Modulation of fixation stiffness from flexible to stiff in a rat model of bone healing

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Background and purpose — Constant fixator stiffness for the duration of healing may not provide suitable mechanical conditions for all stages of bone repair. We therefore investigated the influence of stiffening fixation on callus stiffness and morphology in a rat diaphyseal osteotomy model to determine whether healing time was shortened and callus stiffness increased through modulation of fixation from flexible to stiff.

Material and methods — An external unilateral fixator was applied to the osteotomized femur and stiffened by decreasing the offset of the inner fixator bar at 3, 7, 14, and 21 days after operation. After 5 weeks, the rats were killed and healing was evaluated with mechanical, histological, and microcomputed tomography methods. Constant fixation stiffness control groups with either stiff or flexible fixation were included for comparison.

Results — The callus stiffness of the stiff group and all 4 experimental groups was greater than in the flexible group. The callus of the flexible group was larger but contained a higher proportion of unmineralized tissue and cartilage. The stiff and modulated groups (3, 7, 14, and 21 days) all showed bony bridging at 5 weeks, as well as signs of callus remodeling. Stiffening fixation at 7 and 14 days after osteotomy produced the highest degree of callus bridging. Bone mineral density in the fracture gap was highest in animals in which the fixation was stiffened after 14 days.

Interpretation — The predicted benefit of a large robust callus formed through early flexible fixation could not be shown, but the benefits of stabilizing a flexible construct to achieve timely healing were demonstrated at all time points.

The majority of diaphyseal long bone fractures heal with the formation of an external callus. Known as secondary healing, this process occurs in the presence of interfragmentary movement (IFM) (Willenegger et al. 1971) with the magnitude of IFM being critical to healing outcome (Perren and Cordey 1980, Goodship and Kenwright 1985, Claes et al. 1997, Kenwright and Gardner 1998). Rigid fixation that suppresses IFM limits callus formation and impairs healing (Goodship and Kenwright 1985). The size of the external callus increases with the flexibility of fixation (Claes et al. 2000); however, unstable fixation may lead to a large callus that fails to bridge, known as a hypertrophic non-union (Muller et al. 1968). Moderate axial IFMs are known to reliably produce a timely healing outcome (Goodship and Kenwright 1985, Claes et al. 1997).

Surgical intervention and fracture fixation has become the standard of care to support early mobility and prevent the development of joint stiffness and muscle atrophy. The mechanical environment or IFMs are determined by the fixation stiffness (implant type and configuration) and the degree of weight bearing. Typically, the stiffness of fixation is not purposefully modulated over the course of healing to control the mechanical environment. However, fixation stiffness may change with conversion from initial external fixation to definitive internal fixation (Patterson and Cole 1999). Some fixation systems allow dynamization to increase the flexibility of fixation when there are no early signs of healing (Rubinstein et al. 1992). The results of large animal studies have suggested that there is an optimal fixation stiffness (Epari et al. 2007), but there are no clinical means of configuring a fixation device to take account of fracture type and weight bearing and knowingly achieve ideal mechanical conditions for healing. A recent clinical study of locked plating found that one-third of all fractures had little or no callus formation, which was attributed to overly stiff fixation (Bottlang et al. 2010).

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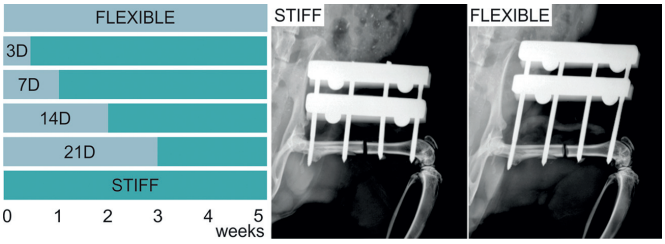

Figure 1. Left panel: The study consisted of 2 control groups, stiff and flexible, for the duration of the 5-week healing period and 4 experimental groups where flexible fixation was converted to stiff fixation on day 3, 7, 14, or 21. Right panels: Postoperative in vivo radiographs of a stiff fixation construct with a 6-mm offset and a flexible construct with an offset of 12 mm.

It is not known whether mechanical stimulation is needed during all stages of repair, nor how the optimal magnitude of IFM may differ at various stages of repair. The ability to modulate the stiffness of fixation has the potential to enable advantageous mechanical stimulation at all periods of healing, promoting tissue formation and remodeling without exceeding tolerated tissue strain. We recently published our hypothesis (Epari et al. 2013) that the optimum form of fixation of a fracture is flexible fixation during the early phases of healing to promote larger callus formation, and rigid fixation during the later stages of repair to reduce tissue strains, enabling callus maturation and bridging. In this study, we investigated this hypothesis with different time periods of conversion from flexible to stiff fixation in a rat model of bone healing.

