Published in final edited form as:

Obes Surg. 2011 December ; 21(12): 1937–1946. doi:10.1007/s11695-011-0377-2.

Impaired Laparotomy Wound Healing in Obese Rats

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Abstract

Background—Obesity increases the risk of laparotomy dehiscence and incisional hernia. The aim of this study was to measure the biological effect of obesity on laparotomy wound healing and the formation of incisional hernias.

Methods—Normal-weight Sprague–Dawley (SD) and obese Zucker rats were used in an established laparotomy wound healing and incisional ventral hernia model. Mechanical testing was performed on abdominal wall strips collected from laparotomy wounds. Hernia size was measured by digital imaging. Picrosirius staining for collagen isoforms was observed with polarized microscopy. Abdominal wall fibroblasts were cultured to measure collagen matrix remodeling and proliferation.

Results—Laparotomy wound healing was significantly impaired in obese rats. Mechanical strength was lower than in normal-weight rats. Yield load was reduced in the obese group at all time points. Picrosirius red staining showed increased immature type III collagen content and disorganized type I collagen fibers within laparotomy wounds of obese rats. Wound size was significantly larger in the obese group. Collagen matrix remodeling was impaired with fibroblasts from obese rats, but there was no difference in fibroblast proliferation between the obese and normal-weight groups.

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Conflicts of interest None

Conclusions—We observed for the first time that laparotomy wound healing is impaired in obese rats. The recovery of laparotomy wound strength is delayed due to abnormal collagen maturation and remodeling, possibly due to a defect in fibroblast function. Strategies to improve outcomes for laparotomy wound healing in obese patients should include correcting the wound healing defect, possibly with growth factor or cell therapy.

Keywords

Wound healing; Hernia; Collagen; Fibroblast; Laparotomy; Obesity

Background

Laparotomy wound failure and incisional hernia formation is the most frequent complication of abdominal surgery [1]. Imaging studies observe a primary laparotomy wound dehiscence rate of 11% leading to incisional hernias in abdominal surgery patients [2, 3]. Controlled clinical trials and large administrative database studies confirm that obesity is a major risk factor for laparotomy wound failure [4–8]. The incidence of incisional hernia is three times greater in obese patients when compared with normal-weight patients [8]. The important systemic risk factors consistently identified for fascial wound dehiscence and incisional hernia are obesity, malnutrition, diabetes, and extracellular matrix disease like arterial aneurysm. Risk factors local to the wound include infection and recurrent hernia, likely due to the pathology of scar formation [4, 5, 7, 9–19]. Many of these risk factors are common among obese patients, especially wound infection. The rate of hernia recurrence following repair ranges from 24% to 54% in the short-term, suggesting a potential acute wound healing defect [20–22].

Previous groups suggested that obesity impairs tissue repair [23, 24]. An experimental study with diet-induced obese rats found that skin healing was impaired when compared with rats on a normal calorie diet. It is also reported in a skin wound model that lean mice healed completely while obese mice developed a thin layer of epithelium and wound contraction was significantly delayed [25]. With regard to the abdominal wall and laparotomy, Levin et al. suggested that diet-induced obesity reduced the strength of the aponeurotic scars, but not the dermal scars [26]. Prolonged or increased inflammation in the obese state was suggested to contribute to wound healing delay by a failure to progress to normal fibro-proliferation.

The hypothesis of the current study was that obesity causes increased laparotomy wound failure due the high mechanical loads placed across the abdominal wall. The primary purpose was to establish the time-course for early laparotomy wound healing and the recovery of mechanical wound strength in the setting of obesity. The secondary purpose was to quantify the expected increase in laparotomy wound failure and incisional hernia formation in the obese rats and to correlate the findings with fibroblast function and collagen synthesis.

Materials and Methods

Materials

Low glucose Dulbecco's modified Eagle's medium (DMEM) and penicillin/streptomycin antibiotics were purchased from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was purchased from Hyclone Laboratories, Inc. (Logan, UT). Saturated aqueous solution of picric acid and Sirius red F3B (C.I. 35782) were purchased from Sigma-Adrich Corp. (St. Louis, MO). Cell Counting Kit-8 (CKK-8) was purchased from Dojindo Molecular Technologies, Inc. (Gaithersburg, MD).

