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Investigation of the biphasic effect of lithium ions on HUVEC proliferation in bioactive glass extracts for burn wound regeneration

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ABSTRACT

In the current work, we developed a simplified empirical formula to isolate and define the effect of lithium ions (Li^+) released from Li-BG on the proliferation of human umbilical vein endothelial cells (HUVECs), based on experiments by Wei et al. We incorporate both culture time and concentration of Li^+ (0–65.4 ppm), while providing a hormesis term to partition the characteristic biphasic (bell-shaped) cellular response to lithium. Nonlinear regression to discover best-fit parameters resulted in $R^2 = 0.8707$, and RMSE = 0.0404. Models predict maximum stimulatory effects would occur at ~15 ppm Li^+, which corresponds with our in vitro results that enhanced HUVEC viability occurred at 1/8–1/16 dilutions of 2.5Li-BG and 3.75Li-BG nanostructured glasses. Additionally, Monte Carlo simulation determined, for the probability of desired cellular outcomes (>80% probability HUVEC $OD_{450} > 0.3$), there exists a therapeutic window (10–15 ppm) with maximum likelihood of achieving desired cellular consequences for probabilistic models. Although the full bioactivity of Li-BG is derived from the combined expression of Li^+ and Si^{4+} ions, as demonstrated in the original experimentation, this framework focused exclusively on Li^+ allows some predictive modeling to optimize subsequent ion release profiles for next-generation wound dressings.

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1. Introduction

Deep burns present challenges to healing, excessive scar development and complications with neurological and metabolic function [1-4]. Poor nerve regrowth brings into question the integrity of cutaneous sensory innervation [4-6]. While excision of burns and placement of skin grafts are the traditional clinical methods to correct and repair the skin, there is an important gap in the literature for next generation skin scaffolds that evolve nerve regeneration and may restore sensory function. To date, studies have incorporated bioengineered scaffolds that have utilized wound healing-related cells to enhance nerve restitution, in cases of deeper and larger burns [7, 8]. However, these studies found that low cell viability presents a problem for clinical translation. Therefore, we need new methods of being successful with wound closure and restoring skin sensation function, as early as practicable.

Angiogenesis is a crucial mechanism for enhancing wound healing by supplying oxygen and nutrients, which are needed to maintain cell survival and function during tissue repair. Bioactive glasses (BGs) have been used to manage chronic wounds because they release bioactive ions that induce angiogenesis in human umbilical vein endothelial cells (HUVECs) and fibroblasts through angiogenic growth factor upregulation [9-12]. In addition, BGs can also be multifunctional, by incorporating therapeutic ions such as copper and zinc into innovative multifunctional biomaterials. Schwann cells (SCs) are important pathology cells for peripheral nerve repair and aquatic sensation of the skin during regeneration. Recent studies found that lithium can benefit peripheral nerve repair anatomy and function, especially as LiCl at concentration, promotes nerve regeneration in rodent animal studies [13-17].

Wei et al. [18] manufactured lithium-doped bioactive glass (Li-BG) with different Li_2O concentrations via the sol–gel process aimed to concurrently facilitate nerve repair and angiogenesis

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during burn wound healing. They obtained ionic extracts of Li-BG according to ISO/EN 10993–12, and then evaluated the impact of the extracts on the proliferation of human umbilical vein endothelial cells (HUVECs) and Schwann cells (SCs) over 1–7 days at different dilutions (1/4 to 1/64). To additionally study the combined effects of lithium and silicon ions, the authors conducted a scratch assay comparing the either 3.75Li-BG extract (1/8 dilution) or the same concentrations of lithium (Li^+) or silicon (Si^{4+}) ions applied separately.

