PROJECT SUMMARY

Cutaneous wound healing is a complex process that involves several physiological and molecular changes surrounding the damaged skin tissue. Environmental contaminants that impede wound healing place individuals at risk for infection and suffering. Steroid hormones, and estrogen in particular, positively influence cellular and tissue processes involved in wound healing, leading to changes in skin structure and physiology and increased shifts in the rate and capacity for wounds to close and heal, but contaminants that interfere with estrogen signaling may impede healing. Inorganic arsenic is an environmental contaminant that interferes with estrogen signaling processes. While there are several potential routes for human exposure to arsenic, in many parts of the world water resources provide a significant exposure route. This risk is real for many Native Americans living in tribal communities where challenges to accessing drinking water with arsenic levels meeting current USEPA standards exist. Health effects resulting from high arsenic exposure are well documented, but recent studies find that even moderate levels of arsenic exposure, such as those identified in some Southwestern Native American communities, are associated with negative health-related outcomes. Much of this exposure is not removed through current water treatment processes on Native lands. Given that Native Americans are at particularly high risk of diabetes and, therefore, diabetes-related wounds determining whether hormonal therapies can improve wound outcomes has the potential to provide translational outcomes useful both to this vulnerable population, and to others experiencing wounds in arsenic contaminated regions globally. The overarching hypothesis of this proposal is that *environmentally relevant levels of arsenic will act to inhibit wound healing processes as well as steroid signaling processes. Furthermore, exogenous administration of estradiol will reverse the effects of arsenic exposure.* Aim 1 determines the full dose response of arsenic on an *in vitro* model for cutaneous wound healing, Aim 2 determines whether estrogen exposure reverses the effects of arsenic exposure using the same *in vitro* model, and Aim 3 determines not only whether an *in vivo* model of wound healing validates the results found in the *in vitro* aims, but also determines whether estrogen treatment in a pre-clinical model supports the use of topical hormone therapy on wounds for individuals exposed to arsenic in their food and water resources.

SPECIFIC AIMS

Cutaneous wound healing is a complex process that involves several physiological and molecular changes surrounding the damaged skin tissue. Environmental contaminants that impede wound healing place individuals at risk for infection and suffering. Steroid hormones, and estrogen in particular, positively influence many processes involved in wound healing, leading to changes in skin structure and physiology and increased shifts in the rate and capacity for wounds to close and heal (Brincat et al., 2005; Campbell, et al., 2010), but contaminants that interfer with estrogen signaling may impead healing. Inorganic arsenic is an environmental contaminant that interferes with estrogen signaling processes. While there are several potential routes for human exposure to arsenic, in many parts of the world water resources provide a significant exposure route. This risk is real for many Native Americans living in tribal communities where challenges to accessing drinking water with arsenic levels meeting current USEPA standards exist. Health effects resulting from high arsenic exposure are well documented, but recent studies find that even moderate levels of arsenic exposure, such as those identified in some Southwestern Native American communities, are associated with negative health-related outcomes (Moon et al., 2013;Newman et al., 2016). Much of this exposure is through geologically released arsenic that is not removed through current water treatment processes on Native lands.

Because arsenic is known to inhibit steroid signaling processes, environmentally relevant arsenic exposure should influence wound healing with estrogen treatment reversing these effects. Our preliminary results using an *in vitro model* system support the hypothesis that arsenic slows the healing process and concomitant estrogen exposure reestablishes wound closure (see Preliminary Data). Studies document that even low ppb exposure levels (below current drinking water limits) cause shifts in expression of genes responsible for numerous cellular processes including oxidative stress, inflammation, proteotoxicity, proliferation, DNA repair, cell cycle control and apoptosis (Gentry et al., 2010). Similarly, estrogen influences these processes to improve outcomes associated with wound closure (Mukai et al., 2016). Given that Native Americans are at particularly high risk of diabetes and, therefore, diabetes-related wounds (Young et al., 2003), determining whether hormonal therapies can improve wound outcomes has the potential to provide translational outcomes useful both to this vulnerable population, and to others experiencing wounds in arsenic contaminated regions globally.

This project is a collaboration between the MPI (Kellar) with expertise in wound healing and Co-PI (Propper) with expertise in endocrinology and environmental toxicology. Our preliminary work is the first to demonstrate that arsenic-inhibited wound healing is mitigated by concomitant treatment with estrogen. If these observations are substantiated and mechanistic pathways more fully elucidated, results will contribute to the potential for translational medicine for at-risk populations including arsenic exposed populations and the elderly women where endogenous estrogen levels are low. We propose to use *in vitro* and *in vivo* models of wound healing, to test the hypotheses that: 1) arsenic exposure slows cutaneous wound healing, and 2) that estrogen treatment can reverse these effects. Specifically, since inorganic arsenic has been shown to act as an estrogen disruptor, *we hypothesize that environmentally relevant levels of arsenic will act to inhibit wound healing processes as well as steroid signaling processes. Furthermore, exogenous administration of estradiol will reverse the effects of arsenic exposure.* The proposed research fills a *critical knowledge gap* in that the deleterious effects of arsenic exposure on wound healing and the potential therapeutic role of estrogen in mitigating these harmful effects.

Specific Aim 1: To determine the influence of arsenic on a model for cutaneous wound healing. Using a well-established *in vitro* wound healing assay (IVWHA), we will characterize the effects of arsenic exposure across a range of doses on wound closure. Cells in this assay will be stored for quantitative polymerase chain reaction (qPCR) for genes involved in estrogen signaling and wound healing.

Specific Aim 2: To determine whether estrogen exposure reverses the effects of arsenic exposure. Using the IVWHA, cells will be exposed to the doses of arsenic demonstrated in Aim 1 to influence wound closure along with increasing doses of estradiol. Scratch closure will be monitored as in Aim 1 and qPCR will be used to evaluate molecular mechanisms involved in the effects of arsenic and estrogen on the wound healing process. The same markers as in Aim 1 will be evaluated.

Specific Aim 3: To determine whether the results found in Specific Aims 1 and 2 are replicated in and applicable to an *in vivo* wound healing model. The ability for estradiol to reverse the deleterious effects of arsenic exposure will be evaluated in full thickness wound healing model.

The proposed innovative study has the potential to uncover basic mechanisms of responses to environmental stressors, provide translational science to inform public health decisions, and define environmental factors that impact health disparities. Furthermore, these studies will provide interdisciplinary research opportunities for undergraduate and graduate students, and will specifically target underserved Native American and Hispanic students whose communities have been impacted by environmental exposures to arsenic.