Freeze dried chitosan/platelet-rich-plasma implants improve marrow stimulated cartilage repair in rabbit chronic defect model

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INTRODUCTION

Bone Marrow Stimulation (BMS) by drilling or microfracture improves knee joint function but elicits incomplete repair. Liquid chitosan (CS)-glycerol phosphate/blood clots have previously been shown to promote cell recruitment, transient vascularization, subchondral bone remodeling and improve cartilage repair following BMS in acute cartilage repair models [1,2]. Platelet-rich-plasma (PRP) contains four-fold concentration of growth factors and cytokines and has been shown to improve recruitment and chondrogenic potential of subchondral mesenchymal stem cells (MSCs). We hypothesize that augmentation of MS with implants composed of freeze-dried chitosan solubilized in PRP would improve repair response in a more clinically relevant rabbit chronic defect model.

METHODS

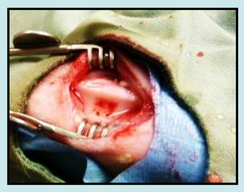
❖ Model development-n=3, early repair time point

Surgery 1
Retain CC

Defect creation
New Zealand White rabbits
Skeletally mature
Bilateral, 4X4 mm, Trochlear
(N=8 animals, 16 knees)

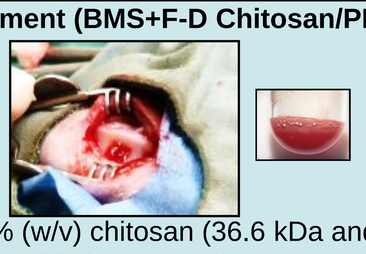
4 weeks

Surgery 2
Debride CC



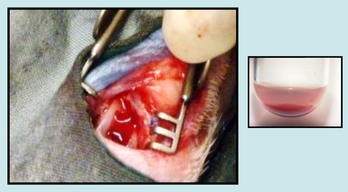
Repair of chronic defect
BMS by drilling
(0.9mm wide X 6 mm deep)

Treatment (BMS+F-D Chitosan/PRP)



(1% (w/v) chitosan (36.6 kDa and 80.2% DDA)+1% (w/v) trehalose as lyoprotectant and 42.2 mM $CaCl_2$ as clot activator)

Control (BMS+PRP)



8 weeks

Repair analysis
Macroscopic, histological and micro-CT

RESULTS

Characterization of chronic defect

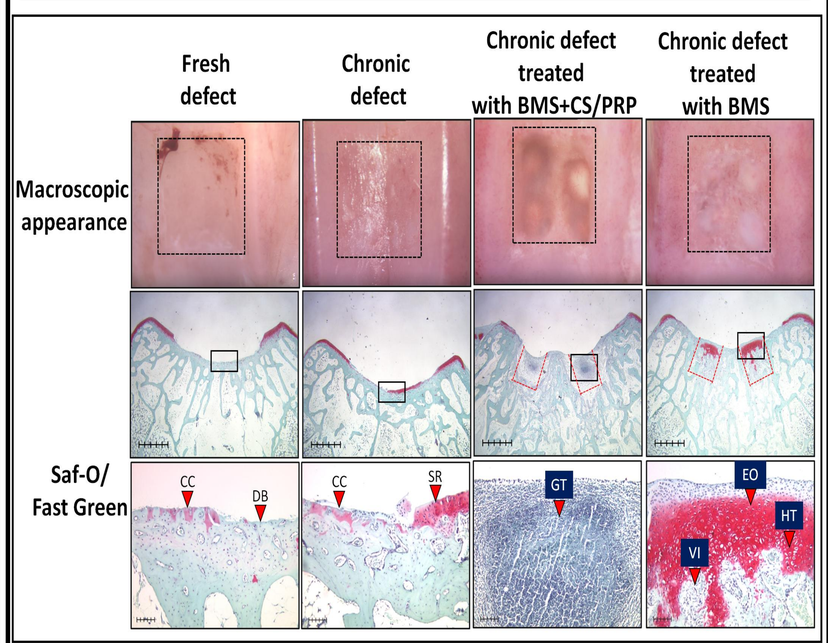


Fig. 2. Assessment of fresh defect, chronic defect after 4 weeks development, chronic defect treated with BMS+CS/PRP implant and chronic defect treated with BMS alone, both 3 weeks after treatment of the chronic defect by macroscopic and histopathological analysis.

