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ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

The Collagenase of the Bacterium *Clostridium histolyticum* in the Treatment of Irradiation-Induced Capsular Contracture

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Abstract

Background Irradiation therapy is an important pillar in the treatment of breast cancer. However, it can trigger capsular fibrosis, the most significant complication of implant-based breast reconstruction. As collagen is the main component of fibrotic capsules, the collagenase of the bacterium *Clostridium histolyticum* poses a potential treatment option for this pathological condition.

Methods Thirty-six rats received miniature silicone implants on their backs. On day 1, the implant sites of two groups were irradiated with 10 Gy. On day 120, one irradiated group received collagenase injections into the implant pockets ($n = 12$). Non-irradiated ($n = 12$) and irradiated capsules ($n = 12$) were injected with plain solvent solution serving as controls. Data were analyzed by means of in vivo imaging, histology, immunohistochemistry and gene expression analysis.

Results Compared with both controls, the injection of collagenase led to significantly thinner capsules. This was verified by in vivo imaging and histology. Although irradiation provoked alterations in capsule collagen structure and vessel wall thickness, the application of collagenase resulted in a significant reduction of collagen density. This was accompanied by an up-regulation of VEGF-A gene expression. Of note, hematoma formation inside the implant pocket occurred in two cases after collagenase injection.

Conclusions The collagenase of the bacterium *Clostridium histolyticum* is effective in degrading irradiation-induced capsular fibrosis around silicone implants. Hematoma formation occurred most likely because of irradiation-induced alterations in vessel wall architecture and capsule vascularization. Further studies need to be performed to address the clinical safety of this novel treatment option.

Keywords Breast augmentation · Capsular contracture · Silicone implants · Collagenase of the bacterium *C. histolyticum* · Capsular fibrosis

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Introduction

The insertion of silicone implants for reconstructive or aesthetic purposes is one of the most frequently performed procedures in plastic surgery [1]. The most prevalent complication after this procedure is the formation of capsular contracture [2–4]. Its incidence rate is heavily influenced by factors like site of implant placement, follow-up period, implant surface and especially indication for implant insertion [5–9]. Interestingly, capsular contracture

occurs three times more frequent in patients who received an implant for breast reconstruction after breast cancer treatment compared with aesthetic indications [10]. Nevertheless, approximately 70% of women undergoing reconstruction after mastectomy decide on silicone implants, and of those, up to 50% develop capsular contracture [11–13]. To date, revision surgery is the only viable treatment option for clinically relevant capsular contracture [14–16].

As the fibrotic capsule mainly consists of collagen, the collagenase of the bacterium *Clostridium histolyticum* (CCH) might be a viable treatment option for capsular contracture. This drug is already FDA-approved for the treatment of Dupuytren's contracture, as well as Peyronie's and Ledderhose diseases [17–19].

In our previous studies, CCH injections led to a dose-dependent reduction of fibrotic capsules around silicone implants [20, 21]. Despite these encouraging results, translation of this therapy to clinical application poses a barrier. Post-mastectomy irradiation can lead to significant alterations in morphology, thickness and collagen organization of the fibrotic capsules, which could potentially influence the effectiveness and safety of CCH application [22].

The aim of this study was to investigate the effect of CCH on irradiation-induced capsular contracture tissue in a standardized rat model. The primary endpoint of this study was the effect of CCH on capsule thickness and density after irradiation. Analyses of gene expression and immunohistochemistry were considered secondary endpoints.

Methods

Study Design

Sample size calculation was based on our previous studies [20, 23]. With the assumption of equal standard deviations, type I error < 0.05, type II error < 0.2 and effect size of 1.1, the number of animals needed is 12 per group. Therefore, we included 36 rats in this study, divided into three groups (CG[Øirrad], CG[irrad] and CCH[irrad]). All animals received silicone implants in a sub-muscular pocket on their backs on day 0. On day 1, rats of groups CG[irrad] and CCH[irrad] received irradiation with 10 Gray (Gy). After stable capsule formation on day 120, a collagenase solution of 0.3 mg/ml (CCH[irrad]) or 0.0 mg/ml CCH (CG[Øirrad] and CG[irrad]) was injected into the implant pockets. On day 130, animals underwent MR imaging and were euthanized and subsequent analyses were performed as described below. According to a study by Sengupta et al. [24], 1 month of an adult rat's life equates to 3 human years. Therefore, the follow-up period of this manuscript equates to one human year.

Animal Model

The research protocol was approved by the Harvard Medical Standing Committee on Animals (AEP No.: 05086). Female Lewis rats received miniature silicone implants with textured surfaces (Polytech Health and Aesthetic, Dieburg, Germany). Implants were inserted in a sub-muscular pocket through a 4-cm incision on the back of each rat [23]. Ten days after CCH injections, animals were euthanized and silicone implants with surrounding capsules were explanted en bloc.

Irradiation

One day after insertion of silicone implants, study groups CG[irrad] and CCH[irrad] received a single irradiation treatment at the implant site with 10 Gy. This is based on a protocol by Katzel et al. [22] in which, except for the increase in fibrotic tissue, no other harmful side effects of the irradiation are reported. Irradiation was administered using a Mark I 68A irradiator (JL Shepherd and Associates, San Fernando, CA) with a Cesium 137 irradiation source.

