Exploring the Effects of a Lack of EGF on the General Growth and Development of Human Skin Organoids

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Abstract

Skin organoids are artificially-generated tissues which serve as model systems to further the understanding of dermatology without needing live samples or donors. A number of molecules are responsible for their growth, and EGF (epidermal growth factor) is one such molecule. This experiment determined the effect of removing EGF on an organoid system. Various measurements of growth – such as thickness – were taken from three sample organoids treated with low-EGF media and three organoids treated with EGF-containing media, and the data was compared to check for significant variations in development. The initial hypothesis was that the no EGF group would likely have more growth, considering that an excess of EGF could lead to early cell differentiation and therefore stagnate overall development of the organoid. After analysis of the post-lab data, however, it was found that EGF, in fact, did *not* lead to premature differentiation, and thus did not inhibit overall growth. It was rather beneficial to growth, so removing it led to reduced tissue development.

Background

EGF is responsible for the growth, differentiation, and the epithelial-mesenchymal transition (EMT) of epithelial cells[1]. It has been long suspected that an overabundance of EGF can cause early differentiation of keratinocytes, which does not provide enough time for the keratinocytes to proliferate and fill the epidermis. Most notably, we suspected that it overstimulated EMT and led to excessive translocation and differentiation of epithelial cells into mesenchymal stem cells, which migrate more frequently than their predecessors. An excess of such mobile cell types likely prevents proper cell-cell attachment within organoids, which is crucial for cell growth[2]. The organoids that were previously created consisted of certain regions that were less dense than the surrounding tissue. In order to counter this problem and make the tissues more viable as human skin replacements, we experimented to see if removing the EGF would reduce the stress on the system and yield organoids that resemble real human skin.

Methodology

1. Take six samples of devitalized dermis (the remnants of skin when all the living cells are removed) and seed a layer of human keratinocytes, cells with the ability to transform into a number of other skin cells, on top.
2. Place the primitive tissues in a 6-well plate and cover the bottom with matrigel to assist the attachment of the keratinocytes to the devitalized dermis.
3. Treat the samples with KGM (keratinocyte growth medium) containing standard levels of EGF and incubate at 37ºC for three days.
4. Switch three organoids to no-EGF KGM and keep the other three on the standard KGM, replacing the expended media.
5. Incubate for three more days at 37ºC, replace the used media with a fresh batch once more, and then incubate one last time for another three days.
6. Cryo-freeze the tissues, section them into tiny strips, and then view the images through fluorescent microscopes under 10X and 40X lenses. To image the organoids, use H&E staining.

Results

Image 1: Control Organoid (With EGF) with 10X Zoom

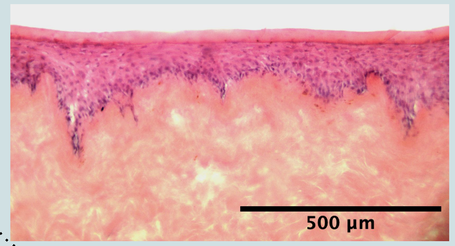
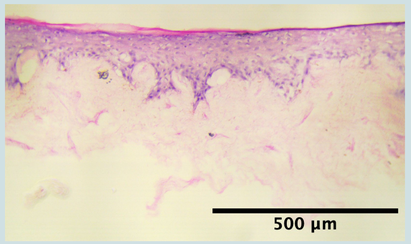


Image 2: Test Organoid Without EGF with 10x Zoom



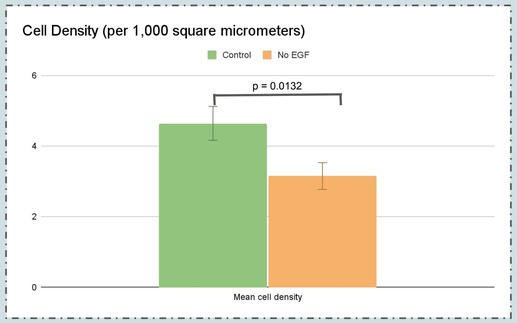


Figure 1: Representation of the mean density of cells in the live part of the epidermis. On average, the cell density for the control group is 1.50 cells/µm^2 higher than that of the no EGF group. Since the p value is 0.0132, this is a highly statistically significant measurement; the cell density is considerably lower for the no EGF group, and this is *not* due to random happenstance.

