Novel In Vitro Technologies for Testing Compounds for Chronic Wound Care

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Clinicians still require new drugs

Many potential drugs do not even reach phase 1 testing

Only 1 in 10 drugs entering phase 1 gets approved

Therefore, we need to improve preclinical testing for better testing overall

Drug Development Pipeline (for new compound for treating chronic wounds):

- Discovery phase
- Lead optimization phase
- Pre-clinical testing phase
- Several phases of clinical testing
- Approval submission
- Only then it reaches patients

The pre-clinical phase is where we have to try and improve the methods

InVitro models:

Create a model to answer a particular research question

Types of questions vary. It might be a mechanism question, risk assessment (is it safe for humans?) or mode of action (how can you predict what it's going to do on a patient?)

Keep the model AS SIMPLE AS POSSIBLE. Only introduce complexity where it's necessary.

Take into account limitations: (can't test, 3,000 compounds simultaneously!)

Also take into account high or low throuput, physiological relevance, and method of application (topical, intradermal, systemic)

WOUND HEALING:

Compare an acute wound bed and a chronic wound bed

In chronic wound bed: prolonged inflammation, different composition of provisional matrix, enhanced matrix degredation, senescent bigroblases, low angiogenesis

Look at the wound (*without defining it)

What cells are present there?

1. adipose MSC
2. dermal fibroblasts
3. endothelial cells

We can culture them

Also keratinocytes and melanocyte co-cultures
We can isolate all the different cell populations to study them

When we increase complexity, we look at 3D organotypic models

In wound healing, we introduce immune cells

Skin co-culture model for topical or "systemic" application includes:

- Reconstructed epidermis in upper well
- Immune cells like DC, PBMC in lower well
- cells and culture supernatants (genomics / proteomics / metabonomics for the identification of novel biomarkers

Compare wound healing in acute vs. chronic wounds

Chronic wound characteristics:

- Prolonged inflammation
- Different composition of provisional matrix
- Enhanced matrix degradation
- Senescent fibroblasts
- Low angiogenesis

How to mimic a chronic wound environment in the lab?
Characterize chronic would bed environment
Collect tissues and analyze what's in it
Found it full of inflammatory mediators, grow factors, lots of proteins. It indicates that the chronic would bed is in inflammatory status

Look at the inflammatory response of a skin equivalent to chronic wound
Also look at the migration of cells in a chronic wound exudate
(we're looking at HEALTHY cells in these studies)

the skin equivalent is the reconstructed epidermis
supplement it with the chronic would exudate. We found that the background secretion of inflammatory proteins is quite low. Culture them with 10% of the exudate from the chronic wound bed, and they go sky high. The chronic wound exudate is extremely bioactive.

What about the endothelial cells?

If we supplement the culture medium, we get NO migration. We then stimulate them with potent grow factors and we added the chronic would exudate. It had NO affect on the migration.

Then, looked at the endothelial cell proliferation - chronic would exudate has NO effect on endothelial cell proliferation

Another – sprouting – angiogenesis
Make a fibrin gel and make a confluent layer of endothelial cells. Then give them a grow factor. They digest it and penetrate into the gel. You can quantify that from above. And also from cross section.
In vitro sprouting is NOT stimulated by chronic wound exudate.
Growth factor induced endothelial sprouting is REDUCED by chronic wound exudate.

Chronic wound exudate:

- Contains active factors which can be studied *in vitro*
- Different responses with keratinocytes, fibroblasts and endothelial cells
- Reduces the angiogenic response of endothelial cells

Conclusion:

The chronic would environment inhibits revascularization in the wound area.

In vitro models provide options to study novel treatments.