Material and methods

Animals and surgical procedures

48 male Wistar rats (weight 400–450 g) were randomly divided into 6 groups (n = 8) consisting of stiff (S) and flexible (F) control groups and 4 experimental groups labeled 3D, 7D, 14D, and 21D. The experimental groups were flexibly fixed for 3, 7, 14, and 21 days, followed by stiff fixation for the remainder of the 5-week healing period.

The unilateral fixator, described previously (Willie et al. 2009, Recknagel et al. 2011), consists of a custom-made bar element that clamps onto 4 threaded stainless steel pins (Jagel Medizintechnik, Bad Blankenburg, Germany) spaced 8 mm apart (Figure 1). For a stiff fixation (axial stiffness 120 N·mm-1), the inner bar of the fixator was set at an offset of 6 mm from the lateral aspect of the femur. For the flexible configuration (30 N·mm-1), the offset was set to 12 mm.

The rats were anesthetized with isoflurane (2% with 2 $L \cdot min^{-1}$ O_2, by face mask). Preoperatively, a 5-mL subcutaneous injection of normal saline was administered along with an antibiotic and analgesic injection. The antibiotic clindamycin-2-dihydrogenphosphate was administered subcutaneously at 45 $mg \cdot kg^{-1}$ prior to surgery and at 3 days postoperatively. The analgesic tramadol was administered subcutaneously at 20 $mg \cdot kg^{-1}$ and was diluted in the animals' drinking water at 25 $mg \cdot L^{-1}$ for 3 days postoperatively.

A lateral incision of 3–4 cm was made through the skin to expose the shaft of the femur. An external fixator bar with drill guides was used to permit reproducible positioning of 4 drill holes to accommodate the fixator pins. After the fixator was in place, a small circular saw was used to create a 1-mm osteotomy. Each animal was housed in its own cage, given unrestricted access to food and water, and monitored daily for infection and mobility. In the experimental groups, the fixator offset was decreased under anesthesia after 3, 7, 14, and 21 days respectively.

Mechanical testing

After 35 days, the rats were killed by CO_2 asphyxiation and the femurs were dissected to remove all soft tissue and the fixators. Mechanical testing was performed according to a previously established protocol (Claes et al. 2009). The femurs were potted in cylinders with polymethylmethacrylate, creating a 30-mm free length (l) between the bending supports for the bone, centered on the osteotomy line. Bending was applied at the level of the osteotomy, in the anterior-posterior direction. The load was applied in 3-point bending with a materials testing machine (5848 MicroTester; Instron, Norwood, MA) at a deflection rate of 1 $mm \cdot min^{-1}$ and a maximum force of 10 N. During the tests, the bones were kept moist with 0.5% NaCl solution. The bending load (F) was applied to the callus and was recorded continuously against sample deflection (d). Flexural rigidity (EI) was calculated from the slope of the linear region of the load-deflection curve (k). As the callus was not always located at the middle of the supports (l/2), the distances between the load vector and the proximal support (a) and the distal support (b), respectively, were considered for calculating the flexural rigidity according to: $ka^2b^2/3l = EI$. The bending load was applied 3 times, with 2 cycles being necessary to condition each sample and the third application being used for the measurement. Flexural rigidity was reported as a percentage of that in the intact contralateral limb.

Microcomputed tomography

Femora were scanned using a microcomputed tomography (μCT) scanner (μCT40; Scanco Medical, Bassersdorf, Switzerland), with a 20-μm isotropic voxel size and an integration time of 250 ms, at 70 keV of energy. The volume of interest (VOI) included the callus between the 2 inner pins of the fixator, subtracting the bone of the cortices. Total callus volume (TV), mineralized bone tissue volume (BV), and the ratio of bone volume to total volume (BV/TV) were quantified using the μCT evaluation software (version 6.5-3; Scanco Medical, Bassersdorf, Switzerland). Bone mineral density (BMD) was calculated after conversion of the gray-level values using a correction algorithm (Claes et al. 2009) in a second VOI that encompassed only the callus formed at the level of the osteotomy.

