MICROGELS BASED ON POLYSACCHARIDES OBTAINED BY ENZYMATIC CROSSLINKING IN EMULSION FOR DRUG DELIVERY SYSTEMS

Virginie Dulong
University of Rouen Normandie - PBS UMR 6270 CNRS, France

Microgels are cross-linked polymer networks combining the interesting characteristics of hydrogels such as softness and high-water content with those of particles as high surface area and small size. Thus, microgels found multiple applications and especially in drug or biomolecule delivery because of their high capacity to protect sensitive (macro)molecules from adverse environments. The use of polysaccharides as polymer network for microgel synthesis is of great interest due to their biocompatible and biodegradable character.
In this study, carboxymethylpullulan grafted with ferulic acid (FA) is enzymatically cross-linked with laccase (by formation of FA dimers forming cross-linking nodes) by the reverse water-in-oil emulsion technique to obtain microgels with size from 40 to 200 µm. We obtain microgels with perfect spherical shape with size depending on laccase activity used and on dispersion time. We show that if the reaction kinetics is too slow or too fast, the size distribution of microgels is wide as well as with dispersion time lower than 5 min. Since the size of microgels depends on the size of water droplets, when the cross-linking reaction is too fast, microgels are formed before the end of dispersion time and before standardization of droplets size. Fluorescence spectroscopy of different probes (pyrene, Nile red and curcumin) encapsulated in microgels shows the non-polar characteristics of hydrophobic microdomains formed by FA and its dimers. Encapsulation and release of curcumin or lidocaine used as drug models are studied in different buffers. Curcumin is well encapsulated but retained in microgels while lidocaine is released at 65-70% in 2h30 in buffer simulating the gastrointestinal tract and at 75-85% in 1h in acetate buffer pH 5.6 (skin pH) or PBS pH 6.9 (mouth pH). These microgels of natural origin produced by a green method could be used as drug delivery systems for oral administration or local skin applications.

DUAL-RESPONSIVE CHITOSAN-BASED MICROGELS FOR ORAL DRUG DELIVERY PURPOSES

Adley Rubira
State University of Maringá, Brasil

Chitosan (CS) is a natural polymer widely used in the production of materials due to its biocompatibility, biodegradability, and non-toxicity. This positively charged polysaccharide usually yields pH-responsive materials. The addition of magnetic nanoparticles to chitosan-based materials leads to multi-stimuli-responsive materials. This work employed water-in-oil emulsion polymerization to synthesize low-molecular-weight CS microparticles containing cobalt ferrite (CoFe2O4) for oral drug delivery purposes. Both CS and CoFe2O4 were previously modified with glycidyl methacrylate (GMA) to allow the formation of crosslinked microparticles, and CS was also functionalized with folic acid (FA) to improve the treatment. Scanning Electron Microscopy analysis confirmed the production of homogeneous round-shaped magnetic microparticles of diameter equal to 1.71 ± 0.61 μm, and FTIR results confirmed the formation of covalent bonds between the precursors. The zeta potential of the magnetic microparticles ranged from (19.6 ± 0.51) mV to (-13.2 ± 0.61) mV, reaching neutrality at pH ≈ 6.3. The controlled release assays showed that the magnetic field has little effect on the release of vitamin-B12 (used as a model drug) at pH 7.4. In this condition, the magnetic field led to a two-step release that lasted for 80 minutes. In the absence of a magnetic field, the matrix reached the release equilibrium within 30 minutes. The matrix displayed an intriguing behavior at pH 1.2. The presence of a magnetic field successfully controlled the Fickian diffusion of vitamin-B12 from the CS matrix, reaching the equilibrium after 60 minutes. In the absence of a magnetic field, the matrix released the drug in a complex and uncontrolled way. It confirms the efficiency of the magnetic field in controlling the drug release, especially at pH 1.2. Therefore, the matrix would possibly be an effective oral drug delivery system, especially for the treatment of ulcers. Furthermore, the high cell viability confirms the non-toxicity of the microgels.
Keywords: smart material; pH-responsive; magnetic-responsive; drug delivery device; controlled drug delivery; natural polymer; inorganic nanoparticle.

