Anti-inflammatory effect of methotrexate conjugated boronate-PAMAM dendrimers for potential arthritis therapy

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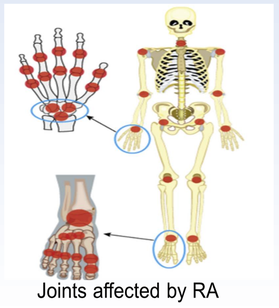
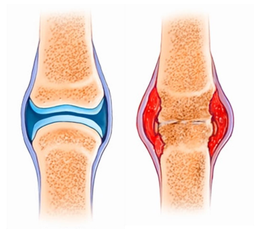
Introduction

- Rheumatoid arthritis (RA) is a chronic disease that affects about 78 million people worldwide[1].
- RA cannot be cured but is managed by the systemic administration of anti-inflammatory or anti-rheumatic drugs, with methotrexate (MTX) as the gold standard[2,3].
- Considering the adverse effect of systemic administration, transdermal drug delivery will be more advantageous for arthritis treatment[4].
- Dendrimers are polymeric nanoparticles with distinctive features beneficial for controlled and targeted drug delivery. Increased generation of dendrimers on their own can however be toxic, without any chemical modifications[5].
- Hence this study is focused on modified PAMAM dendrimers, to increase the biocompatibility of the nanoparticle, with potential for the development of a transdermal drug delivery system for RA.


Joints affected by RA


Healthy joint Rheumatoid arthritis

Hypothesis

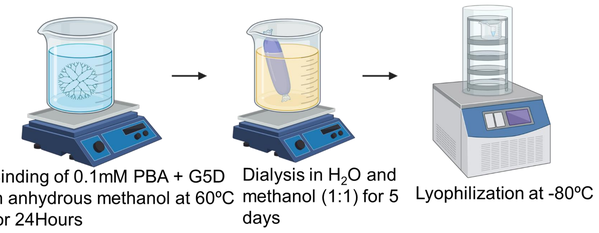
- We hypothesize that Phenyl-boronic-acid-polyamidoamine (PBA) modified generation 5 PAMAM dendrimers (PBA-G5D) will be effective for the transdermal delivery of Methotrexate and provide a better therapeutic efficacy for RA.

Aims

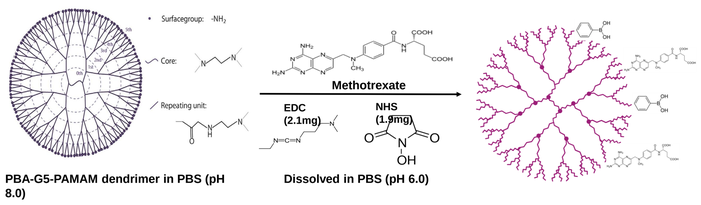
- To synthesize and characterize the PBA-PAMAM dendrimers
- To study the cytotoxic and anti-inflammatory effects of MTX loaded PBA-modified dendrimers.

Methods/Study Design

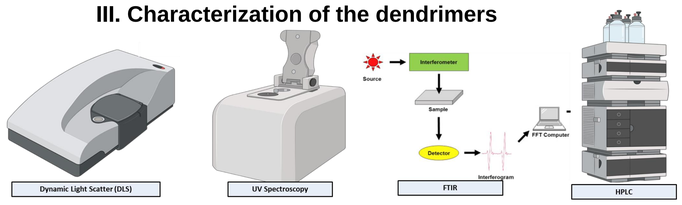
I. Modification of commercial dendrimers



II. Synthesis and loading of MTX on PBA-PAMAM dendrimer



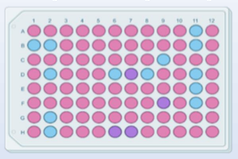
III. Characterization of the dendrimers



IV. In-vitro Evaluation of LSF loaded dendrimers on Murine macrophage cells (RAW 264.7)

Cells cultured in a T25 flask; cells seeded and treated with MTX and MTXPBAG5D; Treated cells incubated at 37°C, 5% CO_2

Cytotoxicity study


MTT Cell Viability Assay

Anti-inflammatory study


Cytokine Bead Array (CBA); Flow Cytometry

Results

I. Size Distribution Profile

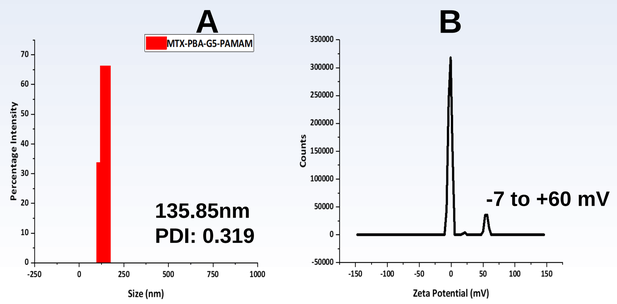


Figure I: DLS revealed the average size distribution of the drug loaded dendrimer (MTX-PBA-G5-PAMAM) **A.** The average size and polydispersity index; **B.** The surface charge of the drug-loaded dendrimer.