Collagenase Injection

We used the collagenase of the bacterium *Clostridium histolyticum* (CCH, Xiaflex, Endo Pharmaceuticals, Malvern, USA). CCH (0.1 ml) was injected at a concentration of either 0.0 mg/ml (CG[Øirrad] and CG[irrad]) or 0.3 mg/ml (CCH[irrad]) 120 days after surgery, based on previously established protocols [20, 23]. Solutions were injected through a small incision on top of the implant between capsule and implant shell with a blunt needle.

Clinical Evaluation

Daily after injections, all animals were monitored for the occurrence of systemic and local side effects like skin perforation or hematoma formation.

Magnetic Resonance Imaging

Animals underwent magnetic resonance imaging (MRI) on day 130 based on previously established protocols [9, 20, 23]. We used an ultra-high-field MRI preclinical scanner, 7.0T Bruker BioSpec® (Billerica, MA). Imaging parameters were set as previously described [20, 23]. Data were analyzed via ImageJ (v. 1.46, NIH, USA) and 3D Volume measurement plug-in "VOLUMEST" [25]. Capsule thickness was measured in three planes from the outer

margin of the implant to the outer side of the skin (Fig. 1). Capsule volume was measured as previously described using "VOLUMEST" plug-in for ImageJ [9]. Results are expressed as mean ± standard deviation (SD).

Histology

After fixation in formaldehyde, capsule specimens were subsequently embedded in paraffin for histologic analysis.

Capsule thickness was assessed by two blinded investigators using Masson trichrome staining. Thickness of fibrotic capsules was measured at the thickest part of the capsule between the surface of the implant and the first tissue that did not belong to the capsule (fat, muscle) [20, 23]. Collagen fiber density was quantified and depicted by Picrosirius red staining. Under circularly polarized light, collagen thick, thin and total fiber density was quantified by counting of stained pixels with ImageJ and evaluation as the percentage of the whole image pixel count. Measurements were taken at 10 randomly chosen sites.

Additionally, wall thickness and diameter of intracapsular vessels were assessed on hematoxylin & eosin-stained slides. Measurements were taken at 20 randomly chosen vessels at a 400 × magnification. A ratio of wall thickness to vessel diameter was formed to ensure comparability between groups. All results are given as mean ± standard deviation.

Immunohistochemistry

Immunohistochemistry was performed using antibodies against collagen subtypes I–IV and TGFβ1 (Abcam, Cambridge, UK) as recommended by the manufacturer. After heat-mediated antigen retrieval, slides were incubated with primary and secondary antibodies. Diaminobenzidine was used for visualization, and cell nuclei were counterstained with hematoxylin. For each sample, three randomly chosen sites of the capsule were digitalized at a 20× magnification. Antigen density was measured as described by Schon et al. [26]. Densities are calculated as percentage of brown-colored pixels in relation to the whole image pixel count.

Quantitative Real-Time PCR

Total cellular RNA was extracted using the RNeasy Mini Kit (Qiagen, USA) and transcribed with the ABI PRISM TaqMan reverse transcription method (Applied Biosystems, USA). Table 1 shows a list of all analyzed markers with corresponding article numbers. Quantitation of gene expression was determined by means of the ABI PRISM 7900HT System (Applied Biosystems, USA). To normalize relative gene expression to expression levels of the housekeeping gene β2-microglobulin, we applied the comparative CT (ΔΔCT) method. Results of expression analysis are given in relative quantitation (RQ).

