On the Use of Dexamethasone Loaded Liposomes to Induce the Osteogenic Differentiation of Human Mesenchymal Stem Cells

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Introduction: Liposomes are well-established carrier systems, presenting significant advantages over other nanoparticle-based drug delivery systems. The advantages include a high load carrying capacity, low cytotoxicity, and a versatile nature in terms of possible formulations and functionalization. Mesenchymal stem cells (MSCs) have received considerable attention by the scientific community because of their potential of expansion and the ability to differentiate into various mesodermal tissues. The main objective of the present study is to evaluate the efficacy of liposomes as carriers of a functional bioactive agent, dexamethasone (Dex). In particular, we aim at validating those carriers in the induction of the osteogenic differentiation of mesenchymal stem cells (MSCs).

Methods: Six Dex-loaded liposome formulations (Table 1) were produced by the lipid film method: formulations A to D were used to study the effect of adding cholesterol (Chol) and Dex in the liposome bilayer. Formulation E was used to perform the release study of Dex from liposomes. Formulation F was used for biological assays.

Table 1- Liposome formulations

	Dex	HSPC	Chol	DSPE-PEG	PE-Rho
A	0.25	2	-	-	-
B	0.50	2	-	-	-
C	0.25	2	0.1	-	-
D	0.25	2	1	-	-
E	0.25	2	0.1	0.1	-
F	0.25	2	0.1	0.1	0.02

Particle size distribution was determined by dynamic light scattering. The liposome morphology was analyzed by SEM and STEM. The concentration of Dex was determined by UV-VIS spectroscopy at 247 nm. The release of Dex from the loaded liposomes was studied using a dialysis method in PBS at 37ºC. The effect of Dex-loaded liposomes was assessed by the study of viability, proliferation, protein synthesis and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs).

Results: We optimized the lipid formulation to successfully encapsulate Dex. The liposomes showed a monodisperse distribution of sizes. Dex encapsulation studies demonstrate that the presence of Chol decreases the Dex loading capacity of the liposome. An in vitro release study demonstrated an initial burst release within 12 hours (Figure 1). Following the initial release, a slower release was observed until 6 days. Afterwards, Dex continues to be released at a slower but steady rate until day 21. The biological results showed that the Dex-loaded liposomes do not have any cytotoxic effect and, more importantly, are able to promote an earlier induction of hBMSCs differentiation into the osteogenic lineage, as demonstrated by the expression of osteoblastic markers at the phenotypic (Figure 2) and at the genotypic levels.

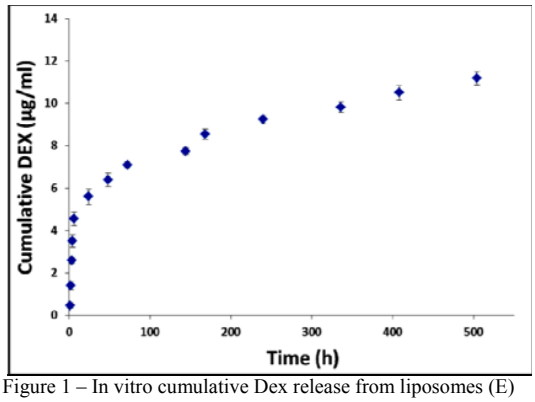

Figure 1 – In vitro cumulative Dex release from liposomes (E)

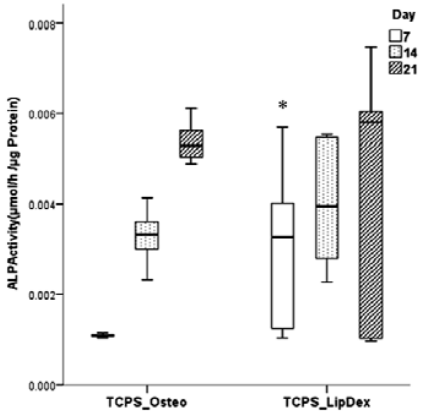

Figure 2 – ALP activity by hBMSCs cultured on TCPS_Osteo and TCPS_LipDex.

Conclusions: We conclude that liposomes are effective as a bioactive agent release system. Furthermore, the Dex-loaded liposomes represent a biological or nature-inspired nanoparticle strategy with potential interest for tissue engineering and regenerative medicine.

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