II. UV-Spectroscopy

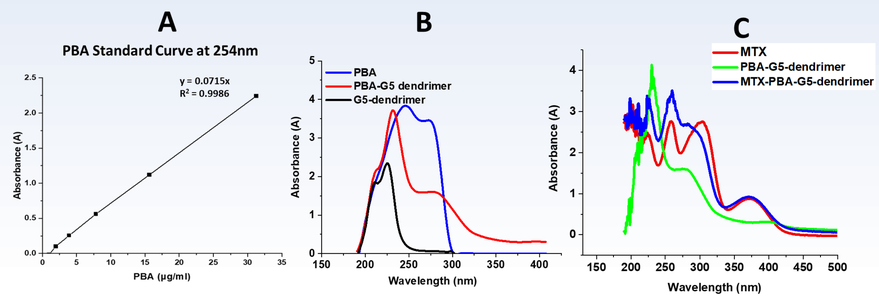


Figure II: UV Spectroscopy; A. The standard curve of UV absorbance revealed by PBA dilutions, and the values were used to determine the binding efficiency of PBA to G5D as 30%; **B.** UV absorbance spectrum of PBA, unmodified-G5 dendrimer, and PBA-G5-PAMAM dendrimer. **C.** UV absorbance spectrum comparing MTX, PBAG5D and MTX-PBA-G5-PAMAM dendrimer. The absorbance levels of the compounds were compared to determine if there was binding of PBA to G5D, and MTX to PBAG5D.

III. FTIR

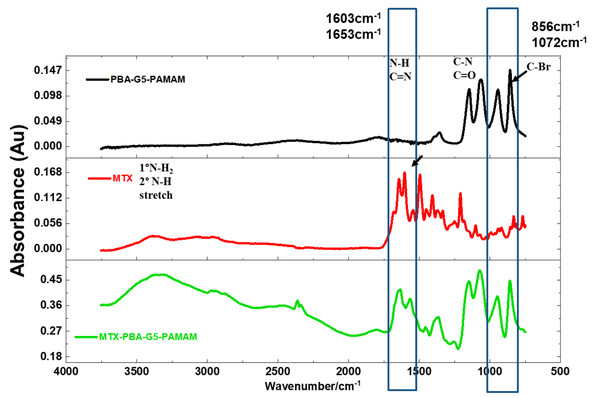


Figure III: Fourier Transform Infrared Spectroscopy. FTIR spectroscopy confirmed the binding of MTX to the modified dendrimer. This is confirmed by the presence of the functional groups specific to each molecule which is seen in the conjugate.

IV. MTX Release Kinetics

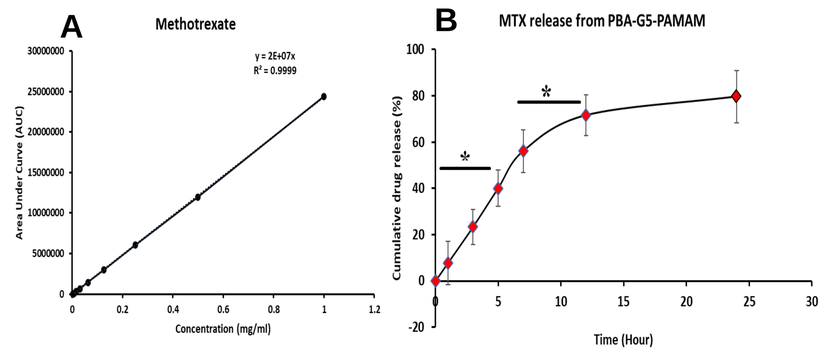


Figure IV: Release Profile of MTX from PBA-G5-PAMAM dendrimers. A. Standard curve of MTX dilutions used to determine the concentration of MTX in the dendrimer. **B.** A sustained release of MTX was observed, with 80% of the encapsulated MTX released after 24 hours using the dialysis bag method.