Debridement was not homogenous and varying levels of calcified cartilage (CC) and debrided bone (DB) were seen in freshly debrided defects.

After 4 weeks, chronic defects showed evidence of partial spontaneous repair (SR) in some areas along with tufts of calcified cartilage (CC). Granulation tissue formation (GT) and enlarged drill holes were seen in presence of CS/PRP implants.

Fibrocartilagenous repair and endochondral ossification (EO) process were seen after 3 weeks of BMS alone, associated with chondrocyte hypertrophy (HT) and vascular invasion (VI).

Red dotted lines represent original drill holes- hole enlargement and wound bloom effect is apparent in defect treated with BMS+CS/PRP.

8 week repair-Macroscopic assessment

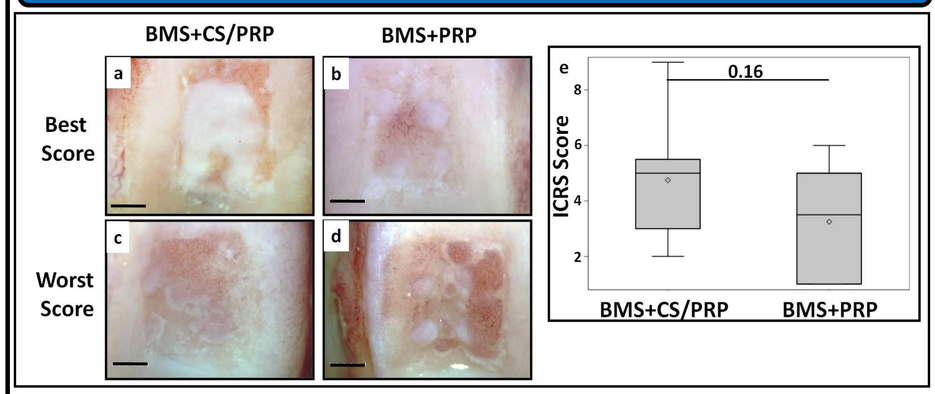


Fig. 3. (a-d): Macroscopic assessment of best and worst repair with BMS+CS/PRP (a,c) and BMS+PRP (b,d). Control defects showed evidence of fibrocartilagenous or fibrous repair and improved repair was observed in defects treated with BMS+CS/PRP. **(e)**: Mean macroscopic ICRS score was higher in BMS+CS/PRP group versus BMS+PRP group.

