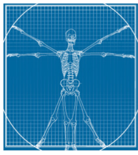
Stem Cells



Critical review

Bone allograft with mesenchymal stem cells: A critical review of the literature

G Grabowski*, RN Robertson

Abstract

Introduction

In orthopaedic surgery, autograft, most commonly from iliac crest, has long been the gold standard for bony augmentation for the treatment of skeletal fractures and fusions.***Clinically, bone allograft with mesenchymal stem cells is readily available, has no donor site morbidity and has been shown to be non-immunogenic. Based on the current, albeit limited, literature, bone allograft with mesenchymal stem cells appears to be equal if not superior to autograft, thus making it an accept-able alternative in joint fusions and the treatment of non-unions and frac-tures with metabolic concerns or large defects. The aim of this critical review was to discuss the characteristics of bone allograft with mesenchymal stem cells and its clinical applicability.

Conclusion

Bone allograft with mesenchymal stem cells looks like a promising augmentation technology. It is as good, if not better, than autogenous graft material. We call for further studies to increase our understanding and improve our techniques.

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Introduction

A continual field of growth in orthopaedic surgery is biologic augmentation of skeletal fractures and arthrodesis procedures. The gold standard remains autograft because it contains the three pillars of bone growth: osteoconductive scaffold, osteoinductive cytokines and osteogenetic cells. Additionally, by definition, autograft is histocompatible and non-immunogenic. However, autograft is influenced in quality and quantity by age, metabolic abnormalities and smoking. Additionally, it comes with its own set of concerns including increased surgical time, limited available volume, surgical morbidity, potential blood loss and infection[1–4]. This has led to increased time and resources being devoted to studying skeletal healing and to the creation of a myriad of commercially available bone graft substitutes, bone graft extenders and osteobiologic materials in an attempt to increase the healing capacity for fractures and production of bony growth. The historical alternatives have been demineralized bone matrices, bone morphogenic proteins, synthetic fillers and other similar products that possess osteoconductivity and/or osteoinductivity but none contained an osteogenic cellular component. All of these products relied on the body's local cells or the addition of bone marrow aspirate or autograft to provide osteogenic cells comprised of mesenchymal stem cells (MSCs) and osteoprogenitor cells (OPCs). This lack of osteogenic cells leads to the creation of allogenic bone grafts with MSCs to provide all three aspects of bony healing but none of the drawbacks of autograft[1–3,5–7]. Allogenic bone graft with MSCs provides the surgeon with a graft with osteogenic properties with a known quality and quantity to allow confidence in bony healing. The aim of this critical review was to see if bone allograft of MSCs is a good alternative for autograft.

Discussion

MSCs

Since their discovery in 1966, MSCs have been widely studied and have been shown to be a key component in bony healing. MSCs are able to self-renew and are multipotential with the capacity to differentiate into bone, cartilage, fat, nerve, muscle, tendon and mature stromal cell lineages, depending on both intrinsic and extrinsic cues in the local environment[2,8–22] (Figure 1).

MSCs can be isolated from umbilical cord blood, connective tissue, skin, synovium fluid, fat, placenta and even teeth, but most commonly, they are isolated from bone marrow[8]. When cultured *ex vivo*, MSCs have been shown to undergo many passages without overt expression of a differentiated phenotype and are able to replicate as many as 38 times before degenerating[11].

For MSCs to be directed into osteogenesis, they require the appropriate density, spatial organization, mechanical forces and bioactive nutrients/cytokines like BMPs, TGF-β, osteogenin, osteogenic growth peptide, ascorbic acid and endogenous

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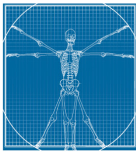

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glucocorticoids[12,13]. It has been shown that even though the density of MSCs that leads to the creation of bone is less than the density that leads to cartilage production, the concentration of MSCs is directly related to the amount of mineralized matrix produced. The cells have also been shown to have autocrine and paracrine functions that may be essential for lineage progression[10,13,14]. These secreted bioactive factors inhibit scar formation/fibrosis and apoptosis, thus limiting the field of injury, suppressing local immune function, enhancing angiogenesis and stimulating division and differentiation of nearby stem/progenitor cells[10,15].

