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Commentary: Systematic review on the application of 3D-bioprinting technology in orthoregeneration: current achievements and open challenges

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The potential impact of bioprinting on orthoregeneration is significant and could revolutionize the way that damaged or diseased tissue in the musculoskeletal system is treated. We sought to gain an understanding of the current state (2011-2022) of this rapidly changing field. Our systematic review focused on bioprinting of cartilage, bone, blood vessels, and composite tissues of bone and cartilage (osteochondral) and vascularized bone[1]. For reviews on bioprinting of other musculoskeletal tissues such as skeletal muscle, tendon and ligament please refer to those by Samandari et al.[2], Khalak et al.[3] and Bakirci et al.[4] respectively.

3D bioprinting is a relatively new field, with the first example being reported in 2003[5,6]. The databases, PubMed and Web of Science were queried with terms such as "3D bioprinting cartilage" and "3D bioprinting bone" returning over 1300 results. These were systematically filtered to remove duplicates, reviews, non-primary research and articles which did not meet the definition of 3D bioprinting – printing with live cells. Our systematic review included 114 articles, 52 of which were on cartilage, 35 on bone, 11 on vasculature, 10 on osteochondral and 12 on vascularized bone. One of the main advantages of bioprinting is the ability to create complex, three-dimensional tissue structures with high levels of precision. This allows for the creation of replacement tissue tailored to the patient's specific needs, potentially resulting in better outcomes and reduced complications. This, custom manufacture is one of the greatest strengths of 3D bioprinting. We see the potential for 3D bioprinting to address major challenges in orthoregeneration of large or complex defects, poor regenerative capacity of cartilage, the need for vascularity in a large implant, and the ability to promote regenerative repair. Therefore, development of printable bioinks is an active area of investigation for all tissues. Gelatin, alginate, and their derivatives, such as gelatin methacrylate were the most used bioinks across all tissues due to their ease of use and high biocompatibility.

Another potential benefit of bioprinting is its ability to use the patients' own cells, known as autologous cells, in the tissue production process. This eliminates the need for tissue matching and the associated risks of rejection, potentially improving the success rate of transplant procedures and reducing the need for immunosuppressive drugs. Just over half of the studies (56%) used human cells, with mesenchymal stromal cells (MSCs) being the most prevalent cell type. The remaining studies used cells isolated from a

range of animals including mouse, pig, rabbit, rat, cow and horse. Animal studies used a broader range of cell types. These studies demonstrate both the applicability to human treatment, through the use of human cells, and the potential for autologous transplant in several of the animal studies[1].

Looking at the outcome metrics used, measurement of cell viability was the predominant assay across all tissues[4]. The next most common analysis was to look at gene expression, defining increases or expression of genes relevant to each tissue type. Many researchers also looked at biochemical characterization of the tissue, assessing glycosaminoglycan, collagen and calcium content. Histology, immunohistochemistry and electron microscopy techniques were all used to determine localization of relevant proteins and tissue structure. A diverse range of characterization techniques have been used within each tissue, more extensive common metrics within each tissue would be beneficial for comparison between studies.

Many cartilage studies assessed the biomechanics of the bioprinted tissue, primarily using unconfined compression measurements. In cartilage bioprinting we see the opportunities or challenges to be: achieving a zonal architecture; defining what the optimal stiffness is for a construct at implantation to achieve integration and regeneration; long term studies in large animal models; repair in a load bearing area; more extensive repair – total joint resurfacing; rehabilitation regime optimization/ implementation. However, even with these challenges there has been significant success with focal repair having been achieved in rabbit studies[7,8]. These two studies are among the few studies that have progressed into *in vivo* experiments.

Significant preclinical success has also been achieved in the area of bone regeneration using 3D bioprinting, particularly in cranial defect repair. In bone bioprinting, a common area of innovation is the incorporation of inorganic particles such as hydroxyapatite or bioactive glasses into the bioink. Many similar opportunities/ challenges exist within bone 3D bioprinting as there are in cartilage 3D bioprinting: determining an optimal tissue strength with/without degradable support to allow growth and maturation of the native bone; tuning the degradation of any support material to stimulate bone regrowth; incorporation of and tuning of growth factor release to achieve integrated repair tissue. Surprisingly, mechanical assessment of the bone tissue either at implantation or after maturation in vivo was lacking, with no 3-point bending assessments having been reported.

Orthoregenerative vascular 3D bioprinting was the most immature area we reviewed. Little preclinical translation has been achieved so far. The production of vascular channels has been achieved, facilitated by both co-axial printing and support or sacrificial structures. Significant challenges in cell production and differentiation are evident. Human umbilical vein endothelial cells (HUVECs) were the most common cell type used often combined with support cells including MSCs. Microvessels were not printed but had formed in some culture experiments. To address the formation of capillary structures we recently developed a bioprinting method using capillary alginate gel[9]. Several studies used histology to assess their constructs[2]. Some groups also looked at perfusion and leakage from the vessels[6]. None of the groups assessed burst pressure, something that has been performed in other tissue engineered vessel studies.

Composite tissues were the most progressed to pre-clinical in vivo studies. Osteochondral constructs were predominantly made with alginate or gelatin methacrylate often as a composite with a polymeric scaffold of poly(caprolactone) or poly(lactic acid). MSCs were the most commonly used cell for both the osteo and chondral components. Implants were assessed for viability, histology and gene expression for each compartment. Vascularized bone has also seen some success with cranial and femoral defects being filled. These tissues had the greatest diversity of bioinks and commonly included MSCs and HUVECs. Outcome metrics again included viability, histology and qPCR. Interestingly, we did not see any bioreactor cultured tissue within the studies we reviewed which could be an area for development.

Overall, the use of bioprinting in orthoregeneration has the potential to greatly improve the treatment of damaged or diseased tissue in the musculoskeletal system. It offers the potential for more precise and personalized tissue production, greater control over tissue manufacturing, faster and more efficient tissue production, and improved success rates for tissue regeneration procedures. Funding for musculoskeletal research needs to be a focus enabling development of these complex models. Co-ordination of regulatory approval pathways with the developing technology is critical in translating these breakthrough technologies to the clinic. 3D bioprinting is a multi-disciplinary science requiring diverse connections between engineers, biologist and clinicians. While the technology is still in its early stages and further research is needed, the potential impact of bioprinting on orthoregeneration is significant and worth exploring further.

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