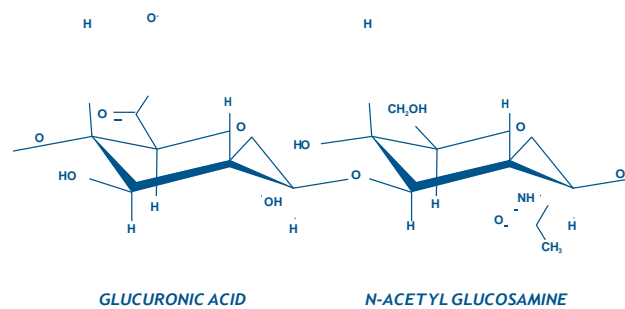


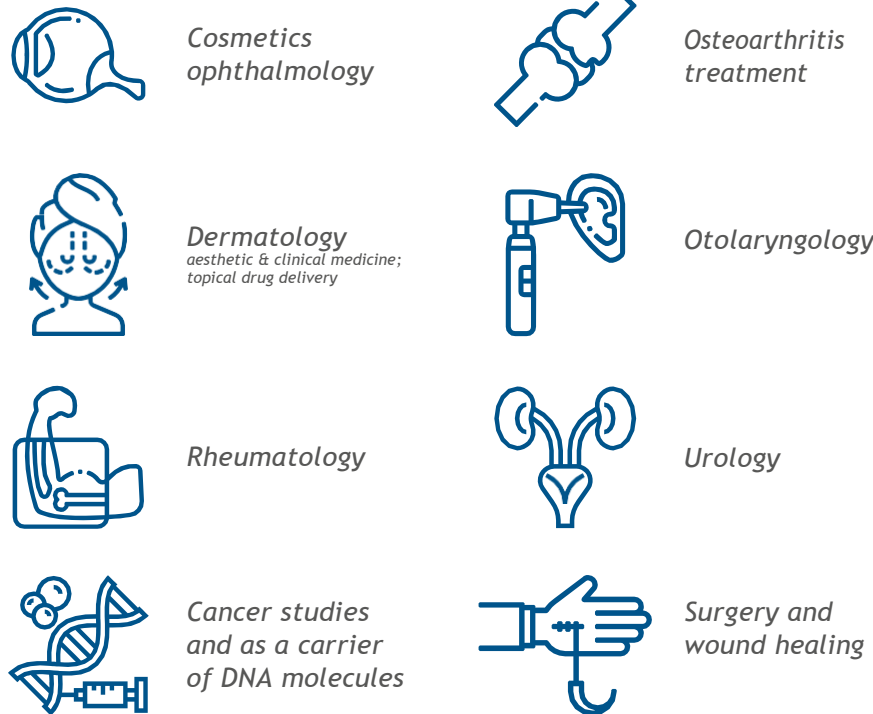
Bioavailability Study: Evaluation of the Hyaluronic Acid Absorption through a human reconstructed intestinal model

1 - INTRODUCTION

Hyaluronic Acid (HA), a linear glycosaminoglycan, is a major component of the extracellular matrix. It is composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl D-glucosamine linked via alternating β -1, 4 and β -1,3 glycosidic bonds¹. It is a body component present in any connective tissue and organ, such as skin, synovial fluid, blood vessels, serum, brain, cartilage, heart valves and umbilical cord². Hyaluronic acid has a wide range of applications based on its excellent physicochemical properties: biodegradability, biocompatibility, non-toxicity and non-immunogenicity, serving as an excellent tool in biomedical applications (osteoarthritis surgery, ocular surgery, plastic surgery, tissue engineering, and drug delivery)³. Recent data support the use of HA-based food supplements in the treatment of HA deficiency, for example in osteoarthritis⁴.



Hyaluronic Acid uses



2 - AIM OF THE STUDY

The purpose of the study was the evaluation of the absorption of an HA-based supplement through a simulated intestinal wall, represented by an in vitro reconstructed gut epithelium.

Specifically the biological test system adopted to simulate the penetration in the intestinal tract was EpiIntestinal by MatTek, a 3D model of reconstructed human intestinal epithelium incorporating enterocytes, paneth cell, M cells, tuft cells and intestinal stem cells into a highly differentiated, polarized epithelium; it also recapitulates many aspects of normal intestinal function including barrier, metabolism, inflammatory and toxicity responses, similar to native human intestinal tissue⁵. In this study an experimental comparison of the

absorption kinetics through the intestinal epithelium of the HA contained in SYALOX[®] 300 PLUS – River Pharma S.r.l. was performed, by considering both the digested and undigested form of the food supplement.

