Glass Surface Properties and Functionalization to Enhance Biocompatibility and Visibility

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Abstract

Glass made medical microdevices are used extensively in a broad range of biomedical applications. Design constraints for glass-based micro devices include low visibility when used in cells and organisms and non-specific binding of macromolecules to exposed glass surfaces, which often lead to reduced function. The objective of this study is to employ novel silanization strategies to modify glass surfaces to enhance visibility and biocompatibility. The specific aims of this study include (1) Covalently attach gold nanoparticles to glass surfaces to enhance visibility; (2) modify the glass surface with polyethylene glycol containing silanization agents to enhance biocompatibility. These aims were realized by coating the glass surface with polyethylene glycol (PEG) and gold-nanoparticles using EDC coupling chemistry. Successful modification of the glass surface requires that the glass surface be cleaned thoroughly to remove organic material from the glass surface prior to the coupling reaction. The glass surface is then silanized with silanization agents which are terminated with desired functional groups. PEG spacers link between the glass and the silanized gold-nanoparticles to coat the glass with a thin pink/purple layer of gold nanoparticles. Research to test the impact of the surface modification strategy on visibility and reduced non-specific binding of proteins to glass surfaces in model biological solutions and in zebrafish embryos is on-going.

Background

Design Constraints

- Low visibility when used in cells and organisms
- Non-specific binding of macromolecules to exposed glass surfaces, which often lead to reduced function

Biomedical Application

- Intracytoplasmic Sperm Injection (ICSI) as part of the In Vitro Fertilization (IVF) cycle
- Tissue-engineered organs/regenerative medicine
- Transgenic animals/plants

Figure 1: Injection into egg via industry standard needle. A clear, glass microcapillary is used to inject sperm into egg sample during the IVF cycle. The needle is transparent and the tip of the capillary is likely to get clogged with proteins, increasing variability per injection.

Employ Novel Silanization Strategies

- The objective of this study is to modify glass surfaces to enhance visibility and biocompatibility
- Covalently attach nanoparticles to enhance visibility
- Modify the glass with polyethylene glycol (PEG) containing silanization agents to enhance biocompatibility

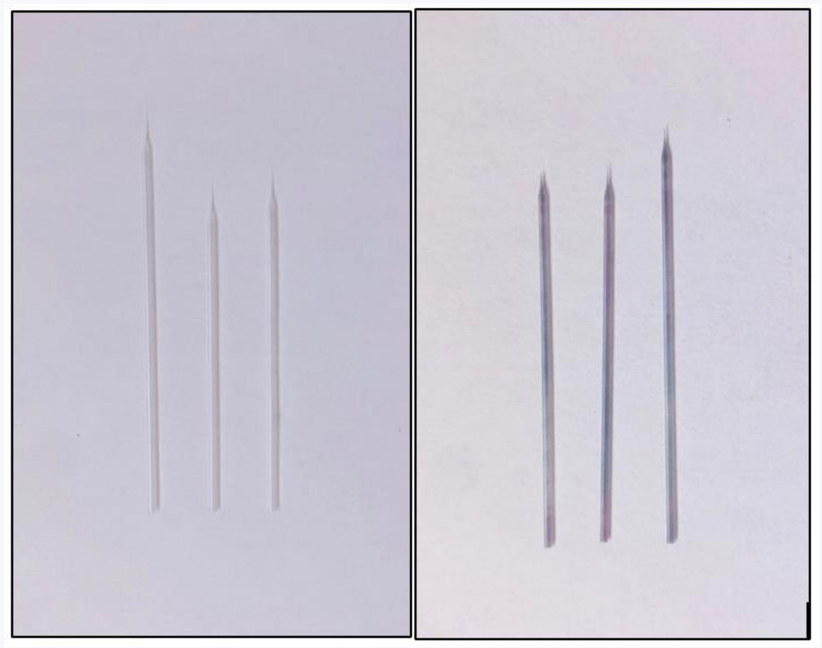


Figure 2: Industry standard needle (left) compared to modified glass with nanoparticles to enhance visibility (right). The transparency and fouling factor of current industry standard microdevices result in low visibility in cell and clogged outlets. The silanized gold-nanoparticle coated glass microcapillary produces a more visible tip in vitro. The color of the coat of gold-nanoparticle varies from light pink to a dark maroon.

Research Methods

Methods

- Coating glass surface with polyethylene glycol (PEG) and AuNP via EDC Coupling chemistry

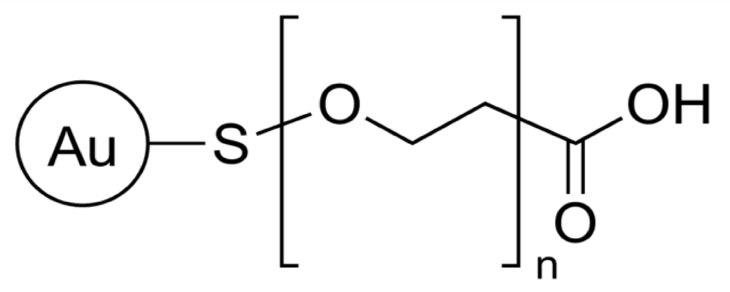


Figure 3: Carboxyl polyethylene glycol (PEG) attached to gold nanoparticle (Au) via thiol bonds. PEG helps in the reduction of protein absorption, reduces nonspecific interactions with cells and disables aggregation of gold nanoparticles.