Laparotomy Wound Healing Model

The rat laparotomy wound healing and incisional hernia models were previously reported [27–29]. Sprague–Dawley (SD) rats (Charles River, Wilmington, MA), age 9 weeks and weighing 250 g, and genetically obese Zucker rats (Charles River, Wilmington, MA), age 9 weeks and weighing 400 g, were acclimated and housed under standard conditions (Fig. 1a). Animals were provided ad libitum intake of standard rat chow and water throughout the study. All animal care and operative procedures were performed in accordance with the United States Public Health Service Guide for the Care of Laboratory Animals (NIH Publication No. 86–23, revised 1985) and were approved by the University Committee on Use and Care of Animals (UCUCA) of the University of Michigan.

Briefly, a 6×3-cm ventral full-thickness skin flap is raised through the avascular prefascial plane, and a 5-cm full-thickness laparotomy incision is made through the linea alba of the musculo-tendinous layer of the abdominal wall. The laparotomy is then repaired in a standardized manner with a continuous running 4-0 polypropylene suture using 0.3 cm suture bites and 0.5 cm progress between stitches. The suture is tied to itself at the caudal end of the wound. The skin flap is returned and sutured in place with 4-0 polypropylene sutures and steel wound clips. After 30 min of recovery under a heat lamp, the rats are returned to fresh individual cages.

Two groups were studied: (1) normal-weight SD rats and (2) obese Zucker rats. On postoperative days (POD) 7, 14, 21, and 28, the rats were killed, and the abdominal walls were excised. The skin layer was removed. Isolated abdominal wall (laparotomy wound, muscle and tendon) strips were then designed for biomechanical testing. Fresh biopsies of the abdominal wall—laparotomy wound interface were also collected for histological staining.

Laparotomy Wound Strength and Biomechanical Properties

Mechanical testing was performed on standardized abdominal wall strips collected from the laparotomy wound healing model as reported [27, 29]. All sutures were removed. Abdominal wall strips were taken perpendicular to the wound healing interface. A cutting template was used to measure the abdominal wall and minimize size variability between specimens. Strips were cut 10 mm in width and 60–80 mm in length. Two strips were collected from each rat and testing was performed within 6 h of necropsy. To standardize tensile measurements, the abdominal wall tissue strip thickness at the laparotomy wound and

the length between load grips were measured with Digimatic calipers (Mitutoyo American Corp, Chicago, IL). Stretch loading facilitated mechanical characterization of the wound-healing interface. Force-extension curves were generated for each abdominal wall strip with the use of an Instron tensiometer (model 5542; Instron Corporation, Canton, Mass) equipped with a 50 Newton static load cell set at a crosshead speed of 10 mm per minute. The abdominal wall strips were mounted into the load frame with the use of pneumatic grips, preloaded to 0.1 Newtons with the gauge length measured between the grips set to approximately 10 mm. The load frame applied testing loads to the abdominal wall strips until mechanical tissue disruption occurred. The anatomic location of the break was noted for each specimen. Force and tissue deformation data were simultaneously recorded and captured on a computer connected to the load frame via a digital interface card. Data analysis was performed with the use of the Merlin materials testing software package (Instron Corp, Canton, Mass) from which breaking strength, the maximum load force ($F_{\max}$) at mechanical failure (N); tensile strength, the maximum stress developed in the specimen per unit area, calculated as $F_{\max}/\text{cross-sectional area}$ (Newtons per cubic millimeter); energy at break (millijoules); yield strength (Newtons); yield energy (millijoules); and stiffness, the slope of the linear elastic region of the force-extension curve (Newtons per millimeter), were generated with the Merlin software package. The strength of the specimen was defined at the yield point where the laparotomy wound irreversibly deformed (beyond the elastic limit) indicating laparotomy wound failure.

Incisional Hernia Model

The model was used as previously described [27, 29]. Again, a 6×3-cm ventral full-thickness skin flap was again raised through the avascular prefascial plane, and a 5-cm full-thickness laparotomy incision was made through the linea alba. In the hernia model, two fast absorbing 5-0 plain catgut sutures were used to close the laparotomy wound. This model results in intentional mechanical failure of the laparotomy wound and progression to incisional hernias. The skin flap is returned and sutured in place with three 4-0 polypropylene stitches and steel wound clips.

