Direct contact cytotoxicity evaluation of CoCrFeNi-based high entropy alloys

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Statement of Purpose: Metallic alloys provide good properties for orthopedic implant applications, but new alloys are needed to improve implant weight and better match the stiffness of bone tissue. High entropy alloys (HEAs) provide the unique attribute of a single-phase microstructure in the presence of several (typically ≥ 4) near equiatomic components. CoCrFeNi-based HEAs have demonstrated excellent corrosion resistance in NaCl and H_2SO_4 [1] and are composed of elements commonly found in 300 series stainless steels (SS), which are well tolerated in the human body. Additionally, the stiffness of CoCrFeNi-based HEAs is significantly lower than that of SS 304 (~160 GPa [2] compared to >200 GPa in SS 304) providing the potential for reduced stress shielding under similar conditions. Several HEAs are being developed via arc-melting in order to investigate their diffusion kinetics, including CoCrFeNi-based HEAs. In addition, CoCrFeNi-based HEAs with Al additions are being investigated for potential vascular stent applications [3]. The purpose of this study is to investigate the cytotoxicity of various CoCrFeNi-based HEAs via direct contact in fibroblast cell cultures. The CoCrFeNi-based HEAs could be explored as a potential new material for orthopedic and vascular stent implants.

Methods: The cytotoxicity of the HEAs was assessed via direct contact evaluation adapted from ASTM standard F813-07. The HEAs were fabricated in 3 compositions from ground powders via arc-melting: $Co_{20}Cr_{20}Fe_{30}Ni_{30}$, $Co_{30}Cr_{30}Fe_{20}Ni_{20}$, and $Co_{20}Cr_{20}Fe_{20}Ni_{20}Mn_{20}$. In addition, commercial SS 304 specimens were prepared as a control eliciting acceptable cytotoxic response. BJ fibroblasts were expanded in α-MEM, 10 v% FBS, 1 v% L-glutamine, 1 v% P/S. The fibroblasts were then stained with DiI stain and seeded into 12-well plates at a concentration of 100k cells/well. Following cell adhesion to the well plates, the metal samples were placed into the center of the wells directly upon the seeded cells. After 24, 96, and 168 hours, the cultures were evaluated using Brightfield and fluorescence imaging and the viability was assessed using AlamarBlue assay compared to an untreated control well (no metal sample).

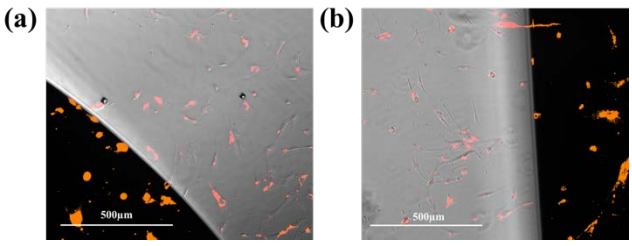

Figure 1. Phase contrast micrograph with DiI fluorescent image overlaid, demonstrating the cell morphology surrounding the (a) $Co_{20}Cr_{20}Fe_{20}Ni_{20}Mn_{20}$ and (b) SS 304 samples after 96 h of direct contact in cell culture. The metal samples are the black regions, cells are red.

Results: Imaging of cultured samples did not indicate changes to cell morphology or other cytotoxic effects for the HEA samples and the SS control. The AlamarBlue assay was used to evaluate the viability of the cells exposed to the metals. After 24 hours, there was no significant reduction in the number of cells exposed to the $Co_{30}Cr_{30}Fe_{20}Ni_{20}$, $Co_{20}Cr_{20}Fe_{30}Ni_{30}$, $Co_{20}Cr_{20}Fe_{20}Ni_{20}Mn_{20}$, or SS samples when compared to the untreated control wells. Additionally, after 168 hours fibroblast proliferation continued to occur in all wells, suggesting that no HEA compositions had acute cytotoxic effects.

Conclusions: The fibroblasts did not demonstrate acute cytotoxicity upon direct contact exposure to three CoCrFeNi-based HEAs. The fibroblasts exposed to the equiatomic $Co_{20}Cr_{20}Fe_{20}Ni_{20}Mn_{20}$ and $Co_{20}Cr_{20}Fe_{30}Ni_{30}$ exhibited the highest viability, suggesting that they may elicit the lowest cytotoxic effect on the fibroblasts. These findings indicate that CoCrFeNi-based HEAs may be a promising material for orthopedic implants. Further in vitro testing with bone marrow stromal cells and animal studies is necessary to confirm the suitability of CoCrFeNi-based HEAs for orthopedic implants.

References: [1] Q. Ye et al., Appl Surface Sci 396;2017;1420-1426
[2] W. Huo et al., Mat Sci and Eng: A 689;2017;366-369
[3] K. Alagarsamy et al., Cardiovascular Eng and Tech 7;2016;448-454