COLONIC DELIVERY OF BUDESONIDE BY SENSITIVE MULTIPLE TRIGGERS MICROSPHERES FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE IN PAEDIATRIC AGE

Angela Lopedota
University of Bari "Aldo Moro", Italy

Background
Drug therapies for inflammatory bowel disease (IBD) involve the use of corticosteroids, including budesonide (BUD). Colon-targeted drug delivery systems use the specific characteristics of the target organ. However, single-trigger release strategies have limitations due to gut variability; only by combining several triggers a colon-specific release can be guaranteed.
Methods
Matrix microspheres were prepared with either sodium alginate (time-responsive polymer) alone or in different ratios with Eudragit® FS 30D (pH-responsive polymer). BUD was loaded in a polymeric mixture as a suspension and was processed by prilling/vibration technique to obtain the microspheres (F0-F4). In addition, inulin (microbiota-responsive polymer) was added to produce further microspheres (F0i-F4i). All formulations were completely characterised.
Results
The microspheres F0-F4 showed very high yields and encapsulation efficiencies (EE% >85.40%). All the microspheres showed good flow properties, diameters smaller than 655 µm, homogeneous sizes and shapes, furthermore the X-ray scattering/microscopy characterization highlighted a homogeneous distribution of the drug in the microspheres. Swelling behaviour and release features were studied at different pH values and, on F3i showing better properties, a release experiment was also conducted in faecal medium. The studies demonstrated that the drug release from F3i was only 12.3%, keeping the formulation in media at pH 1.2 and successively at pH 6.8 for 2 hours respectively. After 2 hours in faecal medium the percentage of BUD released from that formulation was 65.3%.
Discussion (including limitations)
The yield and EE% values demonstrated the effectiveness of the process used; the diameter made the microspheres suitable for paediatric patients. Release studies in media at different pH showed ensured time- and pH-dependent BUD release. The release study in faecal medium performed on F3i showed a robust release of BUD into the colon due to their ability to respond to different independent triggers. Although some conditions present in IBD, as variation of pH, time gastrointestinal transit and unbalanced microbiota, seemed not influence the performance of these microspheres, nevertheless in vivo studies are still required.
Conclusion
BUD-loaded microspheres produced by the prilling technique are a promising system in terms of dosage flexibility, paediatric friendly, and potential colon-specific release thanks to the combination of different triggers.

ANTIMICROBIAL 2D-MATRICES BASED ON ALGINATE AS WOUND DRESSINGS

Benedetta Brugnoli
Sapienza University of Rome, Italy

Background
Several factors can affect wound healing, bacterial infection, above all, significantly contributing to the persistence of chronic wounds. In this context, wound dressings are considered essential devices since they can play an active role in tissue regeneration and wound closure.
Methods
Antimicrobial 2D-matrices based on sodium alginate (Alg), citric acid (CA, 9 mg/cm2) and xylitol (Xy) at different contents (10, 20, 30, 40% w/w) were prepared using solvent casting method and characterized for application as wound dressings.
Antimicrobial and antibiofilm activities were evaluated against four bacterial pathogens, 2 Gram positives and 2 Gram negatives.
Results and discussions
CA significantly improved water stability of the Alg 2D-matrixes presumably because of its ability to establish multipoint interactions with the matrix. Xy increased film ductility (elongation to break up to 35-45 %) and transparency while decreased water vapour transmission rate in a concentration-dependent manner.
AlgCA matrix showed a good in vitro antimicrobial activity against Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Staphylococcus epidermidis (inhibition halo of 12, 14, 26 and 8 mm, respectively). Moreover, a potential synergistic action between Xy and AC was observed in AlgXy30 matrix (containing Xy at 30% and CA at 9 mg/cm2) towards P. aeruginosa and S. aureus.
Conclusions
Among the developed alginate-based 2D-matrices, AlgXy30 exhibited the best physico-chemical and antimicrobial properties. The found synergistic activity between CA and Xy opens interesting perspectives in the development of wound dressings minimizing the risk of drug resistance.