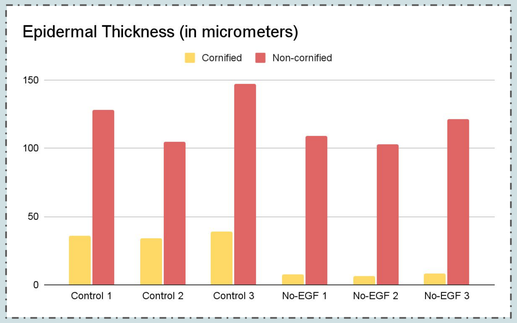


Figure 2: Representation of the average thickness of both the cornified and non-cornified layers for all organoids. With a p-value of 0.0002 for the average cornified thickness, the difference between the two groups is not due to chance. Even at minimum, the difference in thickness is a striking 26 µm. The live region saw more similar thicknesses for both groups, and while the no EGF groups were thinner, a p-value of 0.4319 shows that their differences are insignificant and are not necessarily due to intrinsic qualities.

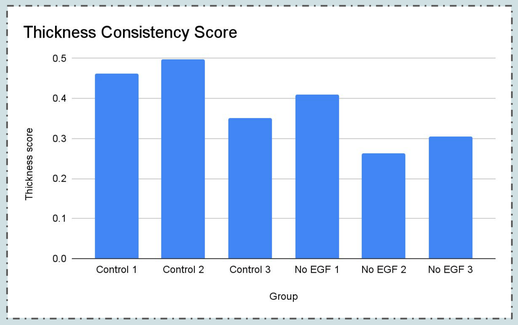


Figure 3: Representation of thickness consistency score, a measure of how consistent the thickness of the epidermis is across the organoid. The lower the resultant value, the more consistent the thickness is for the epidermis. In general, the no EGF group resulted in lower thickness consistency values, meaning that the thickness was much more uniform when compared to the control group. However, the p-value for the two groups was 0.1462, meaning that the variation could be due to chance. As such, both groups saw similar consistencies in epidermal thicknesses.

Figure 4: Representation of the mean difference score. Difference score is the ratio of the cornified layer thickness to the non-cornified layer thickness, and as is evident here, the control group has a much higher value. On average, the control group has a score that is 0.224 higher than that of the no EGF group. The p-value is 0.0004, meaning that the difference is critical.

Discussion

As can be seen from Images 1 and 2, the organoids from both conditions possess some important morphological differences. The control organoid appears generally thicker with distinct, stratified layers along the epidermal region. In contrast, the no EGF organoid appears thinner with a less visible cornified region, indicating worse growth and maturation since a developed cornified layer indicates development of the organoid. These results are reflected in Fig. 4, where the average difference score for the no EGF group is 0.224 less than the control group, suggesting slower maturation and differentiation of the epithelial cells. While slower growth was expected, Fig. 2 suggests that the stratification process has been strikingly interrupted since the no EGF cornified layers are at least 26 µm smaller. Fig. 1 indicates that the cell density of the no EGF groups is also significantly lower than their control counterparts by about 1.50 cells/µm^2. Such results suggest one common theme: the presence of EGF aids in general growth rather than inhibits it.

It should be noted that the differences in the thicknesses of the live regions are not as noteworthy. Fig. 3 suggests that organoids in both groups had similar epidermal growth due to the similar overall thicknesses. This is backed up by Fig. 2, where the thicknesses of the live-cell region, which make up most of the organoid tissue, show no considerable differences. The p-values, being higher than 0.05, confirm this analysis. While the values for no EGF groups were still mostly lower, a lack of EGF does not seem to substantially impact overall organoid thickness, though it does detrimentally affect cell density.

Considering that the no EGF experimental group exhibited significantly lower cell densities and thicknesses, a lack of EGF didn't improve tissue growth and development as initially hypothesized; rather, it simply exhibited slower growth than organoids grown with plentiful EGF. As such, the presence of EGF is clearly beneficial to organoid growth, indicating that EGF does not necessarily overstimulate premature cell differentiation and EMT. It performs its expected job of being an *epidermal growth factor*, yielding more mature, healthy tissue layers and making the organoid more akin to human skin. Perhaps growing the organoids with different levels of EGF would yield more accurate results in the future. These improved human skin models can be used for drug testing, as well as understanding skin development and skin diseases[3]. By making them mimic real skin, the data acquired from them would be more accurate.

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