Hernia Size

The hernia model rats were euthanized on POD 28. The skin was dissected free circumferentially and a 5×10-cm section of the abdominal wall was excised. The specimen was stretched out and pinned down on a dissecting board at the four corners with the peritoneal side up. A ruler was set alongside the wound as a reference for every sample. A standardized image was then captured using a digital camera. Software Spot, Windows: Version 4.5 (Diagnostic Instruments, Inc. University of New South Wales) was used to calculate the hernia size based on the digital image. Calibration was established using the ruler for reference on each image. An outline was drawn along the hernia ring and the hernia size calculated in square centimeters. (Fig. 1b, c)

Laparotomy Wound and Abdominal Wall Histology

Sagittal abdominal wall and laparotomy wound and/or hernia sections were then cut and immediately fixed in 10% neutral-buffered formalin in preparation for histologic analysis.

Specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin or trichrome by the Immunoperoxidase Laboratory at the University of Michigan Comprehensive Cancer Center to confirm the quality of the samples.

Collagen Isoform Synthesis

Picrosirius red staining for collagen was performed as reported by Puchtler et al. [30] with minor modifications. Paraffin sections were de-waxed and hydrated on glass slides. Nuclei were stained with Weigert's hematoxylin for 8 min, and then the slides were washed for 10 min in running tap water. They were then stained in picrosirius red for 1 h followed by washing in two changes of acidified water. Most of the water was removed from the slides by vigorous shaking. They were then dehydrated in three changes of 100% ethanol, cleared by immersion in xylene, and mounted in a resinous medium.

Microscopy and Image Capture

To visualize the bi-refrigent collagen, an Olympus BX-51 upright light microscope with an Olympus DP-70 high-resolution digital camera (Olympus Corporation, Japan) was employed to obtain digital images. The stained collagen fibers displayed as bright green to red color based on collagen isoform subtype. All slides were photographed on the same day to avoid any variability associated with the light source.

Rat Abdominal Wall Musculo-tendon Fibroblast Cell Culture

Biopsies of unwounded linea albas of SD and Zucker rats were treated in 0.0125% trypsin at 37°C for 30 min. The peritoneal side of the biopsies was scraped to remove mesothelial cells. Biopsies were minced in culture medium, spread on the bottom of T-75 cm² flasks and allowed to stand undisturbed for 2 h at 37°C in 5% CO₂ until the pieces adhered to the flask bottom. Medium was then gently drained. DMEM culture media containing 15% FBS and 1,000 µ/ml penicillin G, and 1,000 µg/ml streptomycin sulfate solution was then slowly added to the culture flask, and the tissue samples were incubated at 37°C in 5% CO₂. The medium was changed every 2 days, and the abdominal wall fibroblast primary cell cultures were grown to confluence for passage. Experimental fibroblasts were used at passages 2 to 4. The same source fibroblasts grown to confluence were harvested for fibroblast-populated collagen lattice compaction assays.

Fibroblast-Populated Collagen Lattices (FPCL)

Trypsinized abdominal wall fibroblasts were suspended in cold DMEM. Six-well tissue culture plates were precoated with 4% BSA in PBS for 24 h. For FPCL with fibroblasts cultured on the surface of the collagen gels (FPCL-S), DMEM 1.3 ml plus FBS 0.2 ml plus type I collagen stock solution 0.5 ml were mixed to yield a final concentration of 1.25 mg/ml of collagen. This collagen working solution (2 ml) was added to each well. After polymerization at 37°C, 10⁵ fibroblasts were poured on each collagen gel in 2 ml DMEM containing 10% FBS. The plates were incubated in a cell culture incubator for 3 h until cells adhered to the gel surface. The gels were then detached from the well sidewalls by gently scoring around the circumference with a fine pipette tip.

For the mixed fibroblast collagen gels (FPCL-M), 10^5 fibroblasts in 1.3 ml DMEM suspension plus FBS 0.2 ml plus type I collagen stock solution 0.5 ml were mixed to yield a final concentration of 1.25 mg/ml of collagen. This collagen mixture (2 ml) with three-dimensionally embedded fibroblasts was then added to each well. Gel polymerization progressed at 37°C for 3 h before being circumferentially detached. After polymerization at 37°C, 2 mL DMEM containing 10% FBS was added to each well.

After detachment, gel diameter was measured with a ruler and the gel area was calculated at designated time points.

