

## The basic science of bone marrow aspirate concentrate in chondral injuries

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### Abstract

There has been great interest in bone marrow aspirate concentrate (BMAC) as a cost effective method in delivering mesenchymal stem cells (MSCs) to aid in the repair and regeneration of cartilage defects. Alongside MSCs, BMAC contains a range of growth factors and cytokines to support cell growth following injury. However, there is paucity of information relating to the basic science underlying BMAC and its exact biological role in supporting the growth and regeneration of chondrocytes. The focus of this review is the basic science underlying BMAC in relation to chondral damage and regeneration.

### Introduction

The potential of mesenchymal stem cells (MSCs) to transdifferentiate into different mesenchymal derived tissues has created huge interest in trauma and orthopedic surgery. MSCs are present in numerous tissues in the body including bone, adipose, synovium and blood.<sup>1</sup> There has been great debate in the best way in which to obtain these cells, process them and deliver to the site of injury. *In vitro* culture and amplification of MSCs is associated with significant financial cost, which in the current financial restrictions in healthcare have made this option unfeasible for current clinical use. To overcome this cost, bone marrow aspirate (BMA) as a source of MSCs has been explored.<sup>2</sup> One consideration when using BMA alone is the relatively low percentage of MSCs with only 0.001 to 0.01% of nucleated cells in BMA being MSCs.<sup>3</sup> In order to try and increase the ratio of MSCs in the aspirate the sample can be concentrated. This is most frequently done using a centrifuge to produce bone marrow aspirate concentrate (BMAC).<sup>4</sup> There have been a number of successful ani-

mal models with BMAC showing beneficial results in sheep, rats, rabbit, horses and the mini-pig.<sup>5-9</sup> Initial human trials have also been successful with Gobbi and colleagues showing beneficial functional and health related quality of life scores with patients with grade 4 arthritis and production of hyaline like cartilage.<sup>10</sup> The beneficial effect of BMAC in chondral injuries has also been extended to osteoarthritis with Kim and colleagues showing improved quality of life scores with patients with grade 4 osteoarthritis treated with BMAC.<sup>11</sup> BMAC is a safe treatment with Hendrich and colleagues reviewing 101 patients with an average follow-up time of 14 months with no adverse effects or morbidity from the harvest site reported.<sup>12</sup> However, despite the early promise of BMAC in chondral injuries there is no standardized regime for the harvest or administration of BMAC. There is also paucity of information relating to the basic science underlying BMAC and its exact biological role in supporting the growth and regeneration of chondrocytes. The focus of this review is the basic science underlying BMAC in relation to chondral damage and regeneration. In order to search the literature for the relevant information we used Pubmed with specific search terms. This included basic science, BMAC, chondral injuries and cartilage injuries. From the initial searches we then performed a more detailed search with the key components, growth factors and cytokines contained in BMAC.

### Bone marrow aspirate concentrate composition

#### Cellular composition

The normal composition of BMAC has been analyzed in a variety of methods including light microscopy, laser photometry and flow cytometry.<sup>13-15</sup> Despite the various techniques used the composition has proved to be largely similar. In normal healthy individuals neutrophils and erythroblasts are the dominant cell type. There are some gender differences with males having more erythroblasts than females (28.1 *vs.* 22.5%) but women having more neutrophils than males (37.4 *vs.* 32.7%).<sup>13</sup> Lymphocytes occupy 13%, eosinophils 2.2%, monocytes 1.3% and basophils 0.1%.<sup>13</sup> Platelets show a large variation between individuals highlighting the wide range of what is accepted as a normal range in adults.<sup>14,15</sup> Cassano and colleagues have directly compared the cellular content of whole blood to that of platelet rich protein (PRP) and BMAC.<sup>16</sup> The results found that BMAC contained 11.8x the number of white blood cells, 19.4x the number of neutrophils and 2.5x the

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number of platelets than that of PRP.<sup>16</sup> The number of monocytes, lymphocytes, eosinophils and basophils were largely similar between PRP and BMAC.<sup>16</sup> These ratios are relative to the type of centrifuge system used and serve as an example of the type of differences one could expect between PRP and BMAC.