The density of MSCs is relative to age. When grouped by decades, a dramatic decrease in MSCs per nucleated marrow cell is appreciated with a decrease of 10-fold from birth (10^4) to teens (10^5) and another 10-fold drop from teens to the elderly (10^6)[2,5,8,10]. This decrease with age is shown in Figure 2. It has also been shown that they have decreased ability to proliferate, differentiate and mobilize with age. These characteristics explain the decrease in osteogenic potential as people age, which explains why it is harder to heal bony defects in elderly patients[2,5,8–10,16].

In bony healing, MSCs are a part of the repair blastema (callus) and form a continuous repair tissue that differentiates into bone or cartilage depending on stability[10,12]. For bone formation, cartilage provides the architecture that is replaced by marrow, vascular infiltrates and resorptive tissue that bring in MSCs and direct them towards the osteogenesis pathway[12]. Vasculature is obligatory for osteogenic differentiation, and the growth orientation is dictated by the relationship of the MSCs to the vasculature. In stable constructs, angiogenesis across the fracture is able to cause direct osteogenesis for bridging the bony defect[10,12]. Clearly, in these ways, MSCs have a large role in the healing of fractures,

Figure 1: The mesengenic process. Adult MSCs are able to differentiate into a variety of tissues including bone, cartilage, muscle, marrow, tendon, adipocyte and other stroma connective tissues based on the cues from the surrounding environment. Based on the cues, the MSCs will proceed down a lineage in a sequential progression. (Reproduced from ref.[11].)

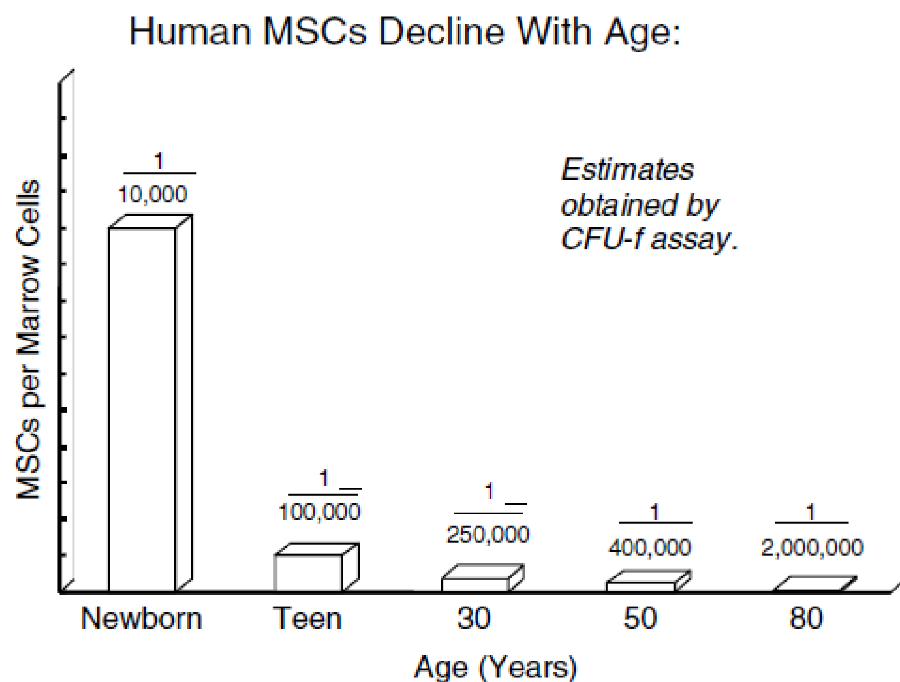

Figure 2: Human MSC concentration based on age. This graph depicts the decline of humans MSCs with age. For the data, Caplan obtained human marrow MSCs and cultured them measuring CFU-F to estimate the titre of MSCs in each marrow sample. The data show a clear decline with age. (Reproduced from ref.[11].)

