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CLINICAL INVESTIGATION

CROSS-LINKED HYALURONAN GEL REDUCES THE ACUTE RECTAL TOXICITY OF RADIOTHERAPY FOR PROSTATE CANCER

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Purpose: To prospectively analyze whether cross-linked hyaluronan gel reduces the mean rectal dose and acute rectal toxicity of radiotherapy for prostate cancer.

Methods and Materials: Between September 2008 and March 2009, we transperitoneally injected 9mL of cross-linked hyaluronan gel (Hylaform; Genzyme Corporation, Cambridge, MA) into the anterior perirectal fat of 10 early-stage prostate cancer patients to increase the separation between the prostate and rectum by 8 to 18mm at the start of radiotherapy. Patients then underwent high–dose rate brachytherapy to 2,200cGy followed by intensity-modulated radiation therapy to 5,040cGy. We assessed acute rectal toxicity using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 grading scheme.

Results: Median follow-up was 3 months. The anteroposterior dimensions of Hylaform at the start and end of radiotherapy were 13 ± 3mm (mean ± SD) and 10 ± 4mm, respectively. At the start of intensity-modulated radiation therapy, daily mean rectal doses were 73 ± 13cGy with Hylaform vs. 106 ± 20cGy without Hylaform ($p = 0.005$). There was a 0% incidence of National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 Grade 1, 2, or 3 acute diarrhea in 10 patients who received Hylaform vs. a 29.7% incidence ($n = 71$) in 239 historical controls who did not receive Hylaform ($p = 0.04$).

Conclusions: By increasing the separation between the prostate and rectum, Hylaform decreased the mean rectal dose. This led to a significant reduction in the acute rectal toxicity of radiotherapy for prostate cancer. © 2009 Elsevier Inc.

Cross-linked hyaluronan gel, Prostate, Toxicity.

INTRODUCTION

Prostate cancer is the most common cancer in men, accounting for 25% of all cancers (1). One in six men will be diagnosed with prostate cancer during their lifetime (2). The American Cancer Society estimates that there were 186,320 new cases of prostate cancer in 2008 (1).

In 74% of cases prostate cancers arise posteriorly in the peripheral zone of the gland (3). The radiation dose at the edge of an intensity-modulated radiation therapy (IMRT) field is only half of the dose at the center of the field. By including the anterior wall of the rectum in IMRT fields, one can increase the radiation dose that is delivered to a prostate cancer, thereby increasing the likelihood of locoregional control (4), biochemical disease-free survival (5–9), and distant metastasis-free survival (10–12).

The rectum is sensitive to radiation therapy. As a result, rectal injury is the dose-limiting toxicity of radiotherapy for prostate cancer (13–15). By increasing the separation between the prostate and rectum, one can reduce the risk of rectal injury.

Cross-linked hyaluronan (*i.e.*, hyaluronic acid) is a sugar that occurs naturally in the skin, cartilage, joints, and eyes. Cross-linked hyaluronan gel has a number of surgical applications including its role as a tissue filler (16).

The purpose of this study is to prospectively analyze whether cross-linked hyaluronan gel can increase the separation between the prostate and rectum and thereby reduce the mean rectal dose and acute rectal toxicity of radiotherapy for prostate cancer.

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Conflict of interest: The Cancer Center of Irvine has a research grant from Genzyme Corporation to study cross-linked hyaluronan gel in patients undergoing radiotherapy for localized prostate cancer.

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METHODS AND MATERIALS

One group in Spain previously studied the ability of cross-linked hyaluronan gel to reduce mean rectal doses and rectal toxicity in prostate cancer patients undergoing brachytherapy with or without IMRT (17, 18). We conducted the first study of cross-linked hyaluronan gel in American men with prostate cancer based on the Spanish group's encouraging results. We needed to obtain an Investigational Device Exemption (IDE) from the Food and Drug Administration (FDA) to conduct the study. As part of our background preparation, we conducted rheological analysis showing that cross-linked hyaluronan gel degrades more quickly after it is irradiated. The FDA then granted the Cancer Center of Irvine (Irvine, CA) an IDE to treat 10 prostate cancer patients with cross-linked hyaluronan gel. The Western Institutional Review Board also granted approval for the single-institution, single-arm, open-label, Phase I study with historical controls. In accordance with the Food and Drug Amendments Act (Title VIII, Section 801), we registered the trial online with ClinicalTrials.gov. All of the prostate cancer patients in our study provided informed consent for treatment with cross-linked hyaluronan gel and radiotherapy.