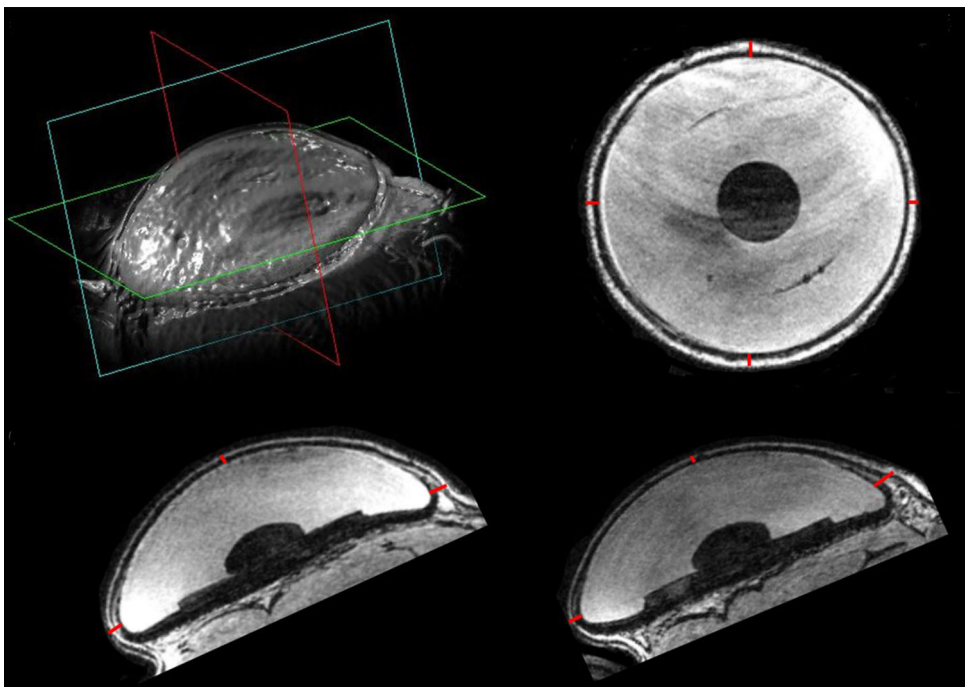

Fig. 1 MRI analysis of in vivo capsule thickness. Top left: 3D-reconstruction, top right: axial plane, bottom left: sagittal plane, bottom right: coronal plane. Red lines indicate capsule thickness measurements. Scale was set by measuring the inner circle which has a diameter of 0.8 cm

Table 1 Primers used for qRT-PCR analysis according to catalog numbers

Primer name	Catalog number
Col1a1	Rn01463848_m1
Col2a1	Rn01533081_m1
Col3a1	Rn01437660_g1
Col4a1	Rn01482927_m1
TGFβ1	Rn00572010_m1
TGFβ3	Rn01517871_m1
CD68	Rn01495634_g1
IL12	Rn01456866_m1
B2m (housekeeping gene)	Rn00560865_m1

Statistical Analysis

Statistical evaluation was performed using the Statistical Package for Social Science SPSS, Version 18.0 (SPSS Inc., Chicago, USA). Data of all groups were assessed using analysis of variance (ANOVA). Post hoc testing was performed by means of *t* test with Bonferroni correction. Results are reported as means ± standard deviations. According to Bonferroni corrections, the statistical significance level was set as $p < 0.017$.

Results

Clinical Evaluation

In two cases of group CCH[irrad], intracapsular hematoma formation occurred one day after collagenase injections (17%). Additionally, rupture of the silicone implant was observed in one animal of group CG[irrad] (8%). We did not detect any signs of skin perforation or skin laceration, implant site infection or other adverse effects.

Magnetic Resonance Imaging

Irradiated capsules of group CG[irrad] appeared to be thicker compared to CG[Øirrad]. However, differences were not statistically significant. Radiologic capsule thickness was considerably lower in group CCH[irrad] after irradiation and CCH injection when compared to both control groups, respectively (1.28 ± 0.25 vs 1.94 ± 0.23 (CG[irrad]) or 1.82 ± 0.42 mm (CG[Øirrad])). Compared to CG[irrad], differences reached statistical significance ($p < 0.017$). Results of in vivo capsule thickness measurements are depicted in Fig. 2.

In terms of capsule volume analysis, we observed a similar data distribution, yet without statistically significant differences (data not shown).

Histologic Capsule Thickness

Results of histologic capsule thickness measurements are shown in Fig. 2. Within control groups, capsule thickness was notably higher after irradiation of the implant site (CG[Øirrad] vs CG[irrad]), yet no statistical significance was detectable. In comparison with both controls, treatment with CCH was able to significantly decrease histologic capsule thickness to mean values of 144.72 ± 30.97 μm ($p < 0.017$).

Capsule Collagen Density

The total collagen fiber density was 24.07 ± 10.02, 35.83 ± 11.01 and 13.23 ± 5.8% for the groups CG[Øirrad], CG[irrad] and CCH[irrad], respectively. Irradiation (CG[irrad]) leads to remarkably denser fibrotic capsules when compared to CG[Øirrad]. In terms of CCH injections, we observed a considerable decrease in capsule collagen content which reached statistical significance when compared to CG[irrad].

Correlating with the results for total collagen, thick and thin fiber density was highest after irradiation of implant sites (CG[irrad]). However, statistically significant differences were not detected in the analysis of thin fiber density. For thick-fiber density, we observed the lowest values after injection of CCH which amounted to 2.74 ± 3.06%. This was significantly less than in the control group CG[irrad], $p < 0017$. Results for collagen density measurements are shown in Fig. 3.

Vessel Wall Thickness/Diameter Ratio

Figure 4 depicts the results of histologic vessel analysis. Injections of CCH (CCH[irrad]) did not lead to any alterations of vessel walls when compared to CG[irrad]. In group CG[Øirrad], we measured a mean thickness/diameter ratio of 0.46 ± 0.28. This was significantly higher than found for both irradiated groups CG[irrad] and CCH[irrad] ($p < 0.017$).

Immunohistochemistry

Antibody staining for collagens I–IV revealed that the CCH leads to a reduction in mainly collagens I and III, whereas it omits collagen IV, as expected from the collagenase properties (data not shown). However, no statistically significant differences between groups could be identified.

