7. Summary and Conclusion

Since the dysregulation of calcium homeostasis following neuronal injury is a crucial factor that contributes to the development and maintenance of neuropathic pain (NP), we have developed a novel inorganic barium-doped bioactive glass (BaBG) that has calcium-regulating properties. The inorganic bioactive glass was amorphous and showed a higher tendency to form hydroxyapatite which affirms its bioactivity. The *in vitro* release profile for Si, Ca and Ba from the BaBG framework in the simulated physiological milieu exhibited controlled release of the dopants which are within the permissible physiological limits. The maximum concentration was observed at 24 h for Ca, Si, and Ba. BaBG exhibited biocompatibility and cytocompatibility, and it enhanced the migration of horizontal cells in the scratch assay, thereby emphasizing its regenerative capacity. In addition, BaBG demonstrated notable anti-inflammatory properties in the *in vitro* LPS model of neuroinflammation. Therefore, BaBG has regenerative and anti-inflammatory potential for treating traumatic injuries.

Further, we examined the pharmacokinetics, biodistribution, and excretion of dopants leached from BaBG in a pre-clinical study following single-dose oral administration to comprehend the fate of the dopants released is crucial for optimizing the dose regimens. The *in vivo* oral pharmacokinetics investigation demonstrated dose-dependent plasma release of network modifiers, with the maximum concentration of Ca, Si, and Ba observed at 24 h (T_{max}). The biodistribution study revealed that Ca, Ba, and Si were primarily accumulated in the excretory organs of the body and feces are the primary route for elimination. Furthermore, we have noted that barium, which was released from BaBG, was found in both the peripheral organs and the central nervous system, specifically the brain. Hence, the therapeutic ions released from BaBG possess the

capability to exert their effects on the central nervous system. Moreover, the safety profile of BaBG was affirmed through an acute and sub-acute oral toxicity study performed in rats in accordance with the OECD guidelines and its LD_{50} was more than 2000 mg/kg b.w. Further, the organ coefficients and the biochemical parameters did not indicate any potential adverse effects on the functioning of vital organs. The neurological safety profile of BaBG was also established as there were no changes in muscle coordination, spontaneous locomotion, or observed anxiety-like behavior in the rats treated with a dose as high as 1000 mg/kg b.w. during the sub-acute toxicity study. Thus, BaBG exhibits greater safety and biocompatibility in preclinical studies and can be used as a therapeutic strategy.

The important finding of our study was establishing the temporal dynamics and significant details about the involvement of intracellular calcium and calcium-binding protein i.e., S100b level in the progression of NP in the chronic constriction injury (CCI) model. There was a notable time-dependent increase in the intracellular calcium and S100b protein levels in both the sciatic nerve and spinal cord acutely 1 h post-injury. However, pentamidine, an S100b inhibitor, mitigated CCI-induced NP sensory and motor impairments, proving that S100b is essential to NP pathogenesis. Pentamidine also reduced astrocyte and microglia hyperactivation in this NP model. By inhibiting glial cell activation, pentamidine reversed NP by breaking the inflammation-pain feed-forward loop. In addition, pentamidine significantly reduced S100b-induced inflammation by reducing CCI-induced TNF-α and IL-6 levels in the sciatic nerve and spinal cord. In conclusion, these data provided substantial evidence supporting the role of S100b in the pathophysiology of NP and emphasized the therapeutic potential of S100b inhibition.

Further, we thoroughly investigated BaBG's pharmacological effects and found that it mitigated CCI-induced sensory hypersensitivity and motor deficits. In the *ex vivo* electrophysiological setup, BaBG resulted in an elongation of the repolarization phase of the action potential. Therefore, BaBG delays action potential propagation and therefore can be used during NP as an increased nociceptors' sensitivity is reported. In the CCI paradigm in rats, BaBG dose-dependently attenuated all NP symptoms. It also hindered the activation of S100b and decreased inflammation post-CCI. BaBG repaired and remodeled dendrites, hence exhibiting disease-modifying properties. Similar to pregabalin, BaBG dose-dependently attenuated the elevated intracellular calcium levels in the SN and SC post-injury in rats. Therefore, BaBG has a disease-modifying and calcium-regulating potential, thus potentially impeding calcium-associated NP pathogenesis. Remarkably, our research stands as the first to demonstrate the pharmacological potential of inorganic materials in the NP treatment.