Fiducial gold seed placement in prostate

Using transrectal ultrasound (TRUS) guidance, a urologist inserted 5 fiducial gold seeds into the patient's prostate gland under anesthesia (19). Urologists placed fiducial markers at the following sites: (1) base, (2) posterior mid gland (3) right mid gland (4) left mid gland, and (5) apex. The gold seeds made it possible to determine the location of the prostate using electronic portal imaging immediately before each IMRT treatment (20). We adjusted the patient's setup each day based on the location of the prostate.

Cross-linked hyaluronan gel

Genzyme Corporation (Cambridge, MA) has several types of cross-linked hyaluronan gels including the one used in this study: Hylaform (21). Hylaform is a safe, strong hydrogel manufactured from rooster combs (22). Rooster combs consist predominantly of hyaluronic acid. The combs are processed to remove as much unrelated material as possible, leaving only hyaluronic acid.

Hylaform is swelled to equilibrium with physiologic saline solution in order for it to act as a tissue filler. The body absorbs irradiated Hylaform over a period of approximately 4 to 8 months and nonirradiated Hylaform over a period of approximately 6 to 12 months.

Injection of cross-linked hyaluronan gel

We placed each patient in the dorsal lithotomy position under spinal or general anesthesia and prepared and draped him. We then inserted a No. 16 Foley catheter into the bladder and inflated its balloon with 5 mL of contrast material. Next, we inserted a 6.5-MHz endorectal ultrasound probe into the rectum. We placed a Tayman-Tokita template against the perineum. We then inserted 16 to 18 high–dose rate (HDR) brachytherapy treatment needles into the prostate transperineally under TRUS guidance.

We removed the TRUS probe from the ultrasound stand and held it by hand. Using TRUS guidance, we advanced a 17-gauge needle transperineally into the anterior perirectal fat. We first placed the needle tip at the apex of the prostate. Care was taken not to perforate the posterior prostatic capsule or the anterior rectal wall. It is easier to inject the gel when a small syringe (*e.g.*, a 3-mL syringe) is used. Consequently, we attached a 3-mL syringe containing cross-linked hyaluronan gel to the needle. After aspirating to ensure that the tip of the needle was not in a blood vessel, we injected 3 mL of cross-linked hyaluronan gel into the anterior perirectal fat extending from the level of the apex of the prostate superiorly along the posterior border of the midline of the lower half of the prostate. We used axial TRUS images to guide placement of the gel. We then attached a second 3-mL syringe containing cross-linked hyaluronan gel to the needle. After aspirating to ensure that the tip of the needle was not in a blood vessel, we injected 3 mL of gel into the anterior perirectal fat extending superiorly along the posterior border of the midline of the upper half of the prostate. Next, we attached a third 3-mL syringe containing cross-linked hyaluronan gel to the needle. After aspirating to ensure that the tip of the needle was not in a blood vessel, we injected 3 mL of gel into the anterior perirectal fat extending superiorly from the base of the prostate along the seminal vesicles. We created an additional 8- to 18-mm anteroposterior (AP) separation between the prostate and the rectum at the start of radiotherapy using 9 mL of cross-linked hyaluronan gel (Fig. 1).

Radiotherapy planning and treatment

Between March 2004 and March 2009, we treated 239 control patients at the Cancer Center of Irvine with no Hylaform, HDR brachytherapy to 2,200 cGy, and IMRT to 5,040 cGy. Between September 2008 and March 2009, we treated 10 early-stage prostate cancer patients with cross-linked hyaluronan gel and the same radiotherapy approach described previously. Patient characteristics are presented in Table 1.

For the HDR brachytherapy, we delivered 550-cGy fractions twice a day on the days of the first and second prostate implants. The two implants were performed 1 week apart, resulting in a total brachytherapy dose of 2,200 cGy in four fractions over a period of 8 days. The brachytherapy dose was prescribed to the 100% isodose line. The prostate gland constituted the clinical target volume (CTV) for the brachytherapy.

