

# Successful outcomes of a new combined solution of hyaluronic acid, chondroitin sulfate and poloxamer 407 for submucosal injection: animal survival study



## Authors

Henry Córdova<sup>1</sup>, Miriam Cuatrecasas<sup>2</sup>, Ana García-Rodríguez<sup>1</sup>, Andrea Montenegro<sup>1</sup>, Jordana Melo<sup>1</sup>, Cristina Rodríguez-de Miguel<sup>1</sup>, Graciela Martínez-Pallí<sup>3</sup>, Rodrigo Garcés-Durán<sup>1</sup>, Josep Llach<sup>1</sup>, Gloria Fernández-Esparrach<sup>1</sup>

## Institutions

- 1 Endoscopy Unit, Gastroenterology Department, ICMDiM, IDIBAPS, CIBEREHD, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain
- 2 Pathology Department, CDB, Hospital Clínic, IDIBAPS, Universitat de Barcelona, Banc de Tumors Biobanc Clínic-IDIBAPS, Barcelona, Spain
- 3 Anesthesiology Department, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

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## Bibliography

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## Corresponding author

G. Fernández-Esparrach, Villarroel 170, 08036 Barcelona, Spain

Fax: +34 932279387

mgfern@clinic.ub.es

## ABSTRACT

**Background and study aims** Endoscopic resection requires use of submucosal injection. This study was conducted to assess efficacy and impact on early healing of hyaluronic acid combined with chondroitin sulfate and poloxamer 407 (Ziverel) when used as a solution for submucosal injection.

**Materials and methods** Prospective and comparative study of gastric endoscopic mucosal resection (EMR) with three groups of two Yorkshire pigs. Six submucosal cushions were created in each animal by injecting 2 mL of Ziverel (Group 1) or succinylated gelatin (SG) (Group 2), enabling 12 EMR in each group. Submucosal cushions were created with Ziverel in Group 3, without resection. Electrosurgery unit settings were the same in all cases. EMR defects and injection sites were marked with clips. The animals were sacrificed 7 days later. EMR specimen size and duration of procedure were recorded. EMR specimens and EMR scars and injection sites were evaluated by a blinded pathologist.

**Results** We successfully performed 24 EMR (15 en-bloc and 9 piecemeal, without differences between groups 1 and 2). Mean EMR specimen dimensions were significantly larger in Group 1 (median 19 mm, range 6–40 vs 16.6 mm, range 5–25;  $P=0.019$ ), without changing the electrocautery unit settings. Blinded histopathologist assessment of EMR specimens showed less fibrosis in the submucosa and a trend to fewer cautery artifacts with Ziverel and did not identify any significant differences in early healing of resection sites.

**Conclusion** The combination of Ziverel enables EMR and does not negatively affect early healing.

## Introduction

Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) are widely used in the West as therapeutic methods for resection of non-polypoid lesions of the gastrointestinal tract. The most effective and simplest way to prevent adverse events during EMR and ESD is to create a sufficiently thick submucosal layer by endoscopic injection of fluid into

the submucosa [1]. Sodium hyaluronic acid is commonly used in Japan to carry out ESD whereas in Western countries different solutions are available [2, 3].

In a recent comparative study of the submucosal injection fluids currently available in the West, a volume expander (6% hydroxyethyl starch) and Eleview (a mixture of medium chain triglycerides, poloxamer 188, polyoxyl-15-hydroxystearate, sodium chloride and methylene blue) were the best-performing

solutions for ESD in a porcine model [4]. Moreover, an ideal solution should be inexpensive and without potential damage of the resected specimen or early healing impairment.

Recently, we have reported that a new treatment for gastroesophageal reflux that combines hyaluronic acid, chondroitin sulfate and poloxamer 407 (Ziverel) could be used as a solution for submucosal injection because it creates a long-lasting cushion, does not induce acute tissue damage and it is not expensive [5].

The objective of this study was to assess the efficacy and impact on early healing of Ziverel as submucosal injection solution for EMR compared with succinylated gelatin (SG), which is the standard solution used in our Unit.