Building on the experimental measures reported in Ref. [18], this paper aims to examine the influence of Li^+ released from Li-doped bioactive glasses (Li-BG) on the proliferation of human umbilical vein endothelial cells (HUVECs), as measured by optical density at 450 nm (OD_{450}) after the cells were exposed to the extracts for 1, 3, and 7 days to examine the Li^+-concentration relationship for 3 glass compositions (Li^- control - undoped BG; 2.5Li-BG; and 3.75Li-BG) with Li^+ concentrations ranging from 0 to 65.4 ppm in the extracts.

An empirical mathematical model was created to describe the interaction of exposure time and lithium concentratin, while capturing the lithium-specific biological response, which is characterized by a bell-shaped curve. Then a Monte Carlo simulation was used to estimate the likelihood of positive biolgial response (OD_{450} is greater than a defined threshold) as a function of Li^+ concentration, while addressing the experimentation variation to define the best therapeutic window.

2. Materials and methods

2.1. Theoretical model

To quantitatively assess the proliferative response of human umbilical vein endothelial cells (HUVECs) to lithium-doped bioactive glass (Li-BG) extracts, a mathematical kinetic model was established based on experimental CCK-8 assay data (OD_{450}) [18]. The model includes two main influences: culture time (t, in days), and lithium-ion concentration [Li^+] (in ppm), determined from ICP-OES measurements, of extracts of the glass, corresponding to Li^+ concentration, shear modulus, and stiffness parameters in the empirical model [18]. Previous literature established a well-known biphasic (bell-shaped) biosystem response to lithium, with stimulation of proliferation starting at low-to-moderate doses, and inhibition of proliferation at cytotoxic levels of lithium. The empirical model described the effective stimulant response to lithium by:

$$Li_{eff} = [Li^+]\exp\left(-\frac{Li^+}{L_0}\right) \quad (1)$$

where L_0 (ppm) is the concentration of lithium at which its maximal stimulatory effect occurs. The projected optical density (OD_{450}), in place of viability, is then expressed as:

$$OD(t; Li^+) = b_0 + b_1 t + b_2 Li_{eff} \quad (2)$$

Where b_0, b_1, b_2, and L_0 refer to the model parameters, which were determined using non-linear least-squares regression against the complete set of experimental data with three glass compositions (BG, 2.5Li-BG, 3.75Li-BG), five dilutions (1/4 - 1/64), and three time points (1, 3, and 7 days). Lithium concentrations for 2.5Li-BG and BG were estimated using measured values (3.75Li-BG) and stoichiometric ratios from the glass formulation (2.61/3.97 ≈ 0.657 for 2.5Li-BG; 0 for BG). To evaluate model performance, the coefficient of determination (R^2) and root mean square error (RMSE) were calculated. By employing the reduced-order model, responder conditions controlled for the effect of lithium on HUVEC proliferation, and demonstrated an optimal therapeutic window for possible lithium delivery during burn wound healing. In addition, a Monte Carlo simulation was performed on the fitted model to further examine variability and uncertainty of experimental conditions., based on the experimental standard deviation (RMSE), was applied and the probability of obtaining a desirable biological response (OD>threshold) at each lithium concentration was calculated. This probabilistic approach could assess the optimal lithium-ion concentration range for improved cell proliferation and offsets experimental variability inherent to the experiment.

3. Results and discussion

The Cell Counting Kit-8 (CCK-8) assay was used to determine the viability of human umbilical vein endothelial cells (HUVECs). HUVECs were seeded at a density of 1,000 cells per well in 96-well plates and cultured in extracts of BG, 2.5Li-BG, and 3.75Li-BG diluted at 1/4 to 1/64 for 1, 3, and 7 days. At each time point, the culture medium was replaced with a 10:1 mixture of fresh culture medium and CCK-8 reagent, and the optical density (OD) of the samples was measured at 450 nm with a microplate reader.