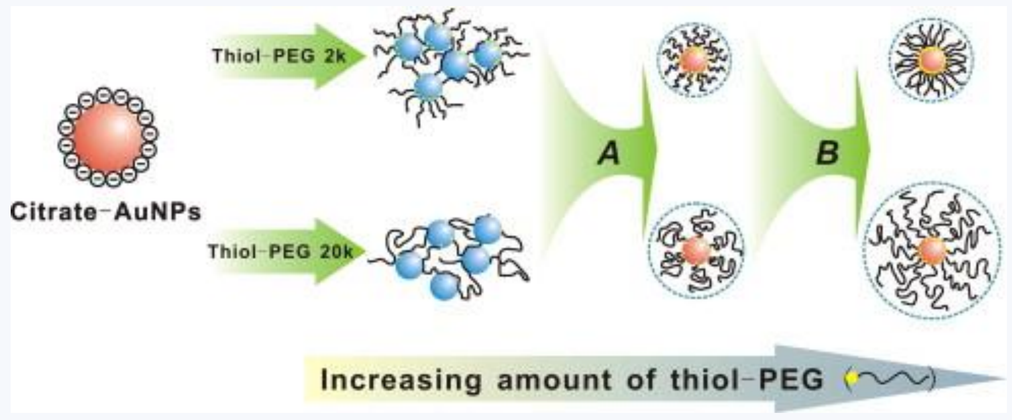


Figure 4: Thiol polyethylene glycol (PEG) attached to gold nanoparticle (Au) via thiol bonds. PEG creates a network of chains that will bind covalently to the glass surface via EDC coupling chemistry.

- Successful modification of the glass surface requires that the glass surface be cleaned thoroughly to remove organic material from the glass surface prior to the coupling reaction
- Silanization agents terminated with functional groups are bonded covalently to the glass surface
- PEG-spacers are covalently bonded to the gold nanoparticles via EDC coupling chemistry

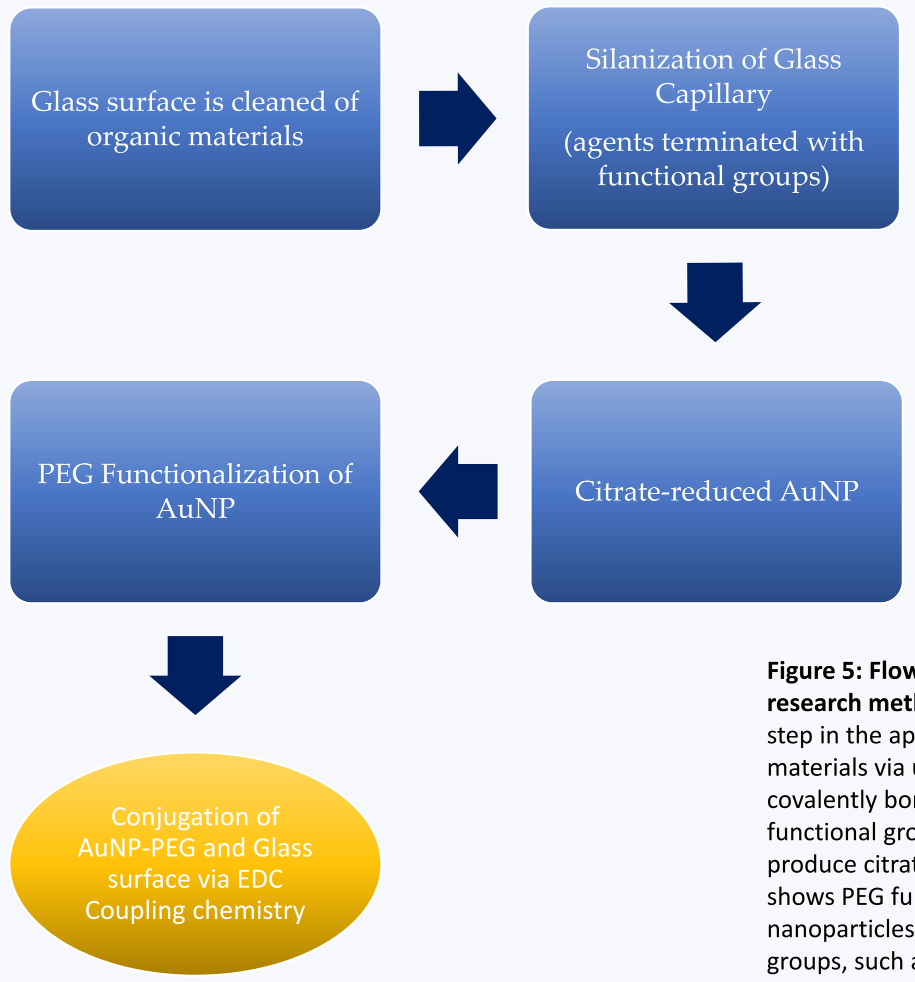


Figure 5: Flowchart diagram depicting generalized overview of research method step-by-step. The diagram shows that the first step in the approach is to clean the glass surface of organic materials via use of a strong acid. The second step is to covalently bond silanizing agents terminated with amine functional groups to the glass surface. The third step is to produce citrate-reduced gold nanoparticles. The fourth step shows PEG functionalization of the citrate-reduced gold nanoparticles. The PEG spacers are terminated with functional groups, such as carboxyl, which conjugate with the functional groups bonded to the glass surface. These groups are covalently attached via EDC/Sulfo-NHS coupling.