Histology and histomorphometry

Femora were fixed in 4% paraformaldehyde for histological analysis. They were then dehydrated in ascending grades of ethanol, infiltrated, and embedded in methyl methacrylate (MMA). Samples were sectioned in the longitudinal direction and stained with paragon (Willie et al. 2011). Quantitative histomorphometry was performed using light microscopy in a region of interest (ROI) that included the complete outer diameter of the periosteal callus in the radial direction and extended 2 mm proximally and distally from the center of the gap. Bone of the cortices were excluded from the ROI. The total callus area, bone area, fibrous tissue area, and cartilage area were measured using OsteoMeasure (OsteoMetrics, Atlanta, GA). The number of animals that achieved bony bridging in the periosteal, intracortical, and endosteal regions was counted and a bridging score from 0 to 4 was calculated based on the number of bridged cortices (Willie et al. 2011).

Statistics

The ANOVA assumption of normality and homogeneity of variance were tested using the Shapiro-Wilk and Levene tests. If the normality assumption was met, then an ANOVA was performed with a Tukey post-hoc test. If the variances were unequal, then a Welch ANOVA with Games-Howell post-hoc test was performed. If normality was not met, a Kruskal-Wallis test was performed, followed by pairwise comparisons using a Wilcoxon-Mann-Whitney U-test corrected according to the Bonferroni procedure. The level of significance was set at 0.05. Analyses were performed using SPSS software version 22.0 and all experimental measures are expressed as mean values (with SD).

Ethics

All procedures were approved by the QUT University Animal Ethics Committee (1100000717) in accordance with guidelines of the Australian National Health and Medical Research Council (NHMRC).

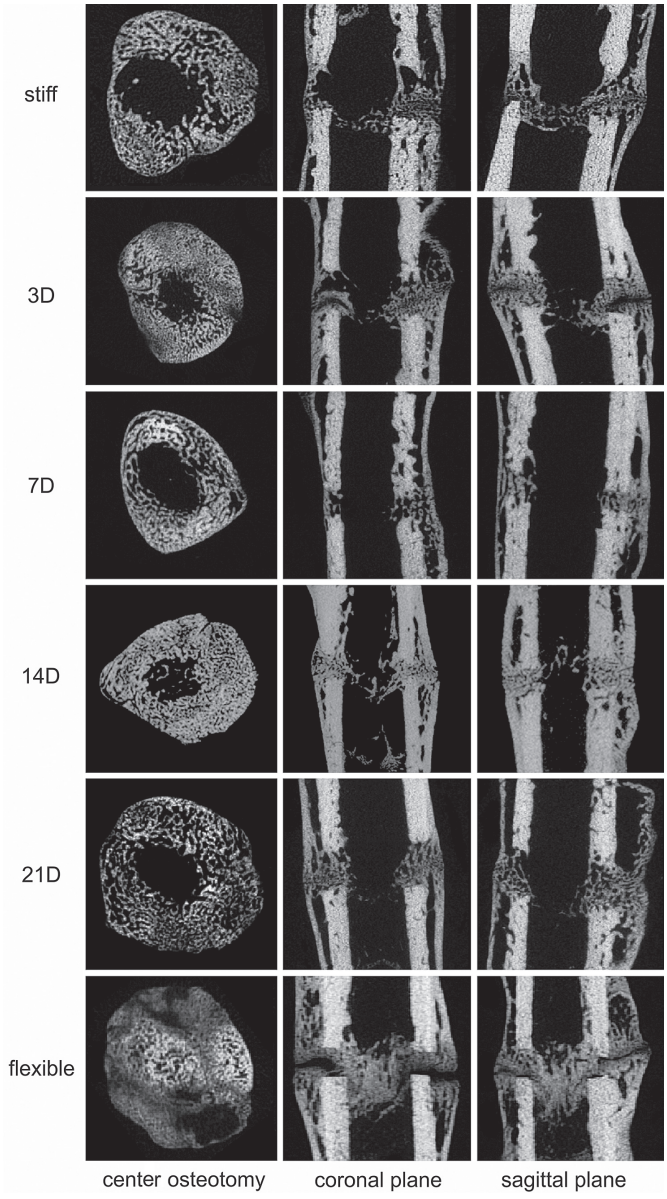

Figure 2. Microcomputed tomography images of osteotomy at 35 days postoperatively after modulation of stiffness from flexible to stiff at 3, 7, 14, and 21 days. Comparison with the stiff and flexible controls.

Results

The rats weighed an average of 438 (SD 26) g at the time of operation, and the average increase in body weight over the course of healing was 20%. Pin breakage occurred in 1 animal in the S, 3D, and 14D groups, reducing the sample size to 7. There was no evidence of pin loosening, which was assessed manually at the time of fixator removal.