Cell Proliferation Assay

Cell proliferation was determined by a cell viability assay. Cell viability was detected using CCK-8. Abdominal wall fibroblasts were seeded 3,000 cells/well in a 96-well plate. At designed time points, culture medium was replaced with 100 μ l fresh FBS-free DMEM containing 1,000 μ g/ml penicillin G, and 1,000 μ g/ml streptomycin sulfate plus 10 μ l CCK-8. The plates were then incubated at 37°C for 1 h and OD values were read at 450 with 690 nm as a reference wave length to determine the cell viability in each well. Fibroblasts from each group were used to generate cell number-cell viability standard curves for calibration.

Statistics

Statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). A *t* test and two-way ANOVA plus Bonferroni post-test were employed. Significance level was set at $P < 0.05$. Data are presented as mean $\pm$ SEM.

Results

Mechanical Properties of the Healing Laparotomy Wounds

Tensiometric analysis was performed on uniform abdominal wall strips with the line of tissue deformation directly perpendicular to the linea alba and laparotomy incision. The laparotomy wounds of the normal-weight SD rats were stronger than those of the obese Zucker rats at all time points. Wound disruption always occurred at the abdominal wall—laparotomy wound interface. As shown in Fig. 2, the breaking strength of laparotomy wounds in SD rats was significantly higher than that of Zucker rats by POD 14 and through POD 28. The differences in tensile strength suggest a significant mechanical defect in the wound healing matrix of the obese Zucker rat, despite its greater size. The lagging energy at break (energy absorbed), stiffness, yield load, and yield energy together suggests a weaker laparotomy wound in the obese rats.

Delayed Laparotomy Wound Collagen Maturation in Obese Rats

Collagen III is the weaker isoform of collagen typically secreted into an early, provisional wound matrix. As wound maturation occurs, increased levels of the stronger collagen I appear, resulting in the normal increase in wound strength [31, 32]. Figure 3 shows the picrosirius red staining for collagen I and III observed under crossing polarization

microscopy in the obese and normal-weight rats. Histological samples were taken of the healing laparotomy wounds between the rectus muscles. Thicker collagen type I fibers stain red-orange with cross-polarization microscopy, and thinner collagen type III fibers stain green. As shown in POD 7 wound images, the early laparotomy wound tissue was loose and stained predominantly for the provisional collagen type III isoform (green). By POD 14, the collagen type III content and wound tissue density in Zucker rat wounds remained similar to that of POD 7 (delayed maturation). A normal pattern of collagen type III decrease was observed in the SD rat wounds by POD 14, and collagen I based wound density increased significantly as the collagen fiber structure became more organized. Together these findings indicate delayed laparotomy wound maturation and remodeling in obese Zucker rats, consistent with the observed weaker mechanical strength of the wounds.

Larger Laparotomy Defects in Obese Rats

To quantify the consequences of laparotomy dehiscence in obese versus normal-weight rats, the rat incisional hernia model was used. On POD 28, all rats were euthanized, and the abdominal walls were excised to measure the extent of laparotomy wound failure. As shown in Fig. 4a, the wound defect size in the obese Zucker group was significantly larger than those in the normal-weight SD rat group, $12.06 \pm 0.62 \text{ cm}^2$ versus $4.22 \pm 0.43 \text{ cm}^2$. Rats in both groups significantly gained body weight over the 28-day postoperative period, indicating no nutritional or intestinal differences (Fig. 4b).

In a human study of incisional hernia formation, it was found that a 12-mm gap in the laparotomy closure on POD 30 would go on to form a clinically significant incisional hernia 94% of the time. Conversely, defects less than 12 mm in size formed clinically significant incisional hernias less than 1% of the time over 3 years [2]. Within limits, small laparotomy defects during the early postoperative period do not tend to form incisional hernias, but larger ones do. The majority of SD rats developed relatively small laparotomy defects, if at all. The largest and smallest hernia or defect sizes were 6.28 and 0.50 cm^2 in SD rat group versus 16.12 and 8.30 cm^2 in the obese Zucker rat group, respectively.

Fibroblast-Mediated Collagen Contraction and Fibroblast Proliferation

Normal fibroblast function is required for effective wound repair [33]. To determine whether fibroblast function is impaired in obese Zucker rats, the intrinsic kinetic activity of fibroblasts derived from the unwounded linea alba of SD and Zucker rats was measured by FPCL for binding to and remodeling a collagen matrix in vitro. Collagen lattice compaction was compared in two matrix models, FPCL-S and FPCL-M.