Table 1: Fewer grade II and more grade IV repair were seen with BMS+CS/PRP

Repair	MS+CS/PRP	MS+PRP
Grade II	1	0
Grade IV	2	4

8 week repair-Histological assessment

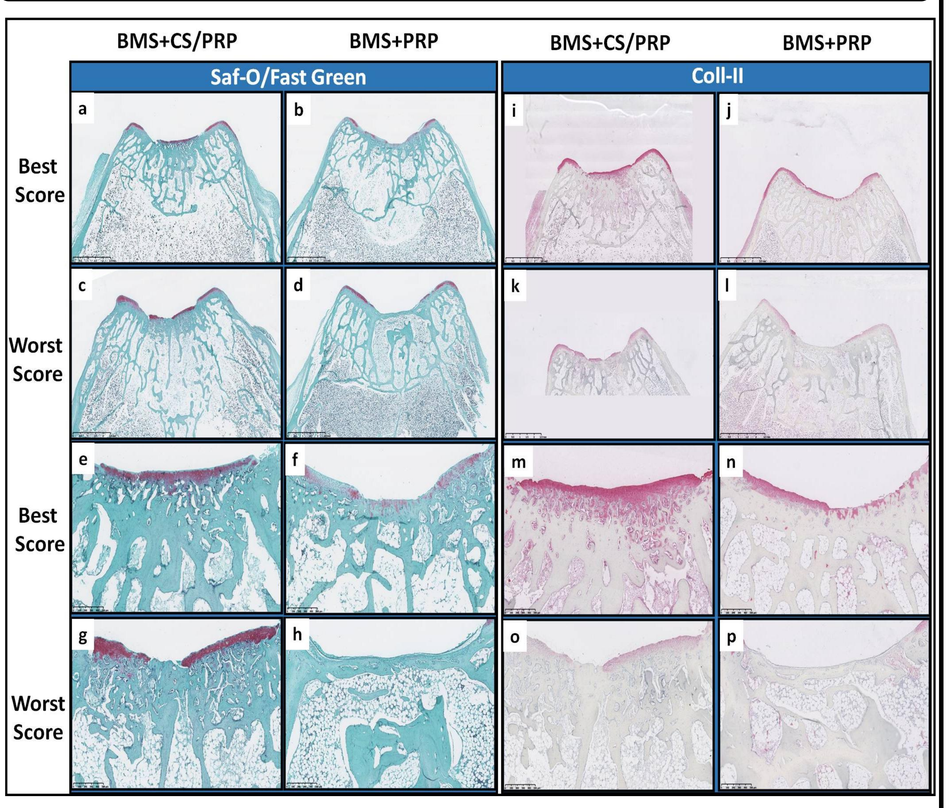


Fig. 5. Histopathological assessment of best and worst repair generated by BMS+CS/PRP and BMS+PRP. **(a-h)**: Saf-O staining for best (a,b,e,f) and worst (c,d,g,h) repair outcomes; **(i-p):** Coll-II immunostaining for best (i,j,m,n) and worst (k,l,o,p) repair outcomes
Better repair was confirmed by higher deposition of collagen type-II and GAGs in defects treated with BMS+CS/PRP implants compared to BMS+PRP.