After 5 weeks, the appearance of all groups was consistent with secondary bone healing showing external callus formation to varying degrees (Figure 2). No group with the most advanced state of healing was clearly observable. The F group showed characteristics of delayed healing and the least advanced healing of all groups, with a prominent band of cartilage remaining in the callus (Figure 3). The F group had the largest amount of callus with the highest proportion of cartilage. The lowest total bridging score and the highest endosteal bridging score indicate that callus remodeling has not progressed (Table). In contrast, most samples from the S group had signs of advanced remodeling with formation of a neo-cortex in the periosteal callus and re-establishment of the marrow canal (Figure 2). The callus and bone volume were lowest in the S group (Table 1). Fractures stabilized with the stiff fixator were almost 150% stiffer than those that were flexibly fixed (Figure 4).

Morphologically, the experimental groups (3D, 7D, 14D, and 21D) were similar in appearance to the S group (Figure

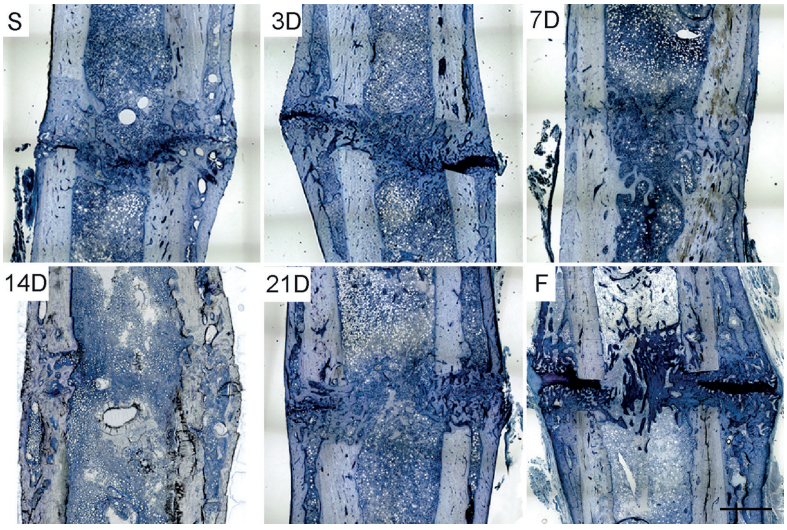

Figure 3. Histological images at 35 days postoperatively after stabilization with stiff (S) and/or flexible (F) fixators and modulation of stiffness from flexible to stiff at 3, 7, 14 and 21 days. Histological sections were stained with paragon: white and blue: fibrous tissue; purple: cartilage; light blue/white: bone.

2). All 4 experimental groups were 100% or more stiffer than the F group (Figure 4). The size of the external callus increased with the number of weeks flexibly fixed. However, a third week of flexible fixation lowered the bridging score by 30% compared to the 14D group, and the callus of the 21D group contained significantly more cartilage (p = 0.02) (Figure 4). Cortical bridging and bone quality in the osteotomy gap (BMD) was highest in the 14D group (Table 1), but this did not give greater callus stiffness.

Discussion

The predicted benefits of inverse dynamization are shortened healing in comparison to very flexible fixation and healing time comparable or faster than optimum fixation with greater callus stiffness (Epari et al. 2013). We found that stiffening of a

Table 1. Data from mechanical testing, microcomputed tomography analysis, and histomorphometry. Mean values (SD)

	Stiff	3D	7D	14D	21D	Flexible
Flexural rigidity (10^3 N·mm^2)						
Operated	112 (39)	96 (21)	107 (33)	87 (12)	76 (29)	42 (16)
Intact	204 (30)	174 (7)	174 (13)	164 (26)	165 (20)	203 (26)
Percent intact (%)	54 (16) [a]	56 (12) [a]	62 (19) [a]	53 (10) [a]	47 (20) [a]	22 (9)
Total volume (mm^3)	69 (26) [a]	73 (14) [a]	82 (16) [a]	74 (17) [a]	97 (22)	106 (22)
Bone volume (mm^3)	49 (15)	53 (10)	57 (12)	49 (11)	64 (22)	69 (24)
BV/TV (%)	73 (9)	73 (4)	70 (5)	66 (5)	65 (10)	63 (13)
BMD (mgHA·cm^{-3})	806 (73)	831 (63) [a]	841 (67) [a]	927 (14) [a,b]	818 (70) [a]	706 (99)
Total area (mm^2)	9.5 (3.4)	13.0 (1.9)	12.9 (3.2)	11.2 (3.1)	15.1 (4.2)	17.7 (2.0)
Bone area (mm^2)	6.1 (2.1)	8.8 (1.4)	8.4 (2.4)	7.7 (2.4)	8.9 (3.2)	10.4 (2.2)
Fibrous area (mm^2)	3.2 (1.3)	3.9 (0.7)	4.3 (1.1)	3.4 (1.2)	5.6 (1.8)	5.3 (0.9)
Cartilage area (mm^2)	0.3 (0.6) [a]	0.4 (0.4) [a]	0.2 (0.3) [a]	0.1 (0.4) [a]	0.6 (0.9) [a]	1.8 (0.7)
Total bridging (%)	79	71	94	96	66	25
Endosteal bridging (%)	43	57	38	14	38	88