References

Zheng et. al, 2018 **Structure–Property Relationships of Amine-rich and Membrane-Disruptive Poly(oxonorbornene)-Coated Gold Nanoparticles,** *Langmuir*

Zheng, 2006 **Superlow Fouling Sulfobetaine and Carboxybetaine Polymers on Glass Slides** *Langmuir*

Data Analysis

- To determine the viability of the product, nanoparticle coated, colored glass was fabricated
  - Determine the polyethylene glycol-based (PEG) passivating ligand formula and adherence protocols to AuNP and AuAGNPs labelled glass microcapillary tubes
    - Presence of functional groups
- Test silanizing process to determine whether amines are bonded to the surface of the glass
  - Hydrophobicity

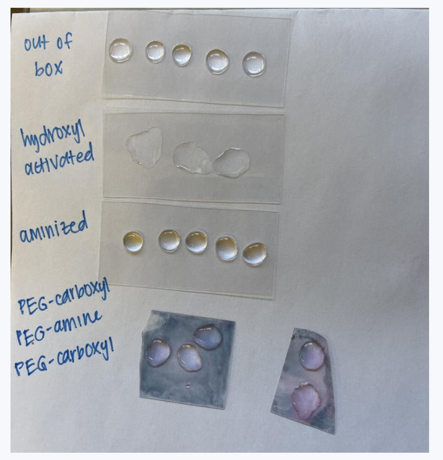


Figure 6: Aerial View

Figure 6 and 7: Hydrophobicity test of glass surfaces. The aerial view (left) shows the four trials used to test the hydrophobicity of the glass. Untreated glass labelled ”out of box” is hydrophobic, therefore causing the droplet too come up from the sides and cause an angle larger than zero degrees. The second trial is hydroxyl activated glass,. The hydroxyl groups cause the glass to act hydrophilic resulting in droplet angles close to zero degrees. The glass treated with silanzing agents, labelled “aminized” should behave in a hydrophobic manner due to the amines on the surface. This can be seen through the contact angle of the droplet too the glass surface. The fourth trial is glass treated with silanizing agent, as well as PEG functionalized to show that the PEG ligands are, in fact, present. The hydrophilicity of the glass surface shows that the PEG ligands are adhered to the surface. The photo on the right is used to show the surface contact angles more clearly.

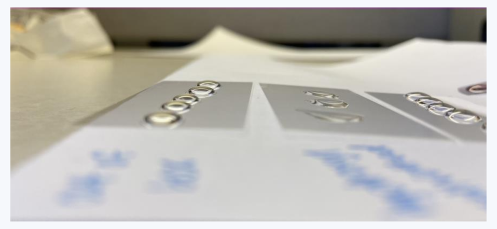
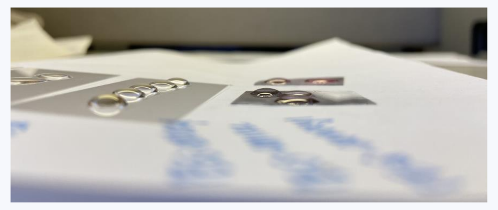


Figure 7: Eye level view. Out of box/untreated and hydroxyl activated (top). Aminized/Silanized and PEG functionalized (bottom)



Conclusion

- Silanization process confirmed to act as expected, binding amines to the surface of the glass
- PEG Functionalization has been confirmed via hydrophobicity testing
- Covalent bonding will not withstand force of mechanical rubbing
- Conjugation will create a network of nanoparticles to produce a darker and more stable stain on the glass

Future Studies

- Testing in Zebrafish embryos
  - Test visualization enhancing properties
  - Test anti-clogging property to improve accuracy by reducing variability in in amount of material delivered

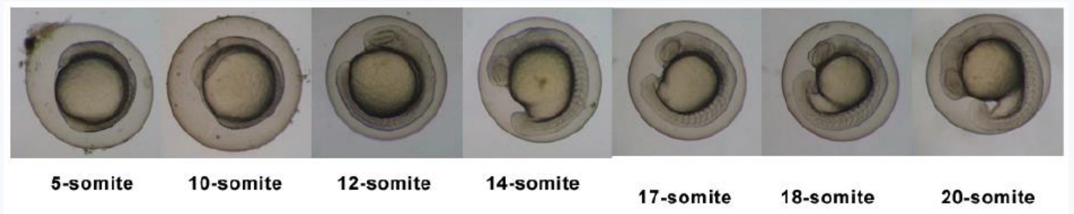


Figure 8: Zebrafish embryo development.

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