To evaluate quantitatively the effects of lithium ions released from Li-doped bioactive glasses on HUVEC proliferation, the empirical mathematical model developed in the previous section, was fit to the CCK-8 assay data reported in [18]. The model was developed using culture time (t), and lithium-ion concentration ([Li^+], ppm), measured using ICP-OES, and included a term for the characteristic bell-shaped biological response of lithium, [Li^+] exp(−[Li^+]/L_0). The fitted model takes the form:

$$OD(t; Li^+) = 0.09 + 0.04t + 0.01 Li_{eff} \quad (3)$$

where

$$Li_{eff} = [Li^+]\exp\left(-\frac{Li^+}{14.80}\right) \quad (4)$$

The empirical Li-only model's predictive efficacy was assessed by comparing predicted OD_{450} values to experimental values for every condition of interest (BG, 2.5Li-BG, 3.75Li-BG; dilutions 1/4–1/64; days 1, 3, and 7). The results are presented in Fig. 1, which shows a high degree of positive association with experimental data, and most of the points are clustered around the line of perfect prediction (R^2 = 0.8707, RMSE = 0.0404). The model accurately represents the overall pattern of increased HUVEC proliferation at medium lithium concentrations and subsequent decline at higher dosages, consistent with an observed biphasic (hormetic) lithium-responsive phenotype.

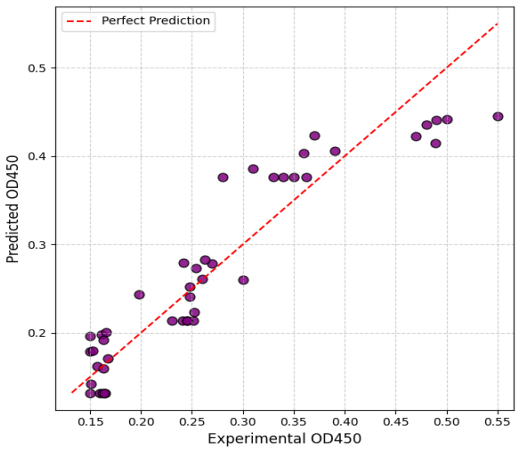

Fig. 1. Validation of the Li-only empirical model against experimental HUVEC proliferation data (OD_{450}) [18].

The model does show some deviation, particularly with low OD values; however, the overall patterns represent a level of predictive validity that supports the use of this simplified framework to identify the optimal lithium concentration window (~15 ppm) with the maximum cellular response.

The predictive accuracy of the Li-only model was subsequently examined by displaying predicted OD_{450} values (as a function of time (1, 3, and 7 days) for all the glass compositions (BG, 2.5Li-BG, and 3.75Li-BG) that were tested and for all the dilutions that were performed (1/4–1/64). As illustrated in Fig. 2, the model adequately captures the overall trends in the data (i.e., increased HUVEC viability over time) across all three experimental conditions. In addition, the model's predicted curves show that high lithium concentrations (e.g., 3.75Li-BG at 1/4 and 1/8) yield increased proliferation relative to BG or even lower dilution treatment conditions. This observation is consistent with previous data from Ref. [18].

The model also supports the dose-dependent effects of lithium, where intermediate dilutions show optimal responses (1/8–1/16), consistent with the bell-shaped biological response integrated into the model in during its development. Although there are minor deviations in predictive accuracy at early time points and at extreme dilutions (1/32 and 1/64), the overall agreement is satisfying, and the model demonstrates sufficient potential to establish favorable conditions for HUVEC proliferation based on lithium-ion concentration.

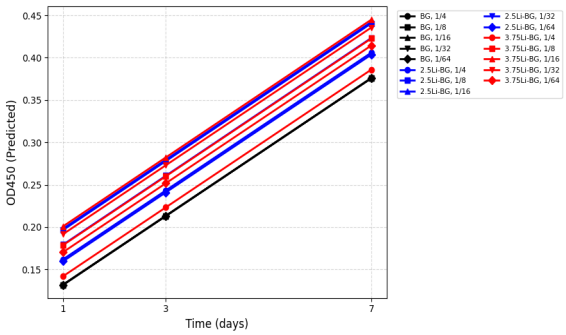

Fig. 2. Predicted OD_{450} values for HUVEC proliferation over time, based on the Li-only model.