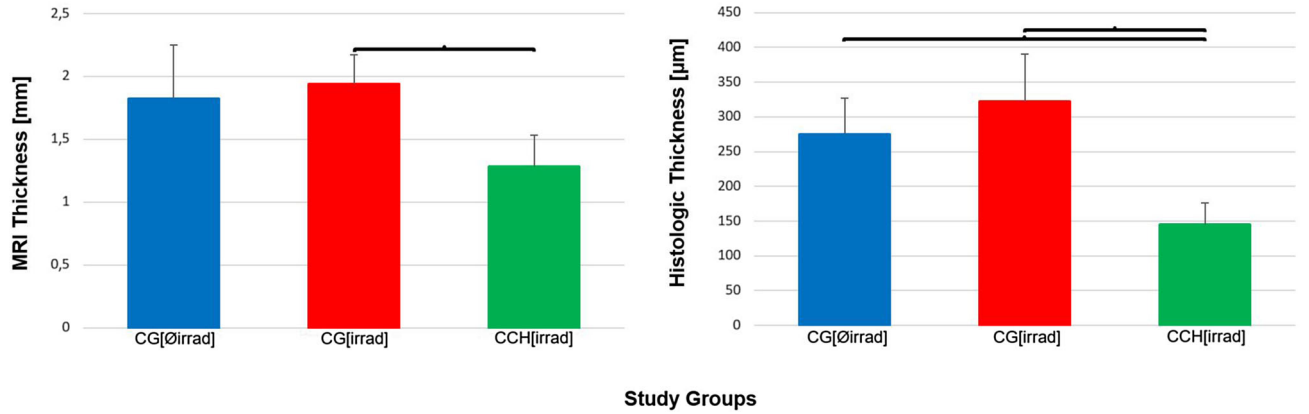

Fig. 2 MRI and histologic capsule thickness measurements. Mean values (bars) with corresponding standard deviations. Brackets indicate statistically significant differences ($p < 0.017$)

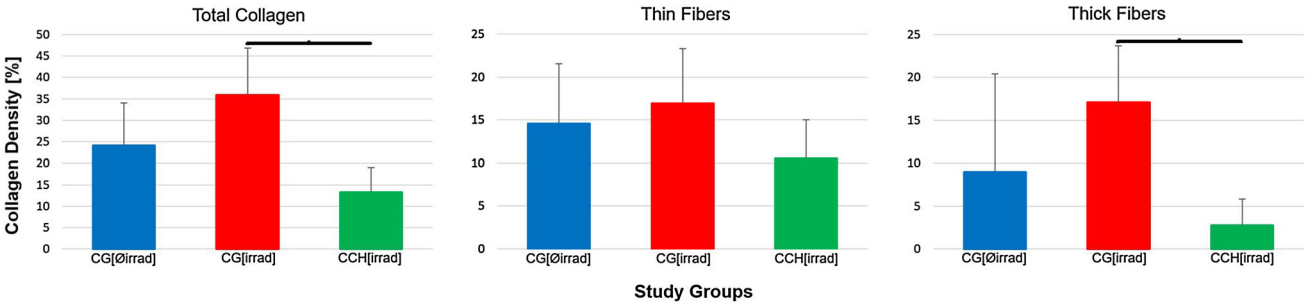

Fig. 3 Capsule collagen density measurements. Left) total collagen, middle) thin fibers, right) thick fibers. Results are given as mean values (bars) with corresponding standard deviation. Brackets indicate statistically significant differences ($p < 0.017$)

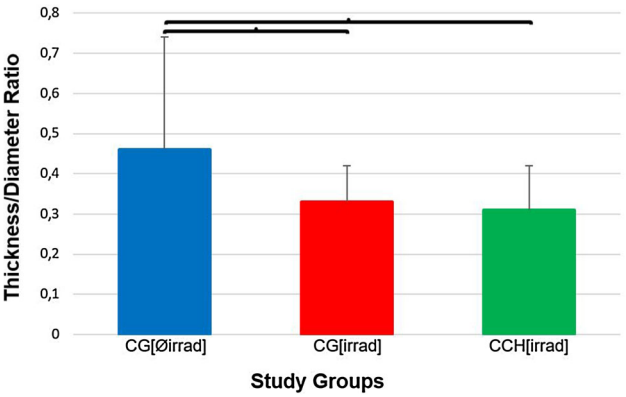

Fig. 4 Results of vessel wall analysis. Mean values (bars) with corresponding standard deviation. Brackets indicate statistically significant differences ($p < 0.017$)

qRT-PCR

A significant overexpression of CD68 was observed in CCH[irrad] compared to CG[Øirrad] ($p < 0.017$; Fig. 5). Although the expression of the profibrotic gene TGFβ1 showed higher levels after irradiation with or without CCH injections, TGFβ3 was under-expressed compared to CG[Øirrad]. However, differences were not statistically significant. With respect to the proangiogenic marker VEGF-A, significant differences were observed between CCH[irrad] and CG[Øirrad] (RQ 5.33 ± 0.14, $p < 0.017$) and between CCH[irrad] and CG[irrad] (RQ 5.33 ± 0.14 vs 1.96 ± 0.27, $p < 0.017$). Significant differences for collagen subtypes I–IV and the inflammatory marker IL-12 have not been detected.

Discussion

This study demonstrates that the CCH is effective in reducing irradiation-induced fibrotic capsules surrounding silicone implants. This was determined by means of in vivo MR imaging, as well as histology. Interestingly, the combination of singular irradiation of implant sites and injection of CCH leads to a down-regulatory trend of TGFβ3 expression, whereas other inflammatory and profibrotic markers displayed a tendency toward higher expression levels. Despite these encouraging results, hematoma formation occurred in 17% of our experimental group.

