Impact of Elemental Compositions of Magnesium Alloys on *Ex Vivo* and *In Vitro* Thrombosis

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Statement of Purpose: Current standards of care use biostable metal stents for percutaneous coronary intervention (PCI). Stents require long term dual-antiplatelet therapy (DAPT) and can fail due to in-stent restenosis, requiring re-intervention. Re-intervention is difficult due to the presence of the prior stent and increased patient bleeding risk due to DAPT. Biodegradable metal stents offer a novel solution to in-stent restenosis by dissolving completely and allowing same-site stent reintervention. Recent evidence showed reduced thrombosis formation on pure magnesium (Mg).[1] Yet, due to rapid corrosion time, alloying of Mg is required for stent applications. However, it is unknown if Mg alloys retain the hemocompatibility of pure Mg. This work sought to compare the thrombotic response on different Mg alloys to consider the effects of alloying on thrombogenicity. Both an *ex vivo* non-human primate (NHP) model and *in vitro* biochemical assays were used complementarily in this study. We hypothesized that changing elemental composition delays corrosion and alters thrombosis onto the material surface. This novel application of *in vitro* and *ex vivo* experimentation on magnesium alloys serves as a first step towards elucidating the effects of elemental composition on surface mediated coagulation biology.

Methods: Mg alloy wires (0.25mm diameter) were obtained from Ft. Wayne Metals: ZX10 (Mg-1Zn-0.3Ca-0.15Mn), WE43 (Mg-47-3ND-0.4Zr), AZ31 (Mg-3Al-1Zn-0.5Mn), LZ21 (Mg-2Li-1.2Zn-0.3Ca-0.3Mn), and RLM (Mg-10Dy-1nd-1Zn-0.2Zr). CoCr (Co40-Cr20-Fe15-Ni15-Mo7-Mn2-C-Be) and NiTi (Ni45-Ti55) were used as a biostable clinical controls and were acquired from Goodfellow Inc. An *ex-vivo* model used NHPs without anticoagulation therapy. Platelets and fibrin were radiolabeled with ^{111}In and ^{125}I, respectively, then quantified over 1hr of flowing whole blood across a material.[1] Additionally, downstream sampling of the blood flow investigated surface-material interactions concerning inflammation (myeloperoxidase assay) and thrombosis (thrombin-antithrombin ELISA and platelet factor 4 ELISA). Finally, *in vitro* assays quantified activation of (F)XIIa and fibrin generation, fully interrogating the start and end of the coagulation cascade contact pathway, respectively.[1]

Results: The Mg alloys retained an anti-thrombogenic effect when compared to biostable controls. In the *ex vivo* loop, all Mg alloys have significantly lower fibrin deposition and platelet accumulation than the biostable clinical controls (**Fig 1A**). The *in vitro* and *ex vivo* results show similarities, as Mg alloys had significantly lower (F)XIIa generation and longer fibrin clotting times than the clinical standards in the biochemical assays (**Fig 1B**).

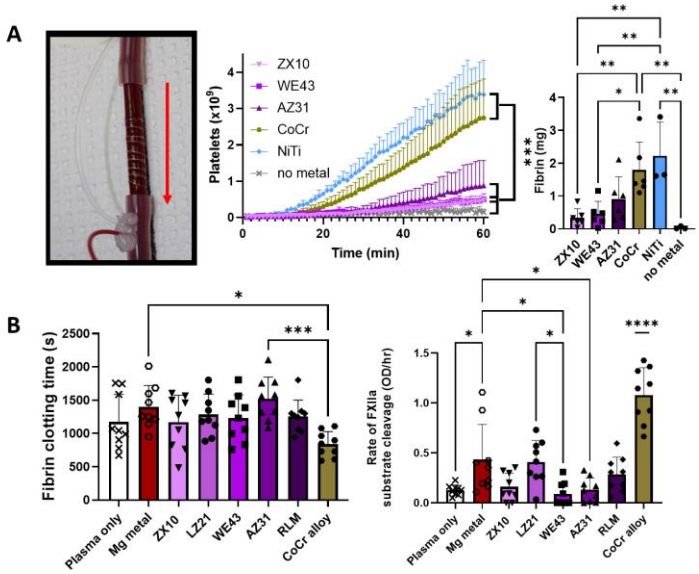

Figure 1. *In vitro* and *ex vivo* testing of magnesium alloys. (**A)** *Ex vivo* NHP flowing blood had significantly less fibrin accumulation and platelet deposition on magnesium alloys than biostable clinical controls. Image shows AZ31 coil in loop, arrow shows flow of blood. (**B**) Magnesium alloys showed significantly lower (F)XIIa generation than biostable controls, while all magnesium alloys had significantly longer fibrin clotting times (* $p > 0.05$, ** $p > 0.01$, *** $p > 0.001$, **** $p > 0.0001$). Data was analyzed with one-way ANOVA and *post-hoc* Tukey test.

Discussion/Conclusions: This study demonstrates a robust anti-thrombogenicity mechanism between all Mg alloys that lowers (F)XIIa activation and fibrin deposition on the Mg alloys, both in an *ex vivo* NHP model and *in vitro* benchtop assay. *Ex vivo* prevention of fibrin and platelet accumulation on Mg alloys and not biostable controls indicates a unique interaction between a Mg surface and whole blood. This is further reinforced by an extended fibrin clotting time and reduced (F)XIIa activation in the *in vitro* assays. The agreement of our *ex vivo* model and *in vitro* testing indicates a robust effect of Mg, unaffected by alloying elements, that prevents surface mediated thrombosis. Alloying Mg can be a metallurgical strategy to control corrosion while still retaining its beneficial hemocompatibility effects. Further studies will examine the mechanisms by which the surface properties of these alloys with their corrosion profile impact hemocompatibility.

References: [1] D.E.J. Anderson, et al. (2024) *Bioactive Materials* 38: 411-421