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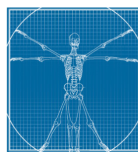

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non-unions and joint fusions, fuelling further research.

Allograft

Bone allograft allows for the uniform loading and retention of MSCs, rapid vascular ingrowth, incorporation into the normal bone remodelling cycle, enhancement of osteoconductive bridging and ease of application[17]. These characteristics lead to the use of bone allograft with associated MSCs as a viable bone augmentation option, which has the potential to decrease surgical time and morbidity and give an unlimited supply of reliable MSCs while providing the needed osteoconduction out of a carrier. This has led to the creation of two bone allografts with MSCs: Trinity Evolution (Orthofix) and Osteocel Plus (NuVasive) that are currently available on the market. These allografts unlike the other non-autograft bone augmentation materials contain all the three essential components of bone growth: osteoinduction, osteoconduction and most importantly osteogenic cells.

Processing

When an autograft is percutaneously harvested, studies have shown that concentrations alone of progenitor cells are too low for reliable bony healing. As a result, *ex vivo* concentration methods have been devised. Despite these possibilities, when trabecular bone is harvested, the yield contains 65-fold more progenitor cells[23,24]. By leaving MSCs attached to the trabecular bone, there is a greater expression of osteogenic genes and increased secretion of osteogenic factors[18]. For this reason, commercially available allograft/MSC products are generated by harvesting cells from trabecular bone and leaving the MSCs adherent to the natural trabecular tissue. In addition to ensuring adequate/optimal osteogenic cells, allografts provide the other components necessary to stimulate and support bony growth, osteoinduction via demineralized cortical bone and osteoconduction via the cancellous bone chips[5].

The graft is harvested from a single donor's bone within 72 hours of death and after serological screening is complete. Donor screening requirements are industry driven and are set so as to exceed those required by the Federal Drug Administration and American Association of Tissue Banks. It includes a medical/social history and physical examination along with comprehensive tissue and blood testing (Table 1). During the processing, they also have rigorous testing for bacterial, fungal and spore contamination, along with the administration of antimicrobial and antimycotic treatments[6,25]. Cancellous bone chips are isolated and then processed, and cortical bone from the donor is milled and demineralized. These bone products are then combined to create the bone graft. The samples during processing also undergo immunodepletion by removing hematopoietic stem cell lineage (e.g. blood cells, lymphocytes and osteoclasts) components from the cancellous bone[6]. Each year, over 1.5 million allografts are implanted with no recorded disease transmission since 2006 and no recorded HIV transmission in over two decades[26]. Also, because of these strict screening methods, allograft-related infection rates are lower than those associated with surgical procedures themselves[2].

Cryopreservation/perisurgical handling

After creation of the allograft to preserve the integrity of the cells, they are combined with a cryoprotectant solution and frozen. Bruder et al.[11] in 1997 showed cryopreservation to have >95% cell recovery and have the same biologic capacity as fresh cells after thawing from cryopreservation. Allograft materials are stored at temperatures less than −80°C for shelf lives from 6 months to 5 years. Samples are tested post-cryopreservation to ensure reliable bone formation potential. The standards comprise cell viability of at least 70%, cell concentration of greater than 50,000 or 250,000 viable cells/cc depending on which product is used and osteogenic and osteoinductive potential by either culturing or looking at BMP-2 levels[5,6]. This ensures the reliability of the allograft for clinical use.

Because bone allograft with MSCs is cryopreserved, attention has been given to perisurgical handling to ensure optimal bone healing with minimal loss of osteogenic potential. The first step is to bring the sample to room temperature. This is optimized by placement of the sample in a 35–39°C water bath. Once at room temperature, it is recommended that the cryoprotectant solution be decanted and replaced with sterile saline or 5% dextrose depending on the product used. Allograft stored perioperatively in these solutions has been shown to have an increased cellular viability at greater than 2 hours of 10–15% when compared with samples left in the cryoprotectant solutions[27]. Bone allograft with MSCs when treated in this manner has a back table life of 2–6 hours depending on the product used.

