Dextrin: a platform for the development of drug delivery systems

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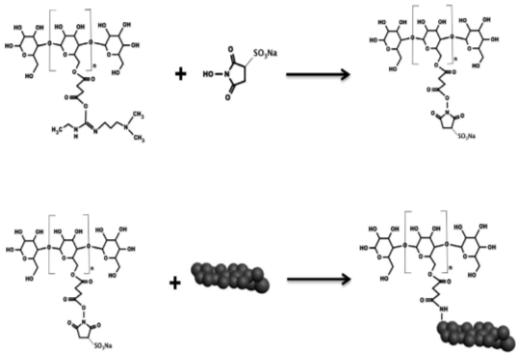

Dextrin is a biocompatible polysaccharide that can be suited for the conjugation with bioactive agents while enabling controlled release at the target site. LLKKK18, an LL37 human cathelicidin analog, has been engineered to enhance antimicrobial properties and decrease toxicity. In this work, chemically modified dextrin bearing a carboxylic group has been produced to allow the conjugation with LLKKK18 for the treatment of osteomyelitis. FT-IR spectra confirmed dextrin modification while maximum degradability was achieved after approximately 2h. A batch of 30% mol modification was used to conjugate with LLKKK18 and incubated *in vitro* with *Staphylococcus aureus*. Conjugates incubated with α-amylase were more effective in killing bacteria when compared to conjugates with intact bonds, emphasizing the protective effect of dextrin and indicating a controlled peptide release.

Dextrin is glucose polymer, generally regarded as safe (GRAS), obtained from partial hydrolysis of starch. It is a biocompatible material, non-immunogenic and degradable *in vivo* by α-amylases. Regarding the biomedical field, the polymer has been shown to envelope a protein thus masking its bioactivity, while enabling controlled restoration of activity at the target site by triggered polymer degradation [1]. Moreover, in previous work, we have demonstrated the conjugation of the antimicrobial LLKKK18 peptide with a carboxyl group created in the dextrin backbone [2].

Antimicrobial peptides (AMPs) are part of the innate immune system with potential as novel therapeutic agents due to its high spectrum of antimicrobial activity and low propensity for bacteria to developing resistance [3]. The bactericidal effect of LL37, the only known human cathelicidin, has been reported [4]. LLKKK18, an LL37 analog, has been engineered to enhance antimicrobial properties and decrease toxicity, being three-fold more effective in the killing of mycobacteria than LL37. Despite these advantages, exogenous administration is limited by enzymatic degradation, ultimately leading to an unsuccessful local delivery of AMPs.

Osteomyelitis is an inflammation of the bone triggered by an infection, which results in inflammatory destruction and tissue necrosis. The antimicrobial effect of LLKKK18 associated to a proper nanocarrier, emerges as an innovative and promising solution to treat this pathological condition.

In this work we hypothesized that the modified polymer would be slowly degraded by amylases, releasing the peptide in a controlled fashion avoiding its early degradation, thus improving antimicrobial effectiveness. The activity of the conjugates obtained was assessed *in vitro*, using a relevant strain mostly responsible for osteomyelitis, *Staphylococcus aureus*.

Dextrin was succinoylated using a modified version of a previously described method [5], based on the introduction of a carboxyl group in the dextrin backbone via an ester linkage, in a 4-dimethylaminopyridine (DMAP)-catalyzed reaction. The ratio of reagents used was optimized to yield a modified dextrin that: (a) would have enough functional groups to bind LLKKK18, and (b) would be able to delay degradation by amylases in order to provide a sustained release of the peptide. Modification % of several batches was confirmed by FT-IR and quantified by titration with sodium hydroxide. Succinoylated dextrin (DexSuc) spectrum showed a transmittance peak around 1729 cm^{-1} that is typical for the ester groups inserted in the dextrin backbone [6], while being absent in the unmodified dextrin. Titration allowed the quantification of the degree of substitution of batches with different degrees of succinoylation. In general, DexSuc degradation by dinitrosalicylic colorimetric method took around 2h, and increasing % modification slowed the degradation rate. LLKKK18 was then conjugated with dextrin [5], based on the binding of the peptide free amines to the free carboxyl groups in the succinoylated dextrin backbone, which results in the formation of an amide (as shown in the Graphical Abstract).

In vitro studies with *S. aureus* showed that incubation of conjugates with α-amylase increased the microbicidal activity as compared to intact conjugates, emphasizing the protective effect of dextrin and indicating a controlled peptide release. In fact, conjugates with α-amylase and intact conjugates corresponding to a peptide concentration of 1.17 μg/mL, were able to reduce viability to 0% and 32.8%, respectively.
These results are promising and will soon be further investigated in a rat model of osteomyelitis.

Acknowledgements
The authors acknowledge the funding from FEDER and NORTE 2020 through the project nº 003262 titled "iBONE therapies: advanced solutions for bone regeneration". This study was supported by Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

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