In the Monte Carlo framework, we introduced random deviations based on the residual noise to account for experimental uncertainty. A limit state function was defined as:

$$g(Li, \varepsilon) = OD_{pred} - OD_{thershold} + \varepsilon \quad (5)$$

where $OD_{pred}(Li)$ is the optically density predicted by the model based upon a lithium concentration of Li, ε accounts for random noise from the experiment, and ($OD_{thershold}$= 0.3) is the optically density threshold that indicates a successful outcome. A positive value of ($g(Li, \varepsilon)$) was defined as a successful response, and a negative value as failure. Subsequently, the probability of success, ($P(g > 0)$), was calculated as the number of successful realizations divided by the total number of Monte Carlo simulations completed for each lithium concentration.

Fig. 3 presents the success probability as a function of Li concentration, and the resulting probability curve shows a clear non-linear pattern: the odds of getting OD > 0.3 start off increasing with lithium, reaching a maximum around 10–15 ppm before declining again at higher concentrations. This is supportive of the biphasic (bell-shaped) biological response of lithium on HUVEC proliferation; specifically, lithium at low (medium) concentrations promotes cell growth, but at high concentrations lithium shows an inhibitory or cytotoxic response. The probabilistic function provides not only an accurate representation of the experimental trends, but provides a means of appropriately addressing the uncertainty inherent with biologically variable systems and postulates a robust probabilistic framework for estimating the possible optimal range of lithium for endothelial cell proliferation.

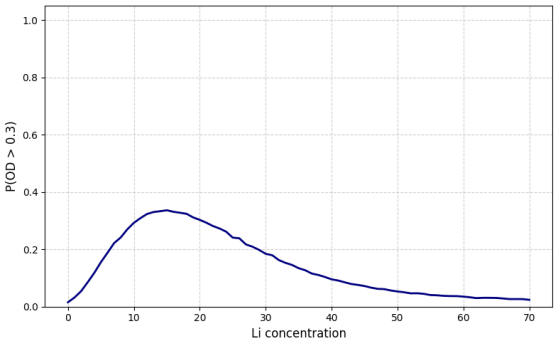

Fig. 3. Success probability of OD_{450} in terms of Li concentration.

4. Conclusion

The research involved creating an empirical mathematical model to isolate and estimate the contribution of lithium (Li^+) released from lithium doped bioactive glasses (Li-BG) conditioning media towards the induced proliferation of HUVECs based on the published study by Wei et al. [18].

Using culture time and Li^+ concentration obtained via ICP-OES from BG, 2.5Li-BG, and 3.75Li-BG extracts (media) as a basis; the model showed the general biphasic (hormetic) growth response of the HUVEC cells to lithium with an estimated peak range of about 15 ppm. The model displayed a strong fit for the entire dataset (R^2 = 0.8707, RMSE = 0.0404). Additionally, the model indicated via Monte Carlo simulation a probabilistic treatment window for optimal desired cellular outcomes (OD_{450} > 0.3) at 10-15 ppm Li^+ concentration. While the study cited indicates that the full bioactivity potential for Li-BG derives from synergy between Li^+ and Si^{4+} ion activity, and neither ion alone has shown improved Schwann cell proliferation or migration in vitro, the analysis done in this Li-centric investigation provides useful insight into lithium's contributions towards inducing endothelial cell responses. The findings support lithium's role as a biopharmacologic agent for modulating HUVEC proliferation and further demonstrate its release could be enhanced by adjusting the Li_2O concentration in the glass matrix.

Author contributions

Azadeh Hasanzadeh: Conceptualization, Writing –original draft, Writing –review & editing; **Mastafa H. Al-Musawi:** Conceptualization, Writing –original draft, Writing –review & editing.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

No data is available.

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