Development of Engineered Microparticles for Probing Insulin Balls

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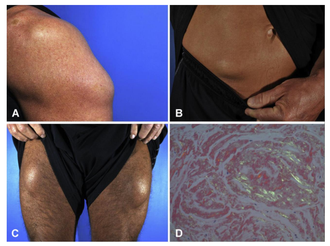
Introduction:

- Patients who chronically inject insulin, or are otherwise insulin dependent, may develop the condition: insulin-derived amyloidosis (Samlaska et al, 2020). This condition creates amyloids (clumps of unused insulin protein fibrils) at sites of injection (Gupta, 2015; Samlaska et al, 2020).



Amyloid deposits from repeated insulin use (Samlaska et al, 2020).

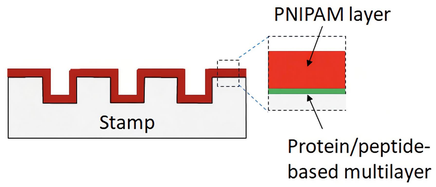
- It is known that at sites of injection, high levels of anti-insulin antibodies were observed (Samlaska et al, 2020). Additionally, it is noted that at sites of injection, monocyte-derived macrophages (blood-derived immune cells) aggregate at amyloid deposits (Gupta, 2015).
- Although it is known that this condition impairs insulin absorption and glycemic control, the cellular mechanisms that cause these impairments are both poorly understood and unknown.
- In attempts to understand the potential cellular-amyloid mechanisms behind insulin resistance, this project plans to utilize macrophages to phagocytose amyloid particles. These macrophages will then be observed for cellular chemical changes and physical membrane alterations.
- If any changes are observed, then it may imply that amyloids have the capability to influence cellular functions that lead to insulin-derived amyloidosis. This can include decreased lysosomal function, proliferated inflammation, increased insulin antibodies, and reduced ability to clear debris, which all may impair insulin efficacy in the body.

Methods:

- To have amyloids transported in macrophages and to observe any physical and chemical changes, a particle was needed that could transport the proteins, be observable inside of cells, be sensitive to pH changes, and be produced uniformly.
- PINIPAM (Poly(N-iso-propylacrylamide)) was used as the base due to its protein-transporting, particle-forming properties for macrophages to engulf. A preliminary PINIPAM and water mixture was used to test the desired ratio required to successfully stamp particles for that day.
- Once the mixture was prepared, the solution was applied to the stamps, which were then spin-coated to evenly distribute the mixture on the surface.
- Once spin coating was complete, particles were then placed on micro glass slides laid on heat pads set to heat at 90°-100° C.
- Stamps were then pressed for 3 seconds, solidifying the mixture and transferring most of the content on to the micro glass slide.
- This micro glass slide was then transferred to a microscope, where the particles would be compared for consistency, shape, and evenness. If the particles had no major deformities and were grossly similar, then the ratio of PINIPAM to water was used when synthesizing the fluorescent particles. If the particles did not seem similar in shape, then a new ratio of PINIPAM to water would be tested using the steps listed above until particles were grossly similar.

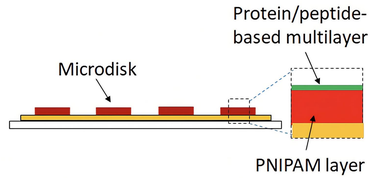
Acknowledgments:

- I would like to thank Masahiro Fukuda for his support and guidance throughout this project.

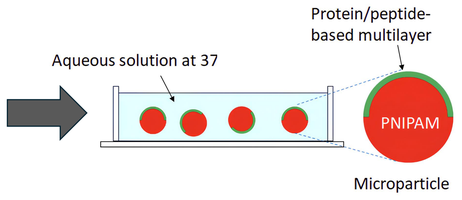


1. The PINIPAM and water solution evenly distributed on a stamp.

2. The PINIPAM and water solution being stamped onto a heated micro-glass slide.



3. Particles transferred to a micro glass slide after stamping.



4. Particles balling up when exposed to biological conditions.

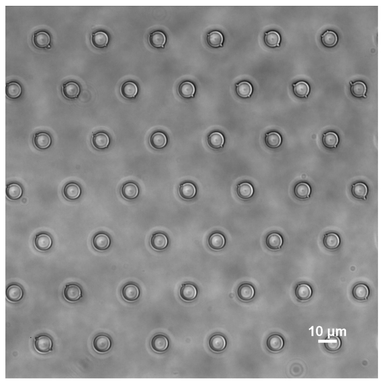


Figure 1: Particles exposed to visible light (no fluorescence).