Immunogenicity

As stated above, because the bone graft and MSCs are from donor stock, significant attention had to be directed towards the immune response that would be elicited by the host. It has been proven, however, that bone allograft with MSCs does not elicit an immune response. This lack of allogenic reaction has been supported by *in vitro* studies as well as by the current clinical data, which has shown no adverse reactions to the graft in human and animal studies.

This lack of immunologic response is partially aided by the processing as stated above to deplete immunogenic cells, like hematopoietic stem cells (HSCs), without harming the native osteogenic cells. This immunodepletion has been shown by cell

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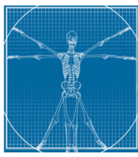

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Table 1 Donor screening and testing criteria for bone allograft with mesenchymal stem cells

Lifestyle Screening	**FDA**	**AATB**	**Bone Allografts with MSCs***
Nonmedical drug use	X	X	X
High-risk sexual history	X	X	X
Incarceration	X	X	X
Aesthetic needle use (e.g., tattooing, piercings)	X	X	X
International travel	X	X	X
Donor Screening/Testing	**FDA**	**AATB**	**Bone Allografts with MSCs***
Hepatitis B Virus	X	X	X
Hepatitis C Virus	X	X	X
HIV-1/HIV-2	X	X	X
HTLV-1/HTLV-2	X	X	X
Tuberculosis	X	X	X
Active genital herpes	X	X	X
Sepsis	X	X	X
Syphilis	X	X	X
Transmissible spongiform encephalopathy	X	X	X
Vaccinia	X	X	X
West nile virus	X	X	X
Clinically active gonorrhea	X	X	X
Encephalitis	X	X	X
Meningitis	X	X	X
Poliomyelitis	X	X	X
Varicella zoster	X		X
Malaria	–	X	X
Clinically significant metabolic bone disease	–	X	X
Leprosy (Hansen's disease)	–	X	X
Polyarteritis nodosa	–	X	X
Rabies	–	X	X
Rheumatoid arthritis	–	X	X
Sarcoidosis	–	X	X
Systemic lupus erythematosus	–	X	X
Systemic mycosis	–	X	X
Clinically active Epstein-Barr virus (mononucleosis)	–	X	X
Multiple sclerosis	–	X	X
Ankylosing spondylitis	–		X
Antiphospholipid syndrome	–		X
Autoimmune hemolytic anemia	–		X
Autoimmune lymphoproliferative syndrome	–		X
Autoimmune thrombocytopenic purpura	–		X
Autoimmune vasculitis	–		X

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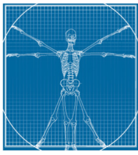

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Table 1 (*Continued*)

Trypanosoma cruzi (Chagas' disease)	–		X
Clostridium difficile infection	–		X
Cold agglutinin disease	–		X
Endocarditis	–		X
Guillain-Barré syndrome	–		X
Methicillin-resistant *Staphylococcus aureus*	–		X
Mixed connective tissue disease	–		X
Myasthenia gravis	–		X
Peritonitis	–		X
Pyelonephritis	–		X
Reactive arthritis (Reiter syndrome)	–		X
Rheumatic fever	–		X
Vancomycin-resistant enterococci	–		X
Wegener's granulomatosis	–		X
Cancer-active malignancy	–		X
Various sexually transmitted diseases	–		X

Prior to processing, all donors must be screened by and held to a set of standards that exceed those required by the Federal Drug Administration (FDA) and American Association of Tissue Banks (AATB). The above graph shows the differing requirements.
*A list of the combined requirements for Osteocel Plus and Trinity Evolution used by MTF and NuVasive in screening.

identification after processing with the samples positive for CD105 and/or CD166 confirming the presence of MSCs/OPCs but negative for CD34 and/or CD45 confirming the lack of immune-competent HSCs[2,5,28]. The processing helps allow the allografts to evade immunogenic reactivity, but the main reason for the lack of immune response is due to the MSCs and how they avoid allogenic rejection[5,6,25].