Depending on tumor size, tumor entity, lymph node metastasis and resection status, radiotherapy is a crucial part of breast cancer treatment. This has to be taken into

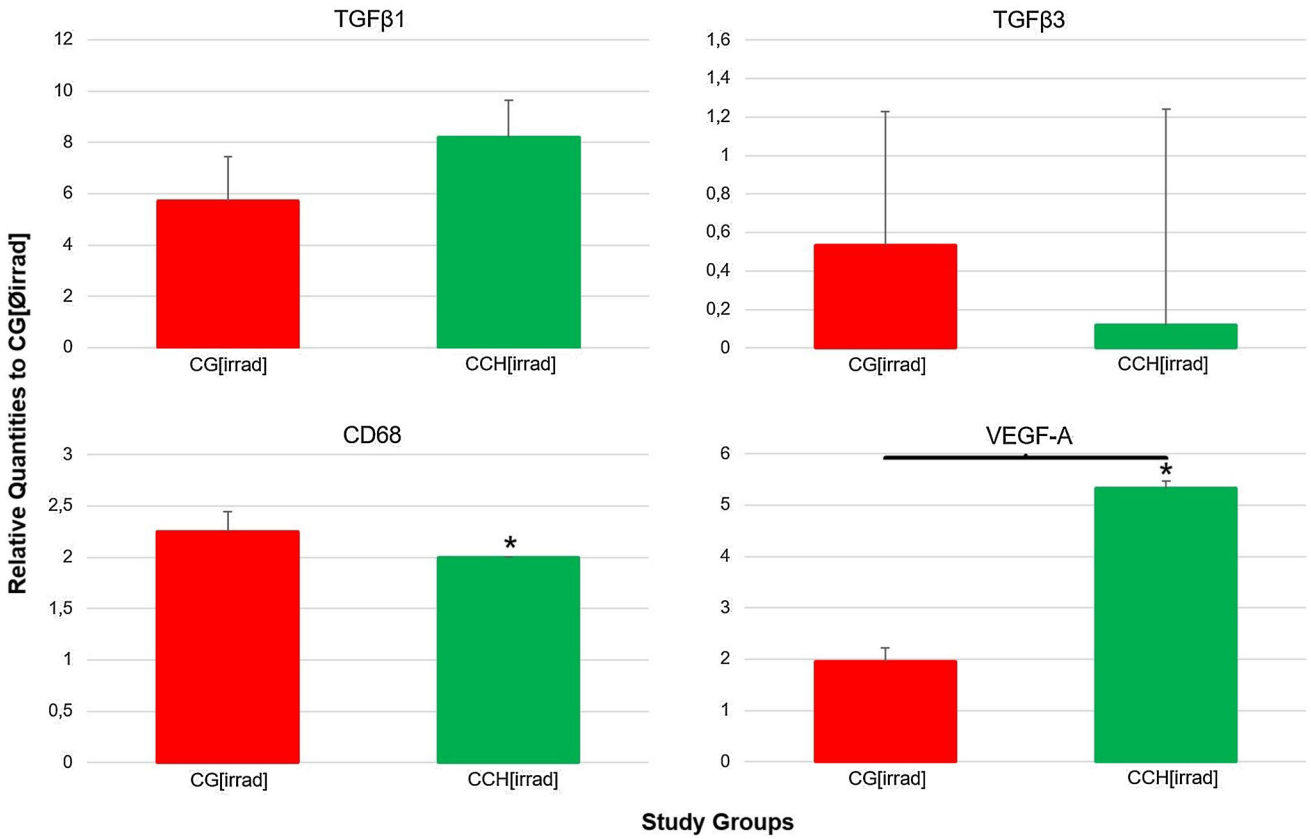

Fig. 5 Results of qRT-PCR analysis. Mean values (bars) with corresponding standard deviation. Asterisks denote statistically significant differences ($p < 0.017$) compared to CG[Øirrad]. Brackets indicate statistically significant differences between groups CCH[irrad] and CG[irrad]

account, especially when considering that approximately 70% of women undergoing reconstruction after mastectomy decide on silicone implants and 50% of those develop capsular contracture [11]. This might be most likely associated with radiation therapy. In a long-term cohort study, Cordeiro et al. [13] evaluated 1415 patients receiving silicone breast implants and demonstrated that incidence rates of capsular contracture were significantly higher in irradiated breasts. Interestingly, radiotherapy does not only affect the occurrence of capsular contracture but also the structure of the fibrotic capsule. Katzel et al. [22] investigated the impact of radiotherapy on capsular contracture formation in a mouse model. The authors detected that capsule tissue was much thicker and disorganized after irradiation. Accordingly, in our study, histologic capsule thickness was higher in the irradiated control group compared to no radiotherapy. This was apparent in MR imaging and histologic analysis. Additionally, we observed changes in collagen structure, such as denser arrangement of collagen fibers. This was accompanied by an overexpression of profibrotic and inflammatory genes like CD68 or TGFβ1.

