THE TIMES THE SUNDAY TIMES GOOD UNIVERSITY GUIDE 2020 INSTITUTE OF TECHNOLOGY OF THE YEAR

AIT Research

Modification of hyaluronic acid for stereolithography 3D printing of hydrogel nerve conduits

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Introduction

The peripheral nervous system transmits signals from the brain to the limbs and organs of the body. Peripheral nerve injuries (PNIs) resulting from trauma, illness or cancer affect approximately 1 million people in Europe and the US per annum. Such injuries may result in gaps/erosion of the nerve which limits or ceases function in that part of the body and are reported in 15-40% of all trauma cases worldwide (1). Of those who undergo surgery, only 25 % regain full function (2). Current therapeutic options such as autografts and allografts involve transplanting a donor nerve to the injured site. However, despite autografts being considered the gold standard for peripheral nerve repair (PNR), the chance of full functional recovery remains low. Since HLA was first demonstrated to enhance PNR *in vivo* over 21 years ago, there has been an increasing focus on the use of HLA in the development of nerve conduits for PNR.

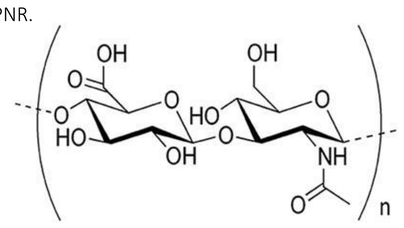


***Figure 1**. Structure of the HLA molecule (3)*

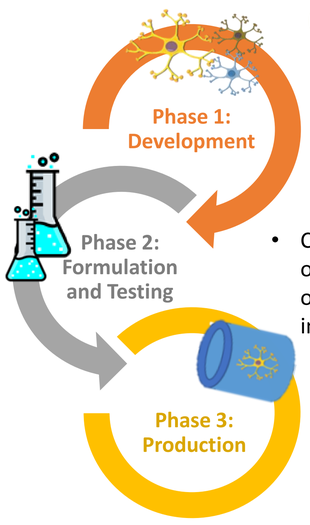
Much of this research has focused on *in vivo* studies due, in part, to the unique rheological properties of HLA posing an issue for *in vitro* analysis. The objective of this first initial phase was to undertake an *in-vitro* biological screen which would identify the optimal molecular weight of HLA to subject to further testing and to establish the cytotoxicity of HLA.

Given the physiochemical properties of HLA it was not possible to examine its cytotoxicity or neuro-proliferative/differentiation effects using standard *in-vitro* assay approaches. Therefore, it was necessary to design and optimise a novel *in-vitro* experimental approach which would allow us to assess the suitability of HLA in the presence and absence of various novel biological agents for nerve conduit design.

Aim

To produce a hydrogel nerve conduit which is inherently bioactive.

Objectives



- Advanced cell culture and bioassays to determine the optimal molecular weight of hyaluronic acid and the toxicity of hyaluronic acid in neuronal and glial cells- Completed.
- Currently in progress and involves the modification of hyaluronic acid and PEGDMA to achieve a degree of substitution which will not affect cell interactions.
- Stereolithography 3D printing of a multilayer hydrogel conduit.

Methods

1. Cell lines

Two continuous cell lines were used in this study, a glial cell line to represent the Schwann cells found in the PNS and a human neuronal cell line. Only data from the neuronal cell line is presented here.

Glial cell line:
The RT4-D6P2T cell line is an immortalized Schwann cell line derived from an N-ethyl-N-nitrosourea (ENU) induced rat peripheral neurotumour (5).

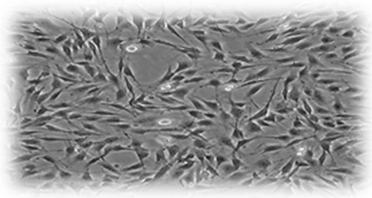


Figure 2. RT4 D6P2T (glial) cell line (5).