The MSC ability to avoid allogenic rejection has been traced to three main factors. First, they are hypoimmunogenic by lacking MHC-II (do have MHC-I expression that helps prevent NK cell deletion) and co-stimulator molecule expression including CD40, CD40L, CD80 or CD86, which are necessary for effector T-cell induction[6,20,22,25]. Second, the cells prevent T-cell response through indirect modulation of antigen-presenting cells such as dendritic cells (DCs) by directing them into a suppressor or inhibitory state and direct disruption of NK, $CD4^+$ and $CD8^+$ cell functions. Specifically, MSCs reduce DC secretion of proinflammatory cytokines IFN-γ, interleukin-12 (IL-12) and TNF-α while increasing the production of suppressive cytokine IL-10[22]. Last, they create a suppressive local microenvironment through the creation of bioactive products including prostaglandins, like PGE-2 that suppresses B-cell activation and induction of regulatory T cells; IL-10, which inhibits T cells; hepatocyte growth factor (HGF), which has been shown to induce mitogenic and apoptotic activity and has a role in wound repair; as well as by the expression of indoleamine 2,3-dioxygenase, which depletes the local milieu of tryptophan which in turn inhibits allogenic T-cell responses[20,22]. This ability of MSCs to create an immune-depleted healing environment has been shown in multiple *in vitro* studies of co-cultured or mixed lymphocyte reactions (MLRs) by the lack of a T-cell response to the co-culturing of cells from two different individuals[2,5,6,20,29]. The lack of immunogenic reaction was shown in vivo by Archambault et al.[30] in 2005, where baboon leukocyte antigen-mismatched allogenic MSCs were able to heal a critically sized defect without rejection or stimulation of the inflammatory response monitored by negative MLR and serum assays for alloantibodies.

Application

While inconclusive, current data suggest that bone allograft with MSCs is equivalent if not superior to autograft[5]. This makes currently available products applicable for fusions, non-unions, fractures with metabolic concerns and large defects. Based on 2010 data, bone allograft with MSCs, also known as cell-based matrices, comprised 17% of all bone grafts and substitutes used[31].

Currently, there are multiple clinical trials dealing with allogenic bone graft with MSCs underway to continue to evaluate the efficacy in posterior

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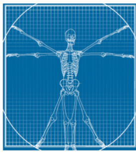
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or transforaminal lumbar interbody fusion, anterior cervical discectomy and fusion, extreme lateral interbody fusion, anterior lumbar interbody fusion and foot and ankle fusion. To date, small case series and case reports exist in the literature that have shown good rates of union[32–37]. Bone allograft with MSCs have been shown to lead to union in foot and ankle surgery by Scott (mean time 11.1 weeks) and Hollawell (mean time 13.5 weeks) in 100% of the patient population and by Rush (mean time 10.4 weeks) in 91.3% of the patient population, which is at the high end of the average rates of fusion of 85–100% in the literature. In all the studies, no graft rejection was appreciated[38–40]. Brosky and Clements also have presented case studies on foot and ankle fusions showing complete fusion at 8 and 12 weeks, respectively[41,42].

Bone allograft with MSCs has also been used widely in spinal surgery and been shown to lead to fusion in 90.2%, 92% and 92.3% in three separate studies by Tohmeh, Ammerman and Kerr, respectively, which can be compared with the weighted average of lumbar fusion using autograft which was shown to be 87.6%[42–45]. In the literature, there also exist multiple case series on the use of bone allograft with MSCs in anterior cervical discectomy and fusion and lumbar interbody fusion, where all patients showed solid fusion without any instances of osteolysis, ectopic bone, postoperative radiculopathy or retropharyngeal swelling with fusion coming at between 3 and 13 months (Figure 3)[46,47]. In these limited studies, a combination of plain radiographs and computed tomography has been used to document fusion and has shown good fusion rates to date.

One possible cause for equality, if not superiority, of allograft with MSCs when compared with autograft is that product testing ensures a reliable graft. At least one study suggests that bone marrow aspirates containing less than 1,000 progenitors/cm^3 or 30,000 progenitors in total are ineffective in the fusion of tibial non-unions, and it also showed that >1,500 progenitors/cm^3 and an average of 50,000 progenitors are required for successful union (Figure 4)[24,38]. As a result, allografts, which can be tested to assure that they exceed 50,000 progenitors, avoid the variable content of autografts; this may explain the equal or superior rate of fusion with allografts.

