Scalable High-Aspect Ratio Bio-Metallic nanocomposites for cellular interactions

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Introduction

• Nanomaterials processing:
• Relevant in toxicity/ environmental studies
• May be utilized for materials transformation (to engineer new materials)
• May provide understanding of aging/stability of materials
• Illustrate the importance of materials-cell transformations
Methods-1

1. Composite Structures:
   • Organo-metallic nano and microstructures obtained from Nanogaia, LLC
     (see: [www.nanogaia.com](http://www.nanogaia.com) under “products”)
   • Contained copper nanoparticles and amino acids (available soon in mg quantities)

2. Cell Cultures:
   • Rat brain tumor cell line (CRL 2303) from ATCC
   • Cell viability by trypan blue exclusion
   • Density of 25,000 cells per well in 24 well plates

3. Nanomaterials:
   • Stock solutions of 1 mg/ml concentration using Locke’s solution
   • Effective concentrations of 50 μg/ml and 25 μg/ml (or as indicated)
   • 18 hour incubation

4. Zeta Potential Measurements:
   • Zeta Plus Zeta Potential Analyzer (Brookhaven Instruments)
   • Diluted 50 μl aqueous samples up to 2 ml with DI water
Methods-2

5. Fluorescent Labelling of Composites:
   • Layer-by-layer polyelectrolyte coating procedures
   • Poly-l-lysine fluorescein isothiocyanate (PLL-FITC) at final concentration of 0.5 mg/ml

6. Cell Viability Measurements:
   • MTT assay
   • Multiskan Spectrum plate reader (Thermo Scientific) at 570 nm
   • Mean percentage viability determined from three experiments

7. Electron Microscopy:
   • Hitachi S-4800 Field Emission Scanning electron microscope (SEM) at 15 kV
   • Zeiss EM912 transmission electron microscopy (TEM) at 15 kV
   • 20 μl drop of structure suspension placed and allowed to dry on carbon coated metallic stage
Example of transformative technology

• Nanoparticles → High-Aspect structures → Resulting in decreased toxicity → Initially discovered as a result of Toxicity testing
Nanoparticle processing by cells in vitro (iron and copper)

From left to right, control cells (left column, media only), iron oxide nanoparticles (middle column), and copper nanoparticles (right column). Top row images = cells at 1DIV and bottom row = at 3 DIV. All images = 100x magnification using 10 microscope objective.
Toxicity testing of starting materials and biocomposites

The metabolic activity of rat glioma cells 18 hours after treatment with composite structures and copper nanoparticles (CuNPs) is shown. Metabolic activity was quantified by MTT assay. Values were normalized with controls. (*P<0.05 and ***P<0.001; two tailed paired t-test).
Control cells (growth over time)
Processing of nanomaterials by cells-1 (75 ug/ml high)
Processing of nanomaterials by cells-2 (25 ug/ml low)
Nano and micro-aspects of our structures

Synthesis of Novel Linear Micro and Nano-Structures

Nano-scale & Micro-scale
SEM morphology of scalable structures

SEM images of starting material copper nanoparticles (left), and metallic biocomposite structures shown at the micro-scale (middle and right panels). Scale bars= 1 micron (left panel) and 5 microns (middle and right panels).
TEM images of composites (nanoscale)

TEM images of the biometallic composites shown at the nanoscale.
Stability of structures

- Stable in water (over 1 year)
- Stable in air (over 1 year)
- Heat stability
- Acid/base stability
The bio-nanomaterial hybrids were found to be stable over a range of pH values (*in the absence of cells*)

| Solution     | Approximate pH value | Stability of bio-nanomaterial hybrid structures | Stability of copper nanoparticles |
|--------------|----------------------|------------------------------------------------|----------------------------------|
| 1 M HCl      | 0                    | Unstable                                       | Unstable                        |
| 1 mM HCl     | 3                    | Stable                                         | Stable                           |
| 1 µM HCl     | 6                    | Stable                                         | Stable                           |
| 1 µM NaOH    | 8                    | Stable                                         | Stable                           |
| 1 mM NaOH    | 11                   | Stable                                         | Stable                           |
| 1 M NaOH     | 14                   | Unstable                                       | Unstable                        |
Material can be taken up by large, 3D cell assemblies (spheroids)

(potential for scaffolding
Or, 3D drug delivery)
Material may be functionalized

- material control, and material visualization
- examples: labeling for binding to surfaces (charge)
Bio-Nanomaterial hybrids can be functionalized by coating them with oppositely charged polymers.

Zeta potential before coating: -30.33 mV

Zeta potential after coating: +30.53 mV

Coating the bio-nanomaterial hybrids by Poly-l-lysine (expected to benefit and control cell-composite interactions)
Charge characterization as measured by Zeta potential

Materials were prepared and Zeta potential measured as described in methods. The starting material was copper nanoparticles (diamond), which was then formed into a composite (squares). This composite was finally labeled with FITC-PLL (triangles). Zeta potential was measured at each step, demonstrating changes in charge distribution.
Examples of functionalization

- Fluorescent labelling (FITC)- interaction with 2D cultures:
  - Time 0
Examples of functionalization
• Fluorescent labelling (FITC)- interaction with 2D cultures:

White light And Epi-Fluorescence Together
Examples of functionalization
• Fluorescent labelling (FITC)- interaction with 2D cultures:
Summary of Interaction of fluorescently labelled biocomposite structures with cells

Images were obtained with both white light and fluorescence illumination. Metal components of the composites (dark linear structures) as well as cells and fluorescent areas could all be seen simultaneously. Scale bar indicates 100 microns.
Future applications

- DNA association/delivery
- Connectors for nano/micro assembly

***Remaining Challenges***

- Optimization of fluorescent labeling (brighter)-
- Bleach test- affinity of label to surfaces (stability and longevity, for example: composites with a combination of 1) FITC bleachable vs. quantum dots).
Summary and Conclusions

• High-aspect ratio and stability of the structures are a benefit as “connectors”
• Structures are degradable when combined with cells, but more slowly than native metal NPs
• Future uses: conductivity? Scaffolding? Light-scattering capabilities?
• And possibly slow drug delivery, with degradable (but toxic) dynamics.
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