3 - INTESTINAL ABSORPTION OF THE TEST ITEM

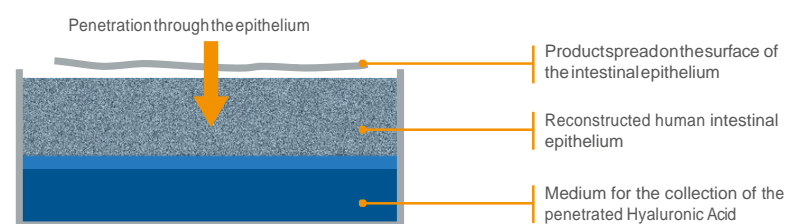


INTESTINAL PHASE

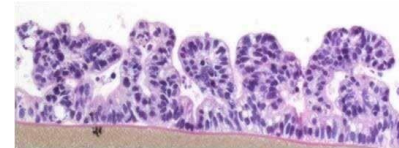
- Simulated duodenal juice
- 1% Pancreatin solution and bile salts
- Were added and stirred for 1 hour at 37°C

Aliquots of the obtained solution (named DIGESTA) and aliquots of undigested product solution were applied on the intestinal epithelium surface.

50 μ L of *Digesta* and 50 μ L *undigested product* solution (containing a theoretical amount of 2500 ng HA), were used for the absorption test, two experimental exposure time points were monitored (8 and 24 hours) by simulating the presence of the product inside the intestinal tract^{6,7}.



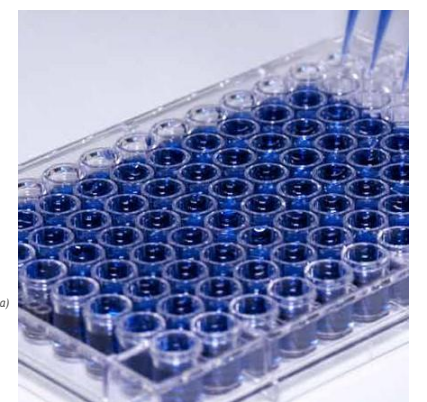
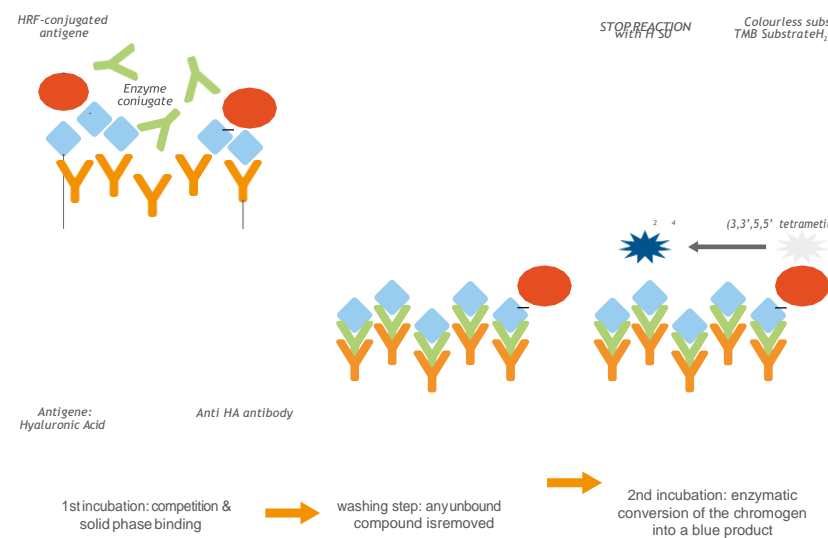
MatTek's EpiIntestinal™



4 - RESULT OF HA DOSAGE

After 8 and 24 hours of application the epithelia-underlying media were collected and the tissues were homogenated. HA dosages were performed on homogenates and media, in order to calculate the quantities penetrated and not released by the epithelium, and penetrated and released into the medium, respectively (bioavailable amounts). The assay was performed using a commercially available ELISA kit.

Enzyme-Linked Immunosorbent Assay (ELISA)



On the basis of applied product aliquots, amounts of collected and theoretical HA applied, as well as volumes of Hyaluronic acid in vitro Absorption test

Application time: 8 hours Dosages in the medium compartment*	HA (ng) without digestion mean st. dev.	Bioavailability without digestion	HA (ng) after digestion mean st. dev.	Bioavailability after digestion
SYALOX [®] 300 PLUS	1675,2 282,3	67,0%	1913,0 221,8	76,5%
Application time: 24 hours Dosages in intestinal epithelium medium*	HA (ng) without digestion mean st. dev.	Bioavailability without digestion	HA (ng) after digestion	Bioavailability after digestion
SYALOX [®] 300 PLUS	1980,9 290,3	79,2%	1996,4 187,9	79,9%

*no significant quantity of hyaluronic acid was recovered in the epithelium homogenate

5 - RESULT DISCUSSION

The results show that the HA included in SYALOX[®] 300 PLUS crosses the intestinal epithelia in this specific experimental in vitro system, appearing highly bioavailable (67.0% after 8 hours and 79.2% after 24 hours for undigested product, 76.5% after 8 hour and 79.9% after 24 hours for digested product). Data recovered after 8 hours suggest a higher absorption and therefore bioavailability for the digested form of the food supplement (76.5% vs 67.0%); after 24 hours the absorption values for both digested and undigested product are similar. Overall the experimental data confirm the high bioavailability of the hyaluronic acid contained in

