Delivery of Engineered *B. subtilis* bacteria for sustainable treatment of pattern baldness

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Introduction

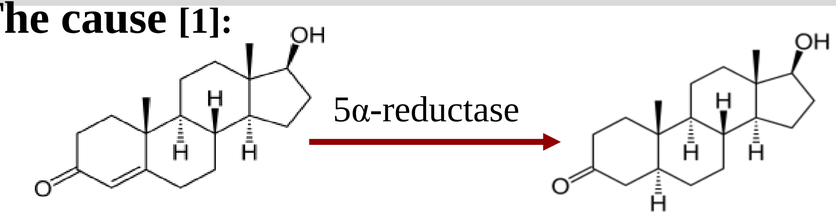
Disease:

Androgenetic Alopecia

It's a genetic disease that affects the hair follicles, it makes them shorter and thinner [1].

80% of males get bald

The cause [1]:



Testosterone (Male Hormone) → Dihydrotestosterone (DHT)

Figure 1: Testosterone conversion into DHT [1].

Current treatments: Minoxidil and Finasteride

Minoxidil: There is disagreement about how well it works.

Finasteride: Side effects include loss of libido and erectile dysfunction [1].

Figure 2: Minoxidil.

Our Solution – Probiotic Formulation

Mixture of a polymer and a bacteria that will prevent the hair from getting shorter.

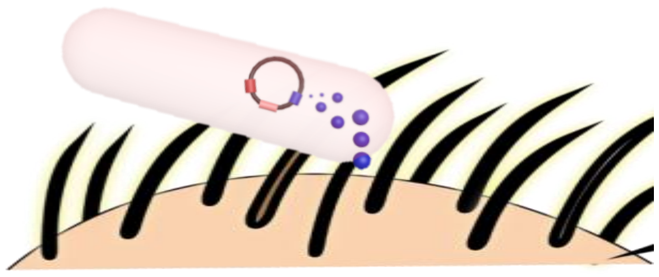


Figure 5: Applying the bacteria on the scalp.

Bacteria:
Bacillus subtilis, genetic modified bacteria, produces 3αHSD enzyme that converts the DHT to less potent derivative (figure 4).

Thermosensitive polymers

PNIPAAm and Pluronic, have the ability to respond to a change in temperature- become gel above 32ºC [2].

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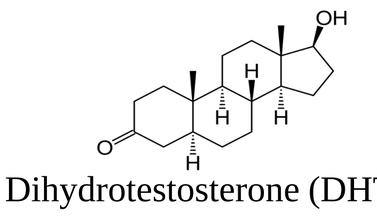
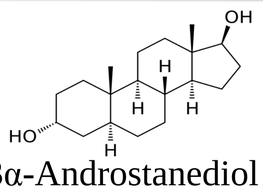


Dihydrotestosterone (DHT)

3α-hydroxysteroid dehydrogenase (3α- HSD)



3α-Androstanediol

Figure 4: DHT conversion into 3α-Androstanediol.

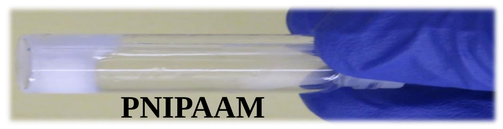


Figure 3: Polymers in their gel form at 37º C. PNIPAAM (left), Pluronic (right).

Gelation Time

Gelation time: Time that takes for the polymer to become a gel. Different concentrations of the polymers solutions were dripped on 37˚C slide (figure 6) and gelation time was measured (figures 7 and 8).

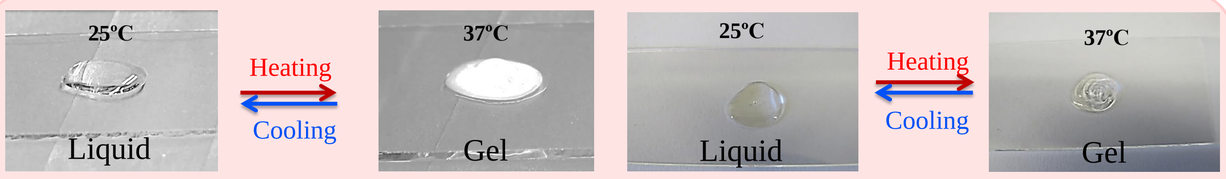


Figure 6: Polymers gelation process. PNIPAAM (left), Pluronic (right).

It can be seen on figure 7 that gelation time of the PNIPAAM increases and reaches its maximum at the 18%, and it starts to decrease on the higher concentrations.

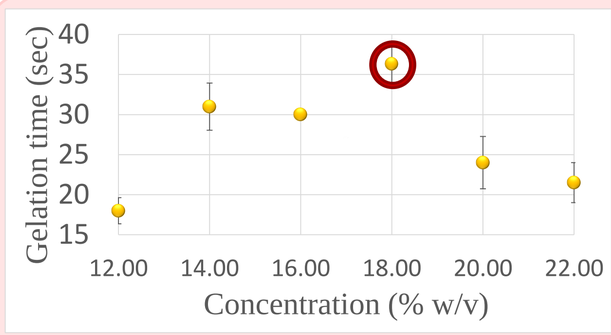


Figure 7: PNIPAAM gelation time at different concentrations.