As shown in Fig. 5a, b, the majority of the fibroblast collagen lattice compaction occurred in the first 24 h in the FPCL-S model, whereas FPCL-M contraction progressed more slowly. Decreased fibroblast kinetic activity was pronounced in fibroblasts derived from obese rats in both FPCL models. Fibroblasts derived from Zucker and SD rats achieved $52.36 \pm 3.93\%$ and $36.18 \pm 5.13\%$ compaction at 24 h in FPCL-M (Fig. 5b), and $42.94 \pm 1.90\%$ and $27.16 \pm 2.24\%$ compaction at 2 h in FPCL-S (Fig. 5a), respectively. Fibroblasts cultured from Zucker rat linea alba were significantly less efficient in causing lattice compaction and achieved less overall lattice compaction when compared with SD fibroblasts. To determine

if the difference in fibroblast collagen matrix compaction between SD and Zucker rats was due to differences in fibroblast number, cell proliferation assays were performed to generate a time-course and FBS dose–response curves for normal and obese rat fibroblasts. As shown in Fig. 5c, d, metabolic proliferation assays revealed no significant differences in proliferation between obese versus **normal-weight** fibroblasts in either an FBS concentration- or time-dependent manner.

Discussion

This study shows for the first time that the high incidence of laparotomy wound failure leading to incisional hernias in obese subjects may be due to impaired laparotomy wound healing. It is clinically assumed that the problem of laparotomy wound failure and incisional hernia in obese patients is due primarily to increased mechanical loading across the obese abdominal wall. Many surgeons even advocate all forms of weight loss prior to considering laparotomy or incisional hernia repair. The results presented here suggest that the high incidence of laparotomy wound failure in obesity is also due to impaired laparotomy wound healing, exacerbating the mechanical disadvantage.

The mechanical strength of an early surgical wound is normally established by a sequential series of coordinated molecular and cellular processes, including inflammatory and repair cell proliferation and the deposition of a provisional extracellular matrix. After an initial inflammatory lag-phase where there is minimal mechanical strength in the wound, the proliferative phase of wound healing predominates, approximately 4 days following tissue injury. Fibroblasts, the most important cellular components during this repair phase, reach their peak biological activity approximately within 14–21 days of wounding [34]. Normally, a provisional or early wound matrix is established to support repair cell ingrowth. This early wound matrix is mechanically weaker than a mature acute wound matrix, expressing higher levels of the thin fibril collagen isoform, collagen type III. In this study, there was higher immature collagen III content in the laparotomy wounds of the obese rats in the early postoperative period. In a skin wound model, it was observed that mice predisposed to obesity, but maintained at normal weight by dietary restriction exhibited normal collagen accumulation, but deficient wound collagen formation and accumulation was seen when allowed to develop obesity [25, 35]. This defect was not reversible and could not subsequently be corrected by insulin treatment and dietary restriction. The same animals when chronologically mature expressed normal wound collagen accumulation if they had never been obese [25, 35]. Other studies also suggest a biological defect in wound healing exists in the setting of obesity. Goodson et al. [25, 35] reported that retarded skin wound contraction and deficient wound collagen formation was observed in obese C57-BL ob/ob mice compared with lean mice. In calorie diet-induced obese Wistar rats, it has been observed that obesity lowered the mechanical strength of the aponeurotic scars but not the skin scars, supporting important differences in the wound healing mechanisms of abdominal wall fascia and skin. In this study of aponeurotic wound healing, obesity did not appear to induce a delayed inflammatory response or impaired collagen density [26]. In wound healing models of tympanic membrane perforation, the Zucker diabetic fatty rats demonstrated a significantly prolonged time to closure compared with **normal-weight** Sprague–Dawley rats [36]. The obese Zucker rat model shares some of the clinical signs of

obesity in humans. For example, these animals are glucose-intolerant, slightly hypertensive, and pass protein in their urine. They have kidney dysfunction even though they are not yet diabetic [37, 38]. It is possible that any or all of these markers for the metabolic disturbance contribute to abdominal wall repair fibroblast dysfunction through systemic signaling or inhibition.

Connective tissue fibroblasts are the most important cell type responsible for the tissue remodeling and wound strength. Fibroblasts synthesize extracellular matrix, can signal angiogenesis, and promote tissue remodeling [39]. In obese mice, contraction of an open skin wound significantly delayed [25]. In the current study, laparotomy wound closure was significantly impaired in obese rats compared with normal-weight rats. Delayed wound contraction suggests a defect in repair fibroblast function. Repair fibroblasts cultured from obese rats were defective in the ability to cause collagen matrix contraction, a measure of wound organization. There was no measurable defect in fibroblast proliferation. Together, these data suggest that abdominal wall fibroblasts from obese rats express a functional defect that results in a lower wound closure rate and lower wound strength.