BaBG attenuates NP phenotypes via calcium-regulatory mechanisms

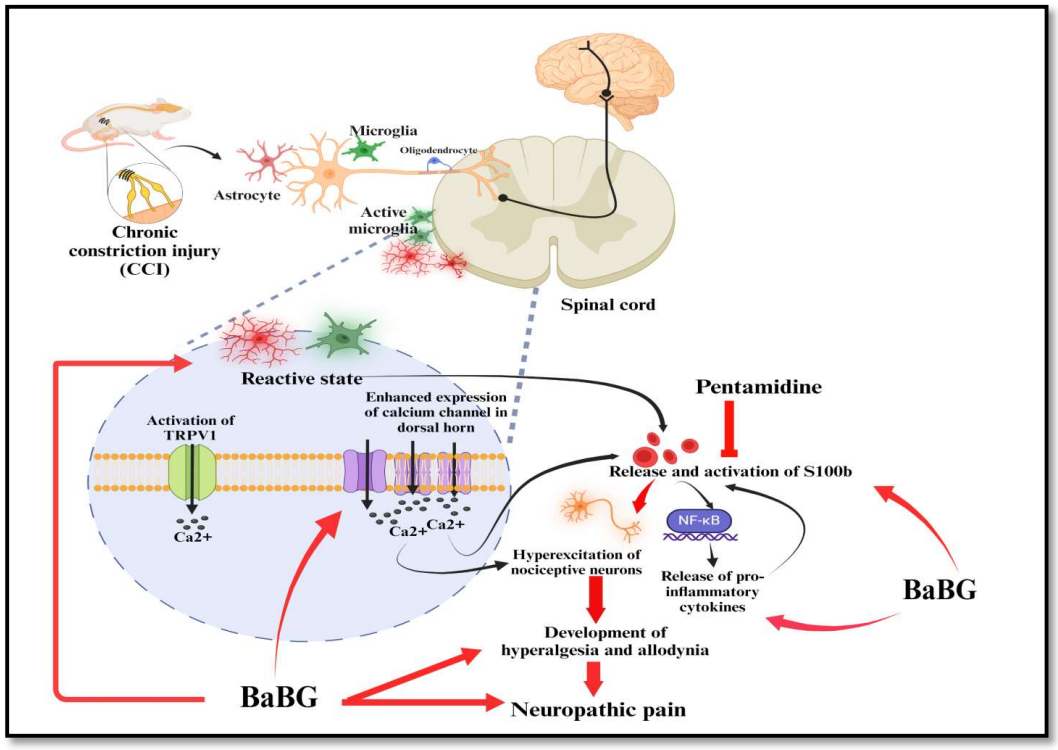

Figure 7.1. Summary and conclusion of the study: red arrow denotes decrease.

The injury to the sciatic nerve results in central sensitization through an increase in the expression of calcium channels (Cav2.2) in the dorsal horn of the spinal cord. In addition, there is an increased expression of heat-sensing TRPV1 channels in the spinal cord. Increased expression of these channels leads to an increase in the influx of calcium ions, causing hyper excitation of the nociceptive neurons and the development of NP phenotypes. In addition, the resident immune cells of CNS i.e., glial cells (astrocytes and microglia) get activated post-CCI leading to an increase in the release of calcium-binding proteins i.e., S100b. S100b gets activated in the presence of calcium ions causing the release of pro-inflammatory cytokines and progression of NP. However, treatment with a specific S100b inhibitor i.e., pentamidine significantly reversed NP phenotypes and lowered S100b-induced downstream inflammatory cascade like NF-

κB. Further, BaBG also ameliorated the CCI-induced sensory hypersensitivity (hyperalgesia and allodynia) and motor deficits. Moreover, it reduced intracellular calcium levels and calcium-binding protein (S100b) in the sciatic nerve and spinal cord post-CCI. BaBG also mitigated the CCI-induced glial cell activation and subsequent release of pro-inflammatory cytokines.

7.1. Important outcomes

- BaBG has regenerative and anti-inflammatory potential.
- There was dose-dependent plasma release of dopants (Ca, Si, and Ba) from BaBG following oral administration which is within the permissible physiological limits.
- Barium released from BaBG was found in both the peripheral organs and the central nervous system, specifically the brain. Hence, the therapeutic ions released from BaBG possess the capability to exert their effects on the central nervous system.
- There was a time-dependent increase in the intracellular calcium and S100b protein levels in both the sciatic nerve and spinal cord acutely 1 h post-injury of the sciatic nerve.
- S100b was found to be involved in the NP pathogenesis in the chronic constriction injury (CCI) model of neuropathic pain.
- BaBG resulted in an elongation of the repolarization phase of the action potential thus delaying the action potential propagation.
- BaBG has a disease-modifying and calcium-regulating potential.
- BaBG dose-dependently attenuated all NP phenotypes.

7.2 Scope for Further Work

Our research has yielded significant insights into the effect of BaBG on calcium currents through *ex vivo* electrophysiological investigations. However, investigating the molecular processes by which BaBG influences calcium currents could yield a precise understanding of its effects on various calcium channel subtypes. The safety profile of BaBG was validated preclinically in the acute and subacute oral toxicity study, and the *in vivo* pharmacokinetic parameters indicated that the concentration of therapeutic dopants released after post-oral administration remained within the physiological limits. Consequently, BaBG is safer and possesses the potential for clinical application, necessitating a clinical trial. Moreover, the optimal pharmaceutical administration form of BaBG will be oral suspensions, owing to enhanced patient compliance. Consequently, addressing these aspects could substantially improve our comprehension of BaBG's effect on calcium channel modulation and its potential role in the treatment of neuropathic pain.