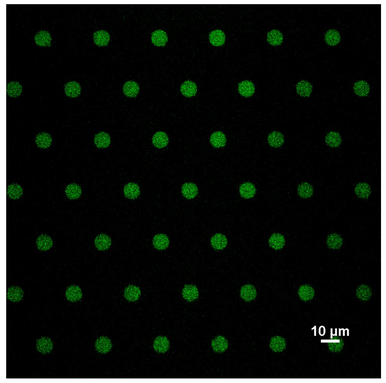


Figure 2: Particles exposed to blue light (FITC fluorescent).

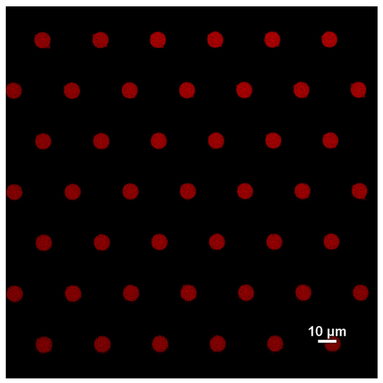


Figure 3: Particles exposed to yellow light (AF594 fluorescent).

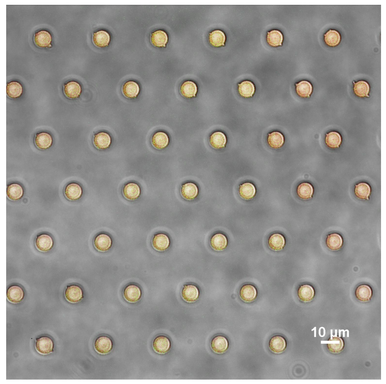


Figure 4: Overlapped images of particles exposed to blue light, yellow light, and visible light.

Methods (Continued):

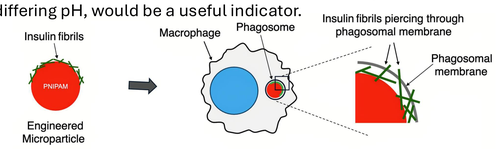
- Using the pre-established ratio, a new mixture composed of water and pre-conjugated PAH with AF594 (Alexa Fluor, red fluorescence) and FITC (Fluorescein isothiocyanate, pH sensitive, green fluorescence) was used to make particles using the previously listed steps.
- Once made, the new particles are imaged using microscopy. Evenness and uniformity are analyzed during this microscopy (Figure 1). In addition to this, the particles are tested for fluorescent capabilities utilizing blue light and yellow light wavelengths to expose fluorescence (Figures 2 and 3).
- To confirm uniform spread of fluorescent material, all images were overlaid on top of one another to compare the spread and evenness of each particle's color (Figure 4).

Results:

- Particles are largely uniform and even with only minor visible deformities.
- Every particle appears to have fluorescent properties utilizing pH-sensitive compounds. The fluorescence appears to be evenly distributed among the particles.
- FITC (pH sensitive), when exposed to blue light, reflects its expected color of green. AF594, when exposed to yellow light, reflects its expected color of red.

Discussion:

- Currently, no testing has been conducted using PINIPAM and amyloid particles for macrophage phagocytosis as the project is ongoing. However, preliminary results show that viable particles that are observable, uniform, able to transport proteins, and pH sensitive can be synthesized.
- As previously stated, PINIPAM can be used to carry a variety of proteins into a cell. With amyloids being proteins, this phenomenon will likely work for amyloids, too. Additionally, macrophages have previously phagocytosed PINIPAM and protein particles, implying that macrophages could do the same to PINIPAM and amyloid particles.
- Furthermore, each particle was successfully synthesized using pH-sensitive (FITC), fluorescent chemicals (AF594 and FITC). This is important for two reasons:
  - First, while untested here, it is very likely that once macrophages engulf the particles, the particles will remain observable due to the vibrant fluorescent characteristics of the chemicals used. This vibrance will likely be able to be seen through the thin membranes of macrophages when observed under procedural microscopy conditions.
  - Second, when tests are done using amyloids, one of the things being measured will be chemical changes within cellular membranes, such as lysosomes, which have a pH of 4.5-5.0. If the chemical contents within this lysosome were to change, for example, a membranous puncture caused by amyloid fibrils, then it would be possible that the pH would change. Having a chemical that can indicate this change, such as FITC vibrancy changes at differing pH, would be a useful indicator.



- With the successful particle synthesis, it is now possible to have amyloids observed under controlled laboratory conditions in conjunction with case studies, allowing for better control of other variables to more accurately assess whether amyloids are the cause behind the alterations in cellular mechanisms responsible for insulin resistance or not.

Resources:

Scan the code for the full list of sources.