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In vitro culture of native jejunal segments for use as 3D intestinal testing system

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Isolated analysis of intestinal uptake is only possible *in vitro*

Effectiveness, quality and safety of new drug candidates meant for oral intake in humans have to be proven previous to clinical studies. Today's most precise method for preclinical studies is animal testing. Additional to ethical problems connected with these methods, metabolic processes and species differences complicate analysis of the experimental results.

Refinement of experiments, reduction of animal testings

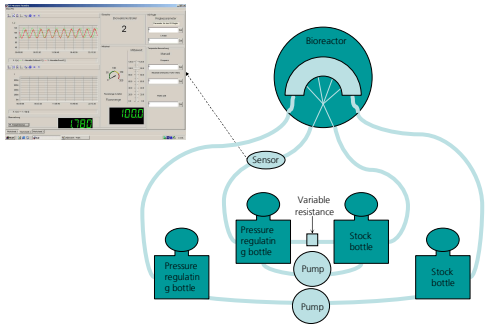
Uptake studies on isolated jejunal segments of different species obtained alive and functional *in vitro* give the option to reduce distracting metabolic processes as well as species differences. It also allows usage of several segments received from just one organ donor.

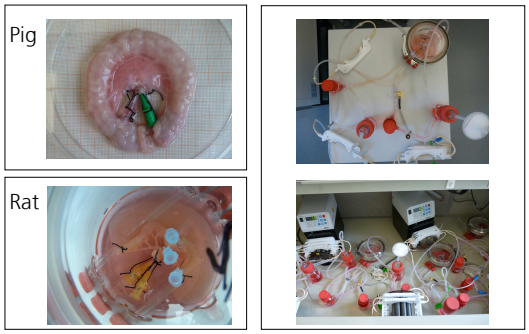
Methods

As soon as possible jejunal segments including their main vessels are explanted from euthanized pigs or rats.

Imitating the *in vivo*-situation the segments are perfused trough their own vascular system using a PC-controlled curve of pressure and a special balanced media.

Rat jejunum is also cultivated under an intermittant lumen perfusion.





Results

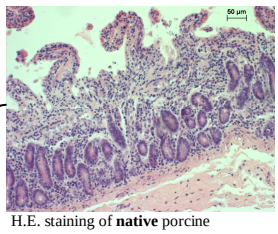
Successful obtaining of an isolated jejunal segment *in vitro* longer than 5 days

Due to our specialized bioreactor module and our high supplemented media composition it was possible to cultivate native porcine and rat jejunal segments *in vitro* for longer than 5 days. Porcine jejunal segments even showed undirected, but clear-cut peristalsis up to 10 days in culture.

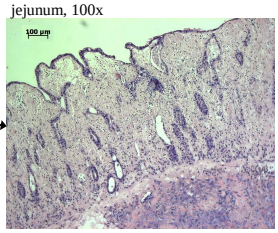
Rat even more than porcine jejunal segments showed enhanced reepithelialisation by vital enterocytes over culture period.

Histological and immuno-histological staining showed markers of typical cell types of small intestine, such as pan-cadherin, SMA and PCNA.

Pig

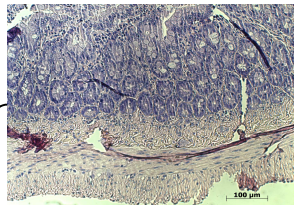


H.E. staining of **native** porcine jejunum, 100x

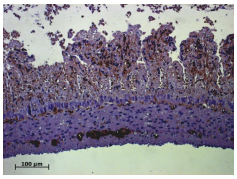


H.E. staining of porcine jejunum, **cultivated for 9 days**, 200x

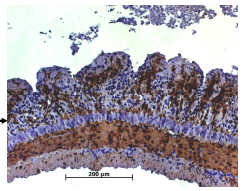
Rat



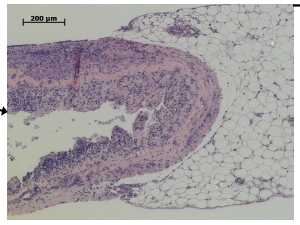
H.E. staining of **native** rat jejunum, 100x



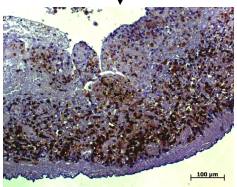
antibody staining against pan-cadherin, 200x



antibody staining against SMA, 200x



H.E. staining of rat jejunum, **cultivated for 6 days**, 50x



antibody staining against PCNA, 200x

Summary

Despite various kinds of animal testings used to explore intestinal resorption of drugs after oral uptake there is still no satisfying method established to estimate these processes in a human body. The difficulty is due to species differences as well in metabolic processes as in actions of drugs, that complicate usage of blood concentration or medical surveillance for analysis.
We succeeded in obtaining isolated jejunal segments of rats and pigs *in vitro*. Longer than 5 days cells still showed typical immuno-histological markers of cell types naturally found in the intestine. Longer than 9 days there still was an obvious peristaltic movement in cultured pig intestine.
By using isolated jejunal segments of different species for uptake studies under well defined conditions it is more easy to analyse uptake amounts, species differences and other influencing factors. Moreover animal testings could be reduced and replaced.

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