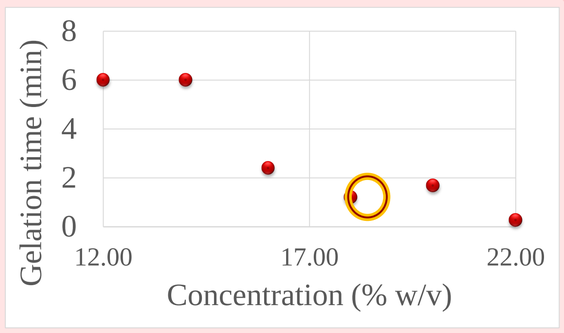


Figure 8: Pluronic gelation time at different concentrations.

It can be seen on figure 8 that at the higher concentrations, Pluronic becomes a gel faster and at the lower concentration it took more the 6 minutes to become a gel.

In order to examine the influence of the bacteria on polymers' gelation time, same experiment was conducted for the same polymers 18% (w/v) mixed with *Bacillus* solution (in LB).

It can be noticed in figure 9 that higher ratio of pluronic resulted in faster gelation while the PNIPAAM's gelation time was approximately consistent.

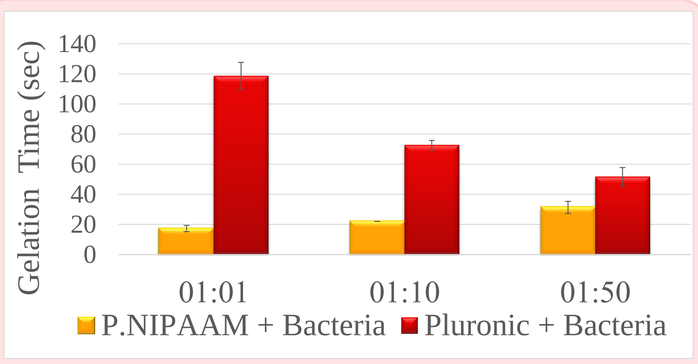


Figure 9: Volume Ratio LB + Bacteria : polymer.

Viability Test

Three different solutions were prepared and incubated for 24 hours : LB + Bacteria, Pluronic + Bacteria and PNIPAAM + Bacteria. Samples were taken and mixed with Luciferin reagent. The reagent combines with ATP molecules that live bacteria produces, this combination creates Luminescence light that was measured in plate reader (figure 10).

After 5 hours, the samples were mixed with SYto9, that dyes the living bacteria, and were observed under microscope (figure 11).

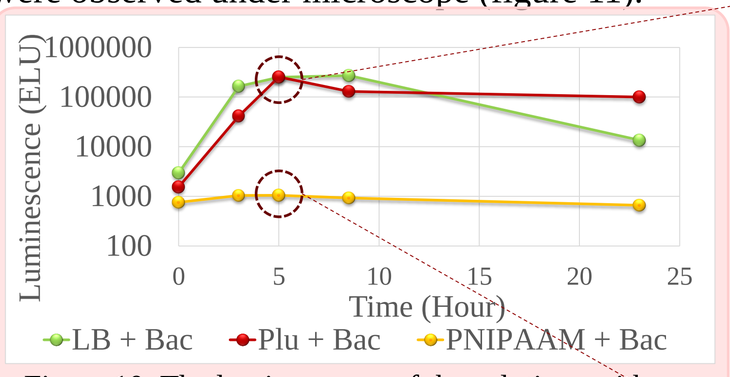
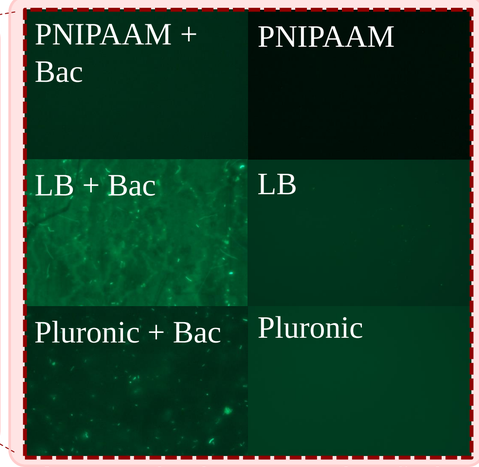


Figure 10: The luminescence of the solutions with the bacteria over 24 hours.

Figure 11: Microscope images X40, SYto9 dye, GFP filter. Comparison between the solutions with (left) and without (right) bacteria, after 5 hours.



It can be seen on figure 10 that until the third point we had a growth of the bacteria, for the LB and the Pluronic, reaching its peak after 5 hours. After that it shows a decrease in luminescence. The PNIPAAM + Bacteria solution doesn't show any bacteria growth.

Conclusions

- Best polymer concentration is 18% - gelation time is slow enough for easy administration and not too fast so it won't drip from the scalp.
- Volume ratio of 1:10 LB + Bacteria : polymer was chosen because the amount of the bacteria doesn't change the gelation time dramatically and it still produces the enzyme.
- The bacteria can grow inside the Pluronic unlike in the PNIPAAM that doesn't show a growth rate of the bacteria.
- Main conclusion: Pluronic is the best polymer for the formulation.

Acknowledgments

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References

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