**Controlled Delivery of Pan-PAD-inhibitor Cl-amidine using Poly (3-hydroxybutyrate) Microspheres.**

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**Abstract:**

This study deals with the process of optimization and synthesis of Poly(3-hydroxybutyrate) microspheres with encapsulated Cl-amidine. Cl-amidine is an inhibitor of peptidylarginine deiminases (PADs), a group of calcium-dependent enzymes, which play critical roles in a number of pathologies, including autoimmune and neurodegenerative diseases, as well as cancer. While Cl-amidine application has been assessed in a number of *in vitro* and *in vivo* models, methods of controlled release delivery remain to be investigated. P(3HB) microspheres have proven to be an effective delivery system for several compounds applied in antimicrobial, wound healing, cancer, cardiovascular and regenerative disease models. In the current study, P(3HB) microspheres with encapsulated Cl-amidine were produced in a size ranging from ~4-5µm and characterized for surface morphology, porosity, hydrophobicity and protein adsorption, in comparison with empty P(3HB) microspheres. Cl-amidine encapsulation in P(3HB) microspheres was optimized and these were found to be less hydrophobic, compared with the empty microspheres and subsequently adsorbed a lower amount of protein on their surface. The release kinetics of Cl-amidine from the microspheres were assessed *in vitro* and expressed as a function of encapsulation efficiency. There was a burst release of ~50% Cl-amidine in the first 24 hours and a zero order release from that point up to 16 days, at which time point ~93% of the drug had been released. As Cl-amidine has been associated with anti-cancer effects, the Cl-amidine encapsulated microspheres were assessed for the inhibition of the Vascular Endothelial Growth Factor (VEGF) expression in the mammalian breast cancer cell line SK-BR-3, also in the presence of the anti-proliferative drug rapamycin. The cytotoxicity of the combinatorial effect of rapamycin with Cl-amidine encapsulated P(3HB) microspheres was found to be 3.5% more effective within a 24 hour time period. The cells treated with Cl-amidine encapsulated microspheres alone, were found to have 36.5% reduction in VEGF expression when compared with untreated SK-BR-3 cells. This indicates that controlled release of Cl-amidine from P(3HB) microspheres may affect tumor vascularization, growth and metastasis via regulation of VEGF. Furthermore, it has synergistic effect with chemotherapeutic agents, such as rapamycin. In addition to putative application in cancer, controlled delivery of Cl-amidine may be relevant ​​for targeted ​​application in​​ CNS pathologies ​​where​​ Cl-amidine application ​​has​​ previously been shown to be neuroprotective. Using controlled drug-delivery of Cl-amidine encapsulated in Poly(3-hydroxybutyrate) microspheres may be a promising novel strategy for application in a number of PAD-associated pathologies.

**Biography of presenting author**

Miss Dina Ahmed Khaled Ahmed, MSc holder in pharmacology and pharmacogenomics from the University of Westminster, London in 2017 where she was invited as a visiting researcher in 2018 where she developed a first generation polymeric delivery system for the anti-neurodegenerative drug, Cl-amidine. Besides, she is a BSc holder in Clinical Pharmacy and Pharmaceutical Sciences from faculty of pharmacy, Cairo University, Egypt in 2014. Dina has worked as a research and teaching assistant to undergraduate pharmacy students at Modern and New Giza Universities in Egypt from 2014-2016, then from 2019-2021. Currently, she is working as a research assistant at the Stem Cell and Regenerative Medicine Department at Egypt Center for Research and Regenerative Medicine (ECRRM) as well as being a registered PhD student at the School of Life Sciences, University of Westminster, London.

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