We obtained a treatment planning pelvic computed tomography (CT) scan before and after the injection of cross-linked hyaluronan gel. Next, we constructed a dose–volume histogram for each CT scan, contouring the rectum as a solid organ from the ischial tuberosities to the rectosigmoid junction. We then calculated mean rectal doses for the IMRT portion of the treatment. We did not calculate mean rectal doses for the brachytherapy portion of the treatment because we preplan with ultrasound. We followed rectal dose constraints in Radiation Therapy Oncology Group protocol 0126 involving IMRT for localized prostate cancer, stating that no more 15%, 25%, 35%, and 50% of the rectal volume should receive 94.7%, 88.4%, 82.1%, and 75.8%, respectively, of the prescribed dose. Next, we defined the rectal wall by assuming a 3-mm wall thickness (23). We constructed hypothetical treatment plans as if we were delivering IMRT alone to a total dose of 8,100 cGy in 45 daily fractions over a period of 9 weeks. We then determined rectal wall volumes that would have received 6,000 cGy (V60) and 7,000 cGy (V70) relative to the total rectal wall volume (23, 24). These values are known as the rectal wall relative V60 and V70.

We administered IMRT to a total dose of 5,040 cGy in 28 daily fractions over a period of 5.5 weeks beginning 1 to 4 days after the completion of brachytherapy. If the risk of pelvic lymph node involvement was 15% or lower according to the formula Percent lymph node risk = 2/3 × Prostate-specific antigen + [(Gleason score –6) × 10] (25), then the CTV for the IMRT was the prostate gland and inferomedial 10 mm of the seminal vesicles. The CTV was treated to 5,040 cGy by use of daily 180-cGy fractions. The planning target volume included 0- to 10-mm margins on the CTV. At least 98% of the planning target volume received 100% of the prescribed dose. If the risk of pelvic lymph node involvement was greater than

