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| **Developing an in situ hyaluronic acid-tyramine hydrogel platform to deliver extracellular vesicles** |
| Yingchang Ma |
| School of Pharmacy, University College London, London, United Kingdom |
| **Background:** Extracellular vesicles (EVs) are small membrane vesicles secreted by cells, and have emerged as a promising alternative to cell-therapies due to their lack of immunogenicity and targeting capabilities. However, there are significant challenges with their low retention and short-lived therapeutic effects. This study aims to develop a hyaluronic acid–tyramine (HA-Tyr) hydrogel platform through in situ enzymatic crosslinking to determine whether incorporating EVs into these hydrogels can improve their retention time while maintaining their structures and bioactivity. |
| **Methods:** HA-Tyr hydrogel was formed using the oxidative coupling of tyramine moieties catalysed by hydrogen peroxide (H2O2) and horseradish peroxidase (HRP). The optimal composition of the hydrogel was determined by tuning the concentrations of H2O2 and HRP to reach the maximum crosslinking with the minimum cytotoxicity. The adult retinal pigment epithelial cell line-19 (ARPE-19) and H9c2 myoblasts were selected as in vitro models of retina and heart disease respectively. EVs harvested from HEK293T cells were isolated using differential ultracentrifugation and characterised by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and simple western blot. The cell internalisation of free EVs and EVs released from the hydrogels was analysed by the luciferase assay. The EV release profile was determined by CD9 ELISA kit. The stability of EVs after loaded into the hydrogels was assessed by NTA, TEM and simple western blot. |
| **Results:** The optimal composition of the hydrogel showed rapid gelation time (1 minute) when injected in situ, and no cytotoxicity to ARPE-19 and H9c2 cell lines. EVs were successfully isolated and incorporated into these hydrogels with evenly distribution. EVs showed extended release from the hydrogel for over 2 weeks in vitro. These EVs released showed improved stability compared to free EVs and can be internalised by the cells.  |
| **Conclusions:** HA-Tyr hydrogel provides a novel approach for the local delivery of EVs and optimises their retention and stability. Therefore, the sustained release mechanisms achieved through manipulating the formation of such hydrogels provide a key to unlocking the therapeutic potential held within EVs. |