Chrono-tailored Drug Delivery: Harnessing Circadian Rhythms in Microneedle-Mediated Nanoparticle Delivery

Christine T. Butler[1], Cloé Payet[1], Brenton Cavangh[1], Oran D. Kennedy[1], Sally Ann Cryan[1], Ryan F. Donnelly[2], Annie M. Curtis[1]

[1]School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland, Dublin, Ireland; [2] School of Pharmacy, Queens University Belfast, Belfast, NI;

Background: The body's central clock coordinates peripheral clocks in our tissues and cells, aligning the circadian rhythm, a 24-hour cycle, with external cues such as light and food. Research has illustrated that our immune system is strongly controlled by our 24-hour circadian clock, and growing evidence shows that time-of-day vaccination or drug administration may lead to higher efficacy and reduced side effects.

Dendritic cells (DCs) and other skin-resident cells, key targets for drug delivery, process and transport therapeutic compounds into the circulation and to target tissues. Our lab has shown that the cellular clock regulates processing pathways in dendritic cells via mitochondrial morphology and metabolism. The skin has a rich network of cells and blood vessels, which can be efficiently targeted with microneedles (MNs) for intradermal drug delivery. We propose dissolvable MNs for time-specific intradermal drug delivery of nanoparticles (NPs), leveraging the circadian properties of skin cells to enhance drug delivery and therapeutic efficacy.

Methods: Bone marrow-derived DCs (BMDCs) and murine fibroblasts were isolated, and the serum shock method was used to synchronise them according to the time of day. The specific cell type was isolated and plated in a 24-well plate at $2.5x10^5$ cells per well in complete DMEM. After 24 hours, cells were shocked with 50% Horse Serum and 50% DMEM for 2 hours. Fresh, complete DMEM was then replaced. Cells were then treated with Cy5-tagged PLA/ PLGA nanoparticles NPs (100ug/ml) at 12, 24 and 36 hours post-serum shock. Unsynchronised cells were also treated. The cells were then placed in the CellDiscoverer7 imaged for cellular uptake of NP every 15 minutes over 5 hours or analysed by flow cytometry for cellular uptake.

Results: BMDCs were serum shocked, and the expression of core clock genes was analysed by qPCR. Expression of clock genes such as *Bmal1, Per2, Rev-erba* and *Cry2* were all shown to oscillate, confirming that our BMDCs were synchronised. Using the CellDiscoverer7, the fold change in mean intensity fluorescence of our Cy5 tagged NP within the cell over 4 hours was calculated for each 15-minute visualisation. Two points were visualized in each well, and the mean intensity was calculated from these two images. We see a significant increase in NP uptake when NPs were added at 12hrs or 36hrs post serum shock compared to unsynchronised cells. This increase in uptake at 12hrs and 36hrs represents subjective dawn. Flow cytometry was also carried on serum shocked DCs treated with NPs for 4 hours. We similarly see an increase in uptake at subjective dawn (12hrs and 36hrs post serum shock). We performed this in murine fibroblasts; however, we did not see the same effect.

Conclusions: Our experiments reveal a novel circadian aspect in nanoparticle-based drug delivery systems. These experiments suggest a role for the clock in NP uptake by immune cells. Specifically, we demonstrate that NP uptake is time-of-day dependent in immune cells but not fibroblasts, with BMDCs showing increased uptake at subjective dawn. This circadian effect appears unique to immune cells, reinforcing the relevance of circadian rhythms in drug delivery. Future work will explore this across various cell types and elucidate the underlying mechanisms. Further, these findings will help to inform our chrono-tailored MN patch containing our NPs to enhance drug delivery and efficacy at the optimal time of day.