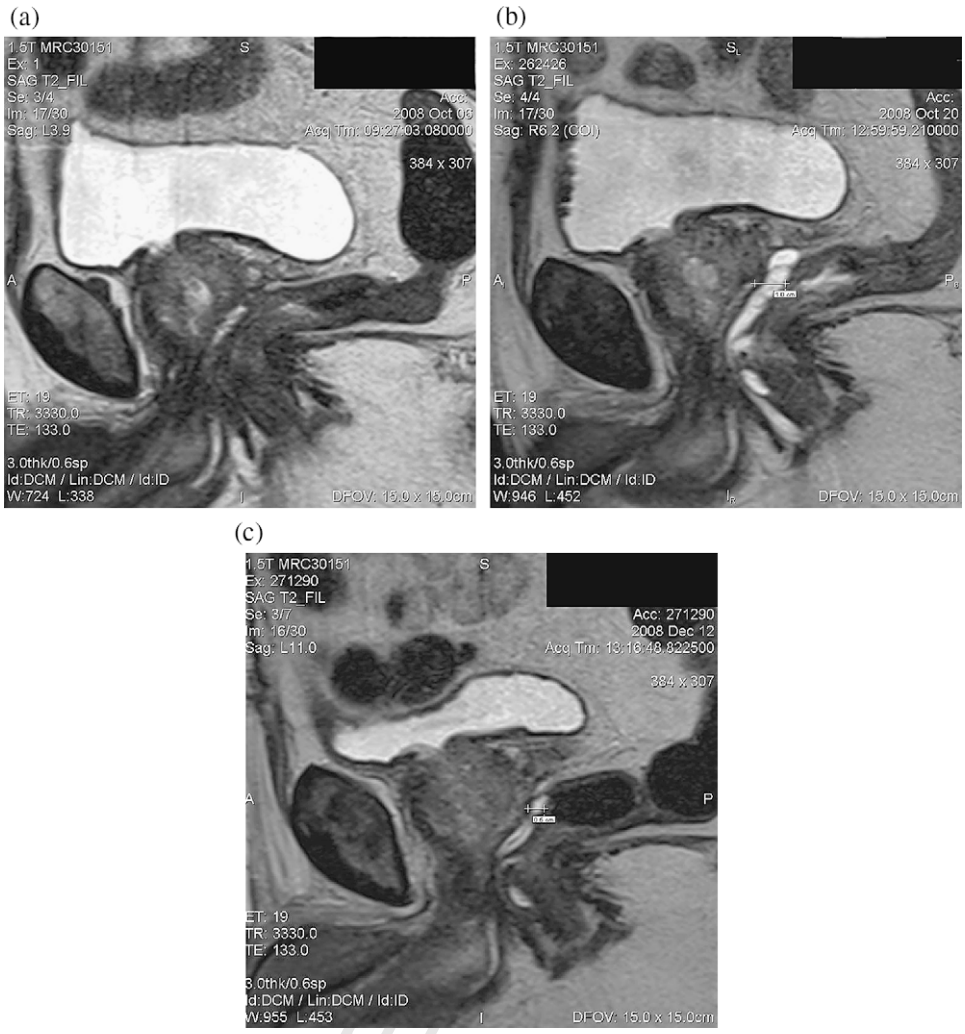

Fig. 1. (a) Sagittal T2-weighted pelvic magnetic resonance imaging scans before injection of cross-linked hyaluronan gel, (b) after injection of cross-linked hyaluronan gel and at the start of radiotherapy, and (c) at the end of radiotherapy. Cross-linked hyaluronan gel between the prostate and rectum appears hyperintense (white). The 10-mm anteroposterior dimension of cross-linked hyaluronan gel (b) at the start of radiotherapy decreased to 5 mm (c) at the end of radiotherapy because of absorption by the body.

15%, the initial IMRT CTV also included the pelvic lymph nodes as defined by Hsu *et al.* (26). We delivered 4,500 cGy to the initial CTV in 25 daily fractions over a period of 5 weeks. We then administered 540 cGy in 3 daily fractions to the final CTV consisting of the prostate and inferomedial 10 mm of the seminal vesicles.

Magnetic resonance imaging

Cross-linked hyaluronan gel is clearly visible on T2-weighted magnetic resonance imaging (MRI) scans without contrast and ultrasound images but not on CT scans. We obtained the first pelvic MRI scan before cross-linked hyaluronan gel injection (Time Point 1). We then obtained a second pelvic MRI scan 2 days after the injection of cross-linked hyaluronan gel (Time Point 2). Next, we obtained a third pelvic MRI scan at the end of the radiation therapy (Time Point 3), which was 7 weeks after the injection of cross-linked hyaluronan gel. The maximum AP dimension of cross-linked hyaluronan gel was measured on MRI scans.

Acute rectal toxicity

The most common acute rectal toxicity due to radiotherapy is diarrhea (27). Acute toxicity is defined here as toxicity occurring within 270 days of the first day of radiotherapy (28). We have observed that diarrhea is most severe during the fifth week of IMRT and quickly improves after the completion of radiotherapy. We scored the severity of diarrhea during the fifth week of IMRT according to the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 grading scheme (29), shown in Table 2.

Statistics

The primary endpoint of this study is mean rectal dose. The null hypothesis is that there is no difference between mean rectal doses with vs. without cross-linked hyaluronan gel. The probability of incorrectly rejecting the null hypothesis, or α error level, is 5%. An α error level of 5% corresponds to a 95% confidence interval. The probability of incorrectly failing to reject the null hypothesis, or β error level, is 12.5%. This results in a sample size of 10 patients for an expected reduction in the mean rectal dose of 10% $\pm$ 12% with cross-linked hyaluronan gel.

We used a 2-tailed Fisher exact test (30) to compare the proportions of patients who received pelvic lymph node irradiation with vs. without cross-linked hyaluronan gel. We used a 2-tailed

Table 1. Patient characteristics

Characteristic	HDR brachytherapy and IMRT (n = 239)	Hylaform, HDR brachytherapy, and IMRT (n = 10)
Age [median (range)] (y)	71 (47–88)	62 (66–83)
Follow-up [median (range)] (mo)	24 (1–53)	3 (1–6)
Clinical T stage		
T1a	0% (1)	0% (0)
T1b	2% (6)	0% (0)
T1c	96% (227)	90% (9)
T2a	1% (2)	0% (0)
T2b–T2c	0% (0)	10% (1)
T3a	1% (3)	0% (0)
Gleason score		
2–6	50% (119)	30% (3)
7	37% (89)	40% (4)
8–10	13% (31)	30% (3)
PSA		
<10 ng/mL	77% (185)	90% (9)
10–20 ng/mL	18% (42)	10% (1)
>20 ng/mL	5% (12)	0% (0)
Androgen deprivation therapy		
Yes	38% (91)	40% (4)
No	62% (148)	60% (6)
NCCN recurrence risk		
Low	42% (99)	20% (2)
Intermediate	42% (99)	50% (5)
High	16% (41)	30% (3)
Pelvic lymph node irradiation	29% (68)	40% (4)
Diabetes mellitus	9% (21)	10% (1)

Abbreviations: HDR = high dose rate; IMRT = intensity-modulated radiation therapy; PSA = prostate-specific antigen; NCCN = National Comprehensive Cancer Network.