SULFATED GLYCOSAMINOGLYCAN-LIKE SEMI-SYNTHETIC POLYSACCHARIDES FOR BIOMEDICAL PURPOSES

Emiliano Bedini
Università degli Studi di Napoli Federico II

Background:
Sulfated glycosaminoglycans (sGAGs) play key roles in many physiological and pathological processes such as immunity, angiogenesis, cancer, infectious diseases etc. Some of these bioactivities are exploited in already well established therapeutic treatments or are currently under development. However, sGAGs are typically obtained from animal sources. This causes several problems related to ethical, economical and ecological reasons as well as to the risk of batch contamination (e.g. heparin crisis in 2007). A solution could be the development of new strategies for the regioselective modification of polysaccharides from eco-sustainable sources (bacteria, algae, fungi) in order to fuel biomedical research with more and more non-animal derived sGAG analogues.
Methods:
The structural modification of natural polysaccharides to give sGAG-like semi-synthetic derivatives has been tackled through chemical methods, both via direct regioselective reactions or by exploiting suitably developed multi-step strategies based upon regioselective installation and cleavage of proper protecting groups. The semi-synthesized polysaccharides have been structurally characterized mainly by 2D-NMR techniques. Some of them have been already investigated for their biological activities.
Results:
A set of sGAG-like polysaccharides has been obtained, differentiating for the native polysaccharide source and for the kind (sulfation, phosphorylation), degree and pattern of derivatization.
Discussion:
The semi-synthetic access to non-animal sourced polysaccharides having a structure similar or analogous to sGAGs has been demonstrated to be feasible by developing tailored chemical strategies. Some of the obtained polysaccharide derivatives have been proved to perform similarly to their native counterparts in in vitro assays testing diverse bioactivities.
Conclusion:
In spite of the enormous availability of polysaccharides from sustainable sources, the exploration of the chemical space achievable by their regioselective modification is still in its infancy era. With our work we contribute to make some steps ahead in the field, in particular in the area of sGAG-like polysaccharides with potential interesting biomedical applications.

THE ACETYL-RICH CAPSULAR POLYSACCHARIDE ISOLATED FROM THE VESICULATING BACTERIUM SHEWANELLA VESICULOSA HM13

Angela Casillo
Università degli Studi di Napoli Federico II

Gram-negative bacteria produce Extracellular Membrane Vesicles (EMVs), small spheres (20–250 nm) released by the bacterial membrane and comprising phospholipids, lipopolysaccharides (LPSs), proteins, peptidoglycans, DNA and RNA. [1] EMVs play different roles in the physiology and pathogenicity of bacteria: biofilm formation, toxin delivery, antibiotic resistance, immunomodulation, stress response, horizontal gene transfer and communication among cells and species. Many studies have been conducted addressing the biogenesis and potential application of the EMVs. [2] However, while the exact function of these nanoparticles has been extensively investigated in pathogens, it is still largely underexplored in the marine and cold environment.[3] In addition, the physicochemical properties of these vesicles have been poorly investigated.
Here, we present our data about the identification and the detailed structural characterization of the capsular polysaccharide from both the cells and EMVs from the psychrotolerant bacterium Shewanella vesiculosa HM13 by NMR spectroscopy, chemical and physicochemical analyses. The polysaccharide consists of a pentasaccharide repeating unit containing neutral monosaccharides together with aminosugars, of which one has never been isolated from a natural source. Our results suggest that the polymer could be involved in biofilm formation through its adhesive properties since DLS measurements indicated a specific affinity for synthetic surfaces, such as those of polystyrene nanoparticles, and lipid bilayer mimics of bacterial membranes. The polymer also activates Caspases on colon cancer cells, making S. vesiculosa EMVs natural nanocarriers for drug delivery.