### Growth factors and cytokines

The study by Cassano and colleagues also reviewed the growth factor and cytokine levels between PRP and BMAC.<sup>16</sup> This found that BMAC had 172.5x the concentration of vascular endothelial growth factor (VEGF), 78x the concentration of interleukin-8 (IL-8), 4.6x the concentration of interleukin-1beta (IL-1β), 3.4x the concentration of transforming growth factor-β2 (TGF-β2) and 1.3x the concentration of platelet derived growth factor (PDGF).<sup>16</sup>

A possible explanation for the increased concentration of growth factors and cytokines in the BMAC samples relates to the concentration of platelets it contains. The alpha granules of platelets contain TGF-β, PDGF, VEGF along with fibroblast growth factor (FGF), bone morphogenic protein (BMP), and insulin-like growth factor (IGF).<sup>17</sup>

## Influence of growth factors and cytokines in bone marrow aspirate concentrate to chondrocyte repair and regeneration

### Interleukin-1

Interleukin-1 (IL-1) is subdivided into IL-1 and IL-1 $\beta$ .<sup>18</sup> The IL-1 has more of an intracellular effect whereas it is IL-1 $\beta$  that modulates the immune response and downstream effects via stimulation of matrix metalloproteinases.<sup>19</sup> IL-1 $\beta$  is produced by local monocytes and macrophages involved in the inflammatory response and is known to contribute to inflammation in human joints and can degrade cartilage via its metalloproteinase action.<sup>19</sup> The effect of this is blocked by the interleukin antagonist IL-1 receptor antagonist (IL-1RA).<sup>19</sup> Abramson and colleagues has shown the beneficial effects of blocking the actions of IL-1 $\beta$  in patients with rheumatoid arthritis with prevention of bone and cartilage loss, highlighting the catabolic role of this cytokine to chondrocytes.<sup>20</sup> Pelletier *et al.* has also shown a similar effect with using gene therapy in an experimental model of osteoarthritis using dogs.<sup>21</sup>

It has been reported that there is a relative imbalance of the IL-1 $\beta$ :IL-1RA ratio with a deficiency of IL-1RA in osteoarthritis allowing IL-1 $\beta$  leading to cartilage degradation.<sup>22</sup> Cassano and colleagues has demonstrated an increase in the IL-1 $\beta$  but also IL-1RA in BMAC, therefore neutralizing the effect of the raised IL-1 $\beta$ .<sup>16</sup>

### Interleukin- 8

IL-8 is a powerful cytokine for neutrophil

chemotaxis and activation as part of the inflammatory response.<sup>16,23</sup> Lotz and colleagues has demonstrated that IL-1 $\beta$  and TNF can stimulate IL-8 release from chondrocytes.<sup>23</sup> Chaffter and colleagues has shown that mechanical stress can also increase IL-8 from chondrocytes.<sup>24</sup> Thus damaged cartilage, which is under increased mechanical stress, would release more IL-8 attracting neutrophils to the affected area. IL-8 has also reported to promote homing of bone marrow derived cells to the site of injury, including MSCs.<sup>25</sup> Hou and colleagues has shown that IL-8 enhances the angiogenic potential of mesenchymal cells via increasing VEGF production.<sup>25</sup> This would appear a clear benefit in promoting angiogenesis and tissue healing especially in full thickness chondral defects.

### Vascular endothelial growth factor

Mature articular cartilage is an avascular structure, which receives its nutrition via diffusion from the synovial fluid.<sup>26</sup> Its unique structure and function is attributable to the dense packing of collagen fibers and mature chondrocytes and lack of blood vessels.<sup>26</sup> Mature articular cartilage contains inhibitors to angiogenesis including Troponin I and Chondromodulin-1 in the avascular layers of the cartilage but are absent in the supporting subchondral bone and spongiosa allowing VEGF to promote a vascular supply.<sup>27-29</sup> Therefore this is clearly important in full thickness chondral defects where the subchondral bone can release growth factors and cytokines to support cartilage repair. Maes and colleagues has clearly shown the importance of VEGF for epiphyseal blood supply and cartilage development using mice as an animal model.<sup>30</sup> VEGF deficient mice

