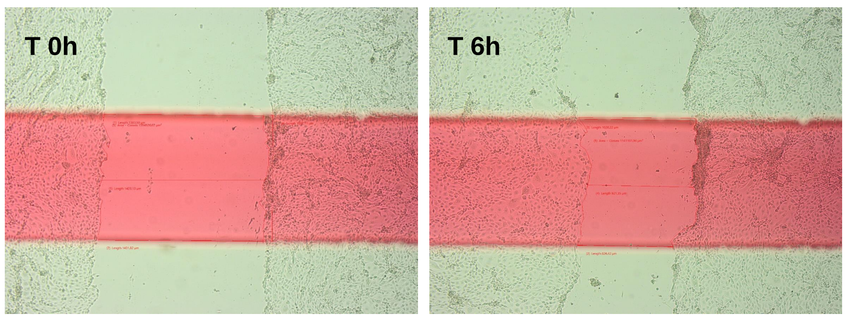
Wound-healing assay for nutritional compounds: not a 'one-size-fits-all' method

P7

L. Van Bockstal, S. Prims, C. Van Ginneken
Comparative Perinatal Development Group
University of Antwerp, Wilrijk, Belgium

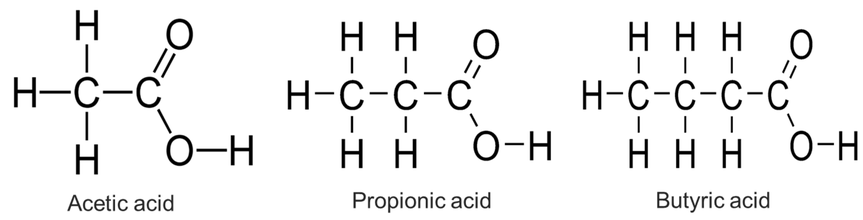
Background

The **wound-healing assay** is a simple, easy and inexpensive method to monitor cell proliferation and migration *in vitro*. A scratch is made in a monolayer of cells and the absolute wound closure rate is determined by comparing different images over time.



Aim

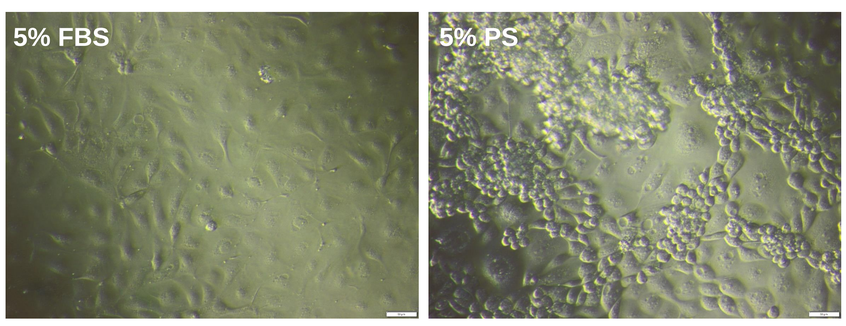
This study aims to determine the **effect of several short-chain fatty acids** (acetate, butyrate and propionate) **on the wound healing capacity of IPEC-J2 cells**, a porcine intestinal cell line.



To achieve our main goal an **optimized protocol** with proper positive and negative controls was developed.

Materials and Methods

1. IPEC-J2 cells display microvilli on their apical side and tight junctions sealing neighbouring cells together. However, usually these cells are being cultured in foetal bovine serum (FBS, 5%) which make them more robust, while supplementation with **porcine serum** (PS, 5%) results in characteristics that **mimic the *in vivo* situation** much better.



2. Additionally, when studying the effect of nutrients, it is advisable that the **negative control** is not too good since this might mask beneficial effects of your studied compound. Therefore, an additional '**starvation step**' (for 18h), where the cells are being exposed to a less rich medium (1%) can increase the gap between the studied compound and negative control.

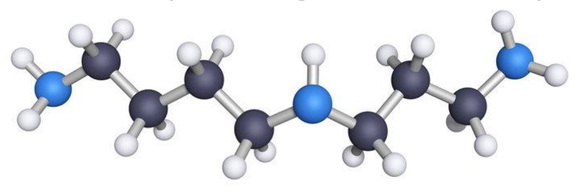
3. A **positive control** is equally important. The epidermal growth factor (EGF) was suggested by literature, however this compound promotes proliferation, while propionate for example, is more associated with cell migration, hence a compound that promotes cell migration rather than proliferation should be preferred. **Spermidine** was proven to be effective as a positive control in our protocol (p = 0.033), while EGF (0.8 nM or 8 nM) failed (p = 0.517 and p = 0.939, respectively).

Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(6): 194
https://doi.org/10.31083/j.fbl2706194

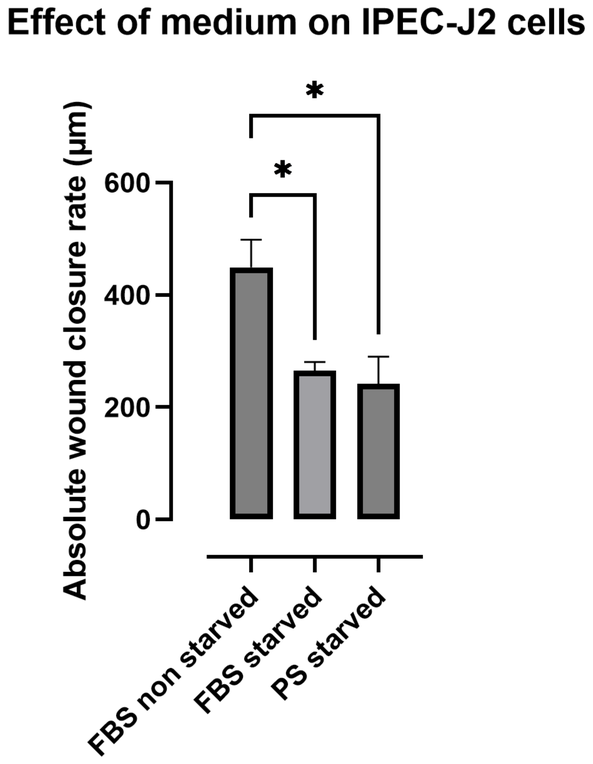
Original Research
Effects of Spermidine on Cell Proliferation, Migration, and Inflammatory Response in Porcine Enterocytes

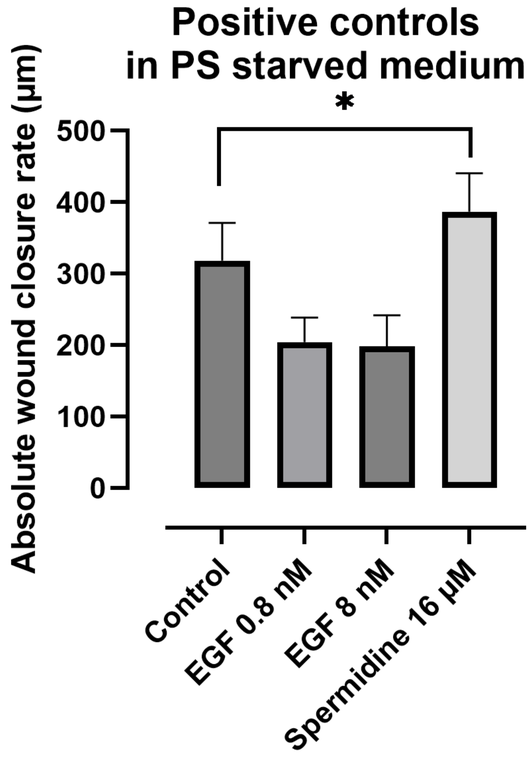
Zi-xi Wei[1,†], Long Cai[1,†], Xue-mei Zhao[1], Xian-ren Jiang[1], Xi-long Li[1,*]

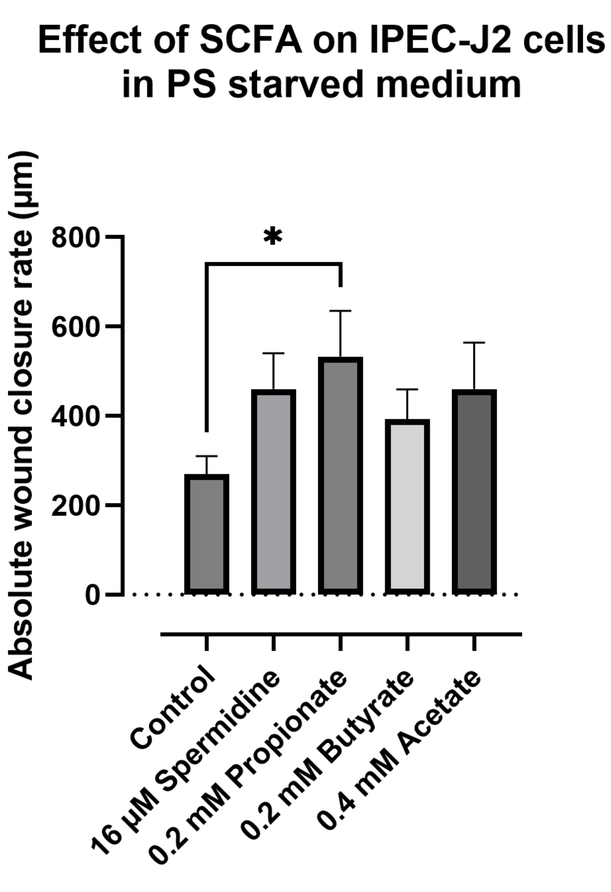


Spermidine
$C_7H_{19}N_3$
metabolic polyamine

Results







Conclusions

1. We were able to optimize our wound-healing assay by using 5% PS on IPEC-J2 cells, which were starved during 18h with 1% PS. We proved that spermidine is a good positive control with regards to cell migration.

2. We tested different short-chain fatty acids on the absolute wound closure rate of IPEC-J2 cells. Propionate at a 2 mM concentration showed to improve the wound healing significantly (p = 0.039) in our optimized wound-healing protocol. No effect of butyrate at a 0.2 mM concentration and acetate at a 0.4 mM concentration was seen (p = 0.584 and p = 0.199, respectively).

University of Antwerp
Faculty of Pharmaceutical, Biomedical and Veterinary Sciences