[a] $p < 0.05$ in comparison to control group flexible.
[b] $p < 0.05$ in comparison to control group stiff.

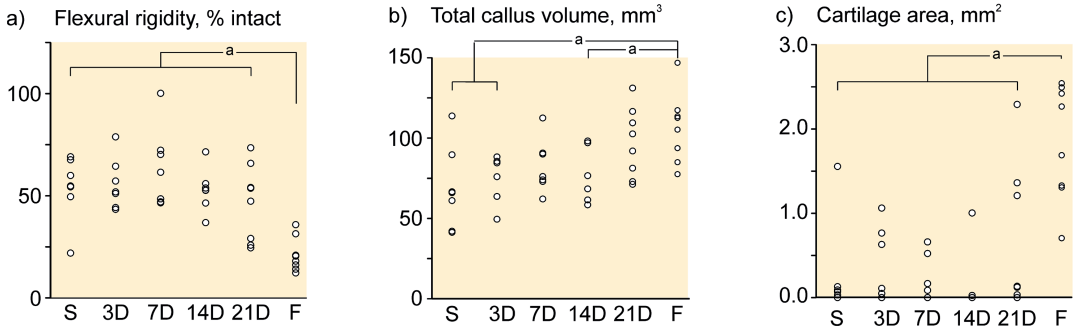

Figure 4. Effects of modulation of fixation stiffness from flexible to stiff after 3, 7, 14, and 21 days on bone healing, evaluated mechanically and by microcomputed tomography and histomorphometry. a) Flexural rigidity of the callus normalized to the contralateral limb. b) Total volume of the fracture callus. c) Cartilage area in the callus. Any p-value of < 0.05 was considered to be significant (a).

flexible fixation construct that would otherwise delay healing improved healing outcome at all time points. Except during the latest stage of repair, the healing outcome was comparable to that with constant, stiff fixation for the duration of healing. Stiffening of an originally flexible fixation over the course of healing did not lead to larger callus and greater callus stiffness in any of the modulated groups. Callus size was determined at the endpoint of the study and, due to callus remodeling, we may not have captured the maximum size of callus formed during healing. It may be a weakness of our study that unlike callus stiffness, strength and fracture energy were not determined from destructive testing. The decision was made so that the same animals could be used for mechanical testing, μCT, and histology without introducing destructive testing artifacts. Callus strength may be a less sensitive measure during the latter stages of healing (Chehade et al. 1997), and a recent study of fracture healing in rats showed no increase in callus stiffness between 30 and 60 days but an almost 2-fold increase in callus strength and fracture energy (Sigurdsen et al. 2009). Hence, in any further studies we recommend that failure strength should also be determined.

While stiffening of a flexible fixation construct that would otherwise delay healing improved healing outcome at all time points, there was an influence of timing of modulation. The 3-day modulation group was included to investigate the importance of the initial phase of bone healing on outcome. It has been suggested that this phase may be particularly sensitive to the mechanical environment (Klein et al. 2003), and instability for as little as 24 h is sufficient to induce cartilage formation 10 days postoperatively (Miclau et al. 2007). The 3D group differed from the stiff control group in that flexible fixation was applied for only the first 3 days. At 5 weeks, the morphological characteristics of 2 groups were indistinguishable, demonstrating no lasting effect of the initial, flexible fixation on healing outcome.

At the other extreme, stiffening of fixation as late as 21 days improved healing with respect to the flexible fixation, but healing was not as advanced as in the stiff fixation group. Callus stiffness tended to be lower, which correlated with a lower bridging score and higher amounts of cartilage in the callus. It has previously been concluded that instability prolongs the latter chondral phase during bone healing (Epari et al. 2006). In this study, it appears that stiffening of fixation, which would be expected to reduce callus strains, permitted mineralization and accelerated bony bridging. Furthermore, this result provides supporting evidence for intervening and increasing stability in the clinical treatment of delayed healing where instability is suspected, and supports the practice in management of hypertrophic non-unions (Ateschrang et al. 2013).