## Materials and methods

This prospective and comparative study was conducted with six Yorkshire pigs allocated into three groups: EMR with Ziverel (Norgine, UK) (Group 1,  $n = 2$ ), EMR with SG (Gelafundin, Braun Medical, Spain) (Group 2,  $n = 2$ ) and injection of Ziverel without resection (Group 3,  $n = 2$ ). Gastroscopy was conducted with the animals under general anesthesia and all the procedures were performed by a single endoscopist who was not blinded to which solution was injected.

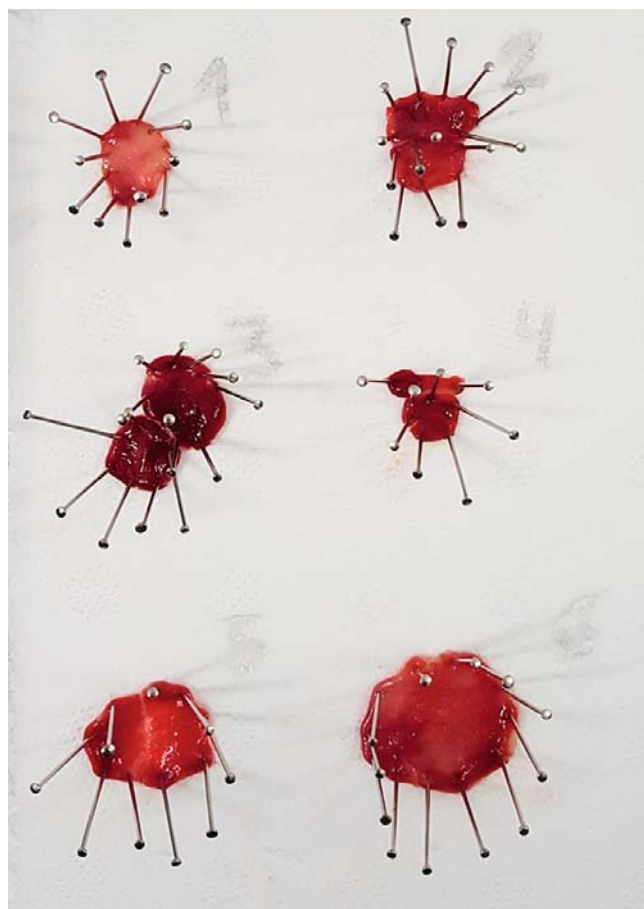
Six submucosal cushions were created in each animal by injecting 2 mL of solution with a 23-gauge catheter injection needle (Interject, Boston Scientific Corp., Marlborough, Massachusetts, United States). Ziverel was diluted with saline solution at a concentration of 50% to ease the injection [4]. In Groups 1 and 2, a total of 24 EMR were performed (12 in each group) using a 25-mm polypectomy snare (SD-210U-25, Olympus Medical Systems Corp., Tokyo, Japan). Each EMR was the largest possible safe en-bloc or piecemeal resection. An ERBE electro-surgery unit was used (VIO 300D, ERBE Co, Tübingen Germany) with the same settings (ENDOCUT Q mode, effect 3, duration 1, interval 6) in all cases. A 2-mL injection volume was chosen because preliminary experimentation showed that a larger volume resulted in a bleb that did not fit into the snare. At the end of the procedure, EMR defects and injection sites were marked with clips (► Fig. 1) and retrieved EMR specimens were pinned to a cork board (► Fig. 2).

EMR specimen size was measured (maximal length  $\times$  maximal width) and EMR duration was measured by time and number of pulses of Endocut needed to completion of snare resection. Note was made of systematic adverse effects, bleeding or perforation.

Animals were observed daily for 6 days and were sacrificed on day 7. The stomachs were retrieved and opened to find the remaining clips, and abdominal cavities were inspected. Stomachs and EMR specimens were fixed with formalin. Selected sections from each area marked with clips or mucosal lesion were cut and processed to obtain formalin-fixed paraffin-embedded tissue blocks. From each paraffin block, 2- $\mu$ m-thick histological sections were stained with hematoxylin and eosin (H&E) and examined with a conventional Olympus BX41 microscope (Olympus, Tokyo, Japan) by a pathologist blinded to the solution used. Pathological features such as reepithelization was scored as 0 = absence and 1 = presence, while fibrosis and



► Fig. 1 EMR defects and injection sites marked with clips.