One limitation of the current study is that the Zucker rats and Sprague–Dawley rats, although of the same species, are genetically distinct and differences in tissue repair gene expression may contribute to the observed delay in wound healing in the obese animals. This could be tested in a similar study using calorie induced obesity model of the same breed of animal, although that study design would be difficult to control for caloric and nutritional intake. It has never been shown that interspecies variation in tissue repair signaling contributes to delays in wound healing of the magnitude observed in this study, favoring the metabolic role for obesity in wound healing. It is also possible that a genotype unique to both obesity and wound healing results in delayed or defective tissue repair.

We observed that obese rats express impaired laparotomy wound healing, most importantly measured as a significant delay in the recovery of wound mechanical strength. A potential cellular defect is identified within fibroblasts isolated from the obese rat. This model may therefore be valuable in the identification of the fundamental mechanism for the delayed laparotomy healing in obese surgical patients. Furthermore, this model and others like it may be used to test whether weight loss or the correction of metabolic derangements like hyperglycemia or insulin resistance will reverse the wound healing defect. There is also the potential to treat the observed impairment in laparotomy wound fibroblast function in obesity, as with growth factor or cell therapy.

Acknowledgments

This project was supported by the Rapid Wound Healing Technology Development Project grant from the US Department of Defense and Stemnion, Inc.

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Fig. 1.

Obese Zucker rats and normal-weight Sprague-Dawley (SD) rats were studied (a). Rats in both groups were 9 weeks old. Obese Zucker rats weighed 390–410 g and SD rats weighed 240–260 g. In the laparotomy wound failure groups, incisional hernias developed, as demonstrated in whole abdominal wall explants of muscle and fascia (b). The size of the abdominal wall defects was quantified using standardized and calibrated digital images (c)

Fig. 2.

Delayed laparotomy wound healing in obese rats. Biomechanical recovery of the abdominal wall following laparotomy was measured with an Instron tensiometer for 28 days. Values are the mean±SEM of 20 abdominal wall samples each from SD and obese Zucker rats. Two-way ANOVA plus Bonferroni post-tests were performed for normal-weight SD versus obese Zucker rats at each time point, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Fig. 3.

Photomicrographs of picrosirius red-stained histological sections of laparotomy wound scar tissue from the linea alba (*midline*) observed under cross-polarized light. The images are oriented so that the laparotomy scar is in the middle with one rectus muscle to the left and to the right. Tissue sections were prepared perpendicular to the linea alba in order to optimize the view of organizing collagen bundles. The thick, strongly bi-refrangent *yellow or red* fibers represent maturing type I collagen in the healing laparotomy wounds. Immature, provisional matrix collagen type III is thinner and weakly bi-refrangent resulting in the green fibers (*arrows*). Over the 14 days shown, there is a delay in the progression from immature green type III collagen to mature red type I collagen in the obese Zucker rats

Fig. 4. Comparison of abdominal wall defect size. **a** Incisional ventral hernia defect size (area) was measured on POD 28. Values are the mean $\pm$ SEM from the SD and obese Zucker rat groups. **b** Body weights on POD 0 and POD 28. *** $p < 0.001$, normal-weight SD versus obese Zucker rats groups at each time point. ### $p < 0.001$, increase in body weight from POD 0 versus POD 28 for both groups

Fig. 5.

Fibroblast-mediated collagen matrix contraction in vitro is impaired with cells derived from obese Zucker rats. **a** Fibroblasts were seeded on the surface of a type I collagen matrix (FPCL-S) or **b** three-dimensionally mixed and embedded into the collagen matrix (FPCL-M) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n = 6$). Fibroblast proliferation in cell culture is not different between SD rats and obese Zucker rats in low glucose DMEM. **c** Proliferation of fibroblasts from SD and obese Zucker rats in the presence of increasing concentrations of FBS for 120 h. **d** Proliferation of fibroblasts from SD and obese Zucker rats in the presence of 10% FBS for 72–120 h. These data suggest a functional defect in the laparotomy repair fibroblasts isolated from the obese rats