REMOVAL Ink.

MICRONEEDLE TATTOO REMOVAL

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Motivation

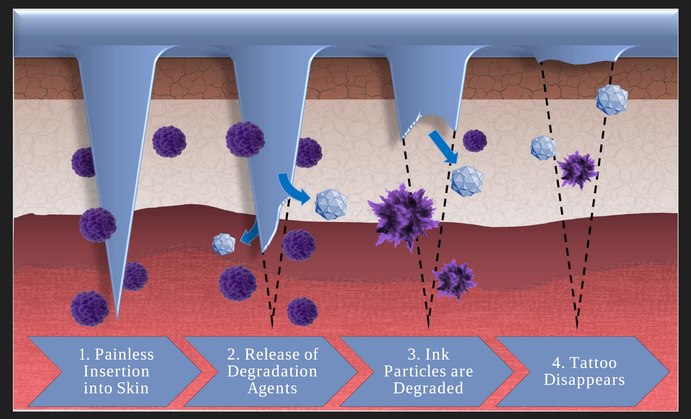
The global tattoo industry is valued at over 1.35 billion dollars and is expected to have a continuous annual growth rate (CAGR) of 10% over the next decade [1-2]. Consequently, the global tattoo removal industry is currently valued at $694 million and has a CAGR of 18% [1,3-4]. According to the Harris Poll conducted in the United States (U.S.) in 2015, 47% of Millennials and 36% of Generation X had at least one tattoo [2]. Of the tattooed population, 23% regret one or more of their tattoos, which translates to 9 million people [5]. Laser tattoo removal owns 66% of the tattoo removal market as of 2016, but associated with it is painful, repetitive and expensive treatment [3].

Design Challenges

- **Biocompatibility** of patch contents and reaction by-products
- **Specificity** of degradation agents to tattoo inks
- Localized **delivery** and adequate **dispersion** of degradation agents in the skin

Our Solution

1. Painless insertion of the microneedles is ensured by optimizing the needle length; they must be long enough to be in proximity of the ink particles but shallow enough not to contact any nerves in the dermis.
2. Release of degradation agents is induced by the dissolution of the microneedle array in the skin's interstitial fluid.



3. The released enzyme then partakes in the degradation of the tattoo ink particles. This process is powered by molecular oxygen and mediated by HOBT.
4. Upon completion of the tattoo ink degradation, the ink particles are rendered colourless in the visible spectrum and "disappear".

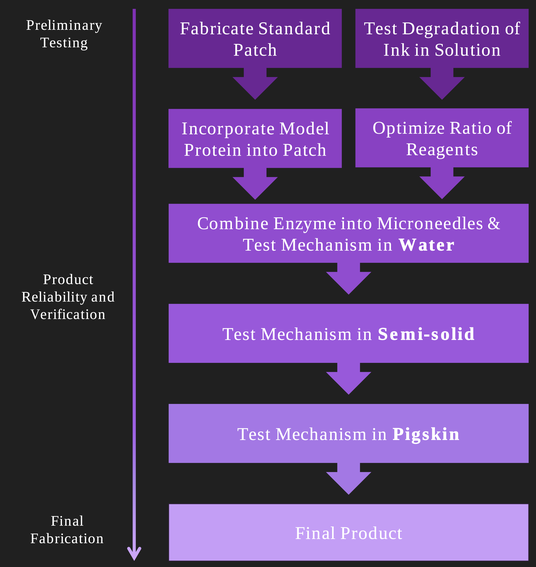
***Figure 1**: Visualization of microneedle breakdown and enzymatic degradation mechanism in the skin.*

Conclusions

Microneedles:
- Successfully fabricated functional microneedles using inexpensive biocompatible materials.
- Determined patch dimensions to confirm that insertion of the needles would be painless to coincide with our design specifications.
- Determined the release rate of encapsulated compounds in the patch to show its ability to deliver high molecular weight compounds.
- Tested the decolourization capabilities of the laccase patches in solution and in semi-solids to better replicate the skin.
- Achieved consistent fabrication of strong microneedles.

Decolourization Mechanism:
- Attempted decolourization of Methylene Blue, but the blue-shift of the absorbance peak was not great enough to reach the UV range.
- Successful decolourization of Allura Red AC was achieved using both 1x and a 5x HOBT (difference lies in decolourization rate).
- Various tattoo inks were studied and complete decolourization could not be achieved within the timeframe of the project.

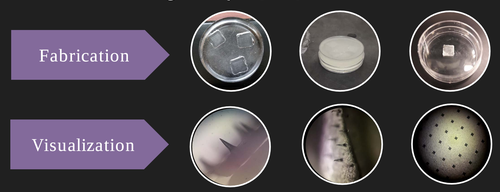
Experimental Design



Methods

Microneedle Fabrication:
1. Combine a 2:10 gelatin and starch mixture with DI water and heat at 90°C for 30 mins.
2. Cool to 40°C , add the enzyme and pour into the mold. Centrifuge and add a thin backing for structural integrity.
3. Incubate at 34°C for 24 hours.