► Fig. 2 Retrieved EMR specimens pinned to cork.

acute and chronic inflammation were semi-quantitatively scored as 0 = absence; 1 mild; 2 intermediate; 3 high. Thickness of the submucosa in the resected specimen and the granulation tissue were measured in mm.

The study was approved by the Ethics Animal Committee of Barcelona University.

► **Table 1** Technical and macroscopic characteristics of EMR.

	Group 1 (Ziverel®)		Group 2 (SG)		P value	
	All EMR N=12	En-bloc N=7	All EMR N=12	En-bloc N=8	All	En-bloc
Maximal length (mm)	19 (6–40)	19 (17–40)	16.5 (5–25)	16.5 (5–22)	0.019	0.035
Maximal width (mm)	16 (5–32)	15 (13–32)	12.5 (3–19)	10 (3–19)	0.060	0.041
Time (minutes)	10.6 (1.5–19.7)	8.5 (1.5–19.7)	10.2 (3.4–19.7)	9.2 (3.4–19.7)	0.95	0.972
Endocut pulses	9.5 (3–15)	7 (3–15)	8.5 (4–18)	7.5 (4–18)	0.8	0.994

EMR, endoscopic mucosal resection.

## Statistical analysis

Quantitative variables are expressed as median plus range. Statistical comparisons between groups 1 and 2 were made by using the Mann-Whitney non-parametric test. A  $P$  value  $<0.05$  was considered significant. Calculations were performed with SPSS software (SPSS 21.0 for Windows, SPSS, Inc., Chicago, Illinois, United States).

## Results

We successfully performed 24 EMRs (15 en-bloc and 9 piecemeal). The median EMR specimen maximal length was significantly larger in Group 1 than in Group 2 (19 mm, range 6–40 vs 16.5 mm, range 5–25;  $P=0.019$ ). Using the same electrocautery unit settings, mean time upon completion of all EMR was not different between groups (► **Table 1**). No perforation or intraprocedural hemorrhage was observed. Animals recovered well and gained weight (median 29.4 kg, range 27.9–20.6 vs 32 kg, range 29.9–33.9).

Microscopic examination of the EMR specimens showed a thicker submucosa with edema and a trend to fewer cautery-related artifacts in Group 1 (► **Table 2**, ► **Fig. 3**). The muscularis propria was not visible in any specimen.

We could identify the 24 EMR scars in the gastrectomy specimens at the time of necropsy. Histological assessment of the scars did not show any significant differences between Group 1 and 2, except for more fibrotic changes in the latter (► **Table 3**, ► **Fig. 4**). With regards to the stomachs in Group 3, only mild fibrosis and chronic inflammation were identified. These findings are commonly described in pigs' normal gastric mucosa (► **Table 4**).

## Discussion

In this study, we compared a new treatment for gastroesophageal reflux that combines hyaluronic acid, chondroitin sulfate, and poloxamer 407 with a common solution used for submucosal injection and evaluated their impact on the outcomes of gastric EMR and early healing in pigs. Use of the new substance was associated with larger and deeper EMR specimens and few-

er cautery artifacts, and did not negatively affect early healing or cause any long-term injury in the normal gastric wall.

SG was used as a control injection substance because it is used for performing EMR in our unit due to its durability, wide availability in our country and low price. Moreover, SG provides EMR specimens with larger surface area than with normal saline solution [3]. Compared with SG, Ziverel provided a better circumscribed elevation that facilitated tissue capture with the snare and a larger specimen.

The capability of Ziverel to separate the mucosal layer from the muscularis propria by increasing the thickness of the submucosa due to edema was already demonstrated in the pilot study performed by our group [5]. Poloxamer 407, which is one of the compounds of Ziverel, is a reverse phase polymer that becomes gel at body temperature, breaking and replacing the connective tissue. Having a thick submucosa layer included in the resected specimen is of paramount importance because it increases the chance of performing a curative resection with free margins, even in the case of deep infiltration of the superficial submucosa.