Besides examining the effectiveness of CCH injections, several authors particularly investigated the safety of CCH in the treatment of Dupuytren's contracture and Peyronie's disease [17, 27–29]. In a structured literature review of 59 clinical studies involving 8809 patients, Peimer et al. [27] compared the safety profile of CCH application with surgical fasciectomy. Although incidence rates for adverse effects like chronic regional pain syndrome or scar hypertrophy were noticeably lower following enzyme treatment, injury of the skin occurred in 16.2% compared to 2.8% after surgery. These skin injuries included less severe side effects like skin discoloration and blistering as well as skin exfoliation and laceration. However, skin damage did not necessitate further special medical treatment. This ties in with reports by Warwick et al. [29] who state that skin injury rates were 13% after CCH injections and that skin tears healed within 21 days without impairment of skin quality. In our previous study, we observed skin injury following collagenase treatment in 17% of cases [20]. Yet, skin tearing occurred more than 48 h after enzyme injection, a timepoint when the collagenase is expected to be inactivated already [30]. Thus, we hypothesized that the cause of skin perforation might be irritation through direct contact between the textured silicone implants and the skin after capsule digestion. This hypothesis is supported by the absence of skin perforation

after incubation of rat and human skin with six times higher CCH concentrations in our in vitro studies [20, 21].

In the present study, the combination of acute irradiation with CCH application led to hematoma formation within the implant pocket in 17%. In this context, Levine et al. [17] investigated the efficacy and safety of collagenase injections in patients with Peyronie's disease. In this phase III study, 347 patients received multiple CCH injections (0.58 mg/ml) into Peyronie's plaques. Although Levine reported overall good clinical outcomes, hematoma formation developed in two cases. Both hematomas were treated conventionally and resolved spontaneously. Of note, both patients received further CCH injections afterward with no re-emerging side effects. In our study, hematoma formation occurred inside the implant pocket in two animals. The development of hematoma despite a low collagenase concentration might be due to irradiation of the implant site. By the induction of endothelial cell necrosis and degenerative changes, irradiation therapy can cause inflammation of vessels [31]. Concurrent, irradiation affects and reduces the oxygen supply of the targeted tissue and thus leads to hypoxia, the most important trigger of proangiogenic factor release [32]. These aspects are reflected by the statistically significant overexpression of VEGF-A in the presented study. VEGF-A is a proangiogenic factor found in almost all vascularized tissues [33]. It is considered a key player in the induction of new vessel formation through angiogenesis and vasculogenesis as well as the increase in vessel permeability [33–35]. In addition to these irradiation-induced changes, several studies described a growth of vessel wall thickness through hyperplasia after radiotherapy [36–38]. Interestingly, an experimental dog model by Powers et al. [39] demonstrated that fractionated radiation results in vascular wall hyperplasia, whereas acute radiotherapy inhibited vascular wall thickening. This is consistent with our observations. We measured a significantly lower vessel wall thickness/diameter ratio after both, irradiation alone and in combination with CCH application. We hypothesize that the combination of thinner vessel walls increased angiogenesis and thus more immature blood vessels, and inflammation might lead to an increased vulnerability of blood vessels and causes hematoma formation after CCH injections. This theory is supported by the fact that we did not observe hematoma formation in our two previous in vivo experiments, despite the application of three times higher collagenase concentrations [20, 23].

There are several limitations to our study. For breast reconstruction or augmentation, two different kinds of silicone implants with smooth or textured surfaces are commonly used in clinical practice. Although the influence of implant surface could not yet be fully investigated, a recent meta-analysis by Liu et al. [40] linked smooth implants to increased rates of capsular contracture. In our study, we only inserted implants with textured surfaces. Therefore, the results presented in this study do not allow conclusions on the influence of different silicone implants on the effectiveness of CCH injections. Additionally, implants were placed under the panniculus carnosus muscle which, compared to the implant coverage with the pectoralis muscle in a clinical setting, poses a much thinner muscle layer.

Capsular contracture is defined by clinical symptoms like hardness, breast deformity or pain. Yet, primary endpoints of this study were radiologic and histologic assessments of the capsule tissue. This is due to the difficult evaluation of corresponding clinical symptoms in animal models. It could be argued that the data acquired in our study do not reflect improvement of symptoms. However, several studies demonstrated a significant correlation between capsule thickness and collagen density with clinical symptoms and Baker's grade [41–43]. Therefore, reductions of these parameters can indirectly suggest clinical improvement.

Furthermore, our model used single-time irradiation to induce capsular fibrosis around the implant. Clinical protocols for breast cancer therapy, however, apply multiple, fractioned irradiation doses which allow for the application of higher total doses while reducing side effects. Thus, our study does not allow conclusions to what extend different irradiation regimen influence capsule formation, capsule quality and effectiveness of CCH Injections. The above-mentioned aspects warrant further experimental analyses before proceeding to clinical trials. Furthermore, if this novel treatment is proven effective, a cost-effect study compared to the current gold-standard, namely capsulectomy with implant exchange, should be performed in the future.

Conclusion

CCH was capable of actively degrading irradiation-induced capsular fibrosis tissue. This novel method poses a viable non-surgical treatment option for the therapy of capsular fibrosis around silicone implants. Despite the overall encouraging results reported in this study, safety issues like hematoma formation and the potential of tumor recurrence that need to be accounted for prior to clinical testing still exist.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All applicable institutional and national guidelines for care and use of animals were followed (AEP No.: 05086).