8 week repair-Microscopic assessment

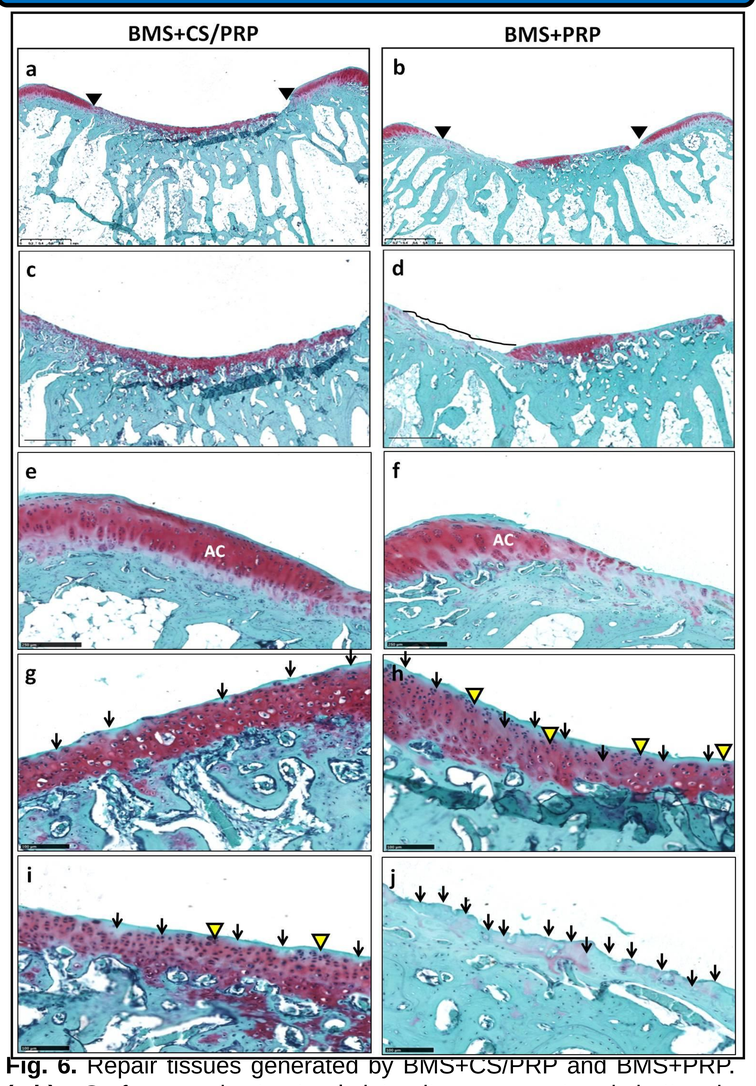


Fig. 6. Repair tissues generated by BMS+CS/PRP and BMS+PRP. **(a,b):** Surface and structural integrity was restored better in BMS+CS/PRP group versus BMS+PRP; **(c,d):** Repair tissue was more uniform in BMS+CS/PRP group versus BMS+PRP; **(e,f):** Adjacent cartilage (AC) showed improved appearance in BMS+CS/PRP group; **(g,h):** Black arrows indicate zones of hypocellularity, yellow arrows indicate cell clusters, both more frequent in BMS+PRP.

8 week repair-Histomorphometric analysis

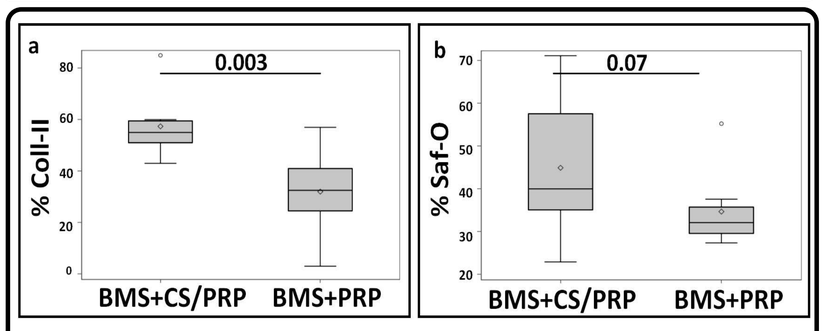


Fig. 4. (a). Mean %Coll-II was significantly higher for BMS+CS/PRP group versus BMS+PRP group.
(b). Mean % Saf-O was higher for repair tissues in defects treated with BMS+CS/PRP versus defects treated with BMS+PRP, although this difference was not significant.

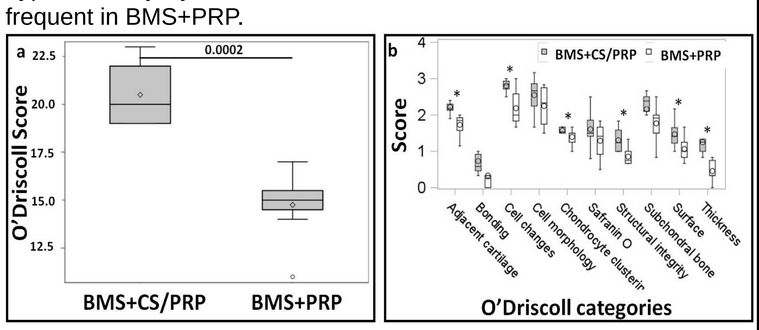


Fig. 7. (a). Mean O'Driscoll score was significantly higher for repair tissues in BMS+CS/PRP group versus BMS+PRP. **(b).** Significant differences (*) were observed between treatments, and scores for adjacent cartilage (p=0.004), cellular changes (p=0.002), cell clusters (p=0.009), structural integrity (p=0.0001), surface integrity (0.05) and thickness of repair tissue (p=0.002) were significantly higher for defects treated with BMS+CS/PRP.

CONCLUSION

Freeze-dried CS formulations, which are expected to have a long shelf life, can be solubilized in PRP to form injectable implants that coagulate *in situ*. CS/PRP implants have been shown to reside for several weeks *in vivo* and to have significant bioactivity, in contrast to PRP implants which are quickly degraded in a day [4]. Chronic defects were more challenging to treat and CS/PRP implants improved cartilage repair compared to BMS+PRP. The superior bioactivity of CS/PRP implants likely arise from negligible clot retraction, sustained release of PRP derived growth factors, increased recruitment and differentiation of MSCs [4].

REFERENCES

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[4] Vaisman *et al* (2012). Cartilage 3-2: 118–127

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