It is of crucial importance to achieve en bloc resection of colorectal laterally spreading tumors or early gastrointestinal neoplasia, because en bloc resection is associated with a low local recurrence rate [6, 7]. In the last few years, endoscopic submucosal dissection (ESD) has emerged as an alternative to EMR when lesions are big and it is not possible to perform en bloc resections. However, piecemeal EMR is still the standard of care in several countries, but even in that case, it is preferable to obtain large specimens to decrease the number of pieces [8]. Although with Ziverel the rate of en bloc EMR was not different from SG, the size of the resected specimens was larger.

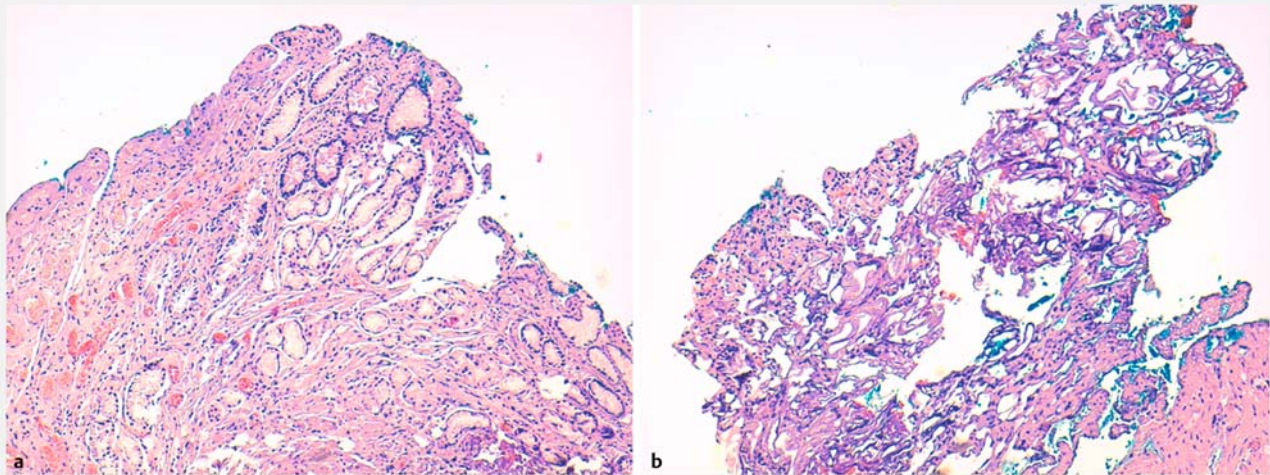
Damage to the resected specimen, either by piecemeal resection or by cauterization artifacts, can limit precise histological diagnosis of a targeted lesion, and hyaluronic acid has shown to be the least harmful [9]. Furthermore, damage to the muscle layer may result in delayed bleeding or perforation. The addition of poloxamer to hyaluronic acid used in the current study did not damage the mucosa of the EMR specimen, confirming its safety, but even more important was the fact that cautery damage of the borders was less evident than with SG.

The impact of potential solutions for submucosal injection on resected ulcer healing has been evaluated in several studies