Decolourization Mechanism:
- Employ Laccase as our principle degradation agent.
- Laccase, when combined with a mediator, HOBT, and O_2, has been shown to decolourize organic dyes [6-7].



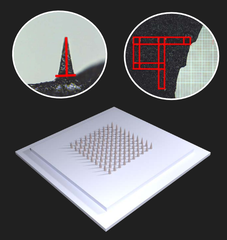
Results and Observations

Microneedle Testing

The patch dimensions, content delivery rate and decolourization potential were determined. The removal mechanism was also tested in a semi-solid.

***Table 1**: Patch dimensions determined using an optical microscope and hemocytometer.*

	Methylene Blue Patch	
Dimension	Length (mm)	σ (mm)
Spacing	0.439	±0.014
Diameter	0.141	±0.014
Length	0.348	±0.026



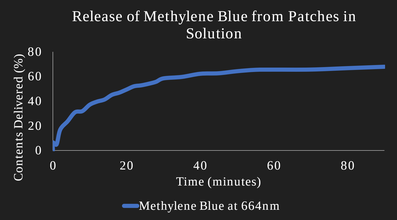


***Figure 2**: Methylene Blue release profile during microneedle dissolution.*

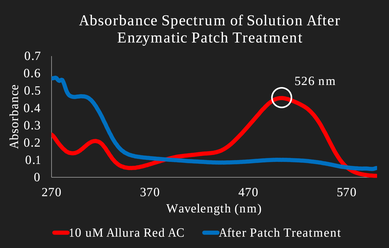


***Figure 3**: Allura Red AC decolourization via patch dissolution in solution after 24 hours.*

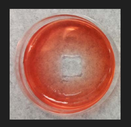


***Figure 4**: 100 µM Allura Red AC decolourization via patch dissolution in a 1% agarose gel after 24 hours. Although decolourization was observed in the vicinity of the patch, the mechanism by which the colour was removed is unclear. Partial decolourization could be a result of the enzyme, but absorption of the dye into the patch is the likely cause.*

Decolourization Testing

The decolourization mechanism was tested on various dyes, and the content ratio was optimized for fast and effective removal of the dye.

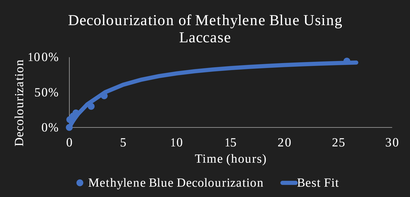


***Figure 5**: Decolourization of Methylene Blue in solution over time.*

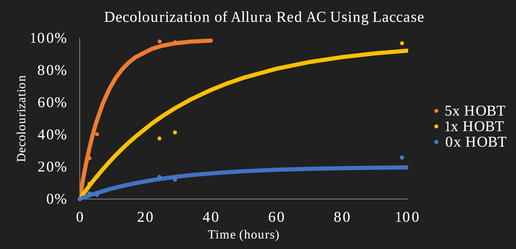


***Figure 6**: Decolourization of Allura Red AC with varying concentrations of HOBT.*

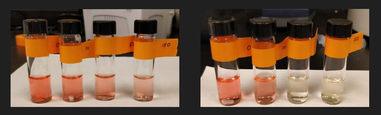


***Figure 7**: Decolourization of Allura Red AC with varying concentrations of HOBT. L to R: 0x HOBT, 1x HOBT, 5x HOBT and 15x HOBT.*

This same mechanism was applied to the array of tattoo dyes depicted below, but given their dissimilar interactions in solution compared to the aforementioned dyes, sufficient decolourization could not be obtained within the scope of the project.

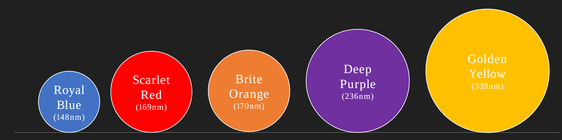


***Figure 8**: DLS results indicating the unique sizes of each of the coloured tattoo inks.*

Future Works

Microneedles:
- Determine the optimal ratio of starch to gelatin to obtain both the ideal flexibility and strength of the microneedles, as well as reasonable dissolution time.
- Up-scale the protocol to fabricate larger microneedle patches.
- Optimize the microneedle density to ensure even decolourization throughout the skin.

Decolourization Mechanism:
- Determine methods to break down tattoo inks prior to treatment with the degradation mechanism (sonication was not successful).
- Optimize factors such as enzyme and mediator concentrations and system pH to achieve maximum enzymatic activity.
- Explore other enzymes and mediators.

Future research and development:
- Explore additional applications such as treatments for birthmarks, melanomas, scars, hyperpigmentation or melasma.

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