showed altered growth plates, ossification centers and joint dysplasia.<sup>30</sup> Oxygen tension is a key factor that is triggering VEGF production via the stimulation of hypoxic inducible factor-1 (HIF-1). Lund-Olesen has shown that after a traumatic effusion there is a reduction in the oxygen tension leading to HIF-1 and VEGF production.<sup>31</sup> Clearly this situation would match a chondral injury pattern with an associated effusion or hemarthrosis and therefore increased VEGF production. Alongside oxygen tension other factors have been shown to stimulate VEGF production including IL-1 via the Mitogen Activated Protein Kinase signaling pathway as demonstrated by Murata and colleagues.<sup>29</sup> Thus, highlighting the complex interplay between the cytokine cascades.

### Transforming growth factor $\beta$

TGF superfamily include a number of important growth factors for cartilage regeneration and repair including TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3 and BMP-2 and BMP-7.<sup>32</sup> The TGF ligands bind type 1 and type 2 TGF- $\beta$  receptors, which are serine/threonine kinases.<sup>33</sup> The type 2 TGF- $\beta$  receptor phosphorylates the type 1 receptor. There are two distinct pathways relating to the TGF or BMP ligands.<sup>34</sup> The TGF ligands lead to phosphorylation of the mothers against decapentaplegic homolog-2 (SMAD-2) and SMAD-3. The BMP ligands use SMAD-1, SMAD-5 and SMAD-8.<sup>34</sup> The end result of these pathways is the proliferation and differentiation of chondrocytes.<sup>35</sup>

Much of the research studying the role of these growth factors has been done in animal models. The study by Cassano and colleagues highlighted the raised TGF- $\beta$ 2 in BMAC. Wang and colleagues has shown bone marrow derived MSCs transfected with TGF- $\beta$ 2 show increased

**Table 1. Summary of the growth factors and cytokines in bone marrow aspirate concentrate.**

Growth factor/cytokine	Principle action	Signaling pathway	Reference
TGF $\beta$ 1, TGF $\beta$ 2, TGF $\beta$ 3	Chondrocyte Proliferation + differentiation	SMAD-2 and SMAD-3	33,65
BMP-2	Chondrocyte proliferation, matrix synthesis and hypertrophy	SMAD-1, SMAD-5, SMAD-8, TAK-1	65
BMP-7	Increase ECM production		
IL-1/IL-1 $\beta$	Inflammatory response- cell migration/ recruitment to site of injury	Mitogen activated kinases (JNK, P38, ERK1/2)	66
IL-8	Inflammatory response; MSC homing to site of injury; Increased VEGF production; chondrocyte hypertrophy	Mitogen activated kinase; P38	16,25,67
VEGF	Promotes angiogenesis to sub-chondral bone and supports cartilage growth	HIF-1, Runx2	29,30,68
PDGF	Wound healing, collagen synthesis, angiogenesis, suppression of IL-1 $\beta$ , enhanced BMP signaling	ERK 1/2, down-regulation of NF-kB signaling	49,51,52,69
IGF-1	Increased synthetic and metabolic activity- increased collagen and proteoglycan synthesis, chondrogenic differentiation	PI-3K, ERK 1/2	55,56,65,70,71
FGF-2	Chondrogenic differentiation, MSC homing	ERK 1/2, STAT1/P21	61-63
FGF-18	Chondrogenic differentiation, enhanced BMP signaling		58-60,72

JNK, C-Jun N-terminal kinase; ERK, extracellular signal-related kinases; TAK-1, TGF- $\beta$  activating kinase 1 (TAK-1); STAT1- signal transducer and activator of transcription-1. PI-3K, phosphoinositide 3-Kinase; Runx2, Runt-domain transcription factor family-2; HIF-1, hypoxia inducible factor-1; NK-kB, nuclear factor kappa beta.