References

1. Surgeons ASoP (2013) Complete plastic surgery statistics report
2. Wick G, Backovic A, Rabensteiner E, Plank N, Schwentner C, Sgonc R (2010) The immunology of fibrosis: innate and adaptive responses. Trends Immunol 31:110–119
3. Wilflingseder P, Hoinkes G, Mikuz G (1983) Tissue reactions from silicone implant in augmentation mammaplasties. Minerva Chir 38:877–880
4. Tamboto H, Vickery K, Deva AK (2010) Subclinical (biofilm) infection causes capsular contracture in a porcine model following augmentation mammaplasty. Plast Reconstr Surg 126:835–842
5. Stevens WG, Nahabedian MY, Calobrace MB, Harrington JL, Capizzi PJ, Cohen R, d'Incelli RC, Beckstrand M (2013) Risk factor analysis for capsular contracture: a 5-year Sientra study analysis using round, smooth, and textured implants for breast augmentation. Plast Reconstr Surg 132:1115–1123
6. Adams WP Jr, Robinson JB Jr, Rohrich RJ (1998) Lipid infiltration as a possible biologic cause of silicone gel breast implant aging. Plast Reconstr Surg 101:64–68 **(discussion 69–71)**
7. Adams WP Jr, Rios JL, Smith SJ (2006) Enhancing patient outcomes in aesthetic and reconstructive breast surgery using triple antibiotic breast irrigation: six-year prospective clinical study. Plast Reconstr Surg 118:46s–52s
8. Henriksen TF, Fryzek JP, Holmich LR, McLaughlin JK, Kjoller K, Hoyer AP, Olsen JH, Friis S (2005) Surgical intervention and capsular contracture after breast augmentation: a prospective study of risk factors. Ann Plast Surg 54:343–351
9. Fischer S, Hirche C, Reichenberger MA, Kiefer J, Diehm Y, Mukundan S, Alhefzi M, Bueno EM, Kneser U, Pomahac B (2015) Silicone implants with smooth surfaces induce thinner but denser fibrotic capsules compared to those with textured surfaces in a rodent model. PLoS ONE 10:e0132131
10. Marques M, Brown SA, Oliveira I, Cordeiro MN, Morales-Helguera A, Rodrigues A, Amarante J (2010) Long-term follow-up of breast capsule contracture rates in cosmetic and reconstructive cases. Plast Reconstr Surg 126:769–778
11. Pomahac B, Recht A, May JW, Hergrueter CA, Slavin SA (2006) New trends in breast cancer management: is the era of immediate breast reconstruction changing? Ann Surg 244:282–288
12. Panchal H, Matros E (2017) Current Trends in Postmastectomy Breast Reconstruction. Plast Reconstr Surg 140:7s–13s
13. Cordeiro PG, Albornoz CR, McCormick B, Hu Q, Van Zee K (2014) The impact of postmastectomy radiotherapy on two-stage implant breast reconstruction: an analysis of long-term surgical outcomes, aesthetic results, and satisfaction over 13 years. Plast Reconstr Surg 134:588–595
14. Gurunluoglu R, Sacak B, Arton J (2013) Outcomes analysis of patients undergoing autoaugmentation after breast implant removal. Plast Reconstr Surg 132:304–315
15. Young VL (1998) Guidelines and indications for breast implant capsulectomy. Plast Reconstr Surg 102:884–891 **(discussion 92–94)**
16. Collis N, Sharpe DT (2000) Recurrence of subglandular breast implant capsular contracture: anterior versus total capsulectomy. Plast Reconstr Surg 106:792–797
17. Levine LA, Cuzin B, Mark S, Gelbard MK, Jones NA, Liu G, Kaufman GJ, Tursi JP, Ralph DJ (2015) Clinical safety and effectiveness of collagenase *Clostridium histolyticum* injection in patients with Peyronie's disease: a phase 3 open-label study. J Sexl Med 12:248–258
18. Peimer CA, Blazar P, Coleman S, Kaplan FT, Smith T, Lindau T (2015) Dupuytren contracture recurrence following treatment with collagenase *Clostridium histolyticum* (CORDLESS [collagenase option for reduction of dupuytren long-term evaluation of safety study]): 5-year data. J Hand Surg 40:1597–1605
19. Hammoudeh ZS (2014) Collagenase *Clostridium histolyticum* injection for plantar fibromatosis (Ledderhose disease). Plast Reconstr Surg 134:497e–498e
20. Fischer S, Hirsch T, Diehm Y, Kiefer J, Bueno EM, Kueckelhaus M, Kremer T, Hirche C, Kneser U, Pomahac B (2015) The Collagenase of the bacterium *Clostridium histolyticum* for the treatment of capsular fibrosis after silicone implants. Plast Reconstr Surg 136:981–989
21. Fischer S, Hirche C, Diehm Y, Nuutila K, Kiefer J, Gazyakan E, Bueno EM, Kremer T, Kneser U, Pomahac B (2016) Efficacy and safety of the collagenase of the bacterium *Clostridium histolyticum* for the treatment of capsular contracture after silicone implants: ex-vivo study on human tissue. PLoS ONE 11:e0156428
22. Katzel EB, Koltz PF, Tierney R, Williams JP, Awad HA, O'Keefe RJ, Langstein HN (2010) A novel animal model for studying silicone gel-related capsular contracture. Plast Reconstr Surg 126:1483–1491
23. Fischer S, Diehm Y, Henzler T, Berger MR, Kolbenschlag J, Latz A, Bueno EM, Hirche C, Kneser U, Pomahac B (2016) Long-term effects of the collagenase of the bacterium *Clostridium histolyticum* for the treatment of capsular fibrosis after silicone implants. Aesthetic plastic surgery 41:211–220
24. Sengupta P (2013) The Laboratory rat: relating its age with human's. Int J Prev Med 4:624–630
25. Merzin M (2008) Applying stereological method in radiology. Volume measurement. Bachelor's thesis University of Tartu
26. Schon LC, Gill N, Thorpe M, Davis J, Nadaud J, Kim J, Molligan J, Zhang Z (2014) Efficacy of a mesenchymal stem cell loaded surgical mesh for tendon repair in rats. J Transl Med 12:110
27. Peimer CA, Wilbrand S, Gerber RA, Chapman D, Szczypa PP (2015) Safety and tolerability of collagenase *Clostridium histolyticum* and fasciectomy for Dupuytren's contracture. J Hand Surg Eur 40:141–149
28. Thomas A, Bayat A (2010) The emerging role of *Clostridium histolyticum* collagenase in the treatment of Dupuytren disease. Ther Clin Risk Manag 6:557–572
29. Warwick D, Arandes-Renu JM, Pajardi G, Witthaut J, Hurst LC (2016) Collagenase *Clostridium histolyticum*: emerging practice patterns and treatment advances. J Plastic Surg d Hand Surg 50:251–261
30. Holzer LA, Holzer G (2009) Injectable collagenase *Clostridium histolyticum* for Dupuytren's contracture. N Engl J Med 361:2579 **author reply 79–80**
31. Tumerdem-Ulug B, Kuran I, Ozden BC, Mete O, Kemikler G, Aktas S, Calik B (2011) Does hyperbaric oxygen administration