As flexible fixation delayed healing, it might be expected that healing quality would decrease with the length of time of flexible fixation. However, stiffening of fixation mid-repair (the 7D and 14D groups) produced morphological characteristics suggestive of the most advanced healing state of all groups, with the highest degree of bony bridging. It is an apparent contradiction that the bone volume to total volume (BV/TV) tended to be higher in the stiff group than in the 14D group. However, the unusual callus remodeling observed in rodents characterized by the formation of a neo-cortex (Gerstenfeld et al. 2006) means that BV/TV peaks prior to the onset of callus remodeling. In contrast, BMD continuously increases and approaches the value of mature cortical bone. These results suggest that modulation of fixation stiffness from flexible to stiff has the potential to shorten healing relative to constant fixation stiffness.

Understanding the degree of stability in the control groups is important for interpretation of the results of this study, and the clinical implications. Interfragmentary strain (IFS) is a useful descriptor of stability relating IFM to fracture gap size (Perren and Cordey 1980). Knowing the fixation stiffness (Recknagel et al. 2011), rat femur loads (Wehner et al. 2010), and gap size (1 mm), the initial IFMs (0.25 and 1 mm) and IFS values (25% and 100%) in the stiff and flexible groups can be estimated. Initial interfragmentary strains within the 7–30% range have been shown to support timely healing (Claes et al. 1997). In another study, callus stiffness as monitored in vivo was found to determine maximum stiffness at 4 weeks with the stiff fixator, as compared to 9 weeks with the flexible fixator (Wehner et al. 2014). Thus, the stiff fixator can be considered to provide stable fixation and timely healing while the flexible fixator can be considered to be too flexible and to delay healing. Clinically, the stiff fixator would be comparable to a unilateral external fixator with a stiffness of approximately 500 N/mm (Bottlang et al. 2010), which under partial weight bearing of 400 N would produce 0.8 mm of axial IFM (with an IFS of 26% in a 3-mm fracture gap).

In experimental models where animals return to full weight bearing relatively quickly, it can be expected that the IFMs will be greatest initially and decrease over time with maturation of tissue. If weight bearing is constant and the fixation is stiffened, IFMs will decrease further at the time of modulation. Clinically, the generally accepted standard of care is to have partial weight bearing initially and for it to increase following radiological signs of callus formation. There is a risk with this approach that if the fixation is too stiff and weight bearing is too low, healing may be understimulated (Bottlang et al. 2010). Our results suggest that this practice may be contrary to that required to create ideal mechanical conditions for callus formation and healing, and should stimulate critical evaluation of potential improvements in clinical treatment. An approach that allows adequate stimulation early (low fixation stiffness and/or increased weight bearing) and stability during the latter stages of healing (high stiffness and/or reduced wright bearing) may provide a greater margin for error in fracture management than attempting to configure fixation stiffness to remain constant over the course of healing.

This is the first study to investigate the controlled stiffening of fixation in a model of fracture healing. Previous studies

have investigated the influence of time to stabilization (Coutts et al. 1982) and conversion from an external fixator to an intramedullary nail (Sigurdsen et al. 2011, Recknagel et al. 2013) on fracture healing. While stability of the fracture is typically increased by the intervention in both of these cases, the surgical intervention disrupts the repair process and has been shown to delay healing (Recknagel et al. 2013). The strength of our study is that stiffness was modulated without the need for surgical intervention, allowing only the variable fixation stiffness to be changed.

Stiffening of a flexible construct improves healing outcome, resulting in comparable tissue formation to that achieved through stable fixation. Although some effects of modulation of fixation stiffness during healing were found, it was not possible to differentiate the effects on callus size itself when assessing only the healing outcome. Further investigation of this phenomenon in large animal models may give more clinically relevant data. While some studies have suggested that the mechanical conditions during the initial stage of healing may define the healing path and outcome, this study has shown—at all time points—the benefits of stabilizing a flexible construct to achieve timely healing.

DE, LC, MS, and AI: design of the study, interpretation of the data, and preparation of the manuscript. NB: data acquisition, analysis, and writing of the manuscript. LK, RB, VG, and RS: operation of animals and data acquisition. MS: clinical input into the design, and interpretation of the data.

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No competing interests declared.

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