type II collagen and aggrecan production after 48 hrs and this persists for up to 4 weeks.<sup>36</sup> This suggests MSCs are activated by TGF- $\beta$ 2 increasing the synthetic activity. Zhao and colleagues has demonstrated that TGF- $\beta$ 1 can stimulate chondrogenic differentiation of MSCs *in vitro*.<sup>37</sup> Draio and colleagues has developed this with an animal model showing beneficial effects of TGF- $\beta$ 1 in chondral defects in the rabbits with improved cartilage repair.<sup>38</sup> Although TGF- $\beta$ 1 has shown good promise there has been concerns with using TGF- $\beta$ 1 due to evidence of side effects including proliferative synovium, fibrosis and osteophyte formation which has hindered its development as a therapeutic target.<sup>39</sup> Joyce and colleagues assessed the role of TGF- $\beta$ 1 and TGF- $\beta$ 2 in chondrogenesis and osteogenesis in rodent femurs by subperiosteal injection.<sup>40</sup> TGF- $\beta$ 2 was more potent than TGF- $\beta$ 1 in stimulating chondrogenesis and osteogenesis but also TGF- $\beta$ 2 increased autocrine TGF- $\beta$ 1 production. Thus highlighting the importance of TGF- $\beta$ 1 and TGF- $\beta$ 2 in chondrogenesis but also in its local autocrine and paracrine role.<sup>40</sup> This work has been supported by Tekari and colleagues using expanded bovine chondrocytes.<sup>41</sup> This study assessed the ability of the chondrocytes to autonomously produce cartilage. After 3 cell passes in cell expansion there was loss of ability for chondrocytes to form cartilage. This was restored when TGF- $\beta$ 1 was added. Furthermore, there was also reduction in TGF- $\beta$  receptors and transcripts for TGF- $\beta$ 2 prior to TGF- $\beta$ 1 administration supporting the paracrine/autocrine role of TGF- $\beta$ .<sup>41</sup> This paracrine/autocrine role of TGF- $\beta$  is also evident in human models with Villiger and colleagues demonstrating that human chondrocytes have receptors for and can secrete TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3.<sup>42</sup> Fan and colleagues has shown that pellet culture of TGF- $\beta$ 3 with MSCs can enhance glycosaminoglycan, collagen and ECM production *in vitro* surrounded by a gelatin scaffold.<sup>43</sup> This has been transferred to an ovine model with Tang and colleagues producing a well-integrated cultured scaffold of MSCs and TGF- $\beta$ 3.<sup>44</sup>

BMP as a component of platelet alpha granules is present in BMAC. BMP-2 has a synergistic effect to TGF- $\beta$  in that it is able to induce chondrogenic differentiation of MSCs *in vitro*.<sup>45</sup> Schmitt and colleagues concluded that BMP-2 initiates chondrogenic lineage development of adult human MSCs.<sup>46</sup> Cultured MSCs with BMP-2 increased type II collagen and ECM production.<sup>46</sup> BMP-7 has been hailed as the *gold standard* growth factor in cartilage repair by Fortier and colleagues in part to its unique function in that the response of BMP-7 is not affected by age or damage to cartilage.<sup>32</sup> Although with normal ageing BMP-7 is reduced.<sup>47</sup> Jung and colleagues used local BMP-7 release from a biological scaffold to repair osteochondral defects in a rabbit model.<sup>48</sup> At 12 weeks post implantation the

grafts were well integrated with new cartilage formation.<sup>48</sup>

### Platelet derived growth factor

PDGF has an established role in wound healing but also functions to promote collagen synthesis and contributes angiogenesis in subchondral injuries.<sup>49</sup> Animal models have shown that PDGF has an active role in chick limb bud development and also has been found to induce chondrocyte proliferation in newborn rats.<sup>50</sup> Although PDGF has a minor role in cartilage repair it does have a synergistic action with suppression of IL-1 $\beta$  cartilage degradation.<sup>51</sup> This synergism is also shown with PDGF promoting osteogenic differentiation by activating BMP related SMAD 1/5/8 signaling.<sup>52</sup>