Neuronal cell line:
SH-SY5Y are an immortalized human catecholaminergic cell line routinely employed as a neuronal model *in vitro* and were used undifferentiated for this study (**6).**

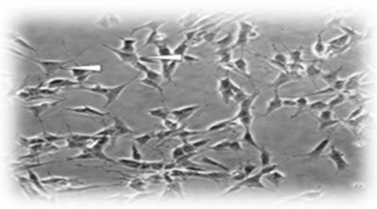


Figure 3. SH-SY5Y (neuronal) cell line **(6)**

2. Identification of optimal molecular weight of HLA

- HLA of molecular weights ranging from 30-50 kilodalton (kDa) to 2200 kDa were screened in neuronal and glial cells using the resazaurin and trypan blue exclusion (TBE) assays.

3. Cytotoxicity of HLA

- Once the optimal molecular weight of HLA was established, this was subjected to further screening with MTT, resazurin and TBE assays. These assays were modified and validated.

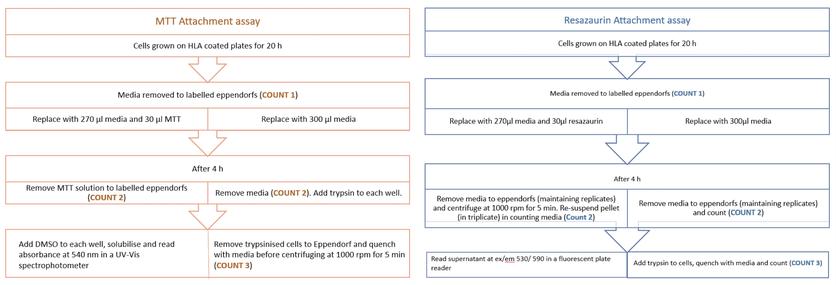


***Figure 4**. Methods flow chart for modified MTT and resazurin assays.*

Results

2. Identification of the optimal molecular weight of HLA

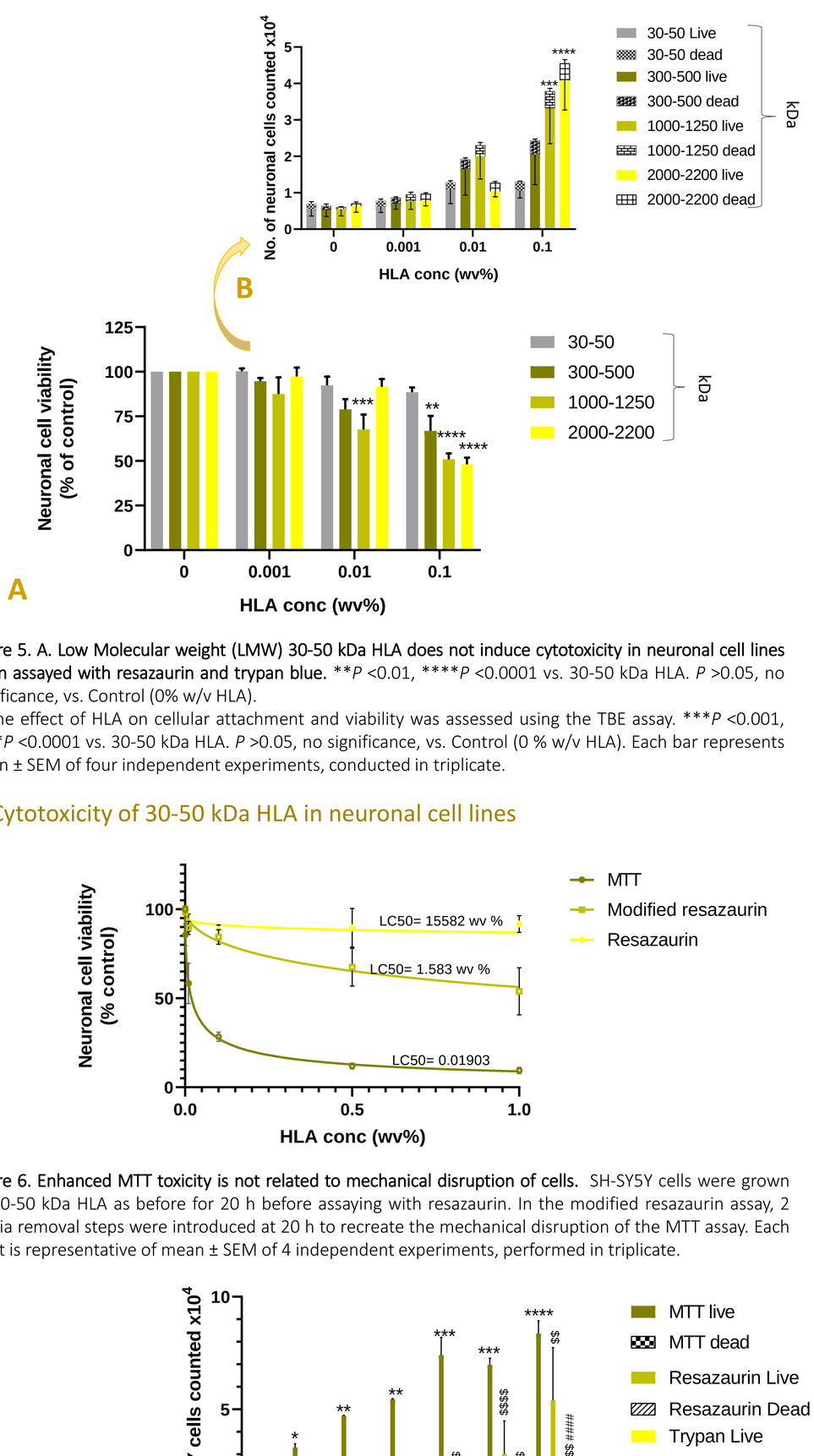


Figure 5. A. Low Molecular weight (LMW) 30-50 kDa HLA does not induce cytotoxicity in neuronal cell lines when assayed with resazaurin and trypan blue. ***P* <0.01, *****P* <0.0001 vs. 30-50 kDa HLA. *P* >0.05, no significance, vs. Control (0% w/v HLA).
B. The effect of HLA on cellular attachment and viability was assessed using the TBE assay. ****P* <0.001, *****P* <0.0001 vs. 30-50 kDa HLA. *P* >0.05, no significance, vs. Control (0 % w/v HLA). Each bar represents mean ± SEM of four independent experiments, conducted in triplicate.

3. Cytotoxicity of 30-50 kDa HLA in neuronal cell lines

Figure 6. Enhanced MTT toxicity is not related to mechanical disruption of cells. SH-SY5Y cells were grown on 30-50 kDa HLA as before for 20 h before assaying with resazaurin. In the modified resazaurin assay, 2 media removal steps were introduced at 20 h to recreate the mechanical disruption of the MTT assay. Each point is representative of mean ± SEM of 4 independent experiments, performed in triplicate.