before or after irradiation decrease side effects of irradiation on implant sites? Ann Plast Surg 67:62–67
32. Shweiki D, Itin A, Soffer D, Keshet E (1992) Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359:843–845
33. Yla-Herttuala S, Rissanen TT, Vajanto I, Hartikainen J (2007) Vascular endothelial growth factors: biology and current status of clinical applications in cardiovascular medicine. J Am Coll Cardiol 49:1015–1026
34. Ferrara N (2004) Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 25:581–611
35. Rissanen TT, Vajanto I, Hiltunen MO, Rutanen J, Kettunen MI, Niemi M, Leppanen P, Turunen MP, Markkanen JE, Arve K, Alhava E, Kauppinen RA, Yla-Herttuala S (2002) Expression of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 (KDR/Flk-1) in ischemic skeletal muscle and its regeneration. Am J Pathol 160:1393–1403
36. Martin JD, Buckley AR, Graeb D, Walman B, Salvian A, Hay JH (2005) Carotid artery stenosis in asymptomatic patients who have received unilateral head-and-neck irradiation. Int J Radiat Oncol Biol Phys 63:1197–1205
37. So NM, Lam WW, Chook P, Woo KS, Liu KH, Leung SF, Wong KS, Metreweli C (2002) Carotid intima-media thickness in patients with head and neck irradiation for the treatment of nasopharyngeal carcinoma. Clin Radiol 57:600–603
38. Steele SR, Martin MJ, Mullenix PS, Crawford JV, Cuadrado DS, Andersen CA (2004) Focused high-risk population screening for carotid arterial stenosis after radiation therapy for head and neck cancer. Am J Surg 187:594–598
39. Powers BE, Thames HD, Gillette EL (1999) Long-term adverse effects of radiation inhibition of restenosis: radiation injury to the aorta and branch arteries in a canine model. Int J Radiat Oncol Biol Phys 45:753–759
40. Liu X, Zhou L, Pan F, Gao Y, Yuan X, Fan D (2015) Comparison of the postoperative incidence rate of capsular contracture among different breast implants: a cumulative meta-analysis. PLoS ONE 10:e0116071
41. Prantl L, Schreml S, Fichtner-Feigl S, Poppl N, Eisenmann-Klein M, Schwarze H, Fuchtmeier B (2007) Clinical and morphological conditions in capsular contracture formed around silicone breast implants. Plast Reconstr Surg 120:275–284
42. Minami E, Koh IHJ, Ferreira JCR, Waitzberg AFL, Chifferi V, Rosewick TF, Pereira MD, Saldiva PHN, de Figueiredo LFP (2006) The composition and behavior of capsules around smooth and textured breast implants in pigs. Plast Reconstr Surg 118:874–884
43. Clugston PA, Perry LC, Hammond DC, Maxwell GP (1994) A rat model for capsular contracture: the effects of surface texturing. Ann Plast Surg 33:595–599