Conclusion

Bone allograft with MSCs appears to be a promising bone augmentation technology. The current clinical and biologic data lends us to believe that it is equivalent if not superior to autogenous graft material. Further, large randomized prospective studies will be required to solidify the role of allograft with MSCs. For the time being, these products appear to be safe and effective options for use particularly when autograft is likely to be compromised or unavailable.

Abbreviations list

DC, dendritic cell; HGF, hepatocyte growth factor; HSC, hematopoietic stem cell; IL, interleukin; MLR, mixed lymphocyte reaction; MSC, mesenchymal stem cell; OPC, osteoprogenitor cell.

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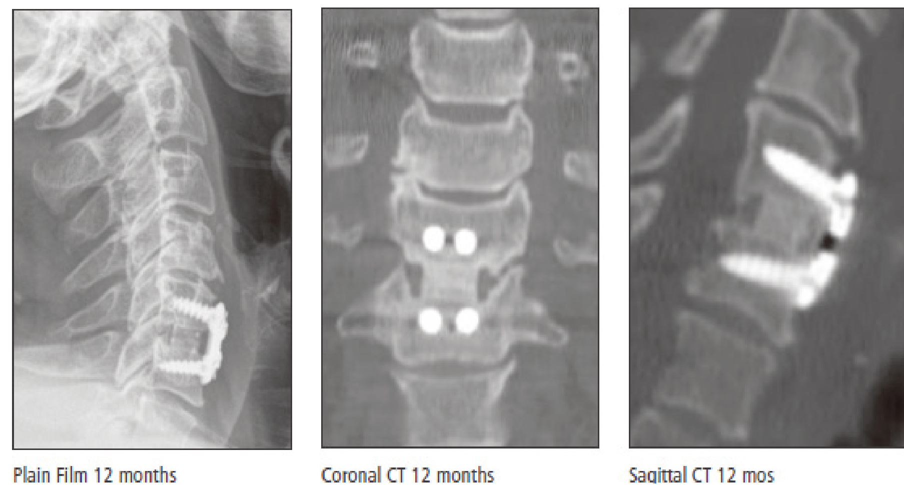

Figure 3: This is an example of bony healing obtained 12 months after anterior cervical discectomy and fusion (ACDF) shown on computed tomography. The patient is a 61-year-old female with left upper extremity radiculopathy and foraminal stenosis and cord compression without signal change shown on MRI. ACDF was performed at C5–6 disk level with bone allograft with MSCs and anterior cervical plate. The post-op imaging demonstrates solid fusion (Reproduced from ref.[48]).

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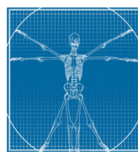

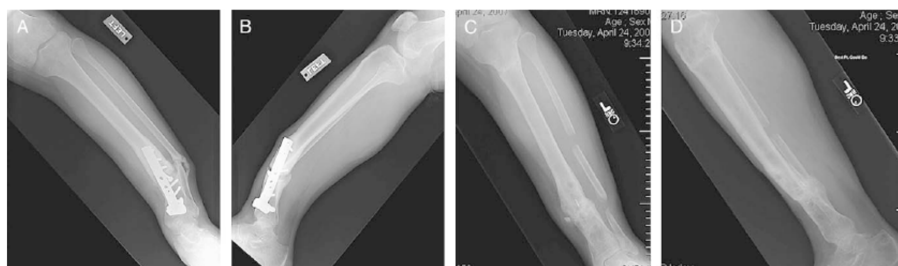

Figure 4: Example of MSC use in non-union. (a, b) A 43-year-old male with distal tibial non-union after open reduction and internal fixation. (c, d) One hundred twenty-three days after revision with circular frame, debridement and MSC grafting. The result was correction of the deformity and complete consolidation of the previous non-union (Reproduced from ref.[39]).

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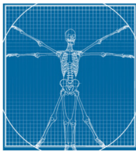

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