V. Cytotoxicity

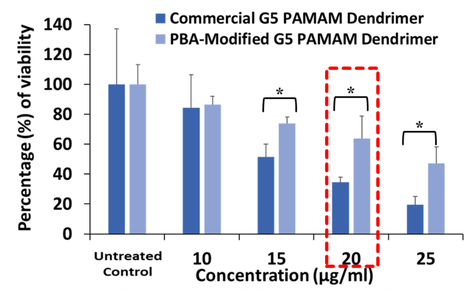


Figure V: Reduction in toxicity of dendrimers after PBA-modification. Significant increase in cell viability was observed after treatment with the same concentrations of the unmodified and modified dendrimers. At 20µg/ml treatment, the PBA-modified dendrimers showed ~2 fold less toxicity than the unmodified dendrimers p<0.05). These results indicate that modification improved the polymer biocompatibility. The data presented is a mean of triplicates ± SD of each sample. *p< 0.05, **p< 0.01, and #p< 0.001.

VI. Anti-inflammatory Effect

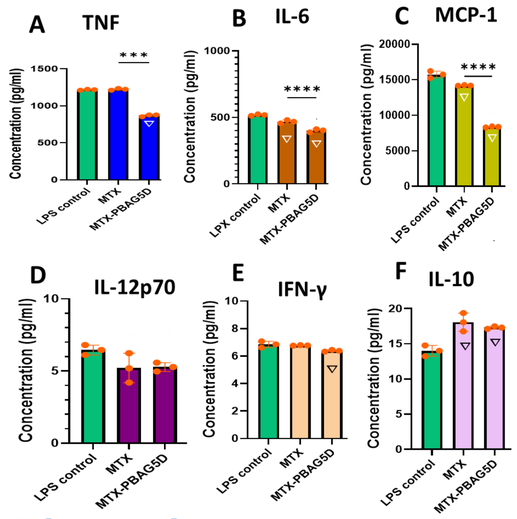


Figure VI: The anti-inflammatory effect of the non-toxic concentrations of MTX (5µg/ml) were tested on LPS-activated murine macrophages. The effect of MTX alone, and MTX in the developed formulation are represented. **A-E**. The anti-inflammatory effect of MTX-PBA-G5D is highlighted and relatively better than MTX alone as seen in the reduction of the pro-inflammatory cytokines TNF, IL-6, MCP-1, IL-12p70 and IFN-γ. **F.** The levels of the anti-inflammatory cytokine IL-10 was increased in both MTX alone and MTX in PBA-G5-D.
The data presented is a mean of triplicates ± SD of each sample. ▾ means significantly different from control, *p< 0.05, **p< 0.01, ***p< 0.001, and ****p<0.0001

Discussion

- Dendrimers have a characteristic architecture suitable for targeted drug delivery. However, higher generation dendrimers are cytotoxic.
- PBA reduced the toxicity posed by the amine groups from unmodified G5-PAMAM dendrimers, thereby increasing the biocompatibility of the dendrimer
- The hydrodynamic size of the PBA-G5D and MTX-PBA-G5D were in the range of 135-200nm in diameter. The modified and MTX-loaded dendrimers showed no cytotoxicity.
- The MTX loaded in PBA-G5D showed better anti-inflammatory properties than MTX alone, which shows that the modified dendrimers may contribute an anti-inflammatory effect in the formulation.
- The significant reduction in levels of TNF, IL-6 and MCP-1 are interesting since these cytokines play important roles in the pathogenesis of RA.
- Further study is in progress to determine the long-term stability and investigate the in-vivo anti-arthritic effect of the developed formulation.
- Overall, the study shows the probability of developing a non-toxic drug delivery system using dendrimers with the potential of topical drug delivery application for RA.

Conclusions

- The outcome indicates that PBA modification significantly decreases the toxicity induced by amine groups of G5 PAMAM and improves its biocompatibility. Furthermore, with the reduction the pro-inflammatory cytokine, and an increase in the anti-inflammatory cytokine levels with MTX-PBA-G5D showed the potential of topical anti-inflammatory drug delivery strategy for RA

Future Directions

1) The stability and release of MTX in the dendrimer should be studied for a longer time and at different pH conditions.
2) More immune cell populations should be investigated, especially cells activated and deregulated based on MTX mechanism of action.

References

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