### Insulin-like growth factor-1

IGF-1 is present in BMAC as it is found in the alpha granules of platelets.<sup>17</sup> Fortier and colleagues reviewed the effect of using *ex-vivo* expanded chondrocytes supplemented with IGF-1 on a fibrin scaffold in horses with full thickness cartilage defects.<sup>53</sup> At 8 months post surgery the animals were slaughtered and found enhanced cartilage regeneration and defect filling with increased type II collagen production.<sup>53</sup> Fortier and colleagues also reviewed the IGF-1 profile in horses following cartilage injury.<sup>54</sup> This study found low levels of IGF-1 at two weeks and increased at four to eight weeks but declined again at sixteen weeks following injury.<sup>54</sup> This suggests that most of the chondrogenic effects of IGF-1 are not immediately following the injury. Pasold and colleagues highlighted the chondrogenic potential of IGF-1 when coupled with nanoparticles cultured with human chondrocytes on a collagen scaffold.<sup>55</sup> The total number of chondrocytes increased over a two week period with integration into the collagen matrix.<sup>55</sup> The metabolic and synthetic activity of the cells also increased with increased type II collagen expression.<sup>55</sup> Mullen and colleagues has also found promising results when using IGF-1 onto a scaffold.<sup>56</sup> The IGF-1 was loaded onto a collagen-glycosamine scaffold containing chondrocytes *in vitro*. The result was increased type II collagen and proteoglycan synthesis by chondrocytes compared to the control where no IGF-1 was used.<sup>56</sup>

### Fibroblast growth factors

FGF represent a large family of growth factors, of which FGF-2 and FGF-18 have been found to be important in cartilage damage and repair.<sup>32</sup> FGF signals through its related tyrosine kinase based receptors (FGFR) to activate

downstream intracellular signaling cascades.<sup>57</sup> FGF-18 in animal studies has been shown to act through FGFR1-4.<sup>57</sup> However, evidence from Ellsworth and colleagues studying the effect of FGF-18 on human articular chondrocytes found increased mRNA for FGFR-2 and FGFR-3 alongside increased proliferation and differentiation activity of chondrocytes. Davidson and colleagues further highlighted the role of FGFR-3 in FGF-18 signaling on mesenchymal cells to promote chondrogenic differentiation.<sup>58</sup> Reinhold and colleagues has demonstrated synergistic actions of FGF-18 signaling with BMP signaling in chondrogenesis by suppressing expression of noggin, an inhibitor of BMP signaling.<sup>59</sup> There is now also evidence for FGF-18 stimulating chondrogenesis and cartilage repair in a rat model of induced osteoarthritis with an FGF-18 dose dependent increase in cartilage thickness at 3 weeks following therapy.<sup>60</sup>

FGF-2 has been found to promote earlier chondrogenic differentiation when compared to FGF-18 as shown by Correa and colleagues using expanded human MSCs with sequential exposure to FGF-2, FGF-9 and FGF-18.<sup>61</sup> The importance of FGF-2 to chondrocyte repair and regeneration has been demonstrated by Henson and colleagues after subjecting explanted equine articular cartilage to a 500 g load from a height of 2.5 cm then culturing in FGF-2.<sup>62</sup> The results indicated that FGF-2 increased the number and speed of transition of chondrocyte progenitor cells to the damaged area.<sup>62</sup> Indeed Chuma and colleagues found that FGF-2 exposure for one day was enough to ensure repair of 5 mm defects in trochlea defects in rabbits.<sup>63</sup> Defects were cultured for 1, 3 and 14 days using an osmotic pump infused with saline (control) or FGF-2. However, culture over 24 hours was enough to fill defects to the same degree as rabbits exposed to FGF-2 for 2 weeks when animals were analyzed at 8 weeks post-injury.<sup>63</sup> Using immunohistochemistry, this study also found FGF-2 promoted migration of MSCs from bone marrow similar to Henson and colleagues.<sup>62,63</sup> Ishii and colleagues also used 5 mm defects in the rabbit knee, which were 4 mm deep and treated them with FGF-2 in a fibrin sealant. These results supported the work by Chuma and colleagues with complete defect filling at 8 weeks.<sup>63,64</sup>

The summary of the growth factors and the cytokines in BMAC is resumed in Table 1.

### Conclusions

Initial experiments with BMAC have demonstrated clear benefit in animal and human models. We have reviewed the constituents of BMAC and its underlying basic science that

supports the positive *in vitro* and *in vivo* findings in cartilage regeneration and repair. What is clear is that a standardized method of BMAC harvest and processing needs to be established. Further clinical trials are needed to establish the long term effects of BMAC in cartilage damage but also other tissues including bone defects, tendon and ligament injuries where BMAC has had good preliminary results.

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