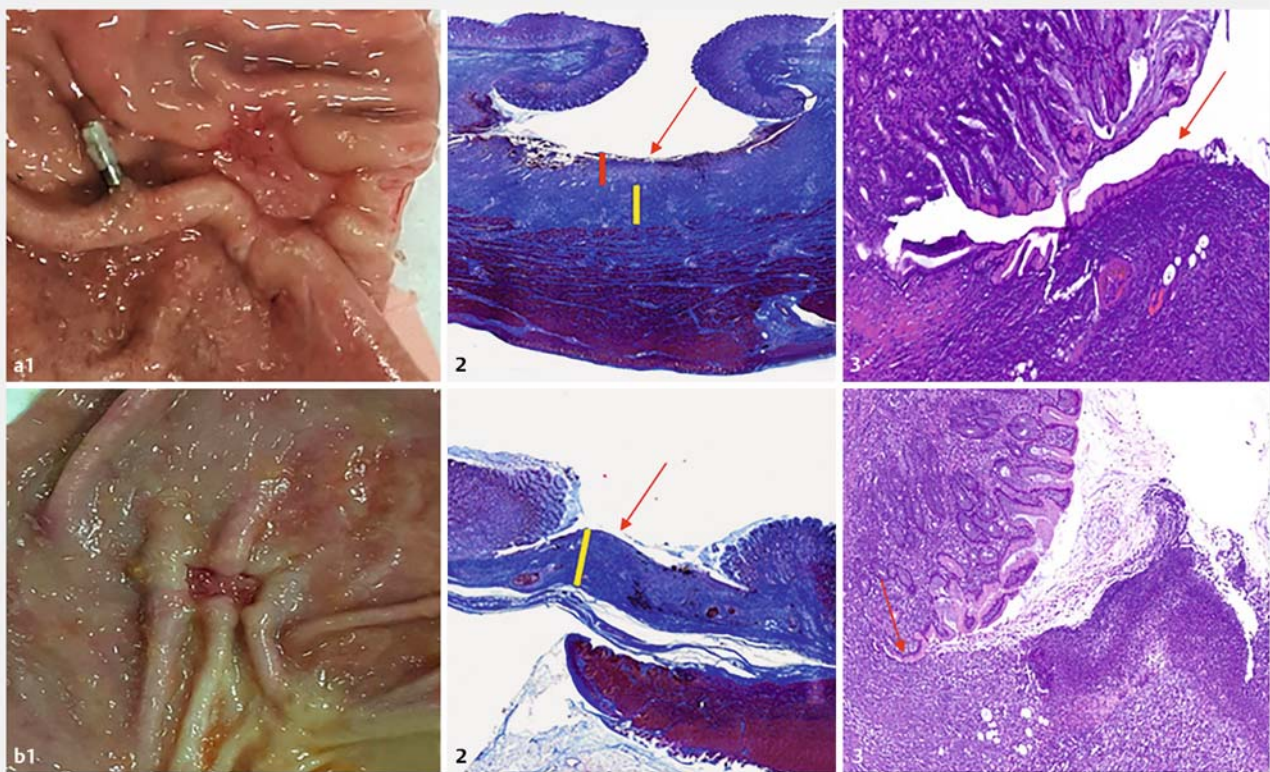
► <b>Table2</b> Histological findings of the EMR specimens.																												
		Group 1 (Zivarel )												Group 2 (SG)												P		
		1	2	3	4	5	6	7	8	9	10	11	12	total	1	2	3	4	5	6	7	8	9	10	11	12	total	
#EMR specimens		0	0	0	0	1	1	0	1	0	0	0	0	3	0	0	1	1	0	0	1	1	1	0	1	0	5	0.7
Acute inflam- mation																												
Congestion		1	1	1	2	1	1	1	1	1	3	1	3	17	0	1	2	3	2	1	1	1	1	3	3	2	20	0.4
Cautery injury		0	0	0	0	1	0	0	0	1	1	0	1	4	1	0	2	3	0	0	0	3	3	0	1	0	12	0.2
Submucosa (mm)		0.5	0.2	0.2	0.2	0.1	0.5	0.1	0.2	0.2	0.3	0.1	0.3	2.9	0.1	0.1	0.2	0.2	0.1	0.1	0	0	0.2	0.3	0.1	0.2	1.6	0.04
EMR, endoscopic mucosal resection.																												

► <b>Table3</b> Histological findings at the level of the gastric scars post-EMR.																											
	Group 1 (Zivarel)												Group 2 (SG)												P		
#EMR scars	1	2	3	4	5	6	7	8	9	10	11	12	total	1	2	3	4	5	6	7	8	9	10	11	12	total	
Reepithelization	0	0	0	0	0	0	0	0	1	1	1	1	4	0	0	0	0	0	0	1	1	1	1	0	1	5	0.7
Granulation (mm)	0.7	2	1	1.2	3	2	1.7	1.5	0.2	0.3	1	0.1	14.7	0.2	1	1.7	2	1.1	2.5	0.1	0.1	0.4	0.1	0.7	0.7	10.6	0.3
Fibrosis	1	0	1	1	1	1	2	1	2	1	2	2	15	2	2	1	1	1	1	2	2	2	2	1	2	19	0.03
Acute inflam- -mation	1	2	0	1	3	2	1	1	0	0	1	0	12	1	1	1	2	1	2	1	1	2	0	2	2	16	0.8
Chronic inflam- -mation	3	2	2	3	1	1	2	2	1	1	2	2	21	2	2	2	2	2	1	2	2	2	2	2	2	23	0.7
EMR, endoscopic submucosal resection.																											





► **Fig. 3** **a** Microscopic examination of the EMR specimens showing less mucosal cautery-related artifacts in the Zivarel group **b** than with SG, where the epithelial glands are not visible and architecture is lost. The muscularis propria is not visible in any specimen.



► **Fig. 4** **a** (1) Gross appearance of the scars with larger mucosectomy area with Zivarel **b** than SG. **a** (2) Microscopic examination of the scars – red arrows – shows residual superficial edema (red line) and less submucosal fibrosis (yellow line) with Zivarel **b** compared with SG. In this latter, there is no residual edema and thicker and more fibrotic changes in the submucosal scar. 3) Reepithelization is seen in both groups (red arrows), being more evident in **a** Zivarel group.

and glycerol has shown more pronounced histological signs of early healing [10]. In our study, Zivarel showed an early healing that was no different from SG, except for less fibrosis that could be explained by changes in permeability of injured mucosa

[11]. Based on these properties, we hypothesize that Zivarel could also facilitate the healing process of the scar left after EMR or ESD and expect an early proliferation of collagen and elastic fibers as described with other solutions [10, 12].

► **Table 4** Histological findings in the stomachs injected with Ziverel without resection.

	Group 3 (Ziverel <sup>®</sup> without resection)												
# Injections	1	2	3	4	5	6	7	8	9	10	11	12	total
Reepithelization	0	0	0	0	0	0	0	0	0	0	0	0	0
Granulation (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibrosis	1	1	1	1	1	1	1	0	0	0	1	2	10
Acute inflammation	0	0	0	0	0	0	0	0	0	0	0	1	1
Chronic inflammation	0	1	1	1	1	2	2	1	2	1	3	3	18

The use of solutions with high osmolality, such as hypertonic saline solution or 20% to 50% dextrose water, induces acute mucosal damage consisting of erosion and congestion of capillary blood vessels that is persistent a week after injection, in the form of submucosal fibrosis [13]. Ziverel is also a hyperosmotic solution with around 2,000 mOsm/L but contrarily, was not associated with any significant abnormality in the gastric wall 1 week after the injection.

Another interesting result is that the new solution did not affect the speed of EMR. Using the same settings of the electro-surgical unit as with SG, there were no differences regarding in time needed to complete EMR. These results are consistent with the initial experience with poloxamer [14].

Among available submucosal injection solutions in clinical practice, so far the most suitable for producing a long-lasting cushion is high-molecular-weight hyaluronic solution [15]. However, the crucial disadvantage of hyaluronic acid in current formulations is the high cost, which limits its use in many countries. Therefore, the combination of hyaluronic acid with Glyceol has been proposed as an acceptable low-cost alternative [15]. Interestingly, the cost of Ziverel is significantly lower than hyaluronic acid alone (0.10 and 11.84 USD per mL, respectively) and very similar to Gelafundin (0.08 USD per mL). Therefore, its use could decrease the final cost of the procedure, making its use widely available.

This study has the strength that the pathologist was blind to the solution used. The endoscopist knew which solution was tested, as noticeable differences in viscosity of the two solutions during the injection and the consistency of the blebs made endoscopist blinding impractical.

With regard to limitations, we only injected 2 mL of solution, which is far from the usual amount needed for a large EMR. We chose this amount because the consistency of the bleb made it impossible to catch a larger bleb with a 25-mm snare.

## Conclusion

In conclusion, the current study demonstrates that the combination of hyaluronic acid, chondroitin sulfate, and poloxamer 407 enables EMR and does not negatively affect early healing.

## Acknowledgement

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## Competing interests

Dr. Fernández-Esparrach has received fees for organizing courses for Norgine Iberia and Olympus Spain in the last 2 years and has been a consultant for a trial design for CDx Diagnostics

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