Degradation and cytocompatibility of magnesium alloys for medical applications – an introduction to methods and observations
Huinan Liu\textsuperscript{1,2}

\textsuperscript{1}Department of Bioengineering, \textsuperscript{2}Interdisciplinary Materials Science and Engineering Program, Bourns College of Engineering, University of California, Riverside, CA. The United States

INTRODUCTION: Magnesium (Mg) alloys are promising biodegradable metallic materials for orthopedic implants due to their many desirable properties. Mg has a mechanical strength and elastic modulus similar to cortical bone \cite{1}, and the degradation products can be naturally metabolized \cite{2}. Furthermore, increased bone growth has been observed surrounding Mg derived implants \textit{in vivo} \cite{3}. However, rapid Mg degradation \textit{in vivo} leads to rapid loss of mechanical properties of implants and acute increase of the local pH, thus limiting clinical translation of Mg alloys to orthopedic implants \cite{4}. Significantly increased pH is often the primary reason for Mg cytotoxicity \textit{in vitro} \cite{5}. Many different experimental techniques have been used to investigate the cytocompatibility of Mg-based biomaterials and their \textit{in vitro} interactions with cells, but the results of different experimental techniques are often not directly comparable to each other, even if the same research question is studied. The ability to compare experimental results among different literature reports is important to advance the field rapidly towards clinical translation. Therefore, this tutorial will first review and compare various \textit{in vitro} techniques used to investigate cellular interactions with Mg-based biomaterials, and then emphasize the urgent need to establish standardized procedures for \textit{in vitro} evaluation of Mg-based biomaterials.

DISCUSSION & CONCLUSIONS: One way to determine the cytocompatibility of Mg and its degradation products is to characterize the effects of Mg on cell proliferation \textit{in vitro}. Both direct contact and indirect contact methods have been reported in literature to describe cell adhesion and proliferation in the presence of Mg alloys. For the direct contact method, cells are incubated directly upon the surface of Mg-based biomaterials. The direct method more closely represents the cell-implant interaction at the interface, which plays a critical role in implant success. Alternatively, for the indirect contact method, Mg-based samples are first degraded in water or buffer solutions since the amount of solubilized degradation products in the cell culture media depends on the degradation rate of Mg-based samples. Cells are then incubated with the soluble degradation products. In contrast to the direct contact method, the indirect contact method precisely controls the exact amounts of degradation products added into the cell culture media, and can allow the media pH values to be normalized across multiple groups. Some extent of standardization should not be difficult to implement in investigations. The combination of direct contact methods (such as PicoGreen or BrdU assay) and fluorescence microscopy to visualize cell adhesion and morphology may provide valuable, comparable information on cytocompatibility and cellular interactions with the biomaterial surface. Moreover, experimental conditions such as the type of cells used and cell-culture protocols (e.g. incubation time, type of media, frequency of media change, etc.) could be standardized to enable direct comparison of results in literature. Finally, consistent controls could provide benchmarks to compare cytocompatibility in different publications. Standardized cytocompatibility testing procedures can potentially enhance comparability of current literature reports on Mg-based biomaterials, promote worldwide data sharing, advance the field of biodegradable metals with more synergy, and accelerate clinical translation of Mg based biomaterials for biomedical implant and device applications.

REFERENCES:

ACKNOWLEDGEMENTS: The author appreciates financial support from Burroughs Wellcome Fund 2012 Collaborative Research Travel Grant, Hellman Faculty Fellowship, and the United States National Science Foundation (CBET 1125801).