References

1. Roier S, et al. (2015) A basis for vaccine development: Comparative characterization of Haemophilus influenzae outer membrane vesicles. Int J Med Microbiol, 305, 298.
2. Toyofuku M, et al. (2017). Membrane vesicle-mediated bacterial communication. The ISME Journal, 11,1504.
3. Ellis, T.N., and Kuehn, M.J. (2010) Virulence and immunomodulatory roles of bacterial outer membrane vesicles. Microbiol Mol Biol Rev 74, 81.

STABILIZATION OF A CHLORELLA FUNCTIONAL EXTRACT INTO A MULTICOMPONENT SPRAY DRIED MICROSYSTEMS BASED ON OSA-MODIFIED STARCH AND INULIN

Tiziana Esposito
University of Salerno, Italy

Introduction

Chlorella, a eukaryotic, rigid cell-walled microalga has a Generally Recognized as Safe (GRAS) status and is approved as a nutraceutical (FSSAI 2016). The cell wall contains various complex substances in the presence of multiple layers such as carbohydrates, protein, lipid, inorganic salts and bioactive compounds. However, bioactive compounds, mainly lutein and various carotenoids, are chemically instable limiting an industrial approach. Encapsulation systems have gained significant interest in designing innovative health products, as they allow for the protection and delivery of bioactive ingredients that have health benefits but are unstable during processing, storage and in the upper gastrointestinal tract. The aim of the present study was to investigate the application of octenyl succinic anhydride (OSA) modified starch and inulin (IN) to stabilize an alcoholic Chlorella extract, rich in carotenoids, within a multicomponent solid microparticulate system by spray drying.

Materials & methods

Chlorella vulgaris biomass has been produced in a photobioreactor experimental system developed within the activities of the Green Mare project. The extract (CHLV) rich in carotenoids has been produced and characterized by the DIFARMA-UNISA research group within the Green Mare activities project. The optimized extract was stabilized by spray drying technology. All formulations have been subjected to chemical-physical, technological and stability studies.

Results

The technological approach applied to stabilize CHLV extract in a microparticulate powder form based on OSA-inulin (OSA-IN) matrix via spray drying led to obtaining high encapsulation efficiency and industrially scalable process yields. Furthermore, scanning electron microscopy revealed OSA/IN microparticles were free of pores or cracks to point out that the combination of wall materials was effective as coating agents. They had few wrinkled, smoother surfaces, providing lower permeability and extract better protection. The so-made spray-dried OSA-inulin microparticles were able to improve the extract stability, without using an antioxidant agent as an additive in formulation, avoiding lutein degradation until 28 days under harsh storage conditions.

GLYCOGEN NANOFACTORY: TOWARDS ADVANCED BIOLOGICAL MATERIALS

Francesca Cavalieri
University of Rome "Tor Vergata" Italy, RMIT University, Australia.

Glycogen is a unique biological polysaccharide nanoparticle fabricated by nature through a bottom-up approach. The physicochemical and biological properties of glycogen, such as hydrophilicity, colloidal stability, degradability, toxicity, immunogenicity, and drug loading capacity, can be tailored using a variety chemical modification and conjugation approaches.
In this work, glycogen nanoparticles, sourced from animals and plants, were engineered to obtain nanoconstructs for intracellular delivery of nucleic acids and chemotherapeutics. These constructs were carefully designed to efficiently penetrate 3D tumour microtissues and deliver the drug payload. The interactions of glycogen-based nanoconstructs with peripheral blood mononuclear cells isolated from human blood and nanoconstructs in vivo biodistribution were also analyzed. In addition, super resolution microscopy techniques enabled the study of glycogen-based nanoconstructs properties inside the cells with improved spatial and temporal resolution. Our work (1-3) highlights the potential of glycogen as biological nanoparticle that can be easily engineered as a functional platform for the safe delivery of nucleic acids and drugs without eliciting immune cell activation and cytotoxic effects.
1. Nanoscale, 2022,14, 3452-3466.
2. Advanced Materials, 2020, 32 (18), 1904625.
3. Biomaterials 2018, 176, 34-49