pectins from citrus waste, the spray drying technique results effective, convenient and easily scalable with less technical effort and costs than the freeze drying process.

TEMPO-OXIDIZED CELLULOSE NANOCRYSTALS FROM NOVEL SUSTAINABLE BIORESOURCE FOR FUNCTIONAL DRUG DELIVERY EXCIPIENT

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Background: The potential of wild Musa spp. pseudostem as a novel sustainable source for the isolation of cellulose nanocrystal (CNCs) was investigated.
Methods: CNCs were isolated from by TEMPO-mediated oxidation under continuous stirring and monitoring the pH at 10 followed by ultrasonication. The isolated CNCs were then characterized through various parameters including TEM, FTIR and Zeta potential. The thermal stability was also investigated by TGA while the crystallinity was determined through XRD spectroscopy.
Results and Discussion: After completion of the oxidation and ultrasonication, a highly viscous cellulose nanocrystal suspension was obtained. The solid content of the CNC was 5.56% w/w. Zeta potential was found to be -51.2 mV with average hydrodynamic diameter 1295 nm. TEM analysis has shown that the isolated CNCs were mostly spherical in shape and some agglomerations were also observed. The spherical particles were found to exhibit diameter less than 200 nm. The FTIR spectra shows carboxylate band at 1608.76 cm-1 and a band at 1772.19 cm-1 attributed to carbonyl group of an ester moiety apart from the characteristic carbohydrate bands. X-ray diffraction study indicate the percent crystallinity to be at 71.56%. Results from the analysis have shown that the pseudostem of wild Musa spp. could be a potential, sustainable source of CNCs for functional excipient in drug delivery.

HYALURONIC ACID-COATED PLGA-BASED NANOSYSTEM FOR CD-44 MEDIATED ANTINOCICEPTIVE ACTIVITY

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Millions of people suffer from chronic pain induced by nerve injury. It has been challenging to understand the neuronal response responsible for this pain. However, nervous and immune systems go hand in hand in causing neuropathic pain. The immune system's first response is to induce inflammation at the site of injury, and macrophages have been recognised to play an important role in the subsequent modulation of neuropathic pain. Hyaluronic acid (HA) is a well-known binder for the CD-44 receptor on classically activated M1-macrophages. This targeted approach of HA can be used with the biocompatibility of PLGA to encapsulate drugs that modulate pain and inflammation. Charge interaction is used for the binding of HA and PLGA with assistance from CTAB. The varied molecular weight and concentration of HA determine the size and charge of the nanosystem. Successful coating of HA on the nanosystem is determined by the final charge and the nanosize is optimised for effective macrophegeal uptake.