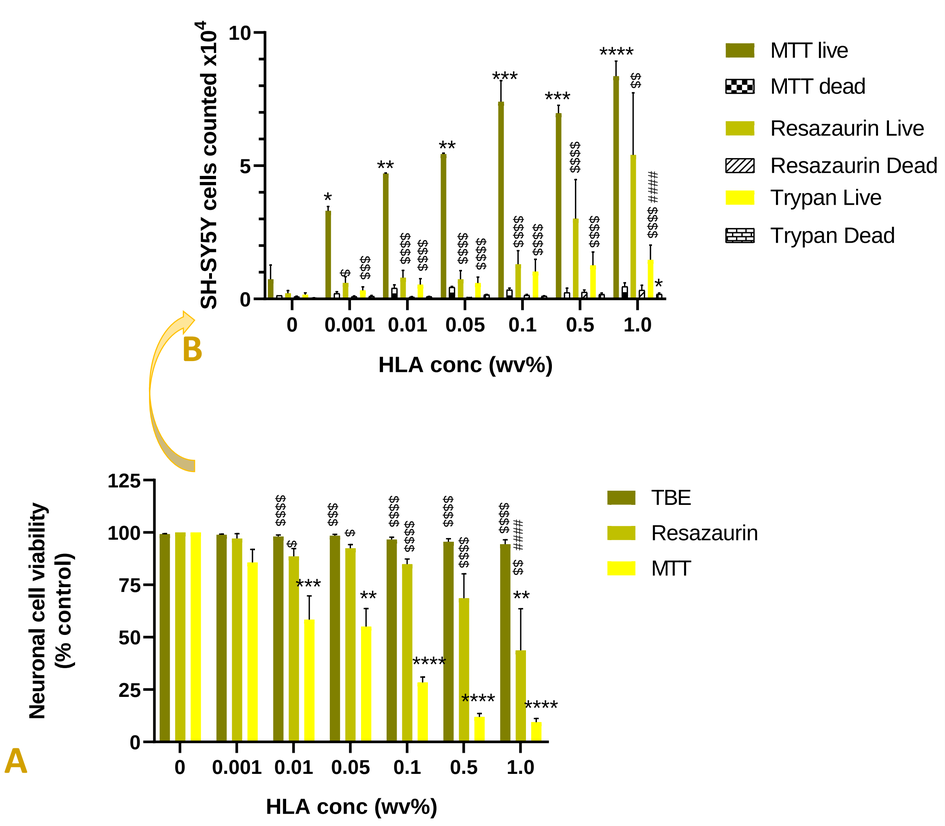


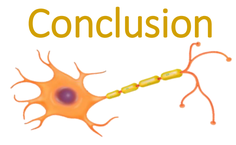
Figure 7. A. MTT enhances HLA toxicity in SH-SY5Y cells. SH-SY5Y cells were grown on 30-50 kDa HLA for 20 h before entering the MTT/resazurin/TBE protocol outlined in Fig. 4 (Count 3). Fig. 7 shows a dose-dependent decrease in cell viability in the presence of MTT and HLA (***P* <0.01, ****P* <0.001, *****P* <0.0001 vs. control). A significant decrease in cell viability was detected by resazurin in the presence of 1 w/v % HLA (***P* <0.01 vs. control). MTT detected significantly less viable cells when compared against both the resazurin reduction assay and the TBE assay ($*P* <0.05, $$*P* <0.01, $$$*P* <0.001, $$$$*P* <0.0001 vs. resazurin/ TBE). No significant decrease in cell viability was detected with TBE (*P* >0.05 vs control).
B. The number of detached cells counted for each condition at count 3 (Fig. 4). Each bar represents the mean ± SEM of at least two independent experiments conducted in triplicate.

Discussion

This poster represents the completion of Phase 1. Development. The purpose of this phase was to elucidate the optimal molecular weight of HLA to subject to further testing and to determine the cytotoxicity of HLA. The results presented here have shown that:

- 30-50 kDa hyaluronic acid was the optimal molecular weight for cell attachment as shown in Fig. 5
- MTT revealed toxicity of 30-50 kDa in both cell lines but SH-SY5Y cells appeared more susceptible
- These results could not be replicated with the resazurin assay despite MTT and resazurin working on a similar method of action, or the TBE bioassay
- It was determined that the overestimation of cytotoxicity was actually due to enhanced cell detachment during an MTT assay, and not cell death (fig. 7 B)
- This result was independent of differences in the basic assay methods.

Conclusion



In conclusion, it was determined that there was a potential synergistic relationship occurring between MTT and HLA which resulted in enhanced toxicity in neuronal cell lines.

Future work

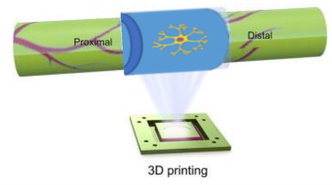
The research shown here is the subject of a manuscript currently in preparation and due for submission to Toxicology *In Vitro* in July 2020.

Phase 2: Formulation and testing is currently underway.

- HLA and PEGDMA are being thiolated and screened in neuronal and glial cell lines.
- Material properties will be evaluated.

Phase 3: Production

- Prototypes will be 3D printed using the modified polymers.
- Potential regeneration enhancing compounds will be screened for inclusion.
- Extensive preclinical testing will be performed.



Acknowledgements

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