Wilcoxon signed rank test (31) to compare mean rectal doses and rectal wall relative V60 and V70 with vs. without cross-linked hyaluronan gel. We used a 2-tailed Mann-Whitney U test (32) to compare the severity of diarrhea in patients who received and did not receive cross-linked hyaluronan gel. If the p value is less than 0.05, there is a significant difference between groups.

RESULTS

Median follow-up was 3 months. The AP dimensions of Hylaform at the start and end of radiotherapy were 13 ± 3 mm (mean ± SD) and 10 ± 4 mm, respectively.

At the start of IMRT, daily mean rectal doses were 73 ± 13 cGy with Hylaform vs. 106 ± 20 cGy vs. without Hylaform (p = 0.005). In patients who received Hylaform, the rectal wall relative V60 and V70 were 12% ± 9% and 4% ± 4%, respectively, at the start of IMRT. These percentages would have increased to 33% ± 13% (p = 0.005) and 25% ± 12% (p = 0.005), respectively, if these patients had not received Hylaform.

There was no significant difference in the proportions of patients who received pelvic lymph node irradiation with vs. without cross-linked hyaluronan gel (p = 0.48) (Table 1). There was a 0% incidence of National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 Grade 1, 2, or 3 acute diarrhea in 10 patients who received Hylaform vs. a 29.7% incidence (n = 71) in 239 controls who did not receive Hylaform (p = 0.04) (Table 3). There were no complications attributable to injection of cross-linked hyaluronan gel into the anterior perirectal fat.

DISCUSSION

Mean rectal dose is a strong predictor of acute rectal toxicity due to radiotherapy for prostate cancer (33). Patients who have acute rectal toxicity are more likely to have late rectal toxicity (23, 34). A dose–wall histogram is slightly better than a dose–volume histogram for predicting the risk of late rectal bleeding (35). In particular, rectal wall relative V60 and V70 are strong predictors of chronic rectal toxicity of Grade 2 or higher (23, 24).

Since another group had previously injected cross-linked hyaluronan gel into the anterior perirectal fat without complications (17, 18), we believed that the risks of the procedure were minimal compared with the benefits that could be achieved. The main risk associated with injection of cross-linked hyaluronan gel was infection (36). Prophylactic antibiotics (cefazolin and gentamicin) were administered, decreasing this risk to less than 5%. There was a less than 5% risk of an allergic reaction because patients who were allergic to avian products were excluded from the study (37, 38). Tenderness and pain at the injection site were also possible (39). In addition, bleeding, bruising, redness, discoloration, or formation of a granuloma or keloid at the injection site were possible (39–41). Lastly, embolization of cross-linked hyaluronan gel through the blood was a potential though unlikely complication (42).

Prada *et al*. (17) in Spain used a different cross-linked hyaluronan gel. This may help to explain why they obtained

Table 2. National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 grading scheme

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Diarrhea	Increase of <4 stools per day over baseline	Increase of 4–6 stools per day over baseline; intravenous fluids for <24 hours; not interfering with activities of daily living	Increase of ≥7 stools per day over baseline; intravenous fluids for ≥24 hours; hospitalization; interfering with activities of daily living	Life-threatening consequences (*e.g.*, hemodynamic collapse)	Death

Table 3. Severity of acute diarrhea in patients who did and did not receive cross-linked hyaluronan gel Q13

	Patient received cross-linked hyaluronan gel		
	Yes	No	Total
Adverse gastrointestinal event (diarrhea) based on NCI CTCAE v3.0 grading scheme			
No increase of stools per day over baseline			
No. of patients	10	168	178
%	100%	70.3%	71.5%
Increase of <4 stools per day over baseline (Grade 1)			
No. of patients	0	65	65
%	0%	27.2%	26.1%
Increase of 4–6 stools per day over baseline (Grade 2)			
No. of patients	0	5	5
%	0%	2.1%	2.0%
Increase of ≥7 stools per day over baseline (Grade 3)			
No. of patients	0	1	1
%	0%	0.4%	0.4%
Total			
No. of patients	10	239	249
%	100%	100%	100%

Abbreviation: NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

a different AP dimension in the perirectal fat. The Spanish group did not observe any side effects from the cross-linked hyaluronan gel in 27 prostate cancer patients treated with two temporary seed implants and external beam radiotherapy based on a mean follow-up of 13 months (range, 9–22 months). Patients did not complain of pain, tenesmus, rectal pressure, or a sensation of rectal filling for the duration of the presence of cross-linked hyaluronan gel *in vivo*. There were no biocompatibility or carcinogenicity issues associated with the irradiated cross-linked hyaluronan gel. By increasing the separation between the prostate and rectum, cross-linked hyaluronan gel significantly reduced the mean rectal dose. Cross-linked hyaluronan gel also significantly decreased the incidence of mucosal damage observed on proctoscopic examinations and macroscopic rectal bleeding in 69 prostate cancer patients treated with a permanent iodine-125 seed implant (18).

Because the FDA limited our study to 10 patients, we did not have adequate statistical power to assess quality of life. Nevertheless, we observed that cross-linked hyaluronan gel significantly decreases the mean rectal dose and rectal toxicity in accordance with Prada *et al.* (17, 18). Because we had 239 historical controls who had undergone HDR brachytherapy and IMRT without cross-linked hyaluronan gel, we did not think that a randomized design was necessary for this Phase I study.

One original aspect of our work is that we conducted rheological analysis of cross-linked hyaluronan gel. We observed a linear relationship between the reduction of the elastic modulus and viscosity of the gel and the radiation dose. The elastic modulus and viscosity decreased at a rate of approximately 0.005%/cGy. After irradiation to a therapeutic dose, the gel maintained an elastic modulus exceeding that of fat *in vivo* (43). Patients absorbed irradiated gel slightly more quickly than the reported results for non-irradiated gel (44, 45). Srinivas and Ramamurthi (46) also observed that irradiation causes cross-linked hyaluronan gel to degrade more quickly. A second original aspect of this study is that we assessed the severity of acute diarrhea due to HDR brachytherapy and IMRT with and without cross-linked hyaluronan gel. Prada *et al.* (17) injected cross-linked hyaluronan gel after the delivery of 1,150 cGy via an HDR brachytherapy implant and 2,000 cGy via external beam radiotherapy. Unlike Prada *et al.*, we injected cross-linked hyaluronan gel before the start of HDR brachytherapy and IMRT in an effort to reduce rectal toxicity as much as possible. Table 3 shows that the gel significantly decreased the severity of acute diarrhea due to radiotherapy. A third original aspect of this study is that we calculated rectal wall relative V60 and V70 values. Cross-linked hyaluronan gel significantly decreased the rectal wall relative V60 and V70. We will continue to observe Q12 patients treated with cross-linked hyaluronan gel to determine whether they have less chronic rectal toxicity of Grade 2 or higher than our historical controls. We will also apply for an IDE from the FDA to conduct a larger, multi-institutional study of cross-linked hyaluronan gel in patients undergoing radiotherapy for localized prostate cancer. We will assess radiation doses delivered to the rectum, rectal toxicity, and quality of life with vs. without cross-linked hyaluronan gel.

Ben-Yosef *et al.* (47) in Israel used an animal model to study an implantable, biodegradable balloon made of polylactic acid and caprolactone copolymers that is 10 to 20 mm in the AP dimension and 35 mm in the lateral dimension when fully inflated. The balloon requires a 2- to 3-mm dilator and a sheath over it for insertion. The Israeli group plans clinical testing in prostate cancer patients undergoing radiotherapy. Although an advantage of their approach is that the tissue spacer has up to a 35-mm lateral dimension,

the balloon requires a relatively large dilator and sheath for insertion and causes a foreign-body reaction. In contrast, hyaluronic acid is injected via a needle with only a 1.5-mm outer diameter and is a naturally occurring polysaccharide (48).

In summary, cross-linked hyaluronan gel decreased the mean rectal dose due to radiotherapy for prostate cancer by increasing the separation between the prostate and rectum. This led to a significant reduction in the acute rectal toxicity of radiotherapy.

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