EFFECTS OF BIOPOLYMERS AND NITRIC OXIDE RELEASE ON HEP G2 LIVER CELLS

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Abstract

Nitric oxide (NO) is endogenous to the body and used for multiple purposes, and as each role is discovered, it opens doors to the possibility for more applications. Research already performed has determined that the natural physiological flux of NO in the blood vessels is 0.5–4.0 × 10^{-10}mol cm^{-2} min^{-1}. Many studies now are focused on the application of NO by creating biopolymers, medicines, and other methods that will distribute NO at amounts within this range to localized areas of the body. Since these distribution values have been defined for some methods of release, it is now important to test the reaction of different cell types to the biomaterials and to the NO released. In this study we used Hep G2 liver cells, a line of liver tumor cells, which are very robust and established. They are also sustainable on a 2D growing surface and were chosen for the different secretions and proteins that can be measured for viability, such as albumin and cytokines. Our hypothesis is that because of the physiological functions of NO, the promotion of angiogenesis and the antibacterial effects may provide a better environment for the regeneration of liver cells and increase the regeneration rate.

Background

- Biomaterials are materials that are safe for use in the body and can be made into different forms for different medical applications. Some biomaterials are polymers such as poly(lactic-co-glycolic acid) (PLGA), which is biodegradable, and carbosil and polydimethylsiloxane (PDMS) which are not biodegradable.
- Nitric oxide (NO) is an endogenous, small gas molecule produced by endothelial cells of vessels in the body. NO acts as an antibacterial agent, promotes angiogenesis, and prevents thrombosis by inhibiting platelet adhesion and activation[1].
- Hep G2 cells are a line of liver tumor cells which are used to study liver function, growth, and regeneration. The liver is able to regenerate naturally by proliferating from current mature cells, while also maintaining normal metabolic functions [2].
- The NO releasing molecule S-nitroso-Nacetylpenicillamine (SNAP) is a synthetic molecule [1]. SNAP releases NO in different concentrations due to the material it is embedded in.
- SNAP is sensitive to light, temperature, and metal ions, each of which increase the rate of NO released [1].

Materials and Methods

- The UV-Vis Spectra was used to determine if UV light sterilization effected films containing SNAP. When SNAP degrades it can be detected by a lower absorbance.
- Films containing SNAP were sterilized under UV light for one hour and then incubated for three days to confirm that UV light exposure was an effective sterilization method.
- Water uptake percentages of multiple polymers were analyzed at 37° C by initial and final weight comparison. The polymers used were carbosil, PDMS, SP60-D60, and SG80A.
- SNAP films were made by solvent evaporation casting. Once dried, nitric oxide release of polymers were measured by chemiluminescence over the course of 3 days to compare polymer release rate at 37° C at 7.4 pH.
- Polymers were attached to plate wells and cells were added with MEM media. After a three day cell study, the DNA analysis was done using Pico Green and a plate reader to analyze cell adherence and determine optimal film. This was done twice to narrow down materials.

Results

UV Light Sterilized SNAP Films

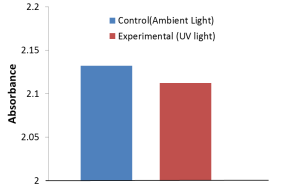

Fig. 1. The absorbance difference of the films signifies the amount of SNAP present in the film solution after one hour in either the ambient light or UV light. The UV light only degraded the SNAP by .02% in comparison to the ambient light.

Water Uptake Study of Biomaterials

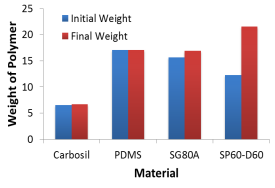

Fig. 2. Comparison of water uptake at 37° C, a characteristic of polymers that effects how NO is released.

Nitric Oxide Flux Comparison

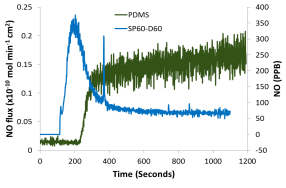

Fig. 3. Comparison of NO release profiles of a hydrophobic polymer, PDMS, versus the more hydrophilic polymer SP60-D60.

Nitric Oxide Flux of Carbosil

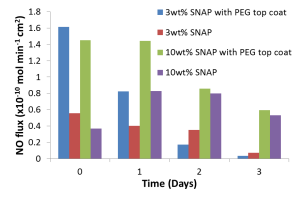

Fig. 4. A 48 well plate with films attached. This was used in the second application of cells to continue to test material characteristics. SNAP was incorporated in these films in different concentration to see the effects NO on Hep G2 cells.

Nitric Oxide Flux of PDMS

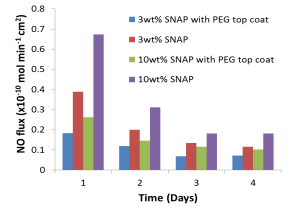

Fig. 5. NO release profiles of carbosil with different weight percentages of SNAP and with PEG, a immobilized protein top coat used for cell adherence.

Films Attached to Plate

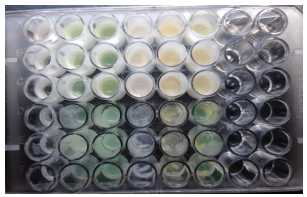

Fig. 6. NO release profiles of PDMS with different weight percentages of SNAP and with PEG, a protein top coat used for cell adherence.

Conclusion/Future Work

The goal of my project was to find the best polymer surface for cell growth and then incorporate SNAP to observe the effect of NO release on Hep G2 cells adhesion and proliferation. We have incorporated SNAP into some of the polymers to see if we could detect an effect, but the adherence of cells to many of these polymers is low because of hydrophobic surfaces so we have worked with immobilized PEG and we are currently working with a poly-L-lysine (PLL) top coat. Future studies are planned to continue working with carbosil and PDMS to find the optimal top coat for cell growth so that the interaction between NO and Hep G2 cells can be researched in greater depth.

References

[1] Brisbois EJ, Handa H, Bartlett RH, Meyerhoff ME., Biomaterials 2013; 34, 6957-6966.
[2] Michalopoulos, G. K., J Cell Physiol. 2007 November; 213(2): 286–300.

Acknowledgements

This material is based upon work supported by a National Science Foundation Research Experiences for